RAGE Gene Polymorphism and Environmental Factor in the Risk of Oral Cancer

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Abstract

Oral squamous cell carcinoma is a common neoplasm that is known to be causally associated with genetic factors and environmental carcinogens. The receptor for advanced glycosylation endproducts (RAGE) is a transmembrane protein of the immunoglobulin superfamily with broad specificity for multiple ligands, and it has been shown to play vital roles in several pathophysiologic processes, including diabetes, Alzheimer disease, renal disease, cardiovascular disease, and cancer. The present study aimed to assess the influences of RAGE gene polymorphisms, combined with environmental carcinogens on the predisposition to oral tumorigenesis. Five polymorphisms of the RAGE gene—including –374T>A (rs1800624), –429T>C (rs1800625), 1704G>T (rs184003), Gly82Ser (rs2070600), and a 63-bp deletion allele (–407 to –345)—were examined from 592 controls and 618 patients with oral cancer. We found that individuals carrying the polymorphic allele of rs1800625 are more susceptible to oral cancer (odds ratio [OR], 1.899; 95% confidence interval [CI], 1.355 to 2.661; adjusted OR [AOR], 2.053; 95% CI, 1.269 to 3.345) after adjustment for age, sex, betel nut chewing, and tobacco consumption. Moreover, we observed a significant association of rs1800625 variants with late-stage tumors (stage III/IV, OR, 1.736; 95% CI, 1.126 to 2.677; AOR, 1.771; 95% CI, 1.101 to 2.851) and large-size tumors (>2 cm in the greatest dimension; OR, 1.644; 95% CI, 1.083 to 2.493; AOR, 1.728; 95% CI, 1.089 to 2.741). Based on behavioral exposure of environmental carcinogens, the presence of 4 RAGE single-nucleotide polymorphisms (SNPs), combined with betel quid chewing and/or tobacco use, greatly augmented the risk of oral cancer. In addition, carriers of particular haplotypes of the 4 RAGE SNPs examined are more prone to develop oral cancer. These results indicate an involvement of RAGE SNP rs1800625 in the development of oral squamous cell carcinoma and implicate the interaction between RAGE gene polymorphisms and environmental mutagens as a predisposing factor of oral carcinogenesis.

Keywords: receptor for advanced glycosylation endproducts, single-nucleotide polymorphisms, haplotype, oral squamous cell carcinoma, betel quid, tobacco

Introduction

Oral cancer is a common neoplasm that can develop in any tissue of oral cavity, with the majority (approximately 90%) being oral squamous cell carcinoma (OSCC; Jemal et al. 2008). The mortality of oral cancer has remained high over the past decades despite the advances in surgical and many other therapeutic modalities (Zini et al. 2010; Jadhav and Gupta 2013). The incidence has not improved, although the etiology of oral cancer has been, to some extent, unveiled in the vast amount of studies (Scully et al. 2000; Scully and Bagan 2009; Gupta and Metgud 2013). It is demonstrated that human papillomavirus (HPV) infection (Gupta and Metgud 2013) and behavioral exposure of environmental carcinogens, such as alcohol and tobacco consumption and betel quid chewing (Awang 1988; Scully and Bagan 2009), are the risk factors of OSCC. In addition, genetic aberrations that alter carcinogen metabolism, DNA repair, and cell cycle are known to mediate oral tumorigenesis (Scully et al.

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2000; Taniyama et al. 2010). Based on the pathogenic mechanism and nature of cancer, all these parameters were interrelated and required to assess the prognosis.

