Virchows Arch (2017) 471:295–307
DOI 10.1007/s00428-017-2118-y

INVITED ANNUAL REVIEW ISSUE

HPV-related carcinomas of the head and neck: morphologic features, variants, and practical considerations for the surgical pathologist

Todd M. Stevens[1] · **Justin A. Bishop**[2]

Received: 23 November 2016 / Revised: 12 March 2017 / Accepted: 3 April 2017 / Published online: 17 April 2017

Abstract Human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma is a distinct tumor entity with clinical, epidemiologic, genetic, histologic, prognostic, and treatment differences from smoking- and alcohol-related head and neck squamous cell carcinoma. This is now well known by the pathology and medical community. What is not yet widely known is that several emerging variants of HPV-related carcinoma of the head and neck exist apart from the prototypical non-keratinizing morphology. Further, there is currently considerable variation in methodologies used and clinical scenarios in which to test for HPV-related head and neck squamous cell carcinoma, and no standard approach has emerged. In this article, we will review the morphology of prototypical HPV-related squamous cell carcinoma of the oropharynx and other HPV-related variants of head and neck carcinoma with an emphasis on their differential diagnosis, grade, and prognosis, as well as outline the current best practices for testing for HPV in head and neck carcinomas.

Keywords Human papillomavirus · Head and neck · Squamous cell carcinoma · Prognostic · Biomarkers

✉ Todd M. Stevens
tstevens@uabmc.edu

[1] Department of Pathology, University of Alabama at Birmingham, 3548 North Pavilion, 1802 6th Ave. S, Birmingham, AL 35249, USA

[2] Department of Pathology, The Johns Hopkins University School of Medicine, 401 N. Broadway, Weinberg 2249, Baltimore, MD 21231, USA

Introduction

Human papillomavirus (HPV) is a causative agent in about 25% of all head and neck squamous cell carcinomas (HNSCCs) [1], and the oropharynx (OP) is the head and neck site most enriched for HPV-related carcinomas, with HPV type 16 causing about 80% of oropharyngeal squamous cell carcinomas (OPSCCs) [2]. These HPV-related OPSCCs carry a better prognosis than HPV-negative head and neck squamous cell carcinoma, arise in younger patients—often males—with certain high-risk sexual behaviors, often with no significant smoking or alcohol use history, and usually display a non-keratinizing morphology [3, 4]. HPV-related OPSCCs show enhanced sensitivity to radiation treatment, which is at least in part related to their wild-type p53 status [5]. One large study showed that HPV-positive OPSCCs have a statistically significant improved overall survival at 3 years as compared to HPV-negative OPSCC (82.4 vs 57.1%, respectively) [4]. This same study also showed an improved progression-free survival at 3 years for HPV-positive OPSCC vs HPV-negative OPSCC (73.7 vs 43.4%, respectively). Therefore, HPV-related OPSCCs are often candidates for treatment "de-escalation," underscoring the importance of accurate identification of this distinct subtype of head and neck squamous cell carcinoma by pathologists [6, 7].

HPV achieves this carcinogenesis largely via its E6 and E7 oncoproteins. E6 inactivates the p53 tumor suppressor protein and activates telomerase activity, while E7 inactivates the retinoblastoma protein (Rb). Inactivation of Rb causes the release of the transcription factor E2F. The net effect of these HPV oncoproteins is therefore cellular proliferation and less reliance on other genetic alterations for carcinogenesis [8]. Indeed, smoking- and alcohol-related/HPV-negative HNSCCs have a greater mutational burden than HPV-related HNSCCs, with the former showing alterations of *TP53*, *NOTCH*, *PIK3CA*, *cyclin D1*, and *CDKN2A*, etc. [5].

The strong predilection of HPV for the oropharynx is due to the microanatomy of the OP, namely the reticulated epithelium of the base of the tongue and tonsils (Fig. 1a, b). For example, the reticulated epithelium of the OP is known to express PDL1, resulting in a reduced cytotoxic T cell response and thus an environment conducive to HPV infection [7]. In addition to the OP, the sinonasal tract is the other head and neck site with a relative predisposition to HPV infection, where about 21% of sinonasal carcinomas are positive for high-risk HPV [2]. While high-risk HPV has been detected in up to 30% of carcinomas of the nasopharynx, a significant subset of these likely represents a direct extension from the oropharyngeal primaries [9]. About 5% of oral cavity, larynx, and hypopharynx carcinomas show the presence of high-risk HPV infection [7, 10, 11]. Therefore, the oropharynx and sinonasal tract are the "hot spots" of HPV-related carcinogenesis in the head and neck. Whereas HPV-related OPSCC is known to carry a better prognosis and allow for treatment de-escalation, it is not currently clear if this same prognostic benefit applies to non-oropharyngeal HPV-related HNSCC [12].

In this review, we will discuss the histologic features of classical HPV-related OPSCC along with morphologic variants of HPV-related carcinoma and their associated differential diagnosis and prognostic import. Histologic grading and prognosis of HPV-related carcinoma variants will be detailed. Finally, best current practices for when and how to test for HPV in head and neck lesions will also be discussed.

Morphologic variants of HPV-related carcinomas

In addition to the classical non-keratinizing, "basaloid" morphology, several other histologic variants of HPV-related head and neck carcinoma have been described, including papillary, lymphoepithelial, adenosquamous, small cell, large cell neuroendocrine carcinoma, spindle cell/sarcomatoid, HPV-related carcinoma with adenoid cystic-like features, ciliated HPV-related carcinoma, and HPV-associated adenocarcinoma of the base of the tongue.

Prototypical non-keratinizing HPV-related squamous cell carcinoma of the oropharynx

The typical, classic HPV-related squamous cell carcinoma of the head and neck arises in the oropharynx and histologically displays solid sheets and broad ribbons of carcinoma cells (Fig. 2a), not uncommonly with central cystic necrosis. The neoplastic cells somewhat resemble the reticulated epithelium of the oropharynx in that they display high nuclear to cytoplasmic ratios, often with penetrating lymphocytes and syncytial cytoplasm, thus giving them an overall basaloid appearance. The nuclei are oval to round and have evenly distributed chromatin (Fig. 2b) [6, 7]. Scattered, random nuclear pleomorphism and anaplasia are common findings (Fig. 2c). Parenthetically, scattered anaplasia has been associated with increased disease recurrence and poorer survival, although this has yet to be widely validated [13]. Keratinization is either absent or focal and when present is often in the form of increased dense orange cytoplasm and/or focal keratin whorls, a pattern referred to by some as *non-keratinizing squamous cell carcinoma* "with maturation" (Figs. 2c and 3a) [14]. These so-called non-keratinizing squamous cell carcinomas with maturation are more likely to harbor high-risk HPV and have better prognosis than overtly keratinizing HNSCC and, therefore, are probably a variation of typical HPV-related OPSCC. Overt widespread keratinization is much less common. Some examples of HPV-related HNSCC may, in addition to

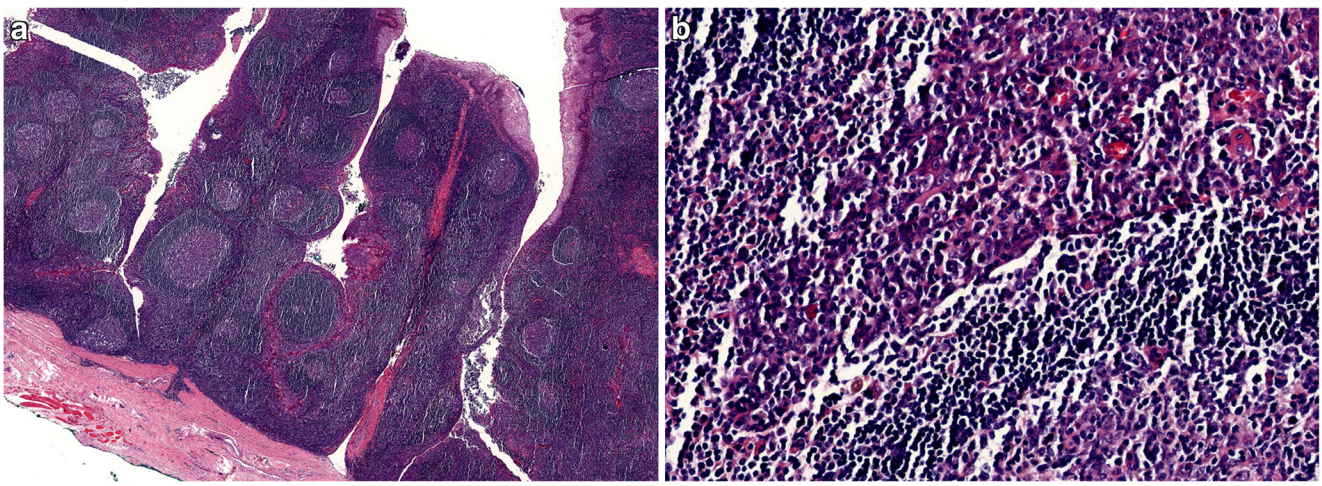

Fig. 1 **a** The normal palatine tonsil displays an undulating mucosal surface with surface invaginations forming crypts, surrounded by lymphoid follicles. **b** The crypts are lined by a reticulated epithelium made up of squamous cells with higher nuclear to cytoplasmic ratios and trafficking lymphocytes that are involved in antigen presentation

