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Interaction of Exo1 genotypes and smoking habit in oral cancer in Taiwan

Ming-Hsui Tsai [a,b], Hsien-Chang Tseng [a,b], Chiu-Shong Liu [a,c], Chia-Lin Chang [a], Chia-Wen Tsai [a,d], Yung-An Tsou [a,b], Rou-Fen Wang [a], Cheng-Chieh Lin [a,c], Hwei-Chung Wang [a,1], Chang-Fang Chiu [a,1], Da-Tian Bau [a,d,1,*]

[a] Department of Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung 40402, Taiwan, ROC
[b] Department of Otolaryngology, China Medical University Hospital, Taichung 40402, Taiwan, ROC
[c] Department of Family Medicine, China Medical University Hospital, Taichung 40402, Taiwan, ROC
[d] Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan, ROC

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SUMMARY

Exonuclease 1 (Exo1) is an important nuclease involved in the mismatch repair system that helps to maintain genomic stability, to modulate DNA recombination, and to mediate cell cycle arrest. Potential polymorphisms in *Exo1* may alter cancer risks by influencing the repair activity of Exo1. Therefore, we hypothesized that single-nucleotide polymorphisms in *Exo1* were associated with the risk of oral cancer. In this hospital-based study, the associations of *Exo1* A-1419G (rs3754093), C-908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P (rs9350) and C3114T (rs851797) polymorphisms with oral cancer risk in a central Taiwan population were investigated. In total, 680 patients with oral cancer and 680 age- and gender-matched healthy controls recruited from the China Medical University Hospital were genotyped. A significantly different distribution is found in the frequency of the *Exo1* K589E genotype, but not the other genotypes, between the oral cancer and control groups. The A allele *Exo1* K589E conferred a significant ($P = 6.18E\text{-}8$) increased risk of oral cancer. Gene–environment interactions with smoking were significant for *Exo1* K589E polymorphism (OR = 2.509, 95% CI = 1.914–3.287). Our results provide evidence that the A allele of the *Exo1* K589E may be associated with the development of oral cancer.

Introduction

Oral cancer, a commonly diagnosed cancer all over the world,[1–4] has the highest incidence of all head and neck cancers in Taiwan.[5] The environmental factors, tobacco, alcohol and betel nuts, are well-known causes of oral cancer in Taiwan, while the genomic etiology of oral cancer is of great interest but largely unknown. Thereafter, the joint effects of environmental and genetic factors may be more comprehensive and less ignorable. Human DNA repair mechanisms protect the genome from various insults caused by endogenous and environmental agents.[6] The DNA repair mechanisms are essential in preventing tumor initiation and progression, and mutations or defects in the DNA repairing systems are thought to be essential for tumorigenesis.[7,8] It is therefore logical to suspect that some genetic variants of DNA repair proteins, such as exonuclease I (Exo1), might contribute to oral cancer pathogenesis.

Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk.[9] Since single-nucleotide polymorphism (SNP) is the most frequent and subtle genetic variation in the human genome and has great potential for application to association studies of complex disease.[10] The DNA damages and genome instability have been thought as the first step of various carcinogenesis. The DNA repair systems are responsible to remove DNA damage and maintaining the genome stability, and each type of DNA injury is repaired via its specific repair pathway. One of the major DNA repair pathways in human cells is the mismatch repair (MMR), which maintains genomic stability, modulates DNA recombination, and mediates cell cycle arrest.[11] This system is important in preventing malignancies, and former reports indicated the deficient mutations of MMR system will lead to various types of cancer.[12–14] The gene *exonuclease 1* (*Exo1*; MIM #606063) belongs to the MMR system, and also belongs to the RAD2 nuclease family. It locates at chromosome 1q42–q43, contains one untranslated exon followed by 13 coding exons and encodes an 846 amino acid protein.[15–17] Exo1 can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells, and with MSH3 in human cells.[18–22]

SNPs of DNA repair genes have been associated with susceptibility to several types of cancer, including oral, breast, gastric,

* Corresponding author. Address: Department of Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung 40402, Taiwan, ROC. Tel.: +886 4 22052121x1543; fax: +886 4 22033295.
E-mail addresses: datain@mail.cmuh.org.tw, artbau1@yahoo.com.tw (D.-T. Bau).
[1] The authors contribute equally to this study.

