A TGF-β1 genetic variant at the miRNA187 binding site significantly modifies risk of HPV16-associated oropharyngeal cancer

Ye Tao1,2, Erich M. Sturgis1,3, Zhigang Huang1,2, Yan Sun1,4, Kristina R. Dahlstrom5, Qingyi Wei5 and Guojun Li1,3

1 Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX
2 Department of Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Key Laboratory of Otolaryngology Head and Neck Surgery, Beijing, China
3 Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX
4 Department of Otorhinolaryngology and Head and Neck Surgery, Yuhuangding Hospital of Qingdao University, Yantai, China
5 Duke Cancer Institute, Duke University Medical Center, Durham, NC

TGF-β1 rs1982073 polymorphism at the miRNA-187 binding site may alter TGF-β1 expression and function, and thereby this polymorphism (genotype CT/CC) increases cancer susceptibility. HPV16 L1 seropositivity is associated with the risk of oral squamous cell carcinoma (OSCC), including oropharyngeal squamous cell carcinoma (OPSCC) and oral cavity squamous cell carcinoma (OCSCC). Thus, we hypothesized that TGF-β1 rs1982073 polymorphism at the miRNA-187 binding site combined with HPV16 L1 seropositivity may have a joint effect on OSCC susceptibility. We determined the genotypes of TGF-β1 rs1982073 and HPV16 status in 325 OSCC subjects and 335 cancer-free controls in the non-Hispanic white population, and used logistic regression models to evaluate the joint effects on OSCC susceptibility. TGF-β1 rs1982073 polymorphism (CT/CC genotype) combined with HPV16 L1 seropositivity increased the risk of OSCC via joint effects, particularly in OPSCC subjects who were never-smokers (OR, 165.9; 95% CI, 28.6–960.4) or never-drinkers (OR, 196.0; 95% CI, 28.2–1,000.0), respectively. Younger subjects had a higher risk of OPSCC than older subjects (OR, 23.5; 95% CI, 6.3–87.0 vs. OR, 6.0; 95% CI, 1.7–17.9, respectively). The significant associations between this polymorphism and HPV16-associated OSCC and OPSCC were also observed. However, OCSCC subjects did not have similar results. Our findings suggest that the joint effects of TGF-β1 rs1982073 and HPV16 L1 seropositivity can increase risk of HPV16-associated oral cancer, particularly in OPSCC subjects who are never-smokers, never-drinkers and young. This result may help us understand the tumorigenesis process and improve early detection, which are critical for prevention and intervention strategies. However, larger studies are needed to validate our findings.

Key words: TGF-β1, genetic variants, HPV, oropharyngeal cancer, susceptibility, biomarkers

Abbreviations: CI: confidence intervals; HPV: human papillomavirus; HR: hazard ratio; OCSCC: oral cavity squamous cell carcinoma; OPSCC: oropharyngeal squamous cell carcinoma; OR: odds ratio; OSCC: oral squamous cell carcinoma; SNP: single nucleotide polymorphism; TGF-β1/TGFB1: transforming growth factor-β1

Conflict of interest: No potential conflicts of interest were disclosed.

Grant sponsor: NIEHS; Grant number: R01 ES-11740; Grant sponsor: NIH; Grant numbers: CA135679, CA133099, CA186261-01A1

DOI: 10.1002/ijc.31530

History: Received 21 Nov 2017; Accepted 29 Mar 2018; Online 16 Apr 2018
Correspondence to: Guojun Li, MD, PhD, Department of Head and Neck Surgery, Unit 1445, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, Tel.: 713-792-0227, Fax: 713-794-4662, E-mail: gli@mdanderson.org; or Yan Sun, Department of Otorhinolaryngology and Head and Neck Surgery, Yuhuangding Hospital of Qingdao University, Yantai, China, Tel.: 11861866056677, E-mail: lenards@163.com