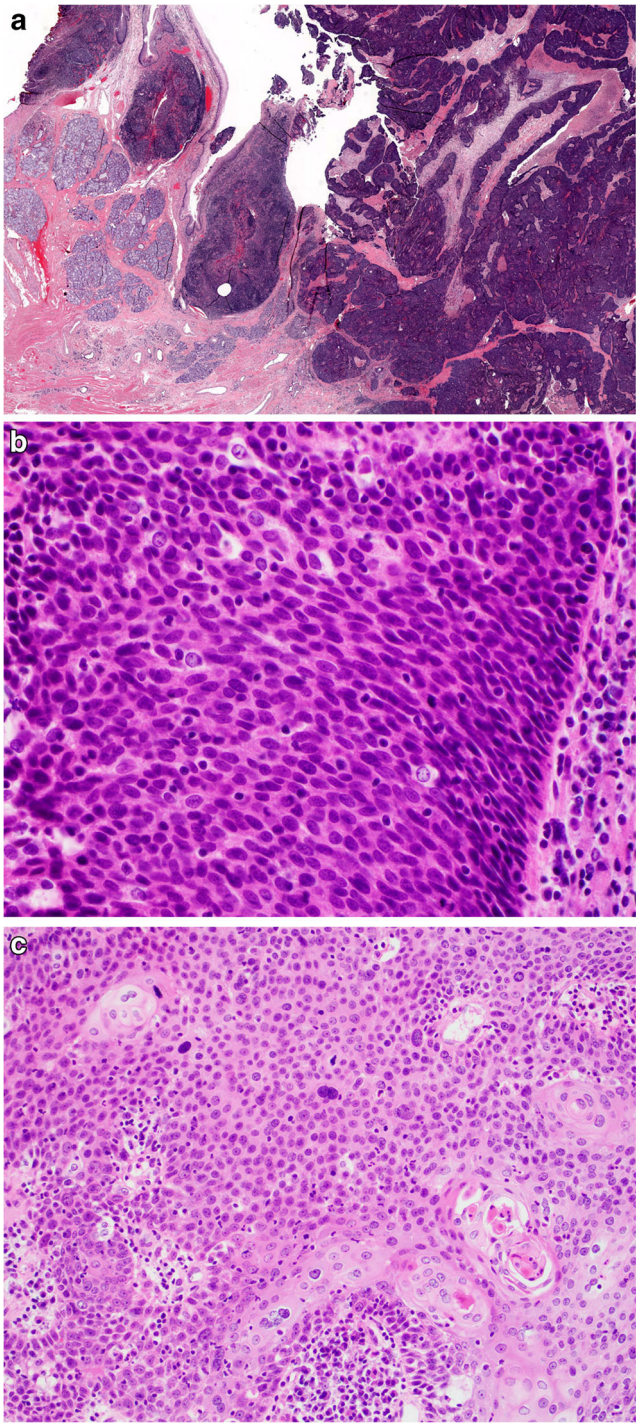

Fig. 2 **a** The typical low-power appearance of prototypical HPV-related squamous cell carcinoma of the oropharynx shows solid sheets and ribbons of blue cells with pushing type invasion. **b** A high-power exam shows oval to round squamous cells with indistinct cell borders and evenly distributed chromatin with admixed trafficking lymphocytes, giving HPV-related squamous cell carcinoma a resemblance to the normal reticulated crypt epithelium, making them well-differentiated neoplasms. **c** Scattered random nuclear pleomorphism is not uncommon in HPV-related head and neck squamous cell carcinoma. This example also shows cells with orange cytoplasm and focal keratinization, so-called non-keratinizing squamous cell carcinoma with maturation

the above features, show peripheral palisading and production of basement membrane material, raising the differential diagnosis of a true basaloid variant of squamous cell carcinoma (Fig. 3b). However, basaloid squamous cell carcinoma, as strictly defined, is an aggressive *HPV-unrelated* carcinoma, typically of the supraglottic larynx and hypopharynx [15]. For this reason, the term basaloid should not be used in pathology reports to refer to p16 and/or HPV-positive non-keratinizing squamous cell carcinoma of the head and neck [6], so as to avoid confusion with the distinct entity basaloid squamous cell carcinoma.

Unlike tobacco- and alcohol-related HNSCCs, where evolution to invasive carcinoma through a dysplasia to carcinoma sequence is accepted, the presence of a precursor, dysplastic stage in HPV-related HNSCC has not yet been fully delineated. HPV-related OPSCC arises from tonsillar crypts, and when surface mucosal involvement of HPV-related HNSCC occurs, it is thought to represent secondary surface colonization by the underlying carcinoma [6, 7].

Diagnosing invasion in classic HPV-related HNSCC is not always straightforward given that it does not elicit the desmoplastic stromal reaction that HPV-negative HNSCC does, and that HPV-related HNSCC arises from the reticulated epithelium, which is already situated deeply within the lamina propria, lacks a continuous basement membrane, and the interface of the epithelium and stroma is often blurred by the trafficking lymphocytes native to the Waldeyer's ring. For these reasons, it is not uncommon to encounter examples of HPV-related HNSCC in the oropharynx that are not clearly invasive but that show metastatic disease in the neck. Therefore, cases of HPV-related OPSCC as described above should be regarded as invasive by definition, even in the absence of clear-cut conventional signs of invasion (i.e., destructive stromal invasion) [6, 7].

HPV-related HNSCC frequently presents as neck metastasis, most typically to levels 2 and 3, and often cystic. The neck metastases often retain the non-keratinizing morphology and often show an undulating, partially cystic architecture which can somewhat resemble a tonsil involved by HPV-related HNSCC (personal observation). Occasionally, the neck node metastasis may be entirely cystic and lined by a flattened, attenuated squamous lining (Fig. 3c, d) and even show focal cilia, invoking the differential diagnosis with branchial cleft cyst [16].

HPV-related squamous cell carcinoma of the head and neck will show diffuse overexpression of p16 by immunohistochemistry (Fig. 3e) and the presence of high-risk HPV by one of the HPV-specific tests such as PCR, DNA, or RNA in situ hybridization.

The presence of extranodal extension in HPV-negative HNSCC is often an indication for chemotherapy. However, in HPV-related HNSCC, extranodal extension is of uncertain clinical significance and interobserver variability for this

