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Telomere Length in Peripheral Blood Lymphocytes Contributes to the Development of HPV-Associated Oropharyngeal Carcinoma

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Abstract

Sexual transmission of human papillomavirus (HPV), particularly HPV16, has been associated with an increasing incidence of oropharyngeal squamous cell carcinoma (OPC). Telomere shortening results in chromosomal instability, subsequently leading to cancer development. Given that HPV16 can affect telomerase activity and telomere length, we conjectured that telomere length in peripheral blood lymphocytes (PBL) might affect the risk of HPV16-associated OPC and tumor HPV16 status in patients. Telomere length in PBLs and HPV16 serologic status were measured in peripheral blood samples in 188 patients with OPC, 137 patients with oral cavity cancer (OCC) and 335 controls of non-Hispanic Whites. Tumor HPV status was determined in 349 OPC cases. ORs and 95% confidence intervals were calculated in univariate and multivariable logistic regression models. Overall, as compared with the long telomere length, short telomere length was significantly associated with a moderately increased risk of OPC but not with increased risk of OCC. When we stratified the data by HPV16 serologic status, using long telomere length and HPV16 seronegativity as the reference group, we found that the risk associated with HPV16 seropositivity was higher among patients with OPC with short telomere length. Notably, such risk was particularly pronounced in never smokers, never drinkers, and those more than 50 years of age. Furthermore, short telomere length was also associated significantly with tumor HPV-positive OPC. Together, our findings suggest that telomere length in PBLs may be associated with higher risk of HPV16-associated OPC and tumor HPV16 status, particularly in certain patient subgroups. Larger studies are needed to validate these findings. *Cancer Res; 73(19); 5996–6003. ©2013 AACR.*

Introduction

Head and neck squamous cell carcinoma, which arises from the mucosa of the upper aerodigestive tract, is the sixth most common cancer worldwide (1). In 2013, approximately 41,380 new oral cavity and pharyngeal cancer cases will be diagnosed and an estimated 7,890 deaths will occur from these cancers in the United States (2). It is well known that tobacco and alcohol use are the principal causes of head and neck cancers and that human papillomavirus (HPV) infection plays an important role in the development of oropharyngeal squamous cell carcinoma (OPC; refs. 1, 3, 4).

Strong evidence from molecular and epidemiologic studies suggests an association between high-risk HPV and OPC (3, 5). Among the known HPV types, high-risk HPV16 is the most common, accounting for approximately 90% or more of HPV-positive OPC cases (5). It is well known that HPV causes human cancers by expressing E6 and E7 oncogenic proteins (6, 7). HPV E6 binds to p53 and inhibits its activity, resulting in reduced protein function and loss of cell-cycle control (6). HPV E6/E7 can affect TERT expression, telomerase activity, and telomere length, subsequently leading to cellular immortalization and cancer development (8–11).

Telomeres consist of several thousand nucleotide repeats (TTAGGG in humans)*n* and a protein complex at the ends of chromosomes, and they maintain genomic stability by protecting chromosomes from degradation, end-to-end fusion, and atypical recombination (12). Human telomeres are approximately 10 to 15 kb in somatic cells and progressively shorten with each cell division (13, 14). Telomere length is a sign of biologic age, and age-dependent shortening of telomeres in human cells impairs cellular function and viability (15). Cells with both very short and very long telomeres exist and may promote carcinogenesis (16, 17). Indeed, several studies have shown an association between telomere length in peripheral blood lymphocytes (PBL) and cancer risk (16–19). For example, telomere shortening has been associated with risk of cancers of the head and neck, lung, skin, bladder, ovaries, and pancreas (16, 20–24), whereas longer telomeres have been associated

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with melanoma and breast cancer (17, 25). Thus, telomere length in PBLs may serve as a common marker of cancer risk.

