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Review Article

Virus-associated carcinomas of the head & neck: Update from the 2017 WHO classification

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ABSTRACT

Virus-associated carcinomas of the head and neck represent an unusual confluence of infections spread by viral transmission and cellular dysregulation resulting in carcinogenesis. While much remains to be elucidated about the exact progression from infection to cancer, a basic framework of viral biology can complement the pathologist's understanding of morphology. This context informs the pathologist's everyday practice, including selecting ancillary studies, communicating prognostically relevant findings, and participating in treatment planning. By comparing and contrasting the salient features of human papillomavirus-associated oropharyngeal carcinoma and Epstein-Barr virus-associated nasopharyngeal carcinoma, this review summarizes recent evidence to guide current practice.

1. Introduction

Virus-driven carcinomas hold a unique place among cancers of the head and neck. Unlike mucosal squamous cell carcinomas (SCCs) with a high mutational burden due to chronic tobacco and alcohol exposure, virus-associated carcinomas have a distinct pathogenesis that incorporates the protein function and genomic material of the virus. Because viral transmission patterns differ from typical carcinogen exposures, the patient population affected by virus-associated carcinomas is also distinct. A basic understanding of viral biology allows the practicing pathologist to fulfill a critical role in identifying these carcinomas and providing prognostic information. Whereas canonical paradigms such as in situ disease and tumor grading are less relevant for virus-driven carcinomas, recognition of the morphologic spectrum of disease in both histologic and cytologic specimens is important in everyday practice, along with judicious application of ancillary studies. Due to their intact apoptotic pathways and immune reaction, these carcinomas are managed with tailored treatment regimens designed to minimize morbidity while providing locoregional control. Key aspects of the biology, diagnosis, and treatment of human papillomavirus-associated oropharyngeal carcinoma and Epstein-Barr virus-associated nasopharyngeal carcinoma are reviewed with an emphasis on theoretical principles that have practical implications.

2. HPV-associated oropharyngeal squamous cell carcinoma

The rapid rise in the incidence of HPV-positive oropharyngeal squamous cell carcinoma (HPV-OPSCC) over the past few decades has paralleled its recognition as a distinct diagnostic entity [1-3]. Initially subsumed under basaloid or nonkeratinizing squamous cell carcinoma (SCC) of the oral cavity, HPV-OPSCC is now separately defined by its anatomic location, characteristic morphology, and improved prognosis [4]. Accurate identification of this tumor may facilitate de-escalated therapy, dependent on the stage.

2.1. Etiology

Long known as an oncogenic agent in cervical cancer, HPV is a non-enveloped, double-stranded DNA virus that is now established as critical in the pathogenesis of HPV-OPSCC [5]. The vast majority of HPV-OPSCCs are associated with HPV type 16, followed distantly by other high-risk HPV types [2,5,6]. High-risk HPV has a predilection for infecting the tonsillar tissues of the oropharynx [7], specifically the palatine and lingual tonsils [8], where lymphoid tissue interfaces with a specialized reticulated epithelium of the tonsillar crypts. With an incomplete basement membrane that facilitates lymphocyte trafficking into the epithelium, tonsillar crypts demonstrate induction of the PD-1:PD-L1 immune checkpoint pathway that may contribute to immune evasion during HPV infection and tumorigenesis [9].

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High-risk HPV initially infects proliferating basal squamous cells, where early replication establishes and maintains the virus in an episomal state. As the infected squamous cells differentiate, productive viral replication begins, and the E6 and E7 viral proteins prevent the cell from entering a post-mitotic state and instead drive cellular proliferation [10,11]. Specifically, the viral E7 protein binds to the Rb family of proteins and prevents interaction with the E2F transcription factor, facilitating unscheduled re-entry into the cell cycle with subsequent arrest in the G2 phase [12-14]. E7-mediated degradation of Rb results in loss of feedback inhibition on $p16^{INK4A}$ (p16), leading to increased expression of p16 detectable by immunohistochemistry [15-17]. Simultaneously, p53 is targeted for degradation by the viral E6 protein [18,19], further driving progression through the cell cycle and obviating the need for a separate p53 mutation.

While persistent high-risk HPV infection is necessary for oncogenesis, it is not sufficient for the development of carcinoma. Integration of the viral genome into the host genome may contribute to carcinogenesis, although episomal viral genomes are also seen in a significant proportion of HPV-OPSCC [20,21]. Additionally, molecular analysis of HPV-OPSCC has demonstrated further genetic and epigenetic changes in tumor cells, including recurring mutations affecting the PI3K pathway [21-23]. The complex sequence of steps leading from cell cycle dysregulation associated with HPV infection to the development of HPV-OPSCC is the subject of ongoing research.

2.2. Epidemiology and clinical presentation

HPV-OPSCC affects a patient population that is overlapping but divergent from patients who develop non-HPV-related SCC. In particular, patients with HPV-OPSCC are more likely to be male, white, of higher socioeconomic status, and 2–4 years younger than their counterparts with HPV-negative carcinomas [24-26]. Within the United States, HPV-OPSCC is more prevalent in Western states and less common in Southern states [26]. Risk factors for developing HPV-OPSCC include behaviors associated with high-risk HPV exposure, including increasing numbers of oral and vaginal sexual partners as well as other measures of sexual activity such as lack of barrier use during sex [25,27]. While alcohol consumption does not appear to increase risk, tobacco and marijuana use have shown a variable association with HPV-OPSCC, and their role in HPV infection and tumorigenesis remains to be fully elucidated [25,28].

At clinical presentation, patients with HPV-OPSCC are more likely to have a low T stage (T1 or T2) with more advanced nodal disease but no distant metastases when compared with non-HPV-related SCC [24,26,29]. In fact, the most common presenting symptom for patients with HPV-OPSCC is a neck mass [30]. Moreover, HPV-OPSCC accounts for a significant proportion of carcinoma of unknown primary presenting with a cervical lymph node metastasis, with specific percentages varying widely but ranging up to 91% in some retrospective studies [31,32]. Many of these cases are found to have a small primary site deep in the crypts of the palatine or lingual tonsils following direct visualization with biopsy and tonsillectomy. The distribution of nodal metastases remains similar to non-HPV-related SCC, with ipsilateral cervical levels II and III being the most common sites of nodal disease [29]. Compared to non-HPV-related tumors, metastatic HPV-OPSCC to cervical lymph nodes is more likely to be cystic than solid or necrotic [33-35].

2.3. Pathologic findings

2.3.1. Histopathology

On histologic evaluation, HPV-OPSCCs have a characteristic appearance that provides a reliable morphologic indication of their etiology and behavior (Fig. 1). HPV-OPSCCs typically do not have a dysplastic surface component, as they do not develop from the superficial keratinizing squamous mucosa. Instead, the tumor arises from the reticulated epithelium (also referred to as lymphoepithelium) of the tonsillar crypts, infiltrating tonsillar tissue in endophytic ribbon-like bands and sheets composed of nonkeratinizing epithelium that may intermingle with lymphoid tissue (Fig. 1A) [36-38]. Comedo-type necrosis as well as apoptotic cells and numerous mitotic figures are often identified (Fig. 1B). Single cell infiltration is notably absent in the majority of cases. Further, despite the presence of an infiltrative carcinoma, a desmoplastic stromal response is typically absent. On higher power, the epithelial cells are relatively uniform, demonstrating a high nucleus to cytoplasm ratio with scant amphophilic syncytial cytoplasm, ovoid to elongated nuclei with hyperchromasia, and coarse chromatin without prominent nucleoli. In one study, this characteristic nonkeratinizing morphology demonstrated a positive predictive value for high-risk HPV mRNA of 100% [39]. In another study of inter-observer reproducibility, nonkeratinizing HPV-OPSCC could be reliably identified by pathologists from multiple institutions, supporting the importance of morphologic recognition in making the diagnosis [38].

While the nonkeratinizing pattern is the most common morphology seen in HPV-OPSCC and most strongly predicts HPV positivity, the majority of oropharyngeal tumors with > 10% squamous maturation (referred to as hybrid carcinomas) are also HPV-related (Fig. 1C) [36,38,39]. Keratinizing SCC with squamous eddies, abundant eosinophilic cytoplasm, intercellular bridges, and keratin pearls is unusual but can also be associated with HPV in 10–30% of cases [36,38-40]. In a small study, keratinizing HPV-OPSCC demonstrated similar biologic behavior to nonkeratinizing HPV-OPSCC [40].