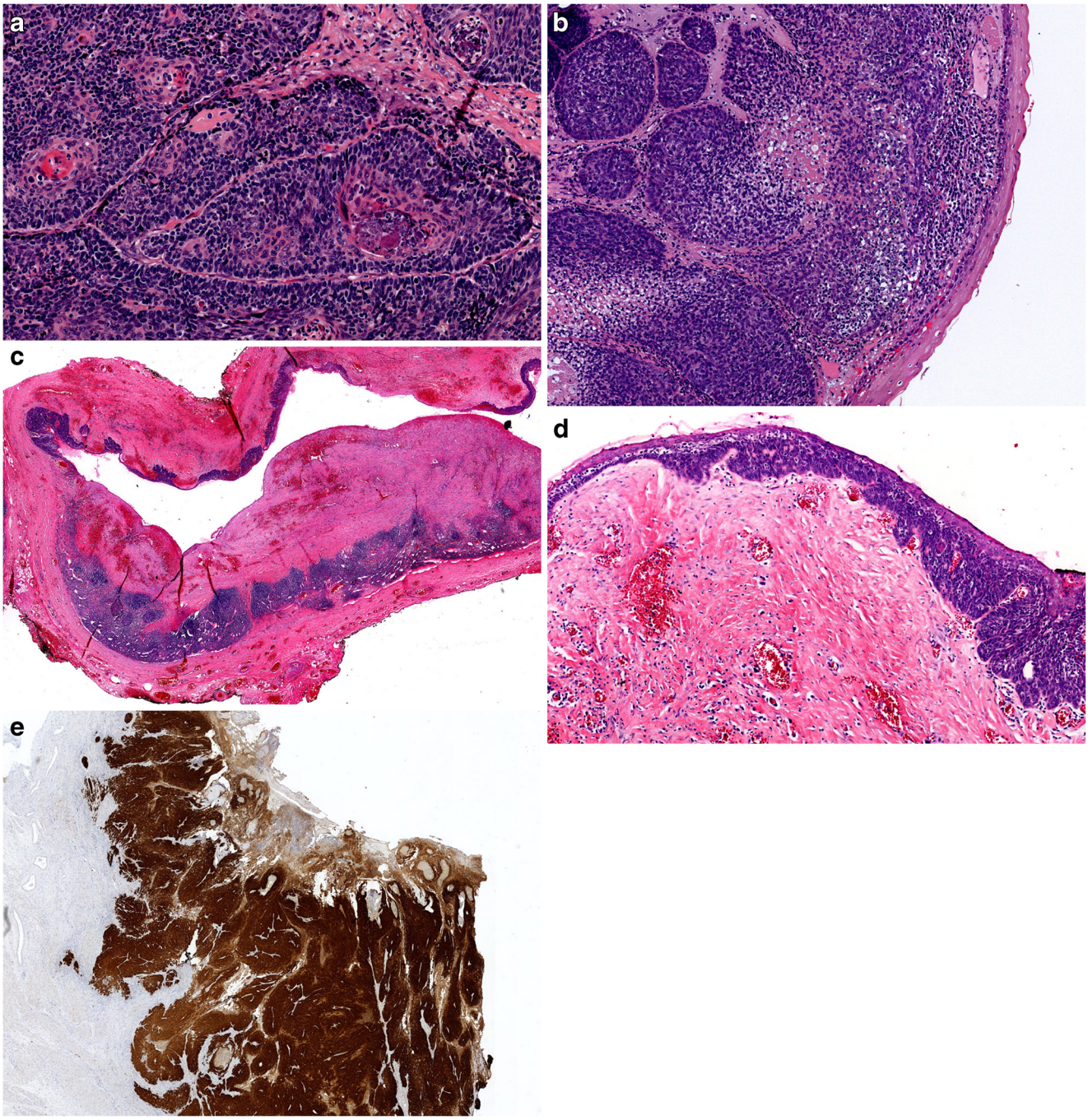

Fig. 3 **a** Example of an HPV-related squamous cell carcinoma showing focal keratinization. Note the lack of a desmoplastic response. **b** HPV-related squamous cell carcinoma showing peripheral palisading and production of pink basement membrane-type material, invoking the differential diagnosis with basaloid squamous cell carcinoma. HPV studies are required to distinguish HPV-related squamous cell carcinoma from the aggressive HPV-unrelated basaloid squamous cell carcinoma, as both can show identical morphology. **c** Lymph node metastasis of HPV-related squamous cell carcinomas is often cystic. **d** The cystic spaces can be lined by an attenuated bland squamous lining (*left side*), inviting confusion with a branchial cleft cyst. More typical nuclear atypia can often be seen in cystic metastasis, however (*right side*). **a** A prototypical HPV-related squamous cell carcinoma of the oropharynx showing diffuse overexpression of p16 by immunohistochemistry

finding is poor [17]. Nevertheless, extensive extranodal extension in HPV-related carcinomas may be clinically significant and should probably be reported [17]. Future studies may shed light on the significance of extranodal extension in HPV-positive HNSCC.

HPV-related head and neck squamous cell carcinoma with papillary features

Approximately 50% of PSCC of the head and neck (HN-PSCC) are HPV-related, and these tend to show non-keratinizing

morphology, unlike the HPV-negative HN-PSCC, which often show keratinization [3, 7]. Most HPV-related HN-PSCC arise in the oropharynx, but also commonly occur in the sinonasal tract, and less commonly in the larynx. In fact, up to 80% of sinonasal PSCC are HPV-related [2]. HN-PSCC, whether HPV positive or negative, are felt to have a better prognosis compared to non-papillary HPV-unrelated HNSCC, and there is limited data that suggests that HPV-positive HN-PSCC show a slight trend toward improved disease-free survival compared to HPV-negative HN-PSCC [3].

HPV-HNSCC with papillary features should be distinguished from verrucous carcinoma. Verrucous carcinoma, unlike HPV-HNSCC with papillary features, shows an endophytic, bland squamous proliferation with a flat, broad deeply pushing base and cells with abundant glassy cytoplasm and little nuclear atypia. HPV-HNSCC with papillary features, however, shows exophytic papillae lined by basaloid, mostly non-keratinizing (Fig. 4a), cells with high nuclear to cytoplasmic ratios. Schneiderian papillomas and carcinomas arising from Schneiderian papillomas also must be distinguished from HPV-HNSCC with papillary features. While there is sinonasal site overlap between benign and malignant Schneiderian papillomas and HPV-HNSCC with papillary features, Schneiderian papillomas will typically show some areas of columnar and ciliated, mucous cell and/or transitional type morphology, often with neutrophilic microabscesses, and will typically be negative for high-risk HPV and p16 (although it should be noted that exophytic Schneiderian papillomas often contain low-risk HPV) [2].

HPV-related lymphoepithelial-like carcinoma of the oropharynx

In 2010, Singhi et al. described a group of 22 oropharyngeal carcinomas that displayed sheets, cords, and individual carcinoma cells with syncytial cytoplasm, vesicular nuclei, and large central nucleoli set in an inflammatory background (Fig. 4b) [18]. These cases, while morphologically indistinguishable from EBV-driven nasopharyngeal carcinoma, uniformly expressed p16, and 86% were positive for HPV-16 by in situ hybridization (ISH) (Fig. 4c) [18]. All were negative for EBV. These HPV-related lymphoepithelial-like carcinomas of the oropharynx (referred to by some authors as "undifferentiated") shared similar clinical associations as those of typical HPV-related HNSCC, in that most occurred in non-smokers and in younger men. Given this newly described variant of HPV-related head and neck carcinoma, any lymphoepithelial carcinoma identified in a neck lymph node or other head and neck site should be tested for HPV and EBV in order to arrive at a correct diagnosis and to facilitate the proper treatment. It should be noted, however, that lymphoepithelial carcinomas that arise in the larynx and hypopharynx are strongly associated with tobacco use and are regarded as a highly malignant disease. HPV-related lymphoepithelial carcinoma of the oropharynx, on the other hand, appears to show the same improved prognosis as typical HPV-related HNSCC [18].

HPV-related head and neck carcinoma with adenosquamous histology

Head and neck adenosquamous carcinoma is an aggressive, rare biphasic neoplasm composed of malignant glandular and squamous elements (Fig. 4d), most commonly occurring in the larynx, followed by the oral cavity, sinonasal tract, oropharynx, and hypopharynx. Bishop et al. [2] found five of six sinonasal adenosquamous carcinomas to harbor HPV type 16 (Fig. 4e), and Masand et al. [19] found two of three oropharyngeal adenosquamous carcinomas and one of three nasal cavity adenosquamous carcinomas to overexpress p16 and show positivity for HPV oncoproteins E6 and E7 by RNA ISH. Experience with HPV-related adenosquamous carcinoma is too limited to determine if it shares the same improved prognosis as that of typical HPV-related HNSCC.

HPV-associated adenocarcinoma of the base of the tongue

Rare examples of adenocarcinomas arising in the tongue base have been described that showed p16 expression and the presence of high-risk HPV; these lesions were coined *HPV-associated adenocarcinoma of the base of the tongue* [20–22]. These cases all showed a malignant gland-forming lesion featuring cuboidal to columnar cells with areas of intraluminal mucin, along with variable cribriform and solid architectures. Cytologically, the cells contained nuclei with vesicular to clumped chromatin with small-to-medium-sized nucleoli, and the cytoplasm was described as eosinophilic and variably abundant [20–22]. All the cases described as HPV-associated adenocarcinoma of the base of the tongue were negative for p63, high molecular weight keratins, and neuroendocrine markers. Before making a diagnosis of HPV-associated adenocarcinoma of the base of the tongue, one must first exclude one of the named salivary gland tumors such as mucoepidermoid carcinoma, mammary analogue secretory carcinoma, polymorphous low-grade adenocarcinoma, and cribriform adenocarcinoma of the minor salivary gland, in addition to metastasis. Too few cases of HPV-associated adenocarcinoma of the base of the tongue have been described to understand its true prognostic import [20–22].

