The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk

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The human OGG1 (hOGG1) gene encodes a DNA glycosylase that is involved in the excision repair of 8-hydroxy-2’-deoxyguanine (8-OH-dG) from oxidatively-damaged DNA. To determine whether hOGG1 plays a role in orolaryngeal cancer, we screened normal orolaryngeal tissue specimens for hOGG1 expression and assessed the role of the hOGG1 Ser326Cys polymorphism in risk for orolaryngeal cancer. hOGG1 expression was determined by reverse transcription-polymerase chain reaction of total RNA from aerodigestive tract tissues, and hOGG1 genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism analysis of buccal cell DNA isolated from 169 Caucasian orolaryngeal cancer cases and 338 race-, sex- and age-matched controls. hOGG1 mRNA was detected in all aerodigestive tract tissues tested including tonsil, tongue, floor of mouth, larynx and esophagus. Significantly increased risk for orolaryngeal cancer was observed for both the hOGG1 326Ser/326Cys (odds ratio [OR] = 1.6, 95% confidence interval [CI] = 1.04–2.6) and hOGG1 326G/C genotype in smokers (as compared with non-smokers), with a correlation observed between levels of 8-OH-dG and the number of cigarettes smoked. A link between 8-OH-dG formation and tobacco smoke carcinogenesis has also been suggested by the fact that increased 8-OH-dG levels were observed in lung DNA of smokers (as compared with non-smokers), with a correlation observed between levels of 8-OH-dG and the number of cigarettes smoked. Furthermore, a 50% increase in the levels of 8-OH-dG was detected in the urine of smokers as compared to non-smokers. Together, these data strongly implicate 8-OH-dG formation in tobacco smoke-induced carcinogenic pathways. These data are also consistent with the fact that G:C \( \rightarrow \) T:A transversions, which are the primary mutational event induced by 8-OH-dG (19,20), are also the primary mutational event occurring in the p53 tumor suppressor gene, a common occurrence for upper aerodigestive tract cancers.

The human OGG1 (hOGG1) gene encodes a DNA glycosylase/AP-lyase that catalyzes the removal of 8-OH-dG adducts as part of the base excision repair pathway (22,23). The hOGG1 gene is expressed as 12 alternatively-spliced isoforms with only the 1α-form containing a nuclear localization signal (24,25). Relatively high levels of hOGG1 expression have been shown in several human tissues including brain, thymus, ovary, testis, kidney, and lung (24). Previous studies have revealed the presence of several polymorphisms at the hOGG1 locus. A C/G polymorphism at position 1245 in the 1α-specific exon 7 of the hOGG1 gene results in an amino acid substitution from serine to cysteine in codon 326 (26). Although no difference in catalytic activities were observed between the 326Cys and 326Ser variants in one study (27), the hOGG1 protein encoded by the wild-type 326Ser allele exhibited substantially higher DNA repair activity than the 326Cys variant in an in vitro Escherichia coli complementation activity assay (26). Recent studies have suggested that the Ser326Cys hOGG1 polymorphism may be associated with smoking-related orolaryngeal cancer risk.

Introduction

The induction of aerodigestive tract neoplasia is highly associated with exposure to tobacco and tobacco smoke carcinogens and the drinking of alcoholic beverages (1–3). In addition to the large number of compounds identified in tobacco smoke that exhibit mutagenic and carcinogenic activity (4), the production of reactive oxygen species may also play an important role in the carcinogenicity of tobacco smoke since both the tar and gas phases of cigarette smoke are well-known sources of these reactive species. Superoxide and H2O2 have been identified in aqueous smoke solutions in vitro (5), while the formation of hydroxyl radicals has been demonstrated in aqueous extracts of cigarette tar (6) and in aqueous smoke solutions after the addition of iron sulfate or iron-containing crocidolite asbestos fibers (7). Reactive oxygen species generated from cigarette smoke has been shown to induce the formation of modified bases and single-strand breaks in the DNA of cultured human cells (8–10). Among the various forms of DNA damage induced by oxygen radicals, 8-hydroxy-2’-deoxyguanosine (8-OH-dG) is a major form of oxidative DNA damage and an important marker of cellular oxidative stress (11). The abundant and highly carcinogenic tobacco smoke carcinogen, benzo[a]pyrene, was shown to induce 8-OH-dG in animal tissues (12). A link between 8-OH-dG formation and tobacco smoke carcinogenesis has also been suggested by the fact that increased 8-OH-dG levels were observed in lung DNA of smokers (as compared with non-smokers), with a correlation observed between levels of 8-OH-dG and the number of cigarettes smoked. In addition, increased levels of 8-OH-dG were detected in both peripheral leukocyte DNA (14,15) and the nuclei of oral mucosa (16,17) from smokers as compared to non-smokers. Furthermore, a 50% increase in the levels of 8-OH-dG was detected in the urine of smokers as compared to the urine of non-smokers (18). Together, these data strongly implicate 8-OH-dG formation in tobacco smoke-induced carcinogenic pathways. These data are also consistent with the fact that G:C \( \rightarrow \) T:A transversions, which are the primary mutational event induced by 8-OH-dG (19,20), are also the primary mutational event occurring in the p53 tumor suppressor gene, a common occurrence for upper aerodigestive tract cancers.

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with increased risk for lung (28) as well as esophageal (29) cancer. In the present study, we examined the potential role of hOGG1 genotype in risk for orolaryngeal cancer by assessing the expression pattern of hOGG1 protein in different aerodigestive tract tissues and the hOGG1 genotypes of individuals with and without orolaryngeal cancer.

**Materials and methods**

### Tissue samples and hOGG1 expression analysis

Samples (2 to 6 g) of normal human liver, esophagus, larynx, tonsil, tongue, and floor of mouth were obtained from the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center (Tampa, FL). All specimens came from subjects undergoing cancer surgery. All tissue samples were quick-frozen at −70°C within 30 min of surgery. Total RNA was isolated from 0.5 g samples of normal liver and orolaryngeal tissues using the guanidinium isothiocyanate/cesium chloride method, followed by treatment with DNase I as previously described (30). Total RNA was stored in aliquots at −70°C.

Three micrograms of total RNA was reverse transcribed in a volume of 20 µl that contained 200 units of AMV reverse transcriptase (Superscript II, GibcoBRL, Rockville, MD) and 0.5 µg of oligo (dT)12–14 primer as outlined in the manufacturer’s protocol. Polymerase chain reaction (PCR) was performed in a volume of 50 µl that contained 5 µl of reverse transcription (RT) reaction mix, 1.5 mM Mg-acetate, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 5 µl TaqMaster buffer (Eppendorf, Hamburg, Germany) 0.2 mM of each deoxynucleoside triphosphate, 10 µmol each of the sense and antisense hOGG1 primers, and 2.5 units of Taq DNA polymerase (Eppendorf). Sense (hOGG1RTF: 5′-GTCCGGTTATGTGAGTTCGAGTG-3′; corresponding to nucleotides 668 through 692 of the hOGG1 complementary DNA [cDNA]; GenBank accession HSU96710) and antisense (hOGG1RTR: 5′-AGAGA-GAGATGGAATGGAGGGGAAGGTG-3′; corresponding to nucleotides 1147 through 1174 of the hOGG1 cDNA) primers homologous to nt2-hOGG1 sequences within exons 4 and 7, respectively, were used in RT-PCR assays to eliminate the possible amplification of contaminating genomic DNA sequences. Reactions were incubated in a Perkin Elmer 9700 thermocycler (Perkin-Elmer Corp, Foster City, CA) at 95°C for 2 min, then for 41 amplification cycles at 94°C for 30 s, 64°C for 30 s, and 72°C for 45 s, followed by a final extension step for 7 minutes at 72°C. RT-PCR assays were performed on pooled tissue samples from each tissue site (n = 3 for floor of mouth, n = 2 for tonsil, n = 5 for tongue, n = 3 for larynx, n = 10 for esophagus, n = 32 for lung, and n = 19 for liver) using equimolar amounts of total RNA. Human β-actin exon 4 sense and exon 5 antisense primers (10 pmol for each primer; see ref. 31 for primer sequences) were added after the 15th amplification cycle as an internal positive control for the presence of RNA in each PCR assay. Reactions without RNA were included as negative controls in all RT-PCR experiments. Ten-µl aliquots were removed from each PCR and resolved by electrophoresis in 8% polyacrylamide gels. PCR-amplified bands were detected after staining of gels with 1 µg/ml ethidium bromide and photography under UV light. The sequences of PCR-amplified bands were confirmed by dyeodeoxy sequencing ([32] performed at the Molecular Biology Core Facility at the H. Lee Moffitt Cancer Center).

