

Educational Case: Human Papillomavirus–Positive Oropharyngeal Squamous Cell Carcinoma

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <http://journals.sagepub.com/doi/10.1177/2374289517715040>.¹

Keywords

pathology competencies, organ system pathology, head and neck, head and neck neoplasia, squamous cell carcinoma of the oropharynx, HPV, p16

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Primary Objective

Objective HN2.3: Causes of Oropharyngeal Squamous Cell Carcinoma: Compare and contrast human papillomavirus (HPV)-driven and alcohol-/tobacco-driven development of squamous cell carcinoma including precursor lesions, tumor formation and progression, anatomic location, and survival rate.

Competency 2: Organ System Pathology; Topic HN: Head and Neck; Learning Goal 2: Head and Neck Neoplasia

Secondary Objective

Objective HN2.2: Squamous Cell Carcinoma of the Oropharynx: Discuss the pathogenesis of squamous cell carcinoma of the oropharynx and the spectrum of histologic findings from normal mucosa to invasive disease.

Competency 2: Organ System Pathology; Topic HN: Head and Neck; Learning Goal 2: Head and Neck Neoplasia.

Patient Presentation

A 53-year-old man presented with a palpable 2-cm right lateral neck mass, which had been present for approximately 2 months.

He had no other significant past medical history. He had been a lifelong nonsmoker and only used alcohol on social occasions. Other than the neck mass, he reported no other symptoms. Physical examination showed a palpable, mobile nodule in the right lateral neck, which was painful on palpation. A course of antibiotics was prescribed by his family practitioner without resolution. Therefore, the patient was referred for evaluation to an otolaryngologist, who ordered a computed tomography (CT) scan of the neck.

Diagnostic Findings, Part I: Imaging

A CT scan of the neck (Figure 1) was performed. The CT shows an enlarged abnormal lymph node in the right lateral neck with cystic degeneration, measuring 1.2 cm on its short

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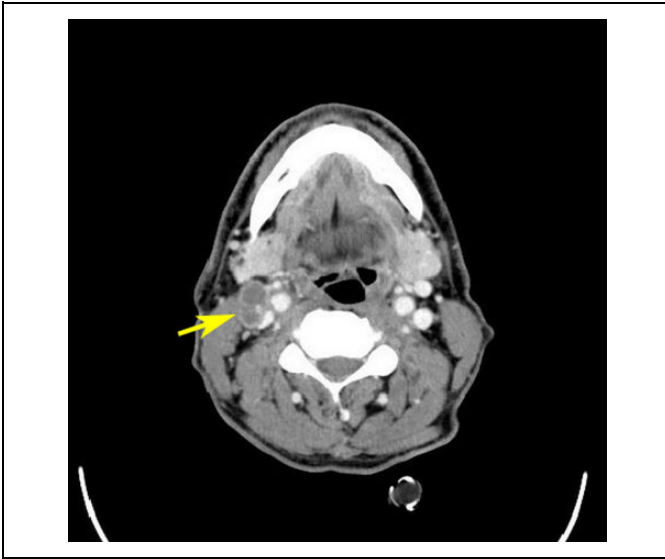


Figure 1. Computed tomography (CT): An enlarged abnormal lymph node in the right lateral neck (yellow arrow) with cystic degeneration, measuring 1.2 cm on its short axis, suspicious for metastatic disease.

axis, suspicious for metastasis, along with subtle asymmetric enhancement of the right palatine tonsil.

Questions/Discussion Points, Part 1

What Is the Differential Diagnosis Based on Computed Tomography?

The presence of central cystic degeneration/necrosis in a lymph node is highly concerning for malignancy. In this age-group, the differential diagnosis would include metastatic carcinoma

(most commonly squamous cell carcinoma [SCC] of the head and neck) and lymphoma. Branchial cleft cysts are benign cystic embryologic remnants that occur in the lateral neck but are typically seen in young adults and children and should not show enhancement.

