Human papillomavirus in head and neck cancers: biology, prognosis, hope of treatment, and vaccines

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Human papillomaviruses (HPVs), especially type 16, are implicated in the development of a subset of head and neck squamous cell cancers (HNSCCs). This subset of oropharyngeal cancers possesses distinct clinical and laboratory features and outcome, and is particularly common in individuals who lack the traditional risk factors of tobacco and alcohol abuse. Moreover, the annual incidence of HPV-related HNSCCs has increased in the USA and Europe in the last few years. As HPV-associated HNSCCs share a better prognosis compared with stage-matched HPV-negative ones, selected patients could be spared the intensive and toxic treatment and be oriented to organ preservation strategies. Preventive HPV vaccines have already been designed against cervical cancer, and a further understanding of HPV-associated carcinogenesis could potentially lead to the development of HPV-targeted therapeutic strategies.

This study summarizes the current knowledge regarding the epidemiology, biology, malignant transformation mechanisms, and prognosis of HPV-associated HNSCCs, and underlines the clinical implications of related treatments and prophylactic strategies. *Anti-Cancer Drugs* 22:586–590 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Human papillomavirus (HPV) is nearly ubiquitously present in humans, but ultimately only a small proportion of infected individuals develop cancer. Epidemiological and molecular data suggest that, besides cervical cancer, which is the most widely accepted HPV-associated malignancy, HPVs, especially type 16, are implicated in the development of a subset of head and neck squamous cell cancers (HNSCCs). This subset of oropharyngeal cancers possesses distinct clinical and laboratory features and outcome, and is particularly common in individuals who lack the traditional risk factors of tobacco and alcohol abuse. This study summarizes the current knowledge regarding the epidemiology, biology, malignant transformation mechanisms, and prognosis of HPV-associated HNSCCs and underlines the clinical implications of related treatments and prophylactic strategies.

Epidemiology

According to Surveillance, Epidemiology and End Results data, the annual incidence of base-of-tongue and tonsil cancers in the USA has increased by 2.1 and 3.9%, respectively, from 1973 to 2001 among White individuals aged 20–44 years, whereas the incidence at other sites has declined [1,2]. An increase in the incidence of tonsillar cancer by 2–3% per year among African–American and White men younger than 60 years through 1998 has also been noted [1–3]. This change in demographics may be explained by the increased incidence of sexual behaviors associated with viral transmission, as is indirectly shown by the 30% increase in herpes-simplex 2 seroprevalence over this time period especially among younger individuals [4]. An analysis conducted on 29 265 adult patients with cancer with HNSCC from 10 European countries (Sweden, Austria, Slovenia, Scotland, Wales, Poland, Germany, The Netherlands, Switzerland, and Italy) in the period from 1988 to 2002 was reported by Licitra *et al.* [5]. The age-standardized incidence of HPV-unrelated cancer sites was higher than that of HPV-related cancer cases (3.8 vs. 2.5/100 000 year). HNSCCs were increased in HPV-related cancer sites and the 3-year survival outcomes were better in those anatomical sites and among women.

As indicated in several studies, oral HPV infection is sexually transmitted and certain types of sexual behavior augment the risk for oral squamous cell cancers (OSCCs) [6,7]. The risk is increased in men and women with multiple sexual partners and in men with a history of anogenital warts and young age of onset of sexual activity [8–11]. Individuals with a history of HPV-associated anogenital cancers and husbands of women with in-situ and invasive cervical cancer are all prone to develop HPV-associated OSCC [1,9,12]. Immunocompromised individuals such as posttransplant patients and HIV-infected men are also at increased risk of developing HPV-associated HNSCCs [13]. Patients with Fanconi anemia also share an increased risk of HPV-associated OSCC because of their inherited genetic instability [6]. It is noteworthy that patients with HPV-associated OSCC are younger, nonsmokers, and nonalcohol users [14].

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Markers of HPV infection have also been associated with increased risk of OSCC. A Swedish study [15] showed that oral infection with high-risk HPVs significantly increased the risk of developing oropharyngeal cancer [odds ratio: 230; 95% confidence interval (CI): 44–1200], after adjustment for alcohol and tobacco intake. A nested case–control study from Norway [16] showed that HPV16 seropositivity conferred a greater than 14-fold increased risk of oropharyngeal cancer. Oral HPV infection and HPV-16 L1 seropositivity were linked to OSCC among patients with or without a history of heavy tobacco and alcohol use in the study by D'Souza *et al.* [6].

