Nonkeratinizing Squamous Cell Carcinoma In Situ of the Upper Aerodigestive Tract: An HPV-Related Entity

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Abstract The clinical and pathologic characteristics of human papillomavirus (HPV)-related premalignant lesions in the upper aerodigestive tract have not been adequately studied. There are a few reports of oral cavity HPV-related severe dysplasia with unique morphology (prominent apoptosis/karyorrhexis imparting a ‘bowenoid’ appearance) and a single case report of HPV-related squamous cell carcinoma in situ with nonkeratinizing histology distinct from the ‘bowenoid’ pattern that extensively involved the upper aerodigestive tract. The aim of this study was to characterize the morphologic and clinical features of HPV-related severe dysplasia/carcinoma in situ. All cases of upper aerodigestive tract severe dysplasia/carcinoma in situ (111 cases from 98 patients) at Washington University from July 2012 to March 2015 were categorized into histologic types: keratinizing, nonkeratinizing, mixed or ‘bowenoid’. There were 83 (85 %) patients with keratinizing, 3 (3 %) nonkeratinizing and 12 (12 %) mixed

patterns. The previously reported ‘bowenoid’ morphology was not identified. All 3 (100 %) nonkeratinizing and 6 (50 %) mixed cases were p16 and HPV RNA in situ hybridization (RNA ISH) positive (100 % concordance between p16 and RNA ISH). Only 2 of 73 keratinizing cases were p16 positive, 1 of which was also HPV RNA ISH positive (1.4 % of keratinizing cases HPV-related). Thus, nonkeratinizing morphology was a strong predictor of transcriptionally-active HPV in severe dysplasia/carcinoma in situ. HPV-related cases most commonly occurred in the floor of mouth and were frequently extensive (≥ 4 cm) or unresectable.

Keywords Dysplasia · Carcinoma in situ · Upper aerodigestive tract · Nonkeratinizing · Human papillomavirus · p16 immunohistochemistry

Introduction

Over the past several decades, human papillomavirus (HPV) has emerged as a major carcinogen in squamous cell carcinoma (SCC) of the upper aerodigestive tract, particularly in the oropharynx, where worldwide a significant minority, if not majority, are HPV-related [1, 2]. Furthermore, the incidence of HPV-related oropharyngeal SCC appears to be on the rise and will likely surpass the incidence of HPV-related cervical cancer in the coming decade [3]. These HPV-related oropharyngeal SCCs have unique clinical and pathologic features that are becoming increasingly important in their diagnosis and treatment. The vast majority are histologically nonkeratinizing, and nonkeratinizing histology is a strong predictor of HPV-positivity [4]. In addition, they tend to be smaller tumors that have a predilection for the tonsils and base of tongue

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[5]. Despite their small size, lymph node metastases are frequent [6]. Yet, even with spread to regional lymph nodes, the prognosis is still much better than for HPV-negative oropharyngeal SCC [6–8]. Tumor HPV status has been recognized as the single greatest determinant of survival in patients with regionally advanced oropharyngeal cancer [8]. As a result, a growing number of clinical trials are aimed at tailoring therapy to tumor HPV status.

Even though patients with HPV-related oropharyngeal SCC have a much more favorable prognosis, the treatments are associated with significant morbidity and subsets of patients do die of disease [8]. Identification of HPV-related pre-invasive lesions in the head and neck would be desirable so that treatment could be initiated before invasive disease and lymph node metastasis develop. Unfortunately, the majority of HPV-related oropharyngeal SCCs arise deep in the invaginated tonsillar crypt mucosa so that early lesions are not clinically visible nor are they cytologically detectable [9]. Furthermore, even very early histologically ‘in situ’ appearing tonsillar crypt lesions have been associated with metastases, calling into question the existence of an HPV-related pre-invasive lesion in the tonsillar crypts altogether [10]. This is in stark contrast to HPV-related SCC of the uterine cervix where there is a clear progression from dysplasia to carcinoma. Early HPV-related cervical lesions can be detected by exfoliative cytology with or without HPV testing, which has led to a marked reduction in morbidity and mortality from cervical cancer ([https://report.nih.gov/nihfactsheets/Pdfs/CervicalCancer\(NCI\).pdf](https://report.nih.gov/nihfactsheets/Pdfs/CervicalCancer(NCI).pdf)).

