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Human Papillomavirus-Related Carcinoma with Adenoid Cystic-Like Features: A Peculiar Variant of Head and Neck Cancer Restricted to the Sinonasal Tract

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Abstract

HPV-related carcinomas of the head and neck are characterized by a predilection for the oropharynx, a non-keratinizing squamous morphology, and infection with the HPV 16 type; but comprehensive HPV testing across all head and neck sites has shown that the pathologic features of HPV-related carcinoma may be more wide-ranging than initially anticipated. In particular, a subset of sinonasal carcinomas are HPV positive, and these include a variant that is histologically similar to adenoid cystic carcinoma (ACC). Cases were identified by retrospective and prospective analyses of head and neck carcinomas with ACC features. HPV analysis was performed using p16 immunohistochemistry and high risk HPV in-situ hybridization. HPV-positive cases were confirmed and typed using HPV type-specific quantitative PCR, and further characterized regarding their immunohistochemical profile and *MYB* gene status. HPV was detected in 8 carcinomas of the sinonasal tract, but it was not detected in any ACCs arising outside of the sinonasal tract. The HPV types were 33 (n=6), 35 (n=1) and indeterminate (n=1). Six patients were women and two were men, ranging in age from 40–73 years (mean 55). The carcinomas were characterized by a nested growth, a prominent basaloid component showing myoepithelial differentiation and forming microcystic spaces, and a minor epithelial component with ductal structures. Squamous differentiation, when present, was restricted to the surface epithelium. The carcinomas were not associated with the *MYB* gene rearrangement that characterizes a subset of ACCs. These cases draw attention to an unusual variant of HPV-related carcinoma that has a predilection for the sinonasal tract. Despite significant morphologic overlap with ACC, it is distinct in several respects including an association with surface squamous dysplasia, absence of the *MYB* gene rearrangement, and an association with HPV, particularly type 33. As HPV positivity confers distinct clinico-pathologic characteristics when encountered in the oropharynx, a more comprehensive analysis of risk factors, response to therapy and clinical outcomes is warranted for HPV-related carcinomas of the sinonasal tract.

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Keywords

Human papillomavirus; sinonasal carcinoma; adenoid cystic carcinoma; squamous cell carcinoma

Introduction

The human papillomavirus (HPV) is a causative agent in approximately 20–25% of head and neck carcinomas.(1) These HPV-related carcinomas have a strong site predilection for the oropharynx where they account for up to 80% of squamous cell carcinomas arising from this location.(2) The likelihood of detecting transcriptionally active HPV in head and neck carcinomas arising outside of the oropharynx is very low, with the notable exception of the sinonasal tract.(3–5) Using an approach that combined HPV DNA in situ hybridization and p16 protein immunohistochemistry, we previously detected high risk HPV in 21% of sinonasal carcinomas, thus establishing HPV as a significant causative factor for carcinomas arising in the nasal cavity and paranasal sinuses.(3) HPV-related carcinomas of the head and neck tend to be squamous cell carcinomas of the non-keratinized type, but HPV positivity is not restricted to this archetypal phenotype when dealing with sinonasal carcinomas. HPV analysis of a large and comprehensive group of sinonasal carcinomas has turned up a peculiar form of HPV-related carcinoma exhibiting features of a salivary gland carcinoma and frequently carrying the pathologic diagnosis of adenoid cystic carcinoma (ACC).(3) The association of high risk HPV with the adenoid cystic phenotype is not without precedence. High risk HPV is frequently detected in the rare ACC arising in the cervix.(6–9) As for the head and neck, Boland et al. detected high risk HPV in 7% of ACCs.(10) Notably, the HPV-positive cases were restricted to the sinonasal tract and exhibited a high grade solid component.(10) Our current study was undertaken to provide a more complete description of HPV-related carcinomas of the head and neck with adenoid cystic-like features.

Methods

Cases

Study approval was obtained from the Johns Hopkins University Internal Review Board. Study cases were identified in three ways: 1) HPV testing was performed on tissue blocks of 161 consecutive primary sinonasal carcinomas diagnosed at the Johns Hopkins Hospital between 1995 and 2011 as previously reported.(3) 2) HPV testing was performed on TMAs constructed from the tissue blocks of 98 head and neck ACCs arising outside of the sinonasal tract, including parotid gland (n=32), oral cavity (n=30), submandibular gland (n=13), larynx/trachea (n=8), periorbital tissues (n=8), ear (n=4), and sublingual gland (n=3). The ACCs consisted of 62 classic cribriform types, 20 solid variants, and 16 tubular variants. Additional non-nasal salivary gland tumors that were present on the TMAs were pleomorphic adenomas (n=31), mucoepidermoid carcinomas (n=30), acinic cell carcinomas (n=18), polymorphous low grade adenocarcinomas (n=5), and adenocarcinomas not otherwise specified (n=5). 3) Finally, selective HPV testing was prospectively performed on primary sinonasal carcinomas with adenoid cystic-like features that were seen in the consultation practice of the study authors (J.A.B. and W.H.W).

Immunohistochemistry

Immunohistochemical studies were performed on five-micron sections prepared from formalin-fixed and paraffin embedded tissue using standard autostaining protocols on a Ventana Benchmark XT autostainer (Ventana Medical Systems, Inc. Tucson, AZ). Deparaffinization and antigen retrieval (i-view detection system; Ventana) were carried out as an automated program of the Ventana autostainer. The primary antibodies and final

dilutions were: p16 (clone INK4a; MTM Laboratories, Heidelberg, Germany); p63 (clone 4A4; Cell Marque Corp., Rocklin, CA); muscle specific actin (clone HHF35; Ventana); calponin (clone CALP; Dako); S100 (clone 4C4.9; Ventana); c-kit (clone YR145; Cell Marque); and AE1/AE3 (clone PCK26; Ventana). As a surrogate marker for the presence of HPV, p16 immunostaining was regarded as positive only if staining was nuclear and cytoplasmic, and present in 70% of the tumor cells.

HPV DNA in situ hybridization

Five-micron sections from formalin-fixed paraffin embedded tumor blocks were evaluated for the presence of HPV DNA by two different methods of in situ hybridization. Each case underwent testing by both an automated protocol utilizing the Ventana HR HPV III probe set that captures HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66 (Ventana Medical Systems, Tucson, AZ). The tumors were also tested using type specific probes for high risk types 16, 18, and 31 and 33 using the catalyzed signal amplification method for biotinylated probes (DAKO GenPoint, Carpinteria, CA). For each method, a positive result was defined by the presence of punctate signals localized to tumor nuclei. HPV-positive controls were cases of HPV-16, HPV-18, and HPV-31/33-positive oropharyngeal cancers, as well as the HPV16-positive SiHa and CaSki cell lines. An HPV negative head and neck squamous cell carcinoma served as a negative control.

An HPV-positive case was defined as those tumors that were p16 positive by immunohistochemistry and were HPV DNA positive by one of the in situ hybridization probes. Quantitative polymerase chain reaction (see below) was used to confirm the presence and type of HPV in the HPV positive cases.

Quantitative polymerase chain reaction

All HPV-related carcinomas with adenoid cystic-like features identified by in situ hybridization were also tested with quantitative HPV-specific PCR. DNA was extracted from slides containing 5 µm-thick formalin-fixed paraffin-embedded tumor samples. Briefly, tissues were deparaffinized using xylene, macrodissected off the slides, and then underwent digestion with 50 µg/mL proteinase K (Boehringer Mannheim) in the presence of 1% sodium dodecylsulfate at 48°C for 2 days. DNA was then extracted using UltraPure Phenol:Chloroform:Isoamyl Alcohol reagents (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

The general consensus primers Gp5-Gp6 and Gp5+-Gp6+ were used to amplify the L1 region in the HPV genome as described previously (11) using the following 30µl PCR solution: 16.6mM Ammonium Sulfate, 67.0 mM Tris-Trizma pre-set crystals (pH 8.8), 6.7 mM Magnesium Chloride, 10.0 mM 2-MercaptoEthanol, 0.1% Dimethyl Sulfoxide, 3.3% DMSO, 20 pmol of each primer and 0.5 U Platinum Taq. The fast PCR program used was: 95°C for 30sec, 44°C for 60sec and 72°C for 90sec for 40 cycles in a Veriti thermal cycler (Applied Biosciences). Furthermore, type-specific primers for the E6 and E7 regions for HPV types 11, 16, 18, 31, 33 and 35 were used as described previously.(12) The PCR conditions used were the same as described above except that the amplification cycle was shortened to 35 and the annealing temperature for the HPV 33 and 35 primers was 57°C for 30 seconds.

Fluorescence in situ hybridization

Nick translated probes produced from bacterial artificial chromosome (BAC) clones were obtained from Empire Genomics as a hybridization-mixture with human cot-1 DNA. Tissue microarray slides were incubated at 56°C overnight, deparaffinized using xylene, and rehydrated using a graded series of ethanol baths. Slides were heated in target retrieval

solution (Dako) at 95°C for 40 minutes, rinsed with distilled water for 5 minutes, digested with Proteinase K at room temperature for 8 minutes, rinsed with distilled water for 5 minutes, then dehydrated with a graded series of alcohol baths. For the MYB break-apart FISH assay, BAC clone RP11-104D9 labeled with 5-Fluorescein and BAC clone RP11-170P19 labeled with 5-carboxy-X-rhodamine (5-Rox) were used. The probe/in situ hybridization buffer mixture was distributed across the microarray area, covered with a glass cover slip, and sealed with rubber cement. Slides were incubated in a humidified chamber at 73°C for 5 minutes to denature the microarray DNA followed by 37°C for 18 hours to hybridize with the probes. After hybridization, slides were washed with 2× SSC at room temperature for 5 minutes, 0.4× SSC/0.3% NP-40 at 73°C for 2 minutes, 2× SSC/0.1% NP-40 at room temperature for 1 minute, and rinsed with distilled water at room temperature for 1 minute. Slides were counterstained using DAPI in Vectashield mounting medium (Vector Laboratories), covered with a glass cover slip, and sealed with CytoSeal XYL. Slides were examined on a fluorescence microscope (Imager A.2, Carl Zeiss Imaging Solutions GmbH) equipped with a 150× Plan-APOCHROMA objective and images captured using a digital camera (AxioCam MRm, Zeiss) controlled by AxioVision software (Release 4.8, Zeiss). A minimum of 100 nuclei were examined per specimen to obtain the consensus probe count.

Results

Eight HPV-related carcinomas with adenoid cystic-like features were identified. All cases arose in the sinonasal tract. All of the 184 salivary gland tumors that arose outside of the sinonasal tract, including 98 ACCs, were HPV-negative. The clinical demographics of these patients are summarized in Table 1. The carcinomas arose in 6 women and 2 men, ranging in age from 40 to 73 years (mean 55 years). Five of the patients were Caucasian, 2 were African-American, and in 1 case the patient's race was not provided. The presenting symptoms included nasal obstruction (n=6), epistaxis (n=5), and epiphora (n=1). Smoking history was available for 5 patients: 2 were current or former smokers (15 pack years for each patient), and 3 were never-smokers. The carcinomas involved the nasal cavity (n=6), the paranasal sinuses (n=5), and the orbit (n=1). The paranasal sinuses involved were the ethmoid (n=4), maxillary (n=2), and sphenoid (n=2). The tumor sizes ranged from 1.1 to 5.4 cm (mean, 3.0 cm). Stage information was available for 7 patients. Interestingly, all of the tumors were localized to the primary site with no evidence of local or distant metastasis. At the time of presentation, the patients were stage T1 N0 M0 (n=3), T3 N0 M0 (n=1) and T4 N0 M0 (n=3).

