CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

P.T. Hennessey[1], W.H. Westra[1,2], and J.A. Califano[1*,3]

[1]Department of Otolaryngology-Head and Neck Surgery and [2]Department of Pathology, Johns Hopkins Medical Institutions, 601 N. Caroline Street, 6th Floor, Baltimore, MD 21287-0910, USA; and [3]Milton J. Dance Head and Neck Center, Greater Baltimore Medical Center, Baltimore, MD 21205, USA; *corresponding author, jcalifa@jhmi.edu

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Human Papillomavirus and Head and Neck Squamous Cell Carcinoma: Recent Evidence and Clinical Implications

ABSTRACT

Over the past 20 years, high-risk human papillomavirus (HPV) infection has been established as a risk factor for developing head and neck squamous cell carcinoma, independent of tobacco and alcohol use. In particular, HPV is strongly associated with the development of oropharyngeal cancer and a small minority of oral cavity cancers. In this review, we summarize what is currently known about the biology of HPV, the mechanisms by which it effects malignant transformation, and the potential impact of HPV status on the clinical management of persons with head and neck cancer.

KEY WORDS: HPV, oral cancer, oropharyngeal cancer.

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INTRODUCTION

In 2008, an estimated 47,500 people were diagnosed with head and neck cancer in the United States, representing approximately 3% of new cancer diagnoses, and an estimated 11,260 people died from this disease (Jemal *et al.*, 2008). The vast majority of these head and neck cancers were squamous cell carcinomas. Over the past 20 years, the overall incidence of head and neck squamous cell carcinoma (HNSCC) has been declining in the United States, a decline which has been attributed to a decrease in the prevalence of smoking (Sturgis and Cinciripini, 2007). Although there has been a reduction in the overall incidence of HNSCC, an analysis of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) data from 1975-1998 found that the incidence of tonsillar cancer increased by 2-3% annually in males under 60 yrs of age from 1975-1998 (Canto and Devesa, 2002). A more recent analysis of SEER data from 1973-2001 showed an annual increase in the incidence of oral tongue, palatine tonsil, and base-of-tongue cancers, by 2.1%, 3.9%, and 1.7%, respectively, in 20- to 44-year-old white patients, while the incidence of HNSCC at other sites declined (Shiboski *et al.*, 2005).

Tobacco and alcohol use are the primary risk factors for HNSCC and are associated with the majority of these tumors worldwide. In addition to these traditional risk factors, high-risk human papillomaviruses (HPV), and in particular HPV-16, are recognized as independent risk factors for a subset of HNSCC and are most strongly associated with oropharyngeal squamous cell carcinomas (OPSCC) (Schwartz *et al.*, 1998; Gillison *et al.*, 2000; Mork *et al.*, 2001; Wiest *et al.*, 2002; Herrero *et al.*, 2003; Hobbs *et al.*, 2006; Ernster *et al.*, 2007; Andrews *et al.*, 2008). HPV has also been associated with the pathogenesis of oral cancer; however, the association of HPV with HNSCC is strongest for oropharyngeal cancer (Gillison *et al.*, 2000; Furniss *et al.*, 2007; Sturgis and Cinciripini, 2007; Chaturvedi *et al.*, 2008; Liang *et al.*, 2008). In this review, we briefly summarize the current, generally accepted knowledge regarding the biology of HPV, and the mechanisms by which it effects malignant transformation, and subsequently focus on presenting recent research relating to the association of HPV with HNSCC, as well as on the future implications this research may have for the clinical management of persons with head and neck cancer.

HUMAN PAPILLOMAVIRUS

Human papillomavirus (HPV) is a ~ 7.9-kD, circular, non-enveloped dsDNA virus that infects squamous epithelial cells. HPV infects the basal layer of

