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Biology of Human Papillomavirus–Related Oropharyngeal Cancer

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Abstract

Recent data show that human papillomavirus–positive oropharyngeal cancer (OPC) has a distinct biological and clinical behavior compared with human papillomavirus–negative OPC. As this subset of head and neck cancer represents an increasing public health concern, a thorough understanding of the causative and mechanistic differences between these diseases and how these distinctions impact clinical treatment is required. In this review, we will summarize recent data in epidemiology, the mechanism of viral carcinogenesis and differences in tumor biology that may provide insights to improve the clinical management of patients with human papillomavirus–positive OPC.

Cancers arising from many distinct anatomical loci (oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx) are collectively termed head and neck cancer (HNC). The clinical behavior of each anatomical site differs to varying degrees along with the treatments. For example, nasopharyngeal carcinoma has long been known to represent a distinct entity from the majority of the HNC with an etiology associated with Epstein–Barr virus infection and exquisite sensitivity to radiation and cisplatin. Consequently, this subset of HNC has been studied in separate clinical trials since the 1980s.^{1,2} Recent data suggest similar research considerations are required for a subset of oropharyngeal cancers (OPCs) caused by high-risk human papillomavirus (HPV) infection. Mounting evidence suggests that HPV-positive OPC (HPV+ OPC) is a biologically and clinically distinct entity compared with HPV-negative OPC (HPV– OPC).^{3–7} In this review, we will summarize recent data in HPV+ OPC epidemiology, the mechanism of viral infection and carcinogenesis, as well as current studies in tumor biology that may provide insight to the future of clinical management for this disease.

Epidemiology of HPV-Related Oropharynx Cancer

In general, the most common risk factors associated with HNC are tobacco use, alcohol consumption, and HPV infection. Because of recent public health awareness and intense tobacco cessation campaigns, the incidence of tobacco-related HNC has been steadily decreasing; however, the overall incidence of HNC has not because of a steady increase in HPV+ OPC.^{8,9} In a recent population-based study, the incidence of HPV+ OPC has increased by 225% between 1988 and 2004, whereas the incidence of HPV– OPC has decreased by 50% (Fig. 1).⁹ Currently, the incidence of HPV+ OPC is equivalent to HPV– OPC because 50%–57% of OPC is associated with HPV infection in North American, European, and Australian studies.^{4,10–14} Furthermore, the number of HPV+ OPC patients is projected to surpass those with cervical cancer by the year 2020 if the trend continues.⁹

Confirming epidemiologic observations, the patient characteristics associated with HPV+ OPC differ significantly from HPV- OPC. Patients with HPV+ OPC are younger, frequently white males, nonsmokers or infrequent smokers, and moderate or nonalcohol drinkers. HPV- OPC patients are older, heavy smokers, and experience a synergistic increase in risk with alcohol consumption. HPV+ OPC is also associated with high-risk sexual practices and marijuana use compared with HPV- OPC.^{5,15} Because of previous studies linking immunosuppression to cannabinoid use,¹⁶ the prevailing hypothesis linking marijuana to HPV+ OPC development involved chronic immunosuppression as a permissive environment for viral-mediated oncogenesis. However, a recent study determined that marijuana was not correlated with an increased risk of cervical neoplasia, a disease also linked to HPV infection.¹⁷ Thus, hypotheses linking HPV+ OPC development to immunosuppression by marijuana use need further investigation. Sexual risk factors for the development of HPV+ OPC also include early age of sexual debut, increased number of vaginal and oral sexual partners, repeated oral-genital and oral-anal contact, and concomitant with a decreased use of barriers during these activities.^{5,18} Consequently, these findings are consistent with HPV infection representing a sexually transmitted disease and a causative factor in OPC development.^{5,15}

