Squamous cell carcinoma of the upper aerodigestive tract is not a single, distinct entity. Several variant histologic patterns are recognized, and some of which have unique prognoses and are associated with unique risk factors. As far as molecular and cytogenetic changes go, the greatest differences between those lesions are seen due to etiology, with virally related tumors, human papillomavirus (HPV)–associated squamous cell carcinoma and Epstein-Barr virus (EBV)–associated squamous cell carcinoma, differing from nonviral (typically related to tobacco and alcohol) tumors.

HUMAN PAPILLOMAVIRUS–ASSOCIATED SQUAMOUS CELL CARCINOMA

During the past decade, molecular and epidemiologic studies have demonstrated a causal relationship between HPV and a subset of head and neck squamous cell carcinomas. These HPV-associated squamous cell carcinomas represent distinct clinical entities because they often portend a better prognosis than the traditional smoking- and alcohol-related squamous cell carcinomas. The epidemiology of these tumors also varies from the non-HPV–associated tumors. These tumors tend to occur in patients who are younger (usually younger by about 5–10 years) and who, on average, have had a greater number of sexual partners, including oral sexual partners. Men develop these tumors more often than women do, and the tumors develop more frequently in blacks.

The HPV-associated squamous cell carcinomas of the upper aerodigestive tract can occur anywhere; however, most occur within the oropharynx, especially at the base of tongue or in the palatine tonsils. These tumors have also been noted in the sinonasal tract, oral cavity, nasopharynx, hypopharynx, and larynx. The HPV-associated oropharyngeal tumors often present at a higher clinical stage with advanced nodal disease, despite being of lower T category (primary tumor size). Despite this advanced clinical stage, the prognosis and overall disease-free survival of patients with these tumors are superior to that of patients with non-HPV–associated tumors.

Because oropharyngeal tumors metastasize early, patients frequently present with neck metastases. These neck metastases can become quite large with cystic degeneration and can sometimes be a diagnostic pitfall for the pathologist on both frozen section and fine-needle aspirates. When a diagnosis of metastatic squamous cell carcinoma in a cervical lymph node is made, a prompt clinical evaluation for the presence of an oropharyngeal primary is needed. However, these primary oropharyngeal tumors are sometimes grossly evasive, and “blind” pharyngeal biopsies from...
patients with clinically apparent neck metastases will frequently identify microscopic, invasive squamous cell carcinomas. Sometimes, no primary tumor is identified, which, likely, is secondary to the lack of sampling of the single microscopic focus of tumor.16

The HPV-related squamous cell carcinomas have a varied, yet histologically distinct, morphologic phenotype.5,15 Despite the many techniques now in use and continually being developed for detecting HPV-associated tumors, the initial assessment of these tumors begins with a thoughtful morphologic approach.17 Most of these tumors are non-keratinizing squamous cell carcinomas (Figure 1) and are sometimes described as having a basaloid phenotype. Some authors9,17–19 argue that the use of the descriptive term basaloid can be misleading because that implies a specific tumor subtype, basaloid squamous cell carcinoma, which has an aggressive course and is not always associated with HPV. As a result, discussions are currently emerging on how all HPV-associated tumors should be reported, and a unified scheme will likely prevail in the future. Undifferentiated and papillary squamous cell carcinomas of the upper aerodigestive tract are also sometimes associated with HPV infection.5,20,21 Uncommonly, glandular or neuroendocrine differentiation in the form of small cell carcinoma can be seen with these tumors.22,23 Recognition of a small cell carcinoma component is important because these tumors have been shown to have poorer outcomes, despite their association with HPV.23

The HPV-related squamous cell carcinomas of the upper aerodigestive tract are immunoreactive with antibodies to cytokeratins and p63, which is similar to other squamous cell carcinomas. The most notable exceptions include the expression proteins specifically related to the molecular workings of HPV. Overexpression of p16 can be identified by immunohistochemistry (IHC) in virtually all head and neck squamous cell carcinomas associated with HPV infection, making it a useful surrogate marker for the detection of HPV (Figure 2, A).24 In these tumors, p16 is overexpressed secondary to depletion of the retinoblastoma protein by the HPV-generated E7 protein.1,24 Cyclin D1 is also down-regulated with decreased retinoblastoma protein. Protein p53 expression, on the other hand, cannot always be demonstrated by IHC with HPV-associated tumors because their genes are usually wild type, and instead, the protein is inhibited by the viral E6 protein.1,24

