

The Association of *Caveolin-1* Genotypes with Oral Cancer Susceptibility in Taiwan

Da-Tian Bau^{1,4,5*}, Ming Hsui Tsai^{1,2*}, Yung-An Tsou^{1,2*}, Chung-Hsing Wang¹, Chia-Wen Tsai¹, Shung-Shung Sun¹, Chun-Hung Hua^{1,2}, Song-Kun Shyue⁵ and Ru-Yin Tsai¹

Departments of ¹Terry Fox Cancer Research Laboratory and ²Otolaryngology, China Medical University Hospital, Taichung; ⁴Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan. ⁵Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

*These authors contributed equally to this study

Correspondence to: Da-Tian Bau, Ru-Yin Tsai, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan.

Tel: +886-422052121 Ext. 1523, Fax: +886-422053366

e-mail: datian@mail.cmuh.org.tw; artbaur@yahoo.com.tw

Key Words: Cav-1, polymorphism, oral cancer, metastasis.

Running title: Bau et al: *Cav-1* Polymorphisms in Oral Cancer

ABSTRACT

Background. Caveolin-1, which has been proposed as a candidate tumor suppressor, plays a regulatory role in several signaling pathways. The aim of this study is to evaluate the association between oral cancer susceptibility and *Cav-1* genotypes. In this hospital—based case—control study, the association of *Cav-1* polymorphisms with oral cancer risk in a Central Taiwanese population was investigated.

Methods. Six hundred patients with oral cancer and 620 age- and gender-matched healthy controls recruited were genotyped and analyzed by PCR-RFLP method.

Results. There were significant differences between oral cancer and control groups in the distributions of their genotypes ($P=1.7*10^{-18}$ and $2.6*10^{-4}$) and allelic frequencies ($P=3.3*10^{-19}$ and $9.5*10^{-6}$) in the *Cav-1* G14713A (rs3807987) and T29107A (rs7804372) polymorphisms, respectively. As for the combined genotype analysis, those who had GG/AT or GG/AA at *Cav-1* G14713A/T29107A showed a 0.72-fold (95% confidence interval=0.52-0.99) decreased risk of oral cancer compared to those with GG/TT, while those of any other combinations were of increased risk. The presence of metastasis was also correlated to both *Cav-1* G14713A AA and *Cav-1* T29107A TT genotypes.

Conclusions. This is the first report providing evidence that *Cav-1* being involved in oral cancer, the A allele of the *Cav-1* G14713A is risky, the A allele of the *Cav-1* T29107A is protective, and AA/TT on these two polymorphisms may be the most risky combined

genotype for the development of oral cancer and may be novel risky markers for early detection and prediction of distant metastasis.

INTRODUCTION

Oral cancer incidence has increased more than 15% from the mid 1970s until the latest (2007) National Cancer Institute Survey. In 2007, the estimated incidence of oral cancer in the United States was 10.3 cases per 100,000 persons, with a mortality rate of 2.5 per 100,000 persons.¹ It is estimated that there will be 36,540 new cases of oral cancer diagnosed in the United States in 2010 and 7,880 deaths due to this disease.² Oral cancer is more common in men than in women and accounts for 3% of new cancer cases in American male.² In Taiwan, where the oral cancer density is highest worldwide, oral cancer is a fatal disease accounting for the fourth highest incidence of malignancy in males and the seventh in females.³ The relatively high prevalence of oral cancer in Taiwan is mainly because there is a high-risk group of 2.5 million people with the habit of smoking, alcohol drinking and betel nut chewing, while the genomic etiology of oral cancer is of great interest but largely unknown.

Caveolae are found primarily in terminally differentiated mesenchymal cells and caveolin proteins (caveolin-1, caveolin-2, and caveolin-3) serve as the structural components of caveolae and also function as scaffolding proteins, which are capable of recruiting numerous signaling molecules to caveolae and regulating their activity. Studies in caveolin-deficient mice have indicated that caveolae and caveolins participate in human disease processes, including diabetes, cancer, cardiovascular disease, atherosclerosis,

pulmonary fibrosis, and a variety of degenerative muscular dystrophies.⁴ Caveolin-1 (Cav-1), a 22- to 24-kDa protein of 178 amino acids, initially was identified as a major substrate for tyrosine phosphorylation in Rous sarcoma virus-transformed chicken embryonic fibroblasts, suggesting that its role of being a candidate tumor suppressor gene.⁵ It has been demonstrated that cav-1 is down-regulated in sarcoma, lung carcinoma, and ovarian carcinoma.⁶⁻⁸ However, elevated expression of cav-1 has been associated with the metastasis of esophageal squamous cell carcinoma and prostate cancer and negatively correlated with patient survival.^{9, 10} These findings indicate that the role of cav-1 may vary considerably, depending on the tissue involved.

