**MicroRNA** Variants Increase the Risk of HPV-Associated Squamous Cell Carcinoma of the Oropharynx in Never Smokers

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**Abstract**

**Background:** Both microRNAs and human papillomavirus (HPV) infection play an important role in the development and progression of oral squamous cell carcinoma (OSCC). In addition, microRNAs affect all facets of the immune/inflammation responses to infection, which may control HPV clearance. We thus hypothesized that microRNA polymorphisms modify the association between HPV16 seropositivity and OSCC risk.

**Methods:** Four single-nucleotide polymorphisms in microRNAs were genotyped and HPV16 serology was determined in 325 cases and 335 matched controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using univariate and multivariable logistic regression models.

**Results:** Overall, each polymorphism had no significant main effect on OSCC risk. Compared with the risk among individuals with both miR146 rs2910164 GG genotype and HPV16 seronegativity, risk of OSCC was increased among those with CG or CC genotype and HPV16 seronegativity (OR, 1.2; 95% CI, 0.9–1.8), GG genotype and HPV16 seropositivity (OR, 3.0; 95% CI, 1.8–5.0), and CG or CC genotype and HPV16 seropositivity (OR, 4.7; 95% CI, 2.3–9.4). Similar results were found for miR149 rs2292832, miR196 rs11614913, and miR499 rs3746444. Analyses stratified by tumor sites and smoking status showed that each polymorphism significantly increased the risk of HPV16-associated squamous cell carcinoma of the oropharynx (SCCOP), and such effect modification was particularly prominent in never smokers.

**Conclusions:** Our results indicate that microRNA polymorphisms modify the risk of OSCC associated with HPV16 seropositivity, particularly in patients with SCCOP and never smokers. Larger studies are needed to verify our findings.

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**Introduction**

Oral squamous cell carcinoma (OSCC; SCC of the oropharynx, SCCOP and SCC of oral cavity) accounts for the majority of head and neck malignant tumors and is one of the most common malignancies worldwide. It has been estimated that approximately 36,540 new cases of OSCC will be diagnosed and 7,880 individuals will die from these cancers in 2012 in the United States [1–3]. Tobacco use and alcohol consumption are considered the most important risk factors for OSCC [4]. Despite decreasing smoking and drinking rates in the United States, the overall incidence of OSCC in young adults has been increasing in recent decades. Epidemiologic evidence implies that this increase is related to an increasing prevalence of infection with human papillomavirus (HPV), partly due to change in sexual behaviors [5–7]. Therefore, HPV may be another important etiologic factor for OSCC in addition to tobacco and alcohol use. Of the more than 130 types of HPV, the high-risk HPV type 16 (HPV16) is by far the most commonly associated with head and neck cancers, accounting for about 90% of HPV-associated OSCCs [8–10]. Although HPV infection may be a major risk factor for OSCC, only a small fraction of individuals infected with HPV eventually...
develop the malignancy, implying that host genetic factors may modify the association between HPV infection and OSCC risk.

MicroRNAs are highly conserved, single-stranded, short, non-coding RNAs of about 22 nucleotides that regulate gene expression through completely or partially base pairing with target mRNAs at the 3’-untranslated region, resulting in mRNA cleavage or translational suppression [11]. To date, emerging evidence has demonstrated that microRNAs play key roles in a broad range of physiologic and pathologic processes [12,13]. Although the biologic functions of microRNAs remain largely unclear, previous studies have indicated that microRNAs may participate in tumorigenesis, functioning as tumor suppressors and/or oncogenes [14–18], and affect the etiology, diagnosis, and prognosis of various cancers [17,19–25]. Furthermore, microRNAs may be involved in regulation of the immune and inflammatory response systems [26,27], which may control HPV clearance and escape from immune surveillance. Therefore, inherited genetic alterations of microRNAs may affect susceptibility to HPV-associated OSCC.