HPV-related small cell carcinoma of the oropharynx

In 2011 and 2012, a series of oropharyngeal carcinomas showing small cell carcinoma morphology, i.e., small-to-medium-sized hyperchromatic cells with nuclear molding, scant cytoplasm, brisk mitotic and apoptotic activity, necrosis, and

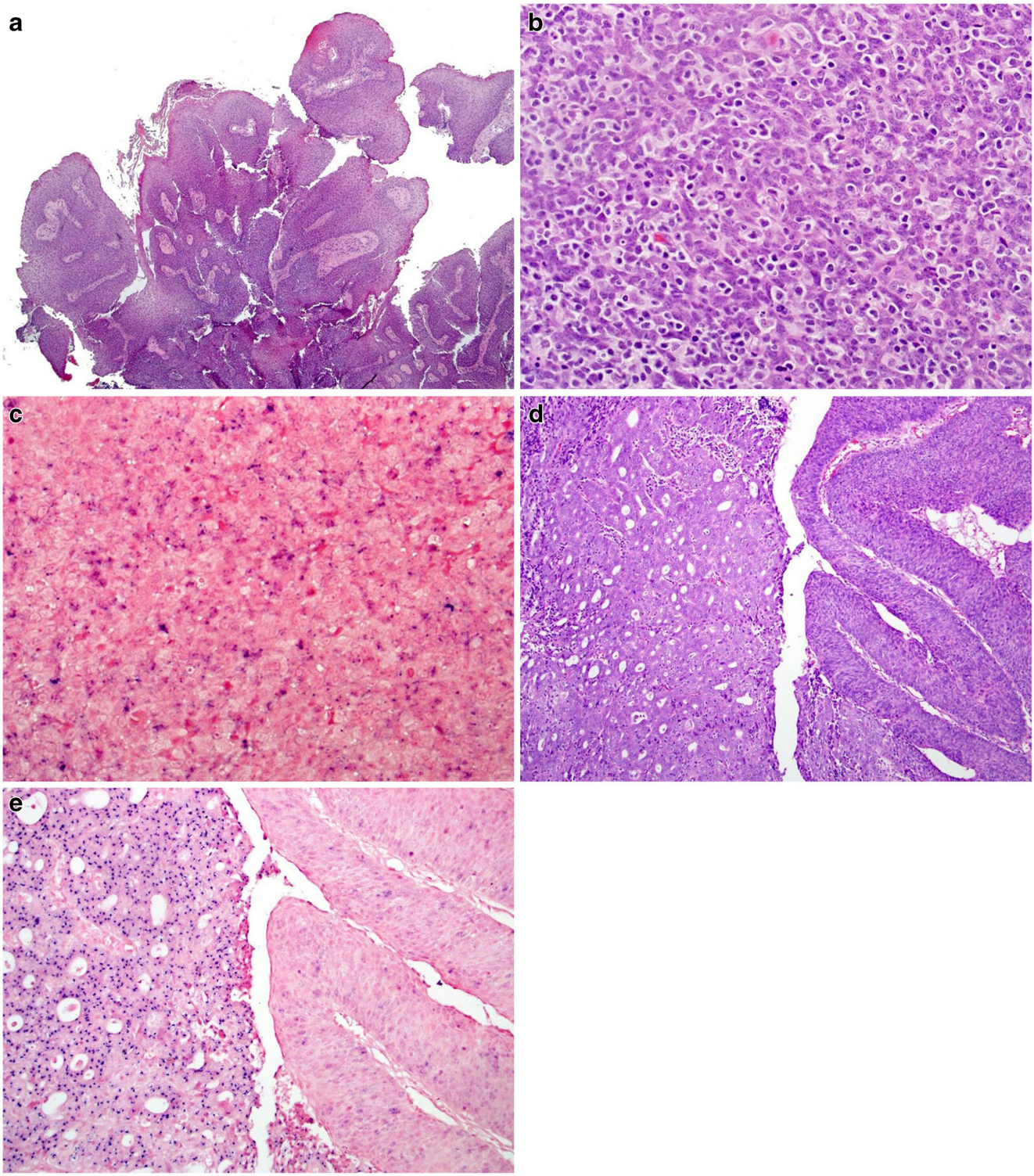

Fig. 4 **a** HPV-related papillary squamous cell carcinoma of the head and neck shows surface papillae and typically a non-keratinizing morphology. **b** HPV-related lymphoepithelial-like carcinoma of the oropharynx shows carcinoma cells with prominent nucleoli and syncytial cytoplasm set in an inflammatory background. **c** HPV-related lymphoepithelial-like carcinoma showing integrated high-risk HPV by DNA in situ hybridization. **d** An HPV-related adenosquamous carcinoma showing malignant glandular and squamous components. **e** Both components of the HPV-related adenosquamous carcinoma show integrated high-risk HPV by DNA in situ hybridization

expression of neuroendocrine markers (Fig. 5a, b, c), were reported [23, 24]. In almost half of these cases, the small cell carcinoma component was juxtaposed to areas of prototypical HPV-related HNSCC. Both small cell and prototypical

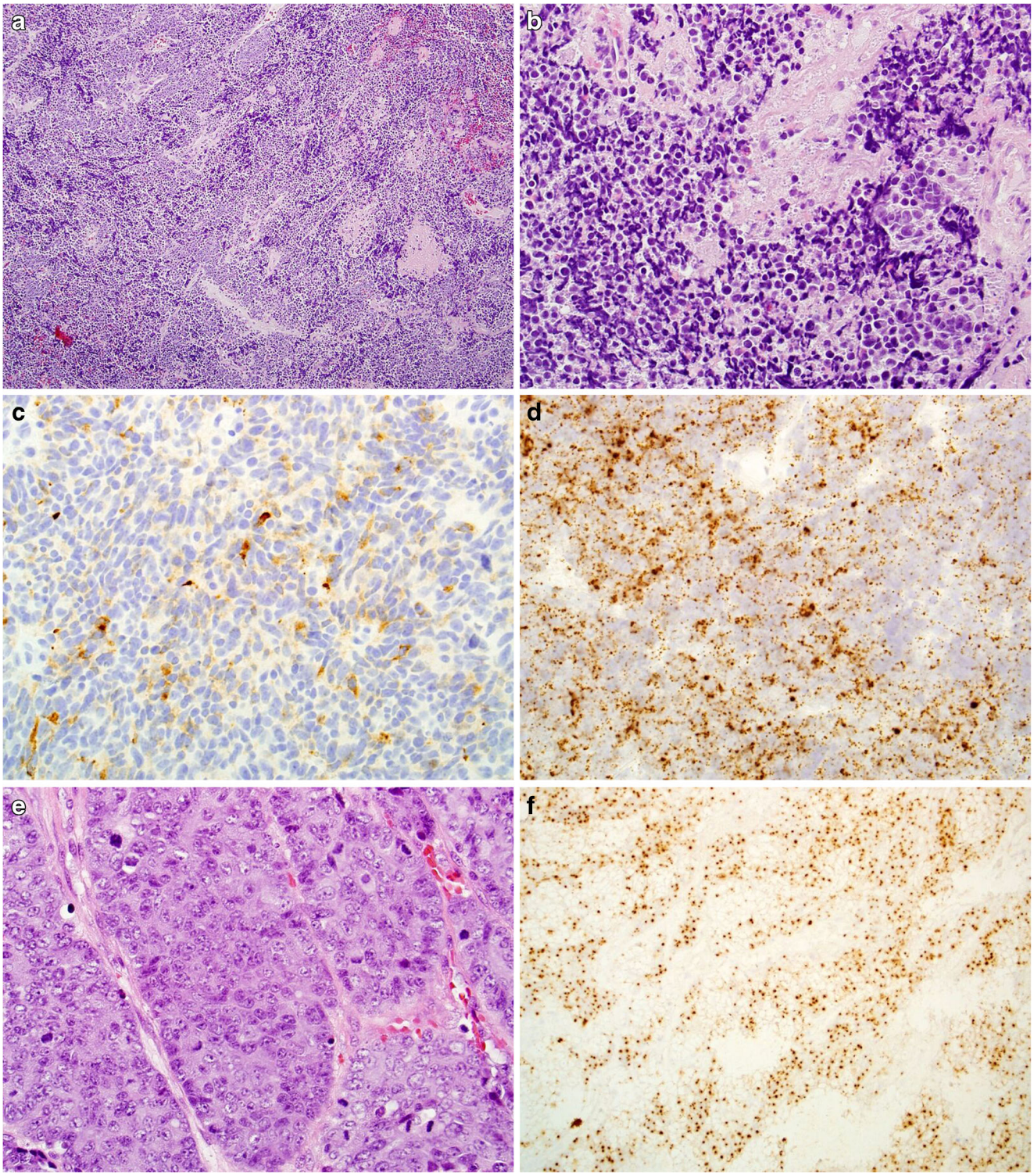

Fig. 5 **a**, **b** HPV-related small cell carcinomas show small-to-medium-sized carcinoma cells with nuclear molding, evenly distributed chromatin, and necrosis. **c** Expression of neuroendocrine markers (synaptophysin as shown here). **d** Integrated high-risk HPV RNA signals by in situ hybridization. **e** An example of a HPV-related large cell neuroendocrine carcinoma showing coarse chromatin and prominent nucleoli. **f** Integrated high-risk HPV RNA signals by in situ hybridization were identified in this HPV-related large cell neuroendocrine carcinoma

