Oral Oncology 69 (2017) 108–114

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Establishment and genomic characterization of primary salivary duct carcinoma cell line

Jie Li [a], Yohitsugu Mitani [a], Pulivarthi H. Rao [b], Laszlo Perlaky [b], Bin Liu [c], Randal S. Weber [d], Adel K. El-Naggar [a,*]

[a] The University of Texas, M.D. Anderson Cancer Center, Department of Pathology, United States
[b] Baylor College of Medicine, Department of Pediatrics, Texas Children's Cancer and Hematology Centers, United States
[c] The University of Texas, M.D. Anderson Cancer Center, Department of Genetics, United States
[d] The University of Texas, M.D. Anderson Cancer Center, Department of Head and Neck Surgery, United States

ARTICLE INFO

Article history:
Received 31 March 2017
Accepted 10 April 2017
Available online 2 May 2017

Keywords:
Salivary duct carcinoma
Cell line development
Androgen receptor
Salivary gland carcinomas
Cell line validation

ABSTRACT

Objectives: To develop and characterize in vitro salivary duct carcinoma as a surrogate for functional studies.
Materials and methods: Cells were dispersed from tumor tissue fragments under sterile conditions in RPMI media. Disassociated cells were cultivated, immortalized with hTERT and propagated for more than 100 passages. Morphologic, linage, cytogenetic and genomic analyses were performed on different passages of cell line and primary tumor. Soft agar growth was performed.
Results: Analysis of cytomorphologic features, growth characteristics and lineage specific markers expression confirmed the epithelial derivation and the neoplastic nature of the cell line. DNA STRs analysis showed identical match of both cell line and primary tumor. Cultivated cells expressed Androgen Receptor (AR), PTEN, and EFGR proteins and the AR-V7 isoform transcript. Comparative exome-sequencing identified common mutated genes in both cell line and primary tumor. In-vitro colony formation of late passages is established.
Conclusion: We report the development of the first human salivary duct carcinoma cell line (MDA-SDC-04) that retains critical biological and genomic features of the donor tumor.

Introduction

Salivary duct carcinoma, a rare highly aggressive epithelial malignancy, commonly presents at advanced stage in elderly patients of both sexes [1–3]. The primary management of SDCs is surgery and/or post-operative radio-therapy while advanced primary and metastatic disease are empirically treated with either conventional chemo-radiotherapy or targeted agents with limited success [4,5]. SDC, like mammary ductal carcinoma, manifest high expression of HER-2 and Androgen Receptor (AR) and frequent alterations of the PI3K signaling pathway [6–13]. Investigations of the biological and therapeutic implications of these, and yet to be identified, targets in patients with SDC have been restrained by the lack of experimental ex-vivo models that faithfully represent primary tumors.

To accelerate cell line developments of this uncommon entity, systematic screening of patients with high grade primary salivary carcinomas for timely in vitro cultivation is necessary. The National Institute of Dental and Craniofacial Research (NIDCR) has established and sustained the Salivary Gland Tumor Biorepository (SGTB) as a biobanking infrastructure for tumor tissues and patients' materials and to develop cell lines representing these tumors [14]. Through the SGTB, a prospective protocol for pre-surgical screening of cytologically and/or clinically diagnosed primary salivary gland carcinomas of consented patients has been implemented for tissue acquisition. In-house patients with suspected primary high grade salivary carcinoma were systemically identified for timely tumor tissue acquisition under sterile conditions for cultivation.

We present the genomic and biological characteristics of the first successfully cultured and characterized cell line from primary untreated SDC.

* Corresponding author at: Department of Pathology, Unit 085, 1515 Holcombe Blvd., The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States.
E-mail address: anaggar@mdanderson.org (A.K. El-Naggar).

http://dx.doi.org/10.1016/j.oraloncology.2017.04.007
1368-8375/

Materials and methods

Primary tumor

Under an approved Institutional Review Board protocol (Protocol ID # 709540), a small portion of the resected fresh tumor mass was promptly harvested from a 5 cm in size primary salivary duct carcinoma of an 89-year-old male patient.

Primary culture and cell line establishment

Tumor tissue was minced and cell suspension was prepared as previously described [14]. Cells were cultured in a DMEM medium (Thermo-Fisher Scientific) containing 15% fetal bovine serum (Thermo-Fisher Scientific), 5 ng/ml epidermal growth factor (Sigma-Aldrich), 10 mg/ml insulin (Sigma-Aldrich), 10 mg/ml hydrocortisone (Sigma-Aldrich) and 100 U/ml penicillin and 100 μg/ml streptomycin (Thermo-Fisher Scientific) in a 37 °C incubator supplied with 95% air and 5% CO_2. Cells were fed by fresh medium 2–3 times a week and sub-cultured at a split ratio 1:2 when they reached 80% confluence. Fibroblasts were depleted with fibroblast-specific antigen attached to anti-fibroblast MicroBeads (Miltenyi Biotec) following the manufacturer's instructions. Cell cultures were tested for the presence of mycoplasma using MycoAlert® assay (Lonza Rockland, Inc.).