Previous studies have demonstrated that hsa-146a was over-expressed in SCCHN cell lines and tumor tissues and hsa-miR-149 was down-regulated in squamous cell carcinoma of the tongue and hsa-miR-149 polymorphism may also affect the miRNA expression [21,28,29]. Recent studies have demonstrated that single-nucleotide polymorphisms (SNPs) of miR146 G>C, miR196 C>T, and miR499 T>C may affect cancer risk and prognosis [30–33]. In one of our previous case-control studies, we explored the association between the four common SNPs (rs14146 vs2910164, rs14149 rs2292832, miR196 rs11614913, and miR499 vs3746444) and risk of head and neck cancers, but no significant association between each SNP and cancer risk was found [33]. Given the roles of microRNAs in regulating the immune and inflammatory response systems and thereby mediating HPV infection, we hypothesized that genetic variants in these four microRNAs alter the association between HPV16 seropositivity and risk of OSCC. To test this hypothesis, we evaluated the joint effect of HPV16 serology and these four common microRNA SNPs on risk of OSCC.

Materials and Methods

Study subjects

Patients with incident OSCC were recruited as part of an ongoing molecular epidemiology study of head and neck cancers through the Head and Neck Center at The University of Texas MD Anderson Cancer Center between December 1996 and October 2002. All patients were recruited without restrictions on age, sex, cancer stage, or histology and had newly diagnosed, histopathologically confirmed, untreated OSCC. Excluded from participation were patients with second primary tumors; primary tumors of the sinonasal tract, nasopharynx, hypopharynx, or larynx; primary tumors outside the upper aerodigestive tract; cervical metastases of unknown origin; and histopathologic diagnoses other than SCC. In addition, patients who had received a blood transfusion in the past 6 months or who were receiving immunosuppressive therapy were excluded. Among patients initially contacted for participation, approximately 95% of eligible incident cases agreed to participate in the study.

A pool of cancer-free subjects was recruited from the Kelsey-Seybold Foundation, a multispecialty physician practice with multiple clinics throughout the Houston metropolitan area, and from healthy visitors who accompanied cancer patients to outpatient clinics at MD Anderson Cancer Center but were genetically unrelated to the cases. In this pool of cancer-free controls, each individual was first surveyed by means of a short questionnaire to determine his or her willingness to participate in research studies. Each eligible subject was subsequently interviewed to collect demographic and epidemiologic information, such as age, sex, ethnicity, smoking status, and alcohol consumption status. The overall response rate for the controls was approximately 78%. Excluded from participation were individuals with history of any cancer, history of immunosuppressive therapy, or blood transfusion in the past 6 months.

Smoking status was categorized as “ever smokers” (those who had smoked more than 100 cigarettes in their lifetime) and “never smokers” (those who had smoked equal to or fewer than 100 cigarettes in their lifetime). Drinking status was categorized as “ever drinkers” (those who had drunk alcoholic beverages at least once a week for more than 1 year) and “never drinkers” (those who never had such a pattern of drinking). Both Institutional Review Boards of UT MD Anderson Cancer Center and Kelsey-Seybold approved the study, and written informed consent was obtained from all study subjects. In this study, 335 cancer-free controls were selected from the pool of potential controls by frequency matching with the patients by age (±5 years), sex, and smoking and drinking status. To avoid confounding factors due to ethnic characteristics, all study subjects included were non-Hispanic whites, who accounted for the vast majority of the cases.

HPV16 serologic detection

HPV16 L1 virus-like particles generated from recombinant baculovirus-infected insect cells were used to test for antibody against HPV16 L1 capsid protein in the plasma of study participants by using a standard enzyme-linked immunosorbent assay, as described previously [34–36]. About 10% of the samples were randomly chosen for the repeated assay, and the results were 100% concordant with results of initial testing.