Human papillomavirus has been identified in head and neck squamous cell carcinomas by polymerase chain reaction (PCR) and in situ hybridization (ISH) (Figure 2, B).25,26 The E6 and E7 transcripts have been identified by reverse transcription–polymerase chain reaction (RT-PCR), ISH, and Northern blot analysis.25,26 Each method of detection has its advantages and limitations, and there is no consensus on the particular method to be employed by clinical laboratories.24 Many researchers advocate for p16 IHC use during initial screening, followed by more-specific detection methods for high-risk HPV subtypes, such as ISH.24

According to the results of several studies,5,27–30 patients with HPV-associated squamous cell carcinomas have better outcomes than do those with non-viral-related tumors. Future clinical trials will be aimed at deintensified treatment regimens for these tumors. Currently, patients are typically treated with induction chemotherapy, followed by surgery or chemoradiation. Patients with HPV-associated tumors are more likely to demonstrate substantial response to induction chemotherapy and, thus, are more likely to be eligible for chemoradiation and organ preservation,31,32 although some patients may still require surgery. In addition, new methods of treatment are being devised that use novel therapeutic vaccines.1,33 Published data regarding the utility of vaccine prevention for HPV oral lesions are currently lacking, but, hopefully, the advent of a preventative vaccination will affect head and neck cancers.2,33

**EPSTEIN-BARR VIRUS–ASSOCIATED SQUAMOUS CELL CARCINOMAS**

Epstein-Barr virus (EBV)–associated squamous cell carcinomas usually involve the nasopharynx. The pathogenesis of these tumors is complex. Infection with EBV is essential; however, given the rather ubiquitous nature of EBV, infection alone does not account for the marked regional differences in the incidence of these tumors (eg, individuals in China are more than 4 times more likely to develop tumors than are individuals in the United States).24–37 Thus, both environmental (eg, nitrosamine from salted fish and preserved food) and genetic factors may be involved in the development of the disease. Consistent evidence for the latter has been reported for HLA class I genes, DNA repair gene RAD51L1, cell cycle control genes MDM2 and TP53, and cell adhesion/migration gene MMP2.38–39

The EBV-associated squamous cell carcinomas occur in a broad age range and sometimes affect children. There is a distinct bimodal age distribution with peaks in the second and sixth decades.40–42 The tumors show a male predilection of approximately 2.5 to 1. As mentioned, almost all occur in the nasopharynx with a high percentage arising near the eustachian tube opening in the fossa of Rosenmüller. Not surprisingly then, presenting symptoms are often related to middle ear obstruction with serous otitis and associated hearing loss. Local invasion may result in headaches, cranial nerve deficits, or epistaxis.40,41,43 Importantly, approximately one-half the patients will have clinically apparent, often bilateral, cervical lymph node metastases as a presenting sign or symptom, with a much less obvious or clinically occult nasopharyngeal primary.40,43

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Figure 1. A nonkeratinizing squamous cell carcinoma of the oropharynx associated with human papillomavirus infection (hematoxylin-eosin, original magnification ×200).
Figure 2. **A, p16 immunostaining of this oropharyngeal, nonkeratinizing squamous cell carcinoma, which was associated with human papillomavirus (HPV) infection. B, High-risk HPV is demonstrated by in situ hybridization of this oropharyngeal squamous cell carcinoma (original magnification ×200 [A]; original magnification ×200 [B]).**

Figure 3. **A, This differentiated nasopharyngeal squamous cell carcinoma shows obvious epithelial growth. B, This undifferentiated nasopharyngeal carcinoma would be impossible to distinguish from other types of high-grade malignancies with only routine stains (hematoxylin-eosin, original magnifications ×200 [A] and ×400 [B]).**

