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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

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Conflict of interest statement

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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 frameshift 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.

Keywords

Oral tongue squamous cell carcinoma; Never-smoker; Cell line; STR DNA fingerprint analysis; Sphere-forming assay; SKY analysis; Whole-exome sequencing; single nucleotide polymorphism; insertion or deletion; DNA copy number aberration

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].

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 150bp and 450bp 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 hours 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 Aldrich) and hybridized with a cocktail of human SKY paint probes tagged with various nucleotide analogues (Applied Spectral Imaging, Inc., Carlsbad, CA). The slides were observed and scored under a fluorescent microscope (Nikon Instruments Inc., Melville, NY).

Whole-exome sequencing (WES)

Genomic DNA was extracted from UCSF-OT-1109 #21 passage 5 cells and the tissue donor's blood cells as described above. DNA library preparation and subsequent targeted enrichment were carried out using TruSeq DNA library preparation kit (FC-121-2001, Illumina Inc., San Diego, CA) and SureSelect 50 Mb exome capture kit (5190-8863, Agilent Technologies, Santa Clara, CA). Exome-enriched products were sequenced using an Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA) by Centrillion Biosciences Inc. (Mountain View, CA) [24, 25]. 100bp paired-end reads were aligned to NCBI build 37 (hg19) of the human genome using Burrows-Wheeler Aligner-Maximal Exact Matches (BWA-MEM) software; duplicate reads were marked, single nucleotide polymorphisms (SNPs) and local insertion or deletion (In/Del) realignment performed, and base-quality scores recalibrated for each sample with PICARD, SAMTOOLS and GATK programs. Novel point mutations and In/Dels were identified (with MuTect and GATK Somatic In/Del Detector, respectively) in tumor samples when compared with the donor's blood cells [26]. Copy number segmentation was performed on the log tumor/donor's blood cells per-exon coverage ratio using CNAKit and the R package 'DNAcopy' [27, 28].

TP53-targeted sequencing

Genomic DNA from UCSF-OT-1109 #21 passage 25 cells was extracted as described above. Genomic DNA from the primary tumor was isolated from microdissected formalin-fixed, paraffin-embedded (FFPE) tissue sections using a QIAamp DNA FFPE Tissue kit (Qiagen). DNA samples were amplified by PCR with the primer set: p53 exon 7 forward: 5′-AAGGCGCACTGGCCTCATCTT-3′; p53 exon 7 reverse: 5′-GAGGTGGATGGGTAGTAG-3′ and proofreading capability Ex Taq DNA polymerase (RR001, Takara Bio USA). Direct sequencing of the PCR product was performed at Quintara Biosciences.

Results

Cell-line tissue donor

The donor, a 44-year-old woman who had never smoked and had no secondary smoke exposure presented with a painful right oral tongue lesion in 2009. On examination, a large right oral tongue mass was noted along with a palpable ipsilateral jugulodigastric lymph node. Biopsy of the lesion revealed invasive SCC. The patient underwent a right partial glossectomy, right neck dissection, and a radial forearm free-flap reconstruction in November, 2009. Final pathology revealed a 5-cm moderately-to-poorly differentiated invasive keratinizing SCC with perineural invasion (Figures 1A and B). Three (of 56) lymph nodes in the right neck were positive for carcinoma with extranodal extension. Final

pathologic staging was T3N2bM0. The patient subsequently underwent adjuvant chemoradiation therapy with weekly cisplatin. She suffered no recurrence and is free of disease eight years later.

Successful development of a novel OTSCC cell line, UCSF-OT-1109

Contamination by host-derived stromal fibroblasts in cultured tumor cells represents a major obstacle in establishing cancer cell lines [29]. Thus, to inhibit overgrowth of fibroblasts, we used a serum-free medium formulated with a low calcium concentration. Among 50 independent colonies cloned from this tumor, five clones (#6, #20, #21, #22, and #23) were able to proliferate after passage 5.

To authenticate the cultured cells, we compared the STR profile of these five clones with that of peripheral blood cells from the tissue donor and found them to be identical, supporting their donor origin (Table 1). After 20 passages; we cultured the cells in regular medium containing FBS. The cells did not show senescence through 50 passages, supporting the successful development of a novel cell line.

UCSF-OT-1109 cells are epithelium-derived, HPV-negative SCC

The appearance of UCSF-OT-1109 clone #21 at passage 25 differed depending on the presence or absence of serum, although both had microscopic characteristics of SCC [30]. In serum-free medium, the cells had a cuboidal epithelioid morphology and grew as a monolayer (Figure 1C); in serum-containing medium, they were pleomorphic, elongated, spread-out, and grew in a stratified pattern (Figure 1D).

To confirm that the cultured cells were of epithelial origin and not stromal fibroblasts, we analyzed expression levels of epithelial cell adhesion molecule (EpCAM; also known as CD326) [31]. We included other OTSCC cell lines SCC-4, SCC-25 and CAL27, the purported cervical cancer cell line HeLa [16], and a colon cancer cell line SW480. All but HeLa expressed EpCAM protein (Figure 1E).