MicroRNA genotyping

From each blood sample, a leukocyte pellet was obtained from the buffy coat by centrifugation of 3 mL of whole blood. The pellet was used for genomic DNA extraction with the DNA Blood Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer’s instructions. A polymerase chain reaction-restriction fragment-length polymorphism assay was used to amplify the fragments that contained SNPs of miR146 vs2910164, miR149 rs2292832, miR196 rs11614913, and miR499 vs3746444 and risk of head and neck cancers, but no significant association between each SNP and cancer risk was found [33]. The distributions of selected demographic variables, tobacco smoking, alcohol consumption, microRNA genotypes, and HPV16 serology between cases and controls were evaluated using the chi-square test. To evaluate the association of HPV16 serologic status and microRNA genotypes with OSCC risk, odds ratios (ORs) and 95% confidence interval (CIs) were calculated using both univariate and multivariate logistic regression analyses. Logistic regression was also used to assess the potential interaction effects by evaluating departures from the models of additive and multiplicative interactions between selected variables. A more-than-additive interaction was suggested when $OR_{11}^{12}>OR_{12}^{11}$ or $OR_{10}^{11}OR_{01}^{12}-1$, for which $OR_{11}=OR$ when both factors were present, $OR_{10}=OR$ when only factor 1 was present and $OR_{01}=OR$ when only factor 2 was present. A more-than-multiplicative interaction was suggested when $OR_{11}^{12} OR_{10}^{11}$.
OR_{CT} \times OR_{TT}

We assessed the interaction by reporting the P values from the Wald test for testing the coefficients (β_{interaction}) for different from 0, where the interaction term consisted of the product of the two variables: microRNA polymorphism and HPV16 seropositivity. We also assessed the joint effects of HPV16 serology and microRNA genotypes on OSCC risk, and the joint effects were further stratified by tumor site and smoking status. Statistical Analysis System software (Version 9.2; SAS Institute, Cary, NC) was used for all statistical analyses. All tests were two-sided, and P<0.05 was considered the cutoff for statistical significance.

Results

Demographics and risk factors for study subjects

The final analysis included 325 OSCC cases and 335 controls. Of the 325 cases, 188 (57.8%) had SCCOP, and 137 (42.2%) had SCC of the oral cavity. The distributions of demographic characteristics and known OSCC risk factors are summarized in Table 2. As a result of frequency matching, there was no significant difference between patients and controls in distributions of age, sex, smoking status, and alcohol use (all P>0.05). However, we observed that HPV16 seropositivity was significantly more common in patients than in controls (P<0.001). HPV16 seropositivity was associated with risk for OSCC (OR, 3.2; 95% CI, 2.1–4.8), particularly for SCCOP (OR, 5.4; 95% CI, 3.7–8.9), but not for oral cavity cancers (OR, 0.8; 95% CI, 0.38–1.48), after adjusting for age, sex, smoking status, and drinking status.

Association of microRNA variants with risk of OSCC

The genotype distributions of the four microRNA SNPs among controls were all in agreement with the Hardy-Weinberg equilibrium (P=0.535 for miR146 rs2910164, P=0.988 for miR149 rs2292832, P=0.783 for miR196 rs11614913, and P=0.489 for miR499 rs3746444). Overall, we did not find any statistically significant association of any of the four microRNA SNPs with the risk of OSCC, but we did find that miR146 rs2910164 and miR499 rs3746444 had a minor effect on the risk of SCCOP (Table 3): After adjustment for age, sex, smoking status, alcohol use, and HPV16 serology, individuals with the combined miR146 rs2910164 CG and CC genotypes had a higher risk of SCCOP than individuals with the GG genotype (adjusted OR, 1.9; 95% CI, 1.3–3.0), and individuals with the miR499 rs3746444 combined CT and CC genotypes had a higher risk of SCCOP than individuals with the TT genotype (adjusted OR, 1.6; 95% CI, 1.0–2.4).

