HHS Public Access

Author manuscript

Oral Oncol. Author manuscript; available in PMC 2020 April 01.

Published in final edited form as:

Oral Oncol. 2019 April ; 91: 92–96. doi:10.1016/j.oraloncology.2019.03.001.

Human papillomavirus DNA detection, pl6^{INK4a}, and oral cavity cancer in a U.S. population

Brenda Y. Hernandez[a,*], **Charles F. Lynch**[b], **Owen T.M. Chan**[a], **Marc T. Goodman**[c], **Elizabeth R. Unger**[d], **Martin Steinau**[d], **Trevor D. Thompson**[e], **Maura Gillison**[f], **Christopher Lyu**[g], **Mona Saraiya**[e], and **The HPV Typing of Cancer Workgroup**

[a]University of Hawaii Cancer Center, University of Hawaii, Honolulu, HI, USA

[b]Department of Epidemiology, College of Public Health, The University of Iowa, Iowa City, IA, USA

[c]Cedars-Sinai Medical Center, Los Angeles, CA, USA

[d]Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

[e]Division of Cancer Prevention and Control, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

[f]The University of Texas MD Anderson Cancer Center, Houston, TX, USA

[g]Rho Federal Systems Division, Inc, Chapel Hill, NC, USA

Abstract

Objectives: The role of HPV in oral cavity cancers was investigated using two markers of viral exposure.

Materials and methods: HPV DNA and pl6^{INK4a} expression were evaluated in tumor tissue from a U.S. population-based sample of 122 invasive oral cavity cancer cases.

Results: HPV DNA was detected in 38 of 122 (31%) oral cavity tumors. Seven genotypes were detected including HPV 16, which was found in 22% of tumors. pl6^{INK4a} was expressed in 30% of tumors and was poorly correlated with HPV DNA detection (Kappa < 0.1). Joint positivity for HPV 16 and/or 18 and pl6^{INK4a} was observed in only 7% of cases. When comparing cases diagnosed in 1993–1999 and in 2000–2004, positivity for HPV DNA 16/18 increased from 19% to 39% ($p = 0.02$) and joint HPV 16/18 - pl6^{INK4a} positivity increased from 0% to 12% ($p = 0.01$). For gingival tumors, HPV 16 and/or 18 positivity was 67% compared to 11–38% for other sites ($p = 0.02$); joint HPV 16/18 - pl6^{INK4a} positivity was 33% compared to 0–8% for other sites ($p = 0.01$). The association of HPV with gingival tumors and more recent diagnosis period remained after adjustment for age and stage ($p < 0.05$). Neither HPV DNA nor pl6^{INK4a} were associated with overall survival.

*Corresponding author at: University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, HI, USA., brenda@cc.hawaii.edu (B.Y. Hernandez).

Conflicts of interest

None declared.

Conclusions: Based on both HPV DNA and pl6^{1NK4a}, HPV is etiologically linked to a limited subset of oral cavity cancers. However, the role of HPV in oral cavity cancer may vary widely by subsite and may have increased over time, similar to trends observed for oropharyngeal cancer.

Keywords

HPV; Human papillomavirus; Oral cavity; Oral cancer; P16^{INK4a}

Introduction

Cancers of the oral cavity comprise a range of tumor subsites including the tongue, gingiva, floor of the mouth, and palate. In the U.S., tobacco and alcohol are the primary risk factors for oral cavity cancers [1,2] and reductions in cigarette smoking may have contributed to declining incidence [3]. An exception is oral tongue cancer for which rising rates have been observed over the past several decades, particularly among younger adults [4]. Oral cavity cancers are etiologically distinct from oropharyngeal cancers, which include the lingual and palatine tonsils and the base of the tongue. In addition to tobacco and alcohol, oropharyngeal cancers are causally linked to human papillomavirus (HPV) [5]. HPV DNA is detected in up to 70% of oropharyngeal cancers in the U.S. [6], and there is evidence that the attributable proportion linked to HPV has increased over time. [7]. HPV is well characterized in oropharyngeal cancer as a major risk factor as well as a predictor of survival [8,9]. In HPV-induced carcinogenesis, the viral E7 oncoprotein binds and inactivates the host's retinoblastoma tumor suppressor gene product, pRb, a negative regulator of pl6^{INK4a}, resulting in overexpression of pl6^{INK4a} [10]. In oropharyngeal cancer, positivity for oncogenic HPV DNA combined with expression of pl6^{INK4a} is indicative of clinically relevant infection [11]. In contrast with oropharyngeal cancers, the role of HPV in the pathogenesis of oral cavity cancers is less well established. We conducted a population-based, cancer registry study to evaluate HPV in invasive oral cavity cancers diagnosed in the U.S. using two markers of viral exposure, HPV DNA and pl6^{1NK4}.