HPV-related SCCs also occur at non-oropharyngeal sites in the upper aerodigestive tract, albeit much less commonly. The sinonasal tract and nasopharynx have recently emerged as other less frequent ‘hotspots’ for HPV-related SCC [11, 12]. However, HPV-related SCC is rare in the oral cavity and larynx [13, 14]. Despite growing amounts of data in the literature, the clinical significance of transcriptionally-active HPV in SCC outside of the oropharynx has not been established. Furthermore, there is very limited data regarding precursor lesions of HPV-related non-tonsillar SCC.

Recently, HPV-related severe dysplasia/carcinoma in situ (CIS) with unique histologic features has been described in a few studies at non-oropharyngeal sites in the upper aerodigestive tract but its clinical significance is not known. Woo et al. [15] and McCord et al. [16] identified HPV-related oral cavity severe epithelial dysplasia characterized by marked karyorrhexis and apoptosis, imparting a ‘bowenoid’ appearance. We also previously reported a single case of HPV-related CIS with nonkeratinizing histology resembling transitional epithelium that was morphologically distinct from the ‘bowenoid’ pattern and extensively involved the upper aerodigestive tract [17]. In

that case, the nonkeratinizing morphology matched the nonkeratinizing morphology that is seen in the majority of HPV-related oropharyngeal SCCs, except that it extensively coated the upper aerodigestive tract surface mucosa rather than originating in the oropharyngeal tonsillar crypts. Here, we undertake a preliminary study to investigate the frequency and clinical and morphologic features of HPV-related severe dysplasia/CIS at different upper aerodigestive tract sites.

Materials and Methods

Case Selection

This study was approved by the Human Studies Protection Office and was conducted using archived material from the Department of Pathology and Immunology at Washington University in St. Louis and Barnes-Jewish Hospital. All cases of upper aerodigestive tract severe dysplasia and CIS from July 2012 to March 2015 with available material (either biopsy or resection specimens) were retrieved and reviewed independently by two authors (LZ and RDC). The study was limited to severe dysplasia/CIS because prior studies have shown a much higher incidence of HPV in these compared to low grade dysplasia and interobserver agreement is more consistent [16]. The cases were categorized into histologic types: keratinizing, nonkeratinizing, mixed, or bowenoid. This morphologic classification departs from the traditional approach which is to consider dysplasia as keratinizing solely on the presence of keratin. Here, the keratinizing type had conventional features of dysplasia with polygonal shaped cells that had eosinophilic cytoplasm and distinct cell borders. Surface keratin was often present *but not required* for diagnosis. The nonkeratinizing type was characterized by dysplastic cells with oval to spindled nuclei, high nuclear to cytoplasmic ratios, indistinct cell borders and little to no surface maturation—resembling transitional epithelium. This type is cytologically akin to nonkeratinizing SCC of the oropharynx which is virtually always HPV-related [18]. Mixed cases contained any amount of nonkeratinizing dysplasia/CIS admixed with any amount of keratinizing dysplasia. There was no minimum percentage of each type required for the mixed category. The bowenoid type was defined according to the histologic features described by Woo et al. [15] as lesions with eosinophilic compact para- or orthokeratosis, epithelial hyperplasia with marked karyorrhexis and apoptosis throughout the epithelium and conventional features of dysplasia involving greater than 2/3 of the epithelium. The following exclusion criteria were applied: (1) superficial biopsies where the base was not visualized, (2) lesions within 1 mm of invasive carcinoma and (3)

dysplasia/CIS continuous with tonsillar or base of tongue carcinoma. The first two exclusion criteria were used simply to exclude direct sampling of an invasive carcinoma, rather than adjacent dysplasia. The third exclusion criterion was to ensure that the dysplasia/CIS did not represent an HPV-related tonsillar carcinoma spreading from the tonsillar crypts with secondary involvement of the surface mucosa, which is a commonly observed pattern of growth unique to tonsil cancers. In addition, dysplasia/CIS arising in other well-characterized neoplasms including Schneiderian papillomas, and verrucous proliferations or papillary squamous cell carcinoma in situ, were also excluded. Clinical data was obtained by chart review.