Diagnostic Findings, Part 2: Pathology

A fine needle aspiration biopsy of the right neck mass was performed, which was positive for metastatic SCC (Figure 2). Fine needle aspiration biopsy is a good initial screening tool for lesions of the neck and can guide further patient workup. This result directed the physician to look for a head and neck primary. Direct laryngoscopy in the office showed an enlarged right palatine tonsil, which was biopsied, and showed invasive SCC (Figure 3A). Additional ancillary testing performed on the biopsy showed the carcinoma was strongly and diffusely positive for p16 immunohistochemistry (Figure 3B), supporting a diagnosis of human papillomavirus (HPV)-associated SCC (HPV-SCC).

Questions/Discussion Points, Part 2

What Are the Most Common Malignancies of the Head and Neck?

Squamous cell carcinoma is the most common malignancy of the head and neck, accounting for 90% of all head and neck cancers. Other malignancies can arise in the head–neck region, including thyroid cancer, salivary gland tumors, and lymphomas. Metastatic malignancies from lung, breast, and colon can also present as neck mass.²

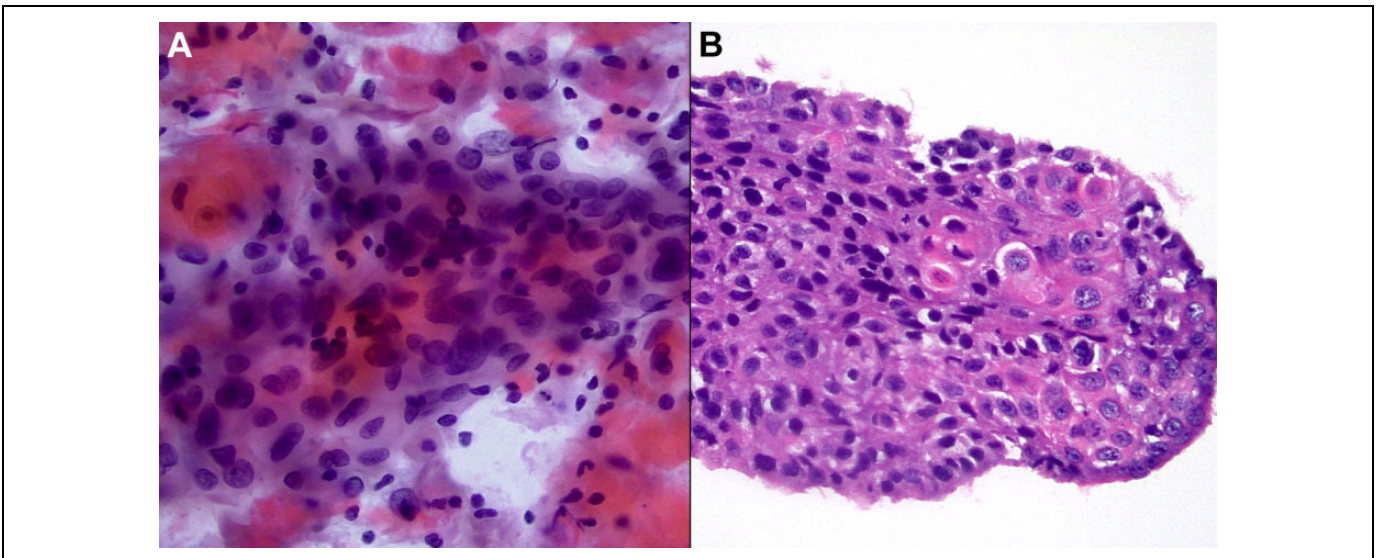


Figure 2. A, Aspirate smears of the fine needle aspiration biopsy show cells in a cluster with enlarged, irregular, hyperchromatic nuclei and abundant dense, pink cytoplasm in a background of keratinized degenerating cells, diagnostic of metastatic squamous cell carcinoma ($\times 400$, Papanicolaou stain). B, The cell block shows a clusters of predominantly nonkeratinizing cells with moderate cytoplasm ($\times 400$, H&E). H&E indicates hematoxylin and eosin.

