Archives of Medical Research 47 (2016) 271–274

Archives of Medical Research

ORIGINAL ARTICLE

Tumor Growth and Cell Proliferation Rate in Human Oral Cancer

Avishai Gavish,[a] Ella Krayzler,[b] and Rafael Nagler[b]

[a]*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[b]*Department of Neuroscience, Faculty of Medicine, Rappaport Family Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa, Israel*

Received for publication January 4, 2016; accepted July 4, 2016 (ARCMED-D-16-00006).

Background and Aims. Oral squamous cell cancer (SCC) has a high rate of morbidity and an overall 5-year survival rate for patients of 50%, statistics that have not changed in the last half century. A better understanding of the biological nature of this aggressive disease is mandatory. The two most studied human oral cancer cell lines—SCC-15 and SCC-25—share some biological characteristics but differ in others and may serve as a platform for further oral SCC analysis. We compared their basic carcinogenic characteristics, cellular proliferation rate and tumor growth, and discussed results according to available data from the literature and our own previous studies.

Methods. We examined doubling time both *in vitro* and *in vivo* of the SCC-15 and SCC-25 cell lines. After seeding the exact same number of cells in a six-well dish we counted them daily. To confirm doubling time differences in cells, we progressed to an *in vivo* model in nude mice using male 9- to 10-week-old BALB/c-nu/nu mice.

Results. In both models (*in vitro* and *in vivo*) SCC-15 multiplied faster than SCC-25 cell line. *In vivo* the difference was more than double and *in vitro* this change was 24%.

Conclusion. Both SCC-15 and SCC-25 cell lines are suitable for further exploration of the oral carcinogenesis process. Based on our currently presented results and on the available literature, it seems that SCC-15 has an increased potential for local tumor growth and cell proliferation, whereas SCC-25 has a higher potential for invasion and metastasis.

Key Words: Oral cancer, Tumor growth, Cell proliferation, Doubling time.

Introduction

Oral squamous cell carcinoma (SCC) is a highly aggressive cancer that destroys adjacent tissues, invades bone and muscle, and often metastasizes. Oral SCC is the sixth most prevalent type of cancer worldwide with ~30,000 new cases diagnosed annually in the U.S. alone. Overall 5-year survival rate for patients is 50%, among the lowest for major types of cancer, and has not changed in the last half century (1–3). Despite therapeutic and diagnostic progress related to this cancer, the disease is characterized by high rates of morbidity and mortality. Thus, a better understanding of the biological nature of this aggressive disease is mandatory (4–10). The two most studied human oral cancer cell lines—SCC-15 and SCC-25— share some biological characteristics but differ in others and as such may serve as a platform for further analysis of oral SCC (11–13). Lee et al. (14), for example, compared both cell lines with respect to their inactivation patterns of p16/INK4A gene, one of the major target genes in carcinogenesis whose inactivation has frequently been reported in other types of tumors. They found that although homozygous deletion was detected in SCC-25, SCC-15 showed hypermethylated promoter region within the p16/INK4A gene. In another example, Min et al. (15) found that both SCC-15 and SCC-25 shared the existence of point mutations in the pivotal p53 tumor suppressor gene in the highly conserved open reading frames of the p53 gene. However,

Address reprint requests to: Rafael Nagler, Prof., Department of Neuroscience, Faculty of Medicine, Rappaport Family Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa 31096, Israel; Phone: 972 77 886687; FAX: 972-4-6541295; E-mail: nagler@tx.technion.ac.il

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2016.07.007

whereas SCC-15 had an insertion of five base pairs between codons 224 and 225, SCC-25 had a deletion of two base pairs in codon 209. Indeed, Gurnani et al. (16) found that adenovirus-mediated p53 gene therapy in combination with cisplatin and paclitaxel showed a significantly enhanced anticancer efficacy in inhibition cell proliferation of both SCC-15 and SCC-25 cell lines.

The purpose of the current study was to compare the basic carcinogenic characteristics of SCC-15 and SCC-25—cellular proliferation rate and tumor growth—and to discuss the results according to the available data in the literature and our own previous studies.