### Study populations and sample processing

Case subjects consisted of 125 patients diagnosed with primary squamous cell carcinoma of the oral cavity (which included cancer of the gingiva, hard palate, dorsal tongue, floor of mouth, inner lip, soft palate, buccal mucosa, tongue, tonsil and oropharynx) and 44 patients diagnosed with primary squamous cell carcinoma of the larynx. Case subjects were identified and recruited from between July 2001 at the H. Lee Moffitt Cancer Center (Tampa, FL), were diagnosed within 1 year of recruitment into the study, and were histologically confirmed by the Department of Pathology at the H. Lee Moffitt Cancer Center. Ninety-eight percent of case subjects who were asked to participate in the study consented.

Community-based controls consisted of subjects who were visiting the cancer screening clinic affiliated with the H. Lee Moffitt Cancer Center known as the Lifetime Cancer Screening Center. At this center, which screens ~20,000 subjects annually, routine screenings are performed for cancers of the breast, prostate, colorectum, cervix and skin. All control subjects were recruited after an initial verbal screening to determine that they had no previous diagnosis of cancer, and none of the controls recruited into this study were diagnosed with any form of cancer or premalignancy after screening. The eligible pool of control subjects was restricted to those individuals with the same age at diagnosis (±5 years), race, and sex as the case subjects, with controls paired matched in a 2 to 1 ratio with cases. Eighty-three percent of the control subjects who were asked to participate in the study consented.

To analyze hOGG1 genotypes in subjects recruited into our study, we collected buccal cell samples from all subjects as previously described (33). For orolaryngeal cancer cases, care was taken to avoid collecting buccal cells from lesion-containing areas. Protocols involving the collection and analysis of buccal cells were approved by the Institutional Review Boards at the Moffitt Cancer Center and the University of South Florida and in accordance with assurances filed with and approved by the US Department of Health and Human Services. Informed consent was obtained from all study subjects.

A questionnaire that contained detailed questions on demographics and lifestyle, smoking and alcohol consumption habits was administered to all study subjects. Tobacco use was categorized into pack-years (py) for smokers of cigarettes (1 py equalled one pack of cigarettes per day for 1 year), cigars (1 py equalled four cigars per day for 1 year), and pipe tobacco (1 py equalled five pipes per day for 1 year) according to the criteria described by Benhamou et al. (34). Study subjects who smoked 100 or fewer cigarettes in their lifetime (the equivalent of 0.014 or fewer py) were categorized as never-smokers. Informative data on tobacco use was obtained from all subjects recruited into this study. Levels of alcohol consumption (determined as the average amount consumed over a minimum of 10 years) were calculated as shots per day, with one shot defined as 12.9 g of 43% alcohol, which is roughly equivalent to 1 oz of 86-proof hard liquor, one 3.6 oz glass of wine, or one 12 oz can of beer. Study subjects were defined as drinkers of alcohol if they reported drinking at least one shot per week for a minimum of 10 years. Subjects were classified as never-drinkers if they consumed one or fewer shots per week, light-drinkers if they consumed 1.1 to 28 shots per week, and heavy-drinkers if they consumed >28 shots per week. Informative data on alcohol consumption were obtained from all subjects recruited into this study except for one control.

