Oral Oncology 47 (2011) 39–44

Contents lists available at ScienceDirect

Oral Oncology

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

Establishment and characterization of a rabbit oral squamous cell carcinoma cell line as a model for *in vivo* studies

Sheng-jiao Li [a,b,1], Guo-xin Ren [a,1], Wu-long Jin [a], Wei Guo [a,*]

[a] The Ninth People's Hospital of Shanghai, Shanghai Jiaotong University, Shanghai, PR China
[b] The Stomatology Hospital of Tongji University, Shanghai, PR China

ARTICLE INFO

Article history:
Received 3 August 2010
Received in revised form 5 October 2010
Accepted 7 October 2010
Available online 11 November 2010

Keywords:
Oral cancer
Tongue squamous cell carcinoma
Rabbit
Cell line
Tumorigenicity

SUMMARY

The incidence of oral squamous cell carcinoma (SCC) is increasing but the long-term survival rate remains low. An animal model would therefore be helpful for evaluation of new treatment modalities for oral SCC. Hamster is small animal, therefore, the cancer of hamster cheek pouch is not optimal for tumor imaging. The VX2 cell line has been used in many carcinoma-related studies, including oral SCC research, but it is derived from cutaneous tissue and not mucosa. We chemically induced tongue squamous cell carcinoma in rabbits and subsequently established a rabbit squamous cell line. The cells grew in multiple layers without contact inhibition for 60 passages over 2 years and were positive for cytokeratin (CK). Electron microscopy revealed that cells were polygonal with rich microvilli on the surface, and there were desmosomes between cells and bundles of tonofibril beside the cell membrane. The chromosome number ranged from 71 to 272, with a modal value of 145 (12.4%). The cells were transplantable into nude mice subcutaneously or rabbit submucosally and produced carcinomas in all the animals. The cell line should be a useful tool for the study of the biological characteristics of oral SCC, especially tongue SCC.

Introduction

It has been reported that oral cavity cancer consistently ranks as one of the 10 most frequently diagnosed cancers in the world. As the incidence of oral cancer continues to rise, the disease becomes an increasingly important public health issue. Presently the overall long-term survival rate from oral cavity cancers remains low. To design more effective therapeutic approaches, an animal model is essential. There are mainly two kinds of animal models for researching oral cancer: hamster cheek pouch cancer in hamster and VX2 tumor in rabbit. In the present study, we were able to chemically induce tongue squamous cell carcinoma in rabbits and subsequently established a squamous cell carcinoma cell line. This cell line should be a better choice for testing the potential of preventive and therapeutic agents, as well as for furthering our understanding of the biological mechanisms of mucosa carcinogenesis and progression.

Materials and methods

Tumor induction

Original tumors were created using a chemical carcinogenesis method as described in detail elsewhere.[1] In brief, New-Zealand rabbits were housed in cages in an air-conditioned room. After 1 week of acclimatization, 0.5% 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in acetone was painted at the posterior left lateral border of the tongue for 16 weeks. If the painted areas were erythematous and/or ulcerative, they were excised. Each specimen was divided in half; one half was prepared for cell culture if the other half was determined to be carcinoma by histological analysis.

Establishment of the cell line

Under sterile conditions, the tumor tissue was rinsed twice with the growth medium and cut with a razor into small pieces of approximately 1 mm^3 in size. Appropriate amounts of fine tissue fragments were seeded into 25-cm^2 flasks. The cultures were maintained in RPMI-1640 medium supplemented with 20% heat-inactivated rabbit serum and at 37 °C in a humidified atmosphere containing 5% CO_2. Culture medium was replaced three times per week. Upon reaching confluence, monolayer cultures were passed

* Corresponding author. Address: The Ninth People's Hospital of Shanghai, Shanghai Jiaotong University, ZhiZao Ju Road 639, Shanghai, PR China. Tel./fax: +86 21 50398070.
E-mail address: guoewishh@163.com (W. Guo).
[1] These authors contributed equally to this paper.

1368-8375/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.oraloncology.2010.10.003

with a solution of 0.25% trypsin. Cells were then collected by centrifugation and subcultured. For purifying cancer cells, the repeated adherence method was used. This method is based on the fact that tumor cells adhere to the container surface more slowly than fibroblasts. In brief, cells were treated with a solution of 0.25% trypsin and washed with phosphate-buffered saline (PBS) medium. After cells were maintained in culture for 20 min, the suspension was then collected and put into another container for further purification. The previous step was repeated until the cells were 100% cancer cells. Further cultivation of these cells was continued only with RPMI-1640 medium. These cells grew without interruption for over 2 years (60 passages) following the initiation of the primary culture. After establishment in long-term culture, the line was cryogenically preserved at every 10th passage.