Oral squamous cell carcinoma (OSCC) consists of oral cavity squamous cell carcinoma (OCSCC) and oropharyngeal squamous cell carcinoma (OPSCC).1,2 Tobacco smoking and alcohol drinking are the major risk factors for OSCC in the U.S. Smoking cessation efforts have reduced the risk of OSCC sharply and decreased the incidence significantly.3 In contrast to the declining trend for OCSCC, the incidence of OPSCC is increasing, particularly among young patients.1–5 This rising incidence trend for OPSCC is mainly attributed to human papillomavirus (HPV) infection.6 The predominant virus type is HPV16, which has a >90% positive rate in HPV-positive OPSCC.7 TGF-β1, a member of the TGF-β family, suppresses tumorigenesis in precancerous tissues and promotes invasiveness in advanced tumors, mainly owing to disequilibrium of TGF-β1 signaling, which features interwoven pathways with...
What's new?
Polymorphisms of transforming growth factor beta (TGF-β) and infection with high-risk human papilloma virus (HPV) types individually modify cancer risk, but the combined effect of both is largely unknown. The authors determined the genotype of a specific TGF-β1 variant that also affects the binding site of miRNA-187 in patients with oral squamous cell carcinoma, a cancer often associated with HPV type 16 infection. They find that this variant combined with HPV16 seropositivity increased oral cancer susceptibility, particularly in young and risk-free individuals, and propose that it could serve as a biomarker for risk of HPV16-associated oral cancer.

Introduction
Polymorphism TGF-β1 rs1982073 has been associated with increased susceptibility to breast cancer and prostate cancer, and our previous study identified the potential effects of TGF-β1 rs1982073 in OSCC subjects. However, no case–control study has evaluated the effect of TGF-β1 rs1982073 on susceptibility to HPV-associated OSCC. Since HPV16 infection is associated with risk of OSCC, and TGF-β1 rs1982073 affects disequilibrium of TGF-β1/Smad2–3 signaling pathway, which facilitates HPV16 E6/7 carcinogenesis, we hypothesized that the TGF-β1 rs1982073 polymorphism at the miRNA-187 binding site combined with HPV16 L1 seropositivity has a joint effect on susceptibility to OSCC. To test this, we determined the TGF-β1 rs1982073 polymorphism genotype and the HPV16 serological status of 325 non-Hispanic white OSCC patients and 335 cancer-free controls to assess the joint effects of these two factors on susceptibility to OSCC.

Material and Methods
Study population
This case–control study included 325 newly diagnosed, untreated patients who had a clinical diagnosis and histopathological confirmation of OSCC. The patients had been recruited at The University of Texas MD Anderson Cancer Center as part of an ongoing molecular epidemiologic study, in which the patient eligibility criteria have been described previously. During that same period, 335 controls were recruited by the Kelsey–Seybold Foundation from a pool of healthy subjects, which included residents of metropolitan Houston, and by MD Anderson Cancer Center from a pool of healthy visitors who had accompanied cancer patients but were genetically unrelated to the patients. The 335 healthy controls were frequency matched to the 325 OSCC patients by sex, age (± 5 years) and smoking and drinking status. All study subjects recruited were non-Hispanic whites. The institutional review boards of both MD Anderson and Kelsey–Seybold approved our study, and every study subject signed an informed consent form. Smoking status was classified as “ever-smokers” (those who had smoked >100 cigarettes in their lifetime) and “never-smokers” (those who had smoked fewer than 100 cigarettes in their lifetime). Drinking status was classified as “ever-drinkers” (those who had drunk more than one alcoholic beverage per day for at least 1 year during their lifetime) and “never-drinkers” (those who never had such a pattern of drinking).

TGF-β1 rs1982073 genotyping
At diagnosis or the recruitment, blood samples were collected and prepared for TGF-β1 genotyping. The criteria for determination of TGF-β1 polymorphism have been previously described. For our study, we extracted genomic DNA from a leukocyte cell pellet using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) in accordance with the manufacturer’s instructions. The polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) method was used for genotyping as previously described. Genotyping was performed by laboratory personnel blinded to the case–control status. Repeated analysis was performed on a randomly selected subset of 10% of the samples, and the results were in 100% concordance with the initial analysis.