Expression of p16 INK4A is frequently upregulated in cervical and oropharyngeal cancers, and is used clinically as evidence of HPV infection [32]. Given that OTSCCs are usually HPV-negative, UCSF-OT-1109 and other OTSCC cell lines do not overexpress p16 INK4A. The absence of HPV infection was confirmed by western blot analysis (Figure 1E), and further validated by a PCR-based method for HPV DNA detection (Figure 1F).

UCSF-OT-1109 cells are capable of forming tumorigenic spheres

We found that cytospins from UCSF-OT-1109 stained strongly positive with CD44, a marker for tumorigenic potential in head and neck SCC (HNSCC) [33] (Figure 2A). However, the tumorigenic potential of HNSCC may also require ALDH1A1 expression [34]. Western blots showed that UCSF-OT-1109 and other OTSCC cell lines, but not 293T cells, expressed both CD44 and ALDH1A1 (Figure 2B). These findings suggest that all five OTSCC cell lines may possess tumorigenic potential.

We therefore evaluated the adherent population of UCSF-OT-1109 #21 passage 25, CAL27 passage 27, and WI-38 passage 5 cells for sphere formation. CAL27 cells are reported to

form tumorigenic spheres [22], but WI-38 lung fibroblasts are non-tumorigenic [35]. Single cell suspensions of the cultured cells were seeded on ultra-low-adherent plate with a serum-free medium containing growth supplements. Sphere formation of UCSF-OT-1109 and CAL27 cells but not WI-38 cells was observed within 4 days; 10 days later, the OTSCC cells and fibroblasts had formed spheres of various sizes (Figures 2C–E). We designated successful sphere populations as those >50 μm in diameter for purposes of quantification, and calculated sphere-forming efficiency as the number of spheres divided by the total number of cells plated. We observed that UCSF-OT-1109, CAL27, and WI-38 cells possess a sphere-forming population of 0.82%, 0.46%, and 0.13%, respectively (Figure 2F).

UCSF-OT-1109 retains numerical and structural chromosomal abnormalities

In elucidating the genomic landscape of UCSF-OT-1109, our SKY analysis of 20 metaphases of the clone #21 passage 25 cells [23] revealed that the cell line has an abnormal human female karyotype with a chromosome number ranging from 75–88 (Figures 3A–D). In addition, numerous structural abnormalities were observed: two copies of a translocation derived from a small portion of chromosome 12 within chromosome 1 [der(1)t(1:12)]; a translocation consisting of portions of chromosomes 1 and 9 [der(1)t(1:9)]; a translocation of portions of chromosomes 1 and 11 [der(1)t(1:11)] (appearing in only one of 20 cells analyzed) (Figure 3C); a translocation of portions of chromosomes 1 and 17 [der(1)t(1:17)] (in only one of 20 cells analyzed) (Figure 3C); two copies of a translocation of a very small portion of chromosome 1 and a larger portion of chromosome 3 [der(3)t(1:3)]; a deletion of a large portion of chromosome 5 [del(5)] (in one of 20 cells) (Figure 3D); one copy of chromosome 9 containing a dicentric centromere [dic(9)]; a translocation of portions of chromosomes 12 and 19 [der(19)t(12:19)]; one copy of chromosome 13 containing a duplication of the entire chromosome [dup(13)] (Figures 3A, B, and D); a translocation of almost equal portions of chromosomes 13 and 14 [der(13)t(13:14)]; a translocation of portions of chromosomes 14 and 19 [der(14)t(14:19)]; one copy of chromosome 16 containing a dicentric centromere [dic(16)]; a translocation of portions of chromosomes 16 and 19 [der(16)t(16:19)]; and a deletion of a portion of chromosome 19 [del(19)], which was observed in 8 of 20 cells (Figures 3B and C).

UCSF-OT-1109 harbors 60 somatic mutations

In WES of genomic DNA extracted from UCSF-OT-1109 #21 passage 5, we used a paired comparison with blood cells from the tissue donor to distinguish somatic mutations and copy number aberrations in the tumor cells [24, 36, 37].

WES yielded mean coverages of 23.72 and 20.28 times per base across the whole exome of UCSF-OT-1109 and control donor blood cells, respectively. We determined significant somatic mutations as variant read frequency (VRF) >10%, and found 60 somatically mutated coding genes (Supplemental Table 1).

Targeted sequencing confirmed the TP53 mutation in both the cell line and primary tumor: coding DNA sequence 743, guanine and adenine are switched in exon 7 (c.743G>A; Figures 4A and D). The reverse read verified the forward read (c.743C>T; Figures 4B and E). The

predicted missense substitution was glutamine (Q) for arginine (R); p.Arg248Gln or R248Q, resulting in the protein stabilization (Figure 4C).

Likewise, mutations of CDKN2A, TP53, SPTBN5, NOTCH2, and FAM136A were found in the Single Nucleotide Polymorphism database (dbSNP) and/or Catalogue of Somatic Mutations in Cancer (COSMIC) (Supplemental Table 1). However, the other 55 mutations were novel. The NOTCH2 mutation suggested an inactive form because of the copy number loss.

