Original

Establishment and Characterization of the Human Tongue Squamous Cell Carcinoma Cell Line

NOKT-1

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Abstract: The squamous cell carcinoma cell line NOKT-1 was successfully established from the right tongue of a 74-year-old Japanese man. Pathological diagnosis of the original tumor was moderately differentiated squamous cell carcinoma. NOKT-1 cells were transplanted subcutaneously into nude mice and xenograft was formed. In addition, the NOKT-1-XG cell line was established from the transplanted tumor of NOKT-1 cells. NOKT-1 cells and NOKT-1-XG cells were epithelial neoplastic and pleomorphic cells, which were similar. Immunocytochemistry revealed that NOKT-1 and NOKT-1-XG cells were CK17 and human mitochondria positive. To authenticate the NOKT-1 cell line and NOKT-1-XG cell line, we examined cross-contamination with other cell lines using short tandem repeat analysis, the results of which showed that NOKT-1 and NOKT-1-XG are new cell lines. Four of the 16 loci, corresponding to 25%, were different between these two cell lines, which indicates that the NOKT-1 genome was altered by transplantation. Moreover, in AM, NOKT-1 did not have a Y chromosome, whereas NOKT-1-XG had. Despite the genetic differences, a collagen gel droplet-embedded culture drug sensitivity test demonstrated that NOKT-1 cells derived from the original tumor and the NOKT-1-XG cell line had the same sensitivity. This cell line could be very useful for the development of immunotherapy and chemotheraphy regimens and research on cancer etiology.

Key words: Cell line, Tongue squamous cell carcinoma, Establishment, Xenograft, Collagen gel droplet-embedded culture drug sensitivity test (CD-DST), Anticancer drug sensitivity test

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy in the head and neck, accounting for over 90% of all oral malignancies1. Nearly 50% of OSCC patients, with the exception of those with distant metastases, have clinical or pathological lymph node metastases, and metastases to the lungs and bones have also been reported2,3. Several tongue-derived squamous cell carcinoma cell lines have been described, and there is an association between tongue cancer and excessive alcohol consumption, smoking history, and human papillomavirus infection4. Commonly used human tongue squamous cell carcinoma cell lines include the highly invasive SAS cells and less invasive clones as well as the highly metastatic LMF3, LMF4, and LMF5 cell lines derived from human oral tongue squamous cell carcinoma. HSC-3 cells have also been established and are frequently used5-10. However, few reports have compared established tumor cell lines with the sensitivity of the original tumor to anticancer drugs, and no previous reports have compared xenografted cells.

The CD-DST method is an anticancer drug susceptibility test conducted in vitro, which combines three-dimensional culture of collagen gel with an image colorimetric assay11-13. This method has advantages in that it allows testing using smaller quantities of cells compared with conventional assays. Furthermore, it eliminates the effects of fibroblasts that can adulterate samples at the time of harvesting and allows the evaluation of physiological drug concentrations14-16. In addition, the CD-DST method can be evaluated by combining multiple anticancer agents. Therefore, CD-DST is a useful method for predicting the efficacy of anticancer drug administration. Previous reports have shown that CD-DST has a clinically successful measurement rate of 87.5% in colorectal cancer, 79.2% in lung cancer, and 84.3% in breast cancer17-19. Similarly, as previously reported, sensitivity tests using cisplatin, fluorouracil, or docetaxel at OSCC showed a success rate of 81.8% in primary culture18. In addition, we conducted anticancer drug susceptibility tests, including those for cetuximab, using OSCC samples with success rates of 83.3% for primary tumors, 100% for metastatic lymph nodes, and 84.0% overall20.

We established a new NOKT-1 cell line from tongue squamous cell carcinoma and used the original tumor and cells xenografted into mice (NOKT-1-XG cells) in a three-dimensional collagen gel droplet-embedded culture drug sensitivity test (CD-DST). We performed single agent contact and additional contact with cisplatin (CDDP), docetaxel (DOC), and 5-flourouracil (5-FU) to verify the change in sensitivity. In addition, regarding cell lines, 18%-36% of cell lines reported misidentification of species or cell type21, whereas 18% of cell lines deposited with the German Cell Bank were misidentified. Moreover, a previous report also indicated that cell cross-contamination had taken place22. Therefore, we
examined the NOKT-1 cell line for the presence of cross-contamination. In addition, one recent concept proposes that changes may occur during cell culture due to the evolution of the cells in vitro. It has also been reported that genetic and transcriptional evolution alters the drug response of cancer cell lines.  