The receptor for advanced glycosylation endproducts (RAGE)—often referred to as a pattern recognition receptor that controls the innate immunity—belongs to a member of the immunoglobulin superfamily of cell surface molecules with a broad spectrum of ligand specificities (Bierhaus et al. 2005). These structurally distinct ligands include the prototype of high-mobility group family proteins (i.e., HMGB1 [high-mobility group protein B1] / amphoterin), members of the S100/calgranulin protein family, extracellular matrix proteins (e.g., collagen types I and IV), β -amyloid, phosphatidylserine, complement C3a, and some advanced glycation endproducts (Xie et al. 2013). Through interacting with its diverse ligand families, RAGE orchestrates many intracellular signaling pathways to control a variety of cellular processes, such as inflammation, apoptosis, proliferation, and autophagy (Xie et al. 2013). In addition to the full-length protein, RAGE undergoes tremendous alternative splicing to generate a myriad of transcripts with distinct functions, including truncated variants lacking cytosolic or ligand binding domains as well as soluble antagonists (Sterenczak et al. 2013). Converging evidence has suggested that RAGE plays crucial roles in several pathophysiologic processes, including diabetes, Alzheimer disease, cardiovascular disease, and cancer (Ramasamy et al. 2009; Park et al. 2011).

The gene for RAGE is located on chromosome 6p21.3 in the class II/III junction of major histocompatibility complex locus and is composed of a 1.7-kb 5' flanking region, 11 exons, 10 introns, and a 3' UTR (Sugaya et al. 1994; Vissing et al. 1994). To date, numerous genetic variants have been identified in the RAGE gene, the majority of which are single-nucleotide polymorphisms (SNPs; Schmidt and Stern 2000). Ample studies have suggested that several RAGE gene polymorphisms, alone or in combination with other factors, are associated with the development or progression of various types of cancer, including gastric, lung, colorectal, breast, cervical, and ovarian cancer (Schenk et al. 2001; Tesarova et al. 2007; Gu et al. 2008; Xu et al. 2012; Zhang et al. 2013; Qian et al. 2014;). Such variations in genomic sequence have a potential to alter the function or expression of RAGE (Hudson et al. 2001; Hofmann et al. 2002), leading to changes in its final bioavailability and, thus, the carcinogenesis. Moreover, it has been demonstrated that blockade of RAGE signaling decreases tumor growth and metastases through regulating tumor proliferation, invasion, and expression of matrix metalloproteinases in animal models (Taguchi et al. 2000).

Many habits related to an individual's lifestyle, with one's genetic background, play a major role in the pathogenesis of oral cancer. Betel nut chewing and tobacco use are 2 key etiologic factors for oral tumorigenesis. However,

little is known regarding the combined effects of behavioral exposure of these mutagens and RAGE gene polymorphisms on the susceptibility to OSCC. Here, we performed a case-control study to evaluate the impact of the interactions of gene variations of RAGE with the exposure of environmental carcinogens on the risk of OSCC.

Materials and Methods

Subjects

This was a hospital-based study encompassing 618 patients with oral cancer and 592 cancer-free controls of Chung Shan Medical University Hospital in Taichung (case, $n = 91$; control, $n = 103$), Changhua Christian Hospital (case, $n = 312$; control, $n = 249$), and Show Chwan Memorial Hospital (case, $n = 215$; control, $n = 240$) in Changhua, Taiwan. Subjects were accrued from 2008 to 2013. Among the 618 cases, tumors were located in the buccal mucosa ($n = 228$), tongue ($n = 221$), gingiva ($n = 74$), palate ($n = 37$), floor of the mouth ($n = 35$), and others ($n = 23$). Oral cancer patients were staged clinically at the time of diagnosis according to the TNM staging system of the American Joint Committee on Cancer (Edge and Compton 2010). Tumor differentiation was examined by a pathologist and rated according to the committee's classification. Subjects with oral precancerous disease, such as oral submucous fibrosis, leukoplakia, erythroplakia, verrucous hyperplasia, and so on, were excluded from the control group. All participants provided informed written consent at enrollment.

Demographic Information

Data on age, sex, betel nut chewing, and tobacco consumption were recorded from each participant. Betel nut chewing is defined as habitual chewing of betel nuts or related products. Tobacco consumption is defined as current smoking of at least 1 cigarette per day during the latest 3 mo. HPV infection was not taken in consideration in the present study, due to a relatively low prevalence in our and others' samples (Lee et al. 2013) in Taiwan, although HPV infections have been causally linked to OSCC.