Materials and methods

This study was approved by the Centers for Disease Control and Prevention (CDC) and the Institutional Review Boards of the University of Hawaii and the University of Iowa. This evaluation was part of a larger study by the CDC partnering with seven U.S. population-based cancer registries to examine the distribution of HPV genotypes in anogenital and head and neck cancers diagnosed in the U.S. prior to the implementation of the prophylactic HPV vaccine [6]. The present study was confined to two cancer registries, the Hawaii Tumor Registry and the Iowa Cancer Registry for which additional archival specimens were available for assessment. Both registries are part of the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End-Results (SEER) program and operate affiliated Residual Tissue Repositories (RTR) [12] comprised of collections of de-identified, formalin-fixed, paraffin-embedded (FFPE) malignant tumor tissue specimens annotated with de-identified, high-quality demographic, clinical, pathologic, and survival data.

Invasive oral cavity cancer was defined based on the International Classification of Diseases for Oncology Version 3 [13]. Oral cavity cancer cases were selected from patients diagnosed in 1993–2004 in Hawaii and Iowa with histologically-confirmed malignancies of the oral tongue (including dorsal, ventral, and anterior 2/3 surfaces and border/ tip) (ICD-O-3 C020-C023); gingiva (upper and lower) (C030-C031); floor of the mouth (including anterior and lateral regions) (C040-C041; C049); hard palate (C050); cheek mucosa (C060); vestibule of the mouth (C061); retromolar area (C062); and overlapping and unspecified parts of mouth (C068). Oropharyngeal subsites were excluded including the base of tongue, tonsils, soft palate, and uvula.

HPV genotyping was conducted at the CDC laboratories. FFPE tumor tissue specimens were prepared following a uniform protocol to avoid cross-contamination. A representative tumor block from each case was selected and sectioned using a new disposable blade for each sample. The first and last tissue sections were stained with hematoxylin and eosin (H&E) and reviewed by a study pathologist (E.R.U.) to confirm the presence of malignancy. DNA was isolated from intervening FFPE tumor sections using high temperature assisted tissue lysis [14] and automated DNA purification with a Chemagic MSM1 (PerkinElmer, Waltham, MA, USA), HPV testing was performed as previously described [15]. Briefly, all samples were tested using the Linear Array HPV Genotyping Test (LA, Roche Diagnostics, Indianapolis, IN), a PCR-based assay targeting a 450-bp consensus region of the L1 HPV genome and detecting 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, IS39). Amplification of the human β-globin gene was included as an internal control. Samples testing negative for β-glohin or HPV in the Roche assay were subsequently tested using the INNO-LiPA HPV Genotyping Assay (LiPA, Innogenetics, Gent, Belgium), a PCR-based assay targeting a 65 base pair region of the HPV L1 gene. INNO-LiPA detects 26 of the same types covered in the Roche assay in addition to 3 additional types (HPV 43, 44, 74). This second assay was included to account for the fixation-related degradation of DNA extracted from FFPE tissue. Specimens testing negative for HPV and the internal control in both assays were considered inadequate for evaluation.

Immunohistochemical evaluation of $p16^{INK4a}$ was conducted at the University of Hawaii Cancer Center Pathology Core laboratory using tumor tissue obtained from the same tumor specimen used for HPV genotyping. A $p16^{INK4a}$ mouse monoclonal antibody (clone JC8; Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:400) was used according to the manufacturer's specifications. A study pathologist (O.C.), who was blinded to the HPV status of cases, evaluated $p16^{INK4a}$ based on staining intensity (mild/weak, moderate, strong), intracellular localization (nuclear, cytoplasmic), and the proportion of tumor cells stained. Specimens exhibiting moderate to strong staining of the nucleus and cytoplasm in ≥ 70% of tumor cells were considered definitively positive for $p16^{INK4a}$ based on established criteria [16,17].

A subset of oral cavity cancer cases testing positive for HPV DNA were evaluated for genotype-specific HPV E6/E7 mRNA via real-time quantitative PCR in the laboratory of Dr. Maura Gillison at Ohio State University according to previously described methods [18]. Briefly, purified tumor RNA was reverse transcribed to cDNA and evaluated for HPV E6/E7

mRNA. All samples were tested for HPV 16 mRNA and individually tested for other carcinogenic types based on the corresponding genotypes detected (HPV 18, HPV 39, HPV 51, HPV 52, and HPV 66). The human sapiens ribosomal protein large P0 gene (RPLP0) was used as an internal control.

