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Ciliated HPV-related Carcinoma: A Well-Differentiated Form of Head and Neck Carcinoma That Can Be Mistaken for a Benign Cyst

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Abstract

Although HPV-related oropharyngeal carcinomas (HPV-OPCs) are generally regarded as “poorly differentiated”, they actually maintain a close resemblance to the lymphoepithelium of the tonsillar crypts from which they arise: they are basaloid, exhibit minimal keratinization, and are often permeated by lymphocytes. In rare cases, the presence of cilia in a primary HPV-OPC and their persistence in lymph node metastasis can confound the distinction between a benign and malignant process. Three cases of ciliated HPV-OPCs were identified from the archives of The Johns Hopkins Head and Neck Pathology consultation service. HPV status was determined using p16 immunohistochemistry and high risk HPV in situ hybridization. All three patients presented with a cystic lymph node metastasis without a known primary carcinoma. One metastasis was originally diagnosed as a branchial cleft cyst only to regionally recur 7 years later. In 2 cases, a primary HPV-OPC was found in the tonsil. The carcinomas exhibited both non-keratinizing squamous epithelium and cystic/microcystic spaces lined by ciliated columnar cells. Both the squamous and ciliated cells were HPV positive. This report draws attention to a novel variant of HPV-related head and neck cancer that exhibits ciliated columnar cells. This variant challenges prevailing notions that 1) HPV-OPCs are uniformly poorly differentiated cancers; 2) cilia are an infallible feature of benignancy; and 3) cilia are a reliable criterion for establishing branchial cleft origin when dealing with cystic lesions of the lateral neck.

Keywords

Human papillomavirus; HPV; cilia; p16

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Introduction

The presence of HPV in an oropharyngeal carcinoma is a favorable prognostic feature that is strongly correlated with improved patient outcomes.(1-3) These HPV-OPCs are widely perceived as poorly or undifferentiated carcinomas based on the immature appearance of a tumor cell that widely diverges from the stratified squamous epithelium that lines the surface of the tonsil.(4) Recently it has been pointed out that HPV-OPCs maintain a striking resemblance to the tonsillar epithelium from which they often arise.(5, 6) Like the specialized epithelium lining the tonsillar crypts, these carcinomas tend to be basaloid, non-keratinized and permeated by lymphocytes. On occasion, the non-neoplastic tonsillar epithelium may be punctuated by patches of ciliated respiratory epithelium.(7, 8) We report 3 cases where the cilia were retained during the process of malignant transformation and tumor spread. The retention of cilia in local and advanced forms of head and neck cancer could have important diagnostic implications, particularly in light of the dogma that the histologic detection of cilia provides indisputable evidence of a benign process.

Methods

Three cases of ciliated HPV-related carcinoma were identified in the files of The Johns Hopkins Hospital head and neck pathology consultation service. For each case, hematoxylin and eosin-stained slides were reviewed and HPV testing was performed. Briefly, immunohistochemistry for p16 (clone INK4a; MTM Laboratories, Heidelberg, Germany; prediluted) was performed on a Ventana BenchMark ULTRA automated stainer with an ultraView detection system according to manufacturer's instructions. HPV testing was performed by two different methods: Cases 1 and 2 were tested by DNA in situ hybridization using a cocktail for high risk types, 18, 33, 35, 45, 51, 52, 56, and 66 (Ventana Inform HPV III Family 16 Probe (B) kit). Case 3 was tested utilizing RNA in situ hybridization for HPV E6/E7 mRNA (a cocktail consisting of types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), performed manually using the RNAscope® HPV kit (Advanced Cell Diagnostics, Inc., Hayward, CA), according to the manufacturer's instructions as previously described.(9) Clinical information was obtained using information provided by the patients' referring pathologists and, when available, the electronic medical records.

Results

Case 1

A 46-year-woman initially presented with a 3 week history of a right neck mass. She was not a smoker or a heavy consumer of alcohol. Physical examination revealed a 5 cm cystic mass deep to the right sternocleidomastoid muscle at the junction of levels II and III. The rest of the head and neck examination was normal. A fine-needle aspiration was non-diagnostic. The mass was excised. Intraoperatively, there was no connection of the cyst to the oropharynx. Pathologic examination revealed a multiloculated cyst lined by flattened squamous and glandular epithelium with focal ciliated cells(Figure 1). The cyst lining was surrounded by a zone of lymphoid tissue with germinal centers. The lining epithelium

lacked overtly malignant cellular features. A diagnosis of branchial cleft cyst was made, and she received no additional therapy at that time.