RAGE Genotyping

Genomic DNA was extracted with QIAamp DNA blood mini-kits (Qiagen, Valencia, CA, USA). Allelic discriminations of the RAGE rs1800625, rs1800624, rs2070600, and rs184003 allelic polymorphisms were assessed by the TaqMan assay with an ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and analyzed with SDS software (Version 3.0; Applied Biosystems). In addition, the 63-bp deletion (−407 to −345) allelic polymorphisms were assessed with polymerase

Table 1. Genotype Distributions of RAGE Gene Polymorphisms in Controls and Oral Cancer Patients.

Variable	Controls ^a (n = 592)	Patients ^a (n = 618)	Odds Ratio ^b (95% CI)	Adjusted Odds Ratio ^c (95% CI)
rs1800625				
TT	532 (89.9)	509 (82.4)	1.00	1.00
TC	57 (9.6)	102 (16.5)	1.870 (1.323 to 2.644)	1.942 (1.178 to 3.201)
CC	3 (0.5)	7 (1.1)	2.439 (0.627 to 9.482)	5.055 (0.756 to 33.800)
TC + CC	60 (10.1)	109 (17.6)	1.899 (1.355 to 2.661)	2.053 (1.260 to 3.345)
rs1800624				
TT	435 (73.5)	461 (74.6)	1.00	1.00
TA	136 (23.0)	136 (22.0)	0.944 (0.719 to 1.238)	0.882 (0.591 to 1.317)
AA	21 (3.5)	21 (3.4)	0.944 (0.508 to 1.752)	1.504 (0.569 to 3.979)
TA + AA	157 (26.5)	157 (25.4)	0.944 (0.730 to 1.220)	0.942 (0.644 to 1.377)
rs2070600				
GG	361 (61.0)	373 (60.4)	1.00	1.00
GA	209 (35.3)	223 (36.1)	1.033 (0.814 to 1.310)	0.933 (0.661 to 1.315)
AA	22 (3.7)	22 (3.6)	0.968 (0.527 to 1.778)	0.753 (0.307 to 1.847)
GA + AA	231 (39.0)	245 (39.6)	1.026 (0.815 to 1.293)	0.915 (0.655 to 1.278)
rs184003				
GG	398 (67.2)	423 (68.4)	1.00	1.00
GT	175 (29.6)	178 (28.8)	0.957 (0.746 to 1.228)	0.837 (0.583 to 1.203)
TT	19 (3.2)	17 (2.8)	0.842 (0.431 to 1.643)	0.893 (0.349 to 2.282)
GT + TT	194 (32.8)	195 (31.6)	0.946 (0.743 to 1.204)	0.843 (0.594 to 1.196)
63bp deletion				
INS/INS	547 (92.4)	577 (93.4)	1.00	1.00
INS/Del	42 (7.1)	41 (6.6)	0.925 (0.593 to 1.445)	0.952 (0.512 to 1.771)
Del/Del	3 (0.5)	0 (0)	—	—
INS/Del + Del/Del	45 (7.6)	41 (6.6)	0.864 (0.557 to 1.340)	0.928 (0.502 to 1.719)

Bold font indicates statistical significance ($P < 0.05$).

CI, confidence interval.

^aValues in No. (%).

^bThe odds ratios with their 95% confidence intervals (CIs) were estimated by logistic regression models.

^cThe adjusted odds ratios with their 95% CIs were estimated by multiple logistic regression models after controlling for age, gender, betel nut chewing, tobacco and alcohol consumption.

chain reaction as described previously (Schenk et al. 2001). The products were separated on a 3% agarose gel and then stained with ethidium bromide.