Statistical analyses were conducted using SAS version 9.4. Overall HPV prevalence was based on the detection of one or more HPV genotypes in tumor tissue. Multiple genotypes detected in a case were not counted more than once in overall prevalence estimates. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were classified as carcinogenic, or high-risk genotypes [19,20]. All other genotypes were considered to be non-carcinogenic, or of low or undetermined risk. Single year age, which was suppressed as part of de-identification for over half of cases, was imputed to the mid-point of 5-year age groups. Chi-square and Kappa statistics were used for group comparisons. Univariate and multivariate unconditional logistic regression were used to evaluate the association of case characteristics with HPV status. Overall survival, which was based on the period from date of cancer diagnosis to date of death or date of last follow-up, was evaluated using the Kaplan-Meier method, the Log-rank test, and Cox proportional hazards regression. All tests were two-sided and a p-value < 0.05 was considered as statistically significant.

Results

Study population

Tumor tissue specimens from 168 cases of invasive oral cavity cancers cases were initially available for the study. Of these cases, 21 were excluded due to absence of tumor in the tissue specimen (n = 14) or sample insufficiency (i.e., negative PCR result for both HPV and the internal control) (n = 7). Of the remaining 147 cases for which HPV DNA results were available, tissue was available to evaluate $p16^{INK4a}$ for 122 cases, which constituted the study population for the present evaluation.

Men and women comprised 49% and 51%, respectively, of oral cancer patients (Table 1). Fifty-nine percent were age 60 years and older at diagnosis. Cases included whites (55%), Asians (31%), and other groups (14%). Sixty-two percent were diagnosed in 2000–2004. Tumors of the oral tongue comprised 48% of cases, floor of the mouth 21%, gingiva 10%, hard palate 5%, and unspecified or overlapping sites 16%. Other oral subsites including tumors of the cheek mucosa, vestibule of the mouth, and retromolar area were not represented in the study population. Sixty-three percent of cases were of moderately differentiated grade. Keratinizing squamous cell carcinoma (SCC) was the most common histological subtype comprising 80% of tumors. Early stage (localized) tumors constituted 56% of cases.

HPV DNA

HPV DNA was detected in 31% (38/122) of oral cavity tumors. Seven different genotypes were detected including HPV 16 which was detected in 22% (27/122) of cases. Other carcinogenic genotypes included HPV 18, HPV 39, HPV 51, HPV 52, and HPV 66. HPV 6

was the sole non-carcinogenic type detected. Thirty-five of the 38 HPV-positive tumors were positive for a single genotype; 3 cases were positive for multiple genotypes.

HPV DNA positivity did not vary by sex, age, race, tumor grade, histology, or stage. Differences by HPV DNA status were observed by year of diagnosis and subsite (Table 1). HPV DNA was detected in tumors of 19% (9/47) of patients diagnosed in 1993–1999 compared to 39% (29/75) of cases diagnosed in 2000–2004 ($p = 0.02$). The highest HPV DNA prevalence was in the gingiva (67%, 8/12) followed by the floor of the mouth (38%, 10/26), oral tongue (29%, 17/59), hard palate (17%, 1/6), and overiapping/unspecified sites (11%, 2/19) ($p = 0.02$).

P16^{INK4a}expression

Overall, pl6^{INK4a} was expressed in 30% (36/122) of tumors characterized by moderate or strong staining of the cytoplasm and nucleus in ≥ 70% of tumor cells (Fig. 1). pl6^{INK4a} expression was observed in 24% (9/38) of HPV DNA positive tumors and 32% (27/84) of HPV DNA negative tumors ($p = 0.34$). pl6^{INK4a} expression was poorly correlated with overall HPV DNA and HPV 16 and/or 18 (Kappa < 0.1). Positivity for both pl6^{INK4a} and HPV DNA was observed in 7% (9/122) of cases. Of the 9 cases positive for both pl6^{INK4a} and HPV DNA, 8 were solely positive for HPV 16 and 1 was positive for HPV 18.

Joint HPV16/18 and pl6^{INK4a} status significantly varied by oral cavity subsite. Positivity for both markers was observed in 33% (4/12) of tumors of the gingiva compared to 0% (0/6)-8% (2/26) for other sites ($p = 0.01$) (Fig. 2). HPV 16/18 and pl6^{INK4a} status also varied by year of diagnoses: None of the 47 cases diagnosed in 1993–1999 were positive for both markers compared to 12% (9/75) of cases diagnosed in 2000–2004 ($p = 0.01$).