Figure 4. **A, The surface of this verrucous carcinoma appears warty. B, The base of this verrucous carcinoma has invaded with broad tongues of squamous epithelium (hematoxylin-eosin, original magnifications ×100 [A and B]).**
Because these tumors are virtually never resected, gross descriptions of primary lesions are rare. Lymph nodes replaced by metastases have a uniform, pale-tan, lymphoma-like appearance. Histologically, the tumors are almost always nonkeratinizing and are frequently undifferentiated. They are characterized microscopically by cytologically uniform cells with vesicular, round to oval nuclei and medium-sized nucleoli. Mitotic figures are typically numerous, usually ranging from 5 to 10 per 10 high-power fields. When the neoplastic cells grow in cohesive nests, the cell borders between adjacent cells are indistinct, creating a syncytial appearance. As mentioned, keratinization or squamous “pearl” formation is only rarely present. An inflammatory infiltrate, primarily consisting of lymphocytes and plasma cells but occasionally with prominent eosinophils, is typically present. Two microscopic growth patterns have been identified in these neoplasms and bear eponyms derived from their descriptors. In the differentiated or Regaud pattern, the neoplastic cells form cohesive nests and cords sharply distinct from the surrounding inflammation (Figure 3, A), which is usually readily identified as a carcinoma. In the undifferentiated or Schmincke pattern, the inflammatory infiltrate permeates the cell nests, resulting in smaller nests of epithelial cells that are more widely separated, as well as single neoplastic cells in a background of inflammation. The result appears distinctly noncarcinomatous and is often confused with lymphoma on initial light microscopic examination (Figure 3, B). This explains this tumor’s previous designation as lymphoepithelioma or lymphoepithelioma-like carcinoma.

Although difficult to distinguish from lymphoma, melanoma, or other poorly differentiated malignancies by histology alone, the advent of sensitive and specific IHC markers for both epithelial and hematolymphoid differentiation has been the greatest factor in improving the diagnostic ease and accuracy of EBV-associated squamous cell carcinomas. It is especially important to distinguish these carcinomas from sinonasal undifferentiated carcinoma, which is a highly aggressive, non-EBV–related malignancy with a dismal prognosis.

The EBV-associated squamous cell carcinomas strongly express cytokeratins and epithelial membrane antigen. Furthermore, antibodies to squamous antigens, such as p63, are also immunoreactive. Evidence of EBV can be demonstrated by both IHC and ISH. Immunohistochemistry with antibodies to LMP1 tends to be insensitive, and most pathologists use ISH for EBV-encoded early RNA for the identification of these tumors. These RNAs are typically expressed in abundance, and nearly 100% of tumors will have obvious hybridization.

The tumors have a “methylator” phenotype. Cyclin D1 overexpression, loss of p16, and inactivation of RASSF1A are key events in the early development of EBV-associated squamous cell carcinomas. Immortalization of the cells may occur via disruption of the p53 and Rb pathways. The p53 and RB mutations are rare; however, chromosomal losses and gains identified with comparative genomic hybridization include losses at 3p14-21, 14q24-qter, and 11q21-qter and gains at 3q21-26 and 12q14-15. The EBV latent gene proteins further drive clonal expansion and other genetic changes, such as TSLC1 and THY1, to add to progression and metastasis of the tumor.

Recent studies have suggested an improved prognosis for these tumors compared with nonviral-related tumors. Radiotherapy with or without chemotherapy remains the backbone of treatment. Because these tumors are highly radiosensitive, radiotherapy alone can result in a 5-year survival of up to 95% for early stage disease. Concurrent chemoradiation therapy remains the standard of care for locoregionally advanced disease, providing a 5-year overall survival rate of 50% to 70%. After treatment, EBV DNA levels have been shown to be a powerful biomarker for monitoring recurrence with improved accuracy over positron emission tomography for surveillance. New treatment options, which include monoclonal antibodies, proton beams, and adaptive radiotherapies among others, are currently under investigation.

VERRUUCOUS CARCINOMA

Verrucous carcinomas are well-differentiated squamous cell neoplasms that lack conventional cytologic features of malignancy but exhibit locally destructive growth. The tumor was first recognized by Lauren Ackerman in 1948 as a distinct, diagnostically problematic squamous cell neoplasm involving the oral cavity. Therefore, many authors refer to this lesion using the eponym Ackerman tumor. Overall, these tumors account for about 1% of squamous neoplasms involving the head and neck region. They show a strong association with tobacco product usage, especially oral tobacco when the tumors arise in the mouth. Alcohol use and poor oral hygiene represent additional risk factors. Several studies have suggested a potential role for HPV as a cofactor in tumor development based on its detection in tissue specimens. However, HPV also has been detected by PCR in 25% of normal larynges, and a causal role has not yet been clearly demonstrated. Also of note, although strains of the virus have been demonstrated by PCR and Southern blot inconsistently, they have generally not been identified by more-specific methods, such as ISH.