Treatment information was available for 7 patients. Two patients underwent surgical resection alone, three patients received surgery with post-operative radiation therapy, one patient received combined chemoradiation therapy, and one patient will be receiving surgery followed by radiotherapy but treatment has not yet commenced. The length of follow-up ranged from 0 to 35 months (median, 15 months). For two patients, no follow-up information was available, in one case because the patient has not yet been treated. Two patients experienced local recurrences which occurred at 23 and 24 months post-treatment. One of these patients initially underwent only surgery; the recurrence was also surgically removed and adjuvant external beam radiation was administered. That patient is currently without evidence of disease. The other patient with recurrent tumor had been initially treated with chemoradiation, and the recurrence was treated with surgical resection followed by further chemoradiation therapy. At last follow-up, the patient is alive but with persistent disease. The remaining four patients are alive and free of disease at last follow-up (2, 6, 33, and 35 months).

Microscopically, the carcinomas were seen as highly cellular proliferations of basaloid cells. The most consistent architectural pattern was solid with rounded nests of varying sizes and shapes separated by thin fibrous bands of collagenized stroma (Figure 1). Small dark cells at the border of the nests demonstrated peripheral pallisading in 4 cases. In 4 of the tumors, the solid pattern occurred together with a cribriform architecture where basaloid cells aligned around cylindromatous microcystic spaces (Figure 2A–B). The microcystic spaces were filled with a basophilic material resembling the mucopolysaccharide material filling the cribriform structures in ACC. Although not a dominant component of any of the tumors, all of the carcinomas demonstrated inconspicuous ductal formations within some of the nests (Figure 2C–D).

The most common cell type was the basaloid type. These basaloid cells were characterized by hyperchromatic and slightly angulated nuclei, scant cytoplasm, and a high nuclear to cytoplasmic ratio. These basaloid cells were tightly packed into the rounded nests and lined the microcystic spaces. The focal presence of cell spindling (n=5), cytoplasmic clearing (n=4), and peripheral orientation around an inner layer of ductal cells suggested myoepithelial differentiation. A second cell type was the true ductal cell. These cells were cuboidal with eosinophilic cytoplasm and were often present at the center of the nests and surrounded by zones of basaloid cells. Although squamous differentiation was not observed in the invasive component, 6 of 8 cases demonstrated squamous dysplasia of the surface epithelium (Figure 3).

The carcinomas had mitotic rates that ranged from 5–82 mitotic figures per 10 high-powered fields (mean 37). Six cases had a mitotic rate of at least 20 mitotic figures per 10 high-powered fields. Three cases had areas of tumor necrosis and two cases exhibited perineural invasion. Lymphovascular invasion was not seen in any of the cases.

The results of immunohistochemical analysis are summarized in Table 2. Each carcinoma was positive for the cytokeratin cocktail AE1/AE3. Cytokeratin staining was strong in the ductal cells, and relatively weaker in the abluminal basaloid cells (Figure 4). In all cases, the basaloid cells expressed two or more of the myoepithelial markers tested (i.e., S100, calponin, p63, and actin), usually in a strong and diffuse fashion (Figure 4). C-kit staining was present in all 8 cases. In 7 of 8 cases, c-Kit expression highlighted just the ductal cells, and in 1 case c-Kit staining was diffuse.

The HPV testing results for the adenoid cystic-like carcinomas of the sinonasal tract are shown in Table 3. P16 showed strong and diffuse immunoreactivity in all cases (Figures 4D and 5B). HPV in situ hybridization signals were present in all 8 cases: Five were positive with the high risk probe cocktail, 5 were positive with the probe for HPV types 31 and 33, and 2 were positive for HPV type 16 (Figures 4D inset and 5C). Hybridization signals were distributed throughout both the basaloid and ductal cells, and in both the surface dysplasia and the invasive component.

In the HPV 16 positive cases, the hybridization signals were weak and focal suggesting either low viral copy numbers, false positive hybridization, or cross reactivity with another viral type. Accordingly, we also performed quantitative PCR to further confirm the presence of high risk HPV and verify the specific HPV type. The presence of high risk HPV was confirmed for all cases using general consensus primers that amplify the L1 region in the HPV genome (Figure 5D). Using type specific probes for HPV 11, 16, 18, 31, 33 and 35, HPV 33 was detected in 6 cases and HPV 35 in 1 case. In one case, the HPV type could not be determined using our panel covering 6 HPV types. Type 16 was not detected, even in the 2 cases that showed weak hybridization signals by DNA in situ hybridization.

