Polymorphism of NQO1 (C609T) in Relation to Susceptibility to Oral Squamous Cell Carcinoma

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The NQO1 gene, encoding the phase II drug-metabolizing enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1), is regarded as an important enzyme for susceptibility to carcinogenesis in humans. Japanese people show a polymorphism (C609T) in NQO1 as C in the wild-type genotype (wt) and T in the variant genotype (vt). In this study of 105 patients with oral squamous cell carcinoma (OSCC), the wt/wt, wt/vt and vt/vt genotypes were found in 42, 49 and 14 cases, respectively, while each genotype was detected in 49, 52 and 1, respectively, of 102 control cases with no carcinoma at any site in the body. The $^2$ test revealed that individuals with the vt/vt genotype were less common in the control cases than in the OSCC patients (P < 0.001) and the odds ratio of the vt/vt to the wt/wt genotype was 16.3. The results indicate that susceptibility to OSCC appears to depend significantly on the NQO1 polymorphism.

Key words: NQO1, nucleotide polymorphism, oral squamous cell carcinoma, PCR-RFLP

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Introduction

Oral squamous cell carcinoma (OSCC) accounts for more than 50% of all oral malignancies, and it has a poor prognosis because therapeutic strategies have only limited effects (1). For effective prevention against this cancer, previous reports have attempted to identify genetically predisposed risk groups since a certain genetic background has been proposed to play an important role in the susceptibility to OSCC carcinogenesis (2-5).

There is substantial evidence that the activities of several drug-metabolizing enzymes are related to susceptibility to carcinogenesis in humans, including the cytochrome P450s (CYPs), glutathione S-transferases (GSTs), N-acetyltransferases (NATs) and quinone oxidoreductases (NQOs) (6-10). Most chemical carcinogens in our environmental surroundings require metabolic activation by phase I drug-metabolizing enzymes, such as the CYPs, and subsequent detoxification by conjugation with the various phase II drug-metabolizing enzymes, such as the GSTs, NATs and NQOs (8). Thus, the coordinated expression and regulation of phase I and phase II enzymes and their metabolic balance may be an important host factor in determining whether or not exposure to carcinogens results in cancer (6).

The NQO1 gene encodes the phase II drug-metabolizing enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) and is regarded as one of the important enzymes for susceptibility to carcinogenesis (11). It is evident that a polymorphism of the NQO1 gene caused by a C to T substitution at nucleotide position 609-bp exists, and while most people have a C at 609-bp as the wild-type genotype (wt) and T in the variant genotype (vt), the nucleotide is occasionally replaced by a T as the variant genotype (vt) (12). Furthermore, vt/vt homozygotes are believed to result in the loss of NQO1 enzyme activity (12-17).

The genomic mutation in NQO1 is frequently found in cancer cases such as colon (12, 13), bladder (14), lung cancers (17) and pediatric leukemias (16). In addition, the mutation has been detected in fibroblasts obtained from a cancer-prone family (15). These observations suggest that the genomic polymorphism in NQO1 could be closely related to susceptibility to general carcinogenesis. However, there is no available information about the frequency of the NQO1 polymorphism in oral cancer cases.

In this study, we investigated the incidence of the NQO1 gene polymorphism in cases with OSCC in comparison with control cases containing no malignancies in the whole body among the Japanese population. The re-
sults clarify that the \textit{NQO1} polymorphism (C609T) should be regarded as one of the genetic risk factors for susceptibility to OSCC carcinogenesis.

\textbf{Materials and methods}

\textit{Patients and control cases (Table 1)}

A total of 105 patients (51 females and 54 males, mean age = 59.7) histopathologically diagnosed with primary OSCC (tongue 51, gingiva 26, buccal mucosa 16, floor of the mouth 5, others 7) were selected from patients who visited the Saitama Cancer Center Hospital and consented to participate in this study. We also collected a total of 102 control cases (53 females and 49 males, mean age = 60.3) from patients who visited the Kagoshima University Dental Hospital with complaints of non-neoplastic diseases, including mucocele, gingivitis, periodontitis, and cariogenic lesions among others. The control cases were well matched to the OSCC patients with respect to age and sex, and aside from the oral diseases, were apparently healthy. At the time of the first visitation to the Hospital, the patients were interviewed using a questionnaire provided by the Hospital and answered that they had no history of cancer diseases at any sites in the body.