To date, few studies have investigated the association between the telomere length in PBLs and HPV in cancer risk, particularly, in OPC. In the present study, we hypothesized that telomere length in PBLs, in combination with past HPV16 infection, increases the risk of OPC. To test this hypothesis, we measured telomere length in PBLs by using quantitative real-time PCR (qRT-PCR) and evaluated the effects of telomere length in PBLs on risk of HPV16-associated OPC and on tumor HPV16 status.

Materials and Methods

Study subjects

Details of this study population were previously described elsewhere (26). Briefly, all patients with histopathologically confirmed squamous cell carcinoma of the head and neck were recruited consecutively through the Head and Neck Center Clinics at The University of Texas MD Anderson Cancer Center (Houston, TX) between April 1996 and June 2002. The response rate of eligible patients who signed an informed consent form for participating in the study was approximately 95%. Excluded patients included those with second primary tumors, primary tumors of the sinonasal tract or nasopharynx, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, and histopathologic diagnoses of tumors other than squamous cell carcinoma; also excluded were patients who had received blood transfusions within the last 6 months or who were receiving immunosuppressive therapy. For the purposes of this study, we included those patients with cancers of HPV-related sites (OPC; $N = 188$) and those with cancers at HPV-unrelated sites (oral cavity cancer; OCC; $N = 137$).

Controls were recruited from a pool of cancer-free subjects who included members of the Kelsey-Seybold Foundation (Houston, TX; a multispecialty physician practice with multiple clinics throughout the Houston metropolitan area) and from healthy visitors who accompanied patients with cancer to outpatient clinics at MD Anderson Cancer Center but were genetically unrelated to the patients with cancer. In this cancer-free control pool, each individual was asked to complete a short questionnaire to determine his or her willingness to participate in the study and was then interviewed. Each eligible subject provided demographic and epidemiologic information including age, sex, ethnicity, smoking history, and alcohol consumption. Exclusion criteria for the control group included having had cancer previously, having received blood transfusions within the last 6 months, and receiving immunosuppressive therapy. The overall proportion of responders was approximately 78%. Finally, 335 cancer-free control individuals were selected from the pool of potential controls by frequency matching to the cases on the basis of age (±5 years), sex, ethnicity, and smoking and alcohol-drinking status.

HPV16 testing

For serologic testing, HPV16 L1 virus-like particles were generated from recombinant baculovirus-infected insect cells to test for antibody against the HPV16 L1 capsid protein in the plasma of study participants; this was done with use of a standard ELISA as described previously (27). Two groups of control sera, one known to be positive and one known to be negative were also tested in parallel with the study samples in duplicate on each plate. For tumor testing, paraffin-embedded tissues were detected for the presence of HPV16 DNA by using PCR-based, type-specific assays for the E6 and E7 regions, as previously reported (28). Assays of the samples were run with positive and negative controls (Siha and TPC-1 cell lines, respectively), and β-actin was used as a DNA quality control.

Measurement of telomere length in PBLs

Relative mean telomere length in PBLs was measured by SYBR Green qRT-PCR measurement of the ratio of telomere repeat units (TEL) to a single-copy gene (CON), as described previously (18, 29). In brief, each sample was amplified for telomeric DNA and for human β2-globulin (HBG), a single-copy control gene that provides an internal control to normalize the starting amount of DNA, by using an Applied Biosystems 7900HT thermocycler in a 384-well format. The telomere reaction mixture contained 5 ng of genomic DNA, 2× SYBR Green Master Mix, 200 nmol/L Tel-1 primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT), and 200 nmol/L tel-2 primer (TCCCGACTATCCCTATCCCTTCCCTATCCCTATCCCTA), and the PCR reaction ran for one cycle at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and then 56°C for 1 minute. The HBG reaction mixture consisted of 2× SYBR Green Master Mix, 200 nmol/L HBG-1 primer (GCTTCTGACACAACTGTGTTCACTAGC), and 200 nmol/L HBG-2 primer (CACCAACTTCATCCACGTTCACC). The HBG PCR reaction ran for one cycle at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and then 58°C for 1 minute. All samples for both the telomere and HBG gene reactions were carried out in duplicate.

