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Establishment and characterization of an oral tongue squamous cell carcinoma cell line from a never-smoking patient

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ABSTRACT

Objective: The rising incidence of oral tongue squamous cell carcinoma (OTSCC) in patients who have never smoked and the paucity of knowledge of its biological behavior prompted us to develop a new cell line originating from a never-smoker.

Materials and methods: Fresh tumor tissue of keratinizing OTSCC was collected from a 44-year-old woman who had never smoked. Serum-free media with a low calcium concentration were used in cell culture, and a multifaceted approach was taken to verify and characterize the cell line, designated UCSF-OT-1109.

Results: UCSF-OT-1109 was authenticated by STR DNA fingerprint analysis, presence of an epithelial marker EpCAM, absence of human papilloma virus (HPV) DNA, and SCC-specific microscopic appearance. Sphere-forming assays supported its tumorigenic potential. Spectral karyotype (SKY) analysis revealed numerical and structural chromosomal abnormalities. Whole-exome sequencing (WES) identified 46 non-synonymous and 13 synonymous somatic single-nucleotide polymorphisms (SNPs) and one frame-shift deletion in the coding regions. Specifically, mutations of CDKN2A, TP53, SPTBN5, NOTCH2, and FAM136A were found in the databases. Copy number aberration (CNA) analysis revealed that the cell line loses chromosome 3p and 9p, but lacks amplification of 3q and 11q (as does HPV-negative, smoking-unrelated OTSCC). It also exhibits four distinctive focal amplifications in chromosome 19p, containing 131 genes without SNPs. Particularly, 52 genes showed >3- to 4-fold amplification and could be potential oncogenic drivers.

Conclusion: We have successfully established a novel OTSCC cell line from a never-smoking patient. UCSF-OT-1109 is potentially a robust experimental model of OTSCC in never-smokers.

Introduction

Squamous cell carcinoma (SCC) of the tongue is classified into two subtypes, according to location [1,2]: oral tongue SCC (OTSCC), arising in the front two-thirds; and base-of-tongue SCC (BTSCC), originating in the posterior one-third (considered part of the oropharynx [1]).

OTSCC afflicts approximately 16,000 people a year in the US [3] and is typically related to a long history of smoking and/or heavy alcohol use [4]. Although US smoking rates continue to decline [5], the incidence of SCC of the oral cavity has remained stable [3]. This may be explained by an increase of OTSCC in patients who have never smoked or whose habit was light. These individuals, accounting for ~10% of OTSCC, are often women in their mid-forties or younger [4,6–9]. Human papilloma virus (HPV) has been implicated in the recent rise in oropharyngeal cancers, including BTSCC [10,11]. However, HPV infection does not explain the increase of never-smoker OTSCC because, regardless of smoking status, OTSCC patients are typically HPV-negative [12].

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We recently reported that the never-smoking cohort may experience decreased survival and more aggressive characteristics than the smoking group [7–9]. However, it remains unclear what factors contribute to this poor clinical outcome [13]. One important way to gain insight into the behavior of OTSCC is to establish validated cell lines.

To date, most OTSCC cell lines have been generated from patients who were smokers, or whose smoking history is unknown, supporting the need for OTSCC cell lines verifiably developed from never-smoking patients. Such cells can be used as a platform to identify diagnostic biomarkers and evaluate novel therapeutic agents.

Materials and methods

Tumor specimen and establishment of UCSF-OT-1109

Under an approved Institutional Review Board (IRB) protocol (10-01635) and with informed consent, a portion of fresh tumor tissue was collected from a 44-year-old woman with invasive, keratinizing OTSCC who had never smoked. The tissue was divided into 3-mm cubes; these were placed into 10-cm dishes and maintained in a serum-free 154CF medium (M154CF500, Thermo Fisher Scientific, Waltham, MA) with 0.07 mM Ca^{2+} and growth supplements (both provided as separate components with the medium) in a 5% CO_2 incubator at 37 °C.

Fifty independent colonies were cloned. They were cultured continuously in serum-free medium Defined Keratinocyte-SFM (10744019, Thermo Fisher Scientific) in addition to growth supplements (10744019, Thermo Fisher Scientific; provided as a separate component with the medium), penicillin, and streptomycin. Each clone was passaged every seven days. After 20 passages, the cells were cultured in RPMI medium (11875-093, Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS) until passage 50 (p50). The established cell line was named UCSF-OT-1109 after the recommended style for cell-line designation of the International Cell Line Authentication Committee (ICLAC) [14].