Cell lines derived from human tumors are very useful for basic and clinical studies such as those focused on elucidating the mechanism of keratinization and the development of chemotherapy or immunotherapy. As the NOKT-1 cell line has anticancer drug sensitivity similar to that of the original tumor, important information about the biological response of specific types of cancer, as well as the therapeutic response, such as anticancer drug sensitivity or immune response, can be obtained. Thus, the NOKT-1 cell line is expected to contribute to the development of effective therapies.

Materials and Methods

Medical history

A 74-year-old Japanese male visited the Oral and Maxillofacial Surgery Department of Niigata Hospital, Niigpon Dental University (Niigata, Japan) for oppressive right side tongue pain. The tumor was 30 × 30 mm in size, and following biopsy, the pathological diagnosis was moderately differentiated SCC of the right tongue. This study was approved by the Ethics Committee of the Niigpon Dental University, School of Life Dentistry at Niigata, Japan (approval no. ECNG-H-119). Informed consent was obtained from the participant.

Histopathological examination

The original tumor and xenotransplanted tumor were fixed with a 10% formalin neutral buffer solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 4°C for 7 days. Following dehydration with graded ethanol, the tissues were immersed in xylene and then embedded in paraffin. Subsequently, 5 μm-thick sections were prepared for hematoxylin and eosin (HE) staining.

Establishment of cell lines (NOKT-1 and NOKT-1-XG)

Small amounts of the tumor tissue from original tumor or xenotransplanted tumor extracted by biopsy were rinsed several times with medium and cut into small pieces with a razor blade. Approximately half of the tissue was used for the anticancer drug sensitivity CD-DST. The fragments were dissociated in 0.1% trypsin (BD, MD, USA) and 0.02% EDTA/PBS (−) for 30 min at 37°C. After pipetting, dissociated cells and small fragments were centrifuged at 430 × g for 5 min at room temperature. The cell pellet was resuspended with growth medium (GM) and cultured in a CO2 incubator (4.7% CO2 and 95.3% O2) at 37°C. Cell cultures were passaged at split ratios of 1:3 at confluency. Aliquots of cultured cells were cryopreserved in GM containing 10% dimethyl sulfoxide (Merck KGaA, Darmstadt, Germany) and stored in liquid nitrogen after every five passages. In addition, NOKT-1-XG cells were established by the same method.

Population doubling time, saturation density, and plating efficiency

Cells at passages 15 and 30 were used to estimate the population doubling time and saturation density. The cells (a total of 2 × 105 cells/ml medium) seeded into each 35-mm dish were cultured for 12 days at 37°C. After seeding, samples were obtained at 48-h intervals from three dishes, and cell counts were performed using a hemocytometer. Growth curves were drawn as the mean of three dishes, and they were used to calculate the population doubling time and saturation density. The plating efficiency is defined as the number of cells that grow into colonies per 100 seeded cells, i.e., the proportion of cells that attach and grow to the number of cells originally plated expressed as a percentage.

Electron microscopy

NOKT-1 cells were cultured on 35-mm dishes. Cultured cells were rinsed with Hank’s balanced salt solution and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 30 min. The cells were rinsed with the same buffer and post-fixed with 1% osmium tetroxide. After dehydration with graded ethanol and propylene oxide, the cells were embedded in Epon. Ultrathin sections were prepared using an LKB Nova ultra-microtome and stained with uranyl acetate and lead citrate. The sections were observed with a Hitachi HU-12A microscope.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured cells using ISOGEN II (Nippon gene Co., Ltd, Tokyo, Japan). Using 1 μg of total RNA, cDNA was synthesized with a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific K.K., Tokyo, Japan), and amplification was performed using a PCR Supermix Platinum kit (Life Technologies Japan Ltd.). PCR amplification was performed using 2720 thermal cycler annealing for each gene, as shown in Table 1. PCR products were analyzed by electrophoresis on a 2% (w/v) agarose gel and stained with ethidium bromide.