Verrucous carcinomas typically develop in an individual’s sixth to eighth decade. The tumors are uncommon in patients younger than 40 years and arise more frequently in men. They arise most commonly in the oral cavity and the larynx, and at those sites, most of the tumors often involve the buccal mucosa and vocal cords. These tumors are slowly growing neoplasms, and they may reach considerable size before being brought to medical attention. Grossly, the tumors are tan to grey, rough, bulky exophytic masses with a shaggy or overtly papillary, wartlike surface. After sectioning the tumor, the margin of invasion is sharp and pushing. Application of strict morphologic criteria is essential for the diagnosis of verrucous carcinoma because true verrucous carcinomas have no metastatic potential. Histologically, these tumors are characterized by dense, superficial keratinization, often forming “church spires” of orthokeratotic and parakeratotic squamous cells extending upward from the surface. Neoplastic cells typically have vesicular nuclei with small nucleoli. Nuclear pleomorphism is absent or minimal and mitotic activity is confined to the basal cell layer. Cells have abundant eosinophilic cytoplasm, which generally undergoes uniform keratinization as the cells migrate toward the surface. Individual cell keratinization (dykeratosis) and keratin pearl formation may be focally present. At the base of the lesion, down-growth is in the form of broad, bulbous, sharply demarcated ridges of well-differentiated, benign-

appearing squamous cells that appear to “push” rather than infiltrate (Figure 4, B). Lymphoplasmacytic infiltrates or foreign body–type granulomas may be found at the base of the tumor. Infiltration by small, irregular nests of cells, vascular invasion, and perineural invasion are not allowed. If seen, the tumor should instead be classified as a conventional squamous cell carcinoma with verrucoid features.

Verrucous carcinoma exhibits characteristic cell kinetics that are more akin to those of normal mucosa than they are to conventional squamous cell carcinoma. In particular, DNA synthesis (S-phase) is confined to the basal cell layer, along with the more conventionally detected mitotic activity. The lack of S-phase cells above the basal cell layer is quite unlike the findings in conventional squamous cell carcinomas. Verrucous carcinomas may be diploid or aneuploid.

In the Choi et al study of microsatellite markers in head and neck squamous cell carcinomas, verrucous carcinomas showed similar losses when compared with conventional, well-differentiated squamous cell carcinomas. Verrucous carcinomas showed high incidences of loss of heterozygosity on chromosomes 9 and lower incidences of marker losses on chromosomes 3, 8, and 17, when compared with other variants. Poh et al found less allelic loss at 17p when verrucous carcinomas were compared with conventional, well-differentiated squamous cells carcinomas. Verrucous carcinomas have been shown to harbor H-ras mutations in 25% of cases tested with different staining patterns and antibodies to Raf-1, ERK1, and ERK2, when compared with conventional squamous cell carcinomas. Overexpression of p53, cyclin D1, p21, MDM2, and c-erb-3 has been shown by IHC, although the findings are more akin to well-differentiated squamous cell carcinomas and even hyperplastic lesions. Retinoblastoma protein has been shown to be normally expressed. Promoter methylation of p16 and MGMT has also been noted. β-Catenin expression is strong and membranous, akin to that seen with normal squamous epithelium or well-differentiated squamous cell carcinomas.

Surgical resection without neck dissection is the treatment of choice for pure verrucous carcinomas because, by definition these tumors lack nodal metastases. Radiotherapy and radiochemotherapy represent treatment options for patients with inoperable verrucous carcinoma or as an alternative to surgical resection. Interestingly, topical 5-aminolevulinic acid–mediated phototherapy has reportedly been successful for treatment of an extraoral verrucous carcinoma with no recurrence at 6-month follow-up.

**SPINDLE CELL CARCINOMA**

The spindle cell carcinoma variant of squamous cell carcinoma is characterized microscopically by a prominent sarcoma-like spindle cell growth, often with a minor component of conventional squamous cell carcinoma or carcinoma in situ. These carcinomas of the head and neck are strongly associated with alcohol use, cigarette smoking, or use of other tobacco products. Some patients with spindle cell carcinomas, particularly outside of the larynx, have had a history of radiation therapy, often for conventional squamous cell carcinoma, raising the possibility of radiation-induced neoplasia or transformation. Clinical features are similar to those of their conventional squamous counterparts with the exception that many spindle cell carcinomas present at low stage and are thus less likely to present with metastatic disease. Complaints are nonspecific and related to tumor location. Common sites of involvement include the larynx, oral cavity, hypopharynx, and sinonasal tract. Most laryngeal cases are glottic or transglottic. Spindle cell carcinomas show a male predominance, and the average age of presentation is around 60 years.† Most spindle cell carcinomas are polypoid. Lesions may range from 1 to 6 cm, with polypoid lesions tending to be larger. The surface may be intact or ulcerated and covered with a fibrinopurulent exudate. On sectioning, the mass is typically firm and tan or white. The spindle cell element almost always predominates and varies considerably in its microscopic appearance (Figure 5, A). Most tumors are low to moderately cellular and have a storiform growth pattern, although a more-fascicular or “solid” appearance may predominate. Areas of overt osteoid or cartilage formation by neoplastic cells are sometimes seen. Multinucleated giant cells are frequently found, most of which are malignant; however, occasional cases have interspersed osteoclast-like giant cells. Necrosis is unusual. Overtly squamous elements may be abundant and evenly distributed throughout the tumor. Most often, however, they are a minor component in an overwhelmingly sarcoma-like neoplasm.