Cell culture

The following cell lines were from the American Type Culture Collection: SCC-4, SCC-25, CAL27, HeLa, SW480, 293T, and WI-38. CAL27, HeLa, SW480, 293T, and WI-38 cells were cultured in RPMI 1640 medium (11875-093, Thermo Fisher Scientific) supplemented with 10% FBS. SCC-4 and SCC-25 were cultured in DMEM: F12 HEPES medium (11330032, Thermo Fisher Scientific) containing 400 ng/ml hydrocortisone (H0888, Sigma Aldrich, St. Louis, MO) with 10% FBS. The cells were maintained in a 5% CO_2 incubator at 37 °C [15]. Images were captured with EVOS cell imaging systems (Thermo Fisher Scientific).

Short tandem repeat (STR) DNA fingerprint analysis

Genomic DNA was isolated from the cultured cells (passage 5) and whole blood cells in a clean environment using the Wizard SV genomic DNA purification system (A2360, Promega Cooperation, Madison, WI) or DNeasy blood & tissue kit (69504, Qiagen, Redwood City, CA). STR analysis was performed at the Johns Hopkins University Fragment Analysis Facility using the Powerplex 1.2 system (Promega Corporation) [16]. The following STR markers were tested: AMEL (Xp22.10-22.3 and Y), CSF1PO (5q33.3-34), D13S317 (13q22-q31), D16S539 (16q24-qter), D5S818 (5q21-q31), D7S820 (7q), TH01 (11p15.5), TPOX (2p23-2pter), and vWA (12p12-pter).

Protein preparation and western blot analysis

Total cellular protein was isolated with cell lysis buffer (9803, Cell Signaling Technology, Danvers, MA). Equal amounts of protein were prepared by adding reducing red loading buffer (7723, Cell Signaling Technology) and were resolved by SDS-PAGE (Novex Tris-Glycine Gels, EC60055BOX, Thermo Fisher Scientific) [15]. Western blots were developed by enhanced chemiluminescence and detected by autoradiography film [15]. The following primary and secondary antibodies were used: EpCAM (NBP2-33051, Novus Biologicals, Littleton, CO), p16 INK4A (4824, Cell Signaling Technology), p53 (DO-1; sc-126, Santa Cruz Biotech), β-actin HRP-conjugated (A3854, Sigma Aldrich), CD44 (3570, Cell Signaling Technology), ALDH1A1 (12035, Cell Signaling Technology), anti-mouse IgG HRP (RPN4201, GE Healthcare Life Science, Pittsburgh, PA), and anti-rabbit IgG HRP (7074, Cell Signaling Technology).

Immunohistochemistry

For preparation of cytospins, single cell suspensions were spun onto slides using a cytocentrifuge (Thermo Fisher Scientific). Immunohistochemistry was subsequently performed on the slides with antibody-based staining kits for CD44 (3570, Cell Signaling Technology) at the UCSF Comprehensive Cancer Center Immunohistochemistry & Molecular Pathology Core Facility. Staining was performed as previously described [17–19].

PCR detection of HPV DNA

Genomic DNA was isolated from the cultured cells. DNA samples were separately amplified by PCR with the primer sets:

GP5+: 5′-TTTGTTACTGTGGTAGATACTAC-3′;
GP6+: 5′-GAAAAATAAACTGTAAATCATATTC-3′, and
MY09: 5′-CGTCCMARRGGAWACTGATC-3′;
MY11: 5′-GCMCAGGGWCATAAYAATGG-3′

with proofreading capability Ex Taq DNA polymerase (RR001, Takara Bio USA, Mountain View, CA). The GP- and MY-PCR systems amplify approximately 150 bp and 450 bp DNA fragments, respectively, in the L1 region of HPV [20,21]. PCR products were sequenced at Quintara Biosciences (South San Francisco, CA) to confirm that they contained the HPV-specific nucleotide sequence.

Sphere-forming assay

Single cell suspensions of UCSF-OT-1109 #21 passage 25, CAL27 passage 27, or WI-38 passage 5 were prepared with TrypLE Express (12605010, Thermo Fisher Scientific) and a serum-free Defined Keratinocyte-SFM medium plus growth supplements (10744019, Thermo Fisher Scientific) [22]. Five thousand cells were distributed per well in sextuplicate using 6-well ultra-low-adherent plates (3471, Corning, Lowell, MA). After 10 days of incubation in a 5% CO_2 incubator at 37 °C, images were captured with EVOS cell imaging systems (Thermo Fisher Scientific) and the percentage of sphere-forming cells was calculated.

Spectral karyotype (SKY) analysis

SKY analysis was performed at the Roswell Park Cancer Institute Cytogenetics SKY Core Laboratory [23]. Exponentially growing UCSF-0T-1109 #21 passage 25 cells were treated with colcemid (D1925, Sigma Aldrich) for 2 h and metaphase chromosomes were air-dried on slides. After sequential digestion with RNase (R6513, Sigma Aldrich) and pepsin (P0525000, Sigma Aldrich), the chromosomal DNA was denatured in 70% formamide (F9037, Sigma