Seven years later, the patient complained of a rapidly growing right-sided neck mass in the same location as the prior cyst. It was excised, revealing a complex cystic mass with a lymphoid cuff. The cyst was lined by squamous cells and columnar cells forming glandular structures (Figure 2). Focally, the columnar cells exhibited cilia (Figure 3). In comparison to the prior cyst, the epithelium demonstrated increased cellular atypia, an elevated mitotic rate, focal necrosis and downward proliferation of solid squamous nests into the cyst wall. In effect, the changes of malignancy were more fully developed in the tumor recurrence.

HPV testing of the recurrent cyst showed that both the squamous and glandular components were diffusely p16 positive by immunohistochemistry, and high risk HPV positive by in situ hybridization (Figure 3 inset). Furthermore, the initial cyst that had been previously diagnosed as branchial cleft cyst was also found to be high risk HPV positive on retrospective testing of the archival material.

Based on the morphologic features and the presence of HPV, the carcinomas were presumed to be of oropharyngeal origin even though the radiographic and clinical workup including biopsies of the tonsils and base of tongue failed to confirm a primary site of origin. The patient was treated with combined chemotherapy and radiation to Waldeyer's ring. She is without evidence of disease after 8 months of follow up.

Case 2

A 46-year-old man presented with a left-sided neck mass that had not resolved with antibiotic therapy or incision and drainage. He was not a smoker or heavy drinker of alcohol. Fine-needle aspiration revealed atypical squamous cells suspicious for metastatic carcinoma, but the clinical and radiographic examination did not reveal a site of tumor origin. The patient underwent a neck dissection along with bilateral diagnostic tonsillectomies and base of tongue biopsies.

Pathologic examination of the neck dissection revealed metastatic non-keratinizing squamous cell carcinoma in 8 of 20 cervical lymph nodes with extra nodal extension. A 1.8 cm non-keratinizing squamous cell carcinoma was discovered in the left tonsil, and a 0.3 cm carcinoma in the left tonsil. The right tonsillar carcinoma and the lymph node metastases were comprised exclusively of non-keratinized squamous cells. The carcinoma in the left tonsil demonstrated a solid component comprised of non-keratinizing squamous cells, and a cystic component comprised of columnar cells with cilia. Both the squamous component and the ciliated glandular components ciliated cells were p16 positive by immunohistochemistry and high risk HPV positive by in situ hybridization. Combined chemoradiation was recommended, but the patient was lost to follow-up.

Case 3

A 68-year-old man presented with a right-sided neck mass. Computed tomography scans revealed two cavitary lesions suspicious for necrotic lymph nodes. A core biopsy was performed. Histologic examination revealed of non-keratinizing squamous cell carcinoma

admixed with ciliated columnar cells forming glandular spaces. HPV testing confirmed the presence of HPV in both the squamous and ciliated glandular components, and a diagnosis of HPV-related carcinoma of probable oropharyngeal origin was rendered.

The patient subsequently underwent a right selective neck dissection, bilateral tonsillectomies and base of tongue biopsies. Pathologic examination disclosed a 4 cm cystic metastasis involving one cervical lymph node, and a 1.6 cm tumor involving the right tonsil. Both the metastasis and the primary carcinoma had a similar histologic appearance. The tumors were comprised of squamous cells as well as a pseudostratified respiratory-type epithelium that lined cystic and microcystic spaces (Figure 4). The columnar cells exhibited basally oriented, pseudostratified nuclei with terminal bars and numerous cilia (Figure 4). HPV testing showed that both tumor components were positive for p16 by immunohistochemistry and positive for high-risk HPV by in situ hybridization (Figure 5). The patient is free of disease with limited follow-up.