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prostate, colorectal, bladder and lung cancers.[23–31] These reports indicated that SNPs of the DNA repair systems may affect the genes' function or expression levels, and the capacity of those gene-related systems will also be affected. Therefore, cancer susceptibility will be higher in people who carry risky genotypes. In order to understand and prevent local oral cancer, we have chosen up to nine SNPs of *Exo1*, A-1419G (rs3754093), C-908G (rs10802996), A238G (rs1776177), C498T (rs1635517), K589E (rs1047840), G670E (rs1776148), C723R (rs1635498), L757P (rs9350) and C3114T (rs851797), and investigated their frequencies in Taiwanese population.

Materials and methods

Study population and sample collection

Six-hundred and eighty cancer patients diagnosed with oral cancer were recruited at the outpatient clinics of general surgery between 1994 and 2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-cancer healthy volunteers as controls were selected by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups finished a short questionnaire which included some indulgences and they were recorded. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consents were obtained from all participants.

Genotyping assays

Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies.[25–30] Briefly, the following primers were used for *Exo1* A-1419G: 5′-AACTGACAGGCACACTTAAG-3′ and 5′-GTAGAGAAGCCTTCTTACAC-3′; for *Exo1* C-908G: 5′-GTTAGGTCTACCATAGCCTT-3′ and 5′-TTCATGGTCACTTGTGGCTA-3′; for *Exo1* A238G: 5′-AGTCTCTTACCTCTCAGATG-3′ and 5′-TACATGCAATCTCTCCACCT-3′; for *Exo1* C498T: 5′-AGCGTAGTAAGAATGGCTGA-3′ and 5′-GATAAGAGAGCAGACGATTC-3′; for *Exo1* K589E: 5′-GACACAGATGTAGCACGTAA-3′ and 5′-CTGCGACACATCAGACATAT-3′; for *Exo1* G670E: 5′-AATATGTCTGATGTGTCGCA-3′ and 5′-TAGCTCGTCATTCACATGTA-3′; for *Exo1* C723R: 5′-ACACCTACAGTCAAGCATAA-3′ and 5′-ACTCTAGGAATCTGATTGCA-3′; for *Exo1* L757P: 5′-CAGAATGGTCTTAAAATGGGTGT-3′ and 5′-TTCAGAATAAGAAACAAGGCAAC-3′; and for *Exo1* C3114T: 5′-CTACTTGACAACATTACAGA-3′ and 5′-GAGAACCTGATTGTGTTATA-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 EcoP15 I, HpyCH4IV, Dpn II, Stu I, Mse I, Ear I, HpyCH4IV, Mnl I, and Mse I, restriction enzymes, respectively, for A-1419G (cut from 386 bp A type into 144 + 242 bp G type), C-908G (cut from 470 bp G type into 225 + 245 bp C type), A238G (cut from 367 bp G type into 178 + 189 bp A type), C498T (cut from 323 bp T type into 150 + 173 bp C type), K589E (cut from 306 bp G type into 110 + 196 bp A type), G670E (cut from 273 bp G type into 71 + 202 bp A type), C723R (cut from 264 bp A type into 66 + 198 bp G type), L757P (cut from 255 bp T type into 102 + 153 bp C type) and C3114T (cut from 602 bp C type into 173 + 429 bp T type).

Statistical analyses

Only those matches with all SNPs data (case/control = 680/680) were selected for final analysis. 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 *Exo1* SNPs in the control subjects from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's two-sided χ^2 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 *Exo1* genotypes between cases and controls. Data were recognized as significant when the statistical *P* was less than 0.05.

