# Human Papillomavirus Infection and Oropharyngeal Cancer

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## Introduction

Squamous cell carcinoma of the head and neck (SCCHN), which affects close to 40,000 individuals each year in the United States, typically involves the oral cavity, oropharynx, hypopharynx, and larynx. Smoking tobacco and alcohol abuse are the major risk factors, and treatment is multidisciplinary, involving the use of chemotherapy, radiation, and surgery. Patients typically present with a sore throat, ear pain, odynophagia, or hoarseness, and on physical exam they often have lymph node involvement in the neck. Many patients with oropharyngeal cancer have no common risk factors, and recent epidemiologic and molecular studies have identified high-risk types of human papillomavirus (HPV), especially HPV-16, as the potential etiologic agents.[1,2] Indeed, the connection between HPV and SCCHN has gained increased attention recently. A number of issues associated with HPV-related SCCHN cancers, including epidemiology, prognostic value, and therapy, were discussed at the 2007 meeting of the American Society of Clinical Oncology (ASCO).

## HPV and SCCHN: Pathogenesis

HPV infection is known to be a necessary element for the development of cervical cancer in women,[3] and is also a risk factor for the development of anal, penile, and vulvar cancers.[4-6] The site that is mostly associated with HPV infection in the head and neck area is the oropharynx, particularly the tonsils and tongue base.[7] It is not clear why the oropharynx is more susceptible to HPV transformation, although its similarity to the uterine cervix in terms of easy access for infection and the same embryonic development from endoderm have been suggested. The tonsils also contain deep invagination of the mucosal surface, believed to favor the capture and processing of antigens, which may facilitate viral access to basal cells. The prognostic significance of HPV infection is a subject of intense debate, with emerging data suggesting a better prognosis for those patients with HPV-related SCCHN than for those with unrelated SCCHN.

The mechanism of HPV carcinogenesis was first characterized in cervical

cancer, where more than 90% of cases are related to HPV infection, mostly types 16 and 18.[3] Two viral genes are mainly responsible for HPV-related malignant transformation. The HPV E6 and E7 oncogenes, which encode proteins consisting of 151 and 98 amino acids, respectively, are largely responsible for the onset and persistence of the malignant process in both ano-genital and head and neck cancers. E6 and E7 are best known for their ability to bind and inactivate the tumor suppressors p53 and pRb, and these respective properties have been associated with their oncogenic potential. The E6 protein contains zinc-binding motif and can form a complex with the p53 tumor suppressor protein of the host cell, inducing p53 degradation. The E7 protein forms complexes with retinoblastoma gene family proteins, which are negative regulators of cell growth. This results in the release of the E2F transcription factors in the cell. The free E2F activates the expression of several host genes involved in the cell cycle progression, and the E6/E7-inactivated p53 and pRb-related proteins permit the cell to escape normal check points, with subsequent loss of DNA replication. The simultaneous effects of loss of both p53 and pRb function may lead to the malignant transformation of epithelial cells.

This absence of genetic or epigenetic alterations in the p53 and pRb pathways in HPV-positive SCCHN is in sharp contrast to what is observed in HPV-negative disease. Typically, p53 mutations are frequently observed in the HPV-negative squamous cell carcinomas. By contrast, HPV-positive carcinomas usually do not contain any p53 mutations, and occur predominantly in patients with no excessive tobacco and/or alcohol consumption history. These distinctions imply that HPV-positive and HPV-negative carcinomas of the head and neck represent distinct entities. Moreover, it has been suggested that the prognosis of patients with HPV-positive tumors is better than that of patients with a smoking related, HPV-negative tumor.

The association between HPV infection and other tumor suppressor genes, such as p16, is also of interest. The p16 protein functions as a tumor suppressor by binding to the cyclin D1 CDK4/CDK6 complex, preventing phosphorylation of the Rb protein. Overexpression of p16 protein has been reported repeatedly in HPV-associated cancers, and in a recently published study,[8] only the HPV-positive/p16-expressing tumors -- close to 25% of tumors analyzed -- were the ones associated with favorable prognosis.

It is well established that the transmission of genital HPV infections is associated with sexual contact and that its prevalence increases among

individuals with multiple sexual partners. The mode of transmission of HPV to the oral cavity is less understood and less defined at this stage, but sexual behavior and practices represent possible modes of transmission.[9]

## HPV-Related Findings From ASCO 2007

Fakhry and colleagues[10] reported the effect of tumor HPV status on treatment response and survival outcomes. This prospective evaluation was performed as part of a multicenter, phase 2 clinical trial conducted by the Eastern Cooperative Oncology Group (ECOG). The clinical results had already been reported, and the purpose of the presentation at ASCO 2007 was to focus on the prognostic value of HPV infection. Patients with newly diagnosed, locally advanced SCCHN of the oropharynx or larynx were uniformly treated with 2 cycles of induction chemotherapy (paclitaxel 175 $mg/m^2$ and carboplatin AUC 6) followed by chemoradiation (weekly paclitaxel 30 $mg/m^2$ administered concurrently with standard fractionated external beam radiation therapy, total dose of 70 Gy in 35 fractions over 7 weeks). Tumor HPV status was determined by in situ hybridization on formalin-fixed, paraffin-embedded tumors. The independent effect of tumor HPV status on survival was evaluated by Cox proportional hazards models.