DNA was isolated from buccal cell samples as previously described (33). Briefly, the cell samples were incubated in 0.1 mg/ml proteinase K in 1% sodium dodecyl sulfate at 50°C overnight, and the DNA was then extracted with a mixture of 24 parts phenol to one part chloroform and precipitated with ethanol. DNA isolations were performed in a location distant from the work station where PCR amplifications were performed to prevent contamination of samples during PCR. All equipment utilized for tissue homogenization were washed in a bath of concentrated chromic-acid:sulfuric acid (1:1), rinsed three times in autoclaved double distilled water and once in 70% ethanol, air-dried, and autoclaved after each tissue sample was processed.

### Genotyping assays

We determined the identity of each study subject’s hOGG1 alleles by PCR-RFLP analysis. Briefly, a 156 bp fragment was amplified by PCR in a 50 µl reaction volume that contained 50 ng of buccal cell genomic DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate, 10 pmol of the hOGG1 sense (hOGG1F: 5′-AGTGTGACTAGTCTCATACAG-3′), corresponding to nucleotides 8974 through 8993 of hOGG1 intron 6 DNA sequences; GenBank accession HSAA131341) and antisense (hOGG1R: 5′-CCTTCGGGCTTGTGAGTG-3′; corresponding to nucleotides 9111 through 9129 of hOGG1 exon 7 sequences; GenBank accession HSAA131341) primers with a reverse primer (Amersham, Boehringer Mannheim, Indianapolis, IN). The reaction mixtures were incubated at 95°C for 3 min, then for 40 amplification cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension step at 72°C for 7 min. Ten µl of each PCR sample was digested with five units of HhaI (Boehringer Mannheim) at 37°C for 4 h and resolved on 8% native polyacrylamide gels to detect differences in RFLP patterns. The gels were stained with ethidium bromide, and examined and photographed under UV light. Three banding patterns were observed by RFLP analysis (Figure 1): a 156 bp fragment that corresponded to the 326Ser/326Ser homozygous wild-type genotype (lanes 1, 3, 4, 5, 8 and 9), 156 bp, 100 bp, and 56 bp bands that corresponded to the 326Thr/326Ser heterozygous genotype (lanes 2, 6, and 7), and 100 bp and 56 bp bands that corresponded to the 326Ser/326His homozygous polymorphic genotype (lane 10). Selected PCR-amplified DNA samples from subjects possessing each of the three potential hOGG1 genotypes were examined by dyeodeoxy DNA sequencing (performed at the Molecular Biology Core Facility in the H. Lee Moffitt Cancer Center) for further confirmation of hOGG1 genotyping results.

### Statistical analyses

Orolaryngeal cancer risk in relation to hOGG1 genotypes was estimated using conditional logistic regression to calculate odds ratios and 95% confidence intervals. For logistic regression analyses, the regression model included sex, age, smoking (py), and alcohol consumption (categorical) as variables. Interactions between genotype and smoking or alcohol consumption were evaluated using the likelihood ratio test to compare the goodness-of-fit of the model with and without the various interaction terms. The chi-square test was used for analysis.
of alcohol drinkers and allelic prevalence while a two-tailed Student's t-test was used to compare cigarette consumption between case and control subjects. The Spearman’s non-parametric correlation test was used to examine the correlation between smoking and alcohol consumption. The chi-squared test for trend was used to examine potential associations between predicted high-risk $hOGG1$ genotypes and orolaryngeal cancer risk. The statistical computer software SPSS (version 10.1) was used to perform all statistical analyses (35). All statistical tests were two-sided.

**Results**

**Expression of $hOGG1$ in aerodigestive tract tissues**

To evaluate the potential physiological importance of $hOGG1$ in aerodigestive tract tissue, we performed RT-PCR analysis to determine whether $hOGG1$ is expressed in normal human tongue, tonsil, floor of mouth, larynx, and esophagus. As shown in Figure 2, RT-PCR amplification of $\alpha$-hOGG1 and $\beta$-actin transcripts from total RNA isolated from human aerodigestive tract tissue specimens. Lane M, DNA marker; lane 1, total RNA from two different tonsil specimens; lane 2, total RNA from 19 different liver specimens; lane 3, total RNA from ten different esophagus specimens; lane 4, total RNA from three different larynx specimens; lane 5, total RNA from five different tongue specimens; lane 6, total RNA from three different floor of mouth specimens; lane 7, total RNA from thirty-two different lung specimens; lane 8, no RNA control. DNA marker band sizes are indicated on left of panel; $hOGG1$ PCR-RFLP band sizes are indicated on right of panel.