Results

The frequency distributions of selected characteristics of 680 oral cancer patients and controls are shown in Table 1. These characteristics of patients and controls are all well matched. All of these differences between both groups are no statistically significant ($P > 0.05$) (Table 1).

The frequency of the genotypes for the *Exo1* A-1419G, C-908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T, between controls and oral cancer patients is shown in Table 2. Genotype distribution of various genetic polymorphisms of *Exo1* K589E is significantly different between oral cancer and control groups ($P = 6.18E\text{-}8$), while those for all the other polymorphisms are not significant ($P > 0.05$) (Table 2). To sum up, the *Exo1* K589E is associated with higher susceptibility for oral cancer. The representative PCR-based restriction analyses for the *Exo1* K589E polymorphisms are shown in Fig. 1.

The frequency of the alleles for the *Exo1* A-1419G, *Exo1* C-908G, A238G, C498T, K589E, G670E, C723R, L757P and C3114T, between controls and oral cancer patients is shown in Table 3. The allele frequency distributions of the *Exo1* K589E showed that A allele of *Exo1* K589E is associated with higher susceptibility for oral cancer, while others are not (Table 3).

Genotype distribution of various genetic polymorphisms of *Exo1* K589E is significantly different between oral cancer and control groups who have smoking habit ($P = 2.41E\text{-}11$) (Table 4), while

Table 1
Characteristics of oral cancer patients and controls.

Characteristics	Controls ($n = 680$)			Patients ($n = 680$)			P^a
	n	%	Mean (SD)	*n*	%	Mean (SD)	
Age (years)			62.1 (8.8)			64.5 (9.3)	0.78
Gender							0.39
Male	490	72.1		505	74.3		
Female	190	27.9		175	25.7		
Indulgence							
Cigarette smokers	485	71.3		512	75.3		0.11
Areca chewers	418	61.5		451	66.3		0.07
Alcohol drinkers	466	68.5		476	70.0		0.60
Histology							
Tongue				337	49.6		
Buccal mucosa				194	28.5		
Mouth floor				38	5.6		
Retromolar trigone				30	4.4		
Alveolar ridge				18	2.6		
Palate				16	2.4		
Lip				14	2.1		
Others				33	4.8		

[a] *P* based on χ^2 test.

Table 2
Distribution of *Exo1* genotypes among oral cancer patients and controls.

Genotype	Controls	%	Patients	%	P^a
A-1419G rs3754093					0.1068
AA	283	41.6	261	38.4	
AG	315	46.3	311	45.7	
GG	82	12.1	108	15.9	
C-908G rs10802996					0.9595
CC	383	56.3	380	55.9	
CG	235	34.6	235	34.6	
GG	62	9.1	65	9.6	
A238G rs1776177					0.5001
AA	319	46.9	309	45.4	
AG	308	45.3	306	45.0	
GG	53	7.8	65	9.6	
C498T rs1635517					0.1139
CC	28	4.1	39	5.7	
CT	218	32.1	241	35.5	
TT	434	63.8	400	58.8	
K589E rs1047840					**6.18×10^{-8}**
AA	15	2.2	45	6.6	
AG	183	26.9	244	35.9	
GG	482	70.9	391	57.5	
G670E rs1776148					0.6749
AA	31	4.6	35	5.1	
AG	138	20.3	148	21.8	
GG	511	75.1	497	73.1	
C723R rs1635498					0.6754
AA	522	76.8	508	74.7	
AG	148	21.8	161	23.7	
GG	10	1.4	11	1.6	
L757P rs9350					0.4759
CC	214	31.5	235	34.5	
CT	313	46.0	297	43.7	
TT	153	22.5	148	21.8	
C3114T rs851797					0.6860
CC	133	19.5	139	20.4	
CT	344	50.6	328	48.2	
TT	203	29.9	213	31.4	

[a] *P* based on two-sided chi-square test without Yate's correction.