There was no evidence of transcriptionally active HPV in the adenoid cystic carcinomas arising outside of the sinonasal tract, or in any of the other types of salivary gland neoplasms. P16 positivity was observed in 7 of 19 (37%) non-sinonasal adenoid cystic carcinomas, 3 of 30 (10%) mucoepidermoid carcinomas, 1 of 31 (3%) pleomorphic adenomas, 0 of 18 (0%) acinic cell carcinomas, 0 of 5 (0%) polymorphous low grade adenocarcinomas, and 0 of 5 (0%) adenocarcinomas not otherwise specified; but all salivary gland tumors, including the 11 p16 positive cases, were HPV negative by wide spectrum in situ hybridization. P16 immunostaining as a sole surrogate marker for transcriptionally active HPV must be used with caution. As has been underscored in a growing experience with squamous cell carcinomas of the head and neck, even strong and diffuse p16 staining can be observed in the absence of HPV E6/E7 mRNA expression.(13)

Because of the strong morphologic resemblance of these cases to ACC, the tumors were evaluated for the presence of the *MYB-NFIB* gene fusion using a FISH assay. Recent studies have shown that a t(6;9)(q22-23;p23-24) translocation resulting in a *MYB-NFIB* gene fusion is specific for ACC, and can be detected in up to 49% of paraffin-embedded ACC specimens.(14, 15) The FISH assay for *MYB* was successfully performed on 6 of 8 of our cases, and all 6 exhibited intact loci consistent with a wild type (i.e. non-rearranged) *MYB*. The assay failed in the remaining two cases due to extensive tissue degeneration. Of note, these were the oldest cases in the series (16 years and 17 years).

Discussion

HPV-related head and neck squamous cell carcinomas are generally characterized by origin from the oropharynx,(1, 16) a non-keratinized squamous morphology,(17) and infection by the HPV 16 type.(1, 2) This study draws attention to a form of HPV-related head and neck carcinoma that departs from this prototype in 3 ways: 1) it exhibits morphologic and immunohistochemical features that are very reminiscent of salivary gland carcinoma, particularly adenoid cystic carcinoma (ACC); 2) it has a site predilection for the sinonasal tract; and 3) it is most commonly associated with HPV 33.

Morphologic resemblance to ACC is based on shared architectural patterns of growth including solid and cribriform patterns; a dual population of ductal cells and basaloid cells; the absence of squamous differentiation (at least in the invasive component); and the presence of a myoepithelial phenotype as evidenced by the immunohistochemical expression of several myoepithelial markers. These similarities can cause diagnostic confusion for the unwary pathologist. Indeed, 3 of the 4 retrospective cases were originally diagnosed as a solid variant of ACC; and the diagnosis of ACC was considered by the submitting pathologists in 2 prospective cases sent in consultation for tumor classification. Morphologic similarities aside, we believe that these HPV-related carcinomas do not represent true ACCs. First, they appear to be highly restricted in their distribution to the sinonasal tract. We did not detect HPV in any of the 108 ACCs arising outside of the sinonasal tract. The work of Boland et al also points to this site selectivity.(10) In their evaluation, ACCs of the head and neck, HPV detection was observed only in “high grade” (i.e. solid) ACCs of the nasal cavity. HPV was not detected in any of the 23 ACCs arising from other head and neck sites.(10) Second, none of the 6 tumors tested harbored the *MYB-NFIB* gene fusion, a genetic alteration that is detected in almost half of ACCs.(14, 15) Third, these HPV-related carcinomas are often associated with squamous dysplasia of the overlying surface epithelium. When dealing with basaloid neoplasms of the head and neck, the presence of surface dysplasia is generally taken as evidence against ACC.(18, 19)