With the approval of the Ethical Committees of both the Saitama Cancer Center and the Kagoshima University Dental School, genomic DNA samples of the OSCC patients and cancer-free controls were isolated from the peripheral lymphocytes in blood samples and/or a stored specimen was fixed in formalin and embedded in paraffin using ISOGEN (Wako, Tokyo) according to the manufacturer’s protocol. The DNA samples were stored at -20°C until use.

\textit{PCR-RFLP analysis}

Genotyping identification of the polymorphism (C609T) in \textit{NQO1} was performed using a PCR-RFLP analysis of the genomic DNA samples from the OSCC patients and the control cases, according to the method of Traver \textit{et al.} (12). Briefly, the PCR reaction was carried out for 10 min for the initial stage at 95°C to activate the DNA polymerase (AmpliTaq Gold™, PE Applied Biosystems, CA), and then subjected to 35 cycles under the following conditions: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 1 min primer extension at 72°C. The sense primer, 5’-CTGTGGCTTCAAGTCTTA, and the antisense primer, 5’-CCAATATTCTCCAGGGTT, amplified a 113-bp oligonucleotide that included the region of the polymorphism (C609T). The PCR products were digested with 5 units

| Table 1: Number of cases in age periods(female/male) |
|---------|-------|-------|-------|-------|-------|-------|-------|
| Age     | 31~40 | 50    | 60    | 70    | 80    | 90    | Total |
| OSCC    | 3 (2/1) | 7 (6/1) | 28 (15/13) | 32 (15/17) | 27 (12/15) | 8 (1/7) | 105 (51/54) |
| Control | 1 (1/0) | 8 (5/3) | 41 (23/18) | 34 (18/19) | 14 (5/9) | 1 (1/0) | 102 (53/49) |

Fig. 1: The three genotypes of the \textit{NQO1} polymorphism (C609T) analyzed by PCR-RFLP. \textit{wt/wt}, homozygous wild-type genotype; \textit{wt/\textit{vt}}, heterozygous genotype; \textit{vt/vt}, homozygous variant genotype.

Fig. 2: The mutation of the \textit{NQO1} gene at nucleotide position 609. The homozygous wild-type genotype has a C at 609-bp (A), but the nucleotide is replaced by a T in the homozygous variant genotype (B).
of Hinf I for 3 hours at 37°C and electrophoresed through a 2.0% agarose gel. Complete digestion of the PCR products with Hinf I yielded one band (113-bp) for the wt/wt genotype, two bands (113- and 93-bp) for the wt/vt genotype and one band (93-bp) for the vt/vt genotype (Fig. 1). The nucleotide polymorphism (C609T) of NQO1 was also confirmed by direct sequencing of the PCR products using an ABI 310 genetic analyzer (PE Applied Biosystems, CA) according to manufacturer's protocol (Fig. 2).

Statistical analysis

Differences in the incidences of each genotype of NQO1 between the OSCC patients and the control cases were evaluated using them \( \chi^2 \) test. The odds ratios for each genotype between the OSCC patients and the control cases were estimated for the susceptibility to OSCC.

Results

The three NQO1 genotypes, wt/wt, wt/vt and vt/vt, were found in 49 (48.0%), 52 (51.0%) and 1 (1.0%) individuals, respectively, in the 102 cancer-free control cases. The result was consistent with the Hardy-Weinberg equilibrium with a gene frequency of 0.698 for the wt and 0.302 for the vt, in which the relative frequencies \( p^2 \), \( 2pq \) and \( q^2 \) of the genotypes estimated from the gene frequencies \( p \) and \( q \) must be equal to that observed. On the other hand, the wt/wt, wt/vt and vt/vt genotypes were found in 42 (40.0%), 49 (46.7%) and 14 (13.3%) individuals, respectively, in the 105 OSCC patients (Table 2). Overall, the difference in the distribution of the vt/vt genotype between the OSCC patients and the control cases was significant (\( P < 0.001 \)), and the frequency of the genotypes in the OSCC patients differed significantly from that in the control cases (\( P = 0.003 \)). The odds ratio estimates revealed that individuals with the vt/vt genotype had an \( ~16.3 \)-fold higher risk of developing cancer than those with the wt/wt genotype (95% confidence interval \( ~2.1-129.5 \)) (Table 3).

There was no difference between the genotype frequencies with respect to sex and age in the OSCC patients and the control cases.