UCSF-OT-1109 has a unique mutational profile

The mutations identified through WES were non-synonymous (46 genes) and synonymous (13 genes) somatic SNPs (Supplemental Table 1). Moreover, we specifically observed one frameshift deletion (Del); insertions (Ins) were not found. The SNPs were distributed throughout the genome (Figure 5A). The non-synonymous SNPs were predominantly found in 13 chromosomes (Figure 5B).

We next searched mutational profiles of other OTSCC cell lines in the Cancer Cell Line Encyclopedia (CCLE) database (https://portals.broadinstitute.org/ccle/home) and compared the mutational burden with UCSF-OT1109. The cell lines SCC-4, SCC-15, SCC-25, and CAL27 have missense mutations in TP53; SCC-9 has a missense mutation in TP53BP1. All of the cell lines lack PI3CA mutations. We found that these cell lines have comparable numbers of mutations in the 25–63 coding genes (Figure 5C). However, they consist of a significant portion (15–20 genes) of small insertions and deletions (In/Dels). In contrast, UCSF-OT-1109 has only one frameshift deletion, but no insertions. Thus, UCSF-OT-1109 contains the highest numbers of non-synonymous and synonymous SNPs.

UCSF-OT-1109 exhibits distinctive focal amplifications in chromosome 19p

WES analysis also revealed the tumor genome to have a high burden of both broad and focal DNA copy number aberrations (CNAs) (Figure 6A and Supplemental Table 2).

Specifically, chromosome 8p loss and 8q gain--frequently found in oral cancer--were observed in UCSF-OT-1109 [38]. Moreover, as in HPV-negative oral cancer [39], the cell line lost chromosome 3p and 9p. However, UCSF-OT-1109 lacks amplification of 3q and 11q--a hallmark of smoking-related HNSCCs [40].

UCSF-OT-1109 has unique focal CNAs in chromosome 19p. The magnified ideogram of chromosome 19 revealed the presence of four focal amplifications (R1–R4) in the short arm (Figure 6B; Supplemental Table 3). The regions contain 131 genes (Supplemental Table 3), which are not somatically mutated (Supplemental Table 1). Among them, 52 genes showed a log2 copy number ratio >1.75, which corresponds to 3- to 4-fold amplification. Particularly, NFIX, LYL1, and TRMT1 demonstrated the highest log2 ratio (3.18), matching 8- to 9-fold increases. Thus, the amplifications were high-level chromosomal segment gains [37].

Interestingly, chromosome 19p lost its copy number throughout the short arm, suggesting that the focal CNAs might occur in the translocated chromosomes but not in the original

location. In fact, we observed various translocations of chromosome 19 in the cell line; der(19)t(12:19), der(14)t(14:19), and der(16)t(16:19) (see above and Figures 3A–D).

Discussion

Tobacco use has been linked to cancers of the upper aerodigestive tract [41]; in fact, approximately 90% of OTSCCs occur in patients who are active or previous smokers [4]. The subset of OTSCC patients who have never smoked (~10%) is significantly younger and more likely to be female [6–9].

Although HPV has recently been linked to oropharyngeal cancers, including BTSCC, it does not appear to play a significant role in OTSCC [10,11]. Our novel cell line UCSF-OT-1109 has no evidence of association with HPV.

Different oncogenic etiologies cause unique mutation patterns [42]. HPV infection induces activity of the APOBEC family of cytidine deaminases [4–9]. Accordingly, HPV-associated HNSCC often generates TCW mutations (C>T and C>G mutations at T-C-A/T [TCW] tri-nucleotides) [43]. As a result, APOBEC activation frequently causes oncogenic E542K (c. 1624G > A) and E545K (c.1633G > A) PIK3CA mutations in HPV-associated HNSCC [44].

UCSF-OT-1109 expresses wild-type PIK3CA. Moreover, as in HPV-negative oral cancer, it harbors a mutant form of p53 and loses chromosome 3p and 9p. Likewise, amplification of 3q and 11q—a hallmark of smoking-related HNSCC--is absent [40]. Thus, UCSF-OT-1109 may demonstrate a unique mutational profile independent of HPV-infection and tobacco smoking. In fact, the cell line has only one frameshift deletion, but no insertions. However, decreased In/Dels found in UCSF-OT-1109 might be attributed to a specific nature of the cell line, not to the common character of OTSCC in never-smokers. A recent report with the TCGA database showed that In/Del frequencies do not differ between smokers and never-smokers [45].

We have reported here that UCSF-OT-1109 exhibits distinctive focal amplifications in chromosome 19p. To the best of our knowledge, this is the first report highlighting 19p CNAs in OTSCC. Detection of focal CNAs--such as MYC and CCND1--often promotes our understanding of the genesis and development of cancer [46]. Moreover, the CNAs have enabled us to discover new driver genes (e.g. HER2 and EGFR) that could identify new subsets of cancer, resulting in development of novel therapeutic interventions [37]. We therefore chose one gene from each amplicon; ARRDC5 (Region 1), RAB3D (Region 2), NACC1 (Region 3), and KLF2 (Region 4) and compared the frequencies of their genetic alterations in cancers using the datasets from the cBioPortal for Cancer Genomics (http://www.cbioportal.org/).