Association of microRNA variants with risk of HPV16-associated OSCC

As shown in Table 4, the association between HPV16 serology and OSCC risk was modified by microRNA genetic variants. Specifically, compared with individuals with both miR146 rs2910164 GG genotype and HPV16 seronegativity, those with both GG genotype and HPV16 seropositivity had an increased risk of OSCC (OR, 3.0; 95% CI, 1.8–5.0), and the risk was even higher among those with both CG or CC genotype and HPV16 seropositivity (OR, 4.7; 95% CI, 2.3–9.4). In contrast, compared with individuals with both miR149 rs2292832 CT or TT genotype and HPV16 seronegativity, those with both CT or TT genotype and HPV16 seropositivity had an increased risk of OSCC (OR, 2.9; 95% CI, 1.7–5.0), and the risk was even higher among those with both CC genotype and HPV16 seropositivity (OR, 3.6; 95% CI, 1.9–6.6). Similar results were observed for the associations between miR196 rs11614913 and miR499 rs3746444 SNPs and risk of HPV16-associated OSCC (Table 4). Considering that the difference in the tumor HPV status between patients with SCCOP and oral cavity cancers might be attributed to different etiologies at the two different anatomic sites, we further investigated the modifying effect of each SNP on the association between HPV16 seropositivity and risk of SCCOP and oral cavity cancers (Table 4). We found that the modifying effects of microRNA variants on the risk associated with HPV16 seropositivity were pronounced for SCCOP but not for SCC of the oral cavity. Moreover, the modification effect may suggest an additive interaction. However, when we further performed tests for interaction between HPV16 seropositivity and microRNA variants for risk of OSCC as shown in Table 5, we found that the interaction between HPV16 seropositivity and each of these microRNA variants on the risk of OSCC was not statistically significant (all P values>0.05).

Stratified analyses of joint effects of HPV seropositivity and microRNA variants on OSCC risk by smoking status

In order to explore whether the effects of microRNA SNPs on the risk of HPV16-associated OSCC were confounded by smoking status, we performed stratified analyses of joint effects of HPV16 seropositivity and microRNA variants on risk of OSCC by smoking status (Table 6). Overall, we observed that for each SNP, the risk of HPV16-associated OSCC was much stronger in never smokers than in ever smokers and such effect modification was particularly pronounced in SCCOP.

Table 1. Genotyping Assays of MicroRNA SNPs.

| MicroRNA | SNP (Base Change) | Primers Used (Sense/Antisense) | PCR Product | Enzyme | Digested Products |
|----------|-------------------|--------------------------------|-------------|--------|------------------|
| miR146   | rs2910164 G>C     | 5'-AGAGGGGCCCTTTCCACACCATC-3' | 113 bp      | PstI   | G allele: 113 bp C allele: 89 bp/24 bp |
| miR149   | rs2292832 C>T     | 5'-AGTCACAGGCACACAACCTCGT-3'  | 337 bp      | PvuII  | C allele: 337 bp T allele: 220 bp/117 bp |
| miR196   | rs11614913 C>T    | 5'-CAATCTGACATGCGGTTAGTG-3'   | 93 bp       | Msel   | T allele: 93 bp C allele: 70 bp/23 bp |
| miR499   | rs3746444 C>T     | 5'-AGCGGAGGAAGAGACGACAGTGG-3' | 52 bp       | BspGI  | C allele: 52 bp T allele: 27 bp/25 bp |

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Specifically, compared with individuals with both miR146 rs2910164 GG genotype and HPV16 seronegativity, those with both CT or TT genotype and HPV16 seropositivity had an increased risk of OSCC (OR, 2.9; 95% CI, 1.7–5.0), and the risk was even higher among those with both CC genotype and HPV16 seropositivity (OR, 3.6; 95% CI, 1.9–6.6). Similar results were observed for the associations between miR196 rs11614913 and miR499 rs3746444 SNPs and risk of HPV16-associated OSCC (Table 4). Considering that the difference in the tumor HPV status between patients with SCCOP and oral cavity cancers might be attributed to different etiologies at the two different anatomic sites, we further investigated the modifying effect of each SNP on the association between HPV16 seropositivity and risk of SCCOP and oral cavity cancers (Table 4). We found that the modifying effects of microRNA variants on the risk associated with HPV16 seropositivity were pronounced for SCCOP but not for SCC of the oral cavity. Moreover, the modification effect may suggest an additive interaction. However, when we further performed tests for interaction between HPV16 seropositivity and microRNA variants for risk of OSCC as shown in Table 5, we found that the interaction between HPV16 seropositivity and each of these microRNA variants on the risk of OSCC was not statistically significant (all P values>0.05).
in this study, overall, we did not observe a significant main effect of each SNP of these microRNAs on risk of OSCC, but we did observe a significant association between miR146 rs2910164 and miR499 rs3746444 and a moderately increased risk of SCCOP. However, we found that the joint effect of HPV16 seropositivity and each of these microRNA SNPs increased the risk of OSCC, although we did not observe any significant interaction for such joint effect on risk of OSCC. Moreover, such effect modification was more pronounced for SCCOP as opposed to SCC of oral cavity and in never smokers than in ever smokers. Our results are in agreement with the characteristics of SCCOP associated with HPV infection, indicating that microRNA SNPs might play a role in the development of HPV16-associated SCCOP.

