Human papillomavirus infection in the etiology of laryngeal carcinoma

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Purpose of review

One fifth of cancers worldwide are associated with viral infection. Indeed, the causal link between human papillomavirus and cervical carcinoma is so well established that it is thought to be the first necessary cause of human cancer ever identified. One of the primary aims of research in this area is to reduce cancer prevalence by vaccination. However, the role that human papillomavirus plays in carcinogenesis of the head and neck region may also have important implications for its prevention and treatment.

Recent findings

Although human papillomavirus was first identified in the larynx 20 years ago, the extent to which it is present in epithelium of the normal population is unclear. Laryngeal papillomas are the most common benign tumors in the larynx. They are associated with a small risk (3 to 7%) of malignant transformation, in which smoking and irradiation appear to be cofactors. The search for alternate risk factors for the development of laryngeal cancer, particularly in those who are nonsmokers and nondrinkers, has led to the hypothesis that human papillomavirus may have a pivotal role. Epidemiologic studies, although not conclusive, strongly suggest its involvement in the etiology of a subset of laryngeal carcinomas. Recent molecular evidence supports this.

Summary

An adequately powered, multicenter case–control study is required to elucidate the full extent of this association and to examine the relation between the virus and other risk factors.

Keywords

laryngeal carcinoma, human papillomavirus, laryngeal papilloma

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Abbreviation

HPV human papillomavirus

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Introduction

The role of human papillomavirus (HPV) in the carcinogenesis of the cervix and anogenital area is now firmly established [1]. The results of the first controlled trial of HPV-16 vaccine show encouraging reductions in HPV-16-related cervical intraepithelial neoplasia [2•]. The extent to which HPV is involved in the etiology of head and neck squamous cell carcinoma is less obvious, with cancers of the oropharynx (particularly the tonsil and the base of tongue) having the strongest association [3].

Evidence of laryngeal HPV infection was first found using immunohistochemistry in squamous papillomas more than 20 years ago [4]. In 1985, the association with verrucous carcinoma of the larynx was first suggested [5]. Since then, a number of studies have found HPV DNA in squamous cell carcinoma of the larynx and yet, despite the enormous growth in molecular biologic techniques, it seems we are still no closer to a definite answer regarding whether HPV actually causes laryngeal cancer.

Herein we review the current epidemiologic data and summarize the relevant clinical and molecular information, primarily to address the question of a causative link between HPV and laryngeal squamous cell carcinoma.

Conventional etiology

Tobacco smoking and the consumption of alcohol are the two major independent risk factors for laryngeal cancer. A recent case–control study has confirmed that they act synergistically, with an odds ratio well over 150 for heavy smokers who drink heavily [6•]. Approximately 5% of cases occur in nonsmokers and nondrinkers [7], which although it represents a relatively small group, is proof that other cofactors must contribute to the development of this disease. For a comprehensive review of this subject, see Wight *et al.* [8].

Human papillomavirus

HPV is a small, double-stranded DNA virus belonging to the Papovaviridae family that replicates within epithelial cells of the host's mucosa and skin. HPV infection is the most common sexