Research Article

Cancer Epidemiology, Biomarkers & Prevention

Cytokeratin 7 in Oropharyngeal Squamous Cell Carcinoma: A Junctional Biomarker for Human Papillomavirus–Related Tumors

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Abstract

Background: Human papillomavirus (HPV)–related oropharyngeal squamous cell carcinoma (SCC) represents a distinct subgroup of head and neck tumors. We analyze the expression of cytokeratin 7, a junctional biomarker with a SEQIKA fragment, which stabilizes HPV-16 E7 transcripts, in oropharyngeal SCCs.

Methods: Archived tumor specimens and epidemiologic data were collected from patients with oropharyngeal SCCs over 10 years. Briefly, DNA was extracted from tissue blocks, and HPV testing was carried out using SPF10 HPV PCR and INNO-LiPA HPV Genotyping. Immunohistochemical staining for CK7 and p16ink4a was performed on the Ventana BenchMark Ultra Immunostainer. Analysis was by light microscopy using the *H*-score. CK7 expression was correlated with epidemiologic data, p16ink4a positivity, and HPV status using SPSS.

Results: CK7 expression was observed specifically and uniformly in the tonsillar crypt epithelium of normal tonsils and tumor specimens. There were 226 cases of oropharyngeal SCCs, with 70 demonstrating both HPV and p16 positivity. Of 216 cases evaluated for CK7, 106 demonstrated some positivity, whereas *H*-score > 60 was seen in 55 of these. CK7 *H*-score > 60 was significantly associated with tonsillar subsite and HPV and p16 positivity.

Conclusions: An association between CK7 and HPV has been demonstrated. CK7-expressing tonsillar crypt cells potentially represent an oropharyngeal subsite susceptible to HPV-related SCC.

Impact: Along with the cervix and anorectum, specific oropharyngeal expression of CK7 in a site predisposed to HPV-related tumors may suggest a role for CK7 in the pathogenesis of this subgroup of tumors. Further research is warranted to characterize the association between CK7 and HPV-related head and neck SCC.
Cancer Epidemiol Biomarkers Prev; 26(5); 702–10. ©2017 AACR.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, and a causative relationship with mucosotropic human papillomavirus (HPV) has been established in a subset of these tumors in the oropharynx. Many aspects of this distinct clinicopathologic entity of tumors remain unclear, and the exact molecular mechanisms of carcinogenesis are not completely described. The putative site for HPV-related tumors in HNSCC is the reticular crypt epithelium of palatine and lingual tonsils (1, 2); however, the reason for this specificity is unknown.

Carcinogenesis in HPV-related oropharyngeal SCC is mediated through expression of E6 and E7 oncoproteins, largely mirroring that seen in HPV-related SCCs of the uterine cervix and anal canal, although more than 90% of cases in the oropharynx are attributed to HPV-16 (3), whereas there is greater genotype heterogeneity in cervical HPV infection. In the cervix, the distinction between a transforming HPV infection and a productive HPV infection is the unnaturally altered expression of cell cycle and DNA repair regulators (4). This mechanism is not well-understood, but it has been suggested that a specific host cell environment that is nonpermissive for viral replication could favor noncanonical regulation of E6 and E7 expression (4). Similarly, a host cell environment particular to the oropharynx could also explain the specificity of HPV-related SCCs at this site.

Recent studies have elicited a panel of biomarkers, including cytokeratin 7 (CK7), that identify a discrete population of cuboidal epithelial cells of embryonic origin at the cervical squamocolumnar junction (SCJ), which represent the putative site of progenitor cells of most cervical SCCs and their precursor lesions (5–7). The expression of this protein biomarker panel is not induced by HPV E6 or E7 in squamous epithelial cells *in vitro*, and their expression is lost if the SCJ is removed by cone biopsy or excision. Therefore, it seems that the SCJ-specific expression profile in lesions of cervical intraepithelial neoplasia and cervical cancers is not acquired during the transformation process and instead reflects the embryonal origin of the cells. Of these biomarkers, only CK7 has been identified as a marker of the anorectal junction (7–9) and demonstrates a specific interaction with

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doi: 10.1158/1055-9965.EPI-16-0619

HPV-16, described below, so was selected for investigation in the oropharynx, another site predisposed to HPV-related tumors.

Cytokeratins are major intermediate filaments of epithelial cells and have strong tissue specificity (10–12). CK7 is a basic-type keratin found in transitional, ductal, and glandular epithelia. It is described as a marker for simple and columnar epithelium and is thus believed to belong to endodermal-derived tissue. It has been suggested that it does not become localized to cells in normal stratified squamous epithelium (13); however, it does appear to be specifically expressed in junctional and transitional sites. Embryologic studies in mice and humans have shown CK7 expression specifically localizing to junctional epithelial sites in the uterine cervix, anorectum, esophagogastric region, and oropharynx (5, 13–15).

A molecular interaction has been described between HPV-16 E7 mRNA and a 6-mer peptide having the amino acid sequence SEQIKA, whereby it binds to and prevents decay of HPV-16 E7 mRNA in *in vitro* experiments (16). It was also demonstrated that the SEQIKA peptide is present in the human CK7 at the amino acid 91–96 position. This HPV-16 E7 mRNA/CK7 interaction, unique among the cytokeratins, could also be demonstrated in the SiHa cancer cell line (17). Further data have suggested that CK7 would function in protecting and storing the E7 transcript (16).

Proteome analysis has been carried out on human keratinocytes immortalized by long-term expression of HPV-16 oncoproteins E6 and E7 to identify features of squamous cell cancer that are attributable to long-term HPV oncoprotein expression (18). Interestingly, it was found that CK7 was one of a small number of proteins (which also included p16) that were directly regulated by HPV-16 viral oncoprotein E7 which may distinguish HPV-driven cancers from cancer in general. Indeed, CK7 is present in up to 87% of cervical SCC and 27% of HNSCC (19).

HPV is specific to junctional sites, and CK7 is a marker of junctional sites, and so in this study, expression of CK7 is analyzed in normal tonsils and in oropharyngeal SCC, correlating this with HPV status and p16 expression. Cases that are both HPV DNA–positive and p16-positive are considered as transcriptionally active for HPV as shown in previous research in relation to E6/E7 mRNA (20).

Materials and Methods

Ethical approval

This study was approved by the Research Ethics Committees of the study sites and is in compliance with the Helsinki Declaration.

Patient selection

This was a retrospective study of archived specimens of histopathologically diagnosed new primary oropharyngeal SCC in 2 head and neck cancer centers between January 2003 and December 2012. Hematoxylin and eosin–stained slides were reviewed by 2 pathologists to confirm the presence of invasive SCC and the degree of differentiation was graded. Patients with no or insufficient tissue from the primary oropharyngeal site, patients with previous oropharyngeal SCC from the same subsite, and patients with noninvasive tumors were excluded. Fifteen patients with tonsil specimens obtained at tonsillectomy for benign conditions of varying age range (19–61 years) were also evaluated. Clinical data were gathered from patient records on tumor subsite within the oropharynx, gender, age, smoking and alcohol consumption, previous or synchronous malignancy, tumor–node–metastasis (TNM) stage, treatment, recurrence, and survival.