Biology

HPVs are double-stranded DNA viruses consisting of a circular virion that is enclosed in a small capsid. Two main regions in the HPV DNA possess a dominant role during HPV infection and replication, namely the early (E) and late (L) open reading frames. The early coding regions (E1–E8) encode for the production of enzymes that control the expression, replication, and in the case of oncogenic viruses, transformation of the host cell. The late coding regions encode the production of structural proteins.

HPV-driven malignant transformation in cervical cancer has been studied extensively, as it is composed of the most extensively studied HPV-associated malignancy. The molecular events in HPV-induced carcinogenesis lead to functional abrogation of p53 and pRb pathways that is mediated through the expression of viral oncoproteins. The HPV DNA is usually integrated into the host cell genome and results in the disruption of expression of the main viral transcription/replication factor E2, the transcriptional repressor of E6 and E7 oncogenes [17]. Consequently, the E6 and E7 oncogenes remain in a continuously expressed state. The E6 and E7 genes of oncogenic HPVs encode oncoproteins that bind with a high affinity to the p53 and retinoblastoma (Rb) tumor suppressor proteins, respectively, inducing their degradation. p53 and Rb genes are wild type in the majority of cervical carcinomas. Thus, the p53 and pRB tumor suppressor pathways are active but dormant in these cells because of the continuous expression of E6 and E7 genes [18]. However, in tonsillar carcinomas, transcription of HPV-16 E6/E7 DNA is not necessarily dependent on viral DNA integration and the virus is predominately present in episomal form [19]. How the virus remains in cancer tissues as episomes with high copy numbers is not fully understood. A study by Van Tine *et al.* [20] showed that the HPV E2 protein may serve as an 'anchor' to bind episomal HPV to cellular mitotic spindles. Introduction of bovine papillomavirus E2 gene into HeLa cervical carcinoma cells through an SV40-based viral vector represses transcription of the HPV18 E6 and E7 oncogenes and induces cells to undergo senescence [18]. Antisense strategies targeting E6 and E7 viral oncogenes in cervical carcinoma cell lines typically yield a several-fold inhibition of proliferation [21].

Establishing the link between HPV and a subset of OSCC has been quite difficult. Since 1985, the first time that HPV16 DNA was detected in an invasive OSCC using Southern blot hybridization [22], HPV DNA has been repeatedly found in a wide range of HNSCCs, from less than 10% to up to 100%, probably depending on the anatomic site of tumors, HPV detection techniques and the population studied. In HPV-associated HNSCCs, E6 binds wild-type p53 and induces its degradation, leading to impaired apoptosis. The product of E7 binds pRb, causing the release of the transcriptional factor E2F that activates cellular proliferation. pRb is a negative regulator of p16 protein at the transcriptional level [23]. Therefore, low pRb levels lead to subsequent p16 upregulation. Consequently, HPV-associated cancers bear high p16 levels, low pRb and cyclin D1 protein levels, and wild-type p53 and pRb genes. Tobacco/alcohol-associated HNSCCs are associated with downregulation of the p16 protein, p53 gene mutation, and overexpression of pRb and cyclin D1 [24–26]. In a study on lymph node metastases in HNSCCs, p16 overexpression proved to be a surrogate marker for oropharyngeal primary site and HPV association [27].