Up to now, to our knowledge, there was no report about the association of *Cav-1* with oral oncology. Thus, the objectives of the current study were to determine the genotypic frequency of six polymorphisms of the *Cav-1* gene at C239A (rs1997623), G14713A (rs3807987), G21985A (12672038), T28608A (rs3757733), T29107A (rs7804372), and G32124A (rs3807992). In addition, the correlations of *Cav-1* genotypes with clinicopathological data, such as recurrence, metastasis, and survival outcome were also investigated. To the best of our knowledge, this is the first study carried out to evaluate the contribution of *Cav-1* genotypes in oral oncology all over the world.

MATERIALS AND METHODS

Study Population and Sample Collection

Six hundred and twenty patients diagnosed with oral cancer were recruited at the outpatient clinics of general surgery between 1998-2009 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The mean age of the oral cancer patients and the controls were 65.5 (SD = 9.7) and 63.5 (SD = 8.5) years, respectively. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The questionnaire administered to the subjects included questions on history and frequency of alcohol consumption, betel quid chewing and smoking habits. Self-reported alcohol consumption, betel quid chewing and smoking habits were evaluated and classified as categorical variables. Information on these factors was obtained as more than twice a week for years as “ever”. The same amounts of non-oral cancer healthy people as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping Conditions

Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous papers.¹¹⁻¹⁷ Briefly, the following primers were used for *Cav-1* C239A (rs1997623):

5'-GTGTCCGCTTCTGC TATCTG-3' and 5'-GCCAAGATGCAGAAGGAG TT-3'; for *Cav-1* G14713A (rs3807987): 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCATAGT-3'; for *Cav-1* G21985A (12672038): 5'-GGTGTTCAGCAAGGCTATGCT-3' and 5'-CCAGACACTCAGAATGTGAC-3'; for *Cav-1* T28608A (rs3757733): 5'-GCTCAACCTCATCTGAGGCA-3' and 5'-GGCCTATTGTTGAGTGGATG-3'; for *Cav-1* T29107A (rs7804372): 5'-GCCTGAATTGCAATCCTGTG-3' and 5'-ACGGTGTGAACACGGACATT-3'; and for *Cav-1* G32124A (rs3807992): 5'-GGTGTCTTGCAGTTGAATG-3' and 5'-ACGGAGCTACTCAGTGCCAA-3'. The following cycling conditions were performed: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. The PCR products were studied after digestion with Avr II, Bfa I, Hae III, Tsp509 I, Sau3AI and Nla III, restriction enzymes for *Cav-1* C239A (cut from 485 bp C type into 170+315 bp T type), *Cav-1* G14713A (cut from 268 bp A type into 66+202 bp G type), *Cav-1* G21985A (cut from 251+43 bp A type into 153+98+43 bp G type), *Cav-1* T28608A (cut from 298 bp T type into 100+198 bp A type), *Cav-1* T29107A (cut from 336 bp A type into 172+164 bp T type), and *Cav-1* G32124A (cut from 213+142+67 bp A type into 142+118+95+67 bp T type), respectively. Ten percent of the samples both in control and patient groups were analyzed of their genotypes by PCR direct sequencing (Genomics BioSci & Tech Co., Taipei).

Sample Preparation and Western Blotting Analysis of Cav-1

The tissues of tumor sites and distal sites (as the internal control for each patient) from oral cancer patients at early stage collected from 2009 to 2010 were homogenized in RIPA lysis buffer (Upstate, USA, Inc), the homogenates were centrifuged at 10,000 g for 30 min at 4°C, and the supernatants were used for Western blotting. The protein concentration of the samples was determined by the BCA method (Pierce, Thermo Fisher Scientific Inc, Waltham, MA) using bovine serum albumin as the standard. Equal amounts of total protein (20 µg) were adjusted to a similar volume with loading buffer (10% SDS, 20% glycerin, 125 mM Tris, 1 mM EDTA, 0.002% bromophenol blue, 10% β-mercaptoethanol), denatured by heating at 95°C for 10 min, then separated on a 10% SDS-PAGE gel, and transferred to a nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% non-fat milk and incubated overnight at 4°C with polyclonal rabbit anti-human Cav-1 (Abcam, Cambridge, UK), then with the corresponding horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Chemicon, Temecula, CA) for 1 h at room temperature. After reaction with ECL solution (Amersham, Arlington Heights, USA), bound antibody was visualized using a chemiluminescence imaging system (Syngene, Cambridge, UK). Finally, the blots were incubated at 56°C for 18 min in stripping buffer (0.0626 mol/L Tris-HCl, pH 6.7, 2% SDS, 0.1 mol/L mercaptoethanol) and re-probed with a monoclonal mouse anti-β-actin antibody (Sigma, Missouri, USA) as the loading control.

