Human papillomavirus (HPV) and oropharyngeal squamous cell carcinoma

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Summary

Numerous reports in recent decades have shown that, in addition to smoking and alcohol, human papillomavirus (HPV) is also associated with the development of oropharyngeal squamous cell carcinoma (OPSCC), predominantly in the tonsils and base of the tongue. In 2007, the International Agency on Research against Cancer (IARC) therefore declared HPV to be a risk factor for OPSCC and noted that 80% of those affected are men. In addition, patients with HPV-positive OPSCC, in particular never-smokers, have a much better clinical response to therapy than patients with HPV-negative OPSCC and other head and neck cancers. Most patients with HPV-positive OPSCC may thus not need the increasingly intensive chemoradiotherapy currently administered today to most patients with head neck cancers and associated with many adverse effects. Furthermore, an increase in the incidence of OPSCC has been observed in many western countries, accompanied by a rise in the proportion of HPV-positive tumors. Patients with HPV-positive OPSCC, like those with cervical cancer, more often have an earlier sexual debut and have more sexual partners. It has therefore been proposed that the increased incidence of OPSCC observed might be due to an epidemic of sexually transmitted HPV. The important issues today regarding this growing cohort of patients with HPV-positive OPSCC are therefore individualized treatment and prevention. More specifically, selected HPV-positive OPSCC patients with biomarkers of good prognosis might be included in randomized trials with less intensive treatment. HPV vaccination should also be considered for boys, in addition to the current recommendations for immunization of girls.

Head and neck squamous cell carcinomas (HNSCC) include cancers of the sinonasal tract, oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. They are the sixth most frequent cancer type globally. Around one-tenth of the cases (roughly 60,000 per year) are oropharyngeal squamous cell carcinomas (OPSCCs), most of them in men [1]. The incidence and localization of HNSCC varies widely in different parts of the world. It is the most common cancer in India, and it is more common in southern European countries than in the United-States and northern Europe. Smoking, drinking alcohol and chewing betel are classical risk factors for HNSCC and OPSCC [2]. Over the past 10–15 years, many studies have established an association between human papillomavirus (HPV) and OPSCC. In 2007, the International Agency for Research on Cancer (IARC)
recognized HPV, especially HPV16, as a risk factor for OPSCC [3–7]. Moreover, many western countries report a rise in the incidence of OPSCC due to an increase in HPV-positive OPSCCs. This increase clearly raises the issue of the administration of HPV vaccine to boys [6–9]. Furthermore, HPV-positive OPSCCs respond much better to therapy than HPV-negative OPSCCs and other head and neck cancers (5-year disease specific survival: 80% vs. 40%) [3–6]. Therefore, most patients with HPV-positive OPSCC may not need the intensive chemo-radiotherapy regimes administered today to most patients and associated with serious side effects. Consequently, both HPV prevention and individualized therapy are important issues in this type of cancer.

**Human papillomavirus**

There are numerous types of HPVs: most of them in skin, although others are found in mucosal tissues [10]. They are divided into high-risk (HR) types with oncogenic capacity and low-risk (LR) types, associated with papillomas and warts, but rarely observed in cancer [10,11]. The association of some HPV types with cervical and other anogenital cancers as well as OPSCC is now well recognized [3–7,10,11]. In addition, individuals with the autosomal recessive disease *Epidermodysplasia verruciformis* have susceptibility to HPV types 5 and 8, which cause verruca-like papillomatous lesions and multiple skin tumors. The association of HPV with other skin cancers is less clear [10,11].

All HPVs have a double-stranded circular DNA genome, around 8 kb in size, enclosed in a 52–55 nm virion (figure 1) [10]. The viral genome is arbitrarily divided into a non-coding control region (NCCR), an early coding region, and a late coding region. The genome encodes the regulatory E1-E2 and E4-E7 proteins, as well as the viral capsid proteins L1 and L2. The early proteins are essential for gene regulation, replication, and pathogenesis and, in HR types, for immortalization and transformation [10,11]. In HR HPVs, E6 and E7 are regarded as oncogenes, and E6 binds to and degrades p53, while E7 binds to and inhibits the function of the retinoblastoma protein (Rb); together these interactions dysregulate the cell cycle [10,11]. Furthermore, when this dysregulation occurs, the cyclin-dependent kinase-inhibitor p16INK4A can be activated and overexpressed. Therefore, overexpression of p16INK4A was previously suggested to be a surrogate marker for the presence of HPV in head and neck cancer [6]. In addition, the major capsid protein L1 is in general not expressed in cancer cells as a consequence of integration of the viral genome into the cell genome. However, when L1 is self-assembled into virus-like particles (VLPs), it induces neutralizing antibodies; it is this characteristic that led to the development of HPV vaccines [6,8–11].