Statistical Analysis

The Hardy-Weinberg equilibrium was assessed by a chi-square goodness-of-fit test for biallelic markers. Mann-Whitney U test and Fisher exact test were used to compare the differences in demographic characteristics between healthy controls and OSCC patients. The adjusted odds ratios (AORs) with 95% confidence intervals (CIs) for the association between genotype frequencies and the risk of OSCC plus clinicopathologic characteristics were estimated by multiple logistic regression models after controlling for other covariates. The interactions of RAGE gene polymorphisms with each demographic characteristic were evaluated as described previously (Chung et al. 2011). In brief, we fitted a logistic regression model with main effects (RAGE genetic polymorphism, smoking status, alcohol

drinking status, and betel quid chewing status) as well as an interaction term between them (RAGE genotypes $\times$ demographic characteristic), as comparing the model against a model with only the main effects. The haplotype-based analysis was conducted through the Phase program (Stephens and Scheet 2005). A P value < 0.05 was considered significant. The data were analyzed by SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of Study Participants

The demographic characteristics of 2 study groups (592 controls and 618 patients with oral cancer) were analyzed (Appendix Table 1). Consistent with the findings from others (Zini et al. 2010; Jadhav and Gupta 2013), significantly different distributions ($P < 0.001$) of age, sex, betel quid chewing, and tobacco consumption were detected between healthy controls and OSCC patients. The mean age of 618

Table 2. Associations of the Combined Effect of RAGE Gene Polymorphisms and Betel Nut Chewing with the Susceptibility to Oral Cancer among 752 smokers.

Variable: Genotype	Controls ^a (n = 224)	Patients ^a (n = 528)	Odds Ratio ^b (95% CI)	Adjusted Odds Ratio ^c (95% CI)
rs1800625				
Without betel nut chewing				
TT	126 (56.3)	57 (10.8)	1.00	1.00
TC or CC	23 (10.3)	20 (3.8)	1.922 (0.978 to 3.779)	2.779 (1.097 to 7.042)
With betel nut chewing				
TT	69 (30.7)	374 (70.8)	11.982 (7.993 to 17.960)	21.697 (12.428 to 37.878)
TC or CC	6 (2.7)	77 (14.6)	28.368 (11.676 to 68.922)	140.898 (30.583 to 649.129)
rs1800624				
Without betel nut chewing				
TT	105 (46.9)	65 (12.3)	1.00	1.00
TA or AA	44 (19.6)	12 (2.3)	0.441 (0.217 to 0.895)	0.670 (0.267 to 1.679)
With betel nut chewing				
TT	58 (25.9)	330 (62.5)	9.191 (6.059 to 13.941)	16.994 (9.580 to 30.146)
TA or AA	17 (7.6)	121 (22.9)	11.498 (6.345 to 20.835)	22.851 (9.443 to 55.302)
rs2070600				
Without betel nut chewing				
GG	91 (40.6)	44 (8.3)	1.00	1.00
GA or AA	58 (25.9)	33 (6.3)	1.177 (0.673 to 2.057)	1.218 (0.555 to 2.672)
With betel nut chewing				
GG	36 (16.1)	278 (52.6)	15.971 (9.686 to 26.333)	30.903 (14.913 to 64.039)
GA or AA	39 (17.4)	173 (32.8)	9.174 (5.563 to 15.129)	16.794 (8.257 to 34.156)
rs184003				
Without betel nut chewing				
GG	92 (41.1)	51 (9.7)	1.00	1.00
GT or TT	57 (25.4)	26 (4.9)	0.823 (0.462 to 1.464)	0.658 (0.287 to 1.506)
With betel nut chewing				
GG	50 (22.3)	311 (58.9)	11.220 (7.125 to 17.670)	16.216 (8.694 to 30.244)
GT or TT	25 (11.2)	140 (26.5)	10.102 (4.246 to 5.851)	21.328 (9.678 to 47.003)

Bold font indicates statistical significance ($P < 0.05$).

CI, confidence interval.

^aValues in No. (%).

^bEstimated by logistic regression models.

^cEstimated by multiple logistic regression models after controlling for age, sex, and alcohol consumption.

patients with oral cancer (54.29 ± 11.28 y) is higher than that of control group (51.00 ± 14.99 y). Additionally, a significantly higher number of males were observed among OSCC patients when compared controls.