HPV16 L1 serologic detection
For serologic testing, we generated HPV16 L1 virus-like particles from insect cells infected with recombinant baculovirus to test for antibodies against the HPV16 L1 capsid protein in the plasma; this was done with the use of a standard enzyme-linked immunosorbent assay (ELISA), as described previously. Two groups of control sera, one known to be positive and the other known to be negative, were tested in parallel with the study samples in duplicate on each plate. About 10% of the samples were randomly chosen for a repeat assay, and the results were 100% concordant with the results of the initial testing.
HPV16 detection in tumor specimens

The samples of DNA were extracted from paraffin-embedded tumor tissues of patients and determined for the presence of HPV16 using polymerase chain reaction and in situ hybridization methods described in our previous studies.15,17 For some patients, tumor HPV16 status was determined by in situ hybridization and p16 immunohistochemical analysis from HPV data in the patient’s clinical records, since the pathology laboratory at MD Anderson had begun classifying all OPSCC specimens as a standard clinical practice.

Statistical analysis

Statistical analyses were performed with SAS software (version 9.4; SAS Institute Inc., Cary, NC). We used χ² tests to test the differences in demographics, including smoking and drinking status, HPV16 L1 serology and TGF-β1rs1982073 genotypes between the patients and controls. To evaluate the associations of HPV16 L1 serology and TGF-β1rs1982073 polymorphism with the risk of OSCC, we used both univariate and multivariable logistic regression analyses to compute odds ratios (ORs) and 95% confidence intervals (CIs). Both HPV16 serological status and TGF-β1rs1982073 genotypes (CT/CC or TT genotype) were evaluated individually or in joint effect in cases and controls. Stratified analyses of the joint effects of HPV16 serology and TGF-β1rs1982073 polymorphism were further performed by taking into account smoking and drinking status and age. In addition, logistic regression analysis was performed to evaluate the association between the TGF-β1rs1982073 polymorphism and HPV16 serological status and OSCC risk stratified by cancer site (OPSCC vs. OCSCC patients). All tests were two sided, and p < 0.05 was considered significant.

Results

Demographics and risk factors

The demographic characteristics and risk factors of the 325 cancer-patient cases and 335 cancer-free controls are shown in Table 1. Age, sex, smoking status and alcohol drinking status did not differ significantly between the cases and controls, and these results further confirmed the frequency-match validity. However, the frequency of HPV16 L1 seropositivity was significantly higher in the cases than in the controls (p < 0.001).

Joint effect of TGF-β1rs1982073 polymorphism and HPV16 serology on risk of OSCC

Regardless of HPV16 serology, subjects with the CT/CC genotype of TGF-β1rs1982073 had about a fourfold higher risk of developing OSCC than those with the TT genotype of TGF-β1rs1982073 (Table 2). The subgroups of OPSCC and OCSCC had similar results (Table 2). When HPV16 serology was taken into account, and the effects were adjusted for age, sex and smoking/drinking status, we found that HPV16 seronegative individuals carrying the TT genotype of TGF-β1rs1982073 had the lowest risk. Therefore, the combination of the TT genotype of TGF-β1rs1982073 and seronegativity of HPV16 was set as the reference group in further comparisons with the other three combinations of genotype (CT/CC or TT) and HPV16 serology (seropositivity or seronegativity). The risk of OSCC was greatest among subjects with the CT/CC genotype and HPV16 L1 seropositivity (OR, 13.6; p < 0.001).

| Variables | OPSCC (n = 188) | OCSCC (n = 137) | All (OSCC) (n = 325) | Controls (n = 335) | p value |
|-----------|----------------|----------------|---------------------|-------------------|---------|
| Age (year) |                |                |                     |                   |         |
| <50       | 54 (28.7)      | 33 (24.1)      | 87 (26.8)           | 87 (26.0)         | 0.183   |
| ≥50       | 134 (71.3)     | 104 (75.9)     | 238 (73.2)          | 248 (74.0)        |         |
| Sex       |                |                |                     |                   | 0.100   |
| Male      | 155 (82.5)     | 86 (62.8)      | 241 (74.2)          | 269 (80.3)        |         |
| Female    | 33 (17.5)      | 51 (37.2)      | 84 (25.8)           | 66 (19.7)         |         |
| Smoking   |                |                |                     |                   | 0.673   |
| Ever      | 125 (66.6)     | 102 (74.5)     | 227 (69.8)          | 239 (71.3)        |         |
| Never     | 63 (33.5)      | 35 (25.5)      | 98 (30.2)           | 96 (28.7)         |         |
| Alcohol drinking | |                |                     |                   | 0.121   |
| Ever      | 150 (79.8)     | 100 (73.0)     | 250 (76.9)          | 240 (71.6)        |         |
| Never     | 38 (20.2)      | 37 (27.0)      | 75 (23.1)           | 95 (28.4)         |         |
| HPV16 serology |        |                |                     |                   | <0.001  |
| Positive  | 87 (46.3)      | 13 (9.5)       | 100 (30.8)          | 42 (12.5)         |         |
| Negative  | 101 (53.7)     | 124 (90.5)     | 225 (69.2)          | 293 (87.5)        |         |