**Methods for HPV detection in tumors**

The presence of HPV DNA in tumors is still investigated by using formalin-fixed paraffin embedded (FFPE) tissue, where the DNA partially degrades over time. Accordingly, polymerase chain reaction (PCR) amplification of the short viral DNA fragment is generally used. Longer HPV DNA fragments are easier to detect in fresh or frozen material than in old FFPE samples, although the newer techniques are more sensitive [6,12]. Early studies were often based on Southern blot techniques with in situ hybridization for detection of HPV in head and neck cancer, or by detection of 16nmK+ overexpression as a surrogate marker [6]. Later, technology based on PCR was used to detect HPV DNA and/or RNA [12–14]. General PCR primers detecting several HPV types have often been used in combination with more specific assays to identify specific HPV types, and a cellular gene was used to test DNA amplifiability. The Digene Hybrid Capture 2 (HC2), the Roche linear array HPV Genotyping Test, and a bead-multiplex method where HPV PCR products are coupled to type-specific probes on beads and analyzed by Luminex can be performed to determine the presence of different specific types of HPV [15–17]. The reference method for detecting HPV biological activity in tumor cells is RT-PCR detection of E6 and E7 mRNA [18]. However, in head and neck cancer, combined testing for the presence of HPV DNA by PCR and for overexpression of p16 by IHC is almost as specific and sensitive as the detection of E6 and E7 mRNA [19]. Thus far, HPV DNA has been detected in 45–100% of OPSCCs, but there are variations depending on OPSCC location, time period of analysis, material available and techniques used [3–6,20–24].

**Oropharyngeal squamous cell carcinoma (OPSCC) and HPV**

OPSCCs include cancers of the tonsil, the base of tongue, the soft palate and the walls of the pharynx. Patients with OPSCC, like those with other head and neck cancers, often do not consult before their tumor is fairly large, since smaller tumors do not generally cause distress early on. Today treatment with intent to cure often uses chemo-radiotherapy and sometimes surgery, in combination with treatment by epithelial growth receptor (EGFR) blockers. These therapies may functional and cosmetic harm [2,5]. Palliative therapy is given to avoid pain and discomfort when curative treatment is not possible. OPSCC survival is poor with an overall 5-year survival of around 25–40% [2,5]. The apparent improvement observed in clinical outcomes for OPSCC in contrast to other HNSCCs was later shown to be due to an increase in the proportion of HPV-positive OPSCCs, which have a better clinical outcome than HPV-negative OPSCC and other head and neck cancers (shown for tonsil cancer in figure 2) [3–6]. In many western countries, the proportion of HPV-positive OPSCCs has been increasing continuously, and it is likely that this cancer will be the most common HNSCC in the decades to come [6,21–30]. The better clinical outcome for HPV-positive OPSCC than for HPV-negative OPSCC suggests that both predictive and prognostic markers would be helpful to improve treatment of OPSCC [6,21–30].
Nevertheless, the extensive characterization of both HPV-positive as well as HPV-negative OPSCC has been important for clarifying and establishing the association between HPV and OPSCC. In this respect, patients with HPV-positive tumors are often younger and lack risk factors such as smoking and alcohol [3–6,20,21]. In HPV-positive OPSCCs, HPV16 accounted for 90% of the cases and was episomal and/or integrated into the cellular genome [6,31,32]. HPV E6 and E7 expression suggest an active role of HPV in OPSCC [18]. The combination of 16\(^{\text{Ink4a}}\) overexpression with HPV was indicative of E7 inhibiting Rb functions and thereby inducing upregulation of the cell cycle and of 16\(^{\text{Ink4a}}\) [10,11,33]. HPV-positive OPSCCs often have normal p53 expression [3], are less often differentiated and more often aneuploid compared to HPV-negative OPSCCs [34]. Like cervical cancer cells, HPV-positive OPSCC tumor cells often have chromosome 3q amplification [35]. Most importantly, detection of HPV is a favorable prognostic factor for OPSCC, independent of tumor stage, age, gender, differentiation, or DNA ploidy [3–6,34]. Furthermore, never-smokers with HPV-positive tonsil cancer have a better clinical outcome than smokers [18,36]. It has been suggested that smoking might abrogate the immune response against HPV, or induce additional genetic alterations in addition to those due to HPV in OPSCCs [18,36]. Nevertheless, the prognostic value associated with the presence of HPV cannot be generalized for all HNSCC and should be investigated for each location [37]. Cancer of the tonsils and base of tongue, both located along the pharyngeal (Waldeyers) lymphatic ring, account for 80–90% of all OPSCCs [6,37]. Furthermore, HPV DNA is found more frequently in these two cancers than in OPSCC of other locations [37].

In summary, the data suggest that there are different types of OPSCC. Some, especially in the tonsil and base of the tongue, may depend mainly on HPV, and others mainly on smoking and...
alcohol, or on combinations of these factors. Nonetheless, clinical outcome is better in patients with HPV-positive than those with HPV-negative OPSCCs [3–6,18,21,36].