Discussion

While the age-adjusted incidence of laryngeal, oral cavity, and hypopharyngeal cancers have been in decline, the incidence of oropharyngeal cancer has been on the rise.(10, 11) The majority of these oropharyngeal carcinomas represent a clinically and biologically distinct form of head and neck cancer that is associated with a more favorable clinical outcome and is characterized by a non-keratinized basaloid morphology – a morphology that is often interpreted as poorly differentiated.(4) This persistent practice of assigning an advanced histologic grade to HPV-OPCs may account for the perplexing epidemiologic trend toward improving patient survival in the face of worsening tumor grade.(12) The notion that HPV-OPCs are poorly or undifferentiated carcinomas has recently been confronted by the observation that these tumors maintain a close morphologic resemblance to the specialized lymphoepithelium lining the tonsillar crypts(5, 6) – an epithelium that has a basaloid appearance, is minimally keratinized, and is sometimes interrupted by tracts of ciliated columnar epithelium.(7, 8) To further highlight this likeness and underscore the highly differentiated nature of some HPV-OPCs, we report 3 cases where the tumor cells retain the presence of ciliated columnar cells.

Cilia are normal microscopic structures that are found primarily in columnar cells of the respiratory and gynecologic tracts. As their presence is restricted to the fully differentiated glandular cell, the microscopic detection of cilia has historically been taken as compelling evidence of a benign process, particularly in cytologic material.(13, 14) Although the presence of cilia in bona fide malignancies is exceedingly rare, ciliated adenocarcinomas and adenosquamous cell carcinomas have been reported in the endometrium, uterine cervix, ovary, esophagus, stomach, and lung.(15-26) To our knowledge, ciliated carcinomas have not previously been reported in the carcinomas of the head and neck.

The presence of cilia in HPV-OPCs is diagnostically problematic, particularly when encountered in regional metastases to the upper lateral neck where the differential diagnosis typically includes a branchial cleft cyst.(27, 28) Confusion may be confounded by various other overlapping demographic, clinical, radiologic and pathologic factors including the

observations that: 1) metastatic HPV-OPCs are often cystic, 2) metastatic spread often occurs in the absence of a detectable primary carcinoma, and 3) HPV-OPCs tend to arise in a population of patients shifted towards a younger age and without traditional risk factors (i.e. smoking) (Table 1). The convergence of these overlapping features culminating in a diagnostic error is underscored in Case 1 where a cystic HPV-related carcinoma was initially misdiagnosed as a branchial cleft cyst based on its cystic nature, the relatively young age of the patient, the absence of a known primary, the lack of overtly malignant cytologic features of the lining epithelium, and the paradoxical presence of ciliated cells. When dealing with cystic lesions of the lateral neck, a high index of suspicion for metastatic carcinoma is needed, particularly for patients over the age of 40. Importantly, the presence of cilia alone is not sufficient evidence to exclude a metastatic oropharyngeal carcinoma. HPV status may be useful as branchial cleft cysts have not been found to harbor biologically active high risk HPV.(29) Of note, p16 is overexpressed in almost one-half of benign branchial cleft cysts, rendering p16 immunostaining of little if any value in this diagnostic scenario.(29) Instead, some other method of HPV detection such as in situ hybridization is necessary to confirm the malignant nature of a squamous-lined cyst in the neck.

Ciliated HPV-related carcinoma is a novel, very well-differentiated form of head and neck cancer. Although rare, its presence cautions against the common practice of taking the presence of ciliated cells as definitive evidence of a benign process. This is particularly relevant for cystic lesions of the lateral neck where traditional indicators of malignancy (e.g. high grade cellular features, aggressive clinical behavior) may not be well developed. The threshold for performing HPV testing should be low and at least considered for cystic lesions of the lateral neck in patients over 40 years of age where the lining epithelium takes on the appearance of the tonsillar epithelium.