The optic density of each specific band was measured using a computer-assisted imaging analysis system (Gene Tools Match software, Syngene, Cambridge, UK). The expression level of Cav-1 in the tumor sites from the patients were normalized by the ratio to each distal control sites and listed in Table IV.

Clinical Outcome Survey

All the patients received regular follow up for 10 years or till the patient expired. Clinical stages were determined according to the criteria used for oral cavity cancer as recommended by AJCC 5th edition of 2002. Histology grading was performed according to the 1991 world health organization grading scheme. We had grouped the patients according to their genotypes in the clinical outcome analysis. Poor outcome was defined as if the patient had any of the following case: a) expired within one year due to persisted tumor after treatment; b) occurrence of distant metastasis within half year; c) tumor recurrence after finishing treatment within half year.

Statistical Analyses

Only those matches with all genotyping data (case/control =620/620) were selected into final analyzing. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype

frequencies of *Cav-1* single nucleotide polymorphisms (SNP) in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *Cav-1* genotypes between cases and controls. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. The analysis of survival outcome was performed by the Kaplan-Meier analysis and log rank test for calculating the significance. Any data was recognized as significant when the statistical *P*-value was less than 0.05.

RESULTS

The patient group was composed of 566 men and 54 women between age 25 and 87 years old. There were 100 clinical stage I, 224 stage II, 121 stage III and 175 stage IV oral cancer patients diagnosed by the AJCC 5th edition. The primary tumor sites were tongue, buccal mucosa, mouth floor, retromolar trigone, alveolar ridge, palate, lip, and other site in 299, 186, 36, 26, 17, 13, 13 and 30 patients. There were no significant differences between both groups in their age, sex, and environmental factors (Table I). The frequencies of the genotypes for the *Cav-1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between controls and oral cancer patients is shown in Table II. Genotype distribution of various genetic polymorphisms of *Cav-1* G14713A and T29107A were significantly different between oral cancer and control groups ($P=1.7*10^{-18}$ and $2.6*10^{-4}$, respectively), while those for *Cav-1* C239A, G21985A, T28608A and G32124A were not significant ($P>0.05$) (Table II). To sum up, the polymorphism of *Cav-1* G14713A and T29107A are associated with oral cancer risk and may be a biomarker for oral cancer detection. The representative PCR-based restriction analyses for the *Cav-1* G14713A and T29107A polymorphisms were shown in Figure 1.

The frequencies of the alleles for the *Cav-1* C239A, G14713A, G21985A, T28608A, T29107A and G32124A between controls and oral cancer patients are shown in Table III. The two SNPs of *Cav-1* found to be associated with oral cancer in Table II, G14713A and T29107A, are also found to be associated with higher oral cancer susceptibility in their allele frequency analysis here. As for other four SNPs, the distributions of their allele frequencies are not

significantly different in controls and oral cancer patients (Table III).

Considering potential interactions between the two significant SNPs of *Cav-1* gene and oral cancer susceptibility, the risk of oral cancer related to combined genotype distributions of *Cav-1* G14713A and T29107A were further analyzed (Table IV). Compared with GG/TT combined genotype of *Cav-1* G14713A and T29107A, the GG/AT or GG/AA group has a 0.72-fold lower risk of oral cancer (95% CI=0.52-0.99). Other combinations of AG/TT, AG/AT or AG/AA, AA/TT, and AA/AT or AA/AA conferred 2.81-fold (95% CI=2.00-3.96), 2.00-fold (95% CI=1.39-2.82), 3.64-fold (95% CI=2.16-6.12) and 1.88-fold (95% CI=1.13-3.12) increased risks compared to the GG/TT combined genotype, respectively (Table IV). The expression levels of

Cav-1 in the patients of each combined genotype were also summarized in Table IV. In all patients, the *Cav-1* levels were much lower in their tumor sites than the distal control sites. The average protein levels were 35.9%, 39.3%, 30.5%, 33.7%, 24.8% and 35.4% of the expression levels in their distal control sites for GG/TT, GG/AT or GG/AA, AG/TT, AG/AT or AG/AA, AA/TT, and AA/AT or AA/AA groups, respectively. The *Cav-1* level of AA/TT group was found to be statistically significant lower than that of GG/TT group ($P=0.0012$) in the tumor sites. The sample sizes for each genotype group were 10, 8, 10, 9, 5, and 3, respectively (Table IV).