1P values of two-sided χ² test between OSCC patients and controls.
This risk was even more pronounced in OPSCC among patients with the CT/CC genotype and HPV16 L1 seropositivity (OR, 26.4; 95% CI, 12.9–53.9). In contrast, the risk of OCSCC was highest among subjects with the CT/CC genotype and negative HPV16 serology (OR, 4.4; 95% CI, 2.8–7.0).

### Stratified analysis of the joint effect of HPV16 serology and TGF-β1rs1982073 polymorphism on risk of OSCC by smoking and drinking status

We evaluated the association between the TGF-β1rs1982073 genotype and the risk of HPV16-associated OSCC stratified by smoking or drinking status. Table 3 shows that the joint

---

| Variables | HPV16 serology | OPSCC \(n = 325\) | OCSCC \(n = 325\) | All (OSCC) \(n = 325\) | Controls \(n = 335\) | Adjusted OR (95% CI) \(^1\) |
|-----------|----------------|----------------|----------------|----------------|----------------|-------------------|
| Overall   |                |                |                |                |                |                   |
| TT        |                | 61 (32.4)      | 30 (21.9)      | 115 (35.4)     | 238 (71.0)     | 1.0               |
| CT/CC     |                | 127 (67.6)     | 107 (78.1)     | 210 (64.6)     | 97 (29.0)      | 4.6 (3.3–6.4)     |
|           |                |                |                |                |                | 4.9 (3.3–7.3)     |
|           |                |                |                |                |                | 4.4 (2.8–6.8)     |