### Table 2. Distribution of Demographic and Risk Factors in OSCC Patients and Controls.

| Characteristic | Cases (n = 325) | Controls* (n = 335) | Overall OSCC | SCCOP | SCC of Oral Cavity |
|---------------|----------------|---------------------|--------------|-------|-----------------|
| Age, years    |                |                     |              |       |                 |
| <40           | 27             | 21                  | 17           | 14    | 10.2            |
| 41–55         | 105            | 126                 | 86           | 45.8  | 29.2            |
| 56–70         | 154            | 119                 | 64           | 34.0  | 55.0            |
| >70           | 49             | 49                  | 21           | 11.2  | 28.0            |
| Sex           |                |                     |              |       |                 |
| Male          | 269            | 241                 | 155          | 82.4  | 86.2            |
| Female        | 66             | 84                  | 33           | 17.6  | 14.8            |
| Tobacco smoking|               |                     |              |       |                 |
| Ever          | 239            | 227                 | 125          | 66.5  | 102             |
| Never         | 96             | 98                  | 63           | 33.5  | 37.2            |
| Alcohol drinking |           |                     |              |       |                 |
| Ever          | 240            | 250                 | 150          | 79.8  | 100             |
| Never         | 95             | 75                  | 38           | 20.2  | 37.0            |
| HPV16 serology* |           |                     |              |       |                 |
| Positive      | 42             | 100                 | 87           | 46.3  | 13.0            |
| Negative      | 293            | 225                 | 101          | 53.7  | 90.5            |

*Controls were selected to be frequency matched to cases on the factors shown in the table.

Table 3. Association of MicroRNA SNPs with OSCC Risk.

| Genotype | Cases (n = 325) | Controls (n = 335) | p | Adjusted OR (95% CI)† |
|----------|----------------|-------------------|---|---------------------|
| miR146 rs2910164 |            |                   | 0.299 |                     |
| GG (Ref.)  | 184           | 203               | 1.0 | 1.0                 |
| CG+CC      | 141           | 132               | 1.3 (0.9–1.8) | 1.9 (1.3–3.0) | 1.0 (0.6–1.4) |

| miR149 rs2292832 |            |                   | 0.947 |                     |
| CC (Ref.)  | 158           | 162               | 1.0 | 1.0                 |
| CT+TT      | 167           | 173               | 0.9 (0.7–1.3) | 1.0 (0.7–1.5) | 0.9 (0.6–1.3) |

| miR196 rs111614913 |            |                   | 0.871 |                     |
| CC (Ref.)  | 95            | 96                | 1.0 | 1.0                 |
| CT+TT      | 230           | 239               | 1.0 (0.7–1.4) | 1.0 (0.7–1.6) | 1.0 (0.6–1.5) |

| miR499 rs3746444 |            |                   | 0.060 |                     |
| TT (Ref.)  | 184           | 214               | 1.0 | 1.0                 |
| CT+CC      | 141           | 121               | 1.3 (0.9–1.9) | 1.6 (1.0–2.4) | 1.1 (0.8–1.7) |

*Genotype distributions for miR146 rs2910164, miR149 rs2292832, miR196 rs111614913, and miR499 rs3746444 between patients and controls.

†ORs were adjusted for age, sex, smoking, alcohol drinking, and HPV16 serology.