We have also analyzed the correlation between the *Cav-1* genotypes and the clinicalpathological and behavioral characteristics of oral cancer patients, including age, gender, alcohol consumption, betel nut chewing, smoking, advanced stages, tumor recurrence and distant metastasis. As shown in the details in Table V. It was found that both the *Cav-1* G14713A AA

genotype and *Cav-1* T29107A TT genotype were correlated to the distant metastasis ($P=0.047$ and 0.050 , respectively) (Table VII). The other clinical parameters such as age, gender, advanced T stage (T3+T4), advanced N stage ($\geq 2b$), advanced tumor stage (stage IV), tumor recurrence within half year were not significant clinical parameters which may correlate to the *Cav-1* G14713A AA or T29107A TT genotypes. The effects of *Cav-1* genotypes on 10-year survival rates were also investigated, and it was found that oral cancer patients with G14713A AA and T29107A TT genotypes, but not others, had poor 10-year survival outcomes ($P=0.006$ and 0.037 , respectively) (Figure 2).

DISCUSSION

Although several investigations have shown that *Cav-1* plays a critical role in many tumors,⁶⁻¹⁰ few data are available which consider *Cav-1* for genetic predisposition to cancers.^{18,19} In 2004, the inactivation of *Cav-1* by mutation models or via reducing its expression was found to involve in the pathogenesis of oral cancer.¹⁹ In that study, the exon 1 and 3 sequences of *Cav-1* were investigated in 74 oral squamous cell carcinomas and 15 oral cancer cell lines, and the expression of *Cav-1* was examined. It was reported that only five mutations (1 missense and 4 silent mutations) of *Cav-1* were identified in so many cases, and they were all found in exon 3.¹⁹ Since sequencing of exonic and promoter regions had not revealed and variants in *Cav-1* that might have been directly involved in disease risk, we selected intronic SNPs from the NCBI database in order to evaluate the role of *Cav-1* polymorphisms, which have never been reported

to be associated with oral cancer risk.

Our study revealed that the *Cav-1* G14713A (rs3807987) and T29107A (rs7804372) polymorphisms were associated with the susceptibility to oral cancer (Table II and III), while the other four polymorphisms were not. Although these genetic variations do not direct result in amino acid coding change, it is plausible to suspect the alternative splicing, intervention, modification, determination or involvement of these SNPs influence the expression level or stability of the Cav-1 protein.

The down-regulated expression of Cav-1 observed in the current study was similar to that reported in studies of mucoepidermoid carcinoma of the salivary glands, sarcoma, lung carcinoma, and ovarian carcinoma.^{6-8, 20} In addition, it has been suggested that transcriptional silencing through hypermethylation of the *Cav-1* promoter may abrogate Cav-1 expression in human cancers.⁴ In our results, the protein levels of tumor sites of early stage oral cancer patients were much lower than the distal control sites, and this down-regulated Cav-1 expression supported the idea that the differential expression may be caused by *Cav-1* genotypes. Furthermore, the combined AA/TT genotype at Cav-1 G14713A/T29107A was found to be of lower Cav-1 expression level than those patients of GG/TT genotype, which correlated well with higher odds ratio of oral cancer susceptibility (Table IV). It is interesting that the Cav-1 expression level may be reactivated in the aggressive esophageal squamous cell carcinoma and prostate cancer.^{9, 10} The patient sample size with both genotype (620) and protein expression level (45) should be enlarged and investigated of their correlation with oral cancer progression to

elucidate the role of Cav-1 in the near future.

There was no correlation among either *Cav-1* G14713A or T29107A genotypes with elder age, gender, alcohol consumption, betel nut chewing, smoking, advanced stages, or tumor recurrence. However, the risky AA genotype of G14713A and TT genotype of T29107A were both correlated with higher distant metastasis rates (Table V). These two risky genotypes were also correlated to poorer 10-year survival outcome among the oral cancer patients (Figure 2). Thus, these two SNP sites, *Cav-1* G14713A and T29107A, may be novel and potential oral cancer early detection biomarkers for Taiwanese. In the future, studies in other ethnicities may examine their application as worldwide oral cancer early detection biomarkers.

Environmental factors including cigarette smoking, alcohol consumption and betel quid chewing, were reported to be closely related to oral carcinogenesis. In this study, the joint effects of *Cav-1* gene and individual behaviors including smoking, alcohol drinking and betel quid chewing habits were also analyzed, but no significance interaction was observed (Table V). The large enough sample size and similar trends of significant data after age- and behavior-adjustments strengthen the accuracy and reliability of our findings, and the frequencies of *Cav-1* polymorphisms variant alleles were similar to those reported in the NCBI website in other Asian population studies. For instance, the minor A allele frequencies of *Cav-1* G14713A are 22.5% in our control group, close to those of 16.7% for Beijing and 22.2% for Tokyo populations in NCBI, which strongly suggest no selection bias for the subject's enrolments in terms of genotypes. Therefore, the need for the present results to be verified in further larger

studies is not so necessary.