Morphological examination and growth kinetics

Morphologic cellular changes were monitored and photographed under a phase contrast microscope (Nikon Instrument Inc.). Cells in early and late passage were studied to calculate the population doubling time (PDT). In brief, 1×10^5 cells at day 0 were plated onto a 6-well plate (Corning Incorporated, USA) in a series of triplicates. Cell numbers were counted at 24 h intervals using coulter counter (Beckman). The growth rate was recorded and plotted and the PDT was calculated from the linear curve regression.

hTERT immortalization

Plasmid pBABE-puro-hTERT (Addgene) was recovered and plasmid DNA was amplified as previously described [14]. The hTERT containing retroviral vector was transfected into Phoenix Retroviral Packaging Eco cell line (Allele Biotechnology) using FuGENE6 transfection protocol (Promega). Viral supernatant was harvested after 48 h of culturing, passed through 0.45 μm syringe filter and used to infect tumor cells in the presence of polybrene (4 μg/mL). Tumor cells were selected in 1000 ng/mL of puromycin 48 h after infection. Total RNA from cells was extracted using the RNeasy Plus Universal Mini Kit (Qiagen, Inc.), according to the manufacturer's instructions. A total of 2 μg RNAs were reverse-transcribed into single stranded cDNA using Super Script III First-strand Synthesis System (Thermo-Fisher Scientific). Amplification program comprised of denaturation at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 45 s, annealing at 58 °C for 45 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 10 min. β-actin was used as a control. Primer designed to amplify the hTERT fragment: hTERT-F (5′-CGTGGTTTCTGTGTGGTGTC-3′) and hTERT-R (5′-TGGAACCCAGAAAGATGGTC-3′). Expected length of the amplification product is 303 bp [14].

RT-PCR

Total RNA was extracted from tissue samples and cell lines using RNeasy Universal Kit (Qiagen, Inc.). First-Strand cDNA synthesis was performed using 2 μg of total RNA, oligo(dT) primer and the SuperScript III reverse transcriptase (Thermo-Fisher Scientific). AR full-length primers (*5′*-TGGATAGCTACTCCGGACCTTA-*3′* and *5′*-GGAGTTGACATTGGTGAAGGAT-*3′*) and AR-V7 specific primers (*5′*-CTACTCCGGACCTTACGGGGACATGCG-*3′* and *5′*-TGCCAACCCGGAATTTTTCTCCC-*3′*) were used following previous report [13].

Immunohistochemical staining

Immunocytochemistry was performed as previously described [14]. Cells were harvested and suspended in cold 1×PBS solution. Cytospin preparation of acetone fixed cells were prepared and stained with Cytokeratin Cocktail (Dako), Anti-Androgen Receptor (Dako), Anti-PTEN (Dako), and Anti-EGFR (Thermo-Fisher Scientific) antibodies.

Cytogenetic analysis

Chromosome preparations and G-banded karyotypes were performed according to standard protocols. Chromosomal abnormalities identified by G-banding were described according to an International System for Human Cytogenetic Nomenclature (ISCN, 2013). Clonality was determined based on the detection chromosome gains in two or more cells and loss in three or more cells.

Short tandem repeat (STR) profiling

Genomic DNA from passage 22 and 46 samples and parental tumor tissues were isolated using the Gentra Puregene Tissue Kit (Qiagen, Inc.). A total of 14 STR loci (AMEL, CSF1PO, D13S317, D16S539, D18S51, D21S11, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA) were used with matching comparison of DNA fingerprinting analysis (CCSG Cell Line Core).

Exome sequencing

DNA from primary tumor tissue and cells of different passages was extracted using Gentra Puregene Tissue Kit. 2 μg genomic DNA was submitted to MD Anderson Cancer Center Genomic Core Facility. The Illumina HiSeq4000 sequencing system (Illumina Inc., San Diego, CA) was used for Exome sequencing analysis.

Soft agar colony formation assay

Cell suspension of 1×10^4/well cells in DMEM rich medium containing 0.3% agarose were seeded onto a base agarose concentration of 0.6% in triplicate in 6-well plate. Plates were incubated at 37 °C for 3–6 weeks. Plates were then stained with 0.05% crystal violet. Colonies with diameter greater than 0.2 mm were counted and photographed.

Xenograft transplantation in nude mice

In-vivo cell line injections were conducted on NSG mice (Jackson Laboratory) as previous described [14]. In brief, $1–5 \times 10^6$ cell suspensions were prepared in 100 μl of 1 × PBS solution with 100 μl of Matrigel Growth Factor Reduced Basement Membrane Matrix (BD Bioscience). Subcutaneous injections were performed on both flanks of anesthetized male 4–6 weeks old mice. Injected mice were monitored and examined for tumor development once a week.