---

| Risk groups | HPV16 serology | OPSCC \(n = 325\) | OCSCC \(n = 325\) | All (OSCC) \(n = 325\) | Controls \(n = 335\) | Adjusted OR (95% CI) \(^1\) |
|-------------|----------------|----------------|----------------|----------------|----------------|-------------------|
| Never-smoker|                |                |                |                |                |                   |
| TT          |                | 6 (9.5)        | 14 (40.0)      | 20 (20.4)      | 58 (60.4)      | 1.0               |
| CT/CC       |                | 21 (33.3)      | 18 (58.6)      | 39 (39.8)      | 30 (31.2)      | 4.5 (2.1–9.7)     |
|             |                |                |                |                |                | 7.8 (2.6–23.0)    |
|             |                |                |                |                |                | 3.4 (1.2–9.2)     |
| TT          |                | 10 (15.9)      | 1 (2.9)        | 11 (11.2)      | 6 (6.3)        | 8.5 (2.6–28.0)    |
| CT/CC       |                | 26 (41.3)      | 2 (5.7)        | 28 (28.6)      | 2 (2.1)        | 60.1 (12.3–293.3) |
|             |                |                |                |                |                | 165.9 (28.6–960.4)|
|             |                |                |                |                |                | 5.0 (0.4–65.6)    |
| Ever-smoker |                |                |                |                |                |                   |
| TT          |                | 25 (20.0)      | 35 (34.3)      | 60 (26.4)      | 152 (63.6)     | 1.0               |
| CT/CC       |                | 49 (39.2)      | 57 (55.9)      | 106 (46.7)     | 53 (22.2)      | 5.2 (3.3–8.2)     |
|             |                |                |                |                |                | 5.6 (3.1–10.1)    |
|             |                |                |                |                |                | 5.0 (2.8–8.6)     |
| TT          |                | 20 (16.0)      | 4 (3.9)        | 24 (10.6)      | 22 (9.2)       | 2.9 (1.5–5.5)     |
| CT/CC       |                | 31 (24.8)      | 6 (5.9)        | 37 (16.3)      | 12 (5.0)       | 8.8 (4.2–18.5)    |
|             |                |                |                |                |                | 15.2 (6.8–34.2)   |
|             |                |                |                |                |                | 2.6 (0.9–7.8)     |
| Never-drinker|               |                |                |                |                |                   |
| TT         |                | 4 (10.5)       | 14 (37.8)      | 18 (24.0)      | 60 (63.2)      | 1.0               |
| CT/CC      |                | 14 (36.8)      | 18 (48.6)      | 32 (42.7)      | 26 (27.4)      | 5.8 (2.5–13.7)    |
|            |                |                |                |                |                | 10.5 (2.9–38.4)   |
|            |                |                |                |                |                | 4.8 (1.6–14.1)    |
| TT         |                | 5 (13.2)       | 3 (8.1)        | 8 (10.7)       | 7 (7.4)        | 4.9 (1.4–16.7)    |
| CT/CC      |                | 15 (39.5)      | 2 (5.5)        | 17 (22.6)      | 2 (2.1)        | 59.9 (10.8–332.9) |
|            |                |                |                |                |                | 196.0 (28.2–1,000.0) |
|            |                |                |                |                |                | 6.5 (0.4–112.6)   |
| Ever-drinker|               |                |                |                |                |                   |
| TT         |                | 27 (18.0)      | 35 (25.0)      | 62 (48.8)      | 150 (62.5)     | 1.0               |
| CT/CC      |                | 56 (37.3)      | 57 (57.0)      | 113 (45.2)     | 57 (23.7)      | 4.8 (3.1–7.4)     |
|            |                |                |                |                |                | 5.2 (3.0–9.1)     |
|            |                |                |                |                |                | 4.7 (2.7–8.1)     |
| TT         |                | 25 (16.7)      | 2 (2.0)        | 27 (10.8)      | 21 (8.8)       | 3.2 (1.7–6.1)     |
| CT/CC      |                | 42 (28.0)      | 6 (6.0)        | 48 (19.2)      | 12 (5.0)       | 10.1 (4.9–20.9)   |
|            |                |                |                |                |                | 17.2 (7.9–37.6)   |
|            |                |                |                |                |                | 2.3 (0.8–6.8)     |

---

1Adjusted for age, sex and smoking and drinking status.

95% CI, 7.1–26.2; Table 2). This risk was even more pronounced in OPSCC among patients with the CT/CC genotype and HPV16 L1 seropositivity (OR, 26.4; 95% CI, 12.9–53.9). In contrast, the risk of OCSCC was highest among subjects with the CT/CC genotype and negative HPV16 serology (OR, 4.4; 95% CI, 2.8–7.0).
effect of TGF-\(b\)rs1982073 polymorphism and HPV16 serology on risk of OSCC was more significant in never-smokers and never-drinkers than in ever-smokers and ever-drinkers, particularly for OPSCC patients. In fact, compared to the reference group, the risk of OSCC was 60.1-fold higher in the CT/CC genotype and HPV16 L1-seropositive never-smokers group and only 8.8-fold higher in the CT/CC genotype and HPV16 L1-seropositive ever-smokers group. Likewise, in the CT/CC genotype and HPV16 L1-seropositive never-drinkers group, the risk of OSCC was 59.9-fold higher, versus 10.1-fold higher in the ever-drinkers group. Similarly, these stratified results demonstrated that the joint effects of HPV16 serology and TGF-\(b\)rs1982073 genotype were much more significant among OPSCC than OSCCC. Specifically, in OPSCC subjects, compared to the reference group, the CT/CC genotype and HPV16 L1 seropositivity resulted in 165.9-fold and 196.0-fold higher risks in never-smokers and never-drinkers, respectively, compared to 15.2-fold and 17.2-fold higher risks in ever-smokers and ever-drinkers, respectively.