**Expression of $\alpha$-hOGG1 in aerodigestive tract tissue specimens.** RT-PCR amplification of $\alpha$-hOGG1 and $\beta$-actin transcripts from total RNA isolated from human aerodigestive tract tissue specimens. Lane M, DNA marker; lane 1, total RNA from two different tonsil specimens; lane 2, total RNA from 19 different liver specimens; lane 3, total RNA from ten different esophagus specimens; lane 4, total RNA from three different larynx specimens; lane 5, total RNA from five different tongue specimens; lane 6, total RNA from three different floor of mouth specimens; lane 7, total RNA from thirty-two different lung specimens; lane 8, no RNA control. DNA marker band sizes are indicated on left of panel; $\alpha$-hOGG1 and $\beta$-actin RT-PCR band sizes are indicated on right.

**Fig. 1.** Representative PCR-RFLP analysis of the $hOGG1$ Ser326Cys polymorphism. Lane M, DNA marker; lane 1, undigested PCR-amplified $hOGG1$ intron 6-exon 7 fragment amplified from genomic DNA from a single subject; lanes 2, 6, and 7, Taq I-digested PCR-amplified $hOGG1$ intron 6-exon 7 fragment from three different subjects with the heterozygous $hOGG1$ genotype; lanes 3, 4, 5, 8, and 9, Taq I-digested PCR-amplified $hOGG1$ intron 6-exon 7 fragment from five different subjects with the homozygous wild-type $hOGG1$ genotype; lane 10, Taq I-digested PCR-amplified $hOGG1$ intron 6-exon 7 fragment from a single subject with the homozygous polymorphic $hOGG1$ genotype. DNA marker band sizes are indicated on left of panel; $hOGG1$ PCR-RFLP band sizes are indicated on right of panel.

**Fig. 2.** Expression of $\alpha$-hOGG1 transcripts in aerodigestive tract tissue specimens. RT-PCR amplification of $\alpha$-hOGG1 and $\beta$-actin transcripts from total RNA isolated from human aerodigestive tract tissue specimens. Lane M, DNA marker; lane 1, total RNA from two different tonsil specimens; lane 2, total RNA from 19 different liver specimens; lane 3, total RNA from ten different esophagus specimens; lane 4, total RNA from three different larynx specimens; lane 5, total RNA from five different tongue specimens; lane 6, total RNA from three different floor of mouth specimens; lane 7, total RNA from thirty-two different lung specimens; lane 8, no RNA control. DNA marker band sizes are indicated on left; $\alpha$-hOGG1 and $\beta$-actin RT-PCR band sizes are indicated on right.

**Table I.** Distribution of orolaryngeal cancer cases and controls according to demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean age (years, range)</th>
<th>Sex (M/F)</th>
<th>Smoking (py) Mean ± SD</th>
<th>No. alcohol drinkersa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>338</td>
<td>60 (29–83)</td>
<td>238/100</td>
<td>29 ± 38</td>
<td>215 (64)</td>
</tr>
<tr>
<td>Cases</td>
<td>169</td>
<td>60 (25–87)</td>
<td>119/50</td>
<td>53 ± 44</td>
<td>127 (75)</td>
</tr>
</tbody>
</table>

a Alcohol drinkers were defined as subjects who consumed ≥ 1 shot per week for a minimum of 10 years as outlined in the Materials and methods.

b Mean smoking was significantly ($P = 0.008$) lower in control versus case subjects.

c The percentage of alcohol drinkers was significantly ($P = 0.01$) lower in control versus case subjects. Informative alcohol history information was not accurately collected for one control subject.