We found that the gene amplifications are often found in the major subsets of neuroendocrine prostate cancer (NEPC), ovarian serous cystadenocarcinoma, and breast and pancreatic cancers, but are rarely found in the TCGA HNSCC cohort (Supplemental Figure 1 A–D). Only two oral cancer cases of 279 HNSCCs show similar amplifications (Supplemental Figure 2). However, NACC1 expression is a molecular biomarker for OTSCC

[47]. Thus, regardless of amplification of NACC1 and other CNA genes, their expression may be elevated by other mechanisms in OTSCCs. Further studies are warranted.

With this successful establishment of a novel OTSCC cell line, we believe that UCSF-OT-1109 can be a robust experimental model of oral cavity carcinoma in never-smokers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- An oral tongue squamous cell carcinoma cell line was developed from a never-smoker.
- The cell line UCSF-OT-1109 was authenticated by STR DNA fingerprint analysis.
- UCSF-OT-1109 harbored 60 somatically mutated coding genes.
- The genomic landscape was distinct from tobacco- or HPV-related oral cancer.
- The cell line exhibited distinctive focal amplifications in chromosome 19p.

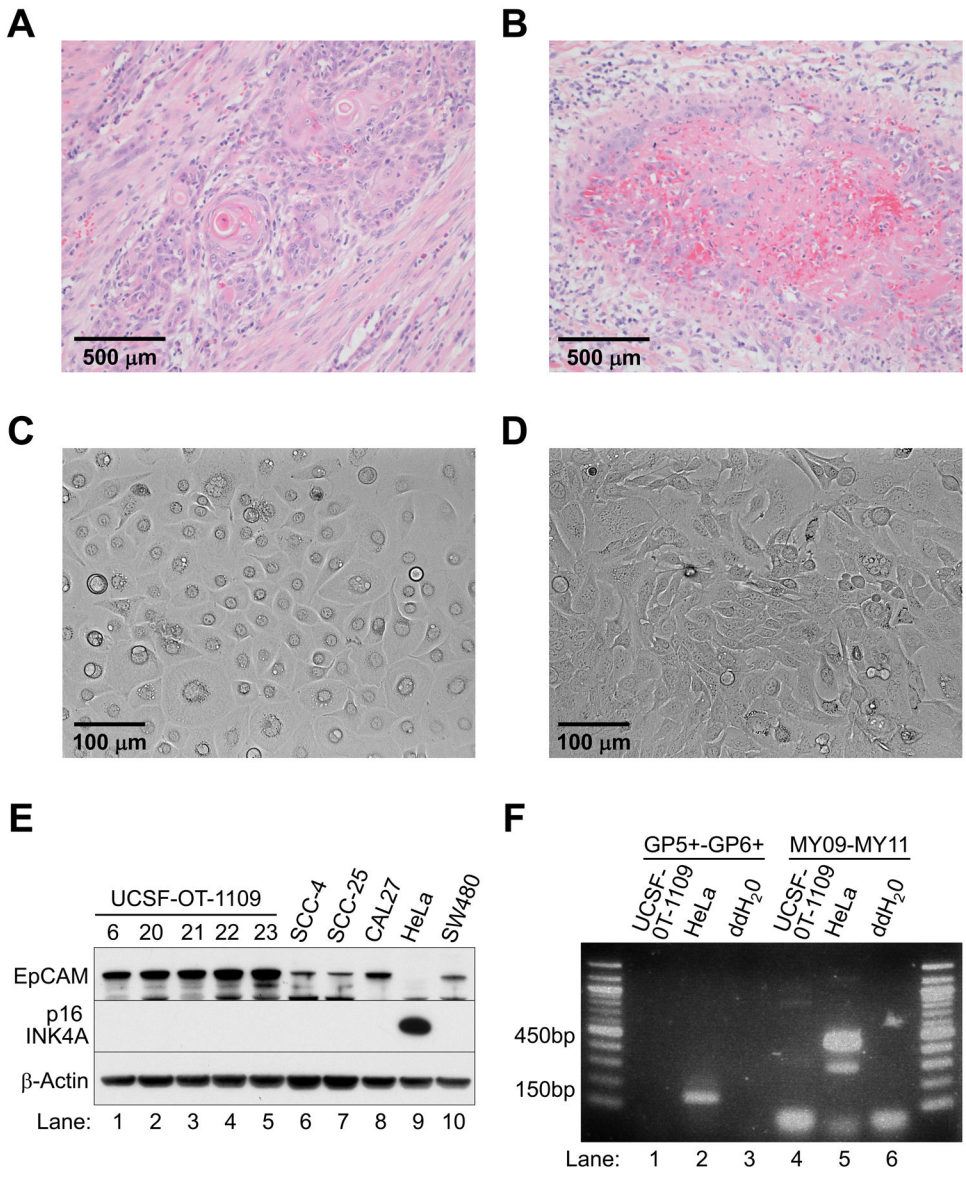

Figure 1. Establishment of UCSF-OT-1109

(A) Histologic sections of the primary oral tongue tumor showing invasive keratinizing squamous cell carcinoma (H & E staining, x200).