In considering in situ versus invasive disease in HPV-OPSCC, the microanatomy of the tonsil provides an essential context. The reticulated tonsillar epithelium supports the specialized functions of tonsillar tissue for antigen surveillance and immunoglobulin secretion. While the surface mucosa is intact, the crypt epithelium is penetrated by numerous lymphocytes, antigen-presenting cells, and occasional intraepithelial capillaries [41]. The basement membrane is discontinuous to facilitate the interaction between epithelial and immune cells. Given this anatomic structure, HPV-OPSCC has immediate access to lymphovascular channels, even at incipient stages. Thus, in situ HPV-OPSCC is not a recognized entity, and even miniscule tumors without morphologic evidence of single-cell invasion are considered invasive due to their potential to metastasize to cervical lymph nodes.

In cervical lymph node metastases, HPV-OPSCC often recapitulates the lymphoepithelial morphology of the tonsillar tissue. On low power, the tumor typically grows in a ribbon-like pattern of nonkeratinizing epithelium of relatively uniform thickness with intervening slit-like spaces or cyst formation [34,42]. The closely associated lymphoid tissue may form germinal centers. The epithelium is often disarmingly bland but at least focally may demonstrate loss of polarity or atypical mitoses. Cystic degeneration and necrosis are common. While patchy maturation can be seen, extensive keratin formation is rare. Diffuse intercellular bridges or keratin pearls should raise the possibility of a metastasis from a non-oropharyngeal primary site [34].

2.3.2. Ancillary testing

While the morphologic features and anatomic location can be highly suggestive of HPV-OPSCC, ancillary testing is recommended to confirm the diagnosis [43]. Based on a number of studies demonstrating a close correlation between p16 positivity by immunohistochemistry and transcriptionally active HPV [44-48], the College of American Pathologists recommends identification of HPV-OPSCC by p16 immunohistochemistry on all newly diagnosed SCCs of the oropharynx [43]. For patients presenting with a neck mass, p16 immunotesting is also recommended during the initial workup of a biopsy showing SCC from cervical level II and III lymph nodes with no clinically apparent primary site. In this setting, p16 positivity strongly suggests an oropharyngeal metastasis [42,43,49]. The high prevalence of disease at these locations allows for optimal sensitivity and specificity for p16 immunohistochemistry, which is available in most diagnostic

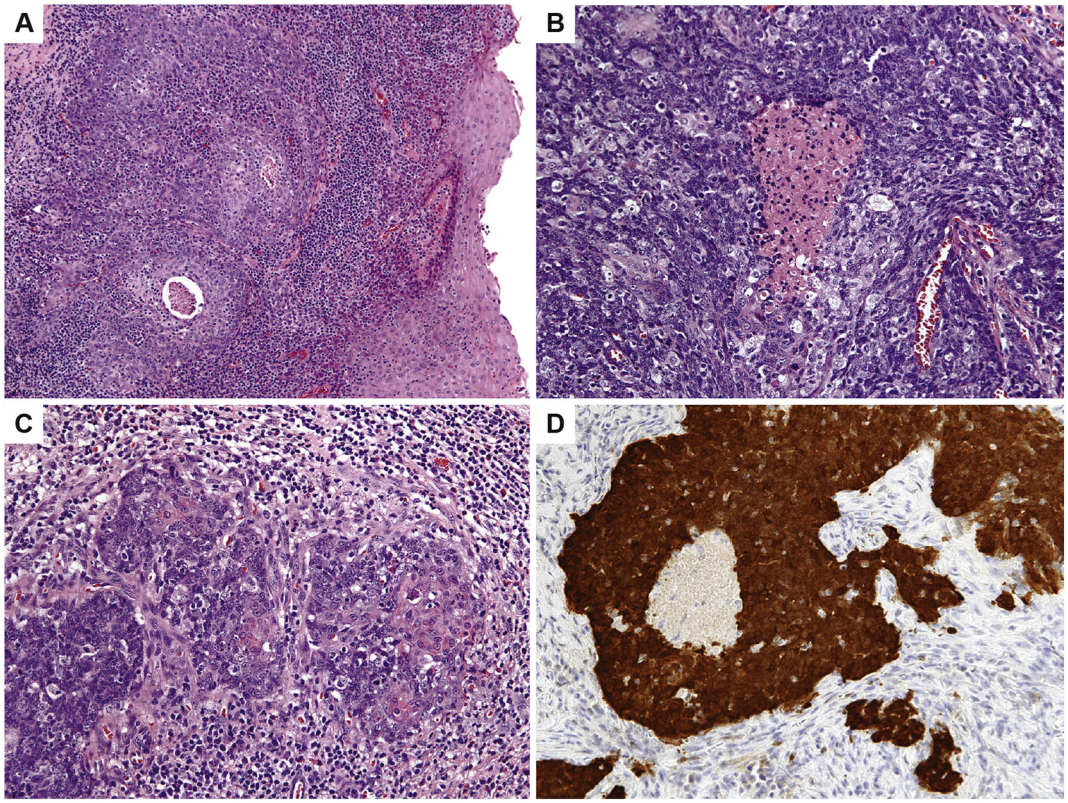

Fig. 1. Classic histomorphologic findings of HPV-OPSCC arising in tonsillar crypt epithelium. The primary carcinoma may be small and typically arises deep to the surface epithelium, which lacks dysplasia (A). Comedonecrosis is common (B). While the morphology is predominantly nonkeratinizing, focal squamous maturation with keratinization can be seen (C). An immunostain for p16 shows > 70% strong and diffuse staining in both the nuclei and cytoplasm of the tumor cells (D), with admixed inflammatory cells and surrounding stroma providing a negative control.

laboratories. In order to score as positive, the tumor must demonstrate > 70% strong and diffuse expression of p16 in both the nuclei and cytoplasm, although areas of keratinization may not stain as extensively (Fig. 1D) [43,50]. The presence of nonkeratinizing morphology in an oropharyngeal SCC in conjunction with strong and diffuse p16 immunopositivity is sufficient to diagnose HPV-OPSCC without further HPV-specific testing [39]. Although a variety of names have been utilized, the diagnostic terminology suggested by the College of American Pathologists is “HPV-positive” or “p16-positive” SCC. If “p16-positive SCC” is used, a comment is recommended to state that the p16 expression is strongly associated with HPV-positive oropharyngeal carcinoma in the appropriate clinical setting.

While the majority of HPV-OPSCC is nonkeratinizing, a small study demonstrated the fidelity of p16 in predicting HPV status for keratinizing SCC of the oropharynx, although this remains to be validated in larger studies [40]. Current guidelines recommend the same algorithmic approach for all histotypes of SCC [43]. Retesting of residual/recurrent or metastatic previously tested tumors is not clinically indicated [43].

Due to the high sensitivity of p16 in comparison with the high specificity of HPV-specific testing, this algorithm will occasionally result in a diagnosis of p16-positive HPV-OPSCC in tumors that do not demonstrate HPV by other methods. However, studies have demonstrated that these tumors retain a similar behavior to tumors that test positively using HPV-specific assays [45,46,51,52]. Some of these tumors harbor high-risk HPV types not covered by type-specific assays, while others are hypothesized to have shed episomal virus that is no longer detectable. Regardless of the mechanism, these tumors are currently treated according to guidelines for HPV-OPSCC based on their similar clinical behavior. Conversely, HPV DNA-positive OPSCCs that are p16-negative behave clinically like HPV-negative cases, obviating the need for HPV-specific testing in p16-negative tumors [51,53]. Further studies are ongoing to clarify the pathogenesis and prognosis of these discordant cases.

Outside of the oropharynx, p16 positivity does not reliably correlate with HPV positivity, and p16 upregulation may be driven by other molecular pathways that are not associated with HPV-OPSCC [54,55]. Specifically, due to the lower prevalence of HPV, p16 demonstrates decreased positive predictive value for HPV at non-oropharyngeal head and neck sites as well as in cervical lymph nodes outside levels II-III [54,56,57]. Furthermore, p16-positive HPV-related tumors outside of the oropharynx do not consistently demonstrate the improved survival and distinctive behavior seen in HPV-OPSCC [55]. Thus, prospective identification of these tumors is currently of limited value and not routinely recommended, although studies of the prognostic value of HPV identification at other anatomic sites are ongoing [43].

In certain settings where an oropharyngeal source is possible but less likely, p16 can be used as a screening assay, followed by confirmatory testing for HPV if p16 is positive [43]. First, this reflex approach is relevant for multisite tumors with involvement of the oropharynx in order to distinguish primary HPV-OPSCC from tumors of other sites extending into the oropharynx. Second, for patients with a cervical lymph node metastasis of unknown primary site, this reflex algorithm is recommended for SCC involving cervical lymph nodes of unspecified anatomic location or lacking the typical nonkeratinizing morphology of HPV-OPSCC. While identification of HPV-OPSCC in these two defined situations is prognostically relevant, the decreased positive predictive value of p16 necessitates HPV-specific confirmation. A number of assays are available to meet this need, including in situ hybridization (ISH) and PCR-based methods, and current guidelines do not favor one method over another [43].

While a comprehensive overview of confirmatory methods for

detecting HPV is beyond the scope of this review, three general approaches can be utilized. PCR-based molecular assays typically demonstrate high sensitivity and correlate well with p16 immunohistochemistry, with options including laboratory-developed tests as well as commercially available kits [45,58,59]. However, since PCR-based assays can be labor-intensive with a risk of cross-contamination, ISH assays for HPV may be more accessible to diagnostic immunohistochemistry laboratories for routine clinical practice. Many initial studies utilized DNA ISH assays, which are highly specific but less sensitive. Thus, correlation with p16 leads to an increased number of discrepant p16-positive but HPV DNA ISH-negative cases compared to PCR-based methods [44,58,60]. More recently, in situ hybridization assays for HPV E6/E7 mRNA have become available [61-63]. Utilizing RNA instead of DNA enables detection of transcriptionally active virus while minimizing cross-reactivity from incidental bystander infections. These RNA ISH assays have demonstrated close correlation with p16 immunohistochemistry as well as PCR-based assays [39,64-66]. Until further studies establish which assays demonstrate superior performance, selection of an HPV confirmation method depends upon the workflow, staffing, and needs of the specific laboratory [43].

2.3.3. Fine needle aspiration cytology

Fine needle aspiration (FNA) plays an important role in the workup of HPV-OPSCCs, which frequently present with cervical lymph node metastases as discussed previously (Fig. 2). In addition to facilitating a morphologic diagnosis of a nonkeratinizing squamous cell carcinoma, an FNA can provide sufficient material for ancillary testing to confirm the specific diagnosis of HPV-OPSCC [67]. In a carcinoma of unknown primary involving a level II-III lymph node, the characteristic morphology in conjunction with p16 positivity or HPV detection strongly suggests an oropharyngeal primary site. This information narrows the clinicians' search and decreases morbidity by limiting the scope of treatment [49,68,69].

On direct smears, HPV-OPSCC can demonstrate several morphologic patterns (Fig. 2) [70]. In the classic pattern of a nonkeratinizing carcinoma, the malignant cells form cohesive sheets with hyperchromatic, mildly irregular nuclei that may demonstrate a streaming pattern with syncytial cytoplasm and nuclear overlapping (Fig. 2A, B). Identification of intercellular bridges or focal cytoplasmic keratinization, which appears turquoise blue on a Romanowsky stain or orange on a Papanicolaou stain, supports morphologic recognition of squamous differentiation. Lymphoid cells may intermingle with the malignant epithelial cells, creating a lymphoepithelial pattern (Fig. 2C). Mitotic figures and necrotic debris are often present. Some cases exhibit a prominent basaloid pattern, with closely packed or loosely aggregated cells with scant or stripped cytoplasm and hyperchromatic nuclei with coarse chromatin lacking nucleoli. Other cases display prominent cystic degeneration with histiocyte-rich proteinaceous fluid that may contain inflammation, bland mature squamous cells, or anucleate keratinaceous debris. While a careful search for atypical squamous cells with hyperchromatic nuclei and irregular nuclear contours can strongly suggest the diagnosis of HPV-OPSCC, ancillary studies play an especially prominent role in cystic nodal metastases that may yield few malignant cells on cytologic preparations.

In the context of supportive morphologic findings, a definitive diagnosis of HPV-OPSCC can be achieved on FNA material from a cervical lymph node using p16 immunohistochemistry, HPV ISH, or HPV PCR on a variety of sample preparations or paraffin-embedded tissue. Current guidelines do not recommend any specific testing methodology, and laboratories may use either p16 immunohistochemistry or HPV-specific assays following validation of diagnostic cut-offs on cytology material [43]. In cases where squamous differentiation is difficult to identify morphologically, immunohistochemistry for p63, p40, or CK5/6 can support a diagnosis of squamous cell carcinoma (Fig. 2D). Immunohistochemistry for p16 has demonstrated reliable positivity in cytology cell block material that correlates with subsequent resection specimens [71,72]. While determining the threshold for p16 positivity can be occasionally problematic in fragmented cell block material, the relative binary distribution of p16 expression implies that most positive samples will demonstrate > 70% unequivocal staining when intact epithelial fragments are present (Fig. 2E). HPV ISH as well as PCR-based RNA and DNA assays have shown similar high fidelity between cytology and subsequent surgical specimens (Fig. 2F) [71-73].

Liquid-based assays for HPV detection that were developed for gynecologic screening are also effective in FNA material from head and neck sites [74-78]. Liquid-based assays can be particularly valuable in cystic or necrotic metastases with limited epithelial cells present for evaluation. In this setting, p16 may be difficult to interpret due to lack of intact epithelium for scoring. Liquid-based detection of HPV in the cyst fluid or identification of HPV by RNA ISH within degenerated keratinocytes strongly favors HPV-OPSCC [79].

2.4. Grading and staging

Although previously mischaracterized as basaloid or poorly differentiated in comparison with keratinizing squamous epithelium, HPV-OPSCC predictably recapitulates the nonkeratinizing tonsillar epithelium from which it originated. Since the basaloid or undifferentiated appearance as well as the lack of keratinization do not convey a worse prognosis, tumor grading is not recommended [5,43].

Similar to other carcinomas of the head and neck, the primary T stage and metastasis to multiple lymph nodes are the staging criteria that most closely correlate with poor survival. Unlike non-HPV-related SCCs, extranodal extension does not necessarily convey a worse prognosis in HPV-OPSCC and is not currently included in staging criteria [80], although some studies have shown worse overall survival in patients with extranodal extension [81]. As tailored treatment strategies are developed for HPV-OPSCC, the role of extranodal extension in prognostication may be refined in future studies.

2.5. Treatment modalities

Compared to HPV-negative oropharyngeal squamous cell carcinomas, patients with HPV-OPSCC have a significantly longer overall survival with a 50–60% decrease in the risks of progression and death [2,5,82-84]. In addition, HPV-OPSCCs demonstrate a higher response rate to chemoradiation with or without induction chemotherapy [82,83]. Furthermore, preliminary studies suggest that a select group of patients with small-volume (stage T1–2) tumors are amenable to endoscopic transoral robotic surgical approaches with or without adjuvant radiation or chemoradiation [85,86]. Similar rates of locoregional control have been reported using intensity-modulated radiotherapy (IMRT) alone [87,88]. Given the improved prognosis compared to HPV-negative tumors, ongoing clinical trials are studying de-escalated therapy protocols for HPV-OPSCC [89]. The goal of these protocols is to preserve disease-free survival while decreasing long-term morbidity, particularly for small tumors.

2.6. Uncommon and recently described variants

2.6.1. Morphologic variants

While the predominant morphologic pattern in HPV-OPSCC is that of a nonkeratinizing squamous cell carcinoma with cystic lymph node metastases, a number of variant histotypes have been described (Figs. 3 and 4). Since the spectrum of morphologic variants has been recently reviewed [90], the salient features of each variant will be briefly summarized here. Despite the wide variety of histologic findings ranging from glandular to basaloid, small studies of morphologic variants have reported a similar prognosis to conventional HPV-OPSCC with the notable exception of neuroendocrine carcinoma, discussed separately below. Thus, an understanding of these alternative morphologies allows the practicing pathologist to recognize HPV-OPSCC in all its patterns,

