Impact of Polymorphisms in Casein Kinase 1 Epsilon and Environmental Factors in Oral Cancer Susceptibility

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Abstract

In Taiwan, the incidence rate of oral cancer is constantly increasing. Polymorphisms and lifestyle habits are major contributing factors to the development of oral cancer in such cases. Casein kinase 1 epsilon (CK1ε) gene expression plays a role in numerous cancers, and the knockdown of CK1ε induces tumor cell-selective cytotoxicity. The present study was designed to determine the effects of CK1ε gene polymorphisms combined with environmental carcinogens on susceptibility to developing oral squamous cell carcinoma and its clinicopathological status. Four single-nucleotide polymorphisms (SNPs) in CK1ε gene (rs135745, rs135764, rs1997644 and rs2075984) from 741 oral cancer patients and 462 healthy controls were analyzed using real-time polymerase chain reaction. Our results shown that variant types (GC) of CK1ε polymorphic rs135745 exhibited a significantly higher risk of 1.41 (95% confidence interval [CI]: 1.036–1.919) for oral cancer than did wild type alleles. Furthermore, these CK1ε gene SNPs along with betel-quid chewing and/or tobacco use further increased susceptibility to oral cancer. Moreover, variant genotypes (GC+CC) of CK1ε rs135745 were significantly associated with lymph node metastasis. These results suggested that the CK1ε gene polymorphism is associated with the clinicopathological development of oral cancer and increases individuals’ susceptibility to environmental carcinogens (e.g., smoking and betel-quid chewing) in terms of developing oral cancer.

Key words: Casein kinase 1 epsilon, oral cancer, polymorphism

Introduction

Oral cancer is estimated to be in the top 10 most common cancers worldwide, and is characterized by poor prognosis and late detection. Since 2003, oral cancer has been the fourth leading cause of cancer deaths among men in Taiwan [1]. The occurrence and growth of oral cancer depends on DNA sequence modifications known as single-nucleotide polymorphisms (SNPs). Along with genetic factors, environmental carcinogens associated with betel-quid (areca nut) chewing, tobacco use, and alcohol intake are major suggested causes of oral squamous cell carcinoma in Taiwan [2].

A member of the casein kinase 1 protein family, the casein kinase 1 epsilon (CK1ε) gene encodes serine/threonine protein kinase. Members of the CK1 gene family (α, β, γ1, γ2, γ3, and δ) have been shown
to be involved in the regulation of cytoplasmic and nuclear processes as well as DNA replication and repair. Furthermore, studies have shown that in colon adenocarcinoma cells, human pancreatic cells, and salivary gland cancer cells, CK1ε phosphorylates vital proteins of the Wnt signaling pathway to maintain cell division and tumor growth [3]. In our previous studies, we have demonstrated that decreased cytoplasmic CK1ε expression correlates with low survival rates in patients with oral and hepatocellular carcinomas [4, 5]. These results have suggested that CK1ε could be utilized as a prognostic predictor for oral and hepatocellular cancers and act as a tumor suppressor agent [5]. Mutations within the coding region of CK1ε were identified in several cancers, including ovarian, head and neck and breast cancers [3, 6]. Relevant studies have reported that CK1 inhibitors may be drug candidates for treating alcoholism [7].

Gene polymorphisms and environmental factors combined determine individuals’ susceptibility to oral cancer [8-13]. Identifying genetic factors and their SNPs associated with the development and progression of cancer are challenging tasks and are often used in early detection to determine the most effective treatment options [14, 15]. To the best of our knowledge, this study is the first to target the CK1ε genetic polymorphism and compare it with environmental factors, such as betel-quid chewing, tobacco use, and alcohol consumption, to determine their effects on oral-cancer susceptibility in Taiwan.

Materials and Methods

Subjects and specimen collection

741 oral cancer patients who were recruited at Changhua Christian Hospital in Changhua and Chung Shan Medical University Hospital in Taichung, Taiwan between April, 2007 and April, 2013. Medical information of the oral cancer group, including the American Joint Committee on Cancer (AJCC) clinical stage, the tumor size, lymph node metastasis, distant metastasis and histologic grade, was obtained from their medical records. This study was approved by the Institutional Review Board of Changhua Christian Hospital (CCH No: 161224) and Chung Shan Medical University Hospital (CSMUH No: CS13214-1).

Selection of casein kinase 1 epsilon Polymorphisms

To obtain adequate power for evaluating the potential association, we investigated rs135745, rs135764, rs1997644 and rs2075984, those with minor allele frequencies greater than 5%. Furthermore, these SNPs of CK1ε gene were selected in this study since these SNPs were associated with the progression of the cancer [16, 17].