(B) Histologic evaluation showing vascular invasion by tumor cells. A blood vessel is occupied by tumor cells at the periphery and fibrin clot (pink staining) containing red blood cells (strong red staining) centrally. The tumor cells form a vague layer around the fibrin clot. The vessel is surrounded by scant smooth muscle (H & E, x400).

(C) (D) Cellular morphology of passage 25 of UCSF-OT-1109 clone #21. The appearance of the cultured cells differs in the serum-free (C) and serum-containing (D) medium.

(E) Western blot analysis with epithelial cell adhesion molecule (EpCAM), p16 INK4A, and β-Actin antibodies: Lanes 1–5, UCSF-OT-1109 clone; Lanes 6–8, OTSCC cell lines; Lane 9, cervical cancer cell line HeLa; Lane 10, colon cancer cell line SW480.

(F) PCR detection of HPV DNA samples from cultured cells were amplified by PCR with the primer set GP5+ and GP6+ or MY09 and MY11. The GP- and MY-PCR systems amplify approximately 150bp and 450bp fragments, respectively, in the L1 region of HPV. PCR was also carried out with double-distilled water (ddH2O) as a no-template negative control.

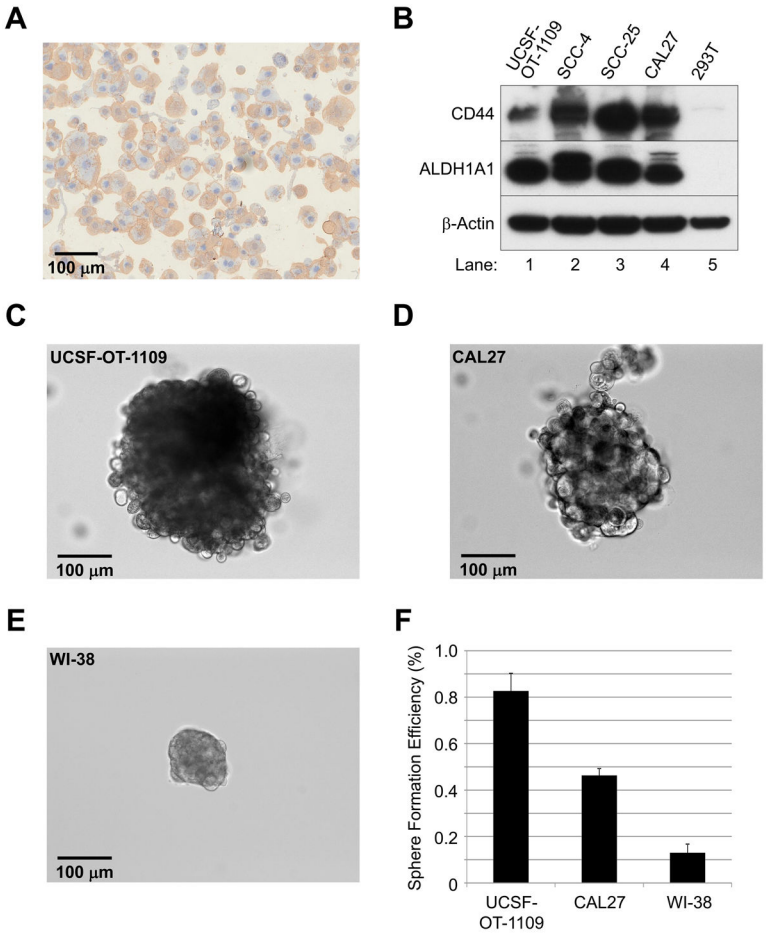

Figure 2. Tumorigenic sphere formation of UCSF-OT-1109

(A) Immunohistochemistry with a CD44 antibody on cytospins of UCSF-OT-1109 cells.

(B) Western blot analysis with CD44, ALDH1A1, and β-Actin antibodies.

(C)–(F) Sphere-forming assay. Single cell suspensions of UCSF-OT-1109 #21, CAL27, and WI-38 were seeded on ultra-low-adherent plate with a serum-free medium containing growth supplements.

(C)–(E) Representative spheres of UCSF-OT-1109 (C), CAL27 (D), and WI-38 (E) cells on day 10.

(F) Sphere-forming efficiency of UCSF-OT-1109, CAL27, and WI-38 (see text).