For each assay, the fractional PCR cycle at which each reaction crossed a predefined fluorescence threshold was determined (C_t value). The amount of the starting template was expected to be proportional to 2^{-C_t}. To correct for variation in genomic DNA concentration, the CON C_t value was subtracted from the TEL C_t value (ΔC_t). The relative "telomere copy number" per genome for each sample should then be proportional to $2^{-\Delta C_t}$. Each plate contained randomly selected samples to have equal representation of cases and controls. The laboratory personnel were blinded to case and control status. In each run, negative (water) and positive controls, a calibrator DNA, and a standard curve were included. The positive controls contained a telomere of 1.2 kb and a telomere of 3.9 kb from a commercial telomere length assay kit (Roche Applied Science). For the standard curve, one reference DNA sample (the same DNA sample from a healthy control for all runs) was diluted by using a 2-fold serial dilution to produce a 6-point standard curve between 20 and 0.625 ng of DNA in each reaction. The R^2 correlation for each standard curve was ≥0.99, with acceptable SDs set at 0.25 for the threshold cycle (C_t) values. If the result was found to be out of the acceptable range, then the sample

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was repeated. To test the interassay variation, two samples with relatively long and short telomere lengths were tested using three different runs, with an interassay variation less than 3.6%. The $2^{-\Delta C_t}$ method was used for calculation of relative values of telomere length in PBLs, and a standard curve was created in each PCR run to monitor PCR efficiency (30, 31).

Statistical analysis

The differences in the smoking and alcohol consumption demographic variables as well as the differences in the HPV16 serologic status between cases and controls were evaluated by using the χ^2 test. Telomere lengths in PBLs were analyzed as categorical variables by setting a cutoff point at the median values among all study subjects including the cases and the controls. The associations of telomere lengths in PBLs with cancer risk were estimated by computing the ORs and their 95% confidence intervals (CI) from both univariate and multivariate logistic regression models. Logistic regression was also used to assess the potential interaction effects by evaluating departures from the models of interactions between selected variables. We assessed the interaction by reporting the P values from the Wald test for testing the coefficients ($\beta_{\text{TL, HPV16 seropositivity}}$) were different from 0, where the interaction term consisted of the product of the two variables: telomere length in PBLs and HPV16 seropositivity. Those subjects who had smoked more than 100 cigarettes in their lifetime were defined as ever smokers; those who had quit smoking for more than 1 year previously were considered as former smokers; and the rest were considered current smokers. Subjects who drank alcoholic beverages at least once a week for more than 1 year in previous years were defined as ever drinkers; of these, those who had quit drinking for more than 1 year previously were defined as former drinkers; and the others were considered current drinkers. We also evaluated the joint effects of HPV16 serology and telomere lengths in PBLs on cancer-risk stratified by tumor site (OPC vs. OCC) and subsequently among OPC cases by smoking and drinking status. Logistic regression analysis was also used to assess potential interactions by evaluating departures from the model of multiplicative interaction between selected variables. All tests were two-sided, and P value less than 0.05 were considered significant. All statistical analyses were conducted with SAS software (version 9.1.3; SAS Institute, Inc.).

Results

Demographic and risk factors for study subjects

The demographic characteristics and risk factors of this study population are summarized in Table 1. The cases and controls seemed to be adequately frequency-matched for age, sex, smoking status, and alcohol use ($P = 0.100$ for age; $P = 0.100$ for sex; $P = 0.673$ for smoking status; and $P = 0.121$ for alcohol use). As expected, cases were much more likely than controls to be HPV16 seropositive ($P < 0.0001$), but HPV

Table 1. Frequency distribution of demographic and risk factors in patients and controls

