Insulin-like Growth Factor II Receptor Gene-167 Genotype Increases the Risk of Oral Squamous Cell Carcinoma in Humans

Athanasis I. Zavras, Waranuch Pitiphat, Tianxia Wu, Vassiliki CARTSOS, Albert Lam, Chester W. Douglass, and Scott R. Diehl

Introduction

Recent reports have implicated the cation-independent M6P/IGF2R or IGF2R in the carcinogenic process. M6P/IGF2R is known to have multiple important biological functions, some of which are critical in the suppression of cell growth. A loss or mutation of the M6P/IGF2R gene (chromosomal location 6q25-q27) could theoretically be associated with increased cancer risk. Such increased cancer risk for certain short tandem repeat genotypes has been described in several types of malignancies, including carcinomas of the breast, liver, endometrium, stomach, colorectum, and Wilms’s tumor. There are currently no reports linking M6P/IGF2R to the risk of human oral epithelial cancer. A hospital-based, case-control study was undertaken to investigate the effect of high-risk genotypes in oral cancer risk.

Materials and Methods

Initially, the institutional review boards of Harvard School of Public Health and the National Institute of Dental and Craniofacial Research, NIH, examined and approved the study protocol. Informed consent was obtained from all subjects participating in the study. Details about the methods have been described previously (1). In brief, a hospital-based, case control study of oral cancer was carried out in Athens, Greece from November 1995 to January 1998. Eligible cases had incident, pathologically confirmed primary squamous cell oral cancer. Controls were patients at the same hospitals, who were being treated for conditions unrelated to cancer. Examples of such conditions included minor surgery (i.e., third molar impaction removal), ear problems, nasal septal deviation, etc. Trauma patients were excluded because accidents are often related to alcohol abuse. To eliminate bias resulting from a change in behaviors caused by a chronic disease (for diet, smoking, or drinking), we required that hospitalization of the controls was attributable to a condition diagnosed during the last 6 months. Controls were matched to cases for sex and age (+/−5 years). A trained interviewer conducted detailed interviews, using a structured questionnaire. The questionnaire was designed for this study to assess demographic and risk factor information, including smoking and alcohol consumption. A small quantity of blood obtained from 93 cases and 94 controls was placed on a 4903 Guthrie card (Schleicher & Schuell) and stored at room temperature.

DNA extractions were carried out using the Puregene DNA isolation Kit (Gentra Systems, Inc.). Exact details about the DNA extraction protocol have been published elsewhere by Zavras et al. (2). Dinucleotide repeat polymorphisms at the IGF2R locus were assessed according to the protocol described by Goto et al. (3) in 1992. The cDNA clone used to determine allele size was similar to the one described by Oshima et al. (4) in 1988. The PCR conditions were as follows: samples were processed through 35 temperature cycles consisting of 1 min at 94°C, followed by 1 min at 55°C, and 45 s at 72°C. Sizes of the alleles were determined by comparison with the size of the amplified products of human Man-6-P/IGF2R cDNA clone mentioned above.

Standard methods of statistical analysis of case-control studies were applied. ORs were estimated using the Mantel-Haenszel method (5) with 95% CIs computed using the Robins et al. (6) method. All tests of statistical significance were two sided. Multivariate logistic regression analyses were performed to control simultaneously for all stratification variables. Multivariate models adjusted for the matching variables sex (males and females), age stratified into two groups (<64 and >64), and referring hospital. In addition, cigarette smoking was analyzed as lifetime exposure (pack-years) stratified into four groups (0, 1–25, >25–50, and >50); alcohol was similarly analyzed as usual use (drinks per week) stratified into four groups (glasses per week: 0, 1–28, >28–42, and >42).

Results and Discussion

The characteristics, demographic information, and main risk factor frequencies have been described previously (1). Briefly, the median age was 63.7 years for cases (range from 26 to 91) and 62.9 years for controls (range from 22 to 90). Most subjects resided permanently in Athens (58%) or other major urban centers (9%); 17% lived in smaller cities and 16% in rural areas. Smoking in men was associated with increased risk for oral cancer (OR, 3.0; 95% CI: 1.2–7.9), whereas relatively few women reported any history of smoking (31% of female cases and 17% of female controls reported either former or current smoking). Drinking >42 drinks/week was associated with a very significant increased risk. As expected, interaction was noted between heavy smoking and drinking, with those at the highest exposure group at a 14 times increased risk compared with abstainers (1).