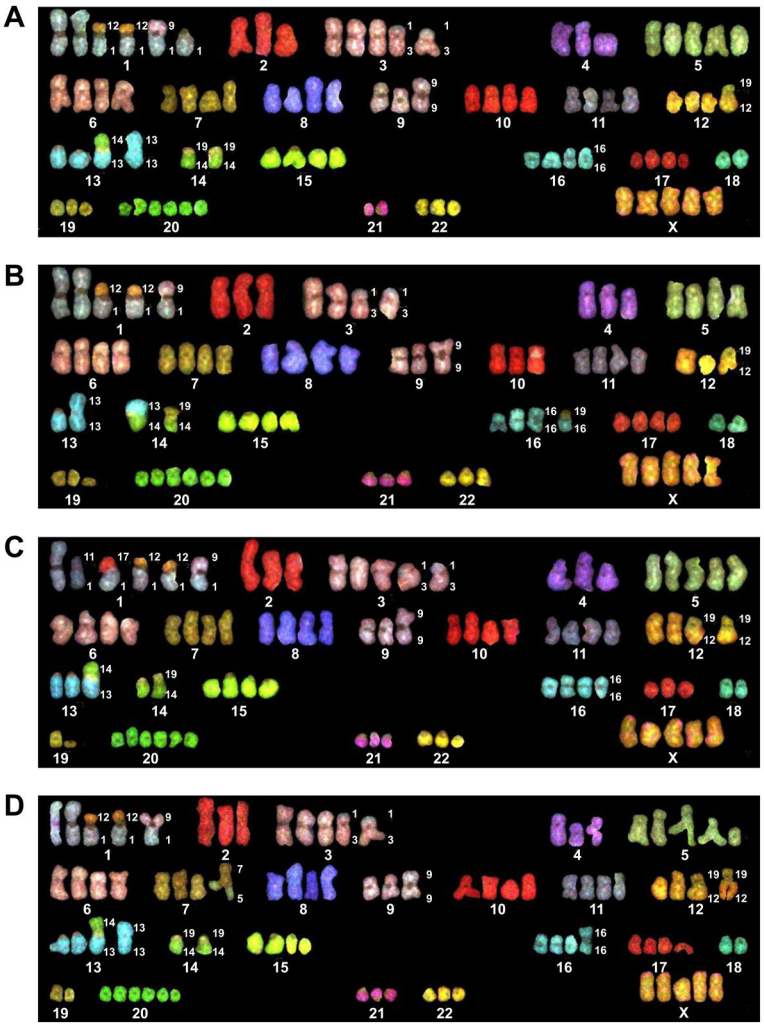

Figure 3. Numerical and structural abnormalities in UCSF-OT-1109 chromosome

(A)–(D) SKY analysis; 20 metaphases of UCSF-OT-1109 clone #21 passage 25 cells were analyzed. The cell line had an abnormal human female karyotype with a chromosome number ranging from 75–88. Numerous structural abnormalities were also observed (see text).