Characteristics	Patients ($n = 325$) No. (%)	Controls[a] ($n = 335$) No. (%)	P[b]
Age, y			0.100
<40	31 (9.5)	27 (8.1)	
41–55	126 (38.8)	105 (31.3)	
56–70	119 (36.6)	154 (46.0)	
>70	49 (15.1)	49 (14.6)	
Sex			0.100
Male	241 (74.2)	269 (80.3)	
Female	84 (25.8)	66 (19.7)	
Tobacco smoking			0.673
Ever	227 (69.8)	239 (71.3)	
Never	98 (30.2)	96 (28.7)	
Alcohol drinking			
Ever	250 (76.9)	240 (71.6)	0.121
Never	75 (23.1)	95 (28.4)	
HPV16 serostatus			
Positive	100 (30.8)	42 (12.5)	0.000
Negative	225 (69.2)	293 (87.5)	
Tumor site			
Oropharynx	188 (57.8)		
Oral cavity	137 (42.2)		

[a]The controls were selected by frequency matching to the patients on the factors shown in this table.
[b]Two-sided χ^2 test.

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Table 2. Association between telomere length in PBLs and risk of OPC and OCC

Telomere length in PBLs	Controls (%)	OPC Cases (%)	OPC OR (95% CI)[a]	OCC Cases (%)	OCC OR (95% CI)[a]
All subjects	335 (100.0)	188 (100.0)		137 (100.0)	
By median					
Long	207 (61.8)	93 (49.5)	1.0	81 (59.1)	1.0
Short	128 (38.2)	95 (50.5)	1.7 (1.1–2.6)	56 (40.9)	0.8 (0.5–1.2)

[a]Adjusted by age, sex, smoking, and drinking status.

seropositivity was associated only with OPC (46.3% seropositive; OR, 6.1; 95% CI, 3.9–9.6 after adjustment for age, sex, smoking, alcohol use, and telomere length in PBLs) and not with OCC (only 9.5% of OCC cases were seropositive; similar to our controls, which were 12.5% seropositive).

Associations between telomere length in PBLs and risk of OPC and OCC

The distribution of telomere length in PBLs and the associations between telomere length in PBLs and the risk of OPC and OCC are shown in Table 2. We found that compared with long telomere length, short telomere length was significantly associated with increased risk of OPC (adjusted OR, 1.7; 95% CI, 1.1–2.6). In contrast, no association was observed between telomere length in PBLs and risk of OCC (adjusted OR, 0.8; 95% CI, 0.5–1.2; Table 2).

Joint effects of telomere length in PBLs and HPV16 seropositivity on OPC and OCC risk

As summarized in Table 3, we combined HPV16 seropositivity and telomere length in PBLs to estimate their joint effects on cancer risk. Compared with the group of long telomere length and HPV16 seronegativity, the group of long telomere length and HPV16 seropositivity had increased OPC risk (adjusted OR, 3.4; 95% CI, 1.9–6.0), and the group of short telomere length and HPV16 seropositivity had the highest OR of 18.4 (95% CI, 8.4–40.3). We found no evidence of a combined effect of HPV seropositivity and short telomere length on risk of OCC. When we further conducted tests for interaction between HPV16 seropositivity and telomere length in PBLs for risk of OPC, we found that the interaction between HPV16 seropositivity and telomere length in PBLs on the risk of OPC was statistically significant ($P_{\text{int.}} = 0.002$).

Stratification analysis of the joint effects of telomere length in PBLs and HPV16 seropositivity on OPC risk

Because we found that the association of telomere length in PBLs with risk of HPV16-associated cancer was evident only for OPC as opposed to OCC, we stratified the associations between telomere length in PBLs and HPV16 serology on risk of OPC by smoking, drinking status, and age (Table 4). We observed that the joint effects of telomere length in PBLs and HPV seropositivity on risk of OPC were much stronger in never smokers than in ever smokers, in never drinkers than in ever drinkers, and in younger subjects (ages $\leq$50 years, median age of controls) than in older subjects and the interaction between HPV16 seropositivity and telomere length in PBLs on the risk of OPC was statistically significant in some subgroups ($P_{\text{int.}} = 0.002$ for never smokers; $P_{\text{int.}} = 0.015$ for never drinkers; and $P_{\text{int.}} = 0.011$ for younger subjects, respectively). However, these results warrant future investigation with larger sample size and appropriate statistical power.

