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CASE REPORT

Ciliated HPV-Related Carcinoma: A Diagnostic Challenge on Frozen Section

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Abstract
Oropharyngeal squamous cell carcinomas associated with high risk HPV show a wide morphological spectrum, including papillary, adenosquamous, lymphoepithelioma-like and sarcomatoid. We report an interesting case of ciliated HPV-related carcinoma arising from tonsillar tissue in a 55-year-old man which was associated with HPV33. This rare variant has been described in only a handful of cases in the literature, and to our knowledge this is the first case specifically associated with HPV33. The presence of cilia is a potential diagnostic problem as it has been traditionally considered a feature of benignancy, and could pose a particular challenge on frozen section. The diagnostic challenges, differential diagnosis of this tumor and the association with HPV33 are discussed.

Keywords Ciliated HPV-related · Mimicker · HPV33

Introduction

Oropharyngeal squamous cell carcinomas associated with high risk HPV are considered a distinct form of head and neck squamous cell carcinoma [1]. The incidence and prevalence of these tumors has increased over the last few decades [2–4]. In contrast to the HPV-negative squamous cell carcinomas, HPV-associated squamous cell carcinomas have a different epithelial cell of origin and risk factors. HPV-associated squamous cell carcinomas are believed to arise from the reticulated epithelium of tonsillar crypts and are associated with sexual practices, while HPV-negative squamous cell carcinomas arise from the surface epithelium and are primarily associated with smoking and alcohol usage. Histologically, HPV-associated squamous cell carcinomas are frequently non-keratinizing, exhibit basaloid-appearing cells with high nuclear to cytoplasmic ratios, indistinct cytoplasmic borders, tumor infiltrating lymphocytes and have high apoptotic and/or mitotic activity. Several variants of oropharyngeal squamous cell carcinomas associated with high risk HPV have been described such as papillary carcinoma, adenosquamous carcinoma, lymphoepithelioma-like carcinoma and sarcomatoid carcinoma [5]. We describe an interesting case of ciliated HPV-related carcinoma, a recently described subtype of HPV-associated squamous cell carcinoma, also reported in the literature as "ciliated adenosquamous carcinoma", which posed difficulty on frozen section evaluation. Additionally, to our knowledge, this is the first case specifically reporting an association with HPV33.

Case Report

A 55-year-old male with a 30–40 pack year smoking history complained of a painful left tonsil for 3 months. CT scan of the neck showed an asymmetrical enlargement of the left tonsil with extension into the ipsilateral tongue base ($2.5 \times 2.4 \times 1.8$ cm) and a hyperenhancing left IIA lymph node ($1.2 \times 1.1 \times 0.7$ cm). Clinical exam revealed a firm and irregular left tonsil which was concerning for malignancy. A tonsillectomy was performed and sent for frozen section to evaluate for the presence of tumor. Although no lesion was grossly identified, frozen section showed tonsillar tissue infiltrated by a diffuse biphasic proliferation of tumor nests with central cystic spaces lined by ciliated epithelium overlying non-keratinizing squamous cells (Fig. 1A–D). The

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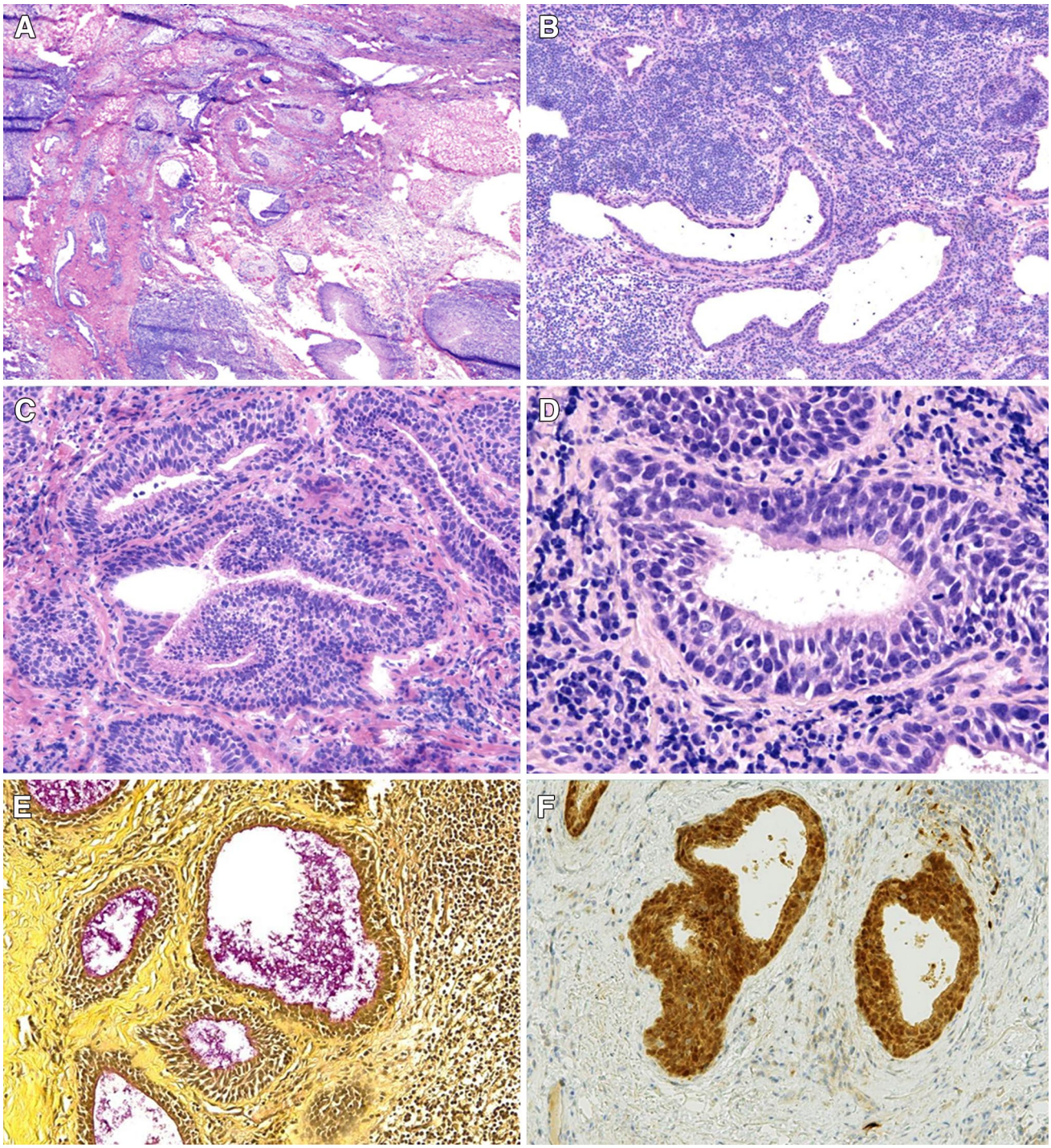

Fig. 1 Ciliated HPV-related carcinoma. **A** (H&E × 25)—frozen section evaluation at low magnification—the neoplasm shows cystic spaces, lined by proliferating epithelium dissecting through the tonsillar tissue and skeletal muscles. **B** (H&E × 100), **C** (H&E × 200), and **D** (H&E × 400)—frozen section evaluation on higher magnifications—the lining epithelium is stratified and shows ciliated epithelium overlying non keratinized squamous cells in a bilayer fashion. The cells appear bland and lack pleomorphism. There is no evidence of necrosis. Mitotic activity is approximately 10/10 hpf. **E** (Mucicarmine × 100)—the tumor cells show lack of intracytoplasmic mucin positivity by mucicarmine stain. **F** (p16 × 200)—the p16 immunostain shows diffuse nuclear and cytoplasmic staining in both the layers of the epithelium

tumor was locally invasive and infiltrated through skeletal muscle. A differential diagnosis of mucoepidermoid carcinoma and adenosquamous carcinoma were considered at the time of frozen section; however, given that mucus cells were not identified, ciliated cells were noted and the lesion was composed of banal appearing cells in an unusual adenoid pattern, a frozen section diagnosis of "neoplasm present" was made, with the final diagnosis deferred pending permanent section. The permanent sections revealed squamous cells with low nuclear grade and lacking pleomorphism. The surface ciliated cells were more prominent on permanent sections compared to the original frozen section. Mitotic activity was 10/10 hpf. No tumor necrosis was identified. A mucicarmine stain (Fig. 1E) was negative. Ultimately, it was concluded that the tumor fit morphologically with ciliated HPV-related carcinoma, a rare variant of HPV-related carcinomas of the head and neck. To confirm the diagnosis, immunohistochemical studies were done that demonstrated tumor cells to have diffuse p16 expression (Fig. 1F). PCR was positive for HPV33. The patient underwent complete surgery and neck dissection a few days later. The lymph nodes were negative for carcinoma. Three months later, imaging studies revealed recurrence of the lesion along the left oral tongue. The lesion was excised, revealing residual carcinoma. Subsequently, the patient underwent chemotherapy and radiation and has been disease-free for 9 months.

Discussion

Ciliated HPV-related carcinomas are rare and relatively recently described subtype of HPV-related carcinomas of the head and neck. The two largest series of ciliated head and neck carcinomas to date by Bishop et al. and Radkay-Gonzalez et al. have described 10 such cases in the head and neck region between them, with proposed nomenclature of "Ciliated HPV-related carcinoma" and "Ciliated adenosquamous carcinoma", respectively [6, 7]. While ciliated cells are typically seen in highly differentiated cells and often regarded as a sign of benignancy, carcinomas with ciliated morphology have been described in other organs including lung, endometrium, ovary, cervix and esophagus [8–12]. Cilia are normally found in columnar epithelium of the respiratory tract. Within the tonsillar tissue, the reticulated epithelium is punctated by patches of ciliated respiratory epithelium [13]. These are believed to be the origin of ciliated HPV-related carcinomas [6].