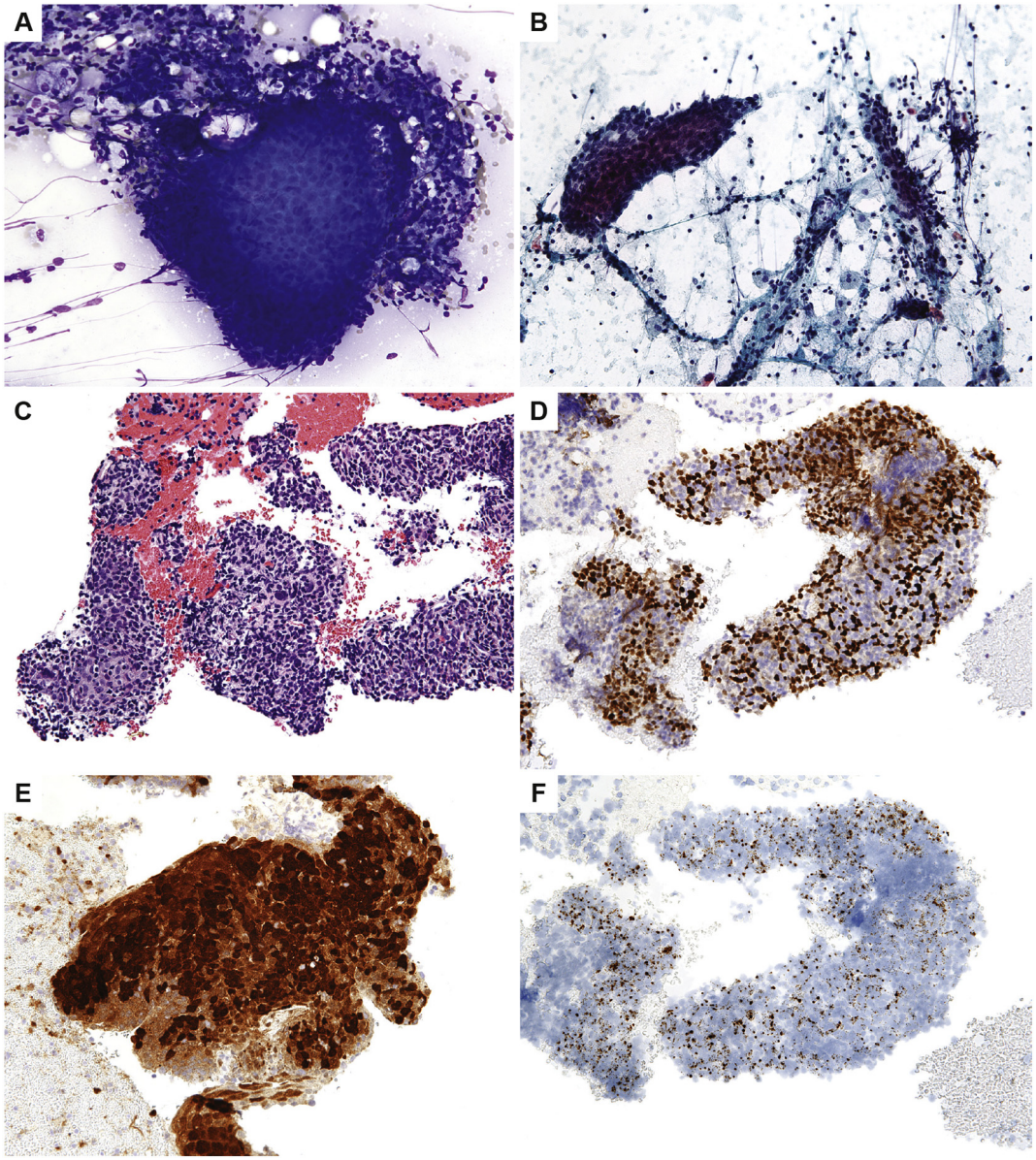

Fig. 2. Fine needle aspiration cytology of a cervical lymph node metastasis of HPV-OPSCC. Diff-Quik (A) and Papanicolaou (B)-stained direct smears show cohesive fragments of epithelium with ovoid, hyperchromatic nuclei and scant cytoplasm. A cell block (C) demonstrates similar fragments of nonkeratinizing squamous epithelium, highlighted by an immunostain for p63 (D). An immunostain for p16 (E) shows strong and diffuse positivity, supporting an HPV-related oropharyngeal primary site. While not necessary for the diagnosis in most cases, RNA in situ hybridization for HPV (F) shows punctate positivity in the tumor cells.

although identification of the specific variant is currently of minimal prognostic significance.

On low-power review of HPV-OPSCC, the majority of cases lack overt keratinization. However, many cases demonstrate > 10% focal or patchy cytoplasmic keratinization and intercellular bridges, particularly at higher magnification. Termed nonkeratinizing squamous cell carcinoma with maturation or hybrid carcinoma, this represents the most common histologic variation (Fig. 3A, B) [39]. In addition, rare cases demonstrate a well-developed keratinizing phenotype with dense eosinophilic cytoplasm, keratin pearl formation, angulated cellular nests, and stromal desmoplasia [39]. Although an extensively keratinizing SCC is more likely to be non-HPV-related, ancillary testing for p16 is recommended in tumors arising from the oropharynx. Positivity for p16 correlates with the presence of HPV in these cases, and initial data suggest that keratinizing HPV-OPSCC retains the survival benefit of its nonkeratinizing counterpart [40].

At the opposite end of the morphologic spectrum, tumors lacking cytoplasmic keratinization can display an undifferentiated phenotype mimicking the lymphoepithelial appearance of a nasopharyngeal carcinoma (NPC) [91,92]. This variant of HPV-OPSCC, termed lymphoepithelial-like or undifferentiated, can be morphologically indistinguishable from NPC [92]. In fact, a subset of Epstein-Barr virus (EBV)-negative keratinizing and nonkeratinizing nasopharyngeal carcinomas are HPV-related, particularly in regions where EBV is nonendemic [93]. Nests and singly distributed malignant cells with enlarged nuclei, open chromatin, prominent nucleoli, and indistinct syncytial cytoplasm are interspersed with abundant mixed lymphocytes and plasma cells (Fig. 3C, D). Clinical and radiographic assessment of the tumor's primary site along with ancillary ISH for HPV and EBV-encoded small RNAs, or EBER, can reliably differentiate these two entities.

In the papillary variant of HPV-OPSCC, the tumor demonstrates a prominent exophytic growth pattern of fibrovascular cores surrounded by SCC (Fig. 3E, F). Invasive growth may be difficult to identify, particularly in a biopsy specimen, as the tumor often invades with a