Using a candidate gene approach, this present study provided evidence supporting the oral tumorigenic contribution of *Cav-1*, of which the genotypes of G14713A and T29107A were the most significantly associated. We have also revealed its correlation with clinicopathological and behavioral indexes. Additional functional analyses of the gene and polymorphisms are worthy to explore the mechanisms by which Cav-1 and its regulated proteins affect oral cancer risk. In conclusion, this is the first report to investigate the association between *Cav-1* polymorphisms and oral cancer. Our findings suggested that *Cav-1* G14713A and T29107A, but not C239A, G21985A, T28608A, or G32124A, were associated with higher susceptibility, higher metastasis, and poorer survival rates of oral cancer. The *Cav-1* G14713A AA and *Cav-1* T29107A TT genotypes might become potential biomarkers for the oral oncology early detection and prediction.

ACKNOWLEDGEMENTS

We are grateful to Wen-Shin Chang, Hsiu-Min Hsieh and the Tissue Bank in China Medical University Hospital for their technical assistance. This study was supported by research grants from the China Medical University and Hospital (DMR-99-049, DOH99-TD-C-111-005), and the National Science Council (NSC 98-2320-B-039-010-MY3).

REFERENCES

1. Altekruse SF, Kosary CL, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2007. Bethesda, Md. *National Cancer Institute*. 2010.
2. American Cancer Society. Cancer Facts and Figures 2010. Atlanta, Ga. *American Cancer Society*. 2010.
3. Department of Health, Taiwan. Cancer registration system annual report. *Taiwan, Department of Health*. 2009.
4. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev*. 2004;84:1341-1379.
5. Glenney JR, Jr. Tyrosine phosphorylation of a 22-kDa protein is correlated with transformation by Rous sarcoma virus. *J Biol Chem*. 1989;264:20163-20166.
6. Belanger MM, Roussel E, Couet J. Caveolin-1 is down-regulated in human lung carcinoma and acts as a candidate tumor suppressor gene. *Chest*. 2004;125:106S.
7. Wiechen K, Diatchenko L, Agoulnik A, et al. Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. *Am J Pathol*. 2001;159:1635-1643.
8. Wiechen K, Sers C, Agoulnik A, et al. Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *Am J Pathol*. 2001;158:833-839.
9. Yang G, Truong LD, Wheeler TM, Thompson TC. Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. *Cancer Res*.

- 1999;59:5719-5723.
10. Kato K, Hida Y, Miyamoto M, et al. Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. *Cancer*. 2002;94:929-933.
 11. Bau DT, Tseng HC, Wang CH, et al. Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. *Oral Oncol*. 2008;44:1047-1051.
 12. Chang CH, Wang RF, Tsai RY, et al. Significant association of XPD codon 312 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. *Anticancer Res*. 2009;29:3903-3907.
 13. Chiu CF, Tsai MH, Tseng HC, et al. A novel single nucleotide polymorphism in ERCC6 gene is associated with oral cancer susceptibility in Taiwanese patients. *Oral Oncol*. 2008;44:582-586.
 14. Chiu CF, Tsai MH, Tseng HC, et al. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. *Oral Oncol*. 2008;44:898-902.
 15. Chiu CF, Wang CH, Wang CL, et al. A novel single nucleotide polymorphism in XRCC4 gene is associated with gastric cancer susceptibility in Taiwan. *Ann Surg Oncol*. 2008;15:514-518.
 16. Hsu CF, Tseng HC, Chiu CF, et al. Association between DNA double strand break gene Ku80 polymorphisms and oral cancer susceptibility. *Oral Oncol*. 2009;45:789-793.

17. Wang HC, Chiu CF, Tsai RY, et al. Association of genetic polymorphisms of EXO1 gene with risk of breast cancer in Taiwan. *Anticancer Res.* 2009;29:3897-3901.
18. Conde MC, Ramirez-Lorca R, Lopez-Jamar JM, et al. Genetic analysis of caveolin-1 and eNOS genes in colorectal cancer. *Oncol Rep.* 2006;16:353-359.
19. Han SE, Park KH, Lee G, Huh YJ, Min BM. Mutation and aberrant expression of Caveolin-1 in human oral squamous cell carcinomas and oral cancer cell lines. *Int J Oncol.* 2004;24:435-440.
20. Shi L, Chen XM, Wang L, Zhang L, Chen Z. Expression of caveolin-1 in mucoepidermoid carcinoma of the salivary glands: correlation with vascular endothelial growth factor, microvessel density, and clinical outcome. *Cancer.* 2007;109:1523-1531.

FIGURE LEGENDS

Fig 1. Genotyping analysis of the T29107A (A) and G14713A (B) polymorphisms of *Cav-1* by PCR-RFLP (A and B) and double-checked by direct DNA sequencing (C-H). (A) M: 100 bp DNA size marker, A/A: indivisible homozygote, A/T: heterozygote, and T/T: divisible homozygote. (B) M: 100 bp DNA size marker, A/A: indivisible homozygote, A/G: heterozygote, and G/G: divisible homozygote. (C) TT genotype of T29107A. (D) AA genotype of T29107A. (E) AT genotype of T29107A. (F) AA genotype of G14713A. (G) GG genotype of G14713A. (H) AG genotype of G14713A.

