Association of TGF-β1 Genetic Variants with HPV16-positive Oropharyngeal Cancer

Xiaoxiang Guan1,3, Erich M. Sturgis1,2, Dapeng Lei2,4, Zhensheng Liu1, Kristina R. Dahlstrom2, Qingyi Wei1, and Guojun Li1,2

Abstract

Purpose: Transforming growth factor-β1 (TGF-β1) plays an important role in inflammation and immune responses, which control the human papillomavirus (HPV) clearance and escape of immune surveillance, and may contribute to genetic susceptibility to HPV16 infection.

Experimental Design: In this case series study, we analyzed the HPV16 status in tumor specimens and genotyped three TGF-β1 polymorphisms using genomic DNA from the blood of 200 squamous cell carcinoma of the oropharynx (SCCOP) cases. We calculated odds ratio (OR) and 95% confidence intervals (95% CI) in univariate and multivariable logistic regression models to examine the association between the TGF-β1 polymorphisms and HPV16 status in SCCOP.

Results: Compared with those with the common homozygous genotype, the TGF-β1 T869C variant genotypes were significantly associated with HPV16-positive tumor status among patients with SCCOP (OR, 1.97; 95% CI, 1.03-3.76), but no significant association was observed for the TGF-β1 C509T or G915C polymorphism. When all variant genotypes were combined, however, SCCOP patients carrying genotypes with any of these TGF-β1 variants were more than twice as likely to have an HPV16-positive tumor (OR, 2.28; 95% CI, 1.16-4.50) as patients with no variant genotypes. The stratified analysis showed that those under 54 years of age, non-Hispanic white patients, never smokers, and never drinkers with any variant TGF-β1 genotypes were also more likely to have HPV16-positive tumors.

Conclusions: TGF-β1 polymorphisms may serve as a susceptibility marker for tumor HPV16 status among SCCOP patients, particularly those who were never smokers and never drinkers. Large studies are needed to validate our findings. Clin Cancer Res; 16(5): 1416–22. ©2010 AACR.
HPV16-positive SCCOP patients seem to have a better response to radiotherapy than do HPV16-negative SCCOP patients. Among HPV16-positive SCCOP patients whose tumors generally lack somatic genetic changes, polymorphisms of inflammation/immune responsive genes may modify response to radiation damage, subsequently modifying SCCOP prognosis. Ultimately, identification of those to receive personalized treatment and those who will be able to benefit from reduction of treatment intensity is critical to treatment with high efficacy and low morbidity.

A number of polymorphisms have been identified that may be functional or that modulate plasma levels of TGF-β1. The C509T polymorphism is located in the promoter region of the TGF-β1 gene, and a previous study found that individuals homozygous for the variant T allele had almost twice the amount of plasma TGF-β1 as those homozygous for the wild-type C allele (18). Two other polymorphisms located in the signal sequence of the TGF-β1 gene, which result in nonsynonymous amino acid changes, have also been associated with variability of TGF-β1 levels. The T869C single nucleotide polymorphism (SNP) is located at codon 10 of exon 1 and results in a leucine-to-proline change. Studies have shown that the variant C allele of T869C is associated with increased production of TGF-β1 (19, 20), whereas it is the wild-type G allele of C915C that is associated with increased production of TGF-β1 (21). Although several studies have investigated the association between HPV and TGF-β1 expression levels in cervical cancer using both in vitro and in vivo methods (22–24), few have investigated whether TGF-β1 polymorphisms are associated with HPV16-associated SCCOP.

We hypothesize that TGF-β1 polymorphisms are associated with risk of HPV16-positive SCCOP.