As HPV DNA detection by itself in HNSCC tissues does not prove a causal association, transcriptional activity of HPV establishes its biological and clinical relevance to the causation of HNSCC. The incidence and clinical implications of biologically relevant HPV16 infection, through p16 protein expression, were studied in a cohort of 107 oropharyngeal squamous cell cancers treated with primary radiotherapy or surgery followed by postoperative radiotherapy at Yale University [28]. HPV16 DNA viral load was determined by real-time PCR. The expression of p53, pRb, and p16 was measured using a quantitative in-situ method of protein analysis after constructing a tissue array composed of these tumors. Results delineated three tumor classes with distinct molecular and clinical features on the basis of the presence of HPV16 DNA and p16 expression status: HPV16-negative/p16 nonexpressing (class I), HPV16-positive/p16 nonexpressing (class II), and HPV16-positive/p16-expressing (class III) oropharyngeal tumors. Overall survival in class III was 79% compared with the other two classes (20 and 18%, $P = 0.0095$, respectively). Disease-free survival for the same class was 75 versus 15 and 13% ($P = 0.0025$), respectively. The 5-year local recurrence was 14% in class III versus 45 and 74% ($P = 0.03$). Only patients in class III had a significantly lower p53 and pRb expression ($P = 0.017$ and 0.001, respectively). Multivariate survival analysis confirmed the prognostic value of the class III model. This study clearly showed that only HPV16-positive/p16-expressing tumors fit the cervical carcinogenesis model and that they are the ones associated with a favorable prognosis.

Recently, Rampias *et al.* [29] showed that E6 and E7 oncogene silencing induces apoptosis and restoration of p53 and pRb tumor suppressor pathways in oropharyngeal

cancer cells. Therefore, the researchers provided further experimental evidence to show that HPV is causally involved in a subset of oropharyngeal cancers.

Prognosis

HPV-associated HNSCCs are associated with a better prognosis compared with stage-matched HPV-negative ones in the majority of studies [14,19,28,30–35]. HPV-positive tumors share a 60–80% reduction in the risk of death from cancer compared with similarly treated HPV-negative tumors. However, some reports in the literature fail to show this superior outcome; this is most likely due to the molecular heterogeneity of the HPV-positive group. Determination of the p16 protein status could be used to clarify delineation of the proportion of HPV-induced HNSCCs [28].

This favorable outcome of HPV-induced HNSCCs may be attributed to the absence of field cancerization and enhanced radiochemosensitivity. As HPV-associated HNSCCs contain wild-type p53, they possess intact apoptotic mechanisms in response to DNA damage caused by radiation and chemotherapy [7].

A retrospective analysis of 743 patients with stage III and stage IV oropharyngeal cancer, enrolled in the RTOG 0129 randomized study, was performed to find an association between HPV status and survival. The study was primarily designed to compare accelerated fractionation radiotherapy with standard fractionation radiotherapy, each combined with cisplatin. Retrospectively, all paraffin-embedded tumor specimens were evaluated for HPV16 DNA positivity and p16 overexpression, with overall survival as the primary end point. HPV-positive HNSCC was identified in approximately 64% of cases and was associated with a 58% reduction in the risk of death. The 3-year overall survival rate was significantly better in patients with HPV-positive tumors (82, 4% vs. 57, 1%, $P < 0.001$) and, after adjustment for age, race, tumor and nodal stage, tobacco exposure, and treatment assignment, had a 58% reduction in the risk of death (hazard ratio: 0.42; 95% CI: 0.27–0.66). Thus, the prognostic significance of HPV status was confirmed in this large HNSCC trial [36]. It is noteworthy that the expression status of p16 protein was even a more significant determinant of prognosis. In unadjusted analyses, the 3-year overall survival rates were 83.6% (95% CI: 78.7–88.6) in the subgroup that was positive for p16 expression and 51.3% (95% CI: 41.5–61.0) in the subgroup that was negative for p16 expression (hazard ratio for death with positive p16 expression: 0.29; 95% CI: 0.20–0.43); the 3-year progression-free survival rates were 74.4 (95% CI: 68.5–80.2) and 38.4% (95% CI: 28.9–47.9), respectively (hazard ratio for relapse or death with positive p16 expression, 0.33; 95% CI: 0.24–0.46). In multivariate analysis, the corresponding hazard ratio for death was 0.33 (95% CI: 0.21–0.53) and the corresponding hazard ratio for relapse or death was 0.42 (95% CI: 0.28–0.64).