HPV E6/E7 mRNA

HPV 16 E6/E7 mRNA was evaluated in 106 of the 122 samples. All were negative for HPV 16 E6/E7 mRNA. The 11 cases positive for HPV 18, HPV 39, HPV 51, HPV 52, and/or HPV 66 were individually tested for E6/E7 mRNA of the corresponding genotypes. All samples were negative. The housekeeping gene, RPLP0, was low in all 106 samples which may be indicative of low levels of RNA. As the RNA quality from these archival FFPE tumor samples may have reduced the ability to detect viral E6/E7 mRNA, these results were not considered in the determination of HPV status.

Subgroup comparisons

Tumor subsite differences were further evaluated (data not shown). Patients with gingiva tumors were older (ages ≥ 60: 92%, 11/12) compared to those with tumors of other oral sites (ages ≥ 60: 55%, 61/ 110) ($p = 0.02$). Cancers of the gingiva were comprised of a greater proportion of advanced tumors (92%, 11/12) compared to other oral cavity subsites (39%, 40/104, respectively) ($p < 0.001$). The distribution of subsites did not significantly differ by sex, race, calendar time, tumor grade, or histology. When HPV 16/18 and pl6^{INK4a} were examined in multivariate logistic regression models that included tumor subsite, age, and stage, the associations of gingival tumors and age with HPV markers remained significant ($p < 0.05$) (data not shown). There was no significant difference in the proportion of oral tumor

specimens requiring testing with the LiPA assay for cases diagnosed before 2000 (30%, 14/67) and those diagnosed in 2000–2004 (38%, 29/75) ($p = 0.32$), suggesting that the observed differences in HPV detection by time period were not attributable to possible degradation of nucleic acid in older specimens.

Overall survival

Overall survival of oral cavity cancer cases did not vary by HPV DNA positivity (any HPV DNA, HPV 16 DNA, HPV 16/18), $p16^{INK4a}$ expression, or joint positivity for both markers (Log-rank p-value > 0.05) (data not shown). None of these markers were associated with overall survival in multivariate models adjusting for age, stage, tumor subsite, and diagnosis year (data not shown).

Discussion

Our study of oral cavity cancers from two U.S. registry-based populations demonstrates wide variability in the estimates of the etiologic role of HPV depending on the measures utilized. The strongest evidence for the etiologic role of HPV was restricted to only 7% of cases based on positivity for HPV DNA 16 and/or 18 accompanied by elevated expression of $pl6^{INK4a}$. In contrast, based on detection of either HPV DNA 16 and/or 18 or $p16^{INK4a}$ alone, our findings suggests the potential for HPV involvement in nearly a third of cases. Our findings are in general agreement with prevalence estimates from international pooled studies which range from 3.5% for joint HPV DNA - $pl6^{INK4a}$ [21] to 24% and 28% for HPV DNA and $pl6^{INK4a}$, respectively [22,18].

HPV DNA and $pl6^{INK4a}$ expression were poorly correlated in oral cavity tumors. PCR-based detection of viral DNA and $pl6^{INK4a}$ expression in tumor tissue each has limitations as measures of viral exposure. HPV DNA measured through PCR has high sensitivity for the presence of viral DNA but cannot distinguish between HPV acting oncogenically within tumor and productive HPV in adjacent benign tissue [23]. Conversely, $pl6^{INK4a}$ is a non-specific measure of viral exposure. E7 inactivation of pRb results in overexpression of $pl6^{INK4a}$ in HPV carcinogenesis [10]. High expression of $pl6^{TNK4a}$ can also result from cellular alterations unrelated to infection [23]. In HPV carcinogenesis, $pl6^{INK4a}$ expression may be lost with increasing host genetic and epigenetic chromosomal instability [21], which may, in part, explain the poor correlation with HPV DNA in oral cavity tumors.

In contrast to oral cavity tumors, $pl6^{INK4a}$ and HPV DNA in oropharyngeal cancer are generally correlated [11]. However, as with oral cavity tumors, HPV DNA detection alone may also overestimate viral involvement in oropharyngeal cancers. In a prior evaluation of oropharyngeal cancers identified through seven U.S. cancer registries, including the two included in the present analysis, overall HPV DNA prevalence was 70% [24]. When combined with $pl6^{INK4a}$ or other markers such as HPV E6/E7 mRNA, the attributable fraction of HPV in oropharyngeal cancer is lower but still substantially higher than for oral cavity cancer [21,22]. HPV E6/E7 mRNA, which was negative for the subset of cases tested, was not considered in our final evaluation given the indications of low quality of RNA yielded from these archival FFPE samples. We observed no association of HPV status, including viral DNA and/or $pl6^{INK4a}$, with overall 5-year survival in oral cavity cancer. This

contrasts with oropharyngeal cancer for which tumor HPV is established as a predictor of favorable survival [8,9].