Although we have designated these tumors in a way that draws attention to their morphologic similarity with adenoid cystic carcinoma, other salivary gland tumors may

enter the differential diagnosis to the degree that individual morphologic features are developed. In those tumors where the ductal component is more conspicuous and intimately admixed with the myoepithelial component, epithelial myoepithelial carcinoma may be a diagnostic consideration. For those tumors dominated by solid nests of basaloid cells with peripheral pallisading, the differential diagnosis may include basal cell adenocarcinoma. Finally, the presence of high grade microscopic features including tumor necrosis and a high mitotic rate may suggest high grade transformation (i.e. “de-differentiation”) of a lower grade salivary gland neoplasm. Indeed, high grade transformation can occur in adenoid cystic carcinomas(20, 21), and in epithelial myoepithelial carcinomas(22). Although we cannot exclude the possibility that HPV infection may be driving high grade transformation, there was no evidence of pre-existing or concurrent low grade conventional salivary gland carcinoma in any of the cases. We suspect that the converse may be more likely. In the absence of a low grade conventional component, high grade carcinomas of the sinonasal tract classified as “dedifferentiated adenoid cystic carcinomas” may actually represent HPV-related carcinomas with adenoid cystic like features.

The presence of squamous dysplasia also raises consideration of the basaloid variant of squamous cell carcinoma. To be sure, a form of HPV-related squamous cell carcinoma with prominent basaloid features is well recognized in the oropharynx.(23) The presence of true glandular structures together with a myoepithelial phenotype, however, does not support classification as a squamous cell carcinoma of surface origin. Instead, these features have been taken as evidence to support an HPV-related form of ACC in other sites such as the cervix.(6–9) In the sinonasal tract, the dual presence of surface dysplasia and an invasive salivary-like carcinoma suggests that HPV infection with HPV-induced tumorigenesis may target the ductal system of intranasal minor salivary glands at or near its transition with the surface epithelium.

In the oropharynx, over 90% of HPV-related squamous cell carcinomas are caused by HPV-16.(1, 2) HPV-16 is also the most common high risk HPV type in the sinonasal tract for those carcinomas taking on a more characteristic squamous morphology.(3, 5) In sharp contrast, HPV 33 was the most common high risk HPV type detected in those carcinomas with the ACC-like morphology. Using type-specific primers for the E6 and E7, HPV 33 was detected in 6 of the 8 HPV-positive cases. Each of these 6 cases exhibited strong p16 staining - a surrogate marker of high risk HPV infection and supportive evidence of transcriptionally active virus - and strong hybridization signals using an HPV31/33 DNA in situ hybridization probe. This finding suggests that a particular tumor phenotype may track with a specific HPV type due to different tissue tropisms of the HPV viral types. Interestingly, HPV 33 has also been detected in ACC of the cervix.(9) In this study we refer to this peculiar variant as “HPV related carcinoma of the sinonasal tract with ACC-like features - a designation that draws attention to its morphologic resemblance to the solid variant of adenoid cystic carcinoma. Admittedly, terminology may of necessity evolve as more is learned about the histogenic origin and behavior of this tumor type.

Although HPV 33 is associated with a histologically distinctive form of sinonasal carcinoma, its clinical relevance remains to be determined. The importance of this open question is highlighted by the clinical significance of HPV in oropharyngeal carcinoma where its detection serves as a powerful indicator of improved clinical outcome. But generalized application of this prognostic factor beyond the oropharynx may not be valid, particularly in these sinonasal carcinomas that depart from other HPV-related head and neck carcinomas with respect to location, microscopic appearance and HPV-type. In the uterine cervix, ACC is recognized as an aggressive form of cervical cancer with a propensity for local recurrences and widespread metastases despite its association with HPV.(24) In our study, there was no documented regional or distant metastasis, however the number of study

cases was small, and clinical follow-up was limited. Awareness of this morphologically distinct form of sinonasal carcinoma, together with the availability and easy application of HPV detection assays, now clears the way for the accumulation of additional cases and meaningful correlation with clinical parameters.