Immunohistochemistry for p16

Immunohistochemistry for p16 (as a surrogate marker for HPV) was performed on formalin-fixed paraffin-embedded, 4 µm tissue sections using an antibody to CDKN2A (p16) (E6H4 clone, CINtec; MTM Laboratories, Westborough, MA; prediluted). Immunostaining was performed on a Ventana Benchmark automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) according to standard protocols with appropriate positive controls. Antigen retrieval, standard on the machine, utilized the Ventana CC1, EDTA-Tris, pH 8.0 solution. Strong diffuse nuclear and cytoplasmic staining in >70 % of tumor cells was used as criteria for p16 positivity. Equivocal patterns were 50–70 % staining and weak diffuse staining; other staining patterns were considered negative.

HPV RNA In Situ Hybridization (ISH)

Automated RNA ISH assay was performed using the ViewRNA eZ-L Detection Kit (Affymetrix, Santa Clara, CA). ISH staining was performed on 5 µm formalin-fixed paraffin-embedded tissue sections utilizing cocktail HPV 16/18 probes (Affymetrix, Santa Clara, CA). Sections were deparaffinized, ISH stained using ViewRNA eZ-L Detection Kit on the Bond RX IHC and ISH Staining System (Leica Biosystems, Leica Microsystems Inc., Buffalo Grove, IL), followed by counterstaining with hematoxylin. The method was previously described by Kerr et al. [19]. Briefly, RNA was unmasked with a 10-min incubation at 95 °C in Bond Epitope Retrieval Solution 1 (Leica Biosystems, Buffalo Grove, IL), followed by 20-min incubation with Proteinase K from the Bond Enzyme Pretreatment Kit at 1:1000 dilution (Leica Biosystems, Buffalo Grove, IL). Hybridization was performed with a cocktail of HPV16 and HPV18 types at a 1:40 probe dilution. Punctate red color hybridization signals in the tumor cell nuclei and cytoplasm defined positivity. Negative cases by cocktail HPV16/18 probe

were additionally assayed for pooled HR-HPV probe set that recognizes 14 HR-HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) using a 1:200 probe dilution.

Results

Histologic Classification

During the study period, there were 111 cases of severe dysplasia/CIS of the upper aerodigestive tract from 98 patients. Subsequent specimens that also contained dysplasia/CIS from the same patients showed the same histologic type, therefore only individual patients were considered. Of the 98 patients, 83 (85 %) had keratinizing type, 3 (3 %) nonkeratinizing, and 12 (12 %) mixed keratinizing and nonkeratinizing severe dysplasia/CIS. None had the previously described HPV-related ‘bowenoid’ features. One of the patients with nonkeratinizing CIS was included in a previous case report [17]. This patient was included in this study because he developed recurrent CIS during the study period. Representative images from the keratinizing and nonkeratinizing cases are shown in Fig. 1 and of a mixed case in Fig. 2. An example of ‘bowenoid’ dysplasia, which was an outside consult case and not among the study cases, is shown for comparison (Fig. 3).

p16 and HPV RNA ISH Results

P16 immunohistochemistry (IHC) was performed on all cases with sufficient material available for testing (Table 1). All 3 (100 %) nonkeratinizing and 6 of the 12 (50 %) mixed histology cases were p16 positive (Fig. 4). Two of 73 (3 %) keratinizing cases with sufficient material for testing were p16 positive. Three additional keratinizing cases were considered equivocal—2 showed partial p16 staining in 50–70 % of cells and 1 showed weak cytoplasmic only staining in >70 % of cells.