**Joint effect of TGF-\(b\)rs1982073 polymorphism and HPV16 serology by age stratification**

We evaluated the joint effects of the TGF-\(b\)rs1982073 genotype and HPV16 serology stratified by younger age (<50 years) or older age (≥50 years), as shown in Table 4. The TT genotype of TGF-\(b\)rs1982073 and HPV16 L1 seronegativity were set as the reference group. In the CT/CC genotype and HPV16 L1-seropositive OSCC younger group, the risk of OSCC was 12.9-fold higher, versus 3.2-fold higher in older subjects. Moreover, the risk of OPSCC was 23.5-fold higher in the CT/CC genotype and HPV16 seropositive younger group and only 6.0-fold higher in the older group. However, this pattern was not observed in OCSCC subjects.

**Association of TGF-\(b\)rs1982073 polymorphism with HPV16-associated OSCC**

Among the 325 cases, there were 170 patients to either have tissue specimens available for tumor HPV determination or have existing tumor HPV status in clinical records. We also included another 40 patients, who were recruited at the same period as those in the current study and had tissue specimens available for tumor HPV determination. These 40 patients were also genotyped for TGF-\(b\)rs1982073 polymorphisms. Therefore, a total of 210 patients were included for this subgroup analysis. The association between TGF-\(b\)rs1982073 polymorphism with HPV16-associated OSCC is presented in Table 5. We found that compared to those with TT genotype, the carriers with CT/CC genotypes of TGF-\(b\)rs1982073 had approximately threefold significantly increased risk of OSCC (OR, 3.2; 95% CI, 1.4–7.4) and OPSCC (OR, 3.3; 95% CI, 1.4–7.8) when our analysis was limited to only HPV16 L1-seropositive individuals. However, such a significantly increased risk was not found for OCSCC (OR, 1.3; 95% CI, 0.8–18.8). Furthermore, the genotype distribution of the TGF-\(b\)rs1982073 polymorphism differed significantly between tumor HPV16-positive and tumor HPV16-negative patients (\(p<0.0001\)). The patients with the CT/CC genotypes of TGF-\(b\)rs1982073 were almost two times more likely to have HPV16-positive tumors than those with the TT genotype among patients with OSCC (OR, 1.9; 95% CI, 1.1–3.4), OPSCC (OR, 2.0; 95% CI, 1.3–6.1) and OCSCC (OR, 1.1; 95% CI, 0.2–2.3), respectively.

**Discussion**

In our current study, we evaluated the association between the TGF-\(b\)rs1982073 genotype and HPV16 L1 serology in 325 non-Hispanic white OSCC patients and 335 cancer-free controls and found that TGF-\(b\)rs1982073 polymorphism...
TGF-β1 positivity and HPV16-associated OSCC and a link between OSCC. To demonstrate a causal link between HPV L1 sero-infection by HPV16 but not presence of a HPV-associated likely to indicate the humoral immune response following to HPV-associated OSCC. Thus, HPV seropositivity is only cooperate in neoplastic transformation and clearly are linked in contrast to antibodies to the proteins E6 and E7, which seems to be only in a proportion of HPV16-associated OSCC.20–21 Therefore, serum anti-HPV16 L1 antibody that are associated with, or predictive of, HPV16-driven recent studies have demonstrated that it is HPV16 E6/E7 polymorphism and tumor HPV16 status were also found. Taken together, our findings suggest that the joint effects of HPV16 L1-seropositive subjects only and between this polymorphism and risk of OSCC and OPSCC.19–21 Therefore, serum anti-HPV16 L1 antibody which are never-smokers or never-drinkers, respectively. Younger patients with OPSCC had a higher risk than older OSCC patients. Similar patterns were not observed in the OCSCC subjects. Moreover, the significant associations between this polymorphism and risk of OSCC and OPSCC among HPV16 L1-seropositive subjects only and between this polymorphism and tumor HPV16 status were also found.12,22,23 The polymorphism of nucleotide C to T transition in breast cancer23 and prostate cancer,24 and the CT/CC allele increases the cancer risk. Since high expression of miR-187 was associated with a trend toward cancer progression in breast cancer23 and prostate cancer,24 and the TGF-β1rs1982073 CC/CT genotype could increase the binding strength of that duplex, we conclude that the TGF-β1rs1982073 CC/CT genotype could potentially increase the risk of and progression of HPV16 L1-seropositive OPSCC.