HPV genotyping

Formalin-fixed, paraffin-embedded tissue samples were sectioned ($7 \times 5\,\mu m$) by microtome with precautions taken to ensure no contamination between cases. Each set of extractions was accompanied by a negative control sample (paraffin block). The samples were deparaffinized, and DNA was extracted by proteinase K digestion with overnight incubation at 56°C. DNA was purified using the QIAamp DNA FFPE Kit (Qiagen Ltd.) according to the protocol recommended by the manufacturer.

Adequacy of DNA quality in extracted samples was measured by PCR using a real-time TaqMan assay for the human β-actin gene (Roche Diagnostics). HPV DNA was amplified by the short PCR fragment (SPF10) HPV primer set (Life Technologies Inc.) described by Kleter and colleagues (21, 22). Briefly, the PCR reaction consisted of 300 nmol/L of each of the 6 SPF10 primer sets (SPF1A, SPF1B, SPF1C, SPF1D, SPF2B, SPF2D; ref. 21), 200 μmol/L deoxynucleoside triphosphates, 10× PCR buffer [containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl], 3 mmol/L $MgCl_2$, and 1 U of Ampli*Taq* Gold Taq polymerase (Life Technologies Inc.) in a final volume of 20 μL. PCR conditions were 5 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 45 seconds at 52°C, and 45 seconds at 72°C. HPV amplicons were detected by 2.2% agarose gel electrophoresis (FlashGel; Lonza). For each PCR, clinical specimens were tested together with negative template controls (distilled water) and positive controls of DNA extracted from HPV-positive HeLa cell lines.

HPV genotyping was performed using the INNO-LiPA EXTRA assay (Inno-LiPA; Fujirebio). Briefly, DNA was amplified using SPF10 primer sets to create biotinylated PCR amplicons which were detected using reverse line blot hybridization.

Immunohistochemistry

Five-micrometer sections were cut from FFPE tissue blocks and stained using a predilute CINtec monoclonal antibody to p16 and using a rabbit monoclonal antibody to CK7 (clone SP52; both Ventana Medical Systems, Inc.) on a Ventana BenchMark Ultra immunostainer (Ventana Medical Systems, Inc.) according to standard protocols. The OptiView DAB IHC Detection Kit (OptiView) was used for detection with light microscopy. The ULTRA Cell Conditioning Solution, ULTRA CC1 (Ventana Medical Systems, Inc.), was used at a temperature of 95°C for 64 minutes, as an antigen retrieval pretreatment step for the p16 and CK7 immunohistochemical (IHC) reactions.

A positive and negative control was used with each set of samples. Samples were interpreted by 2 independent pathologists. For p16 scoring, cases with diffuse nuclear and cytoplasmic staining in more than 70% of tumor cells were considered positive. Cytoplasmic and membranous staining was identified for CK7, which was scored according to the *H*-scoring system (23). The highest intensity of staining present in tumor cells was given a score from 0–3 (0, none; 1, mild; 2, moderate; 3, intense). The percentage of tumor cells staining in the sample was estimated to the nearest 5%. The *H*-score was derived from the cross-product of the intensity and the percentage of tumor cells staining (0%–100%).

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics Version 23.0.0.2 software. Prevalence rates for HPV-related

tumors were determined by combining p16 and high-risk HPV genotype positivity. To select a relevant CK7 cutoff score to identify samples linked to HPV and compare with clinicopathologic variables, receiver operating characteristic (ROC) curve analysis was carried out.

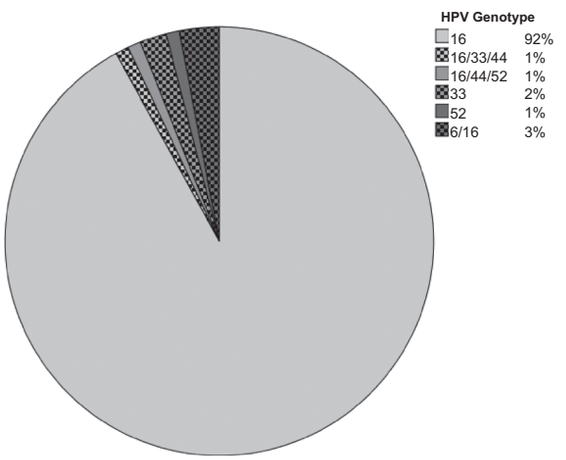
Significance of association tests between clinicopathologic variables and CK7 cutoff scores were performed using the χ^2 test and Fisher exact test for independence. Testing for association with continuous variables was performed using the t test, if normally distributed, or the Mann–Whitney U test, if not normally distributed. The threshold for statistical significance was set at $P \leq 0.05$, and tests were 2-sided. Univariate and multivariate logistic regression were carried out to determine whether CK7 was predictive of HPV-related tumors and tonsillar subsite.

For each patient, the disease-specific survival was measured in days from the date of diagnosis to censoring (either the date of latest follow-up or date of death by other cause) or death from disease, whichever occurred first. Recurrence-free survival was measured in the same way, but considering date of diagnosis of recurrence instead of death from disease. Survival analysis employed the Kaplan–Meier method and the difference between strata was assessed for significance using log-rank tests.

Results

HPV-related tumors

Of 226 cases in total, there were 100 cases that tested positive for HPV. HPV-16 was present in 97% of the HPV-positive cases. HPV-33 was identified in 2 cases and HPV-52 in 1 case. There were 5 cases of multiple HPV infections. Three cases demonstrated HPV-16 and HPV-6 co-infection, whereas 2 cases demonstrated infection with 3 genotypes (HPV-16, 33, 44 and HPV-16, 44, 52). The distribution of genotypes is demonstrated in Fig. 1, which also shows the breakdown of p16 IHC and HPV DNA status. There



	HPV DNA Negative	HPV DNA Positive	Total
p16-Negative	116 (51.3%)	30 (13.3%)	146
p16-Positive	10 (4.4%)	70 (31.0%)	80
Total	126	100	226

Figure 1.
Distribution of genotypes in HPV-positive oropharyngeal tumors and patients according to p16 IHC and HPV DNA status.