epithelial cells through breaks in the epithelial surface and is maintained in the nuclei of infected basal cells (Stubenrauch and Laimins, 1999). As the basal cells divide into squamous epithelial cells, HPV-DNA replicates and attains a high copy number. The infection most often presents clinically as warts or papillomas of the skin and upper respiratory tract and as lesions of the uterine cervix (Bedell *et al.*, 1991; Stubenrauch and Laimins, 1999). Over 120 HPV subtypes have been identified, 33% of which are known to infect the human genital tract (Longworth and Laimins, 2004). HPV is a well-documented cause of anogenital cancers and can be divided into high- and low-risk subtypes based on the propensity to cause malignancy (Walboomers *et al.*, 1999; zur Hausen, 2000). Low-risk HPV subtypes are associated with benign warts, and high-risk subtypes are associated with dysplasia and invasive carcinomas. In cervical HPV infections, the most common low-risk HPV subtypes are HPV-6 and -11, while the most common high-risk subtypes are HPV-16, -18, -31, -33, and -45 (Munoz *et al.*, 2003). HPV-16 and -18 are responsible for the vast majority of cervical cancers, and the mechanism of tumorigenesis has been thoroughly elucidated (Munger *et al.*, 1989; Werness *et al.*, 1990; Scheffner *et al.*, 1992; McDougall, 1994; Cheng *et al.*, 1995; Scheffner and Whitaker, 2003; McLaughlin-Drubin *et al.*, 2005). Briefly, high-risk HPVs produce 2 oncoproteins, E6 and E7, which are necessary for viral replication. The HPV E6 protein binds and promotes the degradation of the tumor suppressor p53 by an ubiquitin-mediated pathway, diminishing the ability of the cell to undergo apoptosis. The HPV E7 protein binds and degrades the retinoblastoma protein (pRb), preventing it from inhibiting the transcription factor E2F, resulting in loss of cell cycle control. The E6 and E7 proteins also interact with a variety of other intracellular targets that have been recently reviewed (Munger *et al.*, 2004). Cells expressing E6 have been shown to undergo structural chromosomal changes, and cells expressing E7 accumulate numerous chromosomal abnormalities and develop aneuploidy (White *et al.*, 1994). The ultimate effect of the activity of the E6 and E7 proteins is dysregulated cell cycle progression and HPV DNA replication in infected squamous epithelial cells, and eventual oncogenesis. In the cervical cancer literature, it has been well-established that expression of viral E6 and E7 oncogenes is necessary, but not sufficient, for progression to dysplasia and, ultimately, to an HPV-associated carcinoma (Crook *et al.*, 1989; Walboomers *et al.*, 1999).

HPV-POSITIVE HNSCC TUMOR BIOLOGY

A model for the genetic and transcriptional development of head and neck cancers related to tobacco and alcohol use has been previously established (Califano *et al.*, 1996; Ha *et al.*, 2003). In HNSCC caused by these traditional risk factors, p53 is commonly mutated (Ahomadegbe *et al.*, 1995; Carlos de Vicente *et al.*, 2004), and 9p21-22 is lost early in carcinogenesis, resulting in loss of the tumor suppressor p16 (Herman *et al.*, 1995; Reed *et al.*, 1996; Worsham *et al.*, 2006; Kim *et al.*, 2007). p16 functions by associating with the cyclin 1-cyclin-dependent kinase 4/cyclin-dependent kinase 6 (CDK4/CDK6) complex to block phosphorylation of pRb, allowing pRb to control cell-cycle regulation by associating with the transcription factor E2F (Serrano *et al.*, 1993). In contrast, HPV-positive HNSCC has decreased expression of wild-type p53, due to inactivation and degradation by E6. Additionally, these tumors do not show p16 depletion, since loss of 9p21-22 does not typically occur in these tumors. Instead, loss of cell-cycle regulation occurs when HPV E7 inactivates host cell pRb, preventing regulation of E2F (Li *et al.*, 1994; Hara *et al.*, 1996; Begum *et al.*, 2005; Licitra *et al.*, 2006; Perrone *et al.*, 2006; Ragin *et al.*, 2006). This represents a distinct molecular phenotype and a unique mechanism of tumorigenesis independent of the mutagenic effects of tobacco and alcohol.