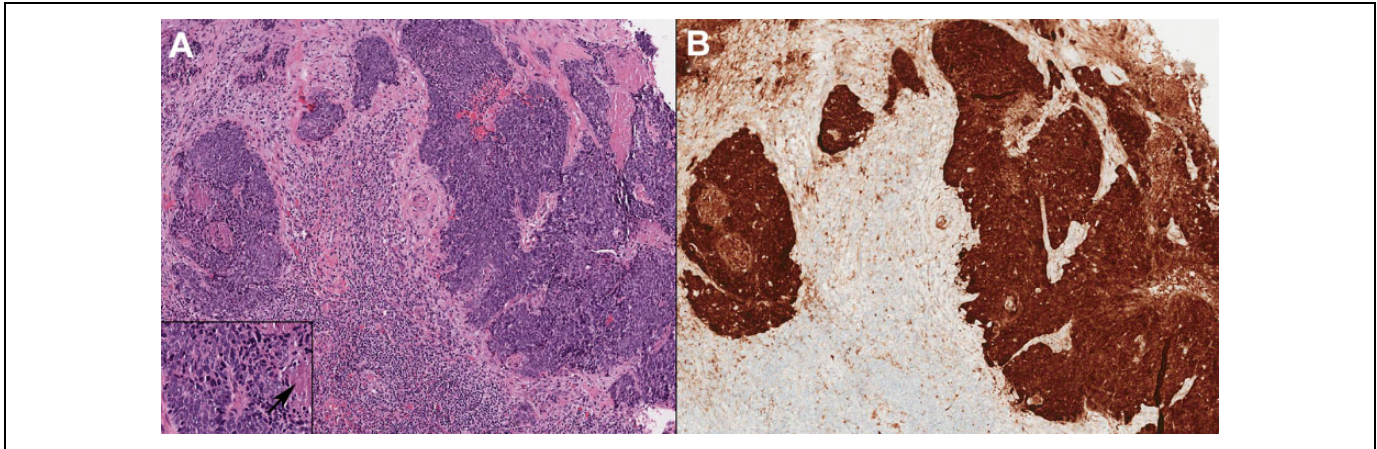


Figure 3. A, Histologic section from the tonsil biopsy shows an invasive nonkeratinizing squamous cell carcinoma in a background of lymphoid tonsillar tissue ($\times 200$, H&E). The inset shows the nonkeratinizing cells morphology with focal necrosis (arrow; $\times 400$, H&E). B, P16 immunohistochemical staining shows strong diffuse ($>70\%$ nuclear and cytoplasmic brown) staining within the invasive carcinoma, consistent with an HPV-associated squamous cell carcinoma ($\times 200$, p16 immunohistochemistry). H&E indicates hematoxylin and eosin; HPV, human papillomavirus.

How Are Squamous Cell Carcinoma and Its Precursor Lesions Distinguished From Benign Squamous Mucosa on Pathologic Examination?

Benign squamous mucosa in the oral cavity demonstrates architectural maturation and polarity from base to top with no cytology atypia (Figure 4A) and is typically nonkeratinizing unless subject to trauma or irritation. In the oral cavity, the most common clinically identified precursors to the development of SCC are leukoplakia (white patches or plaques) and erythroplakia (red, velvety eroded areas), but as many as 50% of oral SCCs can arise from grossly normal mucosa. Leukoplakia generally corresponds, on pathologic examination, to an area of hyperkeratosis, which may or may not be also associated with squamous dysplasia. Erythroplakia is highly associated with dysplasia on pathologic examination.

Keratinizing squamous dysplasia, the pathologic precursor lesion to most tobacco- and alcohol-related SCC of the head and neck, is divided into mild, moderate, and severe/carcinoma in situ grades. In general, all keratinizing dysplasias show hyperkeratosis at the surface of the epithelium. Hyperkeratosis is the presence of increased and abnormal keratin at the surface of a squamous epithelium, recognizable with the deeply eosinophilic color and absence of nuclei. The degree of cytological (nuclear) atypia and abnormal maturation (architectural disorder) is used to determine the overall grade of dysplasia.