components harbored HPV by ISH (Fig. 5d). This entity was coined HPV-related small cell carcinoma of the oropharynx as all such cases were of oropharyngeal origin [23, 24]. Given that typical HPV-related HNSCCs also show hyperchromatic

cells with high nuclear to cytoplasmic ratios and evenly distributed chromatin, identifying small cell carcinoma histology in this context can be challenging. However, small cell carcinoma of the oropharynx will show evidence of neuroendocrine differentiation and typically show loss of squamous markers CK5/6 and p63 [24], unlike areas of prototypical HPV-related HNSCC. It is important to identify the small cell carcinoma component in an HPV-related tumor as this phenotype is associated with highly malignant behavior, like small cell carcinomas from other sites, and the small cell component overrides any prognostic benefit provided by the HPV status. Accordingly, patients with HPV-related HNSCC with small cell features are not offered de-escalation therapy. Further study is needed to determine if HPV-related small cell carcinomas occur in non-oropharyngeal head and neck sites.

HPV-related large cell neuroendocrine carcinoma of the head and neck

Like their more well-known counterparts in the lung and cervix, large cell neuroendocrine carcinomas (LCNEC) of the head and neck show evidence of neuroendocrine differentiation both histologically and immunophenotypically. Unlike the prototypical HPV-related HNSCC, LCNEC (Fig. 5e) show larger, polygonal cells with well-defined cell borders along with a more organoid architecture, peripheral palisading, rosettes, and coarser chromatin with more prominent nucleoli and more well-defined cell borders [25]. LCNEC of the head and neck will show a high mitotic rate (>10 mitosis/10 high-powered fields) and frequent necrosis; however, prototypical HPV-related squamous cell carcinomas will also show a brisk mitotic rate and necrosis. Immunohistochemical expression of neuroendocrine markers (synaptophysin, chromogranin, and/or CD56) and negative or focally positive p63 allows further distinction from prototypical HPV-related HNSCC. Thompson et al. tested ten LCNEC of the head and neck for p16 and HPV ISH and found six to be positive for p16 and three to be positive for high-risk HPV by ISH (Fig. 5f). All three HPV-positive LCNEC were p16 positive, and two were from the oropharynx and one was from the sinonasal tract, the two "hot spots" of HPV-related HNSCC [25]. Importantly, the three HPV-positive LCNEC of the head and neck clinically showed aggressive behavior. Therefore, it appears that HPV-related large cell neuroendocrine carcinomas of the head and neck share a similar dismal prognosis with LCNEC of the lung and cervix.

HPV-related head and neck squamous cell carcinoma with spindle cell/sarcomatoid features

Sarcomatoid carcinomas of the head and neck are most common in the larynx, but can occur at any site, including the oropharynx. Bishop et al. detected high-risk HPV by ISH in three of ten sarcomatoid carcinomas of the oropharynx [26], but no HPV in sarcomatoid carcinomas from other head and neck sites. Watson et al. tested 31 sarcomatoid carcinomas of the head and neck and found that 1/12, 1/14, and 0/5 oral cavity, larynx, and oropharyngeal sarcomatoid carcinomas, respectively, were positive for high-risk HPV E6 and E7 transcripts by RNA ISH [27]. Both patients with HPV-positive sarcomatoid carcinoma showed high-stage disease and death within 2 years. The authors concluded that HPV-related sarcomatoid carcinomas are very rare, and while data is limited, do not appear to carry the same improved prognosis as prototypical HPV-related HNSCC.

HPV-related carcinoma with adenoid cystic-like features

In 2013, Bishop et al. described a group of sinonasal tumors that showed some features of adenoid cystic carcinoma, namely hypercellular proliferations of basaloid carcinoma cells arranged in round nests and solid areas with cribriform and microcystic architecture with fibrous stroma in between (Fig. 6a) [1]. These tumors showed evidence of abluminal myoepithelial differentiation along with microcystic spaces containing basophilic material, similar to that seen in adenoid cystic carcinoma. However, unlike classical adenoid cystic carcinoma, these cases showed squamous dysplasia of the overlying surface mucosa, a lack of *MYB* gene rearrangements, expressed p16, and showed the presence of high-risk HPV by ISH (Fig. 6b, c). Unlike prototypical HPV-related HNSCC, HPV type 33 was most commonly identified. The authors named this entity human papillomavirus-related carcinoma with adenoid cystic-like features. The prognostic significance of this carcinoma type is not yet known.

Ciliated HPV-related carcinoma

While typically indicators of a benign process, the presence of cilia has been described in adenocarcinomas of the gynecologic tract, esophagus, stomach, and lung, and in 2015, two groups described a total of ten cases of HPV-related oropharyngeal squamous cell carcinomas that displayed focal respiratory type columnar cells with cilia (Fig. 7a, b), both in the primary site and in neck metastasis [16, 28]. The columnar ciliated cells were p16 positive and contained high-risk HPV by ISH (Fig. 7c). As neck metastasis from HPV-related HNSCC is often cystic with an attenuated lining, the possible presence of cilia in these tumors further confounds their distinction from branchial cleft cyst. Further, branchial cleft cysts can also express p16. Therefore, in a neck mass where the differential diagnosis is between branchial cleft cyst and cystic metastasis from squamous cell carcinoma, definitive HPV (PCR or ISH) studies can be very helpful, as branchial cleft cysts are negative for HPV [6, 7].

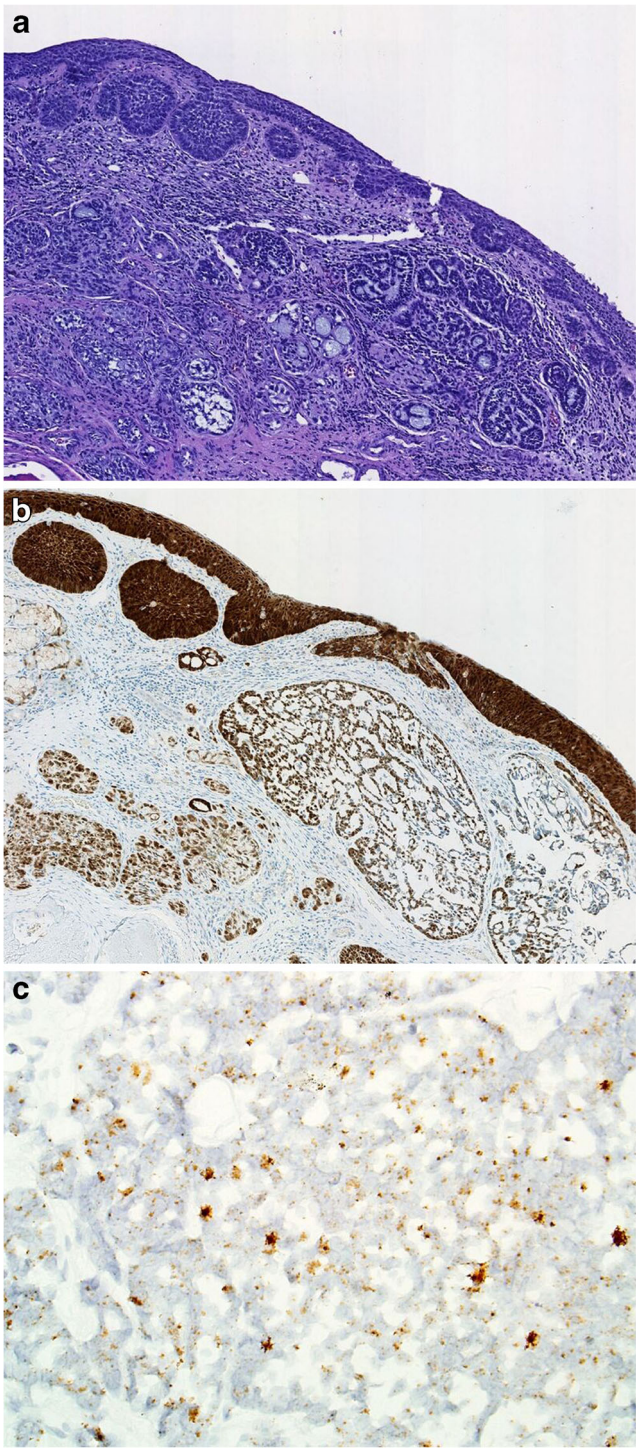

Fig. 6 **a** HPV-related carcinoma with adenoid cystic-like features displays hypercellular nests of blue cells with microcystic and cribriform architecture, surface dysplasia, **b** p16 expression, and **c** integrated high-risk HPV RNA signals by in situ hybridization