In addition to having a distinct etiology, HPV-positive HNSCCs are histologically distinct from HPV-negative tumors. In contrast to the non-HPV-related HNSCC, which are usually moderately differentiated and keratinizing, HPV-associated HNSCC are consistently poorly differentiated and non-keratinizing, and have a distinct 'basaloid' appearance (Wilczynski *et al.*, 1998; Gillison *et al.*, 2000; El-Mofty and Lu, 2003) (Fig. 1). These tumors also differ at the genetic level. A large proportion of HPV-positive HNSCCs have HPV DNA integrated into the host cell genome (Gillison *et al.*, 2000; Wiest *et al.*, 2002; Hafkamp *et al.*, 2003; Kim *et al.*, 2007). HPV-positive HNSCCs demonstrate significantly lower levels of chromosomal mutations and loss than do HPV-negative tumors (Braakhuis *et al.*, 2004; Smeets *et al.*, 2006).

RISK FACTORS FOR HPV-POSITIVE HNSCC

As well as having distinct molecular characteristics, HPV-positive HNSCCs are associated with a risk factor profile that differs from the profile associated with non-HPV-associated HNSCC. Unlike HPV-negative HNSCC, the major risk factors for HPV-positive HNSCC are not tobacco or alcohol, but are instead related to sexual history (Kreimer *et al.*, 2004; Gillison *et al.*, 2008). The risk for HPV-positive HNSCC increases with increasing numbers of both oral and vaginal sexual partners, a history of genital warts, and a younger age at first intercourse (Schwartz *et al.*, 1998; Kreimer *et al.*, 2004). In a recent analysis of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) data from 1973-2002, a diagnosis of cervical cancer was found to confer an increased risk for development of a second primary oropharyngeal cancer (oropharynx SIR = 2.7; tonsil SIR = 3.1), without increasing the risk of developing oral cavity or other cancers (Rose Ragin and Taioli, 2008). Additionally, an increased risk of tonsillar cancer has been observed in the husbands of women with documented history of cervical dysplasia or cancer (Hemminki *et al.*, 2000). Further evidence of the association of HPV-positive cancer with sexual behavior comes from a recent case-control study of 100 persons with oropharyngeal cancer and 200 control individuals, which found that development of oropharyngeal cancer is associated with a high lifetime number of vaginal sex partners (≥ 26) and with a high lifetime number of oral sex partners (≥ 6) (odds ratios 3.1 and 3.4, respectively) (D'Souza *et al.*, 2007). Most recently, a case-control study of 240 individuals with oropharyngeal cancer, 92 of whom had HPV-16-positive HNSCC, found that the risk of developing an HPV-16-positive HNSCC increased with increasing numbers of oral sex partners, as well as with increased marijuana use (Gillison *et al.*, 2008). Neither of these studies found an association between tobacco or alcohol use and the development of HPV-positive HNSCC (D'Souza *et al.*, 2007; Gillison *et al.*, 2008).