Association of telomere length in PBLs with OPC tumor HPV16 status

To further confirm the association of telomere length in PBLs with HPV16-associated OPC, we assessed the association of telomere length in PBLs with patients with HPV16-positive OPC by tumor HPV16 status. We determined the tumor HPV16

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Table 3. Joint effects of HPV16 seropositivity and telomere length in PBLs on risk of OPC and OCC

HPV16 serology	Telomere length in PBLs	Control $n = 335$ (%)	OPC ($n = 188$) Patients (%)	OPC OR[a] (95% CI)	$P_{\text{int.}}$[b]	OCC ($n = 137$) Patients (%)	OCC OR[a] (95% CI)	$P_{\text{int.}}$[b]
−	Long (Ref.)	174 (51.9)	58 (30.8)	1.0	0.002	74 (54.0)	1.0	0.110
−	Short	119 (35.5)	43 (22.9)	1.1 (0.7–1.9)		50 (36.5)	0.7 (0.4–1.1)	
+	Long	33 (9.9)	35 (18.6)	3.4 (1.9–6.0)		7 (5.1)	0.5 (0.2–1.2)	
+	Short	9 (2.7)	52 (27.7)	18.4 (8.4–40.3)		6 (4.4)	1.3 (0.4–4.1)	

[a]ORs were adjusted for age, sex, and tobacco smoking or alcohol drinking in logistic regression model.
[b]$P_{\text{int.}}$, Interaction between telomere length in PBLs and HPV serology.

Table 4. Joint effects of HPV16 serologic status and telomere length in PBLs on risk of OPC stratified by smoking, drinking status, and age

HPV16 serology	Telomere length in PBLs	Never smokers Patients (n = 64)	Never smokers Controls (n = 96)	Ever smokers Patients (n = 124)	Ever smokers Controls (n = 239)	Adjusted OR (95% CI)[a] Never smokers	$P_{int.}$[b]	Adjusted OR (95% CI)[a] Ever smokers	$P_{int.}$[b]
							0.002		0.496
−	Long (Ref.)	17	61	41	113	1.0		1.0	
−	Short	4	27	39	92	0.4 (0.1–1.5)		1.3 (0.7–2.1)	
+	Long	14	7	21	26	6.9 (2.3–20.6)		2.4 (1.2–4.7)	
+	Short	29	1	23	8	120.4 (14.7–987.1)		7.8 (3.2–18.9)	

HPV16 serology	Telomere length in PBLs	Never drinkers Patients (n = 38)	Never drinkers Controls (n = 95)	Ever drinkers Patients (n = 150)	Ever drinkers Controls (n = 240)	Adjusted OR (95% CI)[a] Never drinkers	$P_{int.}$[b]	Adjusted OR (95% CI)[a] Ever drinkers	$P_{int.}$[b]
							0.015		0.088
−	Long (Ref.)	10	52	48	122	1.0		1.0	
−	Short	8	34	35	85	1.5 (0.5–4.9)		1.1 (0.6–1.9)	
+	Long	7	7	28	26	5.7 (1.6–20.8)		2.8 (1.5–5.2)	
+	Short	13	2	39	7	41.8 (7.6–228.4)		13.2 (5.5–31.8)	

HPV16 serology	Telomere length in PBLs	Age ≤50 y Patients (n = 66)	Age ≤50 y Controls (n = 94)	Age >50 y Patients (n = 122)	Age >50 y Controls (n = 241)	Adjusted OR (95% CI)[a] Age ≤50 yr	$P_{int.}$[b]	Adjusted OR (95% CI)[a] Age >50 yr	$P_{int.}$[b]
							0.011		0.140
−	Long (Ref.)	14	65	44	109	1.0		1.0	
−	Short	14	19	29	100	2.7 (1.0–7.2)		0.8 (0.4–1.4)	
+	Long	13	9	22	24	6.9 (2.3–20.6)		2.6 (1.3–5.2)	
+	Short	25	1	27	8	118.2 (14.2–985.8)		9.1 (3.7–22.2)	