The authors found that genomic DNA of oncogenic HPV type 16, 33, or 35 was specifically located within tumor cell nuclei of 61% of oropharyngeal cancer patients. All patients with laryngeal cancer were HPV negative, confirming earlier reports that HPV infection is more of an issue in the oropharynx than in the oral cavity or larynx. Factors associated with HPV-positive tumors in univariate analysis included white race ($P = .02$), improved ECOG performance status ($P = .01$), oropharyngeal primary ($P < .001$), early tumor stage ($P = .02$), and basaloid histology. HPV-positive patients had a higher response rate to induction chemotherapy and to overall protocol therapy. After a median follow-up time of 39.1 months, patients with HPV-positive tumors had a risk of progression that was 72% lower (hazard ratio [HR] = 0.28, 95% confidence interval [CI]: 0.07-1.0) and a risk of death that was 79% lower (HR=0.21, 95% CI: 0.06-0.74) than patients with HPV-negative tumors. The authors concluded that the improved prognosis of HPV-positive SCCHN has been confirmed in a prospective clinical trial, and may be explained in part by enhanced sensitivity to chemotherapy and radiation.

Chaturvedi and his group[11] examined the differences between HPV-related and HPV-unrelated oral squamous cell carcinoma. They did not assess the HPV status of tumors but instead classified them according to

anatomic sites. They used data from the SEER9 program registries to investigate the potential influence of HPV infection on 3 parameters: (1) patient characteristics; (2) incidence trends between 1973- 2003; and (3) overall survival.

Cases of SCCHN (N = 58,158) were classified by anatomic site as potentially HPV related (base of tongue, tonsil, oropharynx; n = 16,712) or HPV-unrelated (lip, tongue, gum, floor of mouth, palate, other mouth, hypopharynx, ill-defined sites of lip, oral cavity, or pharynx; n = 41,446). HPV-related SCCHN was associated with the following patient characteristics:

0. a higher proportion among men;
0. a more advanced initial presentation; and
0. a younger age.

Mean age at diagnosis for HPV-related SCCHN was 61.1 years, compared with 64.5 years for HPV-unrelated ($P < .001$). Incidence trends showed a significant increase in HPV-related SCCHN among white males aged 40-59 years and a decrease in the incidence of HPV-unrelated SCCHN among all races/sexes in patients older than 40 years of age. Improvements in overall survival were observed for HPV-related (all stages) and HPV-unrelated (regional and distant) SCCHN treated by radiotherapy from 1973-2003, but were more marked for HPV-related disease. The absolute increase in 2-year overall survival for regional disease in HPV-related tumors was 24.4% vs 5.8% for HPV-unrelated tumors. The findings from this analysis indicate that the proportion of SCCHN that is potentially HPV-related increased in the United States from 1973-2003, particularly among young males, perhaps due to changing sexual and smoking behaviors.

Rampias and his group[12] looked at retrovirus-mediated delivery of short hairpin RNA targeting HPV16 E6 and E7 oncogenes and its potential to induce apoptosis in oropharyngeal squamous cell cancer cell lines. In their model, small hairpin RNAs targeting E6 or E7 genes were delivered by a retrovirus vector to 93VU147T (bearing integrated HPV16 DNA) and 92VU040T (HPV-negative) oropharyngeal cancer cell lines. Flow cytometry analysis was used to assess apoptosis after the onset of retrovirus infection. The E6 and E7 mRNA downregulation was assessed by reverse transcriptase polymerase chain reaction (RT-PCR). The authors noted an apoptosis rate of over 90% in HPV-positive cell lines 48 hours after infection. By contrast, HPV-negative cells were not affected. RT-PCR demonstrated that HPV16 E6/E7 mRNA levels decreased significantly in HPV-positive cells. The authors concluded that downregulation of E6/E7

gene expression in HPV16-positive oropharyngeal cancer cells results in apoptosis and reactivation of p53 and Rb tumor suppression pathways. These results have significant implications for the treatment of HPV-associated oropharyngeal cancer with HPV-targeted gene therapy.