Association of RAGE Gene Polymorphisms with Oral Cancer

Five polymorphisms of the RAGE gene were examined in this study: -374T>A (rs1800624), -429T>C (rs1800625), 1704G>T (rs184003), Gly82Ser (rs2070600), and a 63-bp deletion allele (-407 to -345). The frequencies of these alleles calculated in our study are mostly compatible with that in dbSNP, with the exception of rs2070600 and rs1800625 (Appendix Tables 2 and 3). Data for genotype frequency and the association between RAGE polymorphisms and the

risk of oral cancer are shown in Table 1. There is no deviation ($P > 0.05$) from Hardy-Weinberg equilibrium in patients and controls for all 5 polymorphisms. To minimize the potential interference of environmental factors, AOR (with 95% CI)—which was calculated by multiple logistic regression models after adjustment for age, sex, betel nut chewing, and tobacco consumption—was used with the odds ratio (OR; with 95% CI) in each comparison. Among these variants tested, the heterozygous genotype (TC) for rs1800625 is significantly associated with the incidence of oral cancer, with the OR being 1.870 (95% CI, 1.323 to 2.644) and AOR being 1.942 (95% CI, 1.178 to 3.201). When combined with the homozygotes (CC) for the variant allele of rs1800625, its association with the risk of oral cancer is increased (OR, 1.899; 95% CI, 1.355 to 2.661; AOR, 2.053; 95% CI, 1.269 to 3.345). However, no difference in

Table 3. Associations of the Combined Effect of RAGE Gene Polymorphisms and Smoking with the Susceptibility to Oral Cancer among 574 Betel Nut Consumers.

Variable: Genotype	Controls ^a (n = 96)	Patients ^a (n = 478)	Odds Ratio ^b (95% CI)	Adjusted Odds Ratio ^c (95% CI)
rs1800625				
Without smoking				
TT	19 (19.8)	23 (4.8)	1.00	1.00
TC or CC	2 (2.1)	4 (0.9)	1.652 (0.272 to 10.024)	1.361 (0.057 to 32.612)
With smoking				
TT	69 (71.9)	374 (78.2)	4.478 (2.315 to 8.660)	6.062 (2.573 to 14.279)
TC or CC	6 (6.2)	77 (16.1)	10.601 (3.788 to 29.673)	49.855 (6.933 to 358.497)
rs1800624				
Without smoking				
TT	15 (15.6)	18 (3.8)	1.00	1.00
TA or AA	6 (6.3)	9 (1.9)	1.250 (0.362 to 4.318)	6.167 (0.319 to 119.208)
With smoking				
TT	58 (60.4)	330 (69.0)	4.741 (2.263 to 9.936)	7.365 (2.723 to 19.923)
TA or AA	17 (17.7)	121 (25.3)	5.931 (2.528 to 13.916)	16.892 (4.155 to 68.669)
rs2070600				
Without smoking				
GG	7 (7.3)	15 (3.1)	1.00	1.00
GA or AA	14 (14.6)	12 (2.5)	0.400 (0.123 to 1.306)	0.219 (0.008 to 6.060)
With smoking				
GG	36 (37.5)	278 (58.2)	3.604 (1.377 to 9.430)	8.218 (1.937 to 34.868)
GA or AA	39 (40.6)	173 (36.2)	2.070 (0.791 to 5.418)	2.713 (0.643 to 11.443)
rs184003				
Without smoking				
GG	12 (12.5)	18 (3.8)	1.00	1.00
GT or TT	9 (9.4)	9 (1.9)	0.667 (0.205 to 2.165)	0.128 (0.007 to 2.348)
With smoking				
GG	50 (52.1)	311 (65.0)	4.147 (1.884 to 9.129)	3.859 (1.443 to 10.321)
GT or TT	25 (26.0)	140 (29.3)	3.733 (1.603 to 8.694)	6.397 (1.582 to 25.875)

Bold font indicates statistical significance ($P < 0.05$).

CI, confidence interval.

^aValues in No. (%).

^bEstimated by logistic regression models.

^cEstimated by multiple logistic regression models after controlling for age, sex, and alcohol consumption.

genotype frequencies for 374T>A (rs1800624), 1704G>T (rs184003), Gly82Ser (rs2070600), and a 63-bp deletion allele (−407 to −345) of the RAGE gene was seen between the case and control groups. Due to the low allele frequency of the 63-bp deletion allele detected in our cases and no association with the disease achieved, this allele is excluded in the subsequent analyses.