Mild dysplasia usually shows mild nuclear pleomorphism and nuclear hyperchromasia with basal cell hyperplasia. Moderate dysplasia shows greater nuclear pleomorphism, and increased and abnormal mitotic figures, with disordered maturation and dyskeratosis (Figure 4B). Severe dysplasia/carcinoma in situ shows significant nuclear pleomorphism including prominent nucleoli, marked variation in nuclear size and shape, abnormal mitotic figures, and architectural abnormalities such as dyskeratosis, loss of polarity, and a drop-shaped interface with the

underlying submucosa (characterized by an irregular interface with the underlying submucosa, often teardrop shaped extending downward; Figure 4C). Squamous cell carcinoma is defined by the presence of invasion by atypical squamous cells into the lamina propria. This is most readily recognized by the irregular, jagged contours of the epithelial groups which lack surrounding basement membrane, paradoxical maturation with deep keratin pearls at an invasive front, and a stromal desmoplastic response (desmoplasia is a stromal change indicating a response to invasive tumor, usually characterized by increased spindle cells and a light-colored or myxoid change; Figure 4D).

Recognition and grading of dysplasia in the head and neck is clinically relevant, as it predicts the risk of malignant transformation. Severe epithelial dysplasia has an overall malignant transformation rate to invasive SCC of about 16%, whereas moderate dysplasia has a relative lower malignant transformation risk (3%-15%), and mild epithelial dysplasia has a very low risk ($<5\%$).³

What Are the Predisposing Factors That Contribute to the Development of Squamous Cell Carcinoma of the Head and Neck?

Tobacco and alcohol use has been strongly established as the primary risk factor in the SCC of the head and neck. Both tobacco and alcohol use in the United States have been steadily declining for the last 5 decades, which is paralleled by decreasing rates of head and neck cancer incidence and mortality. However, despite decreasing rates of tobacco and alcohol use, the rates of oropharyngeal cancer have trended steadily upward for the last decade. This is primarily due to the increase in cancers related to infection with the HPV, which is mainly spread through oral sexual behavior.

Of note, *Areca* (betel) nut chewing is another leading cause of oral SCC in parts of Asia and the Pacific.⁴