Immunohistochemistry and immunocytochemistry

In each sample (NOKT-1, Xenotransplanted tumor, NOKT-1-XG), we performed immunohistochemistry and immunocytochemistry of CK17 (1:20, E3; Dako Denmark A/S, Glostrup, Denmark), CK13 (1:400, DE-K13; Dako Denmark A/S), Ki67 (1:100, ab15580; Abcam PLC, Cambridge, UK), Human mitochondria (1:300, MAB1273; Merck), and p53 (1:100, Pab 240; Abcam), and the samples were observed under a light microscope (BZ-9000, KEYENCE Co., Ltd, Osaka, Japan). For fluorescence immunocytochemistry NOKT-1 cells or NOKT-1-XG cells were fixed with absolute methanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at −30°C for 15 min and then incubated in Blocking One Histo (Nacalai Tesque, Inc., Kyoto, Japan) for 10 min at room temperature. Cultures were incubated with primary antibodies overnight at 4°C and subsequently incubated with the following secondary antibodies for 30 min in the dark at room temperature: Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G (IgG)
(diluted at 1:1,000; Life Technologies). Nuclei were stained with VECTASHIELD® HardSet™ Mounting Medium with DAPI (Vector Laboratories, Inc., CA, USA). Images were obtained under a confocal laser scanning microscope (LSM-510; Carl Zeiss Co., Ltd., Jena, Germany).

Anticancer drug susceptibility tests (CD-DST method) of in vitro cultured NOKT-1 cells derived from the original tumor and NOKT-1-XG cells derived from xenotransplanted tumor

CD-DST was performed according to the method reported by Kobayashi et al. Human tumor cell primary culture system kit (Primaster®; Kurabo Industries Ltd. Osaka, Japan) was used. Each sample was washed with 50 ml saline containing 0.5 mg/ml kanamycin, 2.5 μg/ml amphotericin B, 1.0 mg/ml penicillin and treated with Dispersion Enzyme Cocktail EZ (Primaster® reagent). The resulting cell suspension sample was inoculated into a collagen gel-coated flask (CG flask, Primaster® device) overnight in preculture medium PCM-1 (Primaster® content) at 37°C in 5% CO₂. Next, the collagen gel was digested with 0.05% EZ to obtain live cancer cells. Type I collagen (solution A), 10× F-12 medium (solution B), and reconstitution buffer (solution C) were mixed in ice water at a ratio of 8:1:1 (Primaster® content). The cancer cell suspension was added to the collagen solution at a final density of 1×10⁵ cells/ml. Three drops of collagen-cancer cell mixture (30 μl/drop) were placed in a six-well plate on ice and allowed to solidify at 37°C in a CO₂ incubator. The final cell concentration was approximately 3×10³ cells per collagen gel droplet. After 1 h, DF medium containing 10% fetal bovine serum was overlaid on each well, and the plate was placed in a CO₂ incubator at 37°C overnight. The anticancer drugs were added

| Gene      | Primer sequences, 5′ to 3′ | Product size (bp) | Annealing temp.(℃) | GenBank accession number |
|-----------|----------------------------|------------------|--------------------|-------------------------|
| GAPDH     | S : GTCAAGGCTGAGAACGGGAA   | 613              | 55                 | NM_001256799.1          |
|           | A : GCTTCACCACCTTTGTGATG  |                  |                    |                         |
| keratin 17| S : AAAAGATGCTCACAGCCAC   | 168              | 60                 | NM_000422.3             |
|           | A : CCATTGATGTCGGCCTCC    |                  |                    |                         |
| Ki-67     | S : CGTCCAGTGGAAGATTTGT   | 143              | 59                 | NM_001145966.2          |
|           | A : CGACCCCCGCTCTTGTGATA  |                  |                    |                         |
| TP53      | S : TACAGGCGACCTAGGTTT    | 572              | 55                 | X60011                  |
|           | A : CCTTCTTGCGGAGATCTCT   |                  |                    |                         |