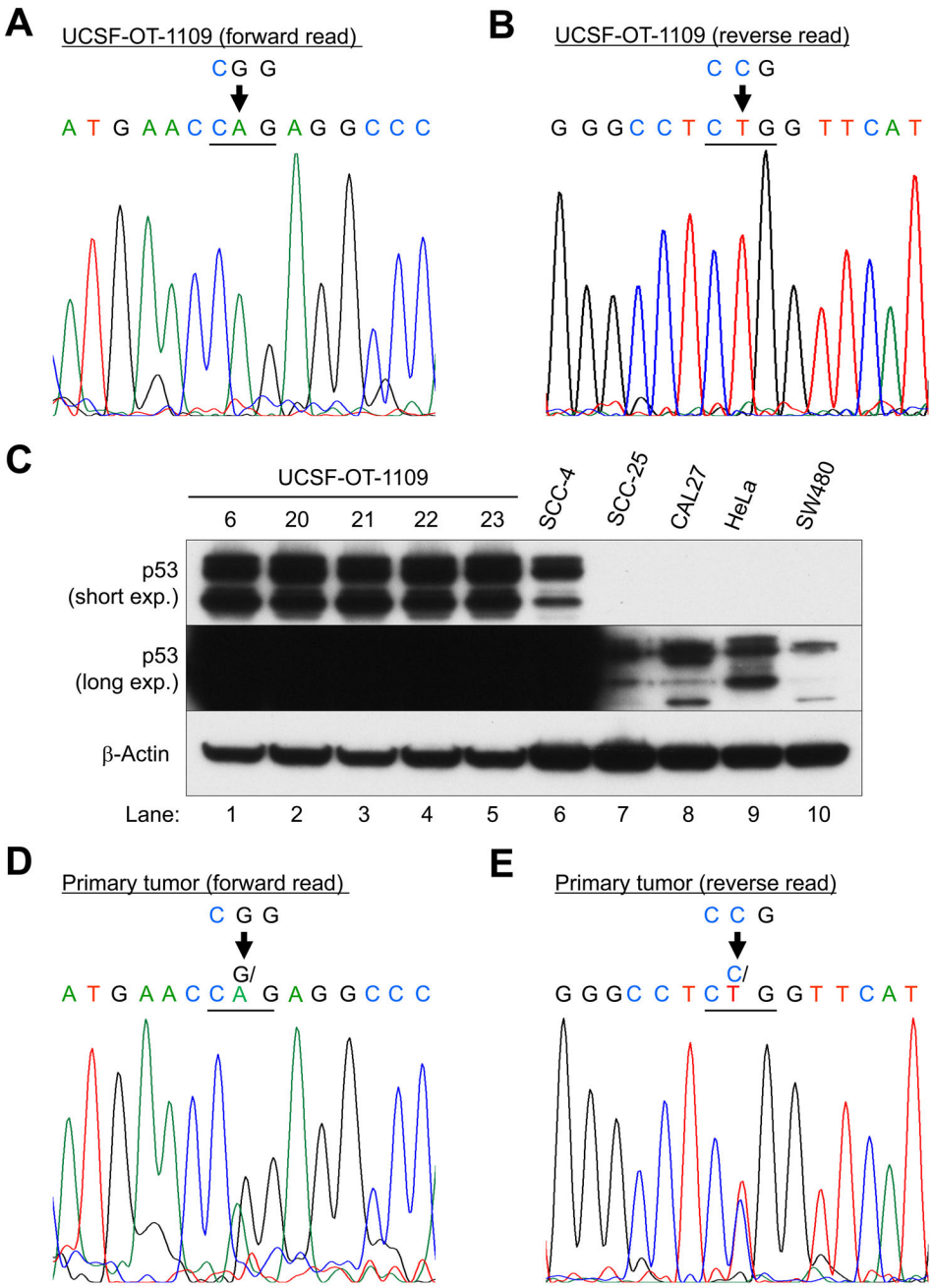

Figure 4. TP53-targeted sequencing of UCSF-OT-1109 (A, forward read; B, reverse read) and the primary tumor (D, forward read; E, reverse read)

(C) Western blot analysis with p53 (short and long exposure) and β-Actin antibodies: Lanes 1–5, UCSF-OT-1109 clone; Lanes 6–8, OTSCC lines, Lane 9, cervical cancer cell line HeLa; Lane 10, colon cancer cell line SW480.

In D and E, genomic DNA was isolated from microdissected FFPE tissue sections.

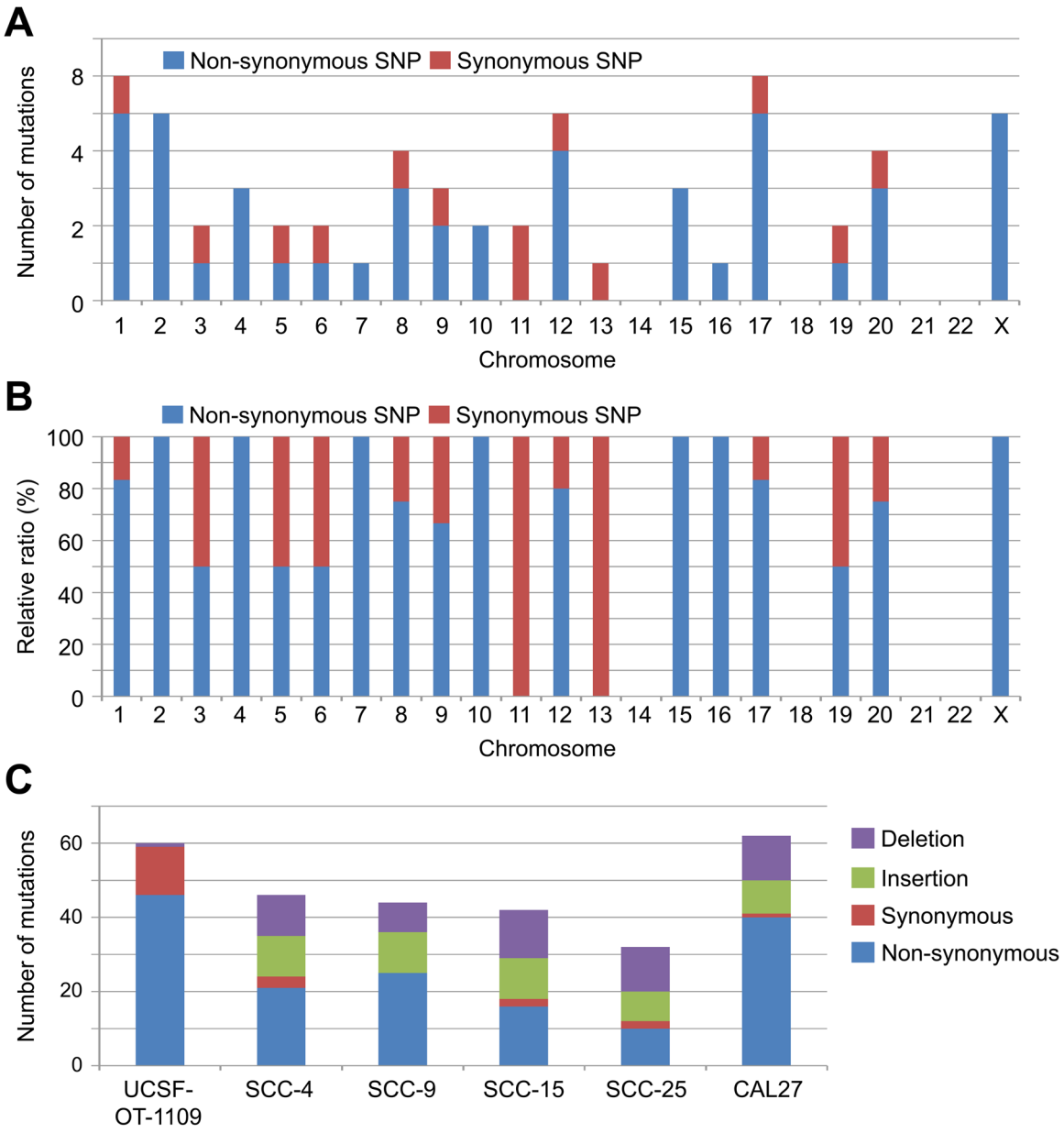

Figure 5. Unique mutational profile of UCSF-OT-1109. Genomic DNA extracted from UCSF-OT-1109 #21 underwent WES

(A) Distribution of non-synonymous and synonymous SNPs.