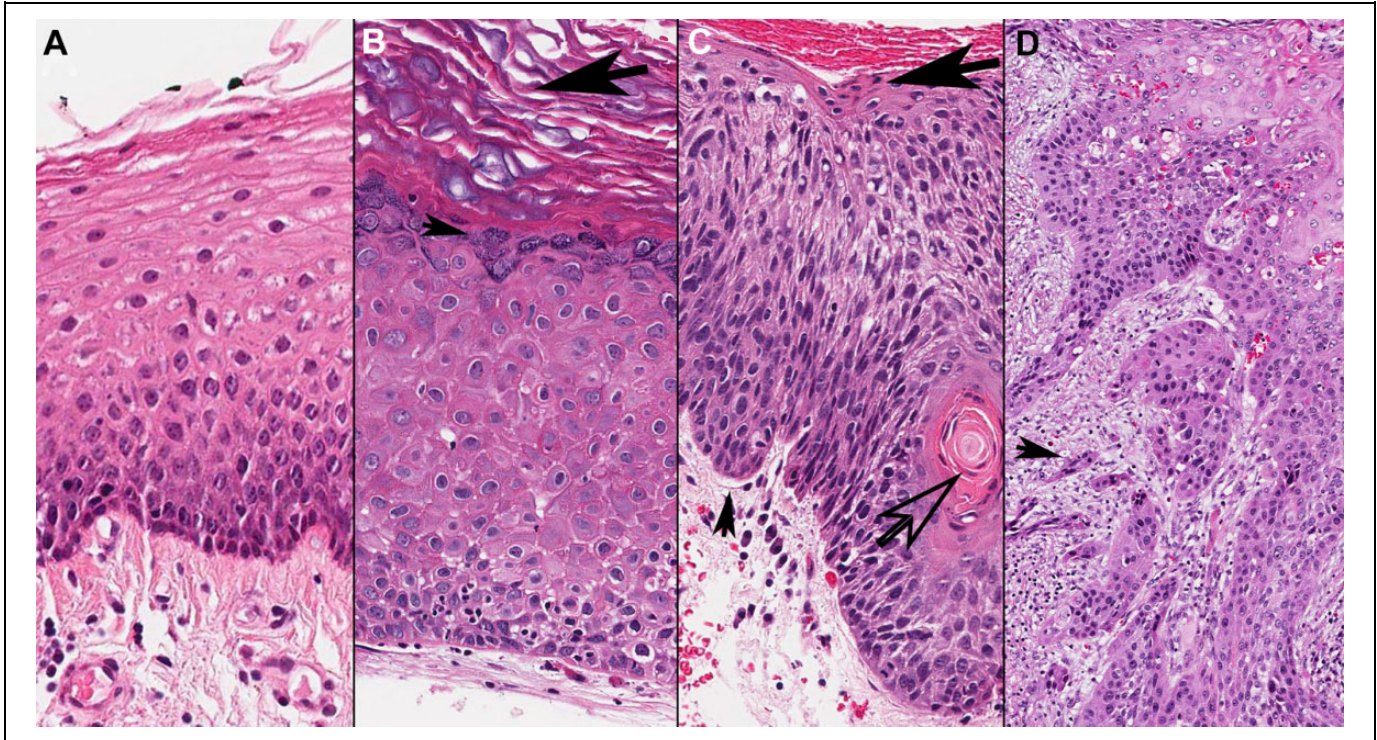


Figure 4. A, Normal squamous mucosa. Note the small uniform nuclei in the basal layer with maturation toward the surface and the absence of keratinization ($\times 200$, H&E). B, Moderate epithelial dysplasia. Note the marked hyperkeratosis (large arrow) and granular cell layer (small arrow) at the surface, as well as abundant pink “glassy” cytoplasm and moderate nuclear variation in size in shape, but a retained flat interface with the underlying stroma and relatively ordered maturation ($\times 200$, H&E). C, Severe dysplasia/carcinoma in situ. The architecture of the epithelium is almost completely disordered with marked nuclear pleomorphism, loss of nuclear polarity, and full-thickness abnormalities. Note the thin layer of hyperkeratotic epithelium on the surface (large arrow), the dyskeratotic keratin pearl deeper within the epithelium (open arrow), and the drop-like interface with the underlying stroma (small arrow; $\times 200$, H&E). D, Invasive squamous cell carcinoma. Note the irregular small islands (small arrow) of squamous epithelium extending into the underlying stroma, which shows a marked desmoplastic response ($\times 100$, H&E). H&E indicates hematoxylin and eosin.

What Are the Classic Clinical and Pathologic Features of Human Papillomavirus–Associated Squamous Cell Carcinoma That Differentiate It From Tobacco- and Alcohol-Related Squamous Cell Carcinoma?

Human papillomavirus–associated SCC is a distinct entity, separated from SCC associated with tobacco/smoking and alcohol consumption by location, patient demographics, and morphologic appearance. Human papillomavirus–associated SCC usually occurs in the oropharynx, most commonly in the tonsils or base of tongue. The oropharynx is a highly specialized area, containing extensive mucosal-associated lymphoid tissue (palatine tonsils, lingual tonsils in the base of tongue, and adenoids/soft palate), which creates a more permissive environment for HPV infection.⁵ Unlike patients with typical SCC, patients with HPV-SCC typically present with advanced clinical stage involving multiple lymph nodes in the lateral upper to mid neck, but often with a small or unrecognized primary tumor. Patients with HPV-SCC are also typically younger, nonsmokers, and lack a history of preexisting dysplasia. Histopathologically, HPV-SCC exhibits a distinctive nonkeratinizing morphology, compared to the usual keratinizing invasive SCCs associated with tobacco and alcohol use (Figure 5). Finally, and perhaps most importantly, despite presenting at a

higher stage, HPV-SCC is associated with better survival outcomes and lower recurrence rates than HPV-negative oropharyngeal SCC (OPSCC; Table 1)⁶ and is treated primarily with radiation and chemotherapy, rather than primary surgical resection.