Our findings also provide evidence for a limited, albeit increasing, role of HPV in cancers of the oral cavity. The joint prevalence of HPV 16/18 and $p16^{INK4a}$ in oral tumors increased from null to twelve percent over the 11-year period of cancer diagnoses. The observed increase in HPV-associated oral cavity cancers over calendar time parallels trends for oropharyngeal cancers for which the increasing incidence in the U.S. appears to be largely driven by HPV. In a study which included tumor samples from two SEER registries included in the present analysis, Chaturvedi et al. [7] reported an increase in HPV-positive or opharyngeal cancers in the U.S. over time but no increase in HPV-negative tumors. The increase in HPV-associated oropharyngeal cancers in the U.S. over time is more pronounced in men than in women and may be attributed to increasing oral HPV exposure through sexual behaviors [25]. A limitation of the present analysis is the lack of information on tobacco and alcohol use, as well as sexual history. HPV-negative head and neck cancers are more closely associated with tobacco and alcohol use, while HPV-positive tumors are related to sexual exposures [26,27].

Although overall prevalence of joint positivity for HPV DNA 16/18 and $p16^{INK4a}$ was low, there was significant variation across subsites. Positivity for both markers was highest in the gingiva, in which 33% of tumors were jointly positive. Our findings are consistent with a metaanalysis reporting pooled HPV DNA prevalence estimates to be higher in the gingival tumors compared to the tumors of the tongue, floor of the mouth, and hard palate [22]. We observed that joint HPV 16/18 - $p16^{INK4a}$ positivity for other oral cavity tumors - including the oral tongue, hard palate, and floor of the mouth - was substantially lower than the gingiva and did not exceed 8%. Tumors of the oral tongue, the most common cancer of the oral cavity, have experienced increasing incidence in the U.S. for several decades, particularly among young whites and are not attributed to changes in exposure to HPV, alcohol, or tobacco [4] suggesting other yet unrecognized risk factors. The bacterial microbiome varies across subsites of the oral cavity. In particular, the gingiva, including the sub-gingiva and supra-gingiva, have been shown to have the most distinct bacterial composition within oral cavity subsites, including the buccal mucosa, hard palate, and tongue [28]. The particular susceptibility of the gingiva to bacterial infection is well-established, clinically manifesting as gingivitis and periodontal disease [29], Whether the gingiva is more susceptible to HPV infection is not known. Given the limited number of tumor samples for distinct subsites, including the gingiva, the confirmation of these findings in larger study populations is needed.

The strengths of our study, including a relatively large study population derived from two population-based cancer registries, are countered by a number of limitations including the use of retrospectively-collected, archival FFPE tissue. Variation in tissue preservation with sample age and fixation conditions may have influenced the detection of HPV DNA and $p16^{INK4a}$ via PCR and IHC, respectively.

We conclude that HPV is etiologically linked to a limited subset of oral cavity cancers overall, but its relative contribution may be substantial for certain subsites. Future research

focusing on specific subsites, including the gingiva, are needed, and prospectively collection of population-based samples would be ideal. Confirmation of the increasing role of HPV in oral carcinogenesis in the U.S. is critical for informing prevention strategies.

Acknowledgements

We thank all members of HPV Typing of Cancers Workgroup for contributions made toward this study.

Funding

This study was funded by the Centers for Disease Control and Prevention with secondary support from the National Cancer Institute, Surveillance, Epidemiology, and End Results (SEER) contracts # HHSN261201800011I (University of Hawaii) and # HHSN261201800012I (University of Iowa) and the National Cancer Institute, Cancer Center Support grants # 2 P30 CA071789 18 (University of Hawaii Cancer Center) and # 2 P30 CA086862 11 (University of Iowa).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

[1]. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988;48(11):3282–7. [PubMed: 3365707]

[2]. Day GL, Blot WJ, Austin DF, Bernstein L, Greenberg RS, Preston-Martin S, et al. Racial differences in risk of oral and pharyngeal cancer, alcohol, tobacco, and other determinants. J Natl Cancer Inst 1993;85(6):465–73. [PubMed: 8445674]

[3]. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008;26(4):612–9. 10.1200/JCO.2007.14.1713. [PubMed: 18235120]

[4]. Tota JE, Anderson WF, Coffey C, Califano J, Cozen W, Ferris RL, et al. Rising incidence of oral tongue cancer among white men and women in the United States, 1973–2012. Oral Oncol 2017;67:146–52. https://doi.Org/10.1016/j.oraloncology.2017.02.019. [PubMed: 28351569]

[5]. D'Souza G, Kreimer AR, Viscidi R. Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356(l9):1944–56, 10.1056/NEJMoa065497. [PubMed: 17494927]

[6]. Saraiya M, Unger ER, Thompson TD, Lynch CF, Hernandez BY, Lyu CW, et al. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. J Natl Cancer Inst 2015;107(6):djv08. 10.1093/jnci/djv08.