In the Thompson et al study of 187 laryngeal spindle cell carcinomas, 68% of cases showed immunoreactivity for antibodies against epithelial antigens within the spindle cell components of the tumor. The immunoreactivity was inconsistent, with antibodies to different cytokeratins, and no single antibody identified more than 41% of the cases. A potentially confusing factor about the immunohistochemical findings in spindle cell carcinomas is the occasional presence of myogenous markers in the neoplasms. Antibodies to p63 can be helpful with the diagnosis of head and neck spindle cell carcinoma because most cases are immunoreactive (Figure 5, B). A recent clinicopathologic review of 103 cases confirmed the findings of Thompson et al.

Molecular and cytogenetic findings have largely been used to prove the clonal nature of both the spindle cell and squamous components of this neoplasm. Overexpression and mutations of p53 are common and correlate well with the spindle and epithelial components of these tumors. Most tumors are nondiploid, which again correlates with both the spindle cell and epithelial components of the tumors. Finally, comparative genomic hybridization studies have shown homology in chromosomal aberrations between the 2 components of the tumors. Choi et al used microdissection and loss of heterozygosity studies of 9 microsatellite markers to show that the spindle cell component of spindle cell carcinomas appears to represent progression from the epithelial component. Spindle cell components frequently showed greater loss of heterozygosity, especially for a focus on band 17p. In a later study, Choi et al investigated 21 microsatellite markers in 48 upper aerodigestive tract squamous cell carcinoma variants. In that later study, spindle cell carcinomas had a greater loss of heterozygosity for markers on chromosomes 123, 124, 130, 132, 134, 139.

† References 123, 124, 130, 132, 134, 139.
Figure 5.  A, Spindle cell carcinoma appears mesenchymal because it is composed predominately of malignant spindled cells. B, p63 expression in a spindle cell carcinoma (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).

Figure 6.  Acantholytic squamous cell carcinomas often appear vascular (hematoxylin-eosin, original magnification ×200).

Figure 7.  A, Some adenosquamous carcinomas have abundant intracellular mucus. B, Other adenosquamous carcinomas have columnar cells that form glandlike structures (hematoxylin-eosin, original magnifications ×400 [A] and ×200 [B]).

Figure 8.  This basaloid squamous cell carcinoma is associated with overlying squamous cell carcinoma in situ (hematoxylin-eosin, original magnification ×200).
4 and 9, compared with other squamous cell carcinoma variants. Some researchers suggest these tumors illustrate the so-called epithelial-mesenchymal transition that occurs with epithelial malignancies as they become more aggressive and capable of metastasis. Spindle cell carcinomas do show increased expression of N-cadherin by IHC.147

In the Thompson et al study, the overall 5-year survival of patients with laryngeal spindle cell carcinoma was 59%. Survival was related to T stage (5-year death rate of T1, 23%; T2, 36%; T3, 57%; and T4, 67%). Also, patients with tumors that lacked immunoreactivity with antibodies to epithelial antigens fared worse. In contrast to the Thompson et al finding that the lung was the most common site for metastasis, Viswanathan et al141 reported lymph node metastasis to be most common. Interestingly, patients treated with radiation and surgery fared worse than those treated with surgery alone. The meaning of that is unclear because several clinical confounders likely had some influence.