DNA extraction and CK1ε genotyping

Genomic DNA was extracted using QIAamp DNA blood mini kits (Qiagen, Valencia, CA, USA) following the manufacturer’s instructions as previously described [18, 19]. Allelic discrimination assessment for CK1ε gene (rs135745, rs135764, rs1997644 and rs2075984) was performed using TaqMan assay with an ABI StepOnePlus™ Software v2.3 real-time PCR system (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Multiple logistic regression models were used to analyze the association between CK1ε gene (rs135745, rs135764, rs1997644 and rs2075984) genotype frequencies, oral cancer risk and clinical characteristics after controlling for age, betel quid chewing, cigarette smoking, and alcohol consumption. The analyses were performed using the SPSS, and a p value less than 0.05 was considered statistically significant.

Results

Demographical characteristics distribution is presented in Table 1. This study found a significant difference (p < 0.001) in the demographical distributions of age, smoking status, alcohol intake, and betel-quid chewing between the control subjects and oral cancer patients (Table 1).

The frequencies of genetic polymorphisms (rs135745, rs135764, rs1997644 and rs2075984) of the control group were in Hardy–Weinberg equilibrium. The people with the GC variants of the rs135745 polymorphism had 1.41-fold (95% CI: 1.036–1.919; p = 0.029) increased risk of developing oral cancer respectively to their corresponding WT gene (Table 2). However, no significant differences were observed between patients with oral cancer having rs135764, rs1997644 and rs2075984 polymorphisms of the CK1ε gene and those with the wild-type (WT) gene.

Tables 3 and 4 demonstrate the associations between environmental risk factors and genetic polymorphisms of CK1ε. Among 821 smokers who had CK1ε polymorphic rs135745, rs135764, rs1997644, or rs2075984 genes and who had a betel-quid chewing habit had 19.031- (95% CI: 9.840–36.807; p < 0.001), 9.196- (95% CI: 5.250–16.108; p < 0.001), 10.572- (95% CI: 6.001–18.627; p < 0.001) and 9.476-fold (95% CI: 5.383–16.681; p < 0.001) higher risks of developing oral cancer, respectively, compared with individuals with the WT gene (Table 3).
To clarify the role of CK1ε genetic polymorphisms in oral cancer clinical statuses, such as tumor size, lymph node or distant metastasis, and histological grade, the distribution frequency of clinical statuses and CK1ε genotype frequencies in patients with oral cancer were estimated. As shown in Table 4, we found that patients with the CK1ε rs135745 variant genotypes (GC+CC) were significantly associated with lymph-node metastasis (1.622; 95% CI: 1.037–2.536; p = 0.034) (Table 4).

Table 3. Associations of the combined effect of CK1 epsilon gene polymorphisms and betel nut chewing with the susceptibility to oral cancer among 821 smokers.

| Variable | Controls (N=187) (%) | Patients (N=634) (%) | OR (95% CI) | AOR (95% CI) |
|----------|----------------------|----------------------|-------------|--------------|
| rs135745 | GG genotype and non betel-nut chewing | 87 (46.5%) | 58 (9.1%) | Reference | Reference |
|          | GC or CC genotype or betel-nut chewing | 87 (46.5%) | 393 (62.0%) | 6.776 (4.518-10.163) | 6.150 (4.069-9.286) |
|          | GC or CC genotype with betel-nut chewing | 13 (7.0%) | 183 (28.9%) | 21.115 (10.986-40.583) | 19.031 (9.840-36.807) |

rs135764
GG genotype and non betel-nut chewing | 84 (44.9%) | 60 (9.5%) | Reference | Reference |
GA or AA genotype or betel-nut chewing | 80 (42.8%) | 422 (66.6%) | 7.385 (4.908-11.111) | 6.988 (4.597-10.621) |
GA or AA genotype with betel-nut chewing | 23 (12.3%) | 152 (24.0%) | 9.252 (5.340-16.030) | 9.196 (5.290-16.108) |

rs1997644
GG genotype and non betel-nut chewing | 47 (25.1%) | 31 (4.9%) | Reference | Reference |
GA or AA genotype or betel-nut chewing | 96 (51.3%) | 287 (45.3%) | 4.533 (2.725-7.540) | 4.366 (2.586-7.369) |
GA or AA genotype with betel-nut chewing | 44 (23.5%) | 316 (49.8%) | 10889 (6.267-18.917) | 10.572 (6.001-18.627) |

rs2059984
AA genotype and non betel-nut chewing | 42 (22.5%) | 32 (5.0%) | Reference | Reference |
AC or CC genotype or betel-nut chewing | 99 (52.9%) | 238 (37.5%) | 3.155 (2.083-5.287) | 2.990 (1.759-5.080) |
AC or CC genotype with betel-nut chewing | 46 (24.6%) | 364 (57.4%) | 10.386 (5.975-18.052) | 9.476 (5.383-16.681) |

The adjusted odds ratio (OR; AOR) with its 95% confidence interval (CI) was estimated by multiple logistic regression models after controlling for age and alcohol consumption.