HPV RNA ISH was performed on all cases of nonkeratinizing and mixed nonkeratinizing/keratinizing cases as well as the 5 p16 positive and/or equivocal keratinizing cases (Table 1). There was 100 % concordance between p16 and HPV RNA ISH confirming the presence of transcriptionally active virus in all 9 of the p16 positive nonkeratinizing and mixed histology cases, whereas all 6 of the p16 negative mixed histology cases were HPV RNA ISH negative (Fig. 5). One of the 2 p16 positive keratinizing cases was HPV RNA ISH positive; none of the p16 equivocal cases were HPV RNA ISH positive. In summary, of 88 patients with upper aerodigestive tract severe dysplasia/CIS and material available for testing, 10 (or 11 %)

Fig. 1 Examples of keratinizing and nonkeratinizing severe dysplasia/carcinoma in situ. Keratinizing severe dysplasia/carcinoma in situ (**a, c, e**) is composed of polygonal shaped cells with eosinophilic cytoplasm and often distinct cell borders. In contrast,

nonkeratinizing carcinoma in situ (**b, d, f**) resembles transitional epithelium and is composed of cells with scant cytoplasm and oval to spindled hyperchromatic nuclei and indistinct cell borders

Fig. 2 An example of a mixed histology severe dysplasia/carcinoma in situ case in which both keratinizing (*top left*) and nonkeratinizing (*bottom right*) areas are present

were HPV-related. The HPV-related cases represent 100, 50 and 1.4 % of the nonkeratinizing, mixed, and keratinizing cases, respectively.

HPV RNA signals were seen in both the nonkeratinizing and keratinizing components in all of the mixed cases (Fig. 5). In addition, an abrupt loss of HPV signals was noted as the epithelium transitioned from dysplasia to histologically normal (i.e. HPV positivity was not seen in the adjacent histologically normal mucosa, Fig. 5). Eight of the 10 HPV RNA ISH positive cases were positive for the HPV16/18 cocktail probes and 2 cases were positive for the pooled high-risk HPV probes only (i.e. HPV types other than 16 or 18). The 2 cases positive for non-16/18 HPV types had mixed nonkeratinizing and keratinizing histology.

Fig. 3 An example of ‘bowenoid’ dysplasia (not from this study) shows severe dysplasia involving greater than 2/3 the thickness of the epithelium with prominent karyorrhexis and a layer of keratin on the surface (**a**, **b**)

Table 1 p16 immunohistochemistry and HPV RNA ISH results in carcinoma in situ/severe dysplasia

Histologic type	p16 IHC positive N (%)	HPV RNA ISH positive N (%)
Nonkeratinizing (N = 3)	3 (100 %)	3 (100 %)
Mixed (N = 12)	6 (50 %)	6 (50 %)
Keratinizing (N = 73)	2 (3 %)	1 (1.4 %)

IHC immunohistochemistry, ISH in situ hybridization

Fig. 4 The vast majority (97 %) of keratinizing dysplasia were p16 negative. A representative example is shown (**a**). All nonkeratinizing and 50 % of mixed keratinizing and nonkeratinizing severe dysplasia/

CIS cases were p16 positive. A p16 positive nonkeratinizing CIS case is shown (**b**)

Clinical and Pathologic Features of HPV-Related Severe Dysplasia/CIS

The clinical and pathologic features of the 10 HPV-related severe dysplasia/CIS cases are summarized in Table 2. Among the 10 cases, there were 7 males and 3 females, with a mean age of 63.4 years (range 45–71). All patients had a history of tobacco use, although one patient had only a remote history of occasional (non-daily) smoking. Cases involved a variety of head and neck sites including oral cavity (5 or 50 %), oropharynx (1 or 10 %), larynx (2 or 20 %) and nasal cavity (1 or 10 %). One (10 %) involved multiple sites. The floor of mouth (FOM) was the most commonly involved subsite (6/10, 60 %). The site distribution of the HPV-related cases was similar to the HPV-unrelated cases. For comparison, 49 of the 88 (56 %) HPV-

unrelated patients had dysplasia involving the oral cavity, 23 (26 %) the larynx, 3 (3 %) the oropharynx, 2 (2 %) the hypopharynx, 2 (2 %) the paranasal sinuses, 2 (2 %) the posterior pharyngeal wall, not otherwise specified, and 7 (8 %) multiple sites.