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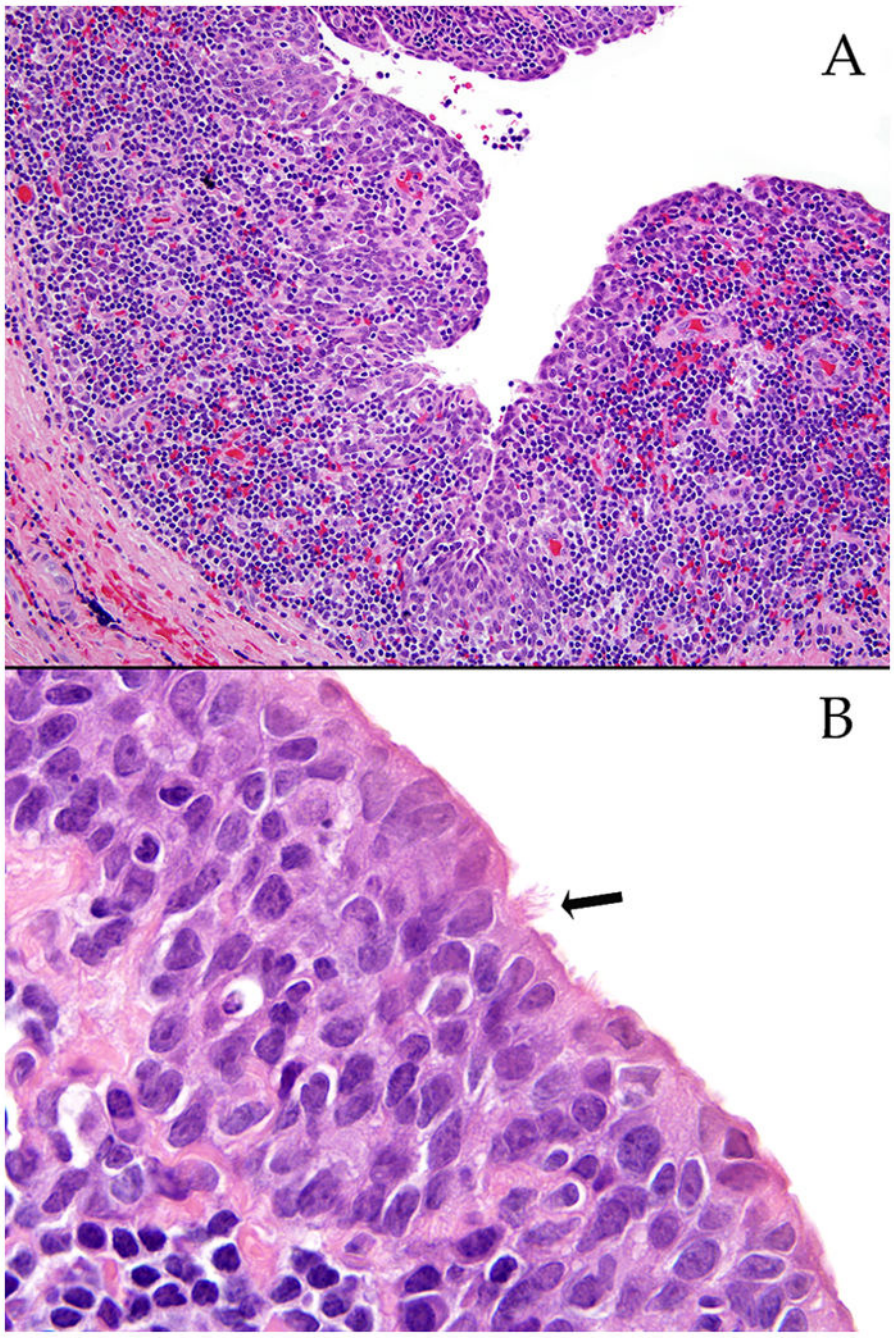

Figure 1.
Case 1 initially presented as a neck cyst that exhibited areas closely resembling normal tonsillar crypts (A). The cyst was lined by cytologically bland squamous and glandular cells which were focally ciliated (B).