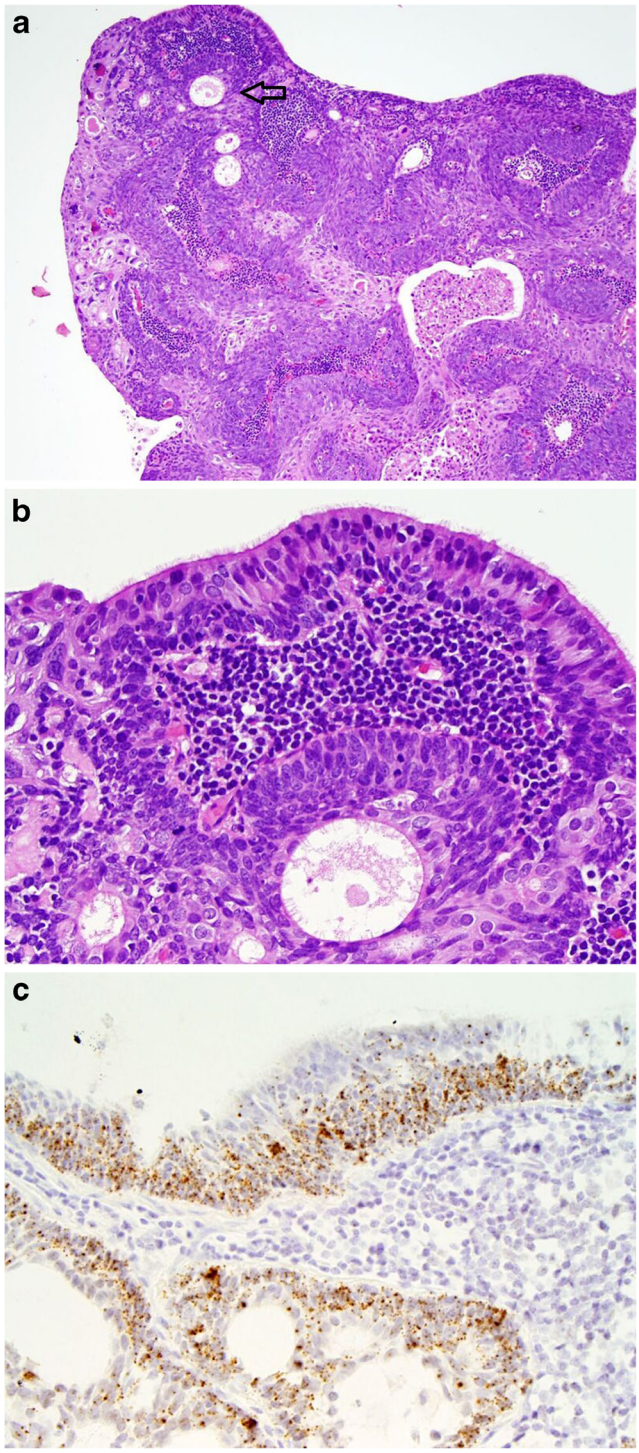

Fig. 7 Like branchial cleft cysts, ciliated HPV-related carcinomas (**a**) contain focal cilia (**b**) and show expression of p16 (not shown), but unlike branchial cleft cysts, ciliated HPV-related carcinomas contain high-risk HPV (positive RNA in situ hybridization) (**c**). Area shown at high power in **b** is marked with an *arrow* in **a**

Nomenclature and grading of HPV-related head and neck squamous cell carcinomas

Given their non-keratinizing and basaloid morphology, HPV-related HNSCCs are often diagnosed as poorly differentiated squamous cell carcinoma. However, this poorly differentiated designation is at odds with the facts that typical HPV-related HNSCC resembles the reticulated crypt epithelium from which most of them arise, and that they carry a better

prognosis than HPV-negative HNSCC. Therefore, HNSCC showing prototypical HPV-related morphology (excluding the small cell and large cell neuroendocrine and sarcomatoid variants) and evidence of HPV infection should be referred to as "squamous cell carcinoma, HPV-related" or "squamous cell carcinoma, p16-positive," and a grade should not be given [6, 7]. The term "non-keratinizing" can be added as appropriate, but as mentioned above, use of the term basaloid is discouraged given the potential confusion with the HPV-unrelated basaloid squamous cell carcinoma that shows a much more aggressive clinical behavior than classic HPV-related HNSCC of the oropharynx.

When and how to test for HPV in head and neck carcinomas

A wide range of methods are available for HPV testing in HNSCC, from routine histology, p16 immunohistochemistry, PCR, and ISH for high-risk HPV DNA, PCR for the E6 and E7 proteins, RNA ISH, and combinations of these methodologies. While, at the current time, there is no universally agreed-upon method or algorithm for testing for HPV in HNSCC, it is understood that testing for HPV in HNSCC should only be directed at the high-risk HPV subtypes and the decision to test should not be influenced by a patient's smoking history.

The E7 protein, a result of transcriptionally active HPV infection, inactivates the Rb protein, which normally inactivates p16. Therefore, HPV-induced tumorigenesis leads to accumulation of the p16 protein, a protein that normally inhibits cyclin-dependent pathways of cellular proliferation. In contrast, smoking- and alcohol-related HNSCCs typically show reduced levels of p16 owing to mutation and hypermethylation of the p16 gene. Therefore, p16 immunohistochemistry is a cheap and readily available surrogate marker for the presence of HPV and in the oropharynx is approximately 100% sensitive for detecting HPV-induced tumorigenesis. While p16 immunohistochemistry shows reduced specificity (~80–85%) as compared to DNA- and RNA-based methods of HPV detection, the presence of p16 overexpression in oropharyngeal squamous cell carcinomas is biological evidence of HPV tumorigenic effect with its resultant prognostic benefits. p16 is considered to be positive in HNSCC when ≥70% of tumor cells show strong nuclear and cytoplasmic staining [29].

PCR for high-risk HPV DNA is highly sensitive but suffers from uncertain specificity given the ability of PCR to amplify small amounts of "passenger" non-tumorigenic HPV DNA. On the other hand, ISH for HPV DNA has the advantage of directly visualizing integrated HPV DNA signals in a tumor cell's nucleus, eliminating the problem of passenger DNA that confounds PCR results. Further, ISH for HPV DNA can be automated and is easily integrated into routine histology laboratories. However, DNA ISH tests suffer from reduced sensitivity compared to p16 immunohistochemistry and PCR-based methods [29, 30]. RNA ISH offers the same localization to tumor cell nuclei but confirms that HPV DNA is being actively transcribed, further confirming its tumorigenic role. However, at the current time, HPV RNA ISH must be performed by hand, limiting its use in most laboratories [31].

Given the reduced specificity of p16 and the reduced sensitivity of ISH-based tests, some have advocated for an approach to HPV testing in HNSCC that uses a combination of methods. For example, if an oropharyngeal SCC shows prototypical non-keratinizing morphology but shows focal or equivocal p16 expression, an additional HPV-specific method such as ISH for HPV DNA or RNA should be employed as a final arbitrator [32]. If such a prototypical non-keratinizing SCC of the oropharynx showed overexpression of p16, however, no additional testing would be needed.

Several different clinical scenarios where HPV testing in HNSCC is used will be presented with recommendations, based on current consensus guidelines [32], for the best platforms used to test for HPV given each scenario.