Seven patients or 70 % had extensive disease (either measuring ≥ 4 cm or clinically unresectable). Of these, 2 patients had severe dysplasia/CIS that measured ≥ 4 cm and 5 had clinically unresectable disease. The patients with unresectable disease are detailed as follows: Patient #1 (previously reported patient) had extensive CIS of the aerodigestive tract clinically manifesting as erythroplakia involving multiple sites in the oral cavity, oropharynx and larynx that was unresectable due to the extent of disease [17]. The patient was treated with high-dose retinoic acid. Patient #2 had an invasive squamous cell carcinoma of the

Fig. 5 HPV RNA ISH was 100 % concordant with p16 status with all p16 positive nonkeratinizing and mixed cases containing transcriptionally active virus. One of 2 p16 positive keratinizing cases was also HPV RNA ISH. An example of positive HPV RNA ISH in a nonkeratinizing CIS case is shown (*red* nuclear and cytoplasmic dots (**a**)). An abrupt transition was seen between HPV RNA ISH positive CIS and HPV RNA ISH negative adjacent normal mucosa (**b**). In mixed cases, HPV RNA ISH was positive in both the keratinizing (*top*) and nonkeratinizing (*bottom*) areas (**c**)

larynx invading the thyroid gland. He underwent a laryngectomy and had incidental CIS extending to the tracheal margin, identified intra-operatively on frozen sections, which was re-excised. The new tracheal margin was also positive for CIS but the surgeon felt that he could not further resect additional trachea. The patient received adjuvant chemoradiation. Patient #4 had a squamous cell

carcinoma of the soft palate excised with CIS at the margins. The patient was then taken back to the operating room for re-excision. On re-excision, the pathologic margins continued to be positive for CIS on intra-operative frozen section and the surgeon felt he could not obtain a negative margin due to ‘field cancerization’. The patient did not receive adjuvant therapy. Patient #5 underwent a right hemiglossectomy for a squamous cell carcinoma. Severe dysplasia/CIS was present at multiple margins, identified on intra-operative frozen section. The surgeon re-excised multiple margins but was unable to clear the ventral tongue margin. The patient underwent post-operative radiation. Patient #6 had a squamous cell carcinoma of the nasal cavity and papillary CIS of the opposite nasal cavity (two separate lesions). There was extensive CIS surrounding the invasive squamous cell carcinoma that was present at the margin, which was re-excised and the re-excision also had CIS at the margin. The surgeon felt that he could not clear the CIS at the margin and the patient chose active surveillance rather than post-operative radiation. These findings show that HPV-related severe dysplasia/CIS is frequently unresectable and often extends beyond the borders of clinically visible lesions.

Clinical follow up information is described in Table 3. Most study patients (7/10, 70 %) had concurrent invasive SCC. The concurrent invasive SCCs were p16 positive except in one patient (Case 5) who had 2 separate primaries, 1 of which was p16 positive and the other p16 negative. There was insufficient tumor remaining for p16 IHC in 1 case. The average length of follow up for the study cases was 24.2 months (range 6.7–68.2). Three patients subsequently developed recurrent invasive SCC, 2 of which were p16 positive and 1 p16 negative. Two patients also developed recurrent, p16 positive nonkeratinizing CIS. The histologic types of the concurrent and recurrent SCCs were variable (4 keratinizing, 4 nonkeratinizing and 2 mixed). In one case, the invasive component was minimal, limiting our ability to characterize the histologic type. At last follow up all patients were alive and free of disease except for one patient who was receiving palliative chemotherapy for recurrent invasive nonkeratinizing SCC involving the oral and base of tongue.