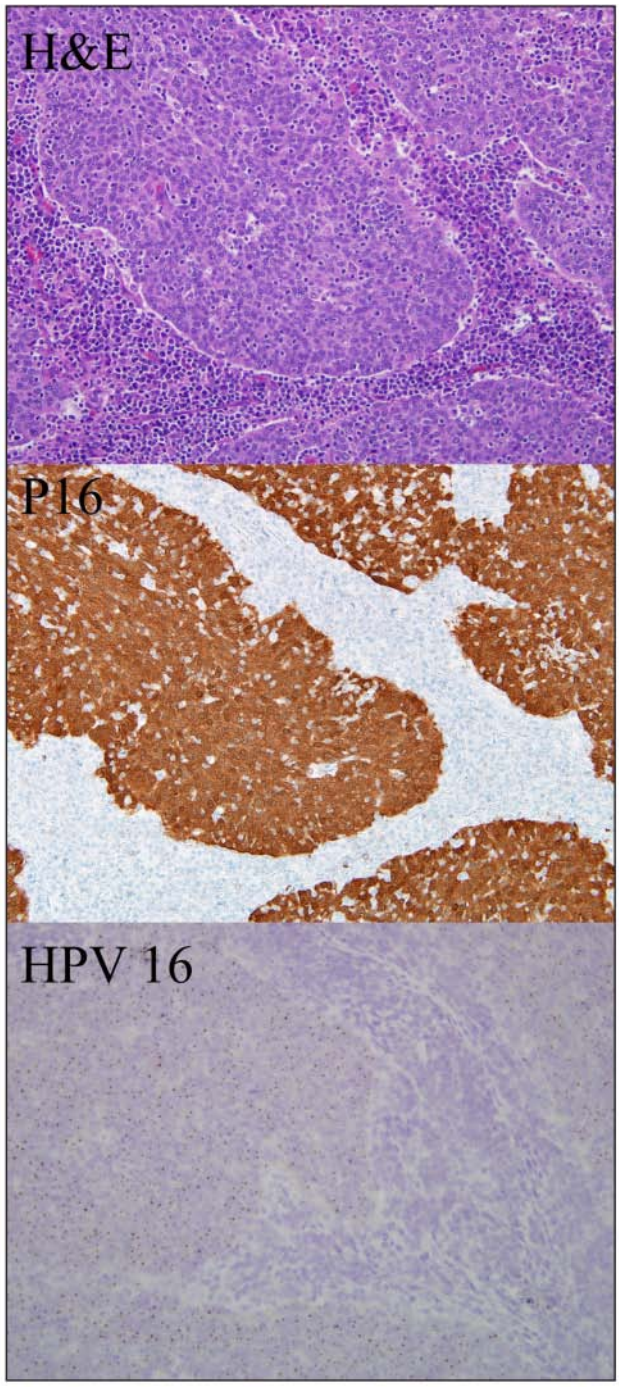

Figure 1. Oropharyngeal squamous cell carcinoma evaluated by routine hematoxylin and eosin staining (box 1), p16 immunostaining (box 2), and HPV-16 *in situ* hybridization (box 3). Tumor islands demonstrate characteristic basaloid appearance and lymphocytic infiltration.

ASSOCIATION OF HPV WITH HNSCC

As previously mentioned, the association between HNSCC and HPV is strongest for OPSCC, specifically for cancers of the palatine and lingual tonsils (Schwartz *et al.*, 1998; Gillison *et al.*, 2000; Mork *et al.*, 2001; Wiest *et al.*, 2002; Herrero *et al.*, 2003; Ernster *et al.*, 2007). A recent meta-analysis of 17 studies found that HPV is most strongly associated with tonsillar cancer (OR 15.1, 95% CI 6.8-33.7), is intermediate for oropharyngeal cancer in general (OR 4.3, 95% CI 2.1-8.9), and is weakest for oral cancer (OR 2.0, 95% CI 1.0-4.2) (Hobbs *et al.*, 2006). Additionally, oral infection with high-risk HPV has been associated with a dramatically increased risk of developing oropharyngeal cancer when adjusted for alcohol and tobacco use (OR 230; 95% CI 44-1200) (Hansson *et al.*, 2005). A recent study by the International Agency for Research on Cancer (IARC) found HPV in 3.9% (95% CI, 2.5-5.3) of oral cavity tumors and 18.3% of OPSCC (Herrero *et al.*, 2003).

HPV-16 seropositivity, as an indicator of HPV exposure, is associated with an increased risk of developing HPV-positive oropharyngeal cancers, as well as a weaker association with oral cancer (Herrero *et al.*, 2003; Furniss *et al.*, 2007). In a landmark paper, researchers performed a nested case-control study using serum samples collected from 900,000 people in Norway, Finland, and Sweden (Mork *et al.*, 2001). From this registry, the study identified 292 individuals diagnosed with HNSCC and compared them with 1568 matched control individuals. After adjustment for smoking, the overall adjusted odds ratio for development of a HNSCC at any site in the setting of HPV-16 seropositivity was modest (OR = 2.1, 95% CI =1.4-3.2). When analyzed for specific subsites in the head and neck, however, HPV-16 seropositivity was most strongly associated with increased risk of oropharyngeal cancer (OR = 14.4; 95% CI = 3.6-58.1) and was more weakly associated with risk of developing a cancer of the oral cavity (OR 3.6, 95% CI = 0.5-26.3) (Mork *et al.*, 2001).

A recent case-control study of persons diagnosed with OPSCC found that HPV-16 seropositivity was strongly associated with OPSCC in people without a history of smoking or drinking (OR = 33.6; 95% CI = 3.3-84.8), as well as in individuals with a history of drinking and smoking (OR = 19.4; 95% CI = 13.3-113.9) (D'Souza *et al.*, 2007).