An HPV induced epidemic of OPSCC

In recent years, many western countries have reported an increase in the incidence of OPSCC [21–30]. Furthermore the proportion of HPV-positive OPSCCs has also risen, and HPV has been suggested to be responsible for it [6,21–25,29,30]. In 2006, a 2.8-fold rise (2.6 in men and 3.5 in women) in the incidence of tonsil cancer was reported in Stockholm, Sweden, during the period 1970–2002 [12]. In parallel, the proportion of HPV-positive tonsil cancer multiplied by 2.9 (from 23 to 68%) [22]. In 2007, concern was raised about the potential emergence of an epidemic of HPV associated head neck cancers [21]. In addition, later studies from Sweden showed a substantial increase between 1998–2006, not only in tonsil cancers, but also in base-of-the-tongue cancers. Moreover, the proportion of HPV positive tumors has continued to rise [21,24,25]. A follow up study of the prevalence of tonsil cancers in Stockholm indicated a 7-fold increase in HPV-positive tonsil cancers between 1970-2007, and a decline of HPV-negative tonsil cancers, most likely due to decreased smoking in men (figure 3) [24]. A similar increase in the proportion of potentially HPV-related OPSCCs, as well as a decrease in HPV-negative OPSCCs, has also been shown in the United-States [30] with the increase in OPSCCs affecting mainly men [23]. An increase was also observed in women, however, as shown in the Stockholm area [22]. We note that smoking has gone down among men in many countries, but that this rate does not always fall as swiftly among women.

The search for reasons for this increase has focused primarily on changes in sexual behavior, with possible increases in the number of sexual partners and the frequency of oral sex. A significant association has been demonstrated between HPV-positive OPSCC and both early sex debut and the number of oral or vaginal partners [38]. The risk of oral HPV infection also increased with the number of lifetime oral or vaginal sex partners [38]. However, it has also been shown that oral-to-oral contact could also account for oral HPV infection, since open-mouth kissing has also been associated with oral HPV infection among university students [39]. Moreover HPV transmission can also occur at birth [40].

In summary, there appears to be an ongoing epidemic of HPV-induced OPSCC, most likely due to genital-to-oral, or oral-to-oral HPV transmission.

Treatment of HPV-associated OPSCC

The increasing incidence of HPV-positive OPSCC in many western countries must be taken into consideration with regard to therapy and prevention, since OPSCCs will likely account for a much larger proportion of all HNSCCs in the coming decades [6]. The poor clinical outcome of patients with HNSCC has resulted in intensification of the treatment of all HNSCCs, by chemoradiotherapy, surgery and sometimes treatment with EGFR blockers. This aggressive treatment leads to more side effects and increases costs. Nonetheless, conventional radiotherapy suffices for 80% of the patients with HPV-positive OPSCC [6]. However, before this therapy can be de-intensified, so to speak, for some patients, it is essential to identify those in whom better-tailored treatment will increase survival and quality of life, while decreasing costs for society.

Several approaches are under study. Patients who respond well to chemotherapy have been offered the possibility of less intensified radiotherapy. However, the patients feel reluctant to downstage treatment without being certain that it will not affect their survival. Simultaneously, several studies have focused on biomarkers, assessing for example, whether any of 16ink4a, CD44, MHC class I expression, CD8+ tumor infiltrating lymphocytes (TILs) or others markers, as well as non-smoking or smoking combined with positive HPV status in OPSCC predict response to therapy [41–43]. In these studies, overexpression of 16ink4a, low expression of MHC class I, and of CD44 and high CD8+ TILs counts, as well as never-smoking were all positive prognostic factors for HPV-positive OPSCC [18,36,41–43]. Moreover, some retrospective studies have shown, although not consistently, that HPV-positive OPSCC responds better to chemotherapy and radiation [5,23].
Notably, in immunodeficient mice, HPV-positive tumors are not more curable despite increased epithelial sensitivity to cisplatin or radiation therapy. Only treatment-induced immune response to the tumors is associated with cure [44]. Presence of HPV in a tumor induced by smoking is a favorable factor, but smoking may weaken the specific immune response and thus the efficacy of the treatment. More molecular and immunological knowledge is needed to improve OPSCC therapy.

In summary, more information is necessary to guide better treatment decisions for individual patients with OPSCC on the basis of their HPV status. As of now, it has been shown that positive HPV status, overexpression of 16ink4a, low MHC class I, and CD44 expression, high CD8+ TIL counts, and never having smoked are each helpful [18,36,41–43]. Prospective clinical studies that assess the efficacy of different therapies according to HPV status and predictive biomarkers will be highly valuable for identifying future better-tailored treatment.

**OPSCC and HPV-prevention**

Vaccines against both HPV16, which is present in 80–90% HPV-positive OPSCCs, and some other types, have been available since 2006. Although it may take decades before the effects of this vaccination on HPV-positive OPSCC become clear, it is possible to monitor the prevalence of oral HPV, and several attempts are currently ongoing: this prevalence has been reported to be around 3–9% [45–49]. However, there are no yet screening programs for OPSCC, because the prevalence of oral HPV is more difficult to monitor than that of genital HPV, in part due to the production of large amount of saliva. Nevertheless, some studies indicate that a high oral HPV load may predict an increased risk of the development of HPV-positive OPSCC [50].

Given that OPSCC is the second most common cancer associated with HPV and that its incidence continues to increase, the efficacy of HPV vaccine in the prevention of this tumor definitely deserves special attention. As HPV-positive OPSCCs are more frequent in men than women, it is obvious that future HPV-vaccination programs should include immunization of both girls and boys.

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