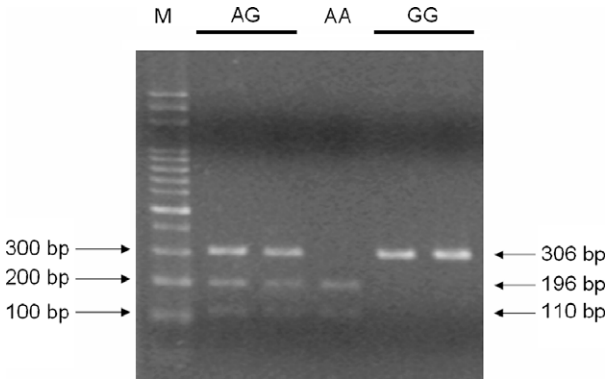

Figure 1 PCR-based restriction analysis of the *Exo1* K589E rs1047840 polymorphism shown on 2.5% agarose electrophoresis. M: 100 bp DNA size marker, G/G: enzyme indigestible homozygote, A/G: heterozygote, and A/A: enzyme digestible homozygote.

those for the other SNPs are not significant ($P > 0.05$) (data not shown). In detail, distributions of *Exo1* K589E A homozygote/heterozygote and G homozygote in controls and oral cancer patients who with smoking habit are 119/366 and 230/282, respectively ($P = 2.41E-11$, OR = 2.509, 95% CI = 1.914–3.287) (Table 4). Distributions of *Exo1* K589E A homozygote/heterozygote and G homozygote in controls and oral cancer patients who with non-smoking habit are 79/116 and 59/109, respectively ($P = 0.344$, OR = 0.795, 95% CI = 0.519–1.218) (Table 4).

Table 3
Distribution of *Exo1* alleles among oral cancer patients and controls.

Allele	Controls	%	Patients	%	P^a
A-1419G rs3754093					0.0566
Allele A	881	64.8	833	61.3	
Allele G	479	35.2	527	38.7	
C-908G rs10802996					0.7946
Allele C	1001	73.6	995	73.2	
Allele G	359	26.4	365	26.8	
A238G rs1776177					0.3628
Allele A	946	69.6	924	67.9	
Allele G	414	30.4	436	32.1	
C498T rs1635517					0.0410
Allele C	274	20.1	319	23.5	
Allele T	1086	79.9	1041	76.5	
K589E rs1047840					**9.45×10^{-9}**
Allele A	213	15.7	334	24.6	
Allele G	1147	84.3	1026	75.4	
G670E rs1776148					0.3386
Allele A	200	14.7	218	16.0	
Allele G	1160	85.3	1142	84.0	
C723R rs1635498					0.4233
Allele A	1192	87.6	1177	86.5	
Allele G	168	12.4	183	13.5	
L757P rs9350					0.3348
Allele C	741	54.5	767	56.4	
Allele T	619	45.5	593	43.6	
C3114T rs851797					0.9079
Allele C	610	44.9	606	44.6	
Allele T	750	55.1	754	55.4	

[a] *P* based on two-sided chi-square test with Yate's correction.

Table 4
Exo1 K589E rs1047840 genotypes and oral cancer after stratified by smoking.

Genotype	Controls (*n*)	Patients (*n*)	P^a	OR (95% CI)[b]
Smokers			**2.41×10^{-11}**[c]	
GG	366	282		1.00
AA + AG	119	230		**2.509 (1.914–3.287)**[c]
Non-smokers			0.3436	
GG	116	109		1.00
AA + AG	79	59		0.795 (0.519–1.218)

[a] *P* based on two-sided chi-square test with Yate's correction.
[b] OR (odds ratio) was estimated with logistic regression analysis.
[c] Statistically identified as significant.

Discussion

In order to find the potential biomarkers of oral cancer, in this study, we selected up to nine SNPs of the *Exo1* gene, and investigated the associations with the susceptibility of oral cancer in the population of central Taiwan. Among these nine polymorphisms, we found that variant genotypes of *Exo1* K589E are significantly associated with a higher risk of oral cancer (Tables 2 and 3).