Discussion

Oral cancer in Taiwan is increasing at an alarming rate compared with other countries, which might be because of the alcohol consumption, tobacco use, and betel-quid chewing habits of the Taiwanese [14, 15, 20]. SNPs in genes, which regulate major pathways in the cells, are associated with greater susceptibility to oral cancer [21]. In this study, we determined the SNPs in the CK1ε gene are associated with clinicopathological statuses of oral cancer. CK1ε is a serine/threonine-specific phosphotransferase that controls major cellular pathways [22]. CK1ε expression was correlated with the c-MYC oncogene in colon, lung, and breast cancer tumors [23]. Varghese et al., reported that CK1ε expression is
associated with glioblastoma cell survival [24]. Gene expression analysis data showed that CK1ɛ is overexpressed in several cancer tissue samples compared with in normal tissues. Furthermore, knockdown of CK1ɛ induces tumor cell-selective cytotoxicity [25].

**Table 4. Distribution frequency of clinical status and CK1 epsilon rs135745 genotype frequencies in 741 patients with oral cancer.**

| Variable                  | GG (N = 497) n (%) | GC + CC (N = 244) n (%) | OR (95% CI) | AOR (95% CI) |
|---------------------------|--------------------|------------------------|-------------|--------------|
| Stage                     |                    |                        |             |              |
| I/II                      | 238 (47.9%)        | 111 (45.5%)            | Reference   | Reference    |
| III/IV                    | 259 (52.1%)        | 133 (54.5%)            | 0.795 (0.480-1.629) | 0.796 (0.481-1.319) |
| T classification           |                    |                        |             |              |
| T1/T2                     | 274 (55.1%)        | 134 (54.9%)            | Reference   | Reference    |
| T3/T4                     | 223 (44.9%)        | 110 (45.1%)            | 1.082 (0.732-1.600) | 1.081 (0.731-1.598) |
| Lymph node metastasis      |                    |                        |             |              |
| No                        | 342 (68.8%)        | 150 (61.5%)            | Reference   | Reference    |
| Yes                       | 155 (31.2%)        | 94 (38.5%)             | 1.623       | 1.622        |
|                           |                    |                        | (1.038-2.537)* | (1.037-2.536)* |
| Distant metastasis        |                    |                        |             |              |
| No                        | 490 (98.6%)        | 242 (99.2%)            | Reference   | Reference    |
| Yes                       | 7 (1.4%)           | 2 (0.8%)               | 0.458 (0.095-2.259) | 0.461 (0.093-2.277) |
| Histological grade        |                    |                        |             |              |
| Well                      | 78 (15.7%)         | 35 (14.3%)             | Reference   | Reference    |
| Moderate/Poor             | 419 (84.3%)        | 209 (85.7%)            | 1.052 (0.679-1.629) | 1.055 (0.995-1.007) |

The OR with its 95% CI was estimated by logistic regression models. The AOR with its 95% CI was estimated by multiple logistic regression models after controlling for age, betel quid chewing, cigarette smoking, and alcohol consumption.

* p<0.05.

In our study, a significant difference was found in demographical distributions of age, smoking status, alcohol intake, and betel-quid chewing between control subjects and oral cancer patients, which have also been observed in most relevant studies. We investigated the rs135745, rs1997644, rs2075984, and rs135764 polymorphisms of the CK1ɛ gene. Our results showed that oral cancer patients with the rs135745 polymorphism of CK1ɛ gene increased the risk of developing oral cancer. Hsu’s group reported that even in the same cancer, CK1ɛ seems to exhibits diverse functions; thus, it could be an onco-protein or a tumor suppressor [26]. We further tested clinicopathological statuses, such as T classification, lymph-node metastasis, distant metastasis, histologic grade, and AJCC cancer stage, and observed significant lymph-node metastases in oral cancer patients with the rs135745-CK1ɛ polymorphic gene. Future studies should investigate the mechanism by which these SNPs modify the clinicopathological status of oral cancer.

Individuals exposed to environmental carcinogens have increased risks of oral cancer [27]. Studies have stated that SNPs and oral cancer-associated environmental risk factors have a synergetic effect that can influence oral-cancer development [28]. The synergistic effects of environmental factors, betel-quid chewing, smoking, and the four CK1ɛ gene SNPs (rs135745, rs135764, rs1997644 and rs2075984) on the risk of oral cancer were studied. Mutations and changes in the expression and/or activity of CK1 isoforms are often identified in various cancers, such as ovarian and breast cancer as well as adenocarcinoma of the pancreas [6]. This study identified the CK1ɛ gene polymorphisms in oral cancer. However, the detailed mechanism of the CK1ɛ genetic polymorphism to oral cancer remained unclear. In the future, some studies are required to elucidate the mechanisms of CK1ɛ genetic polymorphisms in oral cancer progression.

In conclusion, our results suggested that the CK1ɛ-rs135745 G/C gene polymorphism significantly increased the risk of oral cancer, and furthermore, the combined effects of CK1ɛ gene polymorphisms with environmental risk factors increased sensitivity to oral cancer.

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**Competing Interests**

The authors have declared that no competing interest exists.

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