Saraiya and colleagues[13] presented an epidemiologic study looking at squamous cell cancers of the base of tongue, oropharynx, and tonsil in the US population on the basis of age, gender, race/ethnicity, stage, US region, and year of diagnosis. They used data from the Centers for Disease Control and Prevention's (CDC) National Program of Cancer Registries and/or the National Cancer Institute's (NCI) SEER Program for cases diagnosed from 1998-2003, covering 83% of the US population. Incidence rates during the period from 1998-2003 were: base of tongue (1.16 per 100,000); oropharyngeal (0.90 per 100,000); and tonsillar (1.35 per 100,000). When the study population was assessed by race, blacks were found to have the highest incidence of base of tongue, oropharyngeal, and tonsillar cancers (1.25, 1.61, and 1.47 per 100,000, respectively). Whites, Asian/Pacific Islanders, American Indians/Alaska Natives, and Hispanics had the highest incidence rates for tonsillar cancer (1.37, 0.49, 0.85, and 0.89, respectively), while oropharyngeal cancer occurred most often among blacks (1.47). Geographic assessment showed that the US South had the highest incidence of base of tongue, oropharyngeal, and tonsillar cancers (1.24, 1.06, and 1.52 per 100,000, respectively). Annual incidence of tumors of both base of tongue and tonsils increased significantly (2.68% and 2.96%, respectively). The authors concluded that increasing incidence rates among head and neck cancers that are associated with HPV, including those affecting the tonsils and base of tongue, indicate that the HPV vaccine may have a significant impact on these cancers.

## Discussion and Future Directions

Recent studies have clearly established HPV as a definitive risk for oropharyngeal cancer,[14] and HPV-related oropharyngeal cancer is now a well-defined entity with well-known characteristics that include young age, good performance status, male gender, nonsmoking and nondrinking status, basaloid tumor histology, and high-risk sexual behavior. The prognosis for these patients is believed to be better than that for patients who have the "traditional" type of oropharyngeal cancer.

There have been scattered reports over the years suggesting that HPV infection affects prognosis, but the Fakhry[10] presentation represented the first prospective look at prognosis for these patients as part of an

intergroup study. The results reported are quite significant and the implications are important: HPV-related oropharyngeal cancer behaved in a different fashion, had a different response to therapy, was more sensitive to radiation-based therapies, and thus may require a different therapeutic approach compared with HPV-unrelated oropharyngeal cancer. Future studies will be needed to extend and validate these results, and it will be of paramount importance to start using HPV infection as a measure of stratification in head and neck cancer clinical trials.

Another important aspect of these findings is the potential role of HPV vaccination in the prevention of these cancers going forward. Oropharyngeal cancer can now be placed in the category of "virally mediated cancers," along with the HPV-related cervical, anal, vulvar, and penile cancers and the Epstein Barr virus-associated nasopharyngeal cancer and lymphomas. As such, the issue of HPV vaccination[15] will need to be revisited, and future studies will have to take into account all of the HPV-related cancers, not only cervical cancer. Men will potentially need to be vaccinated as well. Indeed, the implications are quite significant from the public health perspective.

This year's ASCO presentations by Chaturvedi and Saraiya[11,13] provided more evidence to support our growing sense that the incidence of tonsillar cancer is rising. A rise in incidence is worrisome, and tonsillar cancer is one tumor on a very small list of cancers with such an increase. It is suspected that high-risk sexual behavior may account for the increase in HPV infection and, ultimately, tonsillar cancer. Since the onset of the HIV epidemic, we have seen an increase in oral sex among teenagers and young adults, probably because this is thought to represent a form of "safe sex" and a worry-free behavior. We now know that this is not the case: Oral sex is not risk free and can result in HPV-related cancer. Public education is of paramount importance. There is a need to disseminate these findings, placing them in context.

In terms of HPV therapeutics, a major focus of the research community has been E6 and E7 targeting. Indeed, an agent that could target these 2 oncogenes would be ideal. Conceptually, this approach is similar to targeted therapies recently developed for chronic myeloid leukemias (bcr-abl targeting) and lung cancer (epidermal growth factor receptor mutations). Research in this area is still preliminary, and previous experience has proven this field to be quite challenging. Nevertheless, newer technologies might make this possible, and the approach described by Rampias and colleagues[12] is promising. If targeting proves feasible,

these findings will have represented a major breakthrough. The ability to treat these cancers without chemotherapy and radiation, but instead with gene therapy and antiviral therapy, is certainly appealing, but we expect that it will be a long time before we see this work come to fruition. In the meantime, our focus should be on fuller utilization of the HPV vaccine and public education measures.

## Conclusion

HPV-related head and neck cancer represents a new entity that is now well defined. The practicing oncologist needs to be aware of these new findings, and HPV testing, with PCR or FISH, should now be performed routinely. For now, results will have significant prognostic though not therapeutic implications. Still, changes come rapidly in this field, and we expect that different treatments will be available to these patients in the near future.