Fig 2. Survival curves of oral cancer patients after diagnosis stratified by genotypes of *Cav-1* T29107A (A) and G14713A (B). Statistical analysis was performed by the log-rank test.

Figure 1

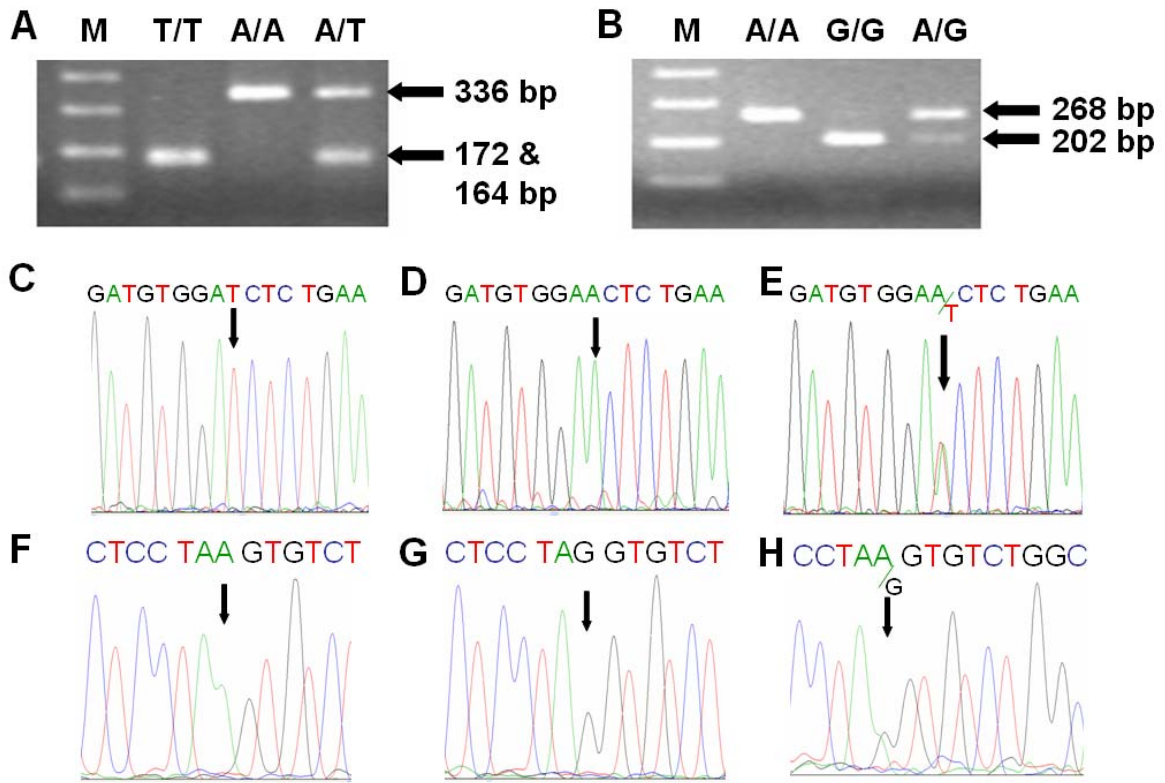
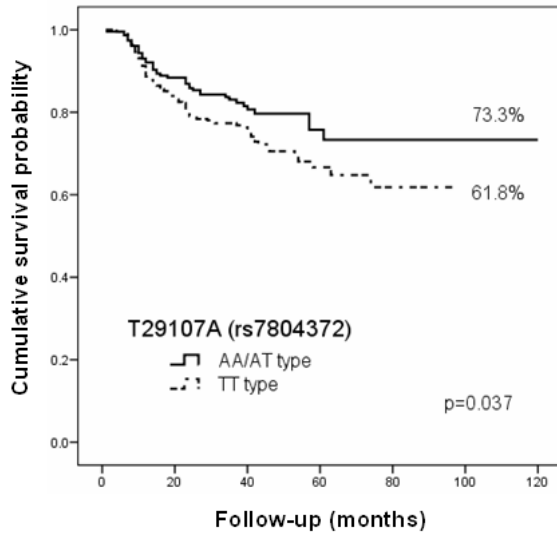