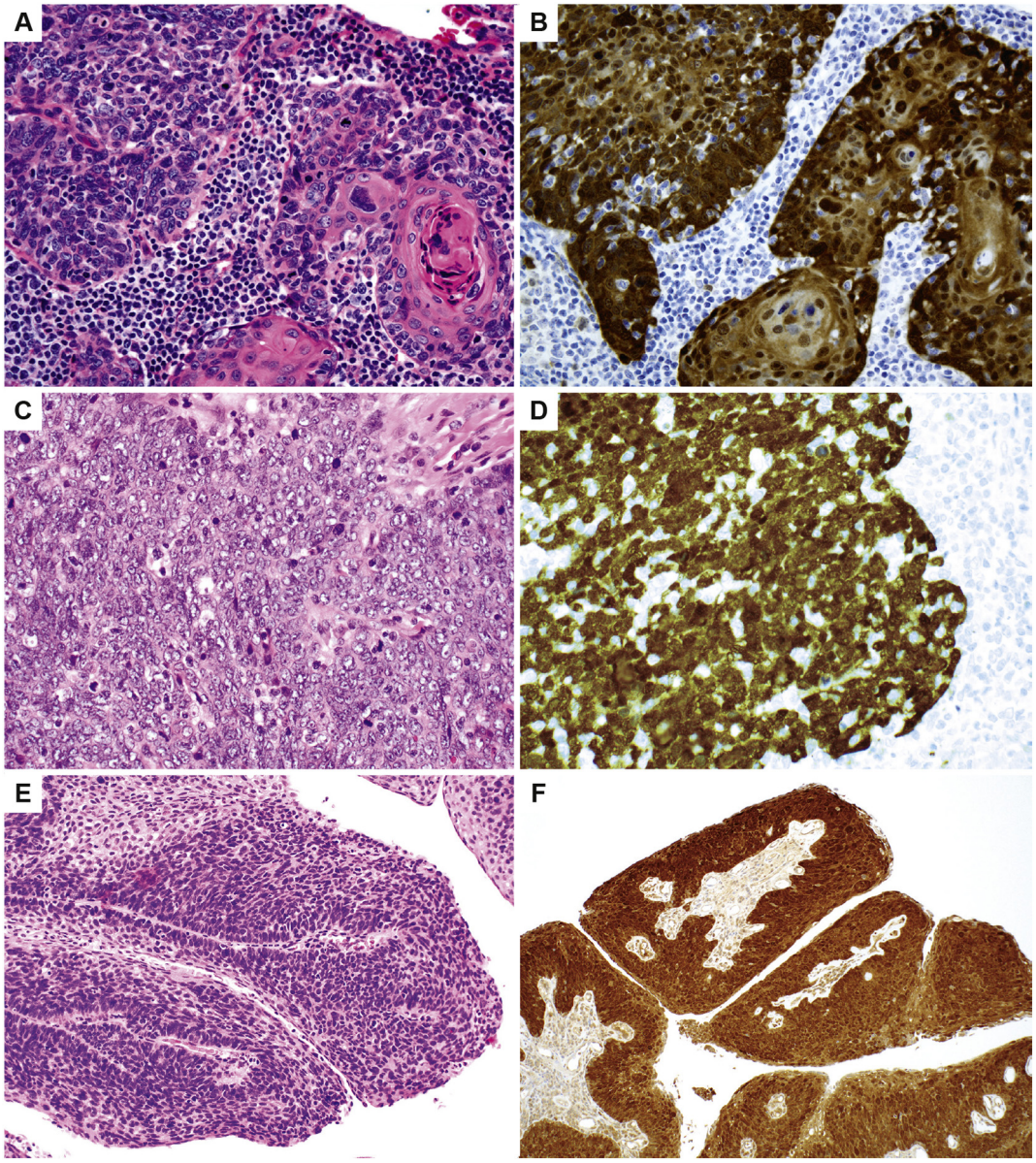

Fig. 3. Morphologic variants of HPV-OPSCC. Oropharyngeal nonkeratinizing squamous cell carcinoma with maturation, also referred to as hybrid carcinoma. Invasive nonkeratinizing SCC with admixed foci of well-developed squamous differentiation (A); p16 immunoreactivity is present, although the staining tends to be less strong in the foci of squamous differentiation. The lymphoepithelial-like variant of OPSCC is histologically similar to its nasopharyngeal counterpart, composed of sheets of malignant cells characterized by enlarged nuclei with vesicular chromatin and enlarged nucleoli (C); tumor cells are diffusely p16 reactive, but the admixed and surrounding benign lymphocytes are negative (D). Papillary SCC is characterized by a papillary architecture with fibrovascular cores surrounded by malignant cells throughout all epithelial layers (E) that are p16 positive (F).

pushing front lacking single cell invasion. The nuclear pleomorphism and hyperchromasia, nuclear contour irregularities, and mitotic activity including atypical mitotic figures differentiate this carcinoma from a benign papilloma. While the morphologic pattern is most commonly nonkeratinizing, keratinizing papillary HPV-OPSCCs have also been described [94,95].

The basaloid variant of HPV-OPSCC can generate confusion, as nonkeratinizing squamous cell carcinoma has been casually described as appearing basaloid due to the lack of abundant cytoplasm. However, basaloid SCC has been historically distinguished as an aggressive malignancy arising in the larynx or hypopharynx with a poor prognosis. Composed of small cells with hyperchromatic, crowded nuclei and scant cytoplasm, the basaloid variant grows in a lobulated architectural pattern associated with a variably myxoid to hyalinized stroma (Fig. 4A). Foci of abrupt keratinization and comedonecrosis can be seen (Fig. 4B). A subset of these tumors located in the oropharynx and expressing p16 with detectable HPV have been identified, and these tumors share the better prognosis of conventional HPV-OPSCC [96,97].

Uncommonly, HPV-OPSCC can also demonstrate an adenosquamous morphology [98]. In these tumors, morphologically distinct areas of well-developed SCC and adenocarcinoma are present in combination (Fig. 4C). The adenocarcinoma can manifest as discrete gland or tubule formation or as goblet cells with intracytoplasmic mucin droplets. While only a minority of adenosquamous carcinomas of the head and neck are HPV-related, these tumors are associated with a good prognosis, unlike their HPV-negative counterparts. These cases demonstrate diffuse p16 expression as well as the presence of HPV DNA or RNA in both the squamous and glandular components. Rarely, the glandular component may be diffusely or focally ciliated (Fig. 4D), refuting the commonly-held belief that cilia are diagnostic of benignity [99,100].

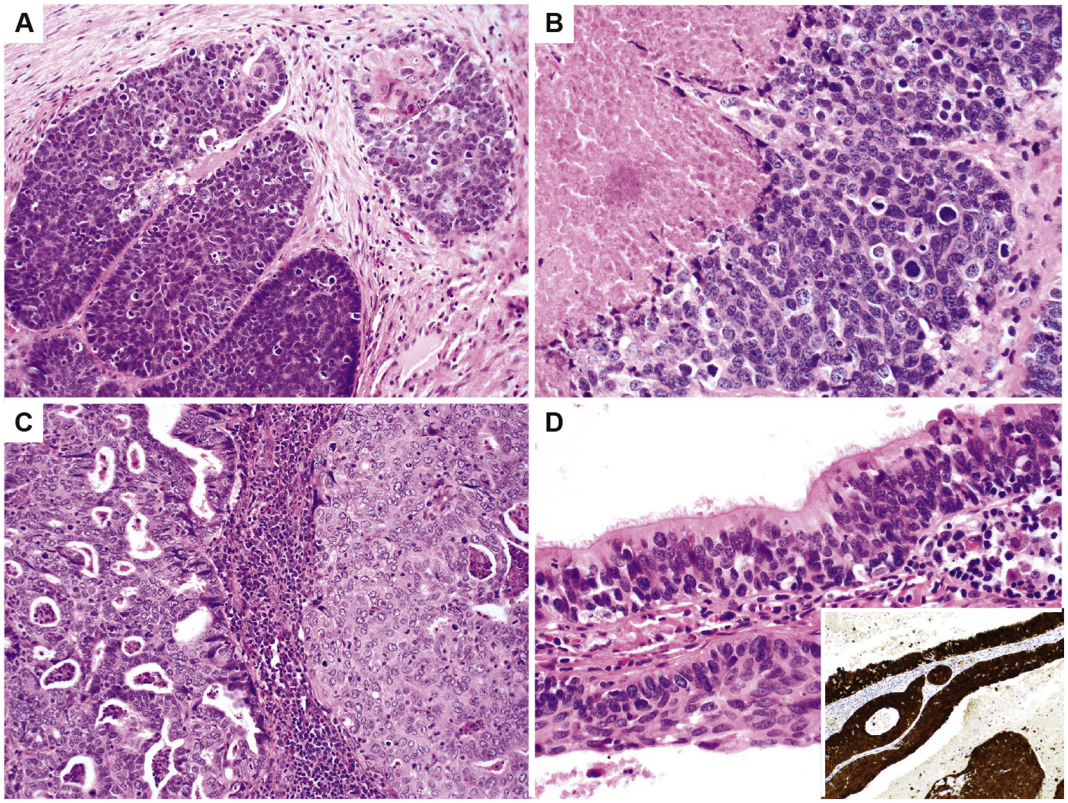

Fig. 4. Less common morphologic variants of HPV-OPSCC. Basaloid SCC with infiltrative nests and lobules composed of markedly pleomorphic basaloid cells with increased mitotic activity and individual cell necrosis, as well as abrupt squamous differentiation (upper right) (A); cystic change with associated comedo-type necrosis often is present (B). Most oropharyngeal basaloid SCCs are p16 positive (not shown), but a subset may be p16 negative. Oropharyngeal adenosquamous carcinoma, composed in part by solid growth and squamous features (right) and areas of glandular differentiation (left and extreme right) (C). In some cases, goblet cells with intracytoplasmic mucin represent the adenocarcinomatous component (not shown). In other cases of adenosquamous carcinoma, ciliated cells may be identified (D); ciliated adenosquamous carcinoma showing diffuse p16 immunoreactivity (D, inset).

While the presence of ciliated columnar epithelium in a neck cyst raises the differential diagnosis of a branchial cleft cyst, p16 positivity (Fig. 4D) and HPV detection have led to the recognition of HPV-related ciliated carcinoma, with reported locoregional recurrences and distant metastases confirming the potential for malignant behavior.

2.6.2. HPV-associated neuroendocrine carcinomas

Unlike the variants discussed so far, which have demonstrated a consistently favorable prognosis in reported case series, HPV-related neuroendocrine carcinomas are significant for their conspicuously worse behavior and potential diagnostic pitfalls [101]. HPV has rarely been identified in both large-cell neuroendocrine carcinomas and small cell carcinomas of the oropharynx [102-104]. The neuroendocrine carcinoma may arise in conjunction with a conventional HPV-OPSCC or in isolation. These tumors demonstrate the same histologic features seen in neuroendocrine carcinomas of other organs, which may overlap with the nonkeratinizing morphology of HPV-OPSCC. Further, these tumors may present with a nodal metastasis in the same cervical node chain (i.e., levels II and III) as HPV-OPSCC in the absence of a known primary carcinoma (cT0) [102,103].