**Table II.** $hOGG1$ allele and genotype prevalences and risk for orolaryngeal cancer

<table>
<thead>
<tr>
<th>$hOGG1$ alleles</th>
<th>Controls</th>
<th>Cases</th>
<th>Crude OR (95% CI)a</th>
<th>Adjusted OR (95% CI)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOGG1326Ser</td>
<td>574 (87)c</td>
<td>262 (78)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>hOGG1326Cys</td>
<td>88 (13)c</td>
<td>72 (22)c</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
</tbody>
</table>

a OR: odds ratio; CI: confidence interval. Significant increase in prevalence of predicted high-risk $hOGG1$ genotypes in cases as compared to controls as determined by trend test ($P = 0.02$).

b Adjusted for age, sex, smoking (py), and alcohol consumption (categorical variables). Analysis did not include seven control and two case subjects for whom informative PCRs were not available, and one control subject with incomplete alcohol consumption data.

c Analysis did not include seven control and two case subjects for whom informative PCRs were not available.

d Numbers in parentheses refer to percentages.

e Case subjects had a statistically significant increase in the prevalence of the $hOGG1326Cys$ allele ($P < 0.001$).

female (Table I). Approximately 81% of case subjects and 67% of control subjects were ever-smokers (including cigar- and pipe-smokers), with a mean smoking dose (in py) of 53 for cases and 29 for controls. Case subjects had a statistically significantly higher level ($P = 0.008$) of smoking than control subjects, and there were a statistically significantly higher number ($P = 0.01$) of alcohol drinkers in cases as compared with control subjects. In addition, a statistically significantly higher percentage of case subjects than control subjects were considered to be heavy drinkers of alcohol (37% of cases versus 9% of controls; $P < 0.001$). A statistically significant correlation ($P < 0.001$) existed between smoking and alcohol consumption in this cohort.

**$hOGG1$ alleles and orolaryngeal cancer risk**

We obtained informative PCR results concerning the identity of the $hOGG1$ codon 326 polymorphism for 167 and 331 of the case and control subjects, respectively. Among the control subjects, the prevalence of the $hOGG1326Ser$ and $hOGG1326Cys$ alleles were 0.87 and 0.13, respectively (Table II), and were similar to those reported for Caucasians in previous studies (28,36) but lower than that reported for Asian populations.
To determine whether the hOGG1 326Cys allele contributed to increased risk for orolaryngeal cancer, we examined the prevalence of hOGG1 alleles in orolaryngeal cancer case subjects versus control subjects. Significantly increased risk for orolaryngeal cancer was observed for subjects with either the heterozygous hOGG1 326Ser/326Cys genotype (odds ratio [OR] = 1.6, 95% confidence interval [CI] = 1.04–2.6) or the homozygous hOGG1 326Cys/326Cys genotype (OR = 4.1, 95% CI = 1.3–13; Table II) as determined by logistic regression analysis. Significant differences were also observed for both the 326Ser/326Cys genotype (OR = 1.7, 95% CI = 1.1–2.6) and the 326Cys/326Cys genotype (OR = 3.6, 95% CI = 1.2–10) when analyzing crude data, a fact that corresponded with the significant trend towards increased risk for orolaryngeal cancer observed with potentially higher-risk hOGG1 genotypes (P = 0.02, trend test; Table II). These data corresponded with the fact that a significantly higher prevalence of the hOGG1 326Cys (P < 0.001) allele was observed in cases as compared to controls (Table II). Significant increases in cancer risk were also observed for the homozygous hOGG1 326Cys/326Cys genotype (OR = 5.3, 95% CI = 1.7–16; data not shown) as determined by logistic regression analysis when laryngeal cancer cases were excluded from this analysis (an analysis of laryngeal cancer risk could not be performed due to insufficient numbers of cases with laryngeal cancer [n = 42] in this cohort).