Treatment/vaccines

As HPV-associated HNSCCs have better prognosis, selected patients could be spared intensive and toxic treatment and be oriented to organ preservation strategies. At present, the standard of care for HNSCCs is determined by tumor stage and anatomical site. The treatment does not differ from site-matched and stage-matched non-HPV-related HNSCC counterparts. Clinical trials that stratify to more or less intense therapies based on HPV status are only now being undertaken. The Eastern Cooperative Oncology Group conducted a trial of taxane-based induction chemotherapy (IC) and chemoradiation therapy (CRT) for organ preservation, and the outcome has been compared for HPV-positive with HPV-negative cases [31]. Patients with locally advanced squamous cell carcinoma of the oropharynx or larynx received two cycles of paclitaxel/carboplatin IC, followed by paclitaxel CRT. HPV status was determined by in-situ hybridization and multiplex PCR. Patients with HPV-positive tumors had superior response rates after IC and CRT. After a median follow-up of 39.1 months, patients with HPV-positive tumors had a risk of progression that was 72% lower and a risk of death that was 79% lower than patients with HPV-negative tumors after adjustment.

Understanding HPV-associated carcinogenesis could potentially lead to the development of HPV-targeted therapeutic strategies. Moreover, as HPV-associated oropharyngeal cancers contain wild-type p53 and pRb tumor suppressor genes, they show an enhanced sensitivity to chemotherapy and radiation.

As HPV-positive cancers constantly express E6 and E7 viral oncogenes, even in the late stages of the disease, inhibition of viral oncogene expression would result in restoration of Rb and p53 tumor suppression pathways and would cause cell growth arrest and apoptosis [18,21]. In addition, therapeutic vaccines that elicit cytolytic response to cells expressing viral proteins could potentially be curative even in advanced stages of the disease [25].

A clinical trial using an HPV16-specific therapeutic vaccine aiming to enhance the cytotoxic T-cell response to HPV16 oncoproteins is closed to accrual and the data are being analyzed (J.H. Hospital, Baltimore). The vaccine was used as adjuvant therapy [37]. At the end of a 2-year follow-up, all 18 patients are alive and cancer-free.

Preventive HPV vaccines have been designed on the basis of recombinant expression and self-assembly of the major capsid protein, L1, into immunogenic virus-like particles that are similar to authentic virions but are noninfectious. Two such commercial vaccines are available nowadays for the prevention of cervical cancer and genital warts. The quadrivalent vaccine Gardasil (Merck & Co. Inc., Collegeville, Pennsylvania, USA) targets HPV subtypes 6, 11, 16, and18, and the bivalent vaccine Cervarix (GlaxoSmithKline, Research Triangle Park, North Carolina, USA) targets subtypes 16 and 18. Both existing vaccines

are able to elicit a robust immune response, much more effective than that elicited by the levels of antibodies acquired after a natural infection, which persists for at least 60 months [38–40]. Several randomized placebo-controlled trials in human volunteers showed that these vaccines significantly decrease the incidence of persistent HPV16 and HPV18 infections and associated moderate-to-high-grade cervical neoplasia CIN2/3 [41,42]. The impact of these vaccines on the incidence of persistent oral HPV infection remains to be identified. Data from animal models immunized against HPV16 have shown a reduction in the development of HPV-negative oral lesions [43]. It is unknown, however, whether a persistent oral HPV infection can lead to premalignant changes in the oropharynx, as it does in cervical carcinoma. The natural history of oral HPV is unknown and routine screening for HPV-associated OSCC is not recommended. In addition, there are as yet no convincing data showing that the vaccines are effective for either sex, against cancers that occur in both men and women [44]. A study which will be nested in a larger study monitoring men to analyze penile and anal HPV infections will examine the persistence of oral HPV infections and determine the natural history of oral HPV infections. The National Cancer Institute is considering introducing an oral HPV component to its follow-up study of women in Costa Rica, who entered a study of GlaxoSmithkline's preventive vaccine (Cervarix). The aim of this oral component will be to compare the prevalence of oral HPV infection in women who received the vaccine with those who did not. Clinical trials to evaluate the efficacy of the quadrivalent HPV vaccine (Gardasil) in protecting against oral HPV infection are currently under way [25].

Recognition and demonstration of causal association of HPV to a substantial subset of HNSCCs could play a significant role, both in reducing the incidence and mortality of HPV-associated HNSCCs and in sparing this good prognosis group of patients the devastating consequences of unnecessary treatment.

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