Discussion

HPV-related premalignant lesions of the head and neck are poorly characterized. Although numerous studies have evaluated squamous dysplasia/CIS of the upper aerodigestive tract for the presence of HPV, the rates are highly variable among studies, ranging from 0 to 100 % [20–27]. This variability may be attributed to differences in inclusion criteria and HPV detection methods, small sample size

Table 2 Clinical and pathologic features of HPV-related severe dysplasia/CIS

Case	HPV type (RNA ISH)	Histologic type	Site	Clinical extent	Age	Sex	Tobacco use
1 ^a	16/18	NK	Oral ^b /orop/larynx	Unresectable	60	M	Yes
2	16/18	NK	Subglottis/Trachea	Unresectable	60	M	Yes
3	16/18	NK	Oral: buccal/FOM/tongue/palate	4 cm	69	M	Yes
4	16/18	Mixed	Soft palate	Unresectable	65	F	Yes
5	16/18	Mixed	Oral: FOM/ventral tongue	Unresectable	71	M	Yes
6	16/18	Mixed	Nasal cavity	Unresectable	67	F	Yes ^c
7	Non-16/18	Mixed	Larynx: vocal cords	Limited to vocal cords	45	F	Yes
8	Non-16/18	Mixed	Oral:FOM/ventral tongue	At least 2.5 cm	71	M	Yes
9	16/18	Mixed	Oral:FOM/ventral tongue	4 cm	60	M	Yes
10	16/18	K	Oral: FOM/ventral tongue	Focal	66	M	Yes

NK nonkeratinizing, Oral oral cavity, Orop oropharynx, FOM floor of mouth, K keratinizing

^a Previously reported patient

^b Including the floor of mouth

^c Remote history of social (non-daily) smoking

and different patient populations [28, 29]. For example, some studies included ‘oral leukoplakia’ without mention of the degree of histologic dysplasia (so some of these cases might not actually be dysplasia at all), while other studies include both oral cavity and oropharynx sites together. Small biopsies of oropharyngeal tonsils that appear histologically to contain CIS may actually represent superficial portions of an invasive SCC, and thus invasive SCCs may have been inadvertently included in studies of ‘pre-malignant’ oropharyngeal lesions. For this reason, we excluded superficial biopsies. We also excluded any case where the clinical lesion was not clearly separate from a tonsil or base of tongue SCC, since these are known to originate predominantly in the tonsillar crypts, without a defined pre-malignant phase, rather than surface mucosa.

The method of HPV detection is also critical, since HPV DNA PCR alone may detect ‘bystander’ virus that is not transcriptionally-active and, further, some DNA ISH methods lack sensitivity for HPV. To our knowledge, our previous case report and the current study represent the only literature on HPV RNA ISH in the evaluation of pre-malignant upper aerodigestive tract lesions [17]. HPV RNA ISH is sensitive and specific for transcriptionally-active HPV [30, 31]. In addition, it has the advantage of being slide-based so that one can visualize viral signals in individual cells. This is especially important in cases of dysplasia/CIS where the lesion of interest may be focal within the examined tissue. In fact, we noted an abrupt transition between HPV RNA ISH positive CIS and RNA ISH negative adjacent normal mucosa.

Despite the above described limitations in the literature, a meta-analysis did show an increased risk of HPV detection in oral cavity pre-malignant lesions and dysplasia relative to controls biopsies (odds ratio of 3.87, 95 % CI 2.22–6.13 and

5.10, 95 % CI 2.03–12.80, respectively) [21]. There is much less data for other head and neck mucosal subsites. Laco et al. [23] found 67 % of laryngeal dysplasias to be HPV positive by DNA ISH but none were strongly and diffusely p16 positive, suggesting that the virus was not transcriptionally active in these cases. p16 immunohistochemistry is a very sensitive surrogate marker of transcriptionally-active HPV, although it lacks specificity in certain situations, especially outside of oropharyngeal SCC. p16 negativity almost always indicates that HPV, if it is present, is not transcriptionally active [6, 30–32].