Figure 2

A



B

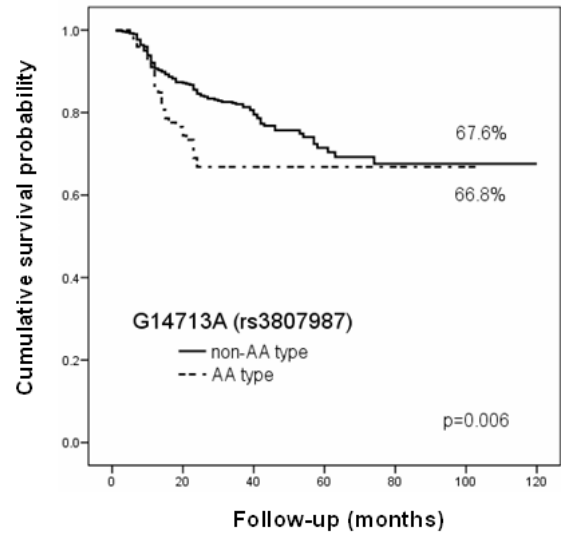


Table I. Characteristics of oral cancer patients and controls

| Characteristics | Controls (n = 620) | | | Patients (n = 620) | | | <i>P</i> ^a |
|--------------------|--------------------|-------|--------------|--------------------|-------|--------------|-----------------------|
| | n | % | Mean (SD) | n | % | Mean (SD) | |
| Age (y) | | | 63.5 (8.5) | | | 65.5 (9.7) | 0.73 |
| Gender | | | | | | | 0.60 |
| Male | 572 | 92.3% | | 566 | 91.3% | | |
| Female | 48 | 7.7% | | 54 | 8.7% | | |
| Indulgence | | | | | | | |
| Cigarette smokers | 452 | 75.3% | | 463 | 77.1% | | 0.52 |
| Areca chewers | 390 | 65.0% | | 408 | 68.0% | | 0.31 |
| Alcohol drinkers | 432 | 70.3% | | 457 | 74.5% | | 0.13 |
| Histology | | | | | | | |
| Tongue | | | | 299 | 48.2% | | |
| Buccal mucosa | | | | 186 | 30.0% | | |
| Mouth floor | | | | 36 | 5.8% | | |
| Retromolar trigone | | | | 26 | 4.2% | | |
| Alveolar ridge | | | | 17 | 2.8% | | |
| Palate | | | | 13 | 2.1% | | |
| Lip | | | | 13 | 2.1% | | |
| Others | | | | 30 | 4.8% | | |

^a*P* based on chi-square test.

Table II. Distribution of *Cav-1* genotypes among oral cancer patients and controls

| Genotype | Controls | % | Patients | % | <i>P</i> ^a |
|--------------------|----------|-------|----------|-------|-----------------------------|
| C521A rs1997623 | | | | | 0.8303 |
| CC | 608 | 98.1% | 610 | 98.4% | |
| AC | 12 | 1.9% | 10 | 1.6% | |
| AA | 0 | 0.0% | 0 | 0.0% | |
| G14713A rs3807987 | | | | | 1.7*10⁻¹⁸ |
| GG | 397 | 64.0% | 238 | 38.4% | |
| AG | 167 | 26.9% | 279 | 45.0% | |
| AA | 56 | 9.1% | 103 | 16.6% | |
| G21985A rs12672038 | | | | | 0.8417 |
| GG | 359 | 57.9% | 368 | 59.4% | |
| AG | 213 | 34.4% | 208 | 33.5% | |
| AA | 48 | 7.7% | 44 | 7.1% | |
| T28608A rs3757733 | | | | | 0.6338 |
| TT | 357 | 57.6% | 367 | 59.2% | |
| AT | 206 | 33.2% | 205 | 33.1% | |
| AA | 57 | 9.2% | 48 | 7.7% | |
| T29107A rs7804372 | | | | | 2.6*10⁻⁴ |
| TT | 306 | 49.4% | 363 | 58.6% | |
| AT | 206 | 33.2% | 193 | 31.1% | |
| AA | 108 | 17.4% | 64 | 10.3% | |
| G32124A rs3807992 | | | | | 0.7814 |
| GG | 306 | 49.4% | 295 | 47.5% | |
| AG | 247 | 39.8% | 259 | 41.8% | |
| AA | 67 | 10.8% | 66 | 10.7% | |

^a*P* based on chi-square test.

Table III. Distribution of *Cav-1* alleles among oral cancer patients and controls