**Materials and Methods**

**Study patients.** Patients with incident SCCOP were recruited consecutively as part of an ongoing molecular epidemiology study of SCCHN at The University of Texas M.D. Anderson Cancer Center from December 1996 to October 2008. All patients recruited without restrictions on age, sex, ethnicity, cancer stage, or histology were newly diagnosed, histopathologically confirmed, and untreated SCCOP. All patients signed an informed consent approved by our institutional review board and prospectively completed at presentation an epidemiologic questionnaire to provide information on demographic and risk factors including smoking and alcohol status. Patients also provided 30 mL of blood for genotyping. The HPV16 status was determined from paraffin-embedded tumor tissue samples. Among the above patient population pool, HPV16 tumor status and TGF-β1 genotypes were available for 200 SCCOP patients. Drinking status was categorized as “ever drinkers” (those who had drunk ≥1 alcoholic beverage/d for at least 1 y during their lifetime) and “never drinkers” (those who never had such a pattern of drinking). Smoking status was categorized as “ever smokers” (those who had smoked ≥100 cigarettes in their lifetime) and “never smokers” (those who had smoked <100 cigarettes in their lifetime).

**HPV16 detection and TGF-β1 genotyping.** DNA was extracted from the paraffin-embedded tissues for HPV16 detection and blood samples for TGF-β1 genotyping. These methods have been previously described for HPV16 detection (2, 25, 26) and for genotyping and selection criteria of the TGF-β1 polymorphisms (27).

**Statistical analysis.** Differences in the distributions of selected demographic characteristics, tobacco smoking and alcohol drinking status, and TGF-β1 genotype frequencies between HPV16-positive and HPV16-negative cases were evaluated using the χ² test. We estimated the association between TGF-β1 genotypes and tumor HPV16 positivity among SCCOP by computing the odds ratios (OR) and 95% confidence intervals (95% CI) using both univariate and multivariable logistic regression analyses. We further stratified the genotype data into subgroups by age, sex, smoking status, and drinking status in multivariable logistic regression models. For logistic regression analyses, the TGF-β1 genotypes were also recoded as a dummy variable. The Statistical Analysis System software (Version 9.1; SAS Institute) was used for all of the statistical analyses. All tests were two-sided, and a P value of 0.05 was considered the cutoff for statistical significance.

**Results**

Of the 200 patients with incident SCCOP, 147 (73.5%) were positive and 53 (26.5%) were negative for tumor
HPV16 DNA. Relevant demographic characteristics as well as smoking and alcohol history for HPV16-positive versus HPV16-negative patients are shown in Table 1. Overall, HPV16-positive patients were more likely to be male, <54 years old, and never smokers and never drinkers compared with HPV16-negative patients; however, these differences were not statistically significant (P = 0.193 for sex, P = 0.489 for age, P = 0.139 for smoking, and P = 0.354 for drinking).

The genotype distributions of the three TGF-β1 polymorphisms, C509T, T869C, and G915C, are shown in Table 2. The genotype distribution for C509T did not vary significantly between HPV16-positive and HPV16-negative patients, with approximately two thirds of the patients having the common CC genotype (P = 0.740). For the TGF-β1 T869C polymorphism, HPV16-positive patients were more likely to have the variant TC/CC genotypes (67.4%) than were the HPV16-negative patients (50.9%);

### Table 1. Distribution of selected variables in SCCOP patients by HPV16 status

<table>
<thead>
<tr>
<th>Variable</th>
<th>HPV16+ patients (n = 147)</th>
<th>HPV16− patients (n = 53)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤54</td>
<td>83 (56.5)</td>
<td>26 (49.0)</td>
<td>0.489</td>
</tr>
<tr>
<td>&gt;54</td>
<td>64 (43.5)</td>
<td>27 (51.0)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>132 (89.8)</td>
<td>44 (83.0)</td>
<td>0.193</td>
</tr>
<tr>
<td>Female</td>
<td>15 (10.2)</td>
<td>9 (17.0)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>137 (93.2)</td>
<td>49 (92.4)</td>
<td>0.856</td>
</tr>
<tr>
<td>Others</td>
<td>10 (6.8)</td>
<td>4 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>77 (52.4)</td>
<td>34 (64.2)</td>
<td>0.139</td>
</tr>
<tr>
<td>Never</td>
<td>70 (47.6)</td>
<td>19 (35.8)</td>
<td></td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>110 (74.8)</td>
<td>43 (81.1)</td>
<td>0.354</td>
</tr>
<tr>
<td>Never</td>
<td>37 (25.2)</td>
<td>10 (18.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Two–sided χ² test.