To examine the relationship between hOGG1 genotype and orolaryngeal cancer risk by exposure to environmental risk factors, we stratified study subjects by hOGG1 genotype and either smoking history or alcohol consumption (Table III). We observed no statistically significant association between hOGG1 genotype and orolaryngeal cancer risk in never-smokers. In contrast, a near-significant increase in risk for orolaryngeal cancer was observed for smokers with the hOGG1 326Ser/326Cys genotype (OR = 1.6, 95% CI = 0.96–2.8) while a significant increase in risk for orolaryngeal cancer was observed for smokers with the 326Cys/326Cys genotype (OR = 4.8, 95% CI = 1.3–18). These data corresponded with the fact that a significant trend towards increased risk for orolaryngeal cancer was observed with potentially higher-risk hOGG1 genotypes in smokers (P = 0.005, trend test) but not in never-smokers (Table III). Similarly, while no association was observed between the hOGG1 326Cys/326Cys genotype and orolaryngeal cancer risk in never-drinkers of alcohol, a significant increase in risk for orolaryngeal cancer was observed for the 326Cys/326Cys genotype in alcohol-drinkers (OR = 6.8, 95% CI = 1.6–29). A significant trend towards increased risk for orolaryngeal cancer was observed with potentially higher-risk hOGG1 genotypes in alcohol-drinkers (P < 0.001, trend test) but not in never-drinkers (Table III). Formal tests for interaction by the likelihood ratio test between hOGG1 genotype and smoking dose or alcohol consumption were not statistically significant in this analysis (results not shown). As expected, we observed a statistically significant increase in risk for orolaryngeal cancer for all subjects, regardless of hOGG1 genotype, as their exposure to tobacco smoke (P < 0.001) or their consumption of alcohol (P < 0.001) increased.

Discussion

8-OH-dG is one of the major forms of DNA adducts induced by oxidative damage, and increased 8-OH-dG formation in DNA is likely to be involved in mutagenesis and carcinogenesis (19,37,38). Previous studies have suggested that hOGG1 is one of the major enzymes involved in the repair of 8-OH-dG adducts in DNA (22,23). In the present study, we demonstrate that hOGG1 is expressed in all aerodigestive tract tissues examined, including esophagus, larynx, tonsil, tongue and floor of mouth. These data are consistent with the hypothesis that hOGG1 plays an important role in the repair of 8-OH-dG adducts in most human tissues (22,24).

Previous studies have implicated the Ser326Cys polymorphism in the hOGG1 gene in risk for smoking- and/or alcohol-related cancers. Significant increases in risk were observed for the homozygous hOGG1 326Cys/326Cys genotype and squamous cell carcinoma of the lung in a Japanese cohort (28). In addition, non-significant increases in the prevalence of the hOGG1 326Cys/326Cys genotype were observed in lung cancer cases as compared to controls in two small studies (26,36). A significant positive association between hOGG1 genotype and cancer risk was also observed for esophageal cancer (29). Although no interaction was observed between hOGG1 genotype and cancer risk was also observed for esophageal cancer including alcohol consumption. In the present study, an association between hOGG1 genotype and risk for orolaryngeal cancer was also observed for esophageal cancer (29).

### Table III. Orolaryngeal cancer risk after stratification by smoking dose, alcohol consumption, and hOGG1 genotypes

| hOGG1 genotypes | Controls | Cases | Multivariate analysis<sup>a</sup> OR 95% CI Trend test (P value)<sup>b</sup> |
|-----------------|----------|-------|-------------------------------------|-----------------------|
| **Never-smokers**<sup>c</sup> |          |       |                                     |                       |
| hOGG1 326Ser/326Ser | 157 (75) | 78 (62) | Referent                            |                       |
| hOGG1 326Ser/326Cys | 49 (23)  | 40 (32) | 1.5 (0.85–2.6)                      |                       |
| hOGG1 326Cys/326Cys | 3 (1.4)  | 8 (6.6) | 4.1 (1.6–29)                        |                       |
| **Smokers**<sup>c</sup> |          |       |                                     |                       |
| hOGG1 326Ser/326Ser | 101 (58) | 56 (46) | Referent                            |                       |
| hOGG1 326Ser/326Cys | 29 (16)  | 23 (14) | 1.7 (0.74–4.0)                      |                       |
| hOGG1 326Cys/326Cys | 0 (0)    | 3 (2.1) | 1.04 (0.00–29)                      |                       |
| **Never-drinkers**<sup>d</sup> |          |       |                                     |                       |
| hOGG1 326Ser/326Ser | 109 (60) | 58 (41) | Referent                            |                       |
| hOGG1 326Ser/326Cys | 35 (19)  | 27 (18) | 1.4 (0.70–2.6)                      |                       |
| hOGG1 326Cys/326Cys | 0 (0)    | 3 (2.1) | 1.04 (0.00–29)                      |                       |

<sup>a</sup> OR: odds ratio; CI: confidence interval. Adjusted for age, sex, smoking (pack-years) and alcohol consumption (categorical variables).