What Is the Mechanism of Human Papillomavirus Carcinogenesis in the Development of Human Papillomavirus–Associated Oropharyngeal Squamous Cell Carcinoma?

Human papillomaviruses are small, nonenveloped double-stranded DNA viruses, with over 170 types identified. The majority of HPV-positive oropharyngeal tumors are caused by high-risk HPV, especially type 16, which is responsible for more than 90% of cases.⁶ Initially, the HPV virus is present within infected cells as circular extrachromosomal particles or episomes (Figure 6A). If the HPV infection is not cleared, over time the HPV DNA can become integrated into the DNA of the host genome. The integration of HPV DNA into host genome is a critical step in the progression to cancer. This integration results in disruption of the HPV E2 messenger RNA (mRNA), which regulates expression of HPV E6 and E7 oncogenes. The overexpression of E6 and E7 binds to and inactivates host cell tumor suppressor p53 and the

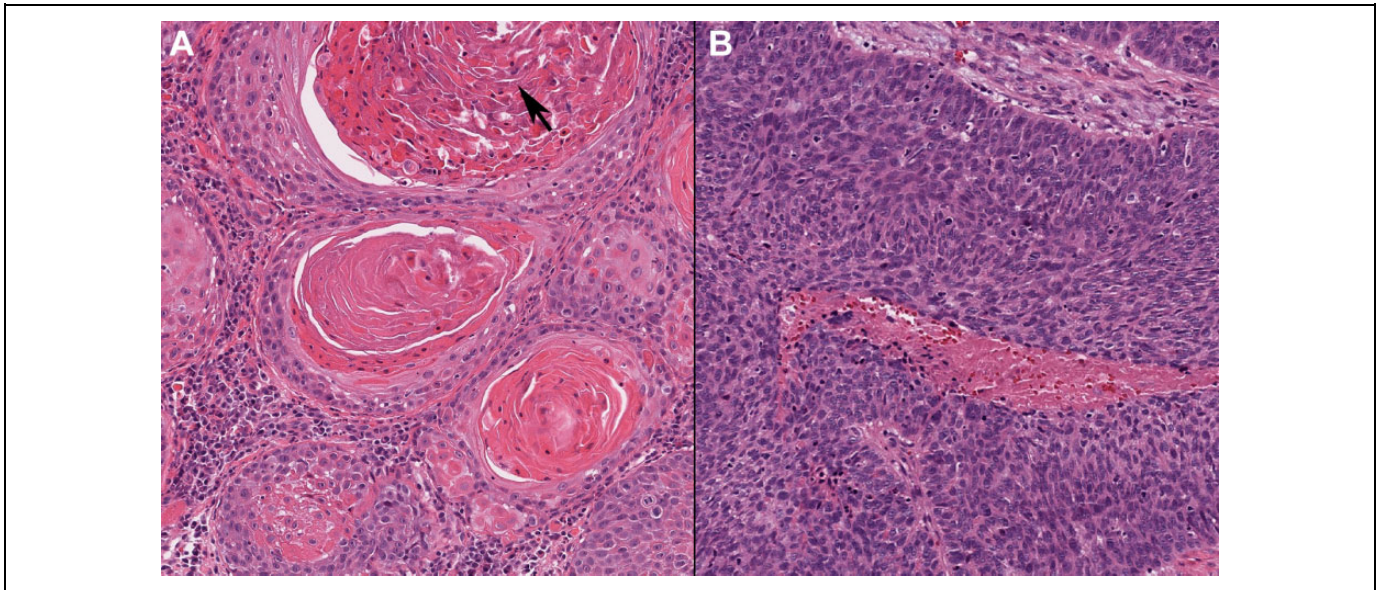


Figure 5. A, Classic, keratinizing invasive squamous cell carcinoma, showing abundant pink cytoplasm and entrapped keratin pearls (arrow; $\times 200$, H&E). B, Human papillomavirus–associated squamous cell carcinoma showing nonkeratinizing morphology, with scant cytoplasm and dark, closely spaced nuclei ($\times 200$, H&E). H&E indicates hematoxylin and eosin.