| Allele | Controls | % | Patients | % | <i>P</i> ^a |
|--------------------|----------|-------|----------|-------|-----------------------------|
| C521A rs1997623 | | | | | 0.6684 |
| Allele C | 1228 | 99.0% | 1230 | 99.2% | |
| Allele A | 12 | 1.0% | 10 | 0.8% | |
| G14713A rs3807987 | | | | | 3.3*10⁻¹⁹ |
| Allele G | 961 | 77.5% | 755 | 60.9% | |
| Allele A | 279 | 22.5% | 485 | 39.1% | |
| G21985A rs12672038 | | | | | 0.5433 |
| Allele G | 931 | 75.1% | 944 | 76.1% | |
| Allele A | 309 | 24.9% | 296 | 23.9% | |
| T28608A rs3757733 | | | | | 0.3785 |
| Allele T | 920 | 74.2% | 939 | 75.7% | |
| Allele A | 320 | 25.8% | 301 | 24.3% | |
| T29107A rs7804372 | | | | | 9.5*10⁻⁶ |
| Allele T | 818 | 66.0% | 919 | 74.1% | |
| Allele A | 422 | 34.0% | 321 | 25.9% | |
| G32124A rs3807992 | | | | | 0.6645 |
| Allele G | 859 | 69.3% | 849 | 68.5% | |
| Allele A | 381 | 30.7% | 391 | 31.5% | |

^a*P* based on chi-square test.

Table IV Distribution of *Cav-1* G14713A/T29107A combined genotypes among oral cancer patients and controls

| G14713A/ T29107A combined genotype | Controls | % | Patients | % | Odds Ratio (95% CI) ^a | Adjusted Odds Ratio (95% CI) ^b | Cav-1 Level in Patients (% of distal control) |
|---------------------------------------|----------|-------|----------|-------|-------------------------------------|--|--|
| GG/TT | 199 | 32.1% | 139 | 22.4% | 1.00 (Reference) | 1.00 (Reference) | 35.9 ± 5.3 % (Reference) |
| GG/AT or GG/AA | 198 | 31.9% | 99 | 16.0% | 0.72 (0.52-0.99) ^c | 0.68 (0.55-0.88) ^c | 39.3 ± 6.0 % |
| AG/TT | 83 | 13.4% | 163 | 26.3% | 2.81 (2.00-3.96) ^c | 2.64 (2.15-3.56) ^c | 30.5 ± 6.7 % |
| AG/AT or AG/AA | 84 | 13.5% | 116 | 18.7% | 2.00 (1.39-2.82) ^c | 2.13 (1.38-3.03) ^c | 33.7 ± 6.4 % |
| AA/TT | 24 | 3.9% | 61 | 9.8% | 3.64 (2.16-6.12) ^c | 3.68 (2.15-5.87) ^c | 24.8 ± 4.0 % ^d |
| AA/AT or AA/AA | 32 | 5.2% | 42 | 6.8% | 1.88 (1.13-3.12) ^c | 1.75 (0.87-2.93) | 35.4 ± 14.1 % |

^a 95% CI, 95% confidence interval.

^b 95% CI, 95% confidence interval, and date were calculated by unconditioned logistic regression and adjusted for age, gender, smoking, alcohol drinking and betel quid chewing behaviors.

^{c,d} Statistically significant.

Table V. Univariate analysis of Cav-1 G14713A and T29107A genotype with clinicopathological and behavioral characteristics in oral cancer patients.

| Variable | Cases (n) | Genotype 1 | Genotype 2 | P-value |
|----------------------------|-----------|------------|-----------------|--------------------|
| G14713A | | AA | AG or GG | |
| Age >45 (yes) | 423 | 73 | 350 | 0.244 |
| Gender (M/F) | 566/54 | 96/4 | 470/50 | 0.080 |
| Alcohol consumption (Yes) | 431 | 69 | 362 | 0.905 |
| Betel nut chewing (Yes) | 534 | 86 | 448 | 1.000 |
| Smoking (Yes) | 550 | 90 | 460 | 0.733 |
| Advanced T stage (T3+T4) | 243 | 45 | 198 | 0.202 |
| Advanced N stage \geq 2b | 70 | 16 | 54 | 0.184 |
| TN stage \geq IV | 175 | 33 | 142 | 0.227 |
| Tumor recurrence (yes) | 172 | 27 | 145 | 0.903 |
| Distant metastasis (yes) | 33 | 23 | 10 | 0.047 ^a |
| T29107A | | TT | AT or AA | |
| Age >45 (yes) | 423 | 264 | 159 | 0.722 |

| | | | | |
|--|--------|--------|--------|--------------------|
| Gender (M/F) | 566/54 | 361/29 | 205/25 | 0.184 |
| Alcohol consumption (Yes) | 431 | 278 | 153 | 0.240 |
| Betel nut chewing (Yes) | 534 | 341 | 193 | 0.231 |
| Smoking (Yes) | 550 | 347 | 203 | 0.318 |
| Advanced T stage (T3+T4) | 243 | 145 | 98 | 0.202 |
| Advanced N stage ≥ 2 ^b | 70 | 40 | 30 | 0.296 |
| TN stage $\geq IV$ | 175 | 109 | 66 | 0.853 |
| Tumor recurrence (yes) | 172 | 110 | 62 | 0.781 |
| Distant metastasis (yes) | 33 | 28 | 5 | 0.009 ^a |

^a Statistically significant.