### Table 2. Risk of SCCOP associated with the TGF-β1 genotypes in HPV16+ and HPV16− patients

<table>
<thead>
<tr>
<th>TGF-β1 genotypes</th>
<th>HPV16+ patients (n = 147)</th>
<th>HPV16− patients (n = 53)</th>
<th>P</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β1 C509T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC†</td>
<td>98 (66.7)</td>
<td>34 (64.2)</td>
<td>0.740</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>CT + TT</td>
<td>49 (33.3)</td>
<td>19 (35.8)</td>
<td></td>
<td>0.90 (0.46-1.73)</td>
<td>0.85 (0.44-1.65)</td>
</tr>
<tr>
<td>TGF-β1 T869C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT†</td>
<td>48 (32.6)</td>
<td>26 (49.1)</td>
<td>0.034</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TC + CC</td>
<td>99 (67.4)</td>
<td>27 (50.9)</td>
<td></td>
<td>1.99 (1.05-3.77)</td>
<td>1.97 (1.03-3.76)</td>
</tr>
<tr>
<td>TGF-β1 G915C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG†</td>
<td>119 (81.0)</td>
<td>46 (86.8)</td>
<td>0.337</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>GC + CC</td>
<td>28 (19.0)</td>
<td>7 (13.2)</td>
<td></td>
<td>1.55 (0.63-3.78)</td>
<td>1.61 (0.65-3.98)</td>
</tr>
<tr>
<td>Combined genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No variants†</td>
<td>33 (22.5)</td>
<td>21 (39.6)</td>
<td>0.016</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>With variants</td>
<td>114 (77.5)</td>
<td>32 (60.4)</td>
<td></td>
<td>2.27 (1.16-4.44)</td>
<td>2.28 (1.16-4.50)</td>
</tr>
</tbody>
</table>

P values for χ² test for genotype distribution.
*Adjusted for age, sex, ethnicity, tobacco smoking and alcohol drinking status in a logistic regression model.
†Reference group.
Although HPV16-positive patients were also slightly more likely to have the variant GC/CC genotype of G915C than were the HPV16-negative patients, this difference did not reach statistical significance (19.0% and 13.2%, respectively; \( P = 0.337 \)).

A significant difference was observed when genotypes were combined; HPV16-positive patients were more likely to have variant genotypes compared with HPV16-negative patients (77.5% versus 60.4%, respectively; \( P = 0.016 \); Table 2). In multivariable logistic regression analyses with adjustment of age, sex, ethnicity, and smoking and alcohol status, a significant difference was observed only for T869C, with HPV16-positive patients twice as likely to have the variant genotype than HPV16-negative patients (OR, 1.97; 95% CI, 1.03-3.76). Although we did not observe a significant association between \( TGF-\beta_1 \)C509T or G915C polymorphism and HPV16-positive SCCOP patients, HPV16-positive SCCOP patients were twice more likely to have any variant genotypes of the three polymorphisms compared with HPV16-negative patients (OR, 2.28; 95% CI, 1.16-4.50; Table 2).

We carried out stratified analyses by age, sex, ethnicity, and tobacco smoking and alcohol drinking status to further explore the association between the combined genotypes of three \( TGF-\beta_1 \) polymorphisms and HPV16 status (Table 3). Among patients <55 years old, those with any variant genotypes of the three SNPs were more likely to have a HPV16-positive tumor than those without any variant genotypes (OR, 4.07; 95% CI, 1.52-10.9); however, no similar association was seen for patients >54 years old. Additionally, patients with any variant genotypes of the three SNPs were more likely to be HPV16-positive SCCOP among non-Hispanic whites (OR, 2.56; 95% CI, 1.26-5.20) and those with no history of smoking (OR, 3.76; 95% CI, 1.15-12.3) or alcohol drinking (OR, 5.01; 95% CI, 1.03-24.3). Because of limited sample size, we could not carry out further meaningful stratification by both age and status of smoking or drinking, although it is well known that patients with HPV16-positive SCCOP tended to be younger or likely to be nonsmokers or non-drinkers (2, 6, 28, 29).