Simply detecting HPV in tumor cells or detecting serum markers for HPV infection, however, does not demonstrate that HPV is involved in the pathogenesis of a given tumor (Snijders *et al.*, 2003). As we described earlier in this review, the cervical cancer literature has established that transcriptionally active HPV is necessary for HPV-associated oncogenesis. This same principle holds true for HPV-associated HNSCC. Markers for transcriptionally active HPV include p16 over-expression, and expression of E6 and E7 proteins (Wiest *et al.*, 2002; Weinberger *et al.*, 2006). HPV has been identified in 0-100% of oral cavity pre-malignant lesions (Chang *et al.*, 1991; Holladay and Gerald, 1993; Fouret *et al.*, 1995; Franceschi *et al.*, 1996; Bouda *et al.*, 2000; Sand *et al.*, 2000; Mork *et al.*, 2001; Ha *et al.*, 2002), as well as in 0-100% of oral cancers (Greer *et al.*, 1990; Mao *et al.*, 1996; Matzow *et al.*, 1998; Miguel *et al.*, 1998; Bouda *et al.*, 2000); however, the majority of studies relied on PCR for detection and did not quantitate HPV viral load or identify markers of HPV transcriptional activity. PCR is a highly sensitive technique that can amplify exceedingly small fragments of HPV DNA, resulting in detection of non-pathologic HPV infections or sample contamination. A variety of other techniques has been used to detect HPV, including Southern blot, quantitative PCR,

and *in situ* hybridization. A meta-analysis of studies using different techniques of HPV detection found higher rates of tumor HPV positivity in studies that relied on non-quantitative PCR for HPV detection, compared with other methods, implying a higher rate of false-positive results (Miller and White, 1996). Recent studies utilizing quantitative PCR and/or *in situ* hybridization have found HPV in only a small proportion of oral cavity squamous cell cancers, while consistently detecting HPV in 40-60% of oropharyngeal tumors (Schwartz *et al.*, 1998; Gillison *et al.*, 2000; Klussmann *et al.*, 2003; Ritchie *et al.*, 2003; Syrjänen, 2005; Kim *et al.*, 2007; Fakhry *et al.*, 2008).

PROGNOSIS OF HPV-POSITIVE HNSCC

In addition to having distinct histopathologic and molecular characteristics, HPV-positive HNSCC carries a better prognosis than HPV-negative HNSCC. Multiple studies have shown that persons with HPV-positive oropharyngeal cancer are more responsive to treatment and have better rates of disease-specific survival than those with HPV-negative oropharyngeal cancer (Gillison *et al.*, 2000; Friesland *et al.*, 2001; Sisk *et al.*, 2002; Li *et al.*, 2003; Ritchie *et al.*, 2003; Licitra *et al.*, 2006; Weinberger *et al.*, 2006; Kumar *et al.*, 2007, 2008).

The molecular markers that differentiate HPV-positive tumors from HPV-negative tumors have been shown to correlate with tumor prognosis. An association between improved survival and transcriptionally active HPV infection exists and has been demonstrated by elevated p16 levels in HPV-infected tumor cells (Klussmann *et al.*, 2003; Licitra *et al.*, 2006; Reimers *et al.*, 2007). Recently, p16 over-expression in OPSCC, as a marker for transcriptionally active HPV, has been associated with 79% 5-year survival, compared with 20% 5-year survival in individuals with HPV-negative tumors, and 18% 5-year survival in persons with HPV-16-positive tumors with normal levels of p16 expression ($P = 0.0095$) (Weinberger *et al.*, 2006). Additionally, a prospective study of 66 individuals with stage III and IV OPSCC study found that tumor p16 over-expression was significantly associated with overall survival ($p = 0.001$) and disease-specific survival ($P = 0.003$) (Kumar *et al.*, 2008). Another recent study demonstrated similarly improved 5-year survival rates in individuals with HPV-positive tonsillar cancers compared with those with HPV-negative tumors (71% *vs*. 36%; $p = 0.023$) (Charfi *et al.*, 2008).