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MicroRNAs act as omnipresent regulators of gene expression and are involved in many cellular processes, including inflammation and immune responses. Recent studies have demonstrated that microRNAs participate in mediating inflammatory and cytokine signaling as well as innate and acquired immune response to viral infection by targeting critical elements in inflammatory signaling pathways [37,38]. MicroRNAs also modify viral-host interactions, which play central roles in the development and progression of infection-associated tumors.

**Table 4. Joint Effect of HPV16 Seropositivity and MicroRNA Genotypes on OSCC Risk.**

| HPV16 Status | Genotype | Cases (n = 325) | Controls (n = 335) | Adjusted OR (95% CI) * |
|--------------|----------|-----------------|-------------------|------------------------|
|              |          | No. | %  | No. | %  | Overall OSCC | SCCOP | SCC of Oral Cavity |
| mIR146 rs2910164 | − | GG (Ref.) | 120 | 36.9 | 173 | 51.6 | 1.0 | 1.0 | 1.0 |
|              | − | CG+CC | 105 | 32.3 | 120 | 35.8 | 1.2 (0.9–1.8) | 1.0 (0.5–1.3) | 1.7 (1.1–2.7) |
|              | + | GG | 64 | 19.7 | 30 | 9.0 | 3.0 (1.8–5.0) | 5.0 (2.9–5.0) | 0.5 (0.2–1.4) |
|              | + | CG+CC | 36 | 11.1 | 12 | 3.6 | 4.7 (2.3–9.4) | 6.3 (2.9–13.3) | 2.4 (0.9–6.4) |
| mIR149 rs2292832 | − | CT+TT (Ref.) | 114 | 35.1 | 148 | 44.2 | 1.0 | 1.0 | 1.0 |
|              | − | CC | 111 | 34.1 | 145 | 43.3 | 1.0 (0.7–1.4) | 1.0 (0.6–1.5) | 1.1 (0.7–1.6) |
|              | + | CT+TT | 53 | 16.3 | 25 | 7.4 | 2.9 (1.7–5.0) | 5.2 (2.9–9.4) | 0.6 (0.2–1.6) |
|              | + | CC | 47 | 14.5 | 17 | 5.1 | 3.6 (1.9–6.6) | 6.0 (3.1–11.6) | 1.0 (0.4–2.5) |
| mIR196 rs11614913 | − | CC (Ref.) | 64 | 19.7 | 82 | 24.5 | 1.0 | 1.0 | 1.0 |
|              | − | CT+TT | 161 | 49.5 | 211 | 63.0 | 1.0 (0.6–1.4) | 1.0 (0.6–1.7) | 0.9 (0.6–1.4) |
|              | + | CC | 31 | 9.6 | 14 | 4.2 | 2.8 (1.4–5.8) | 5.7 (2.6–12.5) | 0.5 (0.1–1.8) |
|              | + | CT+TT | 69 | 21.2 | 28 | 8.3 | 3.2 (1.8–5.6) | 6.0 (3.1–11.3) | 0.8 (0.4–1.9) |
| mIR499 rs3746444 | − | TT (Ref.) | 124 | 38.1 | 186 | 55.5 | 1.0 | 1.0 | 1.0 |
|              | − | CT+CC | 101 | 31.1 | 107 | 31.9 | 1.4 (1.0–2.0) | 1.1 (0.7–1.8) | 1.6 (1.0–2.5) |
|              | + | TT | 60 | 18.5 | 28 | 8.4 | 3.4 (2.0–5.6) | 5.5 (3.2–9.5) | 0.9 (0.4–2.2) |
|              | + | CT+CC | 40 | 12.3 | 14 | 4.2 | 4.1 (2.1–8.0) | 6.9 (3.4–13.7) | 1.0 (0.3–2.8) |

*ORs were adjusted for age, sex, smoking, and alcohol drinking.