Mechanism of HPV-Induced Carcinogenesis

As reviewed previously, HPV-induced carcinogenesis is primarily associated with the oropharynx. This subsite of the head and neck region includes the soft palate, base of tongue, and tonsils. More specifically, HPV infection is most commonly associated with the palatine and lingual tonsils.¹⁹ These secondary lymphoid organs within the lymphoid tissue of Waldeyer ring provide defense against pathogens entering the upper aerodigestive tract. The palatine and lingual tonsils are lined with stratified squamous epithelium, which extends throughout the tonsils, and contain numerous tonsillar crypts. These crypts serve to greatly increase the total surface area of the tonsils, thus dramatically increasing the efficiency of antigen capture and immune surveillance (Fig. 2A).¹⁹ Furthermore, the tonsillar crypts are lined with specialized reticular crypt epithelium that has a disrupted basal cell layer and basement membrane. This structural porosity is designed to facilitate the trafficking of antigens, lymphocytes, and antigen-presenting cells (Fig. 2B). Current theory dictates that deposition of the viral particles across the disrupted basal cell layer and basement membrane facilitates infection of basal cells without the required trauma observed in cervical cancer.¹⁹ Furthermore, the permissive nature of the reticular epithelium to cellular migration may contribute to the early metastasis of HPV+ OPC to cervical lymph nodes, but a complete understanding of the exact mechanism of HPV-mediated malignant transformation is in a relatively nascent stage.^{4,19}

In recent years, high-risk HPV infection (primarily HPV-16) has been established as an important oncogenic driver in OPC.^{20,21} HPV is a circular double-stranded DNA virus consisting of an 8-kb genome encoding viral proteins, including 3 oncoproteins (E5, E6, and E7) and 2 capsid proteins (L1 and L2).²² The genome is encased within an icosahedral structure with a 55-nm diameter consisting of L1 and L2.²³ Most importantly, these capsid proteins mediate the interaction between the virus and the target cell. It is currently thought that capsid affinity for proteoglycan heparin sulfate moieties on the basement membrane serve as the initial binding event for HPV infection.²⁴ Likewise, infectious transfer has been shown to require heparin sulfate.^{25,26} Endocytosis of the virus particle occurs over the course of several hours in cells of epithelial origin.²³ These kinetics are striking, as ligands and most viruses tend to internalize rapidly, which may provide further clues concerning the receptors required for HPV infection.²⁷ Whether these classical studies of HPV infection are applicable to the unique architecture of the tonsillar crypts remains to be seen.

After infection, expression of E6 and E7 targets the tumor suppressors p53 and pRb, respectively. E6 complexes with an E3 ubiquitin ligase (E6AP), thus hijacking E6AP recognition for its natural substrates to inappropriately target p53 for proteasomal degradation.²⁸ In a similar mechanism, E7 binds the cullin 2 ubiquitin ligase complex and silences pRb.²⁰ Recently, HPV viral oncoproteins have also been shown to interact and degrade key signaling components through interactions involving PDZ domains, a common structural domain of 80-90 amino acids found in many signaling proteins.²⁹ This structural motif is present in >400 proteins over 900 times in the human genome,³⁰ providing an incalculable advantage to viral disruption of normal signal transduction mechanisms. In fact, a functional class I PDZ-binding motif has been identified at the C-terminus of high-risk, but not low-risk, HPV E6 proteins.^{31,32} Moreover, the PDZ-binding motifs of HPV-16 E6 and HPV-18 E6 have been shown to promote epithelial-to-mesenchymal transition-like changes in target cells. Consequently, the balance between benign and malignant growth with respect to HPV viral protein targeting is much more complex than originally thought.

Ultimately, selective targeting of critical developmental and growth regulatory pathways results in deregulation of DNA repair, apoptosis, cellular senescence, and cell cycle control (Fig. 3, and reviewed in Chung and Gillison⁶). Not surprisingly, targeted inhibition of HPV-16 E6 and E7 in HPV+ OPC cell lines results in functional restoration of p53 and pRb, leading to apoptosis.²⁰ Conversely, forced expression of HPV-16 E6 and E7 in normal oral keratinocytes results in immortalization, providing in vitro support for the causal association of HPV infection in OPC development.²¹ However, the long latency period between HPV exposures and carcinogenesis suggests that HPV-related OPC development requires additional oncogenic molecular events.¹⁹ In support of this hypothesis, appreciable levels of HNC tumor formation are only achievable in *K14E6E7* bitransgenic mice when a carcinogen (4-nitroquinoline-N-oxide) is added to the drinking water.⁴⁰ Studies with single transgenic mice (*K14E6* vs *K14E7*) have also suggested that E7 is a more potent oncogene compared with E6, as *K14E7* mice exhibit a much higher rate of tumorigenesis than *K14E6* mice.⁴⁰