[7]. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29(32):4294–301. 10.1200/JCO.2011.36.4596. [PubMed: 21969503]

[8]. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363(l): 24–35. 10.1056/NEJMoa0912217. [PubMed: 20530316]

[9]. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100(4):261–9. 10.1093/jnci/djn011. [PubMed: 18270337]

[10]. Munger K, Gwin TK, McLaughlin-Drubin ME. pl6 in HPV-associated cancers. Oncotarget 2013;4(11):1864–5. 10.18632/oncotarget.l523. [PubMed: 24192550]

[11]. Rietbergen MM, Leemans CR, Bloemena E, Heideman DA, Braakhuis BJ, Hesselink AT, et al. Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. Int J Cancer 2013;132(7):1565–71. 10.1002/ijc.27821. [PubMed: 22949073]

[12]. Goodman MT, Hernandez BY, Hewitt S, Lynch CF, Cote TR, Frierson HF Jr, et al. Tissues from population-based cancer registries: a novel approach to increasing research potential. Hum Pathol 2005;36(7):812–20. S0046–8177(05)00161–9 [pii] 10.1016/j.humpath.2005.03.010. [PubMed: 16084952]

[13]. Fritz A, Percy C, Shanmugaratnam K, Sobin L, Parkin DM, Whelan, et al. The international classification of diseases for oncology 3rd ed. Geneva: World Health Organization; 2000.

[14]. Steinau M, Patel SS, Unger ER. Efficient DNA extraction for HPV genotyping in formalin-fixed, paraffin-embedded tissues. J Mol Diagn 2011;13(4):377–81. https://doi.Org/10.1016/j.jmoldx.2011.03.007. [PubMed: 21704270]

[15]. Gargano JW, Wilkinson EJ, Unger ER, Steinau M, Watson M, Huang Y, et al. Prevalence of human papillomavirus types in invasive vulvar cancers and vulvar intraepithelial neoplasia 3 in the United States before vaccine introduction. J Low Genit Tract Dis 2012;16(4):471–9. 10.1097/LGT.0b013e3182472947. [PubMed: 22652576]

[16]. El-Naggar AK, Westra WH. pl6 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: a guide for interpretative relevance and consistency. Head Neck 2012;34(4):459–61, 10.1002/hed.21974. [PubMed: 22180304]

[17]. Pemille Lassen, Hanne Primdahl, Jørgen Johansen, Kristensen Claus A, Andersen Elo, Andersen Lisbeth J, Evensen Jan F, Eriksen Jesper G, Overgaard Jens. Impact of HPV-associated pl6-expression on radiotherapy outcome in advanced oropharynx and non-oropharynx cancer. Radiother Oncol 2014; 113(3):310–6. 10.1016/j.radonc.2014.11.032. [PubMed: 25544647]

[18]. Taberna M, Resteghini C, Swanson B, Pickard RK, Jiang B, Xiao W, et al. Low etiologic fraction for human papillomavirus in larynx squamous cell carcinoma. Oral Oncol 2016;61:55–61. https://doi.Org/10.1016/j.oraloncology.2016.08.009. [PubMed: 27688105]

[19]. IARC. Human papillomaviruses IARC Monograph Eva] Cardnog Risks Hum 2007;90:1–636.

[20]. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24S3:S1–10,

[21]. Castellsague X, Alemany L, Quer M, Halec G, Quiros B, Tous S, et al. HPV involvement in head and neck cancers: comprehensive assessment of biomarkers in 3680 patients. J Natl Cancer Inst 2016;108(6):djv403. 10.1093/jnei/djv403.

[22]. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsague X, Laporte L, et al. HPV DNA, E6/E7 mRNA, and pl6INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol 2014;15(12):1319–31. 10.1016/S1470-2045(14)70471-1. [PubMed: 25439690]

[23]. Combes JD, Franceschi S. Role of human papillomavirus in non-oropharyngeal head and neck cancers. Oral Oncol 2014;50(5):370–9. 10.1016/j.oraloncology.2013.11.004. [PubMed: 24331868]

[24]. Steinau M, Saraiya M, Goodman MT, Peters ES, Watson M, Cleveland JL, et al. Human papillomavirus prevalence in oropharyngeal cancer before vaccine introduction, United States. Emerg Infect Dis 2014;20(5):822–8. 10.3201/eid2005.131311. [PubMed: 24751181]

[25]. Chaturvedi AK, Graubard Br, Broutian T, Pickard RK, Tong ZY, Xiao W, et al. NHANES 2009–2012 findings: association of sexual behaviors with higher prevalence of oral oncogenic human papillomavirus infections in U.S. Men. Cancer Res 2015;75(12):2468–77. 10.1158/0008-5472.CAN-14-2843. [PubMed: 25873485]