Figure 1. Overview of the collagen gel droplet-embedded culture drug sensitivity test method
with the following final concentrations and incubated for 24 h: 0.7 μg/ml fluorouracil (5-FU), 0.1 μg/ml docetaxel (DOC), and 0.5 μg/ml cisplatin (CDDP). For multi-drug anticancer drug contact, each concentration was combined in the same well. The concentration of each anticancer agent in the medium was determined to show an area under the curve similar to that in serum during the first 24 h of intravenous administration at the standard clinical dose of the corresponding drug. After removing the medium containing the anticancer drug, each well was rinsed twice with 3 ml of Hanks’ balanced salt solution, overlaid with 4 ml of PCM-2 medium (Primaster® serum-free medium), and incubated for another 7 days. At the end of the incubation period, a neutral red solution was added to each well to a final concentration of 50 μg/ml. Colonies of collagen gel droplets were stained for 2 h, each collagen droplet was fixed with 10% neutral buffered formalin, washed with water, air dried, and quantified by optical density image analysis using the Primage System (Solution Systems, Inc., Tokyo, Japan) (Fig. 1).

In vitro susceptibility was calculated as the T/C ratio of optical density, where T represents treated sample and C represents control. A T/C ratio of less than 50% was considered to be highly sensitive in vitro. Tumor cell colony volume ratio (tumor growth rate) at time 0 was calculated from the control group. Regardless of the amount of tumor cell colonies in the control group, a value below 0.8 was considered a culture failure (low growth rate).

Short tandem repeat analysis for authentication
To authenticate the established cell line, we analyzed short tandem repeats (STRs) of the NOKT-1 cells and NOKT-1-XG cells. The data were analyzed, and the STR profiles were compared to those recorded in public cell banks, such as the ATCC, DSMZ, RIKEN BRC Cell Bank, and JRCB, for reference matching. The genomic DNA of NOKT-1 and NOKT-1-XG cells was analyzed by STR-PCR, and the results were collated within the database (Promega K.K., Tokyo, Japan).

Confirmation of mycoplasma contamination
Mycoplasma infection of NOKT-1 and NOKT-1-XG cells was assessed by real-time PCR, which was used to test 10 types of mycoplasma and 1 type of acholeplasma (Funakoshi Co., Ltd., Tokyo, Japan).

Results
Histopathological diagnosis
Pathological diagnosis was moderately differentiated SCC, wherein cancer cells were found even in muscle layers. Tumor cells had pleomorphic features and showed clear nucleoli, with mitosis appearing occasionally. Lymphocytic infiltration observed in the interstitial tissue

Figure 2. HE staining of the original tumor (a, b) and xenografted tumor tissue (c, d). The original tumor is generally a moderately differentiated SCC with several cancerous pearls (a. arrows). a*, stratified squamous epithelium; b, high magnification of enclosed area in (a). Xenografted NOKT-1 cell tumors are moderately differentiated squamous cell carcinomas with many nuclear atypia similar to the original tumors, but no cancer pearls (c). d, high magnification of enclosed area in (c).
was of an intermediate degree. Cancer pearls were found in some of the tumors (Fig. 2a, b). NOKT-1 cells were transplanted subcutaneously into nude mice, and xenograft was formed. The size of xenograft was approximately 1 cm at 7 weeks after transplantation. The pathological and histological types were similar to those of the original tumor, and the xenograft was diagnosed as moderately differentiated SCC. Unlike the original tumor, no cancer pearls were found in the xenografts. (Fig. 2c, d). In immunohistochemical staining, the xenotransplanted tumor of NOKT-1 cells was positive for CK-17, Ki67, p53, and human mitochondria (Fig. 3). Similar results were obtained for all three xenografts.