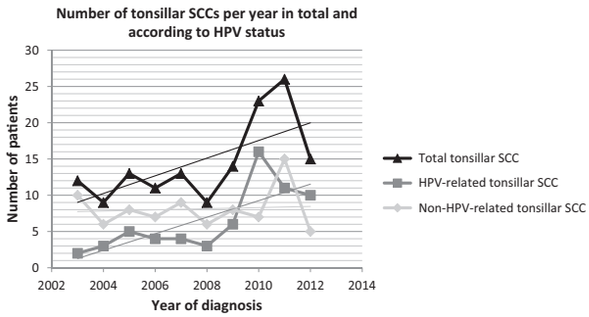


Figure 2.
Total number of tonsillar SCCs over time and according to HPV-related status. Estimated linear regression lines are plotted.

were 70 cases (31%) that were both p16 and high-risk HPV-positive and were considered HPV-related tumors.

The proportion of HPV-related tonsillar tumors increased significantly from 16.7% in 2003 to 66.7% in 2012 ($P = 0.033$) and is shown in Fig. 2. There was a negative average annual percentage change (−0.3%, $P = 0.9$) in non–HPV-related tonsillar tumors compared with a significantly increased average annual percentage change in HPV-related tonsillar tumors (20.2%, $P = 0.01$).

Normal tonsils

All 15 cases of normal tonsillar tissue on which CK7 IHC was carried out demonstrated strong staining specifically in the reticular crypt epithelial cells with no or minimal patchy staining seen in cells of the normal surface stratified squamous epithelium. Examples of these cases are shown in Fig. 3.

Oropharyngeal SCC cases

Tissue was available for IHC for CK7 in 216 cases. The cases were scored by 2 independent pathologists, with discordant results resolved by discussion. CK7 positivity with membranous and cytoplasmic immunostaining was seen in 106 (49.1%) cases. For positive cases, staining intensity was weak in 11 (10.4%) cases, moderate in 31 (29.2%) cases, and strong in 64 (60.4%) cases. The median H-score was 60. Some examples of CK7-positive cases are shown in Fig. 4.

Regarding the correlation between CK7 expression and HPV-related tumor, the mean H-score was significantly different (Mann–Whitney U Test, $P < 0.001$) between HPV-related (mean, 104.4; SD, 110.7) and non–HPV-related tumors (mean, 35.0; SD, 73.5). To determine the most appropriate score for analysis, a ROC curve was plotted for CK7 H-score and HPV-related tumor and the coordinates of the curve evaluated for sensitivity and specificity. The point on the curve with the highest sensitivity and specificity was at 67.5 and so a minimum score of 60 was chosen for further analysis.

Relationship of CK7 to HPV, p16, and clinicopathologic data

IHC results and clinicopathologic findings are shown in Table 1. Expression of CK7 with H-score of 60 or more (CK7 > 60) was seen in 55 of 216 (25.5%) cases. Of these, 36 cases were high-risk HPV-positive and 31 cases were HPV-related tumors. CK7 > 60 was significantly associated with high-risk HPV DNA positivity ($P < 0.001$); p16 IHC positivity ($P < 0.001$); HPV-related tumor ($P < 0.001$); lower age at diagnosis ($P = 0.02$); lower pack-years of

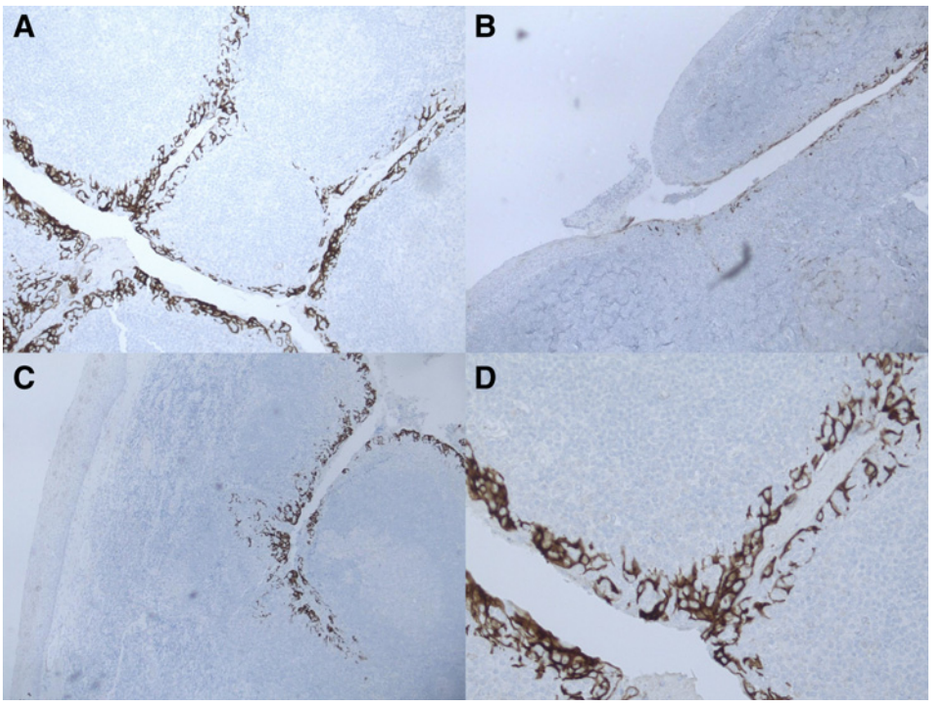


Figure 3.
CK7 staining in normal tonsil crypts. **A,** Positive crypt staining at 10× magnification. **B,** Negative surface staining leading into positive crypt staining at 5× magnification. **C,** Negative surface staining and positive crypt staining at 5× magnification. **D,** Positive crypt staining at 20× magnification.

tobacco ($P = 0.03$); no previous or synchronous malignancy ($P = 0.02$); tumor origin in the tonsillar sites (palatine tonsils or base of tongue; $P = 0.01$).

Predictors of CK7 > 60 were evaluated by multivariate logistic regression analysis, with variables significant by univariate analysis included, including age, smoking status, and a history of previous or synchronous malignancy. The only variable significantly independently predictive of CK7 > 60 was HPV-related tumor [OR, 4.372; 95% confidence interval (CI), 1.994–9.588; $P < 0.001$]. By logistic multivariate forward stepwise regression, CK7 > 60 was a significant independent predictor of HPV-related tumor (OR, 7.206; 95% CI, 2.735–18.983; $P < 0.001$), second only to tonsillar subsite in strength of prediction.

CK7 was significantly associated with tonsillar subsite (Mann–Whitney *U* test, $P = 0.019$), with a mean *H*-score in tonsillar tumors of 70.11 (SD, 103.105) and a mean *H*-score of 30.07 (SD, 60.516) in non-tonsillar tumors. When a cutoff score of 60 is considered as a predictor, the OR of a tonsillar tumor is 2.325 (95% CI, 1.136–4.760; $P = 0.021$) by univariate logistic regression. When considering tonsillar subsite only, CK7 > 60 remains a significant predictor of HPV-related tumor (OR, 5.726; 95% CI, 2.600–12.610; $P < 0.001$); however, this is not true for non-tonsillar sites ($P = 0.639$). When considering HPV-related status, CK7 > 60 was more likely to be found in the tonsillar tumors in both positive and negative cases, with an OR of 3.333 (95% CI, 0.327–33.991) for HPV-related cases and 1.023 (95% CI, 0.426–2.458) in non–HPV-related cases, however, these were not significant ($P = 0.310$, 0.959).