[26]. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008;100(6):407–20. doi: djn025 [pii] 10.1093/jnci/djn025. [PubMed: 18334711]

[27]. Applebaum KM, Fumiss CS, Zeka A, Posner MR, Smith JF, Bryan J, et al. Lack of association of alcohol and tobacco with HPVl6-assodated head and neck cancer. J Natl Cancer Inst 2007;99(23):1801–10. doi: djm233 [pii] 10.1093/jnci/djm233. [PubMed: 18042931]

[28]. Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D, et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol 2012;13(6):R42 10.1186/gb-2012-13-6-r42. [PubMed: 22698087]

[29]. Wade WG. The oral microbiome in health and disease. Pharmacol Res 2013;69(l):137–43. https://doi.Org/10.1016/j.phrs.2012.ll.006. [PubMed: 23201354]

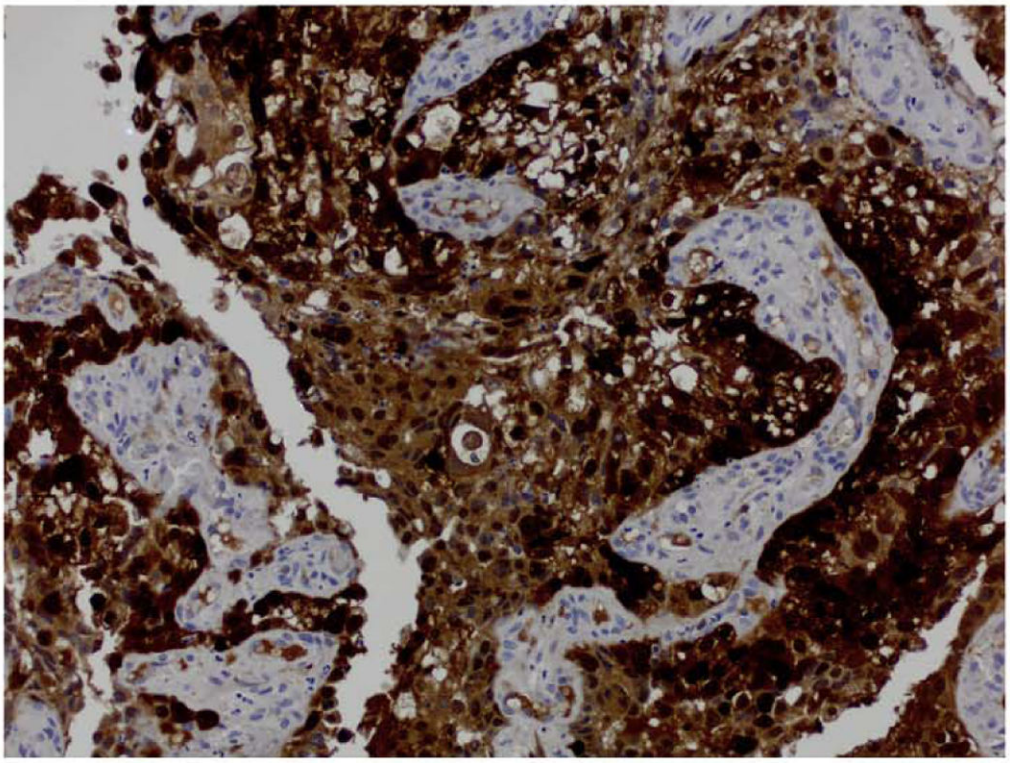

Fig. 1.

Strong expression of pl6 in a gingival tumor positive for HPV 18 (20X). Overall, $p16^{INK4a}$ was expressed in 30% (36/122) of oral cavity tumors characterized by moderate or strong staining of the cytoplasm and nucleus in ≥ 70% of tumor cells. $pl6^{INK4a}$ expression was poorly correlated with HPV DNA and HPV 16 and/or 18.