Discussion

NQO1, formerly referred to as DT-diaphorase (EC 1.6.99.2), is an important phase II drug-metabolizing enzyme that has attracted considerable attention due to its contradictory abilities to detoxify a number of natural and synthetic compounds and, on the other hand, to activate certain procarcinogenic agents (18, 19). It is evident that an association between the NQO1 activity and the benzo[a]pyrene present in the environment is related to

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<th>Table 2: The genotypes of the NQO1 polymorphism in OSCC patients</th>
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<th>Table 3: Incidence of the genotypes of the NQO1 polymorphism</th>
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\(^a\) The odds ratios for each genotype in the OSCC group are indicated against the incidence of the wt/wt genotype in the control group. CI, confidential intervals.

\(^b\) The \( \chi^2 \) value indicates a relationship between the incidence of the wt/wt and wt/vt genotypes in the OSCC group and those in the control group.

\(^c\) The \( \chi^2 \) value indicates a relationship between the incidence of the wt/wt and vt/vt genotypes in the OSCC group and those in the control group.

\(^d\) The \( \chi^2 \) value indicates a relationship between the incidence of all the genotypes in the OSCC group and those in the control group (\( \chi^2 \) analysis for 2 by 3 table).
the risk of carcinogenesis (20). Benzo[a]pyrene is a procarcinogen and a metabolic activation process producing carcinogenic metabolite(s) such as benzo[a]pyrene-quinones is required for its carcinogenic effect (20, 21). NQO1 can decrease the carcinogenic effect of this substance by catalyzing the obligatory two-electron reduction of quinones and quinonoid compounds to the readily excreted hydroquinones using either NADH or NADPH as an electron donor (22). With respect to the role of NQO1 against benzo[a]pyrene, individual data of exposure to tobacco and alcohol were absent in this study. However, it may be the first step for extensive future cohort analysis of genes regarding the susceptibility to OSCC.

A recent study showed that the point mutation C609T in codon 187 causes a single proline-to-serine amino acid substitution in NQO1 and results in the complete loss of the enzyme activity in homozygous (vt/vt) subjects while those with two wild-type alleles (wt/wt and wt/vt) have normal activity (12). The mutation has been found in various cancer cases (12-17). In the present study, we have clarified the observation that the vt/vt genotype of NQO1 is much less common in cancer-free controls compared with OSCC patients (P < 0.001) and that the odds ratio against wt/wt was 16.3. Therefore, the higher OSCC risk observed in individuals with the vt/vt genotype of NQO1 may be consistent with the role of NQO1 in the metabolic detoxification of putative carcinogens for OSCC.

For a long time, attempts have been made to identify whether a given genetic background played some important roles in the susceptibility to OSCC carcinogenesis in humans (2-5). Houch et al. (2) and Foulkes et al. (3) proposed a theory that OSCC carcinogenesis was influenced by a certain genetic background. On the other hand, Goldstein et al. (4) and Johnson et al. (5) discussed the familial and hereditary risks for oral cancer.

To elucidate the host genetic factors in a human disease, studies using appropriate animal models are useful and indispensable. We recently found that the Dark-Agouti (DA) strain of rats had an extremely high susceptibility to 4-nitroquinoline 1-oxide (4NQO)-induced tongue cancers, whereas the Wistar/Furth (WF) strain of rats showed much lower susceptibility (23,24). Further investigation was partly supported by Grants-in-Aid (11470399 and 12470399) from the Ministry of Education, Science, Sports and Culture in Japan. The author is also grateful to the staff members of the Department of Oral Pathology, Kagoshima University Dental School, for their invaluable collaboration. The work was partly supported by Grants-in-Aid (11470399 and 12470399) from the Ministry of Education, Science, Sports and Culture in Japan. The author would like to thank Drs. T. Izumo and H. Shia (Department of Pathology, Suita University Cancer Research Institute) for their kind collaboration in sample collection and technical assistance. I would also like to express my gratitude to Dr. H. Hatano (School of Health Science, Faculty of Medicine, Kagoshima University) for guidance in the statistical evaluation and to Dr. T. Aoyama (Kagoshima University Research Center for the Pacific Islands) for his critical reading of this manuscript. The author is also grateful to the staff members of the Department of Oral Pathology, Kagoshima University Dental School, for their invaluable collaboration. The work was partly supported by Grants-in-Aid (11470399 and 12470399) from the Ministry of Education, Science, Sports and Culture in Japan.

Acknowledgments

In conclusion, it should be noted that knowledge of individual genetic differences, such as the polymorphism in NQO1, with respect to the susceptibility to chemical carcinogens is an important consideration in the risk assessment of OSCC in humans.

References


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