Yet, the exact mechanism of malignant transformation after HPV infection is still unclear, as few descriptive studies have been executed. It is known that a history of extensive smoking in patients with HPV+ OPC is associated with worse prognosis,^{4,41} suggesting there may be an interaction between HPV infection and tobacco-related carcinogen exposure analogous to 4-nitroquinoline-N-oxide initiation of *K14E6E7* bitransgenic tumor growth. Recently whole-exome sequencing of HPV+ OPC samples was completed as a part of two larger HNC tumor sequencing projects.^{42,43} Compared with traditional tobacco-induced HNC tumors, HPV+ OPC had 4-fold fewer gene mutations coupled with a noticeable lack of *TP53* mutations (0% vs 78%). Because of the targeted nature of HPV deregulation, accrual of many mutations to achieve similar levels of genomic instability and deregulated growth is likely unnecessary. Although these results were informative, they did not reveal any specific molecular transformation event inherent to HPV+ OPC to hasten tumorigenesis in the context of high-risk E6/E7 expression. More troubling still was the distribution of mutated oncogenes versus tumor suppressors. Only 18% of the HPV- HNC tumors sequence contained activating mutations in bona fide oncogenes, whereas 89% of these tumors harbored at least one bona fide tumor suppressor mutation.⁴² As the majority of targeted cancer therapies involve inhibition of constitutively active or overexpressed oncogenes (reviewed in Howard et al⁴⁴), the rampant loss of tumor suppressors in HNC represents a unique challenge in drug design moving forward.

Although mutational data has demonstrated a stark difference between HPV+ and HPV- HNC, gene expression analysis has also uncovered striking differences in HPV-related signal transduction. For example, HPV+ HNC demonstrates an upregulation of cell cycle-

related genes compared with HPV– HNC. HPV+ HNC is also associated with increased expression of genes located within chromosomal region 3q24-qter.⁷ Candidate oncogenes within this region include *ZNF639*,⁴⁵ *p63*,⁴⁶ *SCRO* (an RNA component of telomerase),⁴⁷ and most notably *PIK3CA*,⁴⁸ the catalytic subunit of PI3K. It is difficult to determine whether these alterations are the end product of targeted p53 and pRb deletion or drivers of malignant transformation.^{7,49} Therefore, further studies are required to address these critical distinctions.

Recent evidence also suggests HPV infection actively targets the innate immune response to evade detection. Undifferentiated keratinocytes harboring episomal copies of HPV-16 and HPV-18 demonstrate a marked downregulation of antigen-presenting mechanisms, inflammatory components, as well as antiviral, proinflammatory, and chemotactic cytokines.⁵⁰ Although the expression of virus-sensing receptors is unaffected by HPV infection, the components downstream of activated pathogen receptors are dampened. Genes associated with the interleukin-1 β pathway are strongly downregulated, linking the loss of this critical cytokine to a decrease in adaptive immunity as well. Additional studies have shown E7 proteins are also able to downregulate TLR9, facilitating evasion of innate immunity to delay adaptive immune responses.⁵¹ It has been demonstrated in cervical cancer that individuals who do not mount an effective immune response to HPV+ lesions are at risk for progression to high-grade cervical lesions and, ultimately, invasive cancer.¹⁷ Although it is not clear whether HPV+ HNC follows a similar disease-progression model, it is likely that similar immune evasion strategies are in effect.