Impacts of RAGE Gene Polymorphisms Combined with Behavioral Exposure of Carcinogens on Oral Tumorigenesis

We further analyzed the interaction between RAGE gene polymorphisms and environmental factors in determining

the risk of OSCC. Two common environmental carcinogens—betel quid chewing and tobacco use—were selected to investigate their impacts with RAGE gene polymorphisms on the susceptibility to oral cancer. Among 752 smokers, individuals who chew betel nut and carry at least 1 polymorphic allele of rs1800624, rs1800625, rs184003, and rs2070600 (heterozygote or homozygote for the minor allele) are more prone to develop oral cancer than are those neither chewing betel nut nor possessing the polymorphic allele (Table 2). Although the genetic effect alone may be subtle, a significant interaction of betel nut chewing with the presence of at least 1 minor allele of rs1800625 is shown to be associated with OSCC in smokers.

In addition, for 574 betel nut consumers in our cohort, individuals who smoke and carry at least 1 polymorphic

Table 4. Associations between Polymorphic Genotypes of rs1800625 and Clinicopathologic Characteristics of Oral Cancer.

Variable	Genotypic Frequencies		Odds Ratio ^b (95% CI); <i>P</i> Value	Adjusted Odds Ratio ^c (95% CI); <i>P</i> Value
	TT ^a (<i>n</i> = 509)	TC + CC ^a (<i>n</i> = 109)		
Clinical stage				
I/II	240 (47.2)	37 (33.9)	1.00	1.00
III/IV	269 (52.8)	72 (66.1)	1.736 (1.126 to 2.677); 0.012	1.771 (1.101 to 2.851); 0.019
Tumor size				
≤T2	323 (63.5)	56 (51.4)	1.00	1.00
>T2	186 (36.5)	53 (48.6)	1.644 (1.083 to 2.493); 0.019	1.728 (1.089 to 2.741); 0.020
Lymph node metastasis				
No	331 (65.0)	68 (62.4)	1.00	1.00
Yes	178 (35.0)	41 (37.6)	1.121 (0.731 to 1.721); 0.600	1.141 (0.709 to 1.836); 0.587
Distant metastasis				
No	503 (98.8)	107 (98.2)	1.00	1.00
Yes	6 (1.2)	2 (1.8)	1.567 (0.312 to 7.870); 0.582	1.233 (0.108 to 14.058); 0.866
Cell differentiation				
Well	66 (13.0)	17 (15.6)	1.00	1.00
Moderately/poorly	443 (87.0)	92 (84.4)	0.806 (0.452 to 1.438); 0.465	0.719 (0.382 to 1.354); 0.307

Bold font indicates statistical significance (*P* < 0.05).

CI, confidence interval; T2, tumor size in the greatest dimension, ≤ or > 2 cm.

^aValues in No. (%).

^bEstimated by logistic regression models.

^cEstimated by multiple logistic regression models after controlling for age, sex, alcohol consumption, betel nut chewing, and tobacco use.

allele of RAGE gene polymorphisms examined are found to be more susceptible to oral cancer than those homozygous for the reference allele but without smoking, with the exception of rs2070600 (Table 3). While smoking alone remains a considerable risk for OSCC, the presence of at least 1 polymorphic allele of rs1800625 is shown to significantly interact with smoking, causally leading to oral tumorigenesis. These results indicate that alterations in bioavailability of RAGE signaling due to genetic polymorphisms, in combination with betel quid chewing and tobacco use, may contribute to the development of oral cancer.