### Discussion

In this case-case comparison study we found a significant association between \( TGF-\beta_1 \) polymorphisms and HPV16 tumor status among patients with incident SCCOP, indicating that \( TGF-\beta_1 \) polymorphisms may serve as susceptible markers for the tumor HPV16 status, particularly for several subgroups. Normally, HPV infections are transient and are either cleared quickly by the host immune response or are self-limiting. In some instances, however, HPV infection becomes persistent, resulting in cellular abnormalities that may progress and eventually result in the occurrence of malignancy (8, 11). HPV infection normally elicits an immune response involving secretion of various cytokines by T lymphocytes, which are typically divided into two groups known as type 1 and type 2 immunity (13). Type 1 immunity is regulated by T helper type 1 (Th1) lymphocytes that have...
proinflammatory effects characterized by phagocytic activity, whereas type 2 immunity is regulated by T helper type 2 (Th2) lymphocytes that have anti-inflammatory effects ultimately resulting in antibody production (13).

The infection stimulates naive T helper and cytotoxic cells and initiates a primary immune response. T helper cells stimulate different types of immunity, depending on the pattern of cytokines that they secrete. Although Th1 and Th2 cells secrete different types of inflammatory cytokines, most successful immune responses probably require coordination of both type 1 and type 2 responses (30, 31), which have pleiotropic functions. Th1 and Th2 cytokines have a complex and overlapping range of activities, which makes it difficult to isolate individual cytokines. The immune response balanced by the coordination between proinflammatory and anti-inflammatory cytokine activity has more impact on cancer risk than variation at a single locus (32). The perturbation of the balance between proinflammatory and anti-inflammatory cytokine levels can be caused by inherited gene mutations, from which common genetic variants can also alter the expression or function of key genes, disrupting the balance of cytokines, and thus affecting cancer risk and outcome.

TGF-β1, produced by virtually every cell in the body including epithelial cells, is involved in immunosuppression, regulation of cell cycle progression, tumor suppression and progression, angiogenesis, and metastasis (12, 16, 17, 33). As a member of antigen-specific T cells (termed Th3), TGF-β1 is capable of suppressing Th1 and Th2 responses (34). Promotion of imbalances in Th1 and Th2 cytokine levels by Th3 cells may aid in the persistence of viral infections. Evidence for this is provided by studies with HIV, which has been shown to stimulate interleukin-10 and TGF-β1 production from macrophages leading to suppression of Th1 cells and increased differentiation of TGF-β1-secreting Th3 cells (34, 35). Although TGF-β1 is conventionally regarded as an anti-inflammatory agent, its seemingly paradoxical role in biological activities in exacerbating inflammatory response and, thus, promoting autoimmunity, has been reported (36, 37). For example, TGF-β1 inhibits the differentiation of Th1 and Th2 cells, resulting in the uncontrolled differentiation to Th1 and Th2 effector cells, although it also promotes the differentiation of Th17 cells, which links it to a T-cell subset that produces interleukin 17 (38). It became apparent that Th17 cells instead of Th1 cells selectively produce proinflammatory cytokines and are responsible for autoimmunity and responses to pathogens such as viral infection (38).

In addition to TGF-β1-mediated immunosuppression of HPV-infected cells, HPV itself acts to evade the host immune response through close interaction with TGF-β1. The majority of studies investigating the role of TGF-β1 in HPV-mediated carcinogenesis involve cervical cancer, and these studies have shown that as progression from normal tissue to malignancy occurs, there is a corresponding increase in TGF-β1 levels, suggesting an association with severity of HPV infection (24, 39, 40–43). For example, Alcocer-Gonzalez et al. found that cervical cancer biopsies had a Th2/Th3 cytokine expression profile with a reduced expression of Th1 cytokines. Furthermore, HPV-positive cervical cancer cell lines express Th2/Th3 cytokines, whereas HPV-negative cell lines do not, which indicates that HPV is capable of inducing transcription of immunosuppressive cytokines as a means of evading the host immune response (24). Another study found that in HPV-positive cervical intraepithelial neoplasia, a shift from Th1 to Th2 cytokines was correlated with increasing severity of HPV infection (39). Furthermore, more aggressive HPV infection was associated with defective Th1 cytokine production and increased levels of Th2 cytokine production compared with less aggressive HPV infection and HPV-negative controls (39).