TGF-β1rs1982073 may also increase the risk of HPV16 L1-seropositive OPSCC via the Leu10Pro signal peptide substitution that affects TGF-β1 secretion, structure and function. In cytomegalovirus (CMV)-transfected HeLa cell lines, CMV-Pro10 (CC genotype) had a 2.8-fold increase in TGF-β1 secretion compared to CMV-Leu10 (TT genotype), and this increased secretion indicated that TGF-β1rs1982073 (Pro10 homozygosity CC genotype) was associated with an increased risk of breast cancer.9 Likewise, in hepatoma cell lines (HepG2, SMMC7721, LX-2 and L02), cells transfected with CMV-Pro10 had higher capacity for TGF-β1 secretion, greater antiapoptosis effects and stronger enhancement of cell proliferation compared to those transfected with CMV-Leu10 in vitro.25 In vivo, significantly higher serum levels of TGF-β1 have been identified in patients with gastric cancer,10–26 hepatocellular carcinoma25 and prostate cancer;27 however, determining whether there is an association between TGF-β1rs1982073 serum levels and those specific cancers still requires further investigation.

The increased risk of OPSCC associated with TGF-β1rs1982073 depends on the constitutive and extensive cross-talk of TGF-β1 pathways with other signaling pathways (MAPK, PI3K/Akt, Wnt, etc.).28 The crosstalk between the TGF-β1 and HER2/Ras/MAPK pathways often leads to auto-induction of TGF-β1 itself and other growth factors, which in turn promotes epithelial–mesenchymal transition (EMT) and cell invasion.28–35 Likewise, apoptosis and/or cell-cycle arrest were dysregulated by the crosstalk between the TGF-β1 and PI3K/Akt/mTOR pathways, which can enhance cell

| Genetic variants | HPV16 L1 serology | Patients (n = 100) | Adjusted OR (95% CI) | Controls (n = 42) | Adjusted OR (95% CI) |
|------------------|-------------------|-------------------|----------------------|-------------------|----------------------|
|                  | All (OSCC) n (%)  | OPSCC n (%)       | OCSCC n (%)          | All (OSCC) n (%)  | OPSCC n (%)          | OCSCC n (%)         |
| TT               | +                 | 35 (35.0)         | 30 (34.5)            | 8 (61.5)          | 28 (66.7)            | 1.0                  |
| CT/CC            | +                 | 65 (65.0)         | 57 (65.5)            | 5 (38.5)          | 14 (33.3)            | 3.2 (1.4–7.4)        |

$^1$Adjusted for age, sex and smoking and drinking status.
proliferation and induce carcinogenesis. Moreover, TGF-β1/Smads and Wnt/β-catenin are key morphogen pathways that coordinate to influence cell division and cell differentiation so that stem cells can ultimately transform into differentiated cells; however, disrupted coordination in this process can lead to tissue-specific cancers. E6/E7 can consistently activate MAPK, PI3K/Akt, Wnt and many other pathways, and in HPV16 L1-seropositive OPSCC, these activated pathways have pleiotropic crosstalk with TGF-β1 noncanonical pathways in which activation can promote EMT and tumorigenesis. Moreover, TGF-β1rs1982073 genotypes with higher TGF-β1 production can further aggravate that disrupted coordination and disequilibrium.

OPSCC risk increases with the loss of cellular control for apoptosis and homeostasis, and TGF-β1rs1982073 risk genotypes can aggravate that control loss and thereby induce tumorigenesis. p53-mediated cellular apoptosis due to DNA damage can be disrupted in HPV16 L1-seropositive cell lines, where the HPV E6 oncoprotein can bind wild-type p53 to stimulate p53 degradation while E7 can inhibit apoptosis and enhance proliferation via Rp pathway inhibition. Furthermore, E6/E7 can constitutively activate PI3K signaling, and PI3K activation can inhibit p53 apoptosis via Hdm2 and notch1 to sustain cellular transformation, which was induced by HPV16 E6/E7 in Hacat-Neo cells. Therefore, the cellular control of apoptosis and homeostasis is lost in HPV-infected cells in which the effector arm for proliferation (TGF-β1/noncanonical pathways [i.e., PI3K]) was enhanced and the suppressor arm (TGF-β1 canonical pathway) was not strengthened. Since unbalanced arms and loss of apoptosis have constructed the disequilibrium context, and this disequilibrium cannot be compensated for by a higher serum level of TGF-β1 (caused by TGF-β1rs1982073 risk genotypes), that increased level can aggravate the disequilibrium and facilitate tumorigenesis.