Correlation between Polymorphic Genotypes of RAGE and Clinical Status of Oral Cancer

Since rs1800625 is associated with the incidence of oral cancer, we also analyzed the correlations of the RAGE variant genotypes with clinicopathologic features of OSCC patients in this study (Table 4). A significant association was observed of rs1800625 variants with late-stage tumors (stage III/IV; OR, 1.736; 95% CI, 1.126 to 2.677; AOR, 1.771; 95% CI, 1.101 to 2.851) and large-size tumors (OR, 1.644; 95% CI, 1.083 to 2.493; AOR, 1.728; 95% CI, 1.089 to 2.741). Nevertheless, rs1800625 polymorphism failed to associate with lymph node metastasis, distant metastasis, and tumor differentiation, suggesting that rs1800625 variants may affect the tumor cell proliferation but not invasion and differentiation.

Association of RAGE Haplotypes with Oral Cancer

The association of RAGE haplotypes and the risk for oral cancer was also analyzed in our recruited subjects. The frequency distributions of 5 common RAGE rs1800624, rs1800625, rs184003, and rs2070600 haplotypes are shown in Table 5, with the most common haplotype in the control group (T₋₄₂₉T₋₃₇₄G_{82Gly}G₁₇₀₄) being chosen as the reference. Among these common haplotypes, only 2, T₋₄₂₉T₋₃₇₄A_{82Ser}G₁₇₀₄ and C₋₄₂₉T₋₃₇₄G_{82Ser}G₁₇₀₄, were found to be associated with increased susceptibility to oral cancer (OR, 1.324; 95% CI, 1.065 to 1.646; OR, 2.538; 95% CI, 1.780 to 3.620, respectively), while the other haplotypes that we examined failed to exhibit any significant association with a high risk of OSCC.

Discussion

Genetic association studies based on the candidate-gene or genome-wide approach to search for the statistical correlation between genetic variants and a pathophysiologic trait have made important strides in developing numerous diagnostic and therapeutic strategies for various kinds of human diseases (Hirschhorn and Daly 2005; Lunetta 2008). OSCC is a complex malignancy in which genetic alterations, environmental factors, and other risks extensively interact and thus lead to the neoplastic condition (Scully and Bagan 2009). In the present study, we for the first time revealed

Table 5. Estimated Haplotype Frequencies of 4 Examined Polymorphisms in RAGE Gene and the Corresponding Risk for Oral Cancer.

rs1800625T/C	rs1800624T/A	rs2070600G/A	rs184003G/T	Controls ^a (n = 1,184)	Patients ^a (n = 1,236)	OR (95% CI)	P Value
T	T	G	G	521 (44.0)	468 (37.9)	Reference	
T	T	A	G	222 (18.8)	264 (21.4)	1.324 (1.065 to 1.646)	0.011
T	T	G	T	189 (16.0)	209 (16.9)	1.231 (0.975 to 1.554)	0.080
T	A	G	G	158 (13.3)	176 (14.2)	1.240 (0.967 to 1.590)	0.089
C	T	G	G	50 (4.2)	114 (9.2)	2.538 (1.780 to 3.620)	<0.001
Other ^b				44 (3.7)	5 (0.4)	—	—

Bold font indicates statistical significance ($P < 0.05$).

^aValues in No. (%).

^bTTAT (n = 27; control, n = 24; patient, n = 3), CAGG (n = 15; control, n = 13; patient, n = 2), TAAG (n = 7; control, n = 7; patient, n = 0).

that the RAGE gene polymorphism rs1800625 not only conferred an increased risk of oral cancer but was also observed with late-stage and large-size tumors in the Taiwanese population.

RAGE is a transmembrane receptor that contains a V-type domain, which is central to ligand binding, 2 C-type domains, a transmembrane domain, and a C-terminal cytosolic tail, which functions for cell signaling (Neeper et al. 1992). Several truncated forms of RAGE protein that may alter the bioavailability of RAGE signaling are preferably produced through proteolytic cleavage of full-length RAGE or alternative splicing under some circumstances (Xie et al. 2013). Changes in the ratio of a given variant form to the full-length RAGE may serve as a biomarker for the prognoses of many human disorders (Xie et al. 2013).