**ACANTHOLYTIC (“ADENOID” AND “ANGIOSARCOMA-LIKE”) SQUAMOUS CELL CARCINOMA**

The acantholytic microscopic variant of squamous cell carcinoma is characterized by extensive acantholysis resulting in pseudoglandular or pseudovascular spaces. It was first recognized in the skin by Lever,149 and most often, tumors involve sun-exposed regions, including the skin of the head and neck.148–150 Mucosal head and neck lesions have been documented involving the lip, oral cavity, tongue, larynx, sinonasal tract, and nasopharynx.151–161 This change may be seen focally in otherwise typical squamous cell carcinomas from any anatomic site but rarely dominates the microscopic appearance. Acantholytic change might be caused by radiation therapy.153 The tumors occur much more frequently in men.159,162

The gross findings are nonspecific. Oral cavity lesions have been described as ulcerated, nodular, indurated, warty, exophytic, keratotic, or crusted.162 Tumors often measure several centimeters, with a tan or tan-white cut surface.155 These tumors are composed of alveolar or glandlike spaces lined by a peripheral layer of flattened, cuboidal, or “hobnail” neoplastic cells (Figure 6). Exfoliated single cells and cell aggregates are present within the lumenlike spaces.155 Occasionally, the spaces form complex, anastomosing, sinusoidal channels.150,163 The nuclei of the neoplastic cells are pleomorphic and are often hyperchromatic with occasional giant or multinucleated cells. Cytoplasm varies from scant to prominent and, when present, is typically eosinophilic. Small, pearl-like aggregates of cohesivel, overly squamous cells may be present within the lumenlike spaces, and larger areas of overt squamous differentiation may also be seen.150 Mitotic figures are frequently encountered.

Immunohistochemical studies of these neoplasms in the oral cavity, as well as analogous tumors involving the skin and lung, have shown strong reactivity for the epithelial markers cytokeratin and epithelial membrane antigen.162–164 Tumors are typically immunoreactive to p63 antibodies as well.165 The tumors are nonreactive for markers of endothelial differentiation, including CD34, CD31, and factor VIII–related antigens.

Labial tumors have been associated with a good prognosis. In a literature review of 26 adenoid squamous cell carcinomas predominately from the lips, 20 patients (77%) were free of disease, 3 (12%) had died of disseminated disease, and 3 (12%) were lost to follow-up.162 Other mucosal sites, however, fare worse.150,168 Favorable prognosis for tumors of the lips may be primarily due to the relative ease of diagnosis at that location, rather than to any inherent biologic differences in this morphologic subtype. The pattern of disease spread is also analogous to that of conventional squamous cell carcinoma and is dominated by involvement of regional lymph nodes early in the clinical course, with distant dissemination occurring later. The treatment and follow-up for patients with these tumors mimics that of conventional squamous cell carcinoma.

**ADENOSQUAMOUS CARCINOMA**

Adenosquamous carcinoma is an unusual carcinoma of the head and neck that contains components of adenocarcinoma and squamous cell carcinoma in close proximity. Although previously considered by some to be synonymous with mucoepidermoid carcinoma, the tumor can and should be distinguished from the latter neoplasm. Adenosquamous carcinoma is typically high grade and lacks microscopic and cytogenetic features of mucoepidermoid carcinoma. The tumor is thought to arise from the surface epithelium, which often displays high-grade dysplasia.166,167

Most cases of adenosquamous carcinoma arise in men, and the mean patient age is older than 60 years.166 Most cases involve the larynx, but any site within the upper aerodigestive tract can be involved. The disease is related to smoking and alcohol use. Symptoms and complaints are based on the site involved and include hoarseness (when the larynx is involved) or dysphagia when the mouth, tonsils, or hypopharynx is involved. Patients may also present secondary to metastatic disease with a neck mass. These tumors are grossly identical to conventional squamous cell carcinomas. Histologically, they are high grade and composed of mixtures of squamous cell carcinoma and adenocarcinoma. The squamous component usually predominates and can be either invasive or in situ.5,166 The adenocarcinomatous component is always closely associated with the squamous elements, and it lacks the characteristic features of a distinctive form of salivary gland-type adenocarcinoma. Instead, it typically consists of moderately or poorly differentiated, “generic” adenocarcinoma, as evidenced by gland formation, usually with columnar-type cells or the production of intracellular mucin (Figure 7, A and B).167 Typically, this component occurs in the deeper portions of the tumor.166,167 Necrosis, mitotic figures, and perineural invasion are common.

The glandular elements of these tumors have been demonstrated to express carinoembryonic antigen, low–molecular-weight cytokeratins, and CK7.167,168 They are not reactive with antibodies to CK20. High–molecular-weight cytokeratins are present in both the squamous and glandular components. Overexpression of the p53 protein is seen in most cases.22,167 Ten of 12 cases (83%) tested in one study167 were aneuploid. MAML gene rearrangements, typically seen with mucoepidermoid carcinoma, are not seen with adenosquamous carcinomas.169 Because some tumors may be associated with high-risk HPV infection, the molecular changes seen with those tumors (eg, intact p16 expression and overexpression) should be seen with some cases.22
The tumors are typically dense, firm, pale, focally ulcerated, and have prominent nucleoli. Mitotic figures are usually numerous, and atypical nests are often centrally ulcerated with considerable induration of the adjacent nonulcerated mucosa. On cross-section, squamous cell carcinomas are seldom exophytic, and they may show comedonecrosis.