IGF2R allele frequencies are listed in Table 1. The most common allele in cases and controls was the 165 bp (47.8% in cases and 50.5% in controls), followed by the 163- and 167-bp alleles.
growth factors-M6P/IGF2R helps activate the homodimeric cytokine transforming mitogen that normally acts through the IGF1 receptor (7). In this manner, proteins. Two important lysosomal enzymes linked to apoptosis, the main functions of M6P/IGF2R is the binding and transporting of glyco-
theses about the exact mechanism of M6P/IGF2R action. One of the replication in other populations, there are several current proposed hy-
risk; however, interpretation of this finding is difficult because of the very small sample size of subjects with a genotype of 167 of 167 (three cases and three controls).
Our data suggest possible involvement of M6P/IGF2R in the oral carcinogenic process. Although the association of increased risk for heterozygotes containing one copy of the 167-bp allele will require replication in other populations, there are several current proposed hypo-
M6P/IGF2R mediates the internalization and degradation of IGF2, a mitogen that normally acts through the IGF1 receptor (7). In this manner, the receptor serves as a suppressor of IGF2 proliferative actions and thus plays an important role in the suppression of cell growth. In addition, M6P/IGF2R helps activate the homodimeric cytokine transforming growth factor-β1, a potent growth inhibitor (10). In many cells, transforming growth factor-β inhibits cell growth by arresting the cell cycle at the late G1 phase and affects angiogenesis.
M6P/IGF2R is also involved in the antineoplastic pathway of retinoic acid and its analogues the retinoids. Retinoids exhibit diverse biological effects on cells. They control normal growth, fetal develop-
Table 2 lists the ORs and 95% CI obtained from the crude χ² analyses on the effect of genotype on oral cancer risk, as well as the adjusted OR and 95% CI obtained from the multivariate logistic regression. Compared with all other genotypes, being heterozygous for the 167 allele was found to increase the odds of cancer by 2.7 times (95% CI: 1.16–6.48). This adjusted result was statistically significant (P = 0.02). The 167 homozygotes were at no increased risk; however, interpretation of this finding is difficult because of the very small sample size of subjects with a genotype of 167 of 167 (three cases and three controls).

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Table 2 Risk of oral cancer associated with IGF2R genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. cases, controls</th>
<th>Unadjusted OR</th>
<th>95% CI</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>a/b</td>
<td>24, 22</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>165/b</td>
<td>49, 49</td>
<td>0.9</td>
<td>0.45–1.8</td>
<td>1.0</td>
<td>0.48–2.15</td>
</tr>
<tr>
<td>165/165b</td>
<td>20, 23</td>
<td>0.8</td>
<td>0.35–1.83</td>
<td>0.9</td>
<td>0.36–2.15</td>
</tr>
<tr>
<td>a/b</td>
<td>42, 41</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>163/b</td>
<td>41, 43</td>
<td>0.9</td>
<td>0.51–1.71</td>
<td>1.0</td>
<td>0.51–1.86</td>
</tr>
<tr>
<td>163/163b</td>
<td>10, 10</td>
<td>1.0</td>
<td>0.37–2.59</td>
<td>0.9</td>
<td>0.34–2.66</td>
</tr>
<tr>
<td>a/b</td>
<td>67, 80</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>167/b</td>
<td>23, 11</td>
<td>2.5</td>
<td>1.14–5.49</td>
<td>2.7</td>
<td>1.16–6.48</td>
</tr>
<tr>
<td>167/167b</td>
<td>3.3</td>
<td>1.2</td>
<td>0.23–6.11</td>
<td>1.0</td>
<td>0.18–5.69</td>
</tr>
</tbody>
</table>

* Adjusted by alcohol (g/wk: 0, 0–28, 28–42, >42), pack-years of smoking (≥50, >50), sex, age, and hospital (3).
* * denotes “other allele.”

In contrast to work that supports IGF2R’s involvement in cancer development, a critical review of literature on LOH and microsatellite instability contradicts the involvement of M6P/IGF2R in oral cancer. LOH and microsatellite instability seem to be important events during the carcinogenic process in many organs, including squamous cell carcinomas of the head and neck (15–17). Although LOH has been described in the M6P/IGF2R gene in certain tumors (18, 19), the 6q region (where the M6P/IGF2R gene is found) is not included as LOH loci in oral cancer.

In summary, we found that one allele at an IGF2R short tandem repeat polymorphism to be associated with increased risk of oral epithelial cancer. Additional data from other independent study populations will be necessary to confirm our finding. Gene expression profiling and genetic epidemiological investigations of IGF2R in diverse populations are needed to clarify the exact pathways of M6P/IGF2R action during carcinogenesis in the oral epithelium.

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