While conventional HPV-OPSCC and neuroendocrine carcinoma share features such as increased nucleus to cytoplasm ratios, frequent mitoses, and apoptotic/necrotic debris, conventional HPV-OPSCC typically has a streaming pattern with evenly spaced nuclei separated by syncytial cytoplasm. By contrast, a large cell neuroendocrine carcinoma often demonstrates rosettes or trabeculae with nuclear palisading, distinct cytoplasmic borders, coarse chromatin, and prominent nucleoli, whereas a small cell carcinoma displays nuclear molding and stippled chromatin. Neuroendocrine carcinomas reliably express neuroendocrine markers by immunohistochemistry (e.g., synaptophysin, chromogranin), with perinuclear dot-like expression of keratins and a lack of squamous markers such as p63, p40 and CK5/6. Given the morphologic overlap with nonkeratinizing SCC, pathologists should have a low threshold for performing a panel of immunohistochemical stains for neuroendocrine and squamous markers due to the clinical significance of the distinction.

Although only a limited number of cases have been reported in the literature, the behavior and prognosis of HPV-positive neuroendocrine carcinomas appears no better than HPV-negative neuroendocrine carcinomas of the lung or larynx. Patients with these tumors experience frequent recurrence, rapid dissemination, and a high mortality rate. Notably, both HPV-related and HPV-unrelated neuroendocrine carcinomas demonstrate strong and diffuse p16 expression, negating the value of this immunohistochemical marker in the setting of a tumor with neuroendocrine features [105]. HPV-specific assays such as ISH or PCR are necessary to identify HPV-related tumors, although the value of this testing is limited based on the uniformly dismal prognosis.

3. EBV-associated nasopharyngeal carcinoma

In contrast to the relatively recent description of HPV-related oropharyngeal SCC (HPV-OPSCC), EBV-associated nasopharyngeal carcinoma (NPC) is a long-recognized malignant neoplasm whose diagnostic morphology and confirmatory ancillary studies have remained consistent over the past several years. While these tumors share a nonkeratinizing squamous morphology, the pathogenesis, epidemiology, and classification of EBV-associated NPC are distinct from its HPV-associated oropharyngeal counterpart.

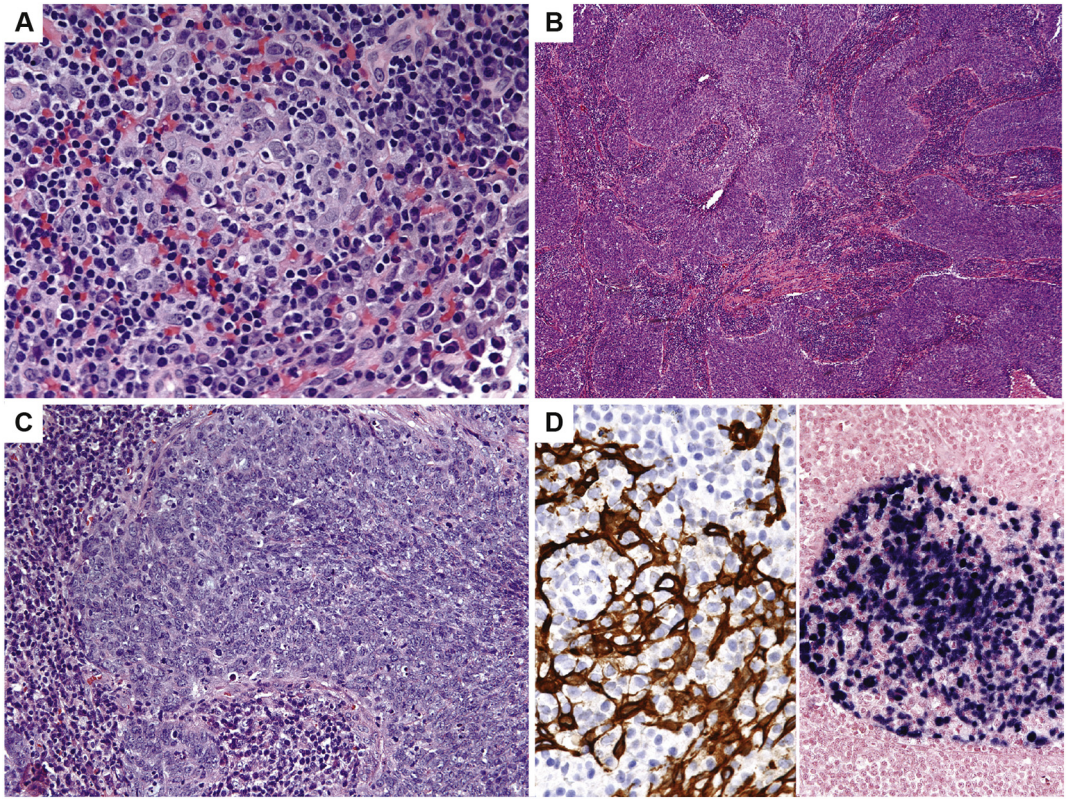

Fig. 5. Classic histomorphologic findings of nonkeratinizing NPC. The nonkeratinizing undifferentiated subtype, which is the most common pattern, demonstrates dispersed malignant epithelial cells with large round nuclei, prominent nucleoli, and indistinct cytoplasm in a background of abundant benign inflammatory cells (A). In the nonkeratinizing differentiated subtype, interweaving trabeculae of epithelial cells are seen in a lymphoid background (B). The tumor cells are smaller than the undifferentiated subtype, with variably condensed chromatin and nuclear grooves (C). In both types, the tumor cells express squamous markers such as CK5/6 by immunohistochemistry (D, left) and are also positive for EBER (D, right).

3.1. Etiology

EBV is an enveloped double-stranded DNA virus that is a member of the herpes virus family. A highly efficient virus, EBV infects the vast majority of people and is transmitted by body fluids, particularly saliva. While EBV readily infects B lymphocytes, causing infectious mononucleosis and leading to a number of EBV-related lymphomas, it can also infect epithelial cells. Although infection of both lymphocytes and epithelial cells by EBV predominantly results in lytic replication, memory B cells develop a latent infection, providing a reservoir of virions that may re-infect epithelial cells. Establishment of a latent phase in epithelial cells has been postulated to represent a critical early step in the development of NPC.

Despite several decades of study, the exact pathway leading from a latent EBV infection to the development of NPC remains unclear. As an oncogenic virus, EBV produces a number of proteins and RNA molecules that dysregulate cellular pathways [106,107]. For example, EBV's latent membrane protein 1 (LMP1) activates the NF-κB pathway, among other oncogenic and anti-apoptotic functions [108]. Furthermore, LMP1-mediated inactivation of p16 allows EBV-infected cells to maintain a stable latent infection, bypassing growth arrest. Additionally, the nuclear antigen 1 (EBNA1) protein blocks the interaction of p53 with its targets, facilitating the survival of cells with DNA damage [109]. Widespread methylation changes as well as chromosomal copy number changes are also observed in NPC [107,110].

Similar to HPV, EBV is thought to be a necessary but insufficient component of the pathogenesis of NPC. While EBV's disruption of cellular pathways may set the stage, additional pre-neoplastic chromosomal changes may precede EBV infection, and subsequent carcinogenic insults in combination with the immune microenvironment appear to play important roles [111]. Studies to date have explored the genetic and epigenetic landscape of NPC as well as the specific interactions of viral proteins and RNAs with host cellular mechanisms. Ongoing research aims to synthesize these complex alterations into a more precise sequence of events.

3.2. Epidemiology and clinical presentation

Although uncommon in many parts of the world, NPC demonstrates a high incidence in the Cantonese-speaking southeastern Chinese population, as well as in southeast Asian, northern African, and Inuit populations [112]. While the reasons for this unusual geographic distribution have not been entirely elucidated, the following carcinogenic, immunological, and viral factors are all thought to contribute: tobacco smoking, alcohol consumption, a diet rich in salted and fermented foods containing nitrosamines, genetic factors such as HLA haplotypes, and possibly specific strains of EBV. The effect of these different factors varies geographically with the tumor histotype. For example, the etiologic agents in high-risk regions, such as dietary nitrosamines, appear more closely linked to the nonkeratinizing undifferentiated subtype. Meanwhile, the carcinogens in low-risk areas, such as tobacco and alcohol, are typically associated with the keratinizing or nonkeratinizing differentiated subtypes [113], discussed in more detail below.

NPC most commonly affects adults aged 45–60 and is seen 2–3 times more often in men than in women [114]. The most frequently reported presenting symptoms are a neck mass; nasal symptoms such as obstruction, postnasal drip, epistaxis, or discharge; aural symptoms such as tinnitus, earache, or hearing changes; and less commonly, headache [114,115]. On physical examination, cervical lymphadenopathy is identified in the majority of patients, corresponding to a locoregionally

advanced stage of disease. A minority of patients demonstrate cranial nerve palsies, typically in cranial nerves V and VI [114].