Non-keratinizing squamous cell carcinoma of the oropharynx In cases of oropharyngeal squamous cell carcinomas that display non-keratinizing histology, p16 positivity carries a near 100% positive predictive value for HPV infection. Therefore, in OP HNSCC with typical HPV morphology, p16 positivity alone can be used to identify the tumor as HPV related.

Keratinizing squamous cell carcinoma of the oropharynx While keratinizing squamous cell carcinomas of the OP that show overexpression of p16 have been shown to carry the same prognostic benefit as confirmed HPV-positive OP HNSCC [33], the numbers of such cases are small. Therefore, any oropharyngeal squamous cell carcinoma that shows histologic deviation from the prototypical HPV-related morphology (e.g., significant keratinization) and is p16 positive should probably be tested for HPV by another HPV-specific methodology (PCR, DNA, or RNA ISH) before diagnosing HPV-related squamous cell carcinoma. However, if a keratinizing squamous cell carcinoma of OP is negative for p16, no further testing is needed.

Squamous cell carcinoma of non-oropharyngeal sites Overall, about 9% of squamous cell carcinomas of non-oropharyngeal sites are positive for HPV, and the presence of HPV in squamous cell carcinomas of non-oropharyngeal sites has not yet been shown to carry the same prognostic benefit as it does in OP sites. Therefore, it is not currently recommended to routinely test HNSCC of non-OP sites for HPV by any methodology. If a clinical scenario or clinical trial demands HPV testing in non-oropharyngeal HNSCC, then a HPV-specific assay such as PCR or ISH should be employed,

given that p16 overexpression in a squamous cell carcinoma from a non-OP site does not show a strong correlation with the presence of HPV infection [6, 7, 29, 31, 34].

Squamous cell carcinoma of unknown primary Thirteen percent of HPV-related OPSCC present initially as neck masses, and demonstrating that a squamous cell carcinoma in a neck node is HPV positive shows a strong correlation with an OP primary, which is important given that many OPSCCs are clinically and radiographically occult [35]. p16 positivity alone in a squamous carcinoma in a level 2 or 3 neck node (jugular chain), especially in the setting of prototypical HPV-related morphology, is probably sufficient to point toward the OP as the primary site. p16 positivity alone in a squamous cell carcinoma in neck nodes outside of level 2 or 3, however, is insufficient evidence to both definitively point to the OP as primary site and label it as HPV-related; in this circumstance (p16 positivity in a squamous cell carcinoma outside of levels 2 and 3), additional HPV-specific testing should be performed .

In patients with newly diagnosed squamous cell carcinoma in the lung with a history of head and neck squamous cell carcinoma, p16 positivity alone would favor a metastasis to lung if the patient had a history of HPV-related OPSCC. In patients with history of non-OP HNSCC with a p16-positive squamous cell carcinoma in the lung, additional HPV-specific testing should be performed if one is to conclude the newly diagnosed squamous cell carcinoma in lung is a metastasis from the head and neck and not a lung primary [6, 7].

Squamous cell carcinoma in head and neck FNA specimens In patients with known OPSCC with unknown HPV status or in cases of squamous cell carcinoma of unknown primary, cell blocks prepared from FNA samples should be tested for high-risk HPV. As cell block samples are often of scant cellularity and of degenerate nature, p16 immunohistochemistry on cell block material suffers from problems with interpretation. Indeed, percent thresholds for regarding p16 as positive are not well established in FNA material as they are in tissue. In addition, branchial cleft cysts, which may be sampled by FNA, are often p16 positive. Therefore, HPV-specific methodologies are recommended for HPV testing on FNA specimens. Liquid-phase HPV assays used in cervical pap smears are increasingly being used in this context, but they have yet to undergo widespread validation [29].

Cystic squamous lesions in the neck It is well known that metastatic HPV-induced head and neck squamous cell carcinomas often form cystic neck metastasis and the epithelium lining these cysts can be quite bland and even show cilia, invoking the differential diagnosis of a branchial cleft cyst [16]. It also has been shown that branchial cleft cysts not uncommonly express p16 [7]. Therefore, in circumstances where the differential diagnosis of a neck mass is between branchial cleft cyst and cystic squamous cell carcinoma, HPV-specific tests are required.

Recurrent and/or metastatic squamous cell carcinoma for which HPV status has already been established In cases of HNSCC where the HPV status is known, it is not currently recommended to retest for HPV should the tumor recur or metastasize.

Small nest of atypical cells in an oropharyngeal specimen In oropharynx specimens that show focal small nests of atypical basaloid epithelial cells that are not clearly recognized as carcinoma, demonstration of HR-HPV would identify the focus as carcinoma [7].

Nasopharyngeal carcinoma vs HPV-related lymphoepithelial-like carcinoma of the oropharynx A lymphoepithelial carcinoma in a head and neck site or neck lymph node is no longer specific for EBV-driven nasopharyngeal carcinoma, given the lymphoepithelial variant of HPV-related OPSCC. Therefore, both HPV and EBV (EBER ISH) testing should be performed when a pathologist encounters a lymphoepithelial carcinoma in a head and neck site. The presence of HPV in a lymphoepithelial carcinoma points to the oropharynx as primary. Further, p16 should be negative in EBV-driven nasopharyngeal carcinoma [18].

Ma in points:

- The oropharynx and sinonasal tract are the head and neck locations most often affected by HPV-related carcinomas.
- HPV-related small cell carcinomas and HPV-related large cell neuroendocrine carcinoma of the head and neck are examples of HPV-related neoplasia of the head and neck where any prognostic benefit afforded by HPV infection is overridden by morphology.
- Prototypical HPV-related squamous cell carcinomas of the oropharynx should not be referred to as poorly differentiated, given their resemblance to the normal reticulated epithelium of the tongue base and tonsil and their overall improved prognosis.
- The term ‘basaloid’ should not be used when referring to an HPV-related squamous cell carcinoma, as it invokes confusion with basaloid squamous cell carcinoma, an HPV-unrelated tumor with dismal prognosis.
- Lymphoepithelial carcinomas of the head and neck should be tested for both HPV and EBV to allow distinction between HPV-related lymphoepithelial-like carcinoma of the oropharynx and EBV-driven nasopharyngeal carcinoma.
- p16 expression by an oropharyngeal squamous cell carcinoma with prototypical HPV-related morphology is sufficient to diagnose it as HPV-related.

- Oropharyngeal squamous cell carcinomas showing morphology suggestive of HPV infection but with negative p16 should be tested for HPV by an HPV-specific methodology.
- p16-positive oropharyngeal squamous cell carcinomas that are heavily keratinizing or show variant morphology should also be tested for HPV by an HPV-specific methodology.
- p16-negative oropharyngeal squamous cell carcinomas that are heavily keratinizing or show variant morphology do not need additional HPV-specific testing.
- Routine HPV testing of a squamous cell carcinoma arising outside of the oropharynx is not currently recommended.
- Branchial cleft cysts can be p16 positive and therefore HPV-specific testing is required when the differential diagnosis of a cystic neck lesion is between a branchial cleft cyst and a cystic well-differentiated HPV-related carcinoma.

Acknowledgements The authors would like to thank Dr. George J. Netto for the opportunity to write this review.