**Table 5. Modifying effect of microRNA variants on association between HPV16 Seropositivity and OSCC risk.**

| Genotype | HPV16+(n = 142) | HPV16−(n = 518) | Adjusted OR (95% CI) * | Pint. |
|----------|-----------------|-----------------|------------------------|-------|
|          | Case | Control | Case | Control |                        |
| mIR146 rs2910164 | 64 | 30 | 120 | 173 | 3.0 (1.8–5.0) |
|          | 36 | 12 | 105 | 120 | 3.9 (1.9–8.2) |
| mIR149 rs2292832 | 47 | 17 | 111 | 145 | 2.9 (1.4–6.1) |
|          | 53 | 25 | 114 | 148 | 3.3 (2.0–5.4) |
| mIR196 rs11614913 | 31 | 14 | 64 | 82 | 3.7 (2.0–6.8) |
|          | 69 | 28 | 161 | 211 | 3.2 (1.8–5.6) |
| mIR499 rs3746444 | 31 | 14 | 64 | 82 | 3.7 (2.0–6.8) |
|          | 40 | 14 | 101 | 107 | 2.8 (1.4–5.6) |

*ORs were adjusted for age, sex, smoking, and alcohol drinking.
Pint.: P values for interaction.

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Table 6. Joint Effect of HPV16 Seropositivity and MicroRNA Genotypes on OSCC Risk Stratified by Smoking Status.

| HPV16 Status | miRNA genotypes | Never Smokers | Ever Smokers | Adjusted OR, (95% CI) * Overall OSCC | Never Smokers | Ever Smokers | Adjusted OR, (95% CI) * SCCOP |
|--------------|-----------------|---------------|--------------|---------------------------------------|---------------|--------------|-------------------------------|
|              | Cases/Controls  | Cases/Controls| Nevever Smokers | Ever Smokers | Nevever Smokers | Ever Smokers | Nevever Smokers | Ever Smokers |
|              | n=98/96        | n=227/239     | n=98/96      | n=227/239 | n=98/96      | n=227/239 | n=98/96      | n=227/239 |
| miR146 rs2910164 |                 |               |               |                        |               |               |                 |               |
| -            | GG(Ref.)       | 32/48         | 88/125       | 1.0                    | 1.0           | 1.0          | 1.0             | 1.0           |
| -            | CG+CC          | 27/40         | 78/80        | 1.0 (0.5–1.9)          | 1.3 (0.9–2.0) | 1.00(2.0–1.3) | 1.1(0.6–1.9) |
| +            | GG             | 28/6          | 36/24        | 8.5 (3.1–23.7)         | 2.0 (1.1–3.7) | 12.3(4.1–37.0) | 3.9(2.1–7.4) |
| +            | CG+CC          | 11/2          | 25/10        | 11.5 (2.3–57.3)        | 4.0 (1.8–9.0) | 17.0(3.2–88.5) | 5.0(2.1–11.9) |
| miR149 rs2292832 | (n=98/96)   | (n=227/239)   | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) |
| -            | CT+TT(Ref.)   | 35/36         | 79/112       | 1.0                    | 1.0           | 1.0          | 1.0             | 1.0           |
| -            | CC             | 24/52         | 87/93        | 0.5 (0.3–1.1)          | 1.4 (0.9–2.1) | 1.00(1.0–10.0) | 1.4(0.8–2.5) |
| +            | CT+TT          | 18/6          | 35/19        | 4.2 (1.4–12.5)         | 2.8 (1.5–5.3) | 5.7(1.7–18.9) | 5.6(2.8–11.1) |
| +            | CC             | 21/2          | 26/15        | 14.0 (3.0–66.8)        | 2.5 (1.2–5.1) | 24.5(4.8–124.5)| 4.0(1.8–8.8) |
| miR196 rs11614913 | (n=98/96)    | (n=227/239)   | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) |
| -            | CC(Ref.)      | 13/25         | 51/57        | 1.0                    | 1.0           | 1.0          | 1.0             | 1.0           |
| -            | CT+TT         | 46/63         | 115/148      | 1.5 (0.6–3.3)          | 0.8 (0.5–1.3) | 3.1(0.8–12.0) | 0.8(0.5–1.5) |
| +            | CC             | 9/4           | 22/10        | 5.7 (1.4–23.7)         | 2.5 (1.1–5.8) | 20.4(3.4–121.3)| 4.6(1.8–11.6) |
| +            | CT+TT         | 30/4          | 39/24        | 19.5 (5.3–71.2)        | 1.8 (1.0–3.5) | 73.8(13.5–402.0)| 3.1(1.5–6.4) |
| miR499 rs3746444 | (n=98/96)   | (n=227/239)   | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) | (n=98/96)   | (n=227/239) |
| -            | TT (Ref.)     | 34/61         | 90/125       | 1.0                    | 1.0           | 1.0          | 1.0             | 1.0           |
| -            | CT+CC         | 25/27         | 76/80        | 1.6 (0.8–3.2)          | 1.3 (0.8–1.9) | 1.1(0.4–2.8) | 1.1(0.6–1.9) |
| +            | TT             | 23/6          | 37/22        | 9.4 (3.4–26.4)         | 2.5 (1.3–4.5) | 16.8(5.4–51.8) | 3.8(2.0–7.4) |
| +            | CT+CC         | 16/2          | 24/12        | 14.9 (3.1–70.9)        | 2.6 (1.2–5.5) | 22.1(4.4–112.0)| 5.0(2.2–11.1) |