Among the DNA repair systems, one of the major roles is played by the MMR system, which is responsible for correcting the mismatch between bases and the small insertion/deletion loops.[32,33] Exo1 is the only exonuclease involved in the human MMR system, playing a critical role as both 5′–3′ and 3′–5′ nucleases and contributing to the overall integrity of the MMR complex.[34] Because the Exo1 plays a distinctive role in the MMR system, the *Exo1* gene has become a famous target gene and widely investigated for its association with risk of colorectal malignants.[35–37] Recent findings indicated that mammalian Exo1 is responsible for mutation prevention and the mice with Exo1 inactivation predisposition have reduced survival time and increased risk for tumor development, specifically lymphoma.[22]

There are already several SNPs of *Exo1* which have been reported as genetic risk factors of cancer. In 2005, a study investigating Japanese population found that two polymorphisms of *Exo1* gene, T439 M and P757L, are associated with colorectal cancer risk.[38] In 2008, the association between SNPs of *Exo1* and lung cancer susceptibility is also examined in a Chinese population, indicating the K589E is associated with lung cancer risk.[39] In this paper, we found that *Exo1* K589E is associated with oral cancer susceptibility in central Taiwan, and the only polymorphism which has positive association is located on exon12 of the *Exo1* gene and its change will cause the 589th amino acid of Exo1 protein product to be altered from lysine to glutamic acid. The amino acid change at codon 589 might influence the products of *Exo1* mRNA, for K589E is located at an exonic splicing enhancer (ESE) region.[39] Our results in Taiwan are consistent with the work in Mainland China, which is also a subpopulation of the Han-nationality, investigation the association of Exo1 polymorphisms with lung cancer.[39] On the contrary, Zienolddiny et al. have found no significant association of *Exo1* K589E polymorphism and risk of non-small cell lung cancer in a Caucasian Norwegian population.[40] Chang et al. have investigated 10,177 non-synonymous SNPs and found that *Exo1* K589E is associated with risk of Caucasian glioblastoma.[41] The reasonable explanation is that the similarity between ours and Jin's findings may be caused by ethnicity; this polymorphism may associate with Mongolian oral cancer, but not that of Caucasians'. The conflict between Zienolddiny's and Chang's may be explained by tissue-specificity. Further investigations of *Exo1* SNPs in various types of cancer and different populations are in need and K589E may be a promising biomarker for specific types of cancers.

Since smoking may be an environmental factor for oral cancer,[42] we have further analyzed the association between K589E genotype and oral cancer risk in patients and controls who have cigarette smoking habits. Interestingly, the interaction between *Exo1* K589E and cigarette smoking habit is obvious, people with the AA or AG genotype have a 2.07-fold higher risk of the oral cancer in than people with the genotype GG (Table 4). We propose that the A allele of K589E may affect the Exo1 activity, slightly influencing its normal function. As the people with A allele(s) get older, the alteration towards carcinogenesis may accumulate via the increasing unremoved DNA adducts. Cigarette smoking, a well-known origin of DNA damage, will release many DNA damage inducers to our respiratory system and cause DNA damages to the cells. Therefore, if people who have risky genetic variant, such as the A allele of K589E, and also have the smoking habit, the joint effect of genetic and environmental factors may synergistically increase their oral cancer susceptibilities. The present study is a comprehensive assessment of the effects of genetic-smoking interaction on oral cancer, adding to previous knowledge an updated and clearer understanding of the factors contributing to the heterogeneity of oral cancer. Our results suggest that smoking is indeed the behavioral factor for oral cancer, and have synergistic effects with genetic factors.

In conclusion, this is a case–control study which focuses on the SNPs of *Exo1* and oral cancer in Taiwan, and the presence of the A allele of K589E is associated with a higher risk of oral cancer. The A allele of K589E may be a useful marker in oral oncology for anticancer application, and early cancer detection.

Conflict of Interest Statement

None declared.

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