**Basaloid Squamous Cell Carcinoma**

The identification of HPV-associated squamous cell carcinomas, which often have a nonkeratinizing and somewhat basoloid morphology, has somewhat blurred the distinction with the basaloid squamous cell carcinoma variant. The morphologic overlap prompted studies of basaloid squamous cell carcinomas (BSCCs) to identify whether there was an HPV subset within this variant. Two recent studies have demonstrated the presence of HPV 16 in a subset of BSCCs. Like most HPV-associated tumors, these HPV-associated BSCCs demonstrated an overall better prognosis and, not surprisingly, most were located in the oropharynx. As such, when a tumor meets the criteria for a BSCC, detection of HPV should be sought to identify this subset. As discussed above, the use of the term basaloid is being called into question when diagnosing HPV-related squamous cell carcinomas exhibit much stronger positivity. Punctate cytokeratin positivity of the type encountered in small cell carcinomas is not seen. Almost all basaloid squamous cell carcinomas show strong and diffuse immunoreactivity with antibodies to p63. Tumors may display abortive neuroendocrine differentiation but are usually negative for synaptophysin and chromogranin. Myogenus antigens, such as smooth muscle actin, are usually not expressed (although there has been some reported variability) nor is CD117 (KIT).

As expected in the HPV-associated subset, there is immunoreactivity with antibodies to p16. In non-HPV-associated tumors, p53 overexpression has been found. Studies detailing the prognosis of these tumors report conflicting results, which will likely evolve as HPV-associated BSCCs are separated from non-HPV-associated BSCCs in follow-up data regarding survival and prognosis.

**Papillary Squamous Cell Carcinoma**

Papillary squamous cell carcinoma is an uncommon variant of squamous cell carcinoma. It demonstrates a predilection for the larynx, oropharynx, and sinonasal tract and is more common in older men. Symptoms are related to the site of involvement and the presence of invasive disease and metastases. Rarely, patients present with a prior history of noninvasive papillomas. However, the relationship of these neoplasms to benign papillary proliferations of the larynx and sinonasal region remains unclear.

These tumors often have a grossly papillary appearance, similar to that of verrucous carcinoma, but they lack the prominent surface keratinization seen in the latter tumors. Tumors are typically friable, soft, and varied from 0.2 to 4.0 cm in one larger study. The noninvasive variant consists entirely of an exophytic proliferation of malignant-appearing squamous cells covering papillae with fibrovascular cores (Figure 9). The epithelium varies in thickness. The epithelial cells are usually immature and atypical forms are easily found.
basal cell-like, resembling squamous cell carcinoma in situ as described in the uterine cervix. Surface maturation and keratinization can be present but are typically limited. The invasive form of the tumor is similar to the noninvasive variant but contains an underlying component of invasive squamous cell carcinoma. The underlying invasive component may be keratinizing or nonkeratinizing. Identifying or excluding the invasive component can be difficult when based on endoscopic biopsy specimens. Ancillary testing is not generally helpful for the diagnosis of papillary squamous cell carcinoma, and the diagnosis should rely on histologic criteria only.

Recent studies have demonstrated HPV positivity in a subset of papillary squamous cell carcinomas. These HPV-related papillary squamous cell carcinomas are, not surprisingly, found in younger patients, display a nonkeratinizing morphology, and occur most often in the oropharynx. Given the rarity of these tumors, limited data are available on the prognosis of this HPV-associated papillary squamous cell carcinoma subtype; however, preliminary studies suggest improved disease-free survival. Current treatment parallels that of conventional squamous cell carcinoma. Authors have suggested that these tumors be treated as potentially invasive until proven otherwise.