Given the high incidence of NPC in geographic regions where the disease is considered endemic, targeted screening programs have been proposed to facilitate early detection. Serologic measurement of IgA antibodies against EBNA1, early antigen (EA), and viral capsid antigen (VCA) as well as plasma EBV DNA or RNA assays have been utilized to identify at-risk patients [116-118]. Nasopharyngeal swabs and brushings for evaluation of EBV DNA have also been proposed [119,120]. Since the presenting symptoms of NPC are non-specific and easily mistaken for benign conditions, prospective identification of patients who could benefit from further diagnostic procedures may enable intervention at earlier stages of disease.

3.3. Pathologic findings

3.3.1. Histopathology

While NPC consistently demonstrates morphologic and immunophenotypic features of SCC, three major subtypes are recognized: nonkeratinizing carcinoma, including undifferentiated and differentiated subtypes, keratinizing SCC, and basaloid SCC (Fig. 5). Although all of the histologic types of NPC are seen throughout the world, the nonkeratinizing undifferentiated subtype is the most common in high-incidence areas such as southeast Asia and is closely associated with EBV [121]. The nonkeratinizing differentiated and keratinizing types are seen more often in low-risk areas and are not always driven by EBV. The pathologist's ability to distinguish the nonkeratinizing subtypes from the keratinizing type is clinically relevant, as the latter carries a worse prognosis. A dysplastic or in situ component may be present in any type of NPC but is often not identified.

Classically described as a lymphoepithelial carcinoma, the nonkeratinizing undifferentiated subtype of NPC contains a robust benign inflammatory infiltrate, including polymorphous lymphocytes, plasma cells, histiocytes, and occasional eosinophils (Fig. 5A). Embedded within this chronic inflammatory background are malignant epithelial cells, often distributed in a loose meshwork. Unlike the typical hyperchromatic, angulated nuclei of an SCC, the tumor cells have large, round to ovoid nuclei with smooth nuclear borders, vesicular chromatin, prominent nucleoli, and indistinct syncytial cytoplasm. A desmoplastic reaction to the tumor is absent. The differential diagnosis for this subtype may include lymphoma or an infectious or inflammatory process, as the malignant epithelial cells may be partially obscured by the inflammatory cells.

Similar to the nonkeratinizing undifferentiated subtype, the nonkeratinizing differentiated subtype often has a rich inflammatory background and is devoid of a desmoplastic stromal reaction. However, the neoplastic cells grow in stratified, cohesive trabeculae that mimic the ribbon-like pattern of urothelial carcinoma (Fig. 5B). While the cells maintain a nonkeratinizing cytoplasm, the nuclei are smaller and often grooved with more condensed chromatin and variable nucleoli (Fig. 5C). Since these tumors are more readily identified as squamous neoplasms, the differential diagnosis may include a squamous papilloma with dysplasia, which lacks the EBV positivity and invasive growth of NPC. Although some published reports have demonstrated a difference in prognosis between the undifferentiated and differentiated subtypes of nonkeratinizing NPC [122], this distinction is not currently thought to be clinically relevant [115]. Indeed, a spectrum including both morphologic patterns may be seen within a single case, limiting the reproducibility of subclassification.

The keratinizing type of NPC, which is morphologically indistinguishable from SCCs of other head and neck sites, demonstrates cytoplasmic keratinization, keratin pearl formation, and intercellular bridges, with hyperchromatic, pleomorphic nuclei. A surrounding desmoplastic stromal reaction is often present. Unlike the nonkeratinizing undifferentiated subtype, which is strongly associated with EBV, only a subset of keratinizing NPCs are driven by EBV. EBV-associated keratinizing NPCs are more commonly seen in endemic regions, while EBV-negative keratinizing NPCs are typically identified in low-risk regions, where tobacco smoking and alcohol play a more prominent role [123]. Keratinizing NPC consistently demonstrates worse overall survival than the nonkeratinizing subtypes, both in endemic and non-endemic regions [124-126]. While nodal and distant metastases are less common in keratinizing NPC, locoregional disease is more aggressive [127]. Thus, pathological classification of NPC into keratinizing versus nonkeratinizing types is relevant for radiation and medical oncologists in clinical decision-making. These findings are in contrast to HPV-OPSCC, in which keratinizing morphology does not appear to convey prognostic significance.

The least common type of NPC is basaloid SCC, which is rarely encountered in practice. Similar to basaloid SCCs at other locations, basaloid NPC is characterized by a lobulated proliferation of basaloid cells with microcystic or trabecular growth, typically accompanied by mucoid secretory material and hyalinized stroma [128,129]. Comedonecrosis and foci of keratinization are often seen. EBV may be identified in basaloid SCC, especially in high-incidence ethnic groups [115]. While the number of cases reported in the literature is small, basaloid NPC may have a better prognosis than basaloid SCC of the larynx or esophagus [129].

3.3.2. Ancillary testing

The nonkeratinizing differentiated and keratinizing types of NPC are often readily recognizable as squamous cell carcinomas by morphology. However, immunohistochemistry to confirm the presence of a carcinoma may be diagnostically helpful in the nonkeratinizing undifferentiated subtype. The malignant cells express cytokeratins and EMA, confirming their epithelial origin. Additionally, they consistently express markers of squamous differentiation such as p63, p40, and CK5/6 (Fig. 5D).

EBV produces small RNAs, termed EBV-encoded small RNAs (EBER) that are present in significant quantities in all phases of EBV infection and latency. Thus, in situ hybridization for EBER is a reliable ancillary study that confirms the presence of EBV within the malignant cells (Fig. 5D) [130]. By contrast, immunohistochemistry for LMP1 is only expressed in a subset of EBV-related lesions, while PCR for EBV may amplify latent virus in memory B lymphocytes that is unrelated to the neoplasm.

Similar to HPV-OPSCC, many NPCs present with a neck mass of unknown etiology. Further, given the shared histomorphologic features of HPV-OPSCC and EBV-NPC, the detection of EBV in a metastatic carcinoma of unknown primary is a consistent indication of nasopharyngeal origin [131,132]. Since the primary tumor may be clinically and endoscopically occult, identification of a metastatic EBV-positive SCC facilitates targeted biopsies of the nasopharynx and narrows the potential radiation field.

Although formal diagnostic terminology has not been proposed for NPC, we recommend using “EBV-positive non-keratinizing SCC” or “EBV-positive basaloid SCC, compatible with nasopharyngeal carcinoma.” This phrasing includes the subtype and EBV result while deferring to the clinical and radiographic impression on the primary site, as rare EBV-driven SCCs have been identified at other sites such as the sinonasal tract that may uncommonly overlap with NPC (discussed below). While tissue-based testing is critical for establishing the initial diagnosis of an EBV-related NPC, blood-based testing for circulating cell-free EBV DNA is utilized in clinical treatment planning and follow-up. The quantity of EBV DNA detected in the patient's plasma correlates with the disease burden of NPC, both before and after treatment [133]. In addition to serving as a useful tool in planning the extent of radiation and chemotherapy, a rising EBV DNA level implies disease recurrence in a previously treated patient, providing a low-cost, minimally invasive method for long-term monitoring [134,135].

Although immunohistochemistry for p16 is not typically indicated in EBER-positive NPCs, a minority of NPCs express p16 [136].

Furthermore, approximately 5–30% of NPCs are recently demonstrated to be HPV-driven instead of EBV-driven, both in endemic and non-endemic areas [137-139]. If a tumor is negative for EBER and strongly and diffusely positive for p16, an HPV-associated nasopharyngeal carcinoma should be considered. This diagnosis can be confirmed by HPV-specific testing such as ISH or PCR; the prognostic and treatment implications for this unique subset of NPCs are not yet clear. If both p16/HPV and EBER are negative in the setting of a nonkeratinizing or lymphoepithelial carcinoma, other diagnostic considerations including but not limited to NUT carcinoma should prompt additional workup.

3.3.3. Fine needle aspiration cytology

As in HPV-OPSCC, fine needle aspiration (FNA) plays a significant role in the accurate diagnosis of a neck mass of unknown etiology. By enabling a diagnosis of squamous cell carcinoma with sufficient cell block material to perform EBER ISH, the FNA provides a definitive tissue diagnosis to support treatment planning.