**Morphological aspects of NOKT-1 cells**

The time between the initial culture and first passage in the culture of NOKT-1 was 4 months. NOKT-1 cells and NOKT-1-XG cells were epithelial neoplastic and pleomorphic cells (Fig. 4a, b), cell deformity was obvious, and the cell arrangement resembled a jigsaw puzzle. Electron microscopy revealed that the cells had a pseudomorphic nucleus and remarkable nucleoli. The nucleus margin has many malformed cells, and some cells have lysosomes. Adhesion between cells was observed due to interdigitation (Fig. 4c), and very well-developed microvilli and interdigitation were also observed (Fig. 4d). In addition, cells with many

Figure 3. Immunohistochemical staining. The xenotransplanted NOKT-1 cell tumor was positive for CK17, Ki67, p53, and human mitochondria. As a negative control, samples were stained without primary antibodies. Bars 100 μm

Figure 4. The epithelial cells NOKT-1 and NOKT-1-XG had neoplastic and pleomorphic features in phase-contrast microscopy (a, b). The nucleus margin is mostly malformed cells, some of which have lysosomes (arrowheads). Adhesion between cells due to interdigitation (arrow) is observed (c). Very well-developed microvilli and interdigitation (arrows) is observed. Cells with many lysosomes (arrowheads) are also observed (d). Intercellular desmosome (arrow), slightly expanded rough endoplasmic reticulum (arrowhead), free ribosome, and irregular mitochondria (white arrow) are observed (e).
lysosomes were found (Fig. 4d), in addition to intercellular desmosomes, slightly enlarged rough endoplasmic reticulum, free ribosome, and irregular mitochondria (Fig. 4e).

**Biological characteristics of the NOKT-1 cell line**

Population doubling time, saturation density, and the plating efficiencies of NOKT-1 cells at passages 15 and 30 were 27 and 23 h, $6.9 \times 10^4$ and $8.7 \times 10^5$ cells/cm$^2$, and 25% and 23% respectively.

**Identities of the NOKT-1 and NOKT-1-XG cell lines**

NOKT-1 cells were CK17 and human mitochondria positive and CK13 negative by immunocytochemistry (Fig. 5a), as were NOKT-1-XG cells (Fig. 5b). Expression of CK17, Ki67, and p53 was confirmed in NOKT-1 and NOKT-1-XG cells by RT-PCR analysis (Fig. 6a, b).

**Cross-contamination test**

It was confirmed that NOKT-1 cells did not correspond to any cells in the JCRB Cell Bank database (a database of data from the ATCC, DSMZ, RIKEN BRC Cell Bank, and JCRB Cell Bank) (Table 2). Of the
16 foci in the NOKT-1 cell line, heterozygosity was detected in only 3 loci, while the other 13 loci showed one allelic pattern including AM. The Y chromosome was not detected. In NOKT-1, the peak derived from Y was recognized as noise level and was not reflected in the results. The slight peaks of Y and X were about 1:60, which was about 1:60. In the NOKT-1-XG analysis, the Y peak was slightly higher than the X peak, and the result was 1:1 because it was derived from normal males. Of the 16 foci in the NOKT-1-XG cell line, heterozygosity was detected in only 3, while the other 13 loci showed one allelic pattern.

Anticancer drug susceptibility test of NOKT-1 and NOKT-1-XG cells

We conducted anticancer drug susceptibility tests on the original tumor, established NOKT-1 cells, and NOKT-1-XG cells derived from the graft of NOKT-1 cells in nude mice. In single-agent contact with CDDP, 5-FU, and DOC, the sensitivity was consistent with both the standard contact concentration and extreme contact. In addition, the standard contact concentration and extreme contact with CDDP + 5-FU + DOC were all sensitive to original tumor, established NOKT-1 cells, and NOKT-1-XG cells transplanted into mice (Table 3).

### Table 3. Anticancer drug susceptibility test

| Drug          | original tumor (T/C %) | NOKT-1 cells (T/C %) | NOKT-1-XG cells (T/C %) |
|---------------|-----------------------|----------------------|-------------------------|
| CDDP          | 69.2                  | 92.7                 | 85.4                    |
| DOC           | 16.0                  | 7.2                  | 31.0                    |
| 5-FU          | 72.0                  | 95.0                 | 72.0                    |
| CDDP (Extreme)| 60.9                  | 57.3                 | 58.5                    |
| DOC (Extreme) | 14.6                  | 0.2                  | 28.4                    |
| 5-FU (Extreme)| 68.4                  | 90.3                 | 63.6                    |
| CDDP+5-FU+DOC | 10.8                  | 8.6                  | 37.4                    |
| CDDP+5-FU+DOC (Extreme) | 7.6    | 0.2                  | 35.2                    |

In vitro drug sensitivity was defined as sensitive when the T/C rate was ≤ 50%.