<sup>b</sup>Trend test assessing correlation between orolaryngeal cancer risk and predicted high-risk hOGG1 genotypes.

<sup>c</sup>Never-smoker = < 100 cigarettes lifetime. Regression analysis did not include four controls and one case subject whom informative PCRs were not available.

<sup>d</sup>Numbers in parentheses refer to percentages.

<sup>e</sup>Non-smokers = > 100 cigarettes lifetime. Regression analysis did not include three controls and one case subject whom informative PCRs were not available, and one control subject with incomplete alcohol consumption data.

<sup>f</sup>Never-drinker = < 1 shot of alcohol/week. Regression analysis did not include one control and one case subject whom informative PCRs were not available.

<sup>g</sup>Alcohol drinker = > 1 shot of alcohol/week. Regression analysis did not include six controls and one case subject whom informative PCRs were not available.
cancer was also observed. Significant increases in orolaryngeal cancer risk were observed for both the hOGG1 326Thr/326Cys heterozygote and the hOGG1 326Cys/326Cys homozygote. Although no association between hOGG1 genotype and orolaryngeal cancer risk was observed in never-smokers, a significant increase in risk was observed for the hOGG1 326Cys/326Cys genotype in smokers. This is consistent with studies demonstrating a link between 8-OH-DG formation and exposure to tobacco smoke carcinogens (12–20), and with the decreased DNA repair activity exhibited by the 326Cys-encoded isoform (26).

The role of oxidative damage and 8-OH-DG induction in alcohol-related carcinogenesis is less clear. Ethanol increased lipid peroxidation and the degradation of hepatic mitochondrial DNA in mice (39) while the feeding of ethanol increased the production of reactive oxygen species as well as lipid peroxidation and DNA fragmentation in the gastrointestinal tract of rats (40–42). Ethanol feeding was also associated with increasing 8-OH-DG adducts in rat hepatic mitochondrial DNA and the induction of DNA strand breaks in rat brain tissue (43). In rats, the consumption of beer was, however, associated with increased resistance to lipid peroxidation (44) and no difference in oxidative status was associated with the consumption of wine (45). Studies in humans are similarly contradictory. Alcohol consumption was linked to the induction of 8-OH-DG in lymphocyte DNA in subjects deficient in aldehyde dehydrogenase-2 (46) and was associated with increased DNA damage as measured by the COMET assay (47). In contrast, no association between alcohol consumption and human lymphocyte 8-OH-DG induction was observed in other studies (15,48,49). As results of the present study demonstrate that the hOGG1 326Cys/326Cys genotype is associated with increased risk for orolaryngeal cancer in alcohol-drinkers but not in never-drinkers of alcohol, it suggests that 8-OH-DG adduct formation may in fact be involved in alcohol-related orolaryngeal tumorigenesis. The fact that contrasting results have been observed regarding a linkage between 8-OH-DG formation and alcohol consumption in different population-based studies may be due to (1) differences in the level of alcohol consumption between subjects in different cohorts; (2) differences in expression of hOGG1 or other enzymes important in the repair of 8-OH-DG in different tissues; or (3) genetic differences in the cohorts studied.

In summary, the hOGG1 DNA repair enzyme is expressed in aerodigestive tract tissues and appears to play an important role in orolaryngeal cancer risk, with the association between hOGG1 genotype and orolaryngeal cancer risk observed in people who smoke or drank alcohol. Further studies are currently underway to better assess the effect of smoking and alcohol consumption on 8-OH-DG adducts in oral tissues in humans, and to better analyze the effect of hOGG1 genotype on these parameters.

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References


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