Supporting evidence for this hypothesis can be found in HNC studies involving HIV-infected patients. HNC is the third most common head and neck malignancy in HIV patients, after Kaposi sarcoma and non-Hodgkin lymphoma.^{52,53} It is well-established that these immunocompromised patients are at an increased risk of developing virally associated cancers.⁵⁴ Thus, HPV+ HNC must be viewed as a successful subversion of a healthy immune system. In fact, the overwhelming responses to standard therapy observed in HPV+ HNC patients may potentially be immune related. A syngeneic mouse model of HPV+ and HPV– HNC was recently developed via transformation of primary mouse tonsil epithelial cells.⁵⁵ Interestingly, neither radiation nor cisplatin was an effective therapy against these HPV+ HNCs in immune-incompetent mice. Thus, perturbing the careful balance of immune evasion mediated by HPV, and unmasking these foreign viral products for clearance presents a compelling mechanism for the successful patient outcomes observed in HPV+ HNC.⁵⁶

Exploiting HPV Status as a Biomarker in OPC

The most common methods of determining HPV status in patient specimens involve detecting high-risk HPV DNA either by in situ hybridization (ISH) or polymerase chain reaction (PCR). Additionally, mRNA expression of high-risk HPV oncoproteins, such as E6 and E7 can be detected by qRT-PCR and indicates active infection. The p16 protein expression determined by immunohistochemistry (IHC) also serves as a surrogate marker for HPV infection (Fig. 4). p16 is commonly silenced in HPV– HNC by multiple mechanisms, including mutation, deletion, and hypermethylation.⁵⁷ In contrast, p16 expression is high in HPV+ OPC because of E7-mediated pRb loss of feedback control leading to a paradoxical upregulation of this potent tumor suppressor.^{6,58} However, E7 also directly activates cyclins A and E downstream of p16, which renders this upregulation inconsequential.^{59,60} Although the observed increase in p16 is theoretically nonfunctional, it serves as an ideal surrogate marker for HPV infection.

Recent studies have evaluated the prognostic significance and concordance of HPV-detection methods in an effort to provide the most accurate and standardized means of predicting patient outcome. When DNA or RNA detection methods are used, the HPV status is determined by the assay readout of presence or absence of detectable high-risk HPV DNA or RNA in the tumor cells. When IHC is used, positive p16 expression is commonly defined as strong and diffuse nuclear and cytoplasmic staining in 70% or more of the tumor cells, although the percentage cutoff can be as low as 10% of the tumor cells staining to be called p16 positive.^{4,12,61,62} A semiquantitative score based on intensity of staining has also been used, such as scoring intensity as 0 (none), 1 (weak), 2 (moderate), or 3 (strong), with 0 or 1 scores defined as negative and 2 or 3 defined as positive.¹³ An investigation of a large Canadian cohort determined E6 mRNA (qRT-PCR), HPV-16 ISH, and p16 overexpression were all significantly correlated with superior overall patient response.⁶³ Additionally, a high degree of concordance was observed between p16 IHC and HPV-16 ISH (92%) or E6 mRNA (86%). A similar study comparing p16 IHC and HPV-specific PCR detection methods demonstrated the same level of concordance (86%).¹³ Although E6 mRNA is the gold standard for detecting an active HPV infection, p16 IHC and HPV-16 ISH are technically less demanding and do not require RNA extraction from formalin-fixed paraffin blocks. Although 90%-95% of HPV+ HNC is caused by HPV-16 infection,^{64,65} an additional advantage of p16 IHC is afforded by its HPV-type independent means of determining HPV infection.