Recurrence and survival

Survival curves stratified by CK7 *H*-score greater or less than 60 were plotted by Kaplan–Meier method, shown in Fig. 5A, and evaluated by log-rank test to compare survival for those with CK7 *H*-score greater or less than 60. There was no significant difference in survival between groups. This was also the case when curatively treated cases only were considered ($\chi^2 = 1.363$, df = 1, $P = 0.243$) or when recurrence-free survival was considered ($\chi^2 = 0.034$, df = 1, $P = 0.853$).

Survival curves stratified by HPV in cases with CK7 > 60 or CK7 < 60 are shown in Fig. 5B. For cases with CK7 > 60, there was a significant difference seen between HPV-related cases and non–HPV-related cases ($\chi^2 = 10.123$, df = 1, $P = 0.001$). For non–HPV-related cases, CK7 > 60/HPV-negative cases have significantly worse survival than the CK7 < 60/HPV-negative cases ($\chi^2 = 4.125$, df = 1, $P = 0.042$). There was no significantly worse survival for CK7 < 60 in HPV-related cases.

Discussion

As with data in this study, rates of HPV-related oropharyngeal SCC are increasing worldwide, implicating a potentially increasing burden on healthcare resources (24, 25). While the proportion of tumors linked to HPV in this European cohort at 31% differs from American figures that are as high as 72% (24), it is consistent with other European figures (26, 27), possibly reflecting the epidemiologic differences between these groups in relation to risk factors for HNSCC (28). Despite this, the increasing rates worldwide indicate the need to further our understanding of this disease and to identify potential biomarkers to improve diagnosis, treatment, and preventative measures.

The biologic explanation as to why the prevalence of HPV is higher in oropharyngeal, and specifically tonsillar, SCC compared with other HNSCCs has remained unclear (27). Despite the almost ubiquitous presence of HPV infection in the vagina and vulva of sexually active women (27), the majority of HPV-related SCCs in the female genital tract arise in the CK7-expressing cells in the SCJ of the cervix. Previous research using exon array analysis identified 5 SCJ-specific biomarkers that were linked to HPV

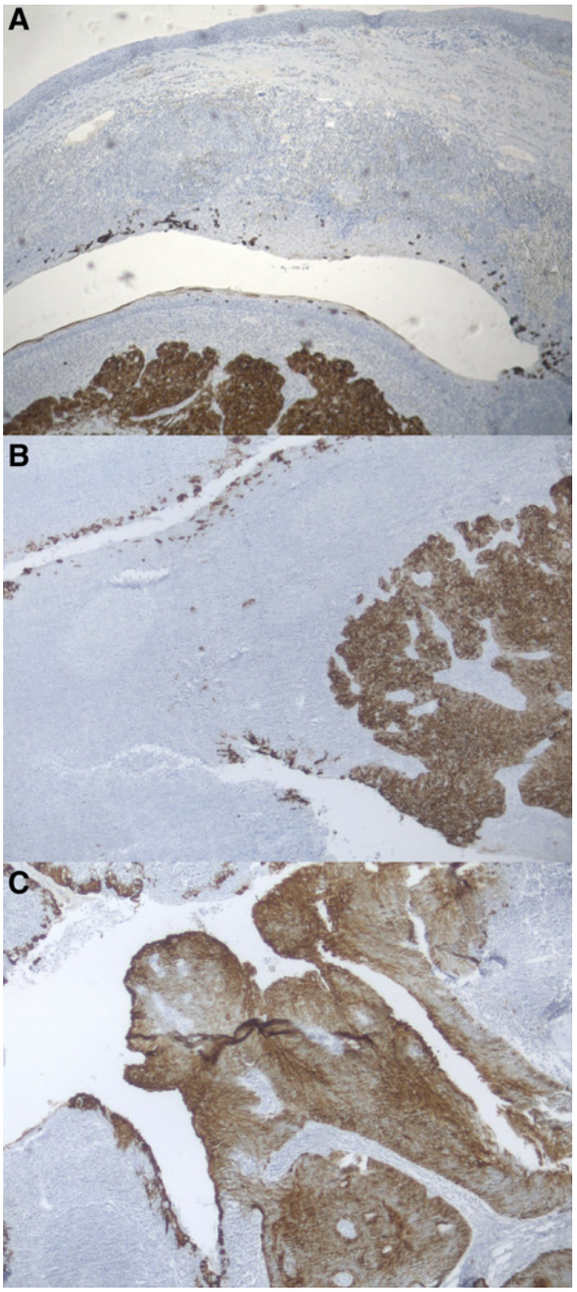


Figure 4.

Typical CK7 staining in oropharyngeal tumors. Staining intensity and percentage of cells staining are in parentheses. Membranous and cytoplasmic staining of tumor cells is seen. **A,** Immunostaining showing negative stain in tonsillar surface epithelium, patchy positive stain in normal tonsillar crypt epithelium, and strong expression in tumor cells (3,100), 5× magnification. **B,** Immunostaining showing patchy positive stain in normal tonsillar crypt epithelium and strong expression in tumor cells arising from crypt epithelium (3,100), 5× magnification. **C,** Immunostaining showing strong CK7 expression in tumor cells that arise from crypt epithelium (3,95), 5× magnification.

infection, with one biomarker, CK7, also identified in the anorectal junction (7), a site also predisposed to HPV-related carcinogenesis. Furthermore, research elsewhere has demonstrated an interaction between CK7 and HPV E7 transcripts (17). We identify a distinct population of CK7-expressing cells isolated in the normal tonsil crypt reticulated epithelium. It is possible that the CK7-expressing tonsillar crypt epithelial cells represent the equivalent "junctional site" of the oropharynx that could be particularly susceptible to malignant transformation by infection with high-risk HPV genotypes.

Higher CK7 expression was associated with a younger age at diagnosis, with a history of no previous or synchronous malignancy and with a history of lower exposure to tobacco; however, these variables are also linked to HPV status. On further investigation, none of these variables were predictive of CK7 > 60 when considering multivariate logistic regression, and in fact, the only variable independently predictive of CK7 > 60 was whether the tumor was HPV-related. CK7 expression has previously been associated with HPV status in tonsillar tumors (29), and a significant association with p16 and HPV is also seen in this study.