[a]ORs were adjusted for age, sex, and tobacco smoking or alcohol drinking in logistic regression model.
[b]$P_{int.}$, Interaction between telomere length in PBLs and HPV serology.

status of 349 patients with OPC whose specimens were available for such testing. We found that short telomere length was significantly associated with tumor HPV16 status. The patients with short telomere length were approximately 2.5 times more likely to have tumor HPV16-positive (OR, 2.4; 95% CI, 1.4–4.2; Table 5).

Discussion

This is the first study to evaluate the association between telomere length in PBLs and risk of HPV16-associated OPC as well as tumor HPV16 status. In this study, we found that short telomere length was significantly associated with only a moderately increased risk of OPC but no increased risk of OCC. In addition, we found that short telomere length may synergize with HPV16 seropositivity to increase the risk of OPC and that such an association was particularly pronounced in subgroups of never smokers, never drinkers, and younger patients. Furthermore, we observed that short telomere length was associated with HPV16 tumor positivity. These findings from the current study are consistent with the characteristics of OPC known to be caused by HPV infection, suggesting that telomere length in PBLs may play a role in the development of HPV16-associated OPC.

Telomeres play an important role in chromosome stability (32). Telomere length is the result of the balance between telomere shortening and lengthening processes, and either very short or very long telomere length may contribute to cancer development if the balance is broken (33). Therefore, telomere length has been recognized as one of the most common tumor markers in the past decade. Recently, although several studies have reported an association between longer telomere length in PBLs' DNA and the risk of several types of cancer (17, 25), most studies have reported that shorter telomere length in PBL-DNA is associated with increased risk of human cancers (16, 20–24). Others have reported a null association between telomere length in PBLs and risk of cancers (19, 29, 30). Reasons for these inconsistent results may be partially explained by differences in study designs, biologic sample collection and processing,

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Table 5. Association of telomere length in PBLs with tumor HPV16 status in patients with OPC

Telomere length in PBLs	HPV(−) OPC cases ($n = 61$) No. (%)	HPV(+) OPC cases ($n = 278$) No. (%)	Adjusted OR, 95% CI[a]
Long	43 (60.6)	105 (37.8)	1.0
Short	28 (39.4)	173 (62.2)	2.4 (1.4–4.2)

[a]ORs were adjusted for age, sex, and tobacco smoking or alcohol drinking in logistic regression model.

and study populations; relatively small sample sizes; and various laboratory techniques for measuring telomere length. To date, only two published studies on head and neck cancers have reported an association between telomere length in PBLs and risk of head and neck cancers (16, 30). Wu and colleagues (16) observed that telomere shortening was significantly associated with head and neck cancers in a Caucasian population of 92 head and neck cancer cases and 92 controls; although our previously published study found no statistically significant association between telomere length in PBLs and risk of head and neck cancers in a non-Hispanic White population of 885 cases of squamous cell carcinoma of the head and neck and 885 cancer-free controls (30). In the current study, we found that telomere length in PBLs was significantly associated with risk of HPV16-associated OPC. The significant association between long telomere length and risk of HPV16-associated OPC was observed and such an association was even higher in several subgroups of never smokers, never drinkers, and younger patients. When the analysis was restricted to those with long telomere length only, HPV16 seropositivity was associated with an increased risk of OPC among such a group (adjusted OR, 3.2; 95% CI, 1.8–5.8). However, the risk of OPC associated with HPV16 seropositivity was higher among those with short telomere length than among those with long telomere length.