Table 1. Comparison of HPV and Alcohol-/Tobacco-Driven Squamous Cell Carcinoma and HPV-Associated SCC.

Characteristics	HPV-Associated SCC	Usual SCC
Median age	50-56 years	60-70 years
Risk factors	Sexual behavior	Tobacco, alcohol
Location	Oropharynx (base of tongue and palatine tonsils)	Oral cavity
Dysplasia	Rarely seen	Often present
Morphology	Nonkeratinizing	Keratinizing
P16 immunohistochemistry	Positive	Negative
Primary treatment modality	Radiation and chemotherapy	Surgical resection with adjuvant radiation and chemotherapy as needed
Overall survival rate (3 years)	82%	57%

retinoblastoma (Rb) proteins, respectively, leading to p16 accumulation, and unrestricted cellular proliferation (Figure 6B).⁷ Human papillomavirus integration has been also associated with selective growth advantage and genomic instability of the infected cells.⁷

What Testing Is Required for the Diagnosis of Human Papillomavirus–Associated Oropharyngeal Squamous Cell Carcinoma?

All newly diagnosed OPSCCs should undergo testing for HPV infection, according to the guidelines developed by the

College of American Pathologists (CAP) and endorsed by the American Society of Clinical Oncology.⁸ Either the oropharyngeal primary tumor or a cervical lymph node metastasis should be tested by immunohistochemistry using p16. In addition, in the setting of a lymph node metastasis involved by SCC but without identification of a primary tumor, HPV testing (by p16 or other HPV assays) is recommended to help guide identification of the primary site and therapeutic options.

A positive p16 result is defined as at least 70% tumor nuclear and cytoplasmic expression with at least moderate-to-strong intensity. If the morphology and p16 immunohistochemistry appear discordant (ie, nonkeratinizing morphology with absent p16 staining or keratinizing morphology with strong p16 staining), additional tests with an HPV-specific assay, such as in situ hybridization for high-risk HPV E6/E7 mRNA, are recommended. At this time, p16 immunohistochemistry is not recommended for routine clinical use in SCCs or other tumor subtypes outside the oropharynx, as the specificity of p16 as a predictor of HPV-associated disease drops significantly in other head and neck subsites and diseases.⁸

Teaching Points

- The most common head neck primary malignancy is SCC.
- Tobacco and alcohol are still leading risk factors for SCC in the head and neck.
- Squamous cell carcinoma usually develops from squamous dysplasia, which is graded from mild, to moderate, to severe/carcinoma in situ.
- The grade of squamous dysplasia is determined based on the degree of nuclear atypia and architectural disarray.