Compliance with ethical standards

Funding None.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Bishop JA, Ogawa T, Stelow EB, Moskaluk CA, Koch WM, Pai SI, Westra WH (2013) Human papillomavirus-related carcinoma with adenoid cystic-like features. Am J Surg Pathol 37(6):836–844. doi:10.1097/PAS.0b013e31827b1cd6
2. Bishop JA, Guo TW, Smith DF, Wang H, Ogawa T, Pai SI, Westra WH (2013) Human papillomavirus-related carcinomas of the sinonasal tract. Am J Surg Pathol 37(2):185–192. doi:10.1097/PAS.0b013e3182698673
3. El-Mofty SK (2012) Human papillomavirus-related head and neck squamous cell carcinoma variants. Semin Diagn Pathol 32(1):23–31. doi:10.1053/j.semdp.2015.02.022
4. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML (2010) Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363(1):24–35. doi:10.1056/NEJMoa0912217
5. Kimple RJ, Smith MA, Blitzer GC, Torres AD, Martin JA, Yang RZ, Peet CR, Lorenz LD, Nickel KP, Klingelhutz AJ, Lambert PF, Harari PM (2013) Enhanced radiation sensitivity in HPV-positive head and neck cancer. Cancer Res 73(15):4791–4800. doi:10.1158/0008-5472.CAN-13-0587
6. Westra WH (2012) The morphologic profile of HPV-related head and neck squamous carcinoma: implications for diagnosis, prognosis, and clinical management. Head Neck Pathol 6(Suppl 1):48–54. doi:10.1007/s12105-012-0371-6
7. Westra WH (2015) The pathology of HPV-related head and neck cancer: implications for the diagnostic pathologist. Semin Diagn Pathol 32(1):42–53. doi:10.1053/j.semdp.2015.02.023
8. Kang H, Kiess A, Chung CH (2014) Emerging biomarkers in head and neck cancer in the era of genomics. Nat Rev Clin Oncol 12(1):11–26. doi:10.1038/nrclinonc.2014.192
9. Singhi AD, Califano J, Westra WH (2012) High-risk human papillomavirus in nasopharyngeal carcinoma. Head Neck 34(2):213–218. doi:10.1002/hed.21714
10. Lingen MW, Xiao W, Schmitt A, Jiang B, Pickard R, Kreinbrink P, Perez-Ordonez B, Jordan RC, Gillison ML (2013) Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol 49(1):1–8. doi:10.1016/j.oraloncology.2012.07.002
11. Chernock RD, Wang X, Gao G, Lewis JS Jr, Zhang Q, Thorstad WL, El-Mofty SK (2013) Detection and significance of human papillomavirus, CDKN2A(p16) and CDKN1A(p21) expression in squamous cell carcinoma of the larynx. Mod Pathol 26(2):223–231. doi:10.1038/modpathol.2012.159
12. Poling JS, Ma XJ, Bui S, Luo Y, Li R, Koch WM, Westra WH (2014) Human papillomavirus (HPV) status of non-tobacco related squamous cell carcinomas of the lateral tongue. Oral Oncol 50(4):306–310. doi:10.1016/j.oraloncology.2014.01.006
13. Lewis JS Jr, Scantlebury JB, Luo J, Thorstad WL (2012) Tumor cell anaplasia and multinucleation are predictors of disease recurrence in oropharyngeal squamous cell carcinoma, including among just the human papillomavirus-related cancers. Am J Surg Pathol 36(7):1036–1046. doi:10.1097/PAS.0b013e3182583678
14. Chernock RD (2012) Morphologic features of conventional squamous cell carcinoma of the oropharynx: 'keratinizing' and 'nonkeratinizing' histologic types as the basis for a consistent classification system. Head Neck Pathol 6(Suppl 1):S41–S47. doi:10.1007/s12105-012-0373-4
15. Begum S, Westra WH (2008) Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. Am J Surg Pathol 32(7):1044–1050. doi:10.1097/PAS.0b013e31816380ec
16. Bishop JA, Westra WH (2015) Ciliated HPV-related carcinoma: a well-differentiated form of head and neck carcinoma that can be mistaken for a benign cyst. Am J Surg Pathol 39(11):1591–1595. doi:10.1097/PAS.0000000000000521
17. Lewis JS Jr, Tarabishy Y, Luo J, Mani H, Bishop JA, Leon ME, Prasad ML, Xu H, Di Palma S (2015) Inter- and intra-observer variability in the classification of extracapsular extension in p16 positive oropharyngeal squamous cell carcinoma nodal metastases. Oral Oncol 51(11):985–990. doi:10.1016/j.oraloncology.2015.08.003
18. Singhi AD, Stelow EB, Mills SE, Westra WH (2010) Lymphoepithelial-like carcinoma of the oropharynx: a morphologic variant of HPV-related head and neck carcinoma. Am J Surg Pathol 34(6):800–805. doi:10.1097/PAS.0b013e3181d9ba21
19. Masand RP, El-Mofty SK, Ma XJ, Luo Y, Flanagan JJ, Lewis JS Jr (2011) Adenosquamous carcinoma of the head and neck: relationship to human papillomavirus and review of the literature. Head Neck Pathol 5(2):108–116. doi:10.1007/s12105-011-0245-3
20. Perez-Ordonez B, Irish JC, Yu ES, Gillison ML (2013) Human papillomavirus-16 associated adenocarcinoma NOS of base of tongue. Head Neck Pathol 7:268–273. doi:10.1007/s12105-012-0404-1
21. Chang AMV, Nikiforova MN, Johnson JT, Bauman JE, Perez-Ordonez B, Seethala RR, Krane JF, Chiosea SI (2014) Human papillomavirus-associated adenocarcinoma of the base of tongue: potentially actionable genetic changes. Head Neck Pathol 8:151–156. doi:10.1007/s12105-013-0508-2
22. Hanna J, Reimann JDR, Haddad RI, Krane JF (2013) Human papillomavirus-associated adenocarcinoma of the base of the

tongue. Hum Pathol 44:1516–1523. doi:10.1016/j.humpath.2012.12.004
23. Bishop JA, Westra WH (2011) Human papillomavirus-related small cell carcinoma of the oropharynx. Am J Surg Pathol 35(11):1679–1684. doi:10.1097/PAS.0b013e3182299cde
24. Kraft S, Faquin WC, Krane JF (2012) HPV-associated neuroendocrine carcinoma of the oropharynx: a rare new entity with potentially aggressive clinical behavior. Am J Surg Pathol 36(3):321–330. doi:10.1097/PAS.0b013e31823f2f17
25. Thompson ED, Stelow EB, Mills SE, Westra WH, Bishop JA (2016) Large cell neuroendocrine carcinoma of the head and neck: a clinicopathologic series of 10 cases with an emphasis on HPV status. Am J Surg Pathol 40(4):471–478. doi:10.1097/PAS.0000000000000580
26. Bishop JA, Montgomery EA, Westra WH (2014) Use of p40 and p63 immunohistochemistry and human papillomavirus testing as ancillary tools for the recognition of head and neck sarcomatoid carcinoma and its distinction from benign and malignant mesenchymal processes. Am J Surg Pathol 38(2):257–264. doi:10.1097/PAS.0000000000000119
27. Watson RF, Chernock RD, Wang X, Liu W, Ma XJ, Luo Y, Wang H, El-Mofty SK, Lewis JS Jr (2013) Spindle cell carcinomas of the head and neck rarely harbor transcriptionally-active human papillomavirus. Head Neck Pathol 7(3):250–257. doi:10.1007/s12105-013-0438-z
28. Radkay-Gonzalez L, Faquin W, McHugh JB, Lewis JS Jr, Tuluc M, Seethala RR (2016) Ciliated adenosquamous carcinoma: expanding the phenotypic diversity of human papillomavirus-associated tumors. Head Neck Pathol 10(2):167–175. doi:10.1007/s12105-015-0653-x
29. Bishop JA, Lewis JS Jr, Rocco JW, Faquin WC (2015) HPV-related squamous cell carcinoma of the head and neck: an update on testing in routine pathology practice. Semin Diagn Pathol 32(5):344–351. doi:10.1053/j.semdp.2015.02.013
30. Stevens TM, Caughron SK, Dunn ST, Knezetic J, Gatalica Z (2011) Detection of high-risk HPV in head and neck squamous cell carcinomas: comparison of chromogenic in situ hybridization and a reverse line blot method. Appl Immunohistochem Mol Morphol 19(6):574–578. doi:10.1097/PAI.0b013e318215248a
31. Bishop JA, Ma XJ, Wang H, Luo Y, Illei PB, Begum S, Taube JM, Koch WM, Westra WH (2012) Detection of transcriptionally active high-risk HPV in patients with head and neck squamous cell carcinoma as visualized by a novel E6/E7 mRNA in situ hybridization method. Am J Surg Pathol 36(12):1874–1882. doi:10.1097/PAS.0b013e318265fb2b
32. Seethala RR, et al. (2013) Protocol for the examination of specimens from patients with carcinomas of the pharynx. College of American Pathologists. http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution Folders/WebContent/pdf/pharynx-13protocol-3300.pdf. Accessed 2 November 2016
33. Cai C, Chernock RD, Pittman ME, El-Mofty SK, Thorstad WL, Lewis JS Jr (2014) Keratinizing-type squamous cell carcinoma of the oropharynx: p16 overexpression is associated with positive high-risk HPV status and improved survival. Am J Surg Pathol 38(6):809–815. doi:10.1097/PAS.0000000000000183
34. Robinson M, Schache A, Sloan P, Thavaraj S (2012) HPV specific testing: a requirement for oropharyngeal squamous cell carcinoma patients. Head Neck Pathol 6(Suppl 1):S83–S90. doi:10.1007/s12105-012-0370-7
35. Singhi AD, Westra WH (2010) Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer 1;116(9):2166–2173. doi:10.1002/cncr.25033