A recent multi-center prospective trial evaluating treatment response and survival in 96 persons with oropharyngeal or laryngeal carcinoma demonstrated that those with HPV-positive oropharyngeal tumors had higher response rates to induction chemotherapy and to chemoradiation therapy than those with HPV-negative tumors (Fakhry *et al.*, 2008). In this study, the overall two-year survival rate for persons with HPV-positive tumors was 95% (95% CI = 87%-100%), compared with a two-year survival rate of 62% (95% CI = 49%-74%) for persons with HPV-negative tumors (Fakhry *et al.*, 2008). Additionally, the study found that individuals with HPV-positive tumors had a better response to induction chemotherapy than those with HPV-negative tumors.

The mechanism of improved survival in HPV-associated HNSCC is still unclear. This survival benefit has been attributed to an enhanced radiosensitivity of HPV-positive tumors (Lindel *et al.*, 2001), an improved apoptotic response secondary to the presence of unmutated p53 in HPV-associated tumors (Butz *et al.*, 1996), and a reduced risk of developing a second primary tumor, since these individuals tend not to have a history of smoking and drinking and therefore lack the field cancerization characteristic of persons with tobacco- and alcohol-related HNSCC (Gillison *et al.*, 2000).

CLINICAL IMPLICATIONS

Although HPV-associated HNSCC has a relatively good disease-free survival rate, a subset of persons develops recurrence of their cancer after treatment and dies from their disease. For these individuals, a screening test that takes advantage of the unique markers associated with HPV infection could be beneficial for the detection of disease persistence or of early disease recurrence. A recent study nested within a longitudinal cohort study of 59 persons with histologically confirmed HNSCC evaluated salivary rinsing as a possible screening test for recurrence of HPV-positive tumors after treatment. In this cohort, detection of HPV E6 and E7 copy number by RT-PCR from salivary rinses had a specificity of 50% and sensitivity of 100% for detection of oropharyngeal cancer recurrence (Chuang *et al.*, 2008). This study, however, encompassed a small number of persons, and a larger study is warranted to determine the value of salivary rinsing as a screening test for tumor recurrence.

Another potentially useful clinical application is in the identification of the site of primary tumor in HNSCC presenting as a neck mass without a known primary site of origin. Neck mass of unknown primary origin constitutes 1-5% of HNSCC diagnoses, and even after complete clinical, endoscopic, and radiographic evaluation, the primary site of origin may not be identified (Schmalbach and Miller, 2007; Wong, 2008). Markers of HPV-associated tumors have been utilized in recent studies addressing this problem. Evaluation of fine-needle aspiration biopsies of neck metastases by *in situ* hybridization for HPV (Begum *et al.*, 2007; Zhang *et al.*, 2008) and by immunohistochemistry for p16 overexpression (Begum *et al.*, 2007) have been shown to be predictive of an oropharyngeal primary tumor (Fig. 2). Additionally, a recent study demonstrated that the finding of cystic lymph nodes correlates strongly with HPV positivity by *in situ* hybridization (Goldenberg *et al*., 2008). These studies demonstrate that detection of HPV in tumor tissue can help localize the primary site of tumor origin for individuals who present with regional metastasis.

Finally, the introduction of the HPV vaccine Gardasil® (targeted against HPV-16, -18, -6, and -11) in June of 2006, and the impending FDA approval of Cervarix® (targeted against HPV-16 and -18), if widely used, could slow or even reverse the trend of increasing prevalence of OPSCC seen in the United States over the past 30 years. These vaccines are highly effective at preventing cervical intra-epithelial neoplasia and cervical cancer (Harper *et al.*, 2004; Villa, 2006; Paavonen *et al.*, 2007), but it remains to be seen how widely they will be used and if their use has an effect on the incidence of HNSCC.