*ORs were adjusted for age, sex, and alcohol drinking.

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Therefore, functional genetic polymorphisms of microRNAs may lead to individual variations in immune function, inflammation, and apoptosis that modify viral immune escape, antiviral defense, and evasion of apoptosis, leading to modification of the risk of infection-associated cancers. Numerous studies have shown that some microRNAs are up-regulated, while other microRNAs are down-regulated in head and neck cancers [39–41]. Such increased or decreased expression of microRNAs may be associated with the development, progression, and prognosis of head and neck cancers [24,42].

Recently, several authors have reported associations between microRNA SNPs and the risk of head and neck cancers. One study indicated that the miR196 rs11614913 variant reduced the risk of head and neck cancers [32]. Another study indicated that miR146 rs2910164, miR149 rs2292832, and miR196 rs11614913 did not modify the risk of head and neck cancers independently of HPV infection but that miR199 rs3746444 moderately reduced the risk of head and neck cancers [33]. The conflicting results may be attributed to many factors, such as different anatomical tumor sites, lack of information about other confounders, and small sample sizes. For example, the previous studies, although larger than our study, had mixed tumor sites without stratification by HPV infection status, whereas in our current study, we assessed the joint effects of microRNA SNPs and HPV16 infection on OSCC risk. HPV16 infection has recently been identified as one of the primary etiologic factors for causes of SCCOP, but oral cavity and laryngeal cancer mainly result from tobacco and alcohol consumption. In our current study, although we had a smaller study size, we found that microRNA SNPs and HPV16 seropositivity may function jointly in the development of OSCC, particularly in patients with SCCOP and in never smokers.

Although the precise mechanism by which the common microRNA genetic variants and HPV16 infection jointly play a role in the development of OSCC has not been fully clarified, a joint effect of microRNAs and HPV16 infection on susceptibility to OSCC is biologically plausible. HPV16 is oncogenic: it encodes viral oncoproteins E6 and E7, which inhibit p53 and Rb cell cycle tumor suppressors. MicroRNA variants might functionally affect expression of genes involved in myriad cellular processes, including inflammation and immune response pathways, subsequently controlling the host’s ability to clear HPV and HPV’s ability to escape immune surveillance [37]. Therefore, our data suggest that HPV and microRNA variants might act jointly in the development of OSCC. However, further studies are required to validate the hypothesized functionality of these SNPs.

The present study, our analysis stratified by tumor site showed that the effect modification was more pronounced for SCCOP than for oral cavity SCC; reinforcing the concept that there are differences in the etiology of cancers of the oropharynx and oral cavity. Additionally, the analysis further stratified by smoking status for each SNP showed that the joint effect of microRNA SNPs and HPV seropositivity on the risk of OSCC was much stronger in never smokers than in ever smokers. These data further support the notion that risk genotypes of the four common SNPs of microRNAs may be involved in the development of HPV-associated OSCC in non-Hispanic white never smokers. However, the modifying effect of each of microRNA SNPs on risk of OSCC associated with HPV16 was not statistically significant. This lack of significance could be either because there was no such interaction...
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