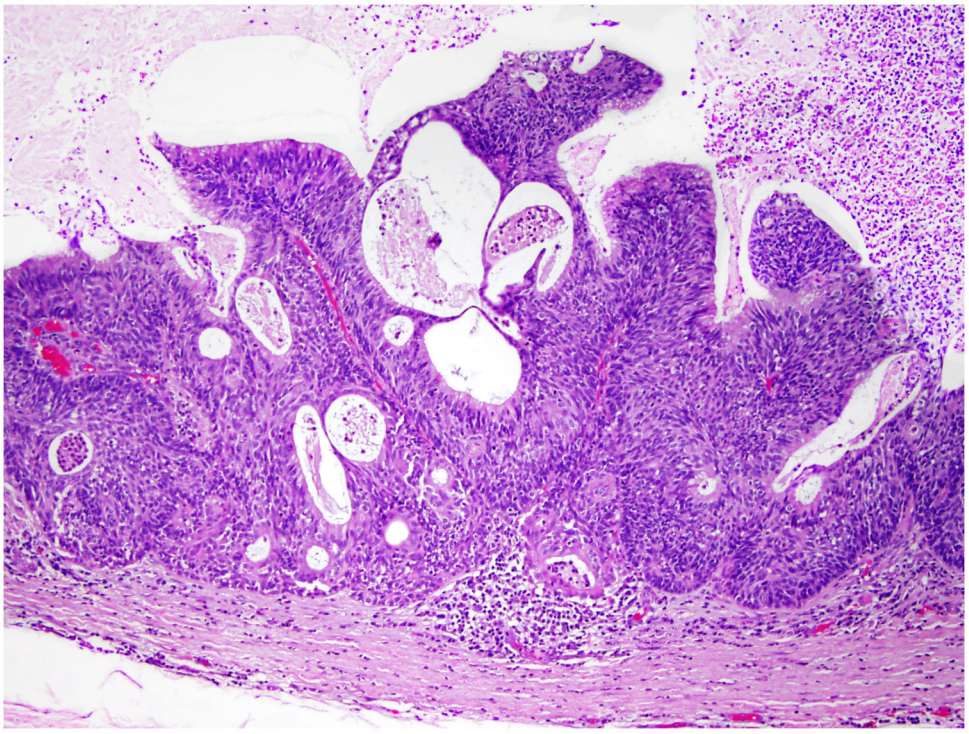

Figure 2.
Case 1 recurred 7 years after the initial excision of the cyst as a cystic tumor lined by proliferative epithelium forming glands and villous structures.

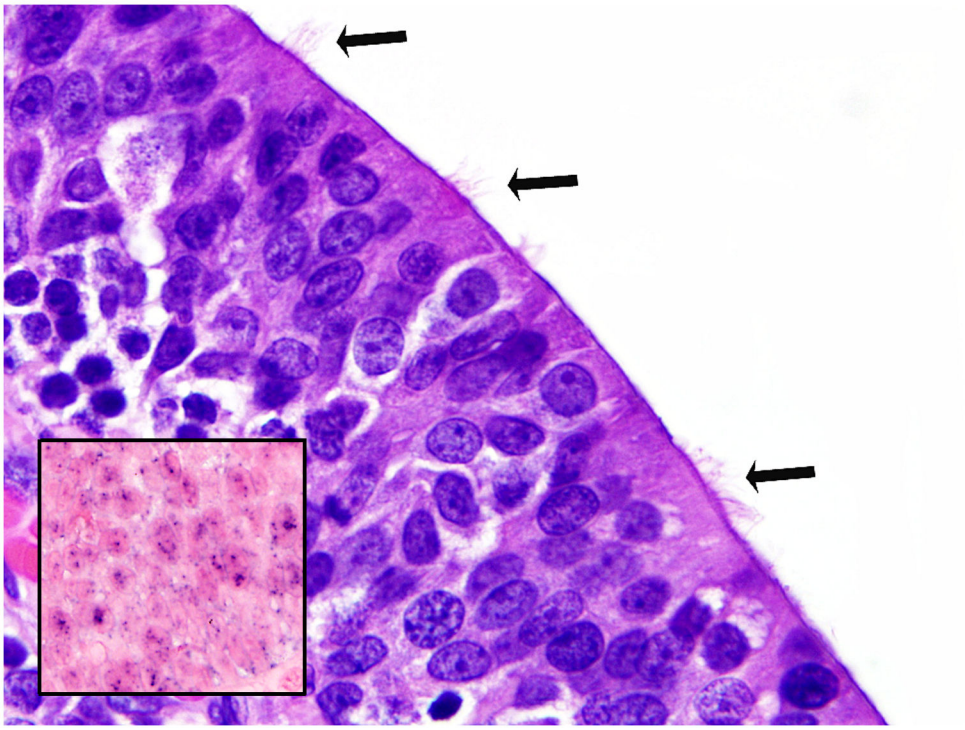

Figure 3.
The recurrent carcinoma in Case 2 exhibited columnar tumor cells that were focally ciliated (arrows). The tumor was positive for high-risk HPV by in situ hybridization (inset).