Materials and Methods

Cancer Cell Lines

In this study we used SCC-15 and SCC-25 human squamous cells, which are epithelial carcinoma cells from the tongue. The cells (American Type Culture Collection, Rockville, MD) were grown in 90% Dulbecco's Modified Eagle Medium: Nutrient Mixture (DMEM) Ham's F-12 media. Cultures also contained 10% heat-inactivated fasting blood sugar (FBS), 2.5 mmol L-glutamine, and penicillin-streptomycin solution (10,000 units/mL penicillin sodium salt and 10 mg/mL streptomycin sulfate; 1% v/v). Cells were grown at 37°C in 95% air and 5% CO_2.

Tumor Cell Implantation and Tumor Growth

In order to compare SCC-15 and SCC-25, we examined the doubling time *in vitro* as an indication of the cell proliferation rate. To clarify doubling time of cells *in vitro* we counted them on a daily basis after seeding the exact same number of cells in a six-well dish.

In order to confirm doubling time differences in cells we progressed to an *in vivo* model in nude mice, male 9–10 week old BALB/c-nu/nu mice. Mice were allowed to acclimatize for 7 d prior to beginning experimentation. Nude mice received a single subcutaneous injection of 4 $\times$ 10^6 SCC-15 or SCC-25 cells into the center of the back. For each cell line, five mice were utilized. The injected cells consistently produced detectable tumors within ~1 week of cell inoculation. Mice were observed daily for the initial appearance of tumor. Tumors were measured three times per week using a caliper. After 52 d, when the tumors had reached an appropriate size, mice were sacrificed and tumors were removed and weighed.

Statistical Analysis

For the *in vitro* model, we fitted linear curves to the number of cells in both cell lines (in log-scale) and calculated the mean doubling time and standard error for both curves.

For the *in vivo* study, experimental and control groups were replicated at $n \geq 5$. Results are presented as the mean ± SD. Determination of statistical significance was performed using Student's *t*-test. When required, one-way analysis of variance (ANOVA) was carried out and the appropriate tests performed; $p < 0.05$ was considered statistically significant.

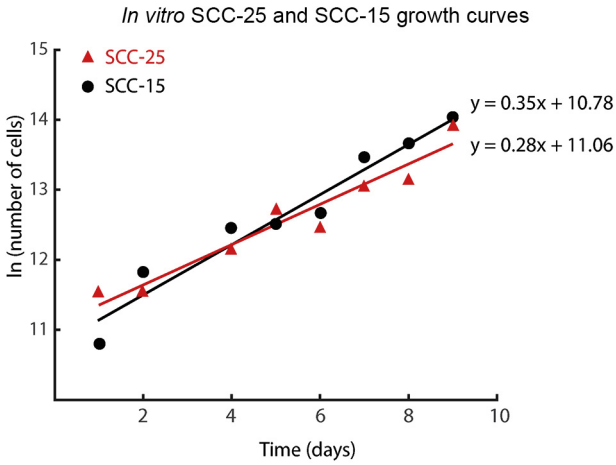

Figure 1. Growth curves of SCC-15 and SCC-25 cell lines *in vitro*. Doubling times is 1.93 ± 0.19 and 2.4 ± 0.16 for SCC-15 and SCC-25 cell lines, respectively. r^2 of black and red fits is 0.957 and 0.954, respectively. (A color figure can be found in the online version of this article.)

Results

In Vitro *SCC-15 and SCC-25 Cell Proliferation Rate and Tumor Growth*—In Vivo *Study*

The *in vitro* experiment showed that the doubling time of the two cell lines is different (Figure 1). SCC-15 cell line had a faster doubling time than the SCC-25 cell line (24% greater, Table 1).

In the *in vivo* experiment, we again found a difference between the SCC-15 and SCC-25 cell lines. The observed increase in the SCC-15 cell proliferation rate may explain the much bigger and heavier subcutaneous growths (2.27 higher) in mice that were *in situ* injected cells with the SCC-15 cell line as compared with the SCC-25 cell line, 19.3 g vs. 8.5 g ($p < 0.05$) (Figure 2, Table 1).

Discussion

We identified the doubling time of SCC-15 and SCC-25 cell lines *in vitro* and their tumor growth rates in nude mice

Table 1. SCC-25 and SCC-15 doubling time (±standard error) and tumor proliferation rate

	Doubling time *in vitro* (d)	**Weight of subcutaneous growth after 11 weeks (mg)**
SCC-15	1.93 ± 0.19	8.5 ± 3.1
SCC-25	2.4 ± 0.16	19.3 ± 3