In addition to being controlled by the host genetic machinery, expression of TGF-β1 is also influenced by HPV16 that is under the control of two viral oncoproteins, E6 and E7, which are known to have antipapoptotic properties as well as an ability to modulate transcription of both viral and cellular genes. Both E6 and E7 have been shown to transactivate the TGF-β1 promoter activity in cervical cancer cells (22). Moreover, several in vitro studies have shown that HPV16-positive keratinocytes are initially inhibited by TGF-β1 but become resistant to the growth inhibitory effects of TGF-β1 as carcinogenesis progresses through E7-mediated blocking of the TGF-β1 tumor suppressor function (44–46).

Our results suggest that the combined variant genotypes of the three TGF-β1 polymorphisms may increase levels of TGF-β1, which subsequently modulate immune and inflammation response to HPV16 infection and lead to immunosuppression, in turn increasing the risk of HPV16 infection (34). We also found that younger HPV16-positive patients (<55 years of age) were more likely to have TGF-β1 variants than HPV16-negative patients, whereas no significant association was found among patients >54 years old. This is consistent with the notion of genetic susceptibility, which is often associated with early age of onset. We also found that HPV16-positive patients who had never smoked were almost four times more likely to have TGF-β1 variants than were HPV16-negative patients who had never smoked. Furthermore, this significant association was not seen among patients who had ever smoked. Likewise, among never drinkers, HPV16-positive SCCOP was significantly associated with TGF-β1 variant genotypes and this association was also not observed among ever drinkers. Previous studies have found that HPV16-positive tumors are more likely to occur among never users of tobacco or alcohol (2, 47). Given that HPV16-positive tumors are more likely to occur among younger age groups and never users of tobacco and alcohol, it is our conclusion that those with TGF-β1 genetic variants are further predisposed to infection with HPV16. It is likely that hosts with these TGF-β1 polymorphisms have a different ability to clear cells infected with HPV. Also, we speculate that smokers and drinkers possibly suppress humoral and cellular immune responses to HPV16 infection but this affects cell cycle regulation.
and apoptosis, leading to clearance of HPV-infected cells. However, this hypothesis needs to be tested in future large studies.

Although we are the first to test the association between these TGF-β1 polymorphisms and HPV16 status in SCCOP patients, our study has several limitations. First, other different characteristics may exist between HPV16-positive and HPV16-negative SCCOP patients, because both diseases are distinct etiologically and clinically; however, it seemed that the two groups were similar with regard to distributions in age, sex, ethnicity, smoking and drinking, and any residual confounding effect from these variables was further adjusted in multivariable analyses. Therefore, the potential confounding effect on the association should be minimal. Furthermore, patients from this study were retrospectively identified from the patient population pool of an ongoing molecular epidemiologic study of head and neck cancers in our institution, and the potential selection bias may exist. Second, the misclassification of HPV16 tumor status due to use of the PCR methods may occur, and this may bias the estimates of association. However, the use of two different methods to confirm HPV detection in this study would serve to minimize the misclassification of HPV16 tumor status. Third, stratified analysis resulted in some of the strata having small numbers, and the positive results could have occurred by chance or some significant associations could not have been detected. Finally, it is possible that selection bias due to the hospital-based nature of the study as well as other unknown confounding factors, such as sexual behavior characteristics, infection with other high-risk HPV types (although unlikely), and oral HPV16 infection may have occurred. In light of these limitations, future prospective and well-designed studies with larger sample sizes are needed to confirm our findings.

Knowing the HPV16 status of SCCOP patients may influence future treatment and prevention strategies. If HPV16-positive patients have a significantly better prognosis, then it may be possible to reduce the intensity of current treatments in HPV-positive SCCOP patients as well as to develop future targeted therapies for such patients. The results from genetic variants could define an individualized molecular profile of HPV16-positive SCCOP leading to individualized prevention and potentially optimizing patient stratification for clinical trials testing HPV16-targeted therapies. The results from this kind of studies would help identify individuals at high risk of HPV16-associated SCCOP and potentially lead to better and individualized treatment to improve both the survival and quality of life of SCCOP patients. Future studies that measure circulating levels of TGF-β1 as well as Th1 and Th2 immune response cytokines are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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