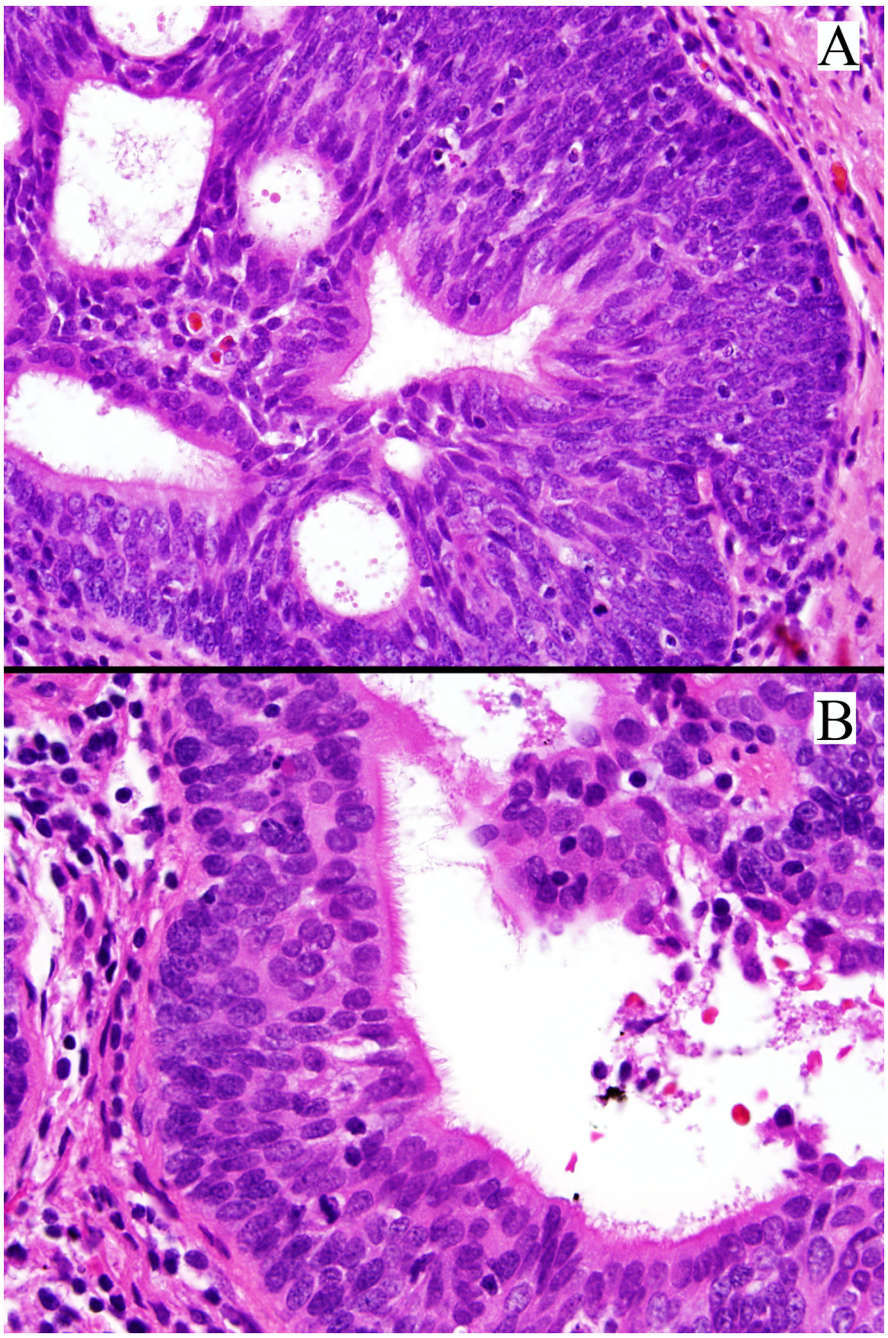

Figure 4.
In Case 3, both the primary oropharyngeal carcinoma (A) and the metastasis to a cervical lymph node (B) demonstrated glandular structures lined by pseudostratified columnar cells with abundant cilia.