Decreased immunity associated with HPV infection increases the risk of OPSCC. For example, HIV/AIDS patients are at higher risk for all HPV-related cancers, including oropharyngeal cancers. In a large population-based study, Chaturvedi et al. found a standardized incidence ratio of 1.6 (95% CI, 1.2–2.1) for oropharyngeal cancer among individuals with AIDS compared to the general population during the period of 1980–2004. Thus TGF-β1rs1982073 can further devastate the immune microenvironment and facilitate cancer immunoeediting and promote tumor evasion. During virus–host interactions, HPV16 infected epithelial cells and depended on epithelial differentiation to complete the virus life cycle. High-risk HPV E6 and E7 can drive epithelial cells into the S-phase and thereby created an environment that is conducive for viral genome replication and cell proliferation. Furthermore, high-risk HPV E7 can blunt or inhibit interferon regulatory factor-1 (IRF-1) in a concentration-dependent manner and thereby decrease production of interferon-γ (INF-γ), which is known for antivirus and antitumor immunity. In contrast, TGF-β1, at high concentrations in the tumor microenvironment, can promote either Th17 or Treg cell lineage differentiation to generate more growth factors, including TGF-β1 itself, to promote tumor invasion. Moreover, growth factor generation can be promoted by TGF-β1rs1982073, and thereby TGF-β1rs1982073 can dampen the microenvironment and promote tumor-infiltrating lymphocytes that result in chronic inflammatory conditions, which enhance the risk of malignancy and disrupt the immune system’s equilibrium to facilitate cancer immunoeediting.

Genetic alterations caused by TGF-β1rs1982073 polymorphism and HPV16 may jointly facilitated OPSCC tumorigenesis. Our results show that HPV16 infection plays a major and independent role in OPSCC susceptibility, whereas OCSCC etiology is mainly associated with tobacco exposure and alcohol use. In our current study, we found that the significant joint effects of TGF-β1rs1982073 and HPV16 L1 seropositivity significantly increased the risk of cancer, particularly OPSCC, in patients who were never-smokers or never-drinkers or young (age < 50). Since the incidence of HPV-seropositive OPSCC has grown in recent decades among subjects without high risk due to advanced age or exposure to tobacco and alcohol, this increased risk may be caused by prevalent oral HPV16 infection in young adults with hereditary susceptibility to cancer development. However, this question still requires further study.

Therefore, in the context of HPV16 infection, E6/E7 dose-dependent carcinogenic effects, and the oropharyngeal infection prevalence, TGF-β1 loss of pathway regulations causes systemic disequilibrium of cell differentiation. This disequilibrium would dysregulate hundreds of genes and increase the risk of OPSCC. However, our results have three main limitations: (1) the exact molecular mechanisms for TGF-β1rs1982073 binding at the miRNA-187 site and its effects on the protein structure of TGF-β1, canonical and noncanonical pathways, the immune system and tumorigenesis have not been evaluated; (2) crosstalk and effects between TGF-β1 and many other pathways require bioinformatics analysis with big data; and (3) in this case–control study, a possible selection bias was generated due to the design limit, with only non-Hispanic whites included in the study so that our results cannot be extrapolated to other ethnic groups.

Taken together, our results suggest that the TGF-β1rs1982073 polymorphism at the miRNA-187 binding site increases OSCC susceptibility, and this polymorphism (genotype CT/CC) combined with HPV16 L1 seropositivity can jointly increase the risk of OSCC, particularly in OPSCC subjects who are never-smokers, never-drinkers, and young. Therefore, this result can help us understand the tumorigenesis process and improve early detection which are critical for prevention and intervention strategies. However, to confirm our findings and elucidate the underlying mechanisms, additional larger population or functional studies are warranted for further validation.

Acknowledgements
The authors gratefully thank Ms. Yingdong Li for laboratory support. They also thank NIEHS (grant R01 ES-11740 to Q.W.) and NIH (CA 135679 to G.L., CA133099 to G.L., CA186261-01A1 to G.L.).