Bishop et al. [6] described 3 cases of ciliated HPV-related carcinoma, one of the cases being initially misdiagnosed as branchial cleft cyst of the neck. The lesion recurred 7 years later as a neck mass. Immunohistochemical studies showed that tumor cells were positive for p16, and tests for high risk HPV DNA by in situ hybridization were positive on both, previous and recurrent cysts. The lesion was presumed to be of oropharyngeal origin, despite the failure of the clinical work up to identify a primary lesion, a common assumption for HPV-associated head and neck carcinomas of unknown primary. The other 2 cases in their series presented with lymph node metastases with primaries in the tonsil. Radkay-Gonzalez et al. described 7 cases of ciliated adenosquamous carcinoma, 3 from palatine tonsil, 1 from base of tongue and 3 from the neck lymph nodes that did not show any tumor in bilateral tonsils. All the cases showed lymph nodal metastasis, p16 positivity and high-risk HPV DNA by in situ hybridization [7].

The differential diagnosis of ciliated HPV-related carcinomas of the oropharynx includes tumors showing at least a focal glandular pattern. Tumors in the differential include primarily mucoepidermoid carcinoma, cystic HPV-related squamous cell carcinomas and, particularly when presenting as a neck mass, branchial cleft cysts. Adenosquamous carcinoma arising in the head and neck is typically high grade in appearance and show pronounced keratinization and prominent nuclear atypia in comparison to the low grade appearance of ciliated HPV-related carcinoma, although they may be considered a subset of adenosquamous carcinomas [7].

Mucoepidermoid carcinoma can be distinguished from ciliated HPV-related carcinoma by the presence of admixture of mucus cells, intermediate cells and squamoid or epidermoid cells with a cystic or solid growth pattern. Mucicarmine stain and Periodic acid–Schiff with diastase demonstrate intracytoplasmic mucin. Of particular interest is a recently described case of ciliated mucoepidermoid carcinoma that harbored *MAML-2* gene rearrangement, wherein the cystic tumor showed prominent cilia along with an admixture of mucus, squamoid and intermediate cells [14]. Our case stained diffusely with p16, harbored HPV33, and lacked mucus cells, hence it was safe to assume that the tumor was surface epithelium derived, and not salivary gland derived. In cases where p16 or HPV genotyping are inconsistent or not helpful, molecular analysis for t(11;19)(q21;p13) *CRTC1-MAML2* fusion or the alternate fusion t(11:15)(q21;q26) *CRTC3-MAML2* that characterize mucoepidermoid carcinomas can be performed [15].

HPV-related squamous cell carcinomas often present with neck metastasis as the initial event. The metastases often undergo cystic degeneration and can be mistaken for benign squamous lined cysts. This differential becomes more complex when there are cilia in cystic metastasis, which can be easily confused with branchial cleft cyst. Interestingly, branchial cleft cysts are p16 positive in more than half of the cases [6]. However, it is important to note that positive staining, if present, is usually localized to the superficial squamous epithelium or the glandular epithelium lining the branchial cleft cyst and that it does not stain full thickness of the epithelium, whereas metastatic

HPV-related squamous cell carcinoma stain with p16 immunostain diffusely and strongly [16]. A high index of suspicion is needed to avoid the pitfall of calling ciliated cystic metastasis as branchial cleft cysts, especially in older population.

HPV33 positivity in this tumor is interesting as most of the HPV-related oropharyngeal squamous cell carcinomas are HPV16 positive. In a study performed by St Guily et al. on oropharyngeal carcinomas, HPV16 was present in 89.7% cases followed by HPV52 in 3.4% cases, HPV33 was identified in just 2.7% cases [17]. HPV33 is the most common HPV subtype identified in HPV-related multiphenotypic sinonasal carcinoma [18], previously called the HPV-related carcinoma with adenoid cystic-like features arising in the sinonasal tract, another unusual morphologic variant of HPV-related carcinoma, raising questions as to possible differential biologic effects of this high risk HPV-variant relative to HPV16 in the head and neck.

Conclusion

Ciliated HPV-related carcinomas present a diagnostic challenge, particularly on frozen section, as traditionally, presence of cilia has been described as a feature of benignancy. Also, owing to the bland appearance of the cell nests on frozen section in our case, the diagnosis was not straightforward. This prompted us to defer the diagnosis to permanent sections for better characterization. Diagnostic clues, which may facilitate the identification of this tumor on frozen sections include a loss of underlying normal architecture and an infiltrative border of cystic spaces lined by multilayered cytologically bland non-keratinizing squamous epithelium, the lumen of which are lined by ciliated cells, at least focally. Our case is associated with HPV33, which is interesting, as most HPV related squamous cell carcinomas are associated with HPV16 and other cases of ciliated HPV-related carcinomas in the literature have been generally reported as "high-risk HPV" without a specific subtype indicated [6, 7].

In summary, we describe a rare case of ciliated HPV-related carcinoma which posed a diagnostic challenge on frozen section. This is the first described case of this rare variant lacking nodal metastases and the first to identify HPV33 specifically.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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