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Amplifications of *TAOS1* and *EMS1* genes in oral carcinogenesis: association with clinicopathological features

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Summary Amplification of chromosomal region 11q13 is one of the genetic alterations most frequently observed in oral squamous cell carcinoma (OSCC). Both *TAOS1*, a recently identified gene, and *EMS1* were thought as two important target oncogenes for driving 11q13 amplification, and their contributions to oral carcinogenesis were hypothesized. Therefore we investigated amplifications of *TAOS1* and *EMS1* genes and their relations to clinicopathological variables in premalignant lesions (leukoplakias) and primary OSCC. *TAOS1* amplification, beginning from mild-dysplastic epithelia, occurred in 33.3% of leukoplakias and 51.5% of OSCC. *EMS1* amplification, beginning from moderate-dysplastic epithelia, occurred in 20% of leukoplakias and 57.6% of OSCC. Both gene amplifications were significantly related to different stages of oral carcinogenesis ($p < 0.05$). During multistage carcinogenesis, no gene amplification was observed in normal tissue and non-dysplastic leukoplakias while, in OSCC with metastasis, amplification frequency increased significantly ($p < 0.005$). Both *TAOS1* and *EMS1* amplifications were significantly associated with larger tumor size, presence of lymph node metastasis, poor histological differentiation and advanced clinical stage. Our data suggested potential roles in oral carcinogenesis and that *TAOS1* might be involved earlier than *EMS1*. Both genes might be candidate biomarkers for diagnosis and prognosis in OSCC.

Abbreviations: *TAOS1*, tumor amplified and overexpressed seqence 1; *EMS1*, ems1 sequence; *ORAOV1*, oral cancer overexpressed 1; OPL, oral premalignant lesion; OLK, oral leukoplakia; OSCC, oral squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; H&E, hematoxylin & eosin.

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Introduction

Gene amplification, one of the major mechanisms for activation of oncogenes, leads to an increase of DNA copy numbers and overexpression of oncogenes in many human tumors, contributing to the growth advantage of cells, which subsequently changes their biological behaviors and causes carcinogenesis.[1–3] Chromosomal band 11q13 is a frequently amplified genomic segment in a large number of human tumors and is thought as a potential biomarker for diagnosis and prognosis.[4,5] Many oncogenes related to oral squamous cell carcinoma (OSCC) reside in the amplified 11q13 and each gene individually may confer different properties to cancers. *TAOS1* was a recently identified gene, which was amplified and overexpressed in 63% of OSCC cell lines.[6] Accumulated evidence indicates that in addition to *CCND1*, *TAOS1* and *EMS1* are the other two important target oncogenes for driving the 11q13 amplification in OSCC.[6] The aberrant regulation of *EMS1* gene contributes to tumor cell invasion and metastasis by organizing the cytoskeleton and cell adhesion structures.[7,8] The dysregulation of cell cycle and cell proliferation due to *CCND1* amplification might play an important role in oral carcinogenesis.[9,10] Given the close proximity of *TAOS1* to *CCND1* (approximately 11.1 kb telomeric to *CCND1* gene) and its high amplification frequency in OSCC cell lines, it could be interesting to hypothesize its cancer-related functions. As yet, until now, the natures and roles of *TAOS1* and *EMS1* genes in oral premalignant lesion (OPL) and primary OSCC are still let to be known.

Leukoplakic oral epithelia are a useful model for monitoring genetic abnormalities and exploring oral carcinogenesis at cellular level. In this study, we set out to investigate *TAOS1* and *EMS1* amplifications in surgically resected primary OSCC and oral leukoplakia (OLK) specimens with various degrees of epithelial dysplasia. In addition, we tried to examine their clinical and prognostic relevance.

Materials and methods

Subjects and samples

A total of 78 subjects, including 30 OLK patients, 33 OSCC patients and 15 healthy persons, were enrolled in the present study from West China Hospital of Stomatology, Sichuan University, China. The subjects were aged between 36 and 83 years (mean ± SD; 57.9 ± 9.5) and they consisted of 46 males and 32 females. Table 1 presents the characteristics of enrolled subjects. 13 OSCC patients accompanied regional lymph node metastasis, and the remaining (n = 20) did not. No subjects had radiotherapy, chemotherapy or other interventional palliative or therapeutic measures prior to sampling. All subjects participated in this study with written consents and the project was approved by the Institutional Review Board.

All surgically resected specimens were formalin-fixed and paraffin-embedded using conventional histopathological techniques. Histopathological evaluation was performed according to the WHO criteria for histological typing of cancer and pre-cancer of the oral mucosa by the Department of Pathology, West China Hospital of Stomatology. Epithelial dysplasia was graded as none (hyperplasia, n = 6), mild (n = 9), moderate (n = 8), and severe (n = 7).

Table 1 Clinical characteristics of the studied populations (n = 78)

Characteristics	Control (n = 15)	OLK (n = 30)	OSCC (n = 33)
Age (yrs)			
Mean ± SD	53.3 ± 5.3	57.7 ± 6.5	60.3 ± 12.4
Range	40—59	40—70	36—83
Gender			
Male	8	14	24
Female	7	16	9
Site			
Tongue	4	19	17
Buccal	1	6	6
Gingiva	3	2	6
Palate	0	2	3
Oral floor	0	1	1
Lip	7	0	0

Microdissection and DNA extraction

Sequential 36 tissue sections (10 μm) stained with hematoxylin were mounted on uncoated slides. Normal/hyperplastic/dysplastic/cancerous epithelia, were dissected and harvested under a stereomicroscope. Target epithelia were confirmed by routine light microscopy of the corresponding 4 μm H&E slide avoiding contamination of adjacent non-target tissues. Samples containing <70% target epithelia were not taken for further analysis. Briefly, DNA was isolated from the harvested epithelia by proteinase K (Merck, Darmstadt, Germany) treatment and ethanol/salt precipitation methods. DNA samples were stored at −20 °C until use.

PCR

The DNA preparation (5 μL) was added to the PCR mixture (20 μL) containing 2.5U of Taq DNA polymerase (Promega, Madison, WI, USA), 1 × PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each deoxynucleotide triphosphate, and 1 μmol/L of each primer. The PCR protocol was then carried out at a PCR system (Eppendorf AG, Hamburg, Germany) with an initial 5-min denaturation step at 94 °C coupled to a repeating cycle of 1 min at 94 °C (denaturation), 30 s at 58 °C (annealing), and 30 s at 72 °C (extension) for 36 cycles, followed by a final cycle at 72 °C for 10 min. A negative reagent control and a negative sample control were included in each PCR run. Two different sets of primers, one for the target gene (*TAOS1* or *EMS1*) and the other for the control gene (*GAPDH*), were present simultaneously in one reaction vessel. Primers of *TAOS1* and *GAPDH* were designed with the PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primers for the *TAOS1* gene were 5′-AAG CAT GTC CGA AAG CAG TC-3′ and 5′-AAA CTC GGC GAC AGA GTG AG-3′, which amplified a sequence of 235 bp. Primers for the *GAPDH* gene were 5′-ATC ACT GCC ACC CAG AAG AC-3′ and 5′-TGA CAA AGT GGT CGT TGA