Most HPV infections result in latency, such as in recurrent respiratory papillomatosis; however, persistent overexpression of high-risk HPV oncoproteins can lead to excessive cell cycling and accumulation of deleterious host gene mutations selected for survival and growth (30). While E6 and E7 can immortalize tonsil epithelial cells *in vitro*, subsequent mechanisms of tumorigenesis are less clear (31), but it is understood that elevated HPV oncogene expression drives neoplastic progression (30), with animal models suggesting that E7 is the dominant HPV oncoprotein in HNSCC (32) as well as cervical SCC (33).

As CK7 expression occurs in epithelial cells in parallel to differentiation (34) and transcript stability is a major determinant of mRNA abundance and therefore the expression level of the relative encoded protein (35), the interaction between the CK7 SEQIKA fragment and E7 mRNA may protect E7 mRNA during keratinocyte differentiation (17). Stabilization of the E7 mRNA transcripts may result in protecting and storing these particular mRNA transcripts to be translated at subsequent stages leading to E7 oncoprotein–mediated carcinogenic events.

The mechanism for this translation may be through expression of CK19 (16), a cytokeratin that exists as a heterodimer with CK7, which correlates with important cell fate decisions in gastrointestinal tract epithelia (36) and has been shown to correlate with tumor progression in the uterine cervix and the upper aerodigestive tract (16). Strong association between CK19 upregulation and HPV-related oropharyngeal SCCs has been demonstrated recently (37), and CK19 is used as a tumor marker in HNSCC and metastatic cervical lymph nodes from HNSCC (38, 39).

There were a number of CK7 < 60, but HPV- and p16-positive, oropharyngeal SCCs (32 cases). There has been mounting evidence of a role for cancer stem cells in HNSCC (40, 41) and, where diffuse positive staining for CK7 was not identified, it is possible that a small focus of CK7-expressing cells exist elsewhere in the tumor acting as cancer stem cells with the expression of CK7 being lost as the cells differentiate. It is also possible that a tumor is biphenotypic with one phenotype expressing CK7, as shown in a previous study (42). Differing roles for E6 and E7 oncoproteins in carcinogenesis are well-known and it is possible that downregulation of CK7 expression by E6-transduced cells mediates its effects on E7 mRNA (18).

Some research suggests that oncogene transcription in tonsillar SCC is not necessarily dependent on viral DNA integration and that viral DNA is predominantly in the episomal form and, in this form, also takes part in carcinogenesis (43, 44), whereas viral

Table 1. Association of clinicopathologic characteristics with CK7 *H*-score, stratified by greater or less than 60

	CK7 *H*-score ≤ 60	**CK7 *H*-score ≥ 60**	**Testing comparison**
High-risk HPV DNA ($n = 216$)			**Fisher exact test, $P < 0.001$**
HPV-negative	105 (84.7%)	19 (15.3%)	
HPV-positive	56 (60.9%)	36 (39.1%)	
p16 IHC ($n = 216$)			**Fisher exact test, $P < 0.001$**
p16-negative	119 (83.2%)	24 (16.8%)	
p16-positive	42 (57.5%)	31 (42.5%)	
HPV and p16 combined ($n = 216$)			**Fisher exact test, $P < 0.001$**
Non–HPV-related	129 (84.3%)	24 (15.7%)	
HPV-related	32 (50.8%)	31 (49.2%)	
Gender/Age ($n = 216$)			Fisher exact test, $P = 0.25$
Male	129 (75.9%)	41 (24.1%)	
Female	32 (69.6%)	14 (30.4%)	
Mean age at diagnosis (SD)	61.35 (9.56)	57.49 (10.75)	**Mann–Whitney *U* test, $P = 0.02$**
Smoking status ($n = 212$)			**Fisher exact test, $P = 0.033$**
Smoker (current or ex)	141 (77.0%)	42 (23.0%)	
Non-smoker	17 (58.6%)	12 (41.4%)	
Average pack-years (SD)	43.83 (30.99)	33.08 (24.84)	**Mann–Whitney *U* test, $P = 0.03$**
Alcohol status ($n = 209$)			Fisher exact test, $P = 0.354$
<10 units/wk	54 (72.0%)	21 (28.0%)	
>10 units/wk	101 (75.4%)	33 (24.6%)	
Previous or synchronous malignancy ($n = 214$)			**Fisher exact test, $P = 0.02$**
No	112 (70.4%)	47 (29.6%)	
Yes	47 (85.5%)	8 (14.5%)	
Family history of cancer ($n = 129$)			Fisher exact test, $P = 0.32$
No	48 (73.8%)	17 (26.2%)	
Yes	39 (68.4%)	18 (31.6%)	
Grade ($n = 216$)			$\chi^2 = 1.27$, df = 2, $P = 0.53$
Well-differentiated	13 (86.7%)	2 (13.3%)	
Moderately differentiated	116 (73.9%)	41 (26.1%)	
Poorly differentiated	32 (72.7%)	12 (27.3%)	
Primary tumor site ($n = 210$)			**Fisher exact test, $P = 0.01$**
Tonsillar (palatine tonsil or base of tongue)	94 (68.6%)	43 (31.4%)	
Non-tonsillar	61 (83.6%)	12 (16.4%)	
T stage ($n = 212$)			$\chi^2 = 2.30$, df = 4, $P = 0.68$
T1	31 (79.5%)	8 (20.5%)	
T2	41 (68.3%)	19 (31.7%)	
T3	47 (78.3%)	13 (21.7%)	
T4a	29 (72.5%)	11 (27.5%)	
T4b	10 (76.9%)	3 (23.1%)	
N stage ($n = 210$)			$\chi^2 = 2.41$, df = 5, $P = 0.79$
N0	51 (81.0%)	12 (19.0%)	
N1	26 (70.3%)	11 (29.7%)	
N2a	17 (68.0%)	8 (32.0%)	
N2b	39 (73.6%)	14 (26.4%)	
N2c	15 (71.4%)	6 (28.6%)	
N3	8 (72.7%)	3 (27.3%)	
M stage ($n = 185$)			Fisher exact test, $P = 0.44$
M0	129 (74.1%)	45 (25.9%)	
M1	9 (81.8%)	2 (18.2%)	
UICC stage ($n = 211$)			$\chi^2 = 3.43$, df = 5, $P = 0.63$
I	19 (86.4%)	3 (13.6%)	
II	8 (61.5%)	5 (38.5%)	
III	33 (75.0%)	11 (25.0%)	
IVA	75 (72.1%)	29 (27.9%)	
IVB	13 (76.5%)	4 (23.5%)	
IVC	9 (81.8%)	2 (18.2%)	

NOTE: Where results of tests of association were significant ($P \leq 0.05$), these are highlighted in bold text.

integration may be necessary at non-tonsillar sites. It is possible that HPV-related carcinogenesis may occur in infected surface rather than reticulated epithelial cells, without a potential selective advantage from CK7 expression. This may explain the lack of any significant difference in previous proteome analysis of oropharyngeal SCC (45–47), and it may in future be worthwhile comparing protein expression between the oropharynx and other sites, especially given the known specificity of HPV to the oropharynx.