Chromosome analysis

In the exponential growth phase, cells (passage 52) were treated with colcemid at a final concentration of 50 ng/ml for 6 h and harvested as a single cell suspension. Cells were treated with 0.075 mol/l KCl solution for 20 min at 37 °C then fixed with methanol: acetic acid (3:1) solution at 0 °C for 10 min. After fixation, the cells were stained with 4% Giemsa solution. Concurrently, air-dried metaphase specimens were prepared from each culture for karyotype analysis.

Light microscopy

Cells were cultivated on a slide in a cell culture dish. When monolayers were confluent, they were rinsed in PBS and dried with a dryer for 10 min. The cells were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunocytochemical staining was performed with primary antibodies against cytokeratin (CK) (1:100).

Electron microscopy

Tumor cells prepared as described above were fixed by immersion in 2% glutaraldehyde then were osmicated for 2 h in 1% OsO_4. After dehydration in a graded series of ethanol (70%, 85%, 95%, 100%; 10 min each), the specimens were divided in half, one for scanning electron microscope (SEM) and the other for transmission electron microscope (TEM). For SEM, dehydrated specimens were soaked in isoamyl acetate, critical-point-dried, and coated with platinum. For TEM, after dehydration the samples were embedded in Epon 812. Ultrathin sections were stained with both uranyl acetate and lead citrate.

Growth characteristics

Growth characteristics were analyzed using growth curve (GC), population-doubling time (DT) and cell cycle detection. Briefly, for

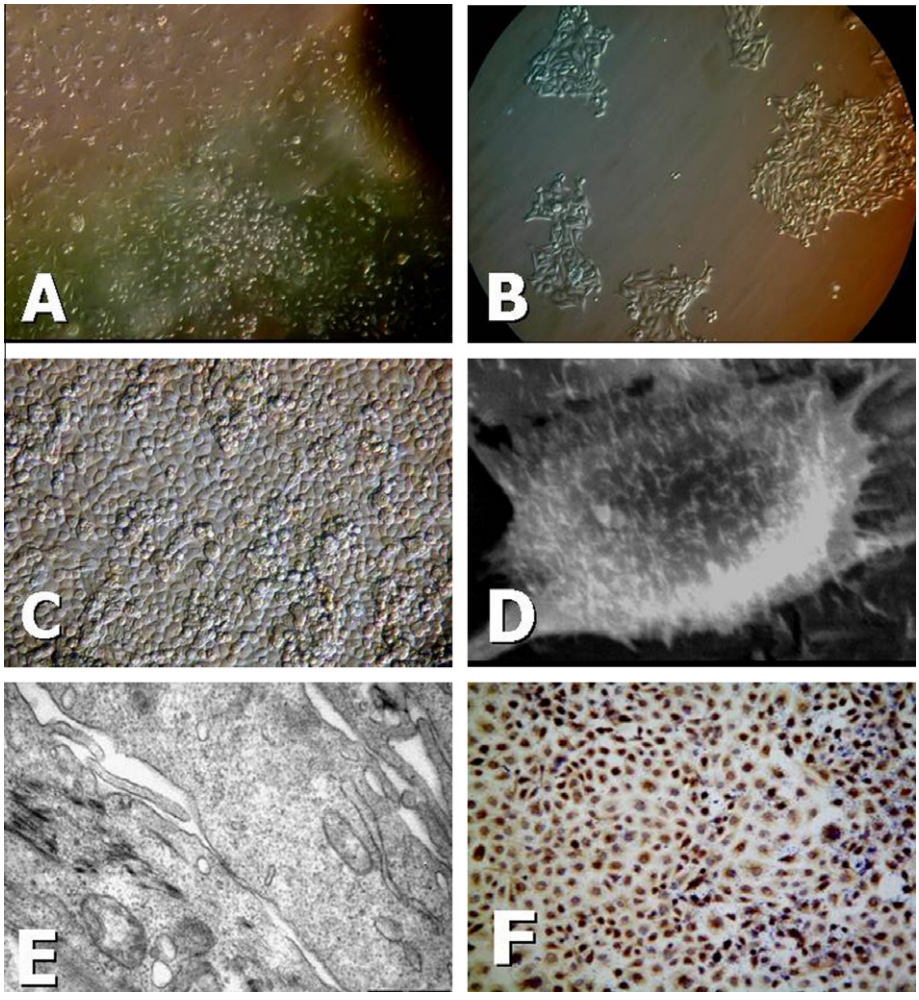

Figure 1 (A) Epithelial colonies and fibroblast-like cells in primary culture (DIC ×100). (B) Pure tumor cells. (DIC ×100). (C) Cancer cells growing in tight clumps (DIC ×100). (D) Cells were polygonal with rich microvilli on the surface (SEM ×6K). (E) Desmosomes between cells and bundles of tomofibril beside cell membrane (TEM ×6K). (F) Immunohistochemical staining for cytokeratin (×200).