Each method has advantages and disadvantages, but they are highly accurate and correlate very well with infection and clinical outcomes if the assays are carefully performed in laboratories proficient in minimizing contamination and quality control.^{4,63} HPV status is currently used as a prognostic biomarker for OPC patients; however, a small number of recent studies suggest it could serve as a predictive biomarker for specific therapies.^{11,66} In patients receiving radiation with and without nimorazole (a hypoxia-activated prodrug), only HPV- HNC patients benefited from the addition of nimorazole. Although it was a small retrospective subset analysis, it suggests that HPV status can be used to exclude patients who would not benefit from nimorazole-based therapies.¹¹ Additionally, a larger study using an alternative hypoxia-specific prodrug (tirapazamine) demonstrated a trend toward improved locoregional control in p16-negative groups in a subset analysis; however, this difference was not statistically significant.¹³ These small, but promising, studies suggest that response could be improved in this population when patients are prospectively selected for p16-negativity concordant with hypoxic primary tumors. Furthermore, combining p16 negativity and additional selection of hypoxic tumors based on currently available imaging studies, such as positron emission tomography,^{67,68} may potentially improve the outcome in this poor prognostic group of patients. With respect to HPV positivity, another retrospective analysis suggested patients with HPV+ OPC experienced better overall survival with radiation therapy combined with an EGFR inhibitor (cetuximab or erlotinib) compared with patients treated with radiation therapy and chemotherapy (2-year overall survival, 80% vs 60%; hazard ratio: 0.15; 95% confidence interval: 0.02-0.77; $P = 0.03$).⁶⁶ Again, this retrospective study was hampered by sample size; however, it suggests that HPV status could potentially be used as a selection criteria for EGFR-targeted therapies.

Conclusions

As the tools used to catalogue the critical differences between HPV+ and HPV- OPC evolve, and the questions we are able to posit as investigational scientists become more sophisticated, the molecular Achilles heel for OPC will eventually present itself. Furthermore, as the costs associated with gene expression analysis, as well as RNA and whole-genome sequencing, continue to fall, the key molecular underpinnings of both these diseases are beginning to be resolved. The data currently suggest that, as in nasopharyngeal

carcinoma, all future clinical trials should stratify HPV+ and HPV– OPC as separate diseases and HPV infection should be included as an integral biomarker for eligibility criteria. Only under these investigational and clinical study parameters will we be able to further our understanding of HPV tumor biology, host-viral interactions, and enable the development of less toxic, HPV-specific targeted therapies.

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Figure 1. Incidence rates for overall oropharyngeal cancer (OPC), human papillomavirus (HPV)–positive OPC, and HPV-negative (HPV–) OPC between 1988 and 2004 in Hawaii, Iowa, and Los Angeles.⁹ (Reprinted with permission from 2008 American Society of Clinical Oncology. All rights reserved.) (Color version of figure is available online.)

Figure 2.

Schematic drawings of tonsillar surface.¹⁹ (A) Topography of the human palatine tonsil. The surface epithelium of the palatine tonsil deeply invaginates into lymphoid stroma with blind endings and ramifying crypts (boxed area), which increase the surface area of the tonsil by nearly 700%. Drawing by Max Brödel. (B) The specialized reticulated epithelium lining the tonsillar crypts. The squamous epithelium zones—basal, intermediate, and superficial layers—are interrupted by migrating nonepithelial cells, including lymphocytes and antigen-presenting cells. Loss of structural integrity leaves the basement membrane exposed to deposition of viral particles. Drawing by T. Phelps. (Reprinted with permission from 2009 Annual Reviews. All rights reserved.) (Color version of figure is available online.)

Figure 3.

Diagram of cellular deregulation resulting from HPV oncoproteins E6 and E7 in keratinocytes. (A) Ubiquitination by the E6/E6AP ubiquitin ligase complex leads to p53 degradation.^{28,33-35} (B) Ubiquitination by E7 and the cullin 2 ubiquitin ligase complex leading to pRb degradation.³⁶⁻³⁸ (C) Increased expression of p16 as a consequence of positive feedback because of the absence of functional pRb.³⁹ (Color version of figure is available online.)

Figure 4.

Representative cases of HPV-16–positive OPC by in situ hybridization (ISH) and p16 immunohistochemistry (IHC) staining.⁴ (A) ISH visualizing multiple punctuated loci per tumor cell nucleus reflecting multiple copies of HPV-16 genome. (B) p16 IHC staining of the corresponding tumor in panel A. (C) ISH visualizing a single-punctuated locus per tumor cell nucleus. (D) p16 IHC staining of the corresponding tumor in panel C. Panel insets demonstrate positive controls performed in all assays derived from cell lines with ~500 (CaSki, inset panel A) or 2 integrated copies of HPV16 (SiHa, inset panel C). (Color version of figure is available online.)