HPV infection has recently been implicated as the most important risk factor for the development of OPC (5); however, it is unknown whether there is interaction between HPV infection and telomere length in PBLs in the development of OPC. Previous studies reported that increased telomerase activity was observed in the presence of HPV in genital condylomata acuminate and that enhanced expression of telomerase activity occurs early during carcinogenesis (34, 35). Recent studies have shown that HPV16 E6/E7 can directly induce *TERT* expression and telomerase activity (8–11). For example, HPV E7 was found to contribute to telomerase activity of immortalized and tumorigenic cells and enhanced E6-induced *TERT* promoter function (8), whereas siRNA knockdown of either E6 or E7 (or both) in HPV-immortalized cells or an HPV-positive cancer cell line reduced *TERT* transcription and telomerase activity (8). In the current study, we observed that telomere length in PBLs was significantly associated with increased risk of OPC (HPV-related), but not OCC (HPV-unrelated), indicating a possible interactive effect between telomere length in PBLs and HPV16 serologic status on the risk of OPC. Indeed, we also observed a significant association between short telomere length and tumor HPV16-positive OPC and detected a significant interaction between telomere length in PBLs and HPV16 serologic status on the risk of OPC.

In the present study, we did find that the associations of short telomere length and HPV16 seropositivity with risk of OPC were even greater in never smokers than in ever smokers and in never drinkers than in ever drinkers. Such results are consistent with other published studies, in which OPC were driven by HPV; and HPV-associated patients with OPC are more likely to be never smokers and never drinkers (36–38). Our observation of more pronounced effects in younger subjects than in older subjects might be partly explained by the increased prevalence of oral HPV16 in young adults, perhaps resulting from changing sexual behaviors or from susceptible groups developing cancer at a younger age (39, 40). Therefore, the results in the current study suggest that smoking or drinking status might also need to be taken into account when assessing the association of telomere length in PBLs with risk associated with HPV16 seropositivity. To investigate and validate the significance and the extent of such associations, further well-designed studies with larger sample sizes are needed.

Although we found that telomere length in PBLs combined with HPV16 serologic status may significantly affect the risk of OPC, there were several limitations in the present study. First, the HPV16 serology tests can only indicate whether the study subjects had previous HPV16 exposure; they cannot specify the anatomic location or time point of viral exposure. In addition, some individuals do not remain seropositive after HPV exposure, and possible false-negative HPV16 cases may result in misclassification of HPV16 status. Second, in current study, the absence of telomere length in tumors did not allow us to evaluate its potential influence on risk of HPV-associated OPC. Although using telomere length in PBLs allows for the inclusion of a cancer-free control group for this case–control study design, the telomere length in PBLs might not reflect actual telomere length in tumors, leading to some misclassification for biased estimates of the association. However, a significant association between short telomere length and tumor HPV16 status in patients with OPC from the current case study did support our findings from the current case–control study, specifically, that short telomere length may synergize with HPV16 seropositivity to increase the risk of

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OPC. Another limitation in the present study was its limited sample size, which meant that our findings could have occurred by chance. Thus, we will closely monitor the role of telomere length in HPV-associated OPC in our future studies when a much larger patient cohort with tumor specimens becomes available.

In conclusion, our results suggest that telomere length in PBLs might synergize with HPV16 infection to increase risk of OPC, and such a risk was particularly high in never smokers, never drinkers, and younger subjects. Furthermore, our findings are consistent with the characteristics of OPC caused primarily by HPV, suggesting that telomere length in PBLs may be a biomarker for risk of HPV16-associated OPC. Large studies are needed to validate our findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Zhang, J. Wen, Q. Wei, G. Li, Z. Liu
Development of methodology: Y. Zhang, E.M. Sturgis, J. Wen, Q. Wei, G. Li, Z. Liu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Zhang, E.M. Sturgis, J. Wen, Q. Wei, G. Li, Z. Liu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Zhang, E.M. Sturgis, J. Wen, H. Liu, Q. Wei, G. Li, Z. Liu
Writing, review, and/or revision of the manuscript: Y. Zhang, E.M. Sturgis, K.R. Dahlstrom, J. Wen, Q. Wei, G. Li, Z. Liu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.M. Sturgis, J. Wen, Q. Wei, G. Li, Z. Liu
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