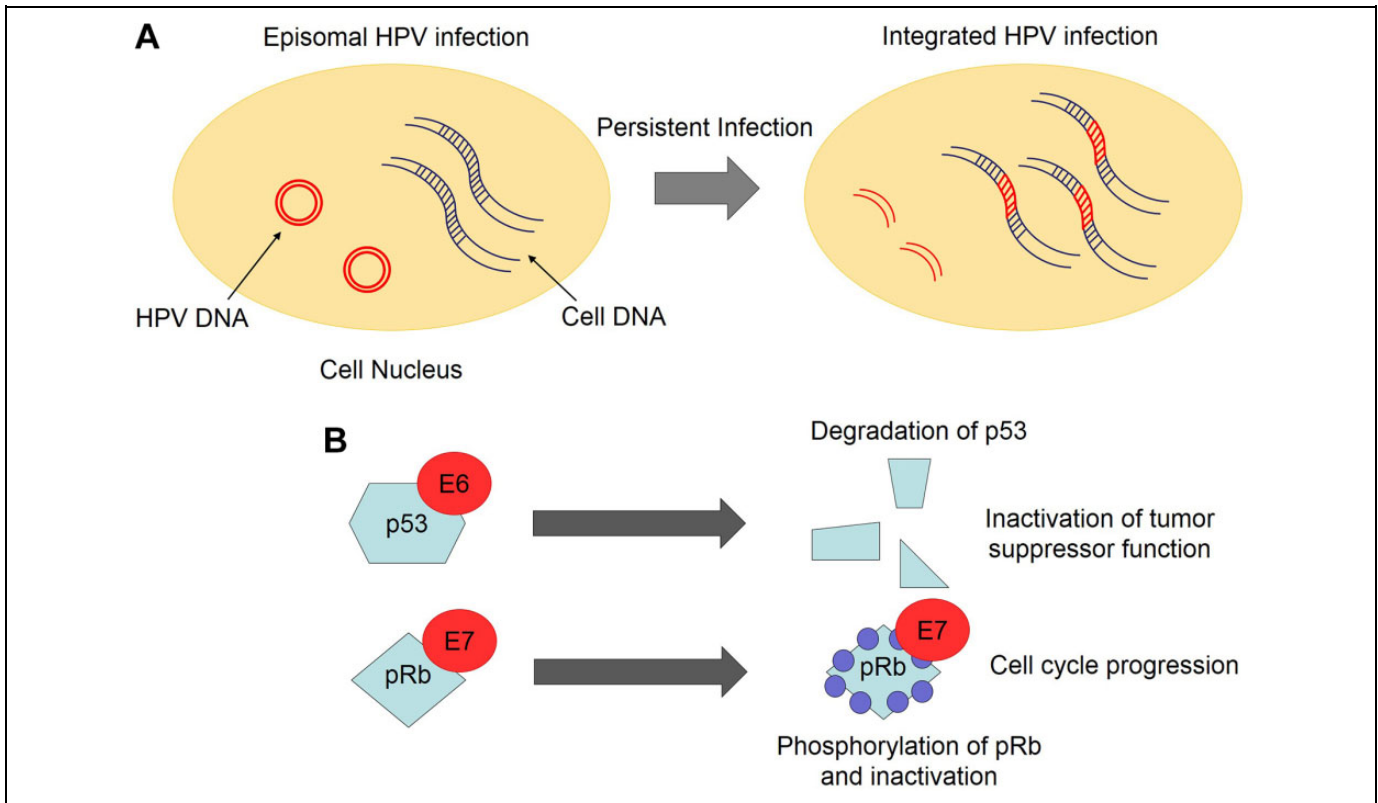


Figure 6. A, Initial HPV infection is episomal, with viral particles in the host cell nucleus, but separate from the native DNA. However, with persistent infection, the circular DNA is broken and integrated linearly into the host genome. B, Human papillomavirus protein E6 binds to the native p53 protein, resulting in degradation of p53 and inactivation of its tumor suppressor function. Human papillomavirus protein E7 binds to the native pRb protein, which then triggers pRb phosphorylation and inactivation, leading to increased cell cycle proliferation. H&E indicates hematoxylin and eosin; HPV, human papillomavirus; pRb, retinoblastoma protein.

- Human papillomavirus–associated SCC is a unique entity that has been increasing within recent decades and has a distinctive clinical, epidemiologic, and pathologic presentation.
- The mechanism of HPV-driven oncogenesis is thought to be largely due to HPV genome integration into host genome, leading to E6 and E7 oncoprotein overexpression and inactivation of host cell tumor suppressor genes p53 and the Rb protein, respectively.
- Human papillomavirus–associated SCC usually occurs among younger men in the oropharynx (tonsils and base of tongue) and presents with an advanced clinical stage, with a small or unrecognized primary tumor and lateral upper to mid neck nodal involvement.
- Despite presenting at higher stage, the overall survival outcomes are better in HPV-associated SCC than usual SCC, and patients respond better to radiation and chemotherapy.
- Human papillomavirus–associated SCC exhibits distinctive predominantly nonkeratinizing morphology, and the diagnosis can be confirmed by strong and diffuse staining for p16 immunohistochemistry. A positive result is defined as at least 70% of tumor cells with nuclear and cytoplasmic expression showing at least moderate-to-strong intensity.
- The CAP guidelines recommend all OSCCs undergo HPV testing. In addition, when nodal metastasis is present with an unknown primary tumor, HPV testing can be helpful in guiding recognition of the primary site and management.

Declaration of Conflicting Interests

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