#### Mycoplasma infection

NOKT-1 and NOKT-1-XG were not infected with mycoplasma according to real-time PCR.

### Discussion

Establishment of an oral cancer cell line from the primary site is particularly difficult, and few successful derivations have been reported. One of the reasons for the difficulty is that bacterial and/or fungal contaminations occur frequently in the primary oral tumor. The majority of previously reported cell lines were established from lymph node metastatic foci or transplanted tumors in nude mice. We have successfully established a tongue-derived cell line without the need for xenotransplantation. However, no previous report has examined whether the cell line has the same sensitivity as the original tumor to the anticancer drug.

In this study, we performed a sensitivity test for anticancer drugs using the CD-DST from biopsy materials, established a NOKT-1 cell line from a part of the biopsy materials. In addition, nude mouse xenotransplantation was performed to establish NOKT-1-XG. CD-DST was also performed on established NOKT-1 and NOKT-1-XG cell lines. CDDP and 5-FU were in contact with the normal dose and 5 times the concentration, and the primary tumor, NOKT-1, and NOKT-1-XG were all in agreement with low sensitivity because the T/C% was 50%. In addition, DOC was in contact with the normal and 5 times the concentration, and the primary tumor, NOKT-1, and NOKT-1-XG were all highly sensitive and consistent because the T/C% was 50% or less. Takahashi et al. reported that the sensitivity of NOCS-1 cell lines established from mandibular gingival carcinoma and cell lines established by xenotransplantation of the cell lines were similar in anticancer drug susceptibility tests. However, in all previous reports, the sensitivity of anticancer drugs is limited to single agents. In the case of clinical administration, administer a combination of multiple drugs. Therefore, we tested simultaneous contact with multiple anticancer agents, which is an advantage of the CD-DST method, and further compared susceptibility. The anticancer drug sensitivity of CDDP + 5-FU + DOC under normal contact concentrations was 10.8% for the T/C% of the primary tumor, 8.6% for NOKT-1, and 37.4% for NOKT-1-XG. Suzuki et al. established cell lines from 15 patients with papillary tubular cancer and compared their susceptibility with that of primary tumors using anticancer drug susceptibility tests. The cell lines and primary tumors demonstrated consistent susceptibility in 13 patients, while the primary tumor and established cell lines exhibited different sensitivities in 2 patients. From this report, it can be assumed that the primary tumor and the established cell line do not necessarily exhibit the same susceptibility. This suggests that data showing similar susceptibility between a cell line and the primary tumor and between NOKT-1, and even xenografted cells, to the established NOKT-1-XG cell line would be valuable.

To authenticate the NOKT-1 and NOKT-1-XG cell line, we examined cross-contamination with other cell lines using STR analysis, the results of which showed that NOKT-1 and NOKT-1-XG is a new cell line. However, in such cases, STR analysis cannot assess cross-contamination between cell lines. However, 4 of the 16 loci, corresponding to 25%, were different between these cells, which indicates that the NOKT-1 genome was altered by transplantation. In addition, NOKT-1 cells do not have a Y chromosome, but NOKT-1-XG cells acquired a Y chromosome. It is not clear how the Y chromosome was acquired, but we predict that the NOKT-1 strain is heterogeneous and that a very small subpopulation of NOKT-1 cells with a Y chromosome is present in mice.
with NOKT-1-XG. This suggests that tumors derived from this cell line may have been selectively formed.

In clinical practice, it is common to administer a combination of drugs. However, previous studies have not reported primary tumors and cell lines in combination with multiple drugs as in our study. In addition, NOKT-1 and NOKT-1-XG showed consistent sensitivity between a single drug and multiple drug combinations, despite genetic alterations. This can contribute to the development of drug discovery and future research.