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Figure 1. HPV-related carcinomas with ACC-like features exhibit a consistent lobular pattern of growth. All four of these cases (A–D) show a proliferation of basaloid cells that are compacted into nests and lobules separated by a fibrous stroma.

Figure 2. Unlike basaloid variants of squamous cell carcinoma, HPV-related carcinomas with ACC often exhibited cribriform structures (A and B) and true ductal formations (C and D).

Figure 3.
Six of the eight carcinomas were associated with squamous dysplasia of the surface epithelium.

Figure 4.

HPV-related ACC-like carcinomas tend to exhibit both myoepithelial and ductal components (A) and these can be highlighted by immunohistochemistry. The ducts show stronger staining for AE1/AE3 (B), while the myoepithelial cells are strongly positive for p63 (C), calponin (D), and muscle specific actin (E). Both components are p16 positive (F) and exhibit hybridization signals for HPV 33 (inset of F).

Figure 5.

An HPV-related adenoid cystic-like carcinoma (A) demonstrating strong p16 immunohistochemical staining (B) and high risk HPV in situ hybridization signals (C). Quantitative PCR using general consensus (GP) and an HPV type specific probes confirms the presence of HPV 33 (lane 1 = HPV 16 positive CaSki cell line; and lane 2 = sinonasal carcinoma).

Table 1

Summary of clinical features HPV-related adenoid cystic-like carcinomas of the sinonasal tract

Patient			Tumor		Treatment		Follow-up (months)
Age	Sex	Presentation	Site	Size (cm)	Stage	1° recurrence	
46	F	epistaxis, obstruction	nasal	4	T1N0M0	surgery RT	NED (35)
48	F	obstruction	ethmoid	3	T1N0M0	surgery RT	LR (24) NED (27)
63	F	epistaxis	maxillary/ethmoid	3	T3N0M0	Surgery RT	NED (33)
61	M	epistaxis, obstruction	nasal	3	unknown	unknown	unknown
57	M	epistaxis	nasal, ethmoid/sphenoid	3	T4aN0M0	surgery	NED (2)
51	F	epistaxis	nasal	1	T1N0M0	surgery RT	NED (6)
40	F	obstruction	nasal, ethmoid/sphenoid	3	T4bN0M0	RT, CT	LR (23) AWD (24)
73	F	obstruction, epiphora	nasal, maxillary, orbit	5	T4aN0M0	pending	NA

F, female; M, male; NED, no evidence of disease; RT, radiation therapy; CT, chemotherapy; LR, local recurrence; NED, no evidence of disease; NA, not applicable

Table 2

Immunohistochemical profiles of the HPV-related ACC-like carcinoma

Case	AE1/AE3	Actin	p63	Calponin	S100	C-kit			
1	+	(B)	+	(A)	Focal	+	(L)		
2	+	(B)	+	(A)	-	+	(L)		
3	+	(B)	+	(A)	+	(A)	+	(D)	
4	+	(D)	Focal	-	+	(D)	+	(D)	
5	+	(B)	-	+	(A)	+	(A)	+	(L)
6	+	(B)	+	(D)	+	(A)	+	(L)	
7	+	(B)	Focal	+	(A)	+	(A)	+	(L)
8	+	(B)	+	(A)	+	(A)	Focal	+	(L)

B, biphasic staining (strong luminal, weak abluminal); A, abluminal staining; L, luminal staining; D, diffuse staining

Table 3

HPV status of HPV-related ACC-like carcinomas

Case	P16 IHC	DNA in situ hybridization wide spectrum*	DNA in situ hybridization		Quantitative PCR Consensus**	type specific
			16	31 and 33		
1	+	+	-	+	+	33
2	+	+	+	-	+	35
3	+	+	-	+	+	33
4	+	-	-	+	+	33
5	+	+	+	-	+	-
6	+	-	-	+	+	33
7	+	-	-	+	+	33
8	+	+	-	+	+	33

IHC, immunohistochemistry;

* HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66;

** HPV types 11, 16, 18, 31, 33 and 35;

w/ weak hybridization signals