There is little description in the literature of the morphology of HPV-related pre-malignant lesions. Daley et al. [33] described a high rate of HPV by DNA ISH in several cases of oral cavity dysplasia that had ‘bowenoid’ histologic features (numerous mitoses and apoptotic debris throughout the epithelium). This unique morphology has been corroborated in two recent studies of oral cavity dysplasia that utilized both HPV DNA ISH and p16 immunohistochemistry [15, 16]. McCord et al. [16] found that a significant subset of high grade (but not low grade) oral cavity dysplasias were HPV-related (7 of 40 or 17.5 %). They noted that the HPV-related case had unique morphology and described them as ‘basaloid’ with mitotic activity and apoptosis throughout the thickness of the epithelium. Woo et al. [15] examined only oral cavity severe dysplasias with ‘marked karyorrhexis/apoptosis’ and found all 20 cases to be HPV-related. Images of the HPV-related oral cavity dysplasias from both studies appear quite similar and show near full-thickness dysplasia with a layer of surface keratin and prominent karyorrhexis/apoptosis throughout. We believe these cases have a ‘bowenoid’ appearance and may be similar to that previously described by Daley et al. [33]. Although we did not

Table 3 Clinical course of HPV-related severe dysplasia/CIS

Case	Prior or Concurrent SCC?	SCC p16 IHC	Stage	Treatment	Recurrence	Recur p16 IHC	Patient status	LOF (months)
1 ^a	No	NA	NA	Surgery + high-dose retinoic acid	K SCC larynx, NK CIS UAT (multiple)	+	AWOD	67
2	Concurrent (mixed K/NK SCC larynx)	+	T4aN1M0	Surgery + chemoradiation	No	NA	AWOD	8
3	Prior (NK SCC BOT) and Concurrent/recurrent (NK SCC oral tongue)	+	T3-4N1M0	Palliative chemotherapy	NA	NA	AWD	6
4	Concurrent (NK SCC soft palate)	+	T1N0M0	Surgery	NK SCC Nasopharynx	+	AWOD	38
5	Concurrent (two primaries: mixed K/NK SCC FOM and K SCC maxillary alveolus)	+	T1N0M0	Surgery + radiation	K SCC BOT	–	AWOD	27
6	Concurrent (NK SCC nasal cavity and NK papillary CIS of opposite nasal cavity)	+	T1N0M0	Surgery	NK CIS nasal cavity	+	AWOD	13
7	Concurrent (minimal invasive SCC larynx)	Insufficient material	T1N0M0	Surgery	No	NA	AWOD	33
8	Concurrent (K SCC oral tongue)	+	T2N0M0	Surgery + radiation	No	NA	AWOD	29
9	No	NA	NA	Surgery	No	NA	AWOD	8
10	No	NA	NA	Surgery	No	NA	AWOD	13

IHC immunohistochemistry, Recur recurrence, LOF length of follow up, NA not applicable, K keratinizing, SCC squamous cell carcinoma, UAT upper digestive tract, CIS carcinoma in situ, AWOD alive without disease, NK nonkeratinizing, BOT base of tongue, AWD alive with disease, FOM floor of mouth

^a Previously reported patient

identify any such ‘bowenoid’ cases in the present study, we have seen a recent oral cavity dysplasia with ‘bowenoid’ morphology (Fig. 1f).

There are several reasons why the current study may not have identified any cases of the previously described ‘bowenoid’ HPV-related dysplasia pattern. First, unlike the prior studies, we included all upper aerodigestive tract sites (of which only half were exclusively from the oral cavity), whereas the prior studies were limited to the oral cavity. ‘Bowenoid’ dysplasia may be unique to the oral cavity and therefore studies limited to the oral cavity would be enriched for these cases. Second, concurrent cancer was frequent among our cases but less common in the prior studies. This may reflect differing patient populations at each referral center. We have a high volume of referrals for surgical management of head and neck cancer compared to oral biopsies.