Xenotransplantation of NOKT-1 cells into nude mice produced histopathological tumors that were same as the original tumor. In addition, the NOKT-1 and NOKT-1-XG cell line seems to inherit the original tumor properties and anticancer drug sensitivity. This cell line could be very useful for studying cancer etiology and developing drug discovery and immunotherapy regimens.

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Conflict of Interest

No competing financial interests exist. The authors declare that there are no conflicts of interest related to the publication of this study.

References

1. Massano J, Regateiro F, Januario G and Ferreira A. Oral squamous cell carcinoma. review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102: 67-76, 2006
2. Som PM. Detection of metastasis in cervical lymph nodes. CT and MRI criteria and differential diagnosis. Am J Radiol 158: 961-969, 1992
3. Yasui A, Okada Y, Mataga I and Katagiri M. An analysis of distant metastasis in oral squamous cell carcinoma. J Hard Tissue Biol 19: 27-32, 2010
4. Gionanni J, Fischel J-L, Lambert J-C, Demard F, Mazeau C, Zanghellini E, Ettore F, Formento P, Chauvel P, Lalanne C-M and Courdi A. Two new human tumor cell lines derived from squamous cell carcinomas of the tongue: establishment, characterization and response to cyto toxic treatment. Eur J Cancer Clin Oncol 24: 1445-1455, 1988
5. Chew E-C, King W-K, Hou H-J and Yam H-F. Establishment and characterization of two new cell lines derived from squamous cell carcinoma of the tongue in Chinese patients. Anticancer Res 12: 1627-1634, 1992
6. King W-W-K, Lam P-K, Huang D-W-S-P, Liew C-T and Li A-K-C. Establishment and characterization of a human cell line from a squamous carcinoma of the tongue. Clin Otalaryngol 20: 15-20, 1995
7. Kim K-M, Park E-J, Yeo Y-H, Cho K-J and Kim M-S. Establishment of a novel HPV-negative and radiosensitive head and neck squamous cell carcinoma cell line. Head and Neck 38: 542-551, 2016
8. Takahashi H, Ishikawa H, Mataga I and Tanaka A. Establishment and characterization of human lingual squamous cell carcinoma cell lines designated Nialym derived from metastatic foci of lymph node, and Nialymx derived from transplanted tumor of Nialym cells. Hum Cell 28: 143-153, 2015
9. Okumura K, Konishi A, Tanaka M, Kanazawa M, Kogawa K and Niitsu Y. Establishment of high- and low-invasion clones derived for a human tongue squamous-cell carcinoma cell line SAS. Cancer Res Clin Oncol 122: 243-248, 1996
10. Momose F, Araida T, Negishi A, Ichijo H, Shiota S and Sasaki S. Variant sublines with different metastatic potentials selected in nude mice from human oral squamous cell carcinomas. J Oral Pathol Med 18: 391-395, 1989
11. Kobayashi H, Tanisaka K, Doi O, Kodama K, Higashiyama M, Nakagawa H, Miyake M, Taki T, Hara S, Yasutomi M, Hanatani Y, Kotake K and Kubota T. An in vitro chemosensitivity test for solid human tumors using collagen gel droplet embedded cultures. Int J Oncol 11: 449-455, 1997
12. Kobayashi H: Development of a new in vitro chemosensitivity test using collagen gel droplet embedded culture and image analysis for clinical usefulness. Recent Results Cancer Res 161: 48-61, 2003
13. Sakuma K, Tamura R, Noda N, Mizutani M, Yamaguchi A and Tanaka A. Collagen gel droplet-embedded culture drug sensitivity testing in hard palate cancer-predicted antitumor efficacy of cetuximab: A case report. Mol Clin Oncol 7: 637-641, 2017
14. Sakuma K, Tanaka A and Mataga I. Collagen gel droplet-embedded culture drug sensitivity testing in squamous cell carcinoma cell lines derived from human oral cancers: Optimal contact concentrations of cisplatin and fluorouracil. Oncol Lett 12: 4643-4650, 2016
15. Sakuma K, Tamura R, Hanyu S, Takahashi H, Sato H, Oneyama T, Yamaguchi A and Tanaka A. Clinical study on collagen gel droplet-embedded culture drug sensitivity test for multidrug combination chemotherapy and super selective intra-arterial infusion chemoradiotherapy in oral squamous cell carcinoma. Mol Clin Oncol 7: 1021-1026, 2017
16. Sakuma K, Hanyu S, Takahashi H and Tanaka A. Identification of the optimal cetuximab concentration that is effective against oral squamous cell carcinoma in collagen gel droplet embedded culture drug sensitivity testing. Mol Clin Oncol 12: 51-56, 2020
17. Takamura Y, Kobayashi H, Taguchi T, Motomura K, Inaji H and Noguchi S. Prediction of chemotherapeutic response by collagen gel droplet-embedded culture drug sensitivity test for colorectal cancer using collagen-gel droplet embedded cultures. Kurume Med J 46: 163-166, 1999
18. Kawamura M, Inoue Y, Oyama T and Kobayashi H. Chemosensitivity test for unresectable non-small cell lung cancer. Nihon Geka Gakkai Zasshi 103: 229-232, 2002 (In Japanese)
19. Kawamura M, Inoue Y, Oyama T, and Kobayashi H. Chemosensitivity test for unresectable non-small cell lung cancer. Nihon Geka Gakkai Zasshi 103: 229-232, 2002 (In Japanese)
20. Identity crisis. Nature 457: 935-936, 2009
21. Chatterjee R. Cell biology. Cases of mistaken identity. Science 315: 928-931, 2007
22. Ben D-U, Siranosian B, Ha G, Tang H, Oren Y, Hinohara K, Strathdee C-A, Dempster J, Lyons N-J, Burns R, Nag A, Kugener G, Cimini B, Tsvetkov P, Maruva K-Y-E, O’Rourke R, Garrity A, Tubelli A-A, Bandopadhayay P, Tsherniak A, Vazquez F, Wong B, Birger C, Ghandi M, Thorner A-R, Bittker J-A, Meyerson M, Getz G, Beroukhim R and Golub T-R. Genetic and transcriptional evolution alters cancer cell line drug response. Nature 560: 325-330, 2018
23. Kawakami M, Ishikawa H, Tachibana T, Tanaka A and Mataga I. Functional trans-plantation of salivary gland cells differentiated from mouse early ES cells in vitro. Hum Cell 26: 80-90, 2013
24. Suzuki M, Ishikawa H, Kawakami M, Nakahara T, Tanaka A and Mataga I. Establishment and characterization of METON myoepithelioma cell line derived from human palatal myoepithelioma: apical reference to the diversified differentiation potential. Hum Cell 26: 170-176, 2013