NUCLEAR PROTEIN IN TESTIS MIDLINE CARCINOMA

Nuclear protein in testis (NUT) midline carcinoma (NMC) is an aggressive, rare variant of squamous cell carcinoma that is defined by its characteristic rearrangement of the NUT gene on band 15q14. The most common translocation involving the NUT gene is t(15;19)(q13;p13.1). This translocation fuses the NUT gene on chromosome 15 to the BRD4 gene. However, other fusion partners, such as BRD3, have been described in about one-third of cases; these NMCs are given the designation NUT-variant tumors. Implicit in its name, the term midline refers to its tendency to arise in the midline with the mediastinum and upper aerodigestive tract being its favored locations. Initially thought to be a pediatric disease, NMCs are now recognized to have no gender or sex predilection. Patients with NMCs frequently present with mass-related symptoms and non-
specific symptoms, such as fever and weight loss have also been noted.\textsuperscript{208–211}

The histologic features are that of a poorly differentiated carcinoma composed of monotonous, medium-sized, round cells with scant eosinophilic cytoplasm (Figure 10, A).\textsuperscript{210,212–215} Nuclei have irregular contours with fine to vescicular chromatin and prominent nucleoli. Mitotic figures and apoptotic bodies are common. Occasional cases may have a more-nested pattern with a desmoplastic stromal response. Large areas of coagulative necrosis may be present. There is often focal abrupt keratinization with sheets of immature cells juxtaposed to well-differentiated, mature, often benign-appearing squamous nests.\textsuperscript{201} A single case of the parotid gland has been described that additionally showed chordoid differentiation.\textsuperscript{215}

This tumor is often misdiagnosed because it can be confused with a variety of poorly differentiated tumors.\textsuperscript{202} Immunohistochemistry and ancillary studies, such as fluorescence in situ hybridization (FISH), cytogenetics, and RT-PCR can assist in the diagnosis. The NUT midline carcinomas are epithelial and react with antibodies to keratins (although staining may be focal).\textsuperscript{212–214} Most of the tumors described are immunoreactive with antibodies to CK7 and sometimes show focal immunoreactivity with antibodies to CK20.\textsuperscript{213} Most tumors express p63, consistent with squamous differentiation. CD34 expression was seen in a little more than one-half of the cases studied in one review.\textsuperscript{215} Immunoreactivity with other antigens expressed in small blue cell tumors of childhood has not been noted. Evidence of EBV and HPV infection has not been identified, although p16 overexpression has been noted in some cases.\textsuperscript{212}

The advent of the commercially available anti-NUT monoclonal antibody has now simplified its diagnosis.\textsuperscript{216} The antibody demonstrates 87% sensitivity and 100% specificity.\textsuperscript{216} A positive result is described as diffuse, speckled nuclear staining in more than 50% of tumor cells.\textsuperscript{216} However, in cases where NMC is high on the differential, and IHC results for NUT are negative, further studies, such as FISH, are warranted to thoroughly exclude NMC (Figure 10, B).\textsuperscript{216} The FISH probes have been developed for the regions flanking the typical breakpoint of the NUT gene on chromosome 15 and for the typical breakpoints with BRD4 and BRD3; however, they are not commercially available. As an alternative to FISH, RT-PCR primers have been developed for both fusion proteins; however RT-PCR remains less sensitive than FISH.\textsuperscript{216}

The function of the NUT protein is not entirely known, although it is constitutively expressed in early germ cells and within the brain (ciliary ganglion).\textsuperscript{199,212} Transcriptionally active chromatin is bound by the BRD proteins.\textsuperscript{199,212} Fusion proteins are thought to be localized to the nucleus secondary to the expressed portions of the BRD4 and BRD3 proteins that are retained.\textsuperscript{199} Further, the BRD-NUT fusion protein is thought to cause a block in differentiation via decreased expression of many genes through decreased histone acetylation.\textsuperscript{199} This process is referred to as sequestration and details regarding this model can be found in a recent review\textsuperscript{199} published in the journal Annual Review of Pathology.

The NMC tumors present at advanced stage and are often unresectable.\textsuperscript{199,200} The treatment approach to NMC is heterogeneous with a combination of surgery, multidrug chemotherapy, and radiation therapy.\textsuperscript{199,200} Although the tumors initially respond to chemotherapy, they subsequent-ly recur and become less responsive to treatment.\textsuperscript{199,200} In the largest NMC study to date, Bauer et al\textsuperscript{200} demonstrated that the median, overall survival was 6.7 months. Bauer et al\textsuperscript{200} found intensive local therapy, such as total gross resection and radiotherapy, was associated with improved survival. However, no chemotherapeutic regimen emerged as having an improved outcome.\textsuperscript{200} As the pathophysiology behind NMCs continues to unravel, new treatment options will likely become available. In vitro research is currently focused on developing small molecule inhibitors (eg, histone deacetylase inhibitors and BET inhibitors) with promising results.\textsuperscript{199}

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