It is also possible that non–HPV-related carcinogenesis may occur in CK7-expressing crypt epithelial cells (29), which could explain those cases with raised CK7 *H*-score, but HPV- and p16-negative oropharyngeal SCCs (24 cases). The expression of CK7 in this study is significantly higher in tonsillar tumors than in

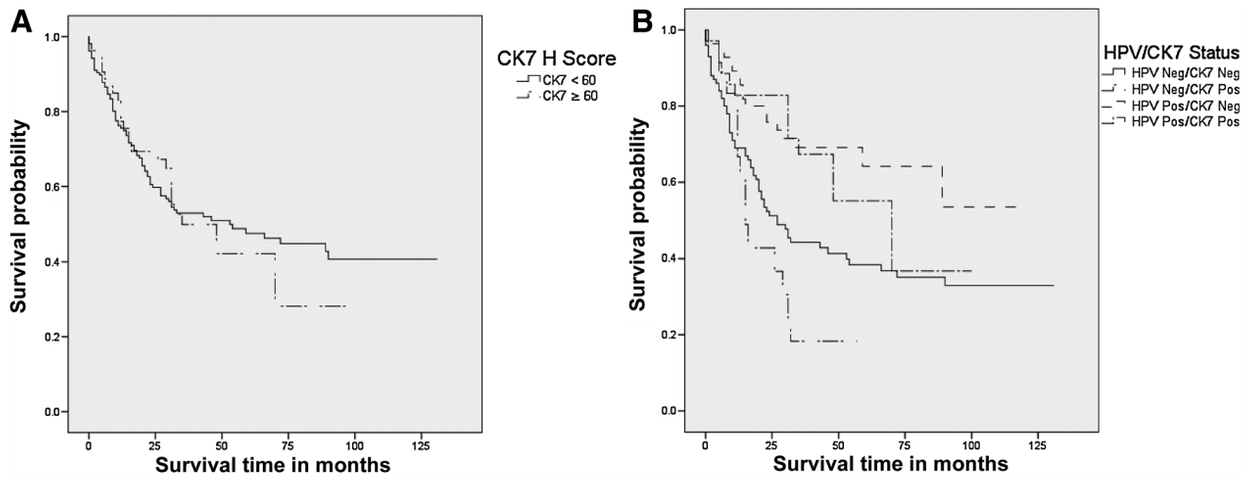


Figure 5.

Kaplan–Meier disease-specific survival curves for (**A**) CK7 *H*-score greater or less than 60 and (**B**) CK7 *H*-score greater than 60 (positive) or less than 60 (negative) stratified by HPV-related status. These were evaluated by log-rank test. **A**, $\chi^2 = 0.034$, df = 1, $P = 0.855$. **B**, $\chi^2 = 18{,}887$, df = 3, $P < 0.001$.

non-tonsillar tumors, and CK7 > 60 is also predictive of tumor origin in the tonsillar subsite within the oropharynx. In some cases, although with a lower mean *H*-score of 30, expression of CK7 was seen within tumors designated "non-tonsillar." Misclassification of tumor site could account for the expression of CK7 within tumors that were designated clinically as non-tonsillar but were within the oropharynx and could have actually arisen from a tonsillar crypt source. Issues related to misclassification of oropharyngeal SCC are well-described in the literature (27, 48–52), and misclassification has even been described as a limitation in relation to site and CK7 expression before (53). Future investigation of CK7 expression in non-oropharyngeal tumors may help further characterize specificity to the tonsillar region.

Nevertheless, a clear association is seen between HPV-related SCCs and CK7 expression in primary oropharyngeal SCCs. As the spatiotemporal E7 protection ensured by CK7-expressing cells (17) could contribute to selectivity to the tonsillar crypts for HPV-related carcinogenic progression in the head and neck, it may be possible that prophylactic tonsillectomy could reduce the burden of this disease, similar to excision of the SCJ in the cervix (54), although lingual tonsils would remain at risk. Indeed recent studies have shown that remote tonsillectomy resulted in a decreased incidence of tonsillar SCC (55, 56). There are, however, no well-defined precancerous lesions or satisfactory biomarkers to identify patients at high risk, and justification for prophylactic tonsillectomy as a cancer preventive strategy is lacking at present due to the associated cost and morbidity (57).

CK7, stratified by *H*-score, had no significant impact on survival. The effect of HPV-related status on survival and recurrence remained despite stratification by CK7 *H*-score. In studies on other tumors, such as esophageal SCC, CK7 expression has predicted a higher risk of death (58–60).

Cystic nodal metastases are typically attributed to a primary tumor located in Waldeyer's ring (61). It is interesting to note the relationship between HPV-related SCCs and cystic nodal metastases (62–64) and also the relationship between cystic nodal metastases and CK7 expression (65, 66). Indeed, it has previously been demonstrated that a subset of tumors arising from Waldeyer's ring and displaying unique clinical features also produce CK7-positive cystic nodal metastases (67). However, these tumors were not assessed for HPV status. It may be that these findings, previously ascribed to salivary type differentiation, are related to CK7-expressing tonsillar crypt SCCs. As shown for non–small cell lung carcinoma, it is possible that nodal disease occurs early and before some mutations have occurred; however, cancers of the head and neck generally maintain their expression profile between primary site and nodal metastases (65), so further research into CK7 expression of HPV-related tumors and cystic nodal metastases is warranted.

Although there is no direct evidence that CK7-expressing cells are the specific cell types associated with HPV in the oropharynx, the specificity of this biomarker to HPV-associated sites, along with the documented interaction of CK7 with HPV-16 E7 warrants further investigation. However, results from this study show there is increasing evidence of an association of CK7 with HPV-related SCCs. To further characterize the association in HNSCC, future research could evaluate HPV-16 E7 mRNA/CK7 interaction in HPV-related oropharyngeal cancer cell lines. CK7 expression in nodal metastases and the relationship to morphology, HPV status, clinical outcome, and primary site warrant further investigation. Future research to determine the susceptibility to HPV-mediated carcinogenesis in CK7-expressing cells is also warranted; however, animal models may not be appropriate, given the interspecies heterogeneity in CK7 (68). Retroviral introduction of oncogenes into CK7-expressing normal epithelial cell lines may offer a more appropriate model for studying this biomarker.

In this study, we discuss the unique propensity of CK7 to bind and stabilize E7 transcripts and demonstrate a significant association between CK7 expression and HPV-related tumors. Specific expression of CK7 in normal tonsil crypt epithelial cells is also demonstrated in this study. The potentially protective effect on E7 transcripts in CK7-expressing tonsillar crypt epithelial cells coupled with the high preponderance of HPV-related tumors in the lingual and palatine tonsil crypts creates an alternative hypothesis to explain the specificity of HPV-related tumors to this site. Further research is warranted to characterize this potential role of

CK7-expressing cells in the selectivity of HPV-16 mediated carcinogenesis to the lingual and palatine tonsils.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

The authors thank Jacqui Barry-O'Crowley, Danielle Costigan, Wafiatul Hamsin, and Muna Mohd Yusoff.