(B) Ratio between non-synonymous and synonymous SNPs in each chromosome.

(C) Mutational burdens of UCSF-OT-1109 and previously established OTSCC cell lines SCC-4, SCC-9, SCC-15, SCC-25, and CAL27 (obtained from the Cancer Cell Line Encyclopedia database). Numbers of non-synonymous and synonymous SNPs and insertion and deletion mutations are indicated graphically.

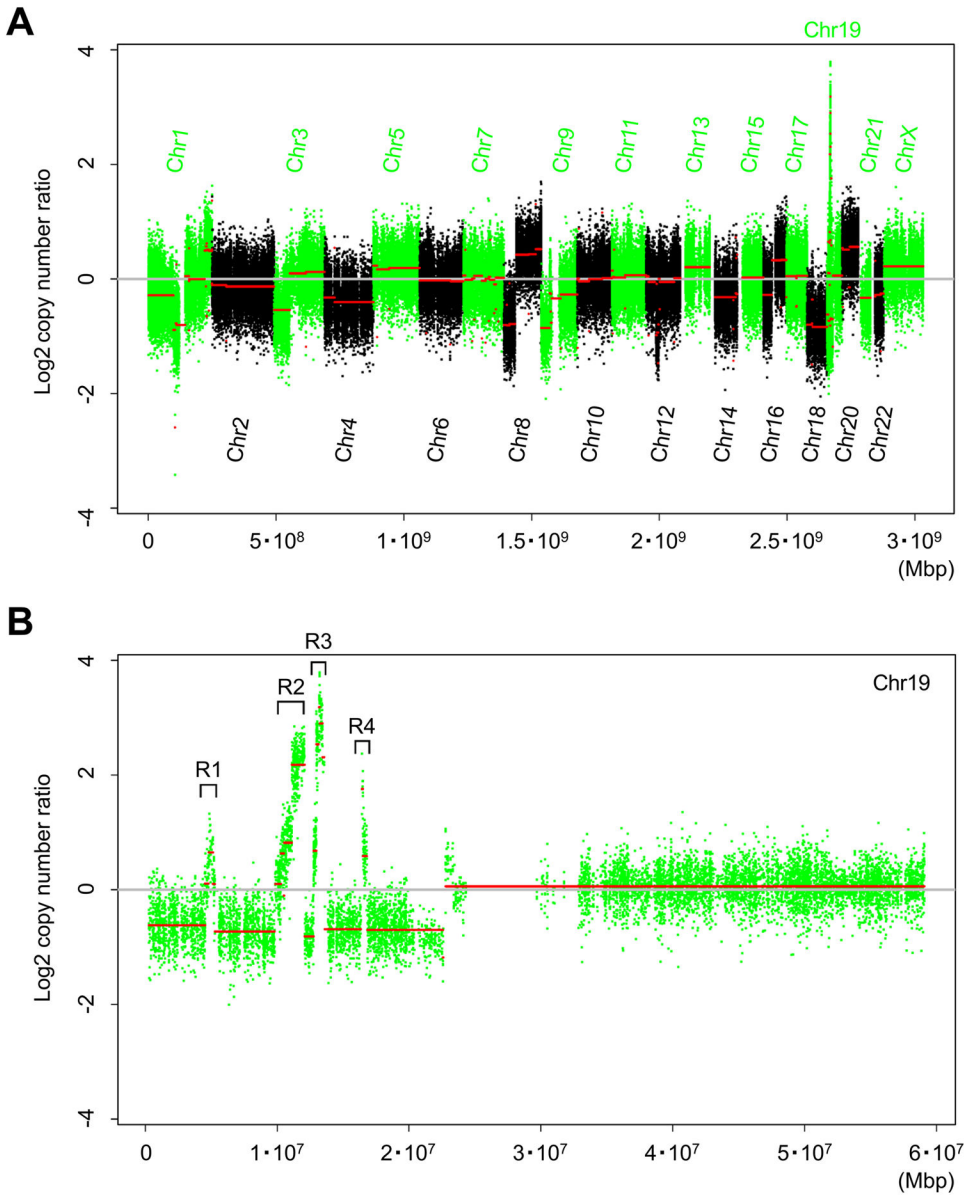

Figure 6. Distinctive focal amplifications in chromosome 19p of UCSF-OT-1109; WES copy number aberration analysis. Each dot represents sequencing probe

(A) The tumor genome with a high burden of both broad and focal DNA copy number aberrations.

(B) The magnified ideogram of chromosome 19. Four focal amplifications (R1–R4) in the short arm are shown.

Table 1

STR DNA fingerprint analysis of UCSF-OT-1109 clones

Clones	STR Locus								
	AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA
Donor Blood	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18
#6	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18
#20	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18
#21	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18
#22	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18
#23	X	11, 12	8, 12	9, 13	12	10, 12	6, 7	8, 11	16, 18