We previously reported a single case that we believed to be unique of HPV-related CIS extensively involving the upper aerodigestive tract that had nonkeratinizing morphology distinct from the previously described ‘bowenoid’ oral cavity dysplasias [17]. Morphologically, this case almost perfectly recapitulated the morphology of HPV-related nonkeratinizing SCC in the oropharyngeal tonsils and resembles transitional epithelium. The dysplastic cells had hyperchromatic, oval to spindled nuclei, scant cytoplasm and indistinct cell borders with little to no squamous maturation. However, the patient did not have invasive oropharyngeal SCC and was status post tonsillectomy for tonsillar hypertrophy. Yet, the CIS extensively coated the surface of the oral cavity, oropharynx and larynx without an invasive cancer. This case lacked the prominent karyorrhexis which defines the ‘bowenoid’ pattern. The cytomorphology was also distinct from the ‘bowenoid’ pattern, in which the dysplastic cells have more eosinophilic cytoplasm, prominent surface keratinization and lack spindled nuclei.

Here, we found the frequency of transcriptionally-active HPV was 11 % among severe dysplasia/CIS of the upper aerodigestive tract. Nonkeratinizing morphology was a strong predictor of transcriptionally-active HPV with all 3 patients (100 %) with pure nonkeratinizing and 6 patients (50 %) with mixed nonkeratinizing and keratinizing severe dysplasia/CIS being HPV-related. In contrast, transcriptionally-active HPV was rare in keratinizing dysplasia (1.4 % of cases). We could not determine a reason, such as subtle morphologic differences between HPV-positive and negative cases, that only 50 % of the mixed histology cases were HPV-related. However, the distribution of HPV across the 3 histologic types of severe dysplasia/CIS is akin to that seen in invasive tonsillar SCC in which nonkeratinizing histology is virtually synonymous with HPV, many but not all mixed (also referred to as ‘nonkeratinizing with maturation’) SCCs are HPV-related and keratinizing SCCs are only HPV-related in a minority of cases [4].

A variety of mucosal subsites were involved by HPV-related severe dysplasia/CIS; the most commonly affected site was the floor of the mouth. Interestingly, the floor of mouth was also the most commonly involved site of the ‘bowenoid’ pattern of HPV-related dysplasia in both the Woo et al. [15] and McCord et al. [16] studies. The reason for the frequent floor of mouth involvement is unclear. Tobacco-related dysplasias also have a predilection for the floor of mouth possibly because carcinogens in tobacco pool in saliva [34, 35]. HPV may have a similar predisposition for saliva or the floor of mouth mucosa, or may simply be acting as a co-factor to a subset of tobacco-related dysplasias. Another feature of the HPV-related cases in our study was the presence of extensive disease. This is different from the HPV-related ‘bowenoid’ dysplasias in the Woo et al. [15] study, which were mostly smaller lesions, almost all less than 4 cm. Interestingly, 2 of the 3 focal/resectable cases in our study were the only 2 cases that harbored HPV subtypes other than 16/18. The third was the only keratinizing type of the HPV-related cases. It is plausible that clinical disease patterns of dysplasia differ among HPV types. An example of such variability is seen in the cervix, where HPV type 18 is associated more strongly with cervical adenocarcinomas and HPV type 16 with squamous cell carcinoma. Further investigation in a larger number of cases is warranted.

In summary, this study expands the spectrum of HPV-related premalignant lesions by defining a new nonkeratinizing pattern of severe dysplasia/CIS. While HPV-related severe dysplasia/CIS is not common, these lesions appear to have characteristic features including nonkeratinizing histology, frequent involvement of the floor of mouth and a propensity for extensive disease. It appears that the floor of mouth may be another ‘hot spot’ for HPV-related disease. Because this is a preliminary study and the clinical implications are unclear, routine HPV testing of head and neck dysplasias/CIS is not indicated at this time. However, further study is warranted.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest or sources of support to disclose.

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