Grant Support

Grants to fund this research were awarded to R.S.R. Woods by the Research Foundations of St. James's Hospital and Royal Victoria Eye and Ear Hospital and by the CERVIVA research consortium.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 31, 2016; revised November 29, 2016; accepted December 6, 2016; published OnlineFirst January 12, 2017.

References

1. Woods RSR, O'Regan EM, Kennedy S, Martin C, O'Leary JJ, Timon C. Role of human papillomavirus in oropharyngeal squamous cell carcinoma: a review. World J Clin Cases 2014;2:172–93.
2. Begum S, Cao D, Gillison M, Zahurak M, Westra WH. Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. Clin Cancer Res 2005;11:5694–9.
3. 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;356:1944–56.
4. Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer 2014;14:395–405.
5. Herfs M, Vargas SO, Yamamoto Y, Howitt BE, Nucci MR, Hornick JL, et al. A novel blueprint for 'top down' differentiation defines the cervical squamocolumnar junction during development, reproductive life, and neoplasia. J Pathol 2013;229:460–8.
6. Herfs M, Parra-Herran C, Howitt BE, Laury AR, Nucci MR, Feldman S, et al. Cervical squamocolumnar junction-specific markers define distinct, clinically relevant subsets of low-grade squamous intraepithelial lesions. Am J Surg Pathol 2013;37:1311–8.
7. Herfs M, Yamamoto Y, Laury A, Wang X, Nucci MR, McLaughlin-Drubin ME, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci U S A 2012;109:10516–21.
8. Williams GR, Talbot IC, Northover JM, Leigh IM. Keratin expression in the normal anal canal. Histopathology 1995;26:39–44.
9. Ramalingam P, Hart WR, Goldblum JR. Cytokeratin subset immunostaining in rectal adenocarcinoma and normal anal glands. Arch Pathol Lab Med 2001;125:1074–7.
10. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11–24.
11. Quinlan RA, Schiller DL, Hatzfeld M, Achtstatter T, Moll R, Jorcano JL, et al. Patterns of expression and organization of cytokeratin intermediate filaments. Ann N Y Acad Sci 1985;455:282–306.
12. Cooper D, Schermer A, Sun TT. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: strategies, applications, and limitations. Lab Invest 1985;52:243–56.
13. Hosoya A, Kwak S, Kim EJ, Lunny DP, Lane EB, Cho SW, et al. Immunohistochemical localization of cytokeratins in the junctional region of ectoderm and endoderm. Anat Rec (Hoboken) 2010;293:1864–72.
14. Fritsch H, Zehm S, Illig R, Moser P, Aigner F. New insights into the development and differentiation of the human anorectal epithelia. Are there clinical consequences? Int J Colorectal Dis 2010;25:1231–42.
15. Wang X, Ouyang H, Yamamoto Y, Kumar PA, Wei TS, Dagher R, et al. Residual embryonic cells as precursors of a Barrett's-like metaplasia. Cell 2011;145:1023–35.
16. Favia G, Kanduc D, Lo Muzio L, Lucchese A, Serpico R. Possible association between HPV16 E7 protein level and cytokeratin 19. Int J Cancer 2004;111:795–7.
17. Kanduc D. Translational regulation of human papillomavirus type 16 E7 mRNA by the peptide SEQIKA, shared by rabbit alpha(1)-globin and human cytokeratin 7. J Virol 2002;76:7040–8.
18. Merkley MA, Hildebrandt E, Podolsky RH, Arnouk H, Ferris DG, Dynan WS, et al. Large-scale analysis of protein expression changes in human keratinocytes immortalized by human papilloma virus type 16 E6 and E7 oncogenes. Proteome Sci 2009;7:29.
19. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000;13:962–72.
20. Jung AC, Briolat J, Millon R, de Reynies A, Rickman D, Thomas E, et al. Biological and clinical relevance of transcriptionally active human papillomavirus (HPV) infection in oropharynx squamous cell carcinoma. Int J Cancer 2010;126:1882–94.
21. Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;153:1731–9.
22. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.
23. Jordan RC, Lingen MW, Perez-Ordonez B, He X, Pickard R, Koluder M, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. Am J Surg Pathol 2012;36:945–54.
24. 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;29:4294–301.
25. Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer–systematic review and meta-analysis of trends by time and region. Head Neck 2013;35:747–55.
26. Combes JD, Chen AA, Franceschi S. Prevalence of human papillomavirus in cancer of the oropharynx by gender. Cancer Epidemiol Biomarkers Prev 2014;23:2954–8.
27. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467–75.
28. Mehanna H, Franklin N, Compton N, Robinson M, Powell N, Biswas-Baldwin N, et al. Geographic variation in human papillomavirus-related oropharyngeal cancer: data from four multinational randomized trials. Head Neck 2016;38 Suppl 1:E1863–9.
29. Morbini P, Capello GL, Alberizzi P, Benazzo M, Paglino C, Comoli P, et al. Markers of squamocolumnar junction cells in normal tonsils and