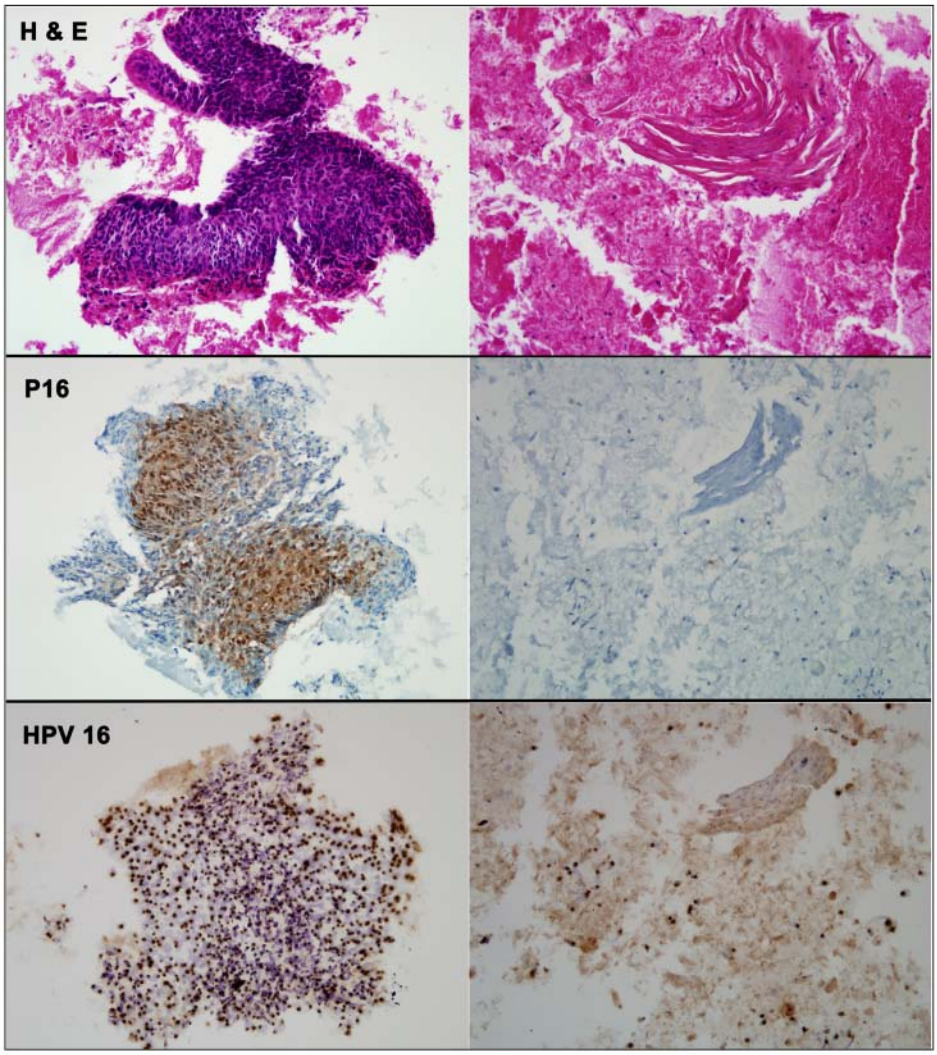

Figure 2. Fine-needle aspirate of a metastatic squamous cell carcinoma evaluated by routine hematoxylin and eosin staining (**row 1**), p16 immunostaining (**row 2**), and HPV *in situ* hybridization (**row 3**). A fragment of viable tumor (**left column**) is strongly p16-positive and HPV16-positive. In areas of cellular degeneration (**right column**), the tumor cells lose their p16 immunoreactivity, but retain their HPV16 hybridization signals. (Previously published in Begum *et al.,* 2007. Used with permission.)

CONCLUSION

Our understanding of the association between HPV infection and HNSCC has been evolving for the past 20 years. Although initial data supported a strong association between HPV infection and both oral cavity and oropharyngeal cancer, it has become clear that, in the head and neck, HPV infection is predominantly associated with carcinogenesis in the oropharynx.

The knowledge gained about the biology of HPV-positive HNSCC is now beginning to be translated clinically and is being used to give patients prognoses, to direct treatment, and to guide follow-up. Further research has the potential to provide reliable screening tests for detection of tumor recurrence in persons treated for HPV-positive HNSCC, and the potential exists for the development of a screening test similar to the Papanicolaou test, which could be used to screen individuals at high risk for developing HPV-associated HNSCC.

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