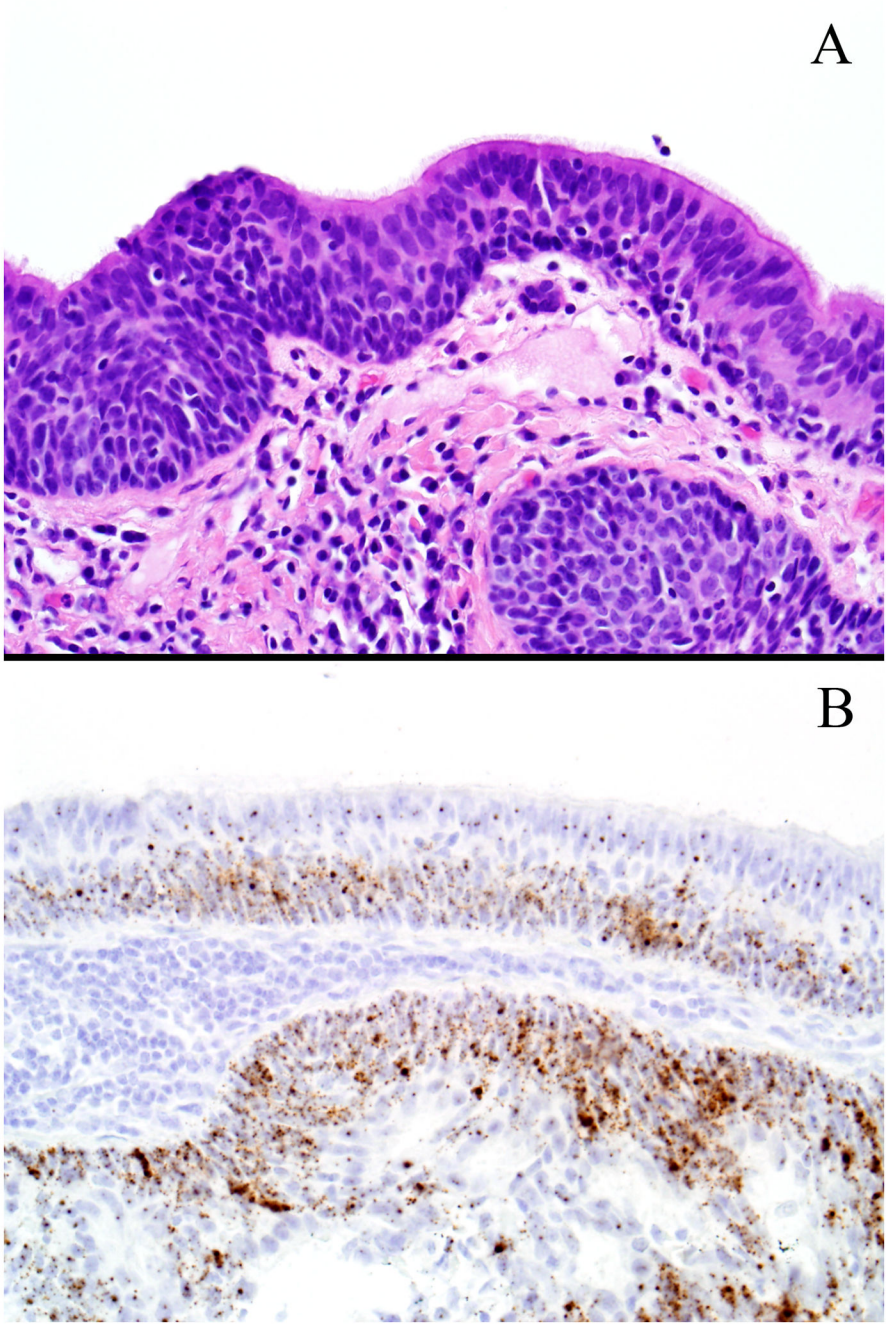

Figure 5.
Both the squamous and ciliated glandular components of the carcinoma in Case 3 (A) were positive for high-risk HPV by in situ hybridization (B).

Table 1

Comparison of clinicopathologic features of branchial cleft cysts and metastatic HPV-related oropharyngeal squamous cell carcinoma

	Branchial cleft cyst	Metastatic HPV-related oropharyngeal carcinoma
location	Upper lateral neck	Upper lateral neck
Cyst formation	present	Often present
Peripheral zone of lymphoid tissue	Present	Present
Squamous lining	Present (90%)	Present
Columnar cells with cilia	Often present (10%)	Rarely present
Age	20s - 40s	40s - 60s
Clinical behavior	benign	can be indolent
Epithelial atypia	variable (reactive atypia when inflamed)	variable (can be minimal)
P16 IHC	sometimes positive	positive
High risk HPV ISH	negative	positive

HPV, human papillomavirus; IHC, immunohistochemistry; ISH, in situ hybridization