oropharyngeal cancer with and without HPV infection. Histol Histopathol 2015;30:833–9.
30. Chow LT, Broker TR, Steinberg BM. The natural history of human papillomavirus infections of the mucosal epithelia. APMIS 2010;118:422–49.
31. Pim D, Banks L. Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs. low-risk human papillomaviruses. APMIS 2010;118:471–93.
32. Jabbar S, Strati K, Shin MK, Pitot HC, Lambert PF. Human papillomavirus type 16 E6 and E7 oncoproteins act synergistically to cause head and neck cancer in mice. Virology 2010;407:60–7.
33. Jabbar SF, Park S, Schweizer J, Berard-Bergery M, Pitot HC, Lee D, et al. Cervical cancers require the continuous expression of the human papillomavirus type 16 E7 oncoprotein even in the presence of the viral E6 oncoprotein. Cancer Res 2012;72:4008–16.
34. Eckert RL, Crish JF, Robinson NA. The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. Physiol Rev 1997;77:397–424.
35. Ross J. mRNA stability in mammalian cells. Microbiol Rev 1995;59: 423–50.
36. Brembeck FH, Moffett J, Wang TC, Rustgi AK. The keratin 19 promoter is potent for cell-specific targeting of genes in transgenic mice. Gastroenterology 2001;120:1720–8.
37. Santoro A, Pannone G, Ninivaggi R, Petruzzi M, Santarelli A, Russo GM, et al. Relationship between CK19 expression, deregulation of normal keratinocyte differentiation pattern and high risk-human papilloma virus infection in oral and oropharyngeal squamous cell carcinoma. Infect Agent Cancer 2015;10:46.
38. Alkotyfan K, Wiegand S, Muller HH, Windfuhr JP, Werner JA, Sesterhenn AM. Cyfra 21–1 as a tumor marker for follow-up of patients with squamous cell carcinoma of the oropharynx. Anticancer Res 2010;30:2291–6.
39. Tao L, Lefevre M, Ricci S, Saintigny P, Callard P, Perie S, et al. Detection of occult carcinomatous diffusion in lymph nodes from head and neck squamous cell carcinoma using real-time RT-PCR detection of cytokeratin 19 mRNA. Br J Cancer 2006;94:1164–9.
40. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A 2007;104:973–8.
41. Major AG, Pitty LP, Farah CS. Cancer stem cell markers in head and neck squamous cell carcinoma. Stem Cells Int 2013;2013:319489.
42. Pitiyage G, Lei M, Guererro Urbano T, Odell E, Thavaraj S. Biphenotypic human papillomavirus-associated head and neck squamous cell carcinoma: a report of two cases. Diagn Pathol 2015;10:97.
43. Mellin H, Dahlgren L, Munck-Wikland E, Lindholm J, Rabbani H, Kalantari M, et al. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. Int J Cancer 2002;102:152–8.
44. Van Tine BA, Dao LD, Wu SY, Sonbuchner TM, Lin BY, Zou N, et al. Human papillomavirus (HPV) origin-binding protein associates with mitotic spindles to enable viral DNA partitioning. Proc Natl Acad Sci U S A 2004;101:4030–5.
45. Slebos RJ, Jehmlich N, Brown B, Yin Z, Chung CH, Yarbrough WG, et al. Proteomic analysis of oropharyngeal carcinomas reveals novel HPV-associated biological pathways. Int J Cancer 2013;132:568–79.
46. Sepiashvili L, Waggott D, Hui A, Shi W, Su S, Ignatchenko A, et al. Integrated omic analysis of oropharyngeal carcinomas reveals human papillomavirus (HPV)-dependent regulation of the activator protein 1 (AP-1) pathway. Mol Cell Proteomics 2014;13:3572–84.
47. Descamps G, Wattiez R, Saussez S. Proteomic study of HPV-positive head and neck cancers: preliminary results. Biomed Res Int 2014;2014:430906.
48. Frandsen VL, Larsen CG, von Buchwald C. Prevalence of human papillomavirus in squamous cell carcinomas of the soft palate. J Clin Pathol 2015;68:942–3.
49. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsague X, Laporte L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol 2014;15: 1319–31.
50. Chaturvedi A, Gillison M. Human papillomavirus and head and neck cancer. In: Olshan A, editor. Epidemiology, pathogenesis, and prevention of head and neck cancer. New York, NY: Springer; 2010. p. 96.
51. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003;95: 1772–83.
52. Colevas AD. Population-based evaluation of incidence trends in oropharyngeal cancer focusing on socioeconomic status, sex, and race/ethnicity. Head Neck 2014;36:34–42.
53. Yang EJ, Quick MC, Hanamornroongruang S, Lai K, Doyle LA, McKeon FD, et al. Microanatomy of the cervical and anorectal squamocolumnar junctions: a proposed model for anatomical differences in HPV-related cancer risk. Mod Pathol 2015;28:994–1000.
54. Herfs M, Somja J, Howitt BE, Suarez-Carmona M, Kustermans G, Hubert P, et al. Unique recurrence patterns of cervical intraepithelial neoplasia after excision of the squamocolumnar junction. Int J Cancer 2015;136: 1043–52.
55. Fakhry C, Andersen KK, Christensen J, Agrawal N, Eisele DW. The impact of tonsillectomy upon the risk of oropharyngeal carcinoma diagnosis and prognosis in the Danish Cancer Registry. Cancer Prev Res (Phila) 2015;8:583–9.
56. Chaturvedi AK, Song H, Rosenberg PS, Ramqvist T, Anderson WF, Munck-Wikland E, et al. Tonsillectomy and incidence of oropharyngeal cancers. Cancer Epidemiol Biomarkers Prev 2016;25:944–50.
57. Misiukiewicz K, Posner M. Role of prophylactic bilateral tonsillectomy as a cancer preventive strategy. Cancer Prev Res (Phila) 2015;8:580–2.
58. Oue N, Noguchi T, Anami K, Kitano S, Sakamoto N, Sentani K, et al. Cytokeratin 7 is a predictive marker for survival in patients with esophageal squamous cell carcinoma. Ann Surg Oncol 2012;19:1902–10.
59. Yamada A, Sasaki H, Aoyagi K, Sano M, Fujii S, Daiko H, et al. Expression of cytokeratin 7 predicts survival in stage I/IIA/IIB squamous cell carcinoma of the esophagus. Oncol Rep 2008;20:1021–7.
60. Yun SP, Seo HI. Prognostic impact of immunohistochemical expression of CK7 and CK20 in curatively resected ampulla of Vater cancer. BMC Gastroenterol 2015;15:165.
61. Goldenberg D, Sciubba J, Koch WM. Cystic metastasis from head and neck squamous cell cancer: a distinct disease variant? Head Neck 2006;28: 633–8.
62. Yasui T, Morii E, Yamamoto Y, Yoshii T, Takenaka Y, Nakahara S, et al. Human papillomavirus and cystic node metastasis in oropharyngeal cancer and cancer of unknown primary origin. PLoS One 2014;9: e95364.
63. McHugh JB. Association of cystic neck metastases and human papillomavirus-positive oropharyngeal squamous cell carcinoma. Arch Pathol Lab Med 2009;133:1798–803.
64. Goldenberg D, Begum S, Westra WH, Khan Z, Sciubba J, Pai SI, et al. Cystic lymph node metastasis in patients with head and neck cancer: an HPV-associated phenomenon. Head Neck 2008;30:898–903.
65. Mokhtari S. Mechanisms of cyst formation in metastatic lymph nodes of head and neck squamous cell carcinoma. Diagn Pathol 2012;7:6.
66. Regauer S, Beham A, Mannweiler S. CK7 expression in carcinomas of the Waldeyer's ring area. Hum Pathol 2000;31:1096–101.
67. Regauer S, Mannweiler S, Anderhuber W, Gotschuli A, Berghold A, Schachenreiter J, et al. Cystic lymph node metastases of squamous cell carcinoma of Waldeyer's ring origin. Br J Cancer 1999;79:1437–42.
68. Smith FJ, Porter RM, Corden LD, Lunny DP, Lane EB, McLean WH. Cloning of human, murine, and marsupial keratin 7 and a survey of K7 expression in the mouse. Biochem Biophys Res Commun 2002;297:818–27.