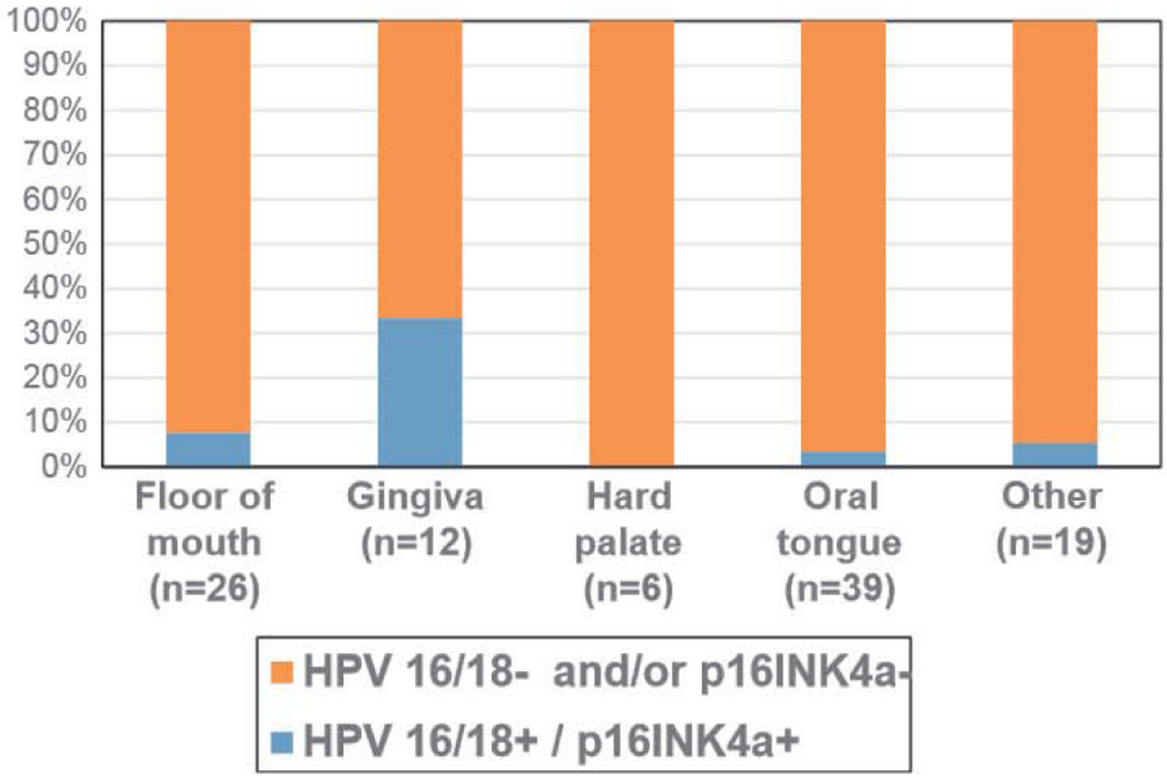

Fig. 2.

HPV 16/18 and pl6^{INK4a} by oral tumor subsite. Positivity for both HPV 16/18 and pl6^{1NK4a} was observed in 33% (4/12) of tumors of the gingiva compared to 0% (0/6)-8% (2/26) for other sites (p = 0.01).

Table 1

Characteristics of Oral Cavity Cancer Cases by HPV DNA[a] Status.

	All		HPV DNA+		HPV DNA−		
	No.	%[b]	No	%[c]	No	%[c]	p value
All	122	100	38	31	84	69	
Sex							
Male	60	49	19	32	41	68	0.90
Female	62	51	19	31	43	69	
Age at diagnosis (years)							
< 60	50	41	16	32	34	68	0.87
≥60	72	59	22	31	50	69	
Race							
Asian	38	31	15	40	23	61	0.40
White	67	55	18	27	49	73	
Other	17	14	5	29	12	71	
Diagnosis year							
1993–1999	47	38	9	19	38	81	0.02
2000–2004	75	62	29	39	46	61	
Subsite							
Floor of the mouth	26	21	10	38	16	62	0.02
Gingiva	12	10	8	67	4	33	
Hard palate	6	5	1	17	5	83	
Oral tongue	59	48	17	29	42	71	
Unspecified/overlapping sites	19	16	2	11	17	89	
Grade[d]							
Well-differentiated	23	20	5	22	18	78	0.43
Moderately differentiated	72	63	23	32	49	68	
Poorly or undifferentiated	20	17	8	40	12	60	
Histology							
SCC Keratinizing	97	80	30	31	67	69	0,39
SCC Non-keratinizing	5	4	2	40	3	60	
SCC, NOS	5	4	3	60	2	40	
Other	15	12	3	20	12	80	
SEER stage[d]							
Localized	65	56	20	31	45	69	0.97
Regional	40	34	12	30	28	70	
Distant	11	10	3	27	8	72	

[a] Includes carcinogenic types HPV 16 (n = 27), HPV 18 (n = 2), HPV 39 (n = 1), HPV 51 (n = 3), HPV 52 (n = 5), and HPV 66 (n = 1); and non-carcinogenic HPV 6 (n = 3). Three cases were positive for multiple genotypes.

[b] Percent total (column).

[c] Percent totals (rows).

[d] Missing data: grade (n = 7); stage (n = 6).