Moreover, the biology of RAGE extends beyond the scope of multiple variant forms expressed and can be largely accounted for by its broad specificity for a variety of ligands. Ligand engagement not only initiates the transduction of intracellular signal but also upregulates RAGE expression (Clynes et al. 2007). The interactions between RAGE and its various ligands (e.g., HMGB1 and certain S100 proteins) play a key role in the pathogenesis and progression of cancer (Sparvero et al. 2009). Of note, we found that RAGE haplotype with the polymorphic allele of Gly82Ser (rs2070600)—a missense SNP that causes a change from glycine to serine within the putative ligand-binding domain of the RAGE protein (Hudson et al. 1998)—is significantly associated with enhanced susceptibility to OSCC (Table 5). While behavioral exposure of environmental carcinogens being taken into consideration, specifically betel nut chewing and tobacco consumption, Gly82Ser polymorphism tends to give rise to oral cancer (Tables 2 and 3). Ser82 isoform has been shown to exert a higher ligand affinity, leading to heightened expression of inflammatory proteins in macrophages isolated from human subjects (Hofmann et al. 2002). Furthermore, recent observations have indicated that the Gly82Ser polymorphism is

relevant to various cancers, including colorectal, gastric, and breast cancer (Tesarova et al. 2007; Gu et al. 2008; Qian et al. 2014). These findings, with our data, suggest that the Ser82 allele may play an important role in oral tumorigenesis by virtue of enhancing ligand affinity.

In addition to altering the ligand affinity or repertoire, changes in the expression level of RAGE appear to be related with an increased risk of OSCC based on our finding that rs1800625 (–429T>C), a RAGE polymorphism located in the promoter region, is associated with the incidence and development of oral cancer (Tables 1 and 4). Such correlation could be magnified if an interactive effect of rs1800625 with betel nut chewing or smoking were assessed (Tables 2 and 3). Our haplotype analysis further supported this observation (Table 5). Under physiologic conditions, RAGE is expressed at low levels in all tissues, with the exception of the lung (Neeper et al. 1992). However, in some pathophysiologic settings, such as diabetes, chronic inflammation, or neurodegenerative disorders, RAGE can be aberrantly expressed in a tissue-specific manner (Yan, Ramasamy, et al. 2009; Yan, Yan, et al. 2009). It has been reported that the expression level of RAGE is lower in OSCC than in normal oral mucosa samples (Landesberg et al. 2008). Nevertheless, a functional investigation has demonstrated that the polymorphic allele (C) of rs1800625 results in augmented expression of RAGE (Hudson et al. 2001). Regardless of these conflicting findings, our data presented here indicate a functional effect of rs1800625 on fluctuations in RAGE expression, ultimately resulting in development and progression of oral cancer.

To date, interactions between polymorphisms of many susceptibility genes and exposure of environmental carcinogens have been reported to affect the risk for OSCC (Chung et al. 2011; Supic et al. 2011; Bharti et al. 2013). Epidemiologic investigations have shown that tobacco smoking and betel nut chewing are 2 of the major risk factors for OSCC development (Awang 1988; Scully and Bagan 2009). It is conceivable that carcinogenic factors derived from these addictive behaviors accumulate over

time and interact with specific genes important for normal cellular development, finally leading to a malignant phenotype. The impacts of environmental risks on the susceptibility of OSCC are limited, and they may be underestimated because of a lack of quantitative definition for betel nut chewing and a potential exclusion of subjects who may have had heavy tobacco use but are not current smokers; however, we consistently found that individuals who carry at least 1 polymorphic allele of rs1800625 are more susceptible to oral cancer if they smoke and chew betel nuts (Tables 2 and 3).

Taken together, our results show that SNP rs1800625 and certain haplotypes of the RAGE gene are associated with the risk and/or progression of oral cancer. In addition, a combined effect of RAGE SNPs (rs1800624, rs1800625, rs184003, and rs2070600) with betel nut chewing and smoking causally contributes to the development of OSCC. These findings indicate a novel genetic–environmental predisposition to oral tumorigenesis.

Author Contributions

S. Su, C. Lin, contributed to conception, drafted the manuscript; M. Chien, contributed to conception, critically revised the manuscript; M. Chen, contributed to analysis and interpretation, critically revised the manuscript; S. Yang, contributed to conception and design, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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