The cytologic features of NPC on direct smears are dependent on the specific tumor subtype. While metastatic keratinizing NPC can be morphologically identical to SCCs from other head and neck sites, the nonkeratinizing differentiated subtype may be indistinguishable from HPV-OPSCC. The nonkeratinizing undifferentiated subtype is morphologically more distinctive, maintaining a lymphoepithelial appearance in direct smears and cell block material (Fig. 6). Admixed with an inflammatory background of lymphocytes and plasma cells with scattered lymphoglandular bodies, the malignant epithelial cells form loose aggregates that may be three-dimensional or flat (Fig. 6A, B). While the nuclei of the nonkeratinizing differentiated subtype are smaller with condensed chromatin, the nuclei of the undifferentiated subtype are larger with open chromatin and prominent nucleoli [140]. In both types, the cytoplasm is delicate and indistinct. Although the malignant epithelial cells of the undifferentiated subtype may mimic the Reed-Sternberg cells of Hodgkin lymphoma, the cells of NPC are more numerous and clustered than Reed-Sternberg cells and demonstrate more fragile, inapparent cytoplasm [141]. Tumor fragments in the cell block show intermingled inflammatory and epithelial cells (Fig. 6C). Frequent mitotic figures, necrotic debris, stripped single cells, and cyst contents may be seen in the background. Eosinophils, neutrophils, or granulomatous inflammation are present in a minority of cases. Rare reports describe stromal and intracellular amyloid deposition associated with NPC, confirmed by Congo red staining and electron microscopy [142].

Immunohistochemistry for p16 or HPV-specific testing may be an appropriate initial work-up to localize the primary site for metastatic nonkeratinizing SCC, particularly in a non-endemic location where HPV-OPSCC is more common than NPC. However, a negative result should trigger EBER to investigate the possibility of metastatic NPC (Fig. 6D). A positive EBER result in FNA material reliably predicts nasopharyngeal origin in both cystic and solid metastases, expediting appropriate treatment [143,144].

3.4. Grading and staging

Similar to HPV-OPSCC, tumor grading in NPC has not demonstrated prognostic significance. However, classification into keratinizing versus nonkeratinizing carcinoma is a relevant prognostic factor for NPC, as discussed above. The significance of subtyping NPC represents a curious contrast to HPV-OPSCC, which appears to have a more uniform prognosis across histologic types.

Multiple prior staging systems have now been unified into the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) staging system [145]. The most significant predictor of prognosis is the stage, followed by the plasma EBV DNA

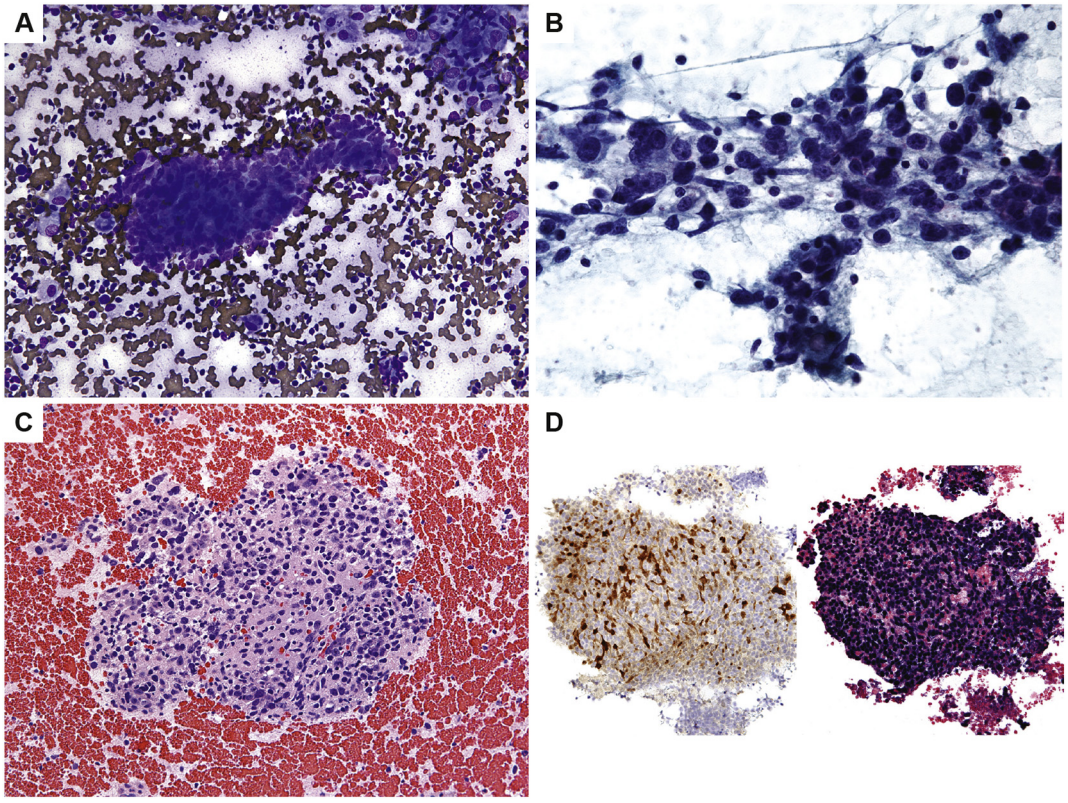

Fig. 6. Fine needle aspiration cytology of metastatic NPC to a cervical lymph node. Depending on the subtype of NPC, the malignant epithelial cells can appear in three-dimensional clusters with condensed chromatin (A, Diff-Quik) or in flat aggregates with open chromatin and visible nucleoli (B, Papanicolaou). Background inflammatory cells can partially obscure the malignant epithelial component (C, cell block). Unlike HPV-OPSCC, immunohistochemistry for p16 will be patchy within tumor fragments (D, left), which should trigger EBER to evaluate for metastatic NPC (D, right).

level [146]. Although stage correlates closely with patient outcome, the majority of patients with NPC are not surgical candidates due to advanced stage at presentation. Thus, pathological staging plays a limited role in the management of NPC, and clinical and imaging data are used for planning therapy.

3.5. Treatment modalities

While surgery can be performed when complete resection of early-stage disease is feasible, the mainstay of treatment for NPC is radiation therapy due to direct tumor extension into anatomically important structures. Similar to HPV-OPSCC, NPC is highly radiosensitive. Ongoing refinement in radiation techniques, including IMRT, has increased the relapse-free survival in treated patients. For advanced locoregional disease or distant metastases, concurrent chemotherapy may also be indicated. The 5-year overall survival for patients with NPC is > 70%, increasing to > 90% for early-stage disease [145]. Ongoing challenges include toxicities such as radiation-related brain damage and secondary malignancies as well as late relapse and distant metastases. Clinical trials are evaluating the role of targeted therapies and immunotherapy to optimize outcomes [147].

3.6. Uncommon sites and variants

Within the nonkeratinizing, keratinizing, and basaloid types of NPC, morphologic variation ranging from spindled to papillary, reticulated, or pleomorphic can be seen [125,148,149]. However, discrete morphologic variants have not been well-defined. While architectural or cytologic variation may raise a broader differential diagnosis, the consistent expression of squamous epithelial markers and positivity for EBER confirms classification as NPC.

Outside of the nasopharynx, EBV-positive lymphoepithelial-like carcinomas have been identified in a number of other head and neck sites, most frequently in the salivary glands [150]. Less common sites include the larynx, sinonasal tract, and middle ear. While these tumors are typically associated with EBV in endemic regions, they are often EBV-negative in non-endemic regions. A metastasis from an occult NPC or HPV-OPSCC should be excluded by clinical exam and imaging studies prior to diagnosing a non-nasopharyngeal lymphoepithelial-like carcinoma.

Although only a handful of cases have been reported in the literature, rare examples of nasopharyngeal large cell neuroendocrine carcinoma have been EBV-positive [151]. Unlike HPV-driven oropharyngeal neuroendocrine carcinomas, the few cases of EBV-associated neuroendocrine carcinoma to date have been highly radiosensitive with a good overall prognosis following combined chemoradiation.

4. Conclusion

HPV-OPSCC and NPC share unique biology and epidemiology as virally-driven carcinomas. The distinctive morphologic features of these tumors enable the practicing pathologist to maintain a high index of suspicion in reviewing specimens from the oropharynx and nasopharynx. Appropriate use of ancillary studies, including p16 immunohistochemistry, HPV-specific assays, and EBER can facilitate a definitive diagnosis, leading to tailored therapy. The overall excellent prognosis of both of these carcinomas underscores the need to avoid overtreatment, cementing the pathologist's role in accurate classification of these intriguing head and neck tumors.

Conflicts of interest

The authors have no conflict of interest to disclose.

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