25. Ji Z-W, Oku N, Umeda M and Komori T. Establishment of an oral squamous cell carcinoma cell line (NOS-1) exhibiting amplification of the erbB-1 oncogene and point mutation of p53 tumor suppressor gene: its biological characteristics and animal model of local invasion by orthotopic transplantation of the cell line. Oral Oncol 37: 386-392, 2001

26. Rupniak H-T, Rowlatt C, Lane E-B, Steele J-G, Trejdosiewicz L-K, Laskiewicz B, Povey S and Hill B-T. Characteristics of four new human cell lines derived from squamous cell carcinomas of the head and neck. J Natl Cancer Inst 75: 621-635, 1985

27. Grooijmans R-P, Schwachofer J-H, Hoogenhout J, Merkx G, Poels L-G, Jap P-H, Ramaekers F-C, Mijnheere E-P, Elprana D and Thomas C-M. Cell lines of human oral squamous-cell carcinomas retaining their differentiated phenotype. Int J Cancer 45: 945-951, 1990

28. Takahashi H, Watanabe M, Ohyama A, Toyomura J, Tachibana T, Tanaka A and Ishikawa H. Establishment and characterization of human gingival squamous cell carcinoma cell line NOCS-1. Hum Cell 32: 75-82, 2019

29. Suzuki M, Ishikawa H, Tanaka A and Mataga I. Anticancer drug sensitivity testing using an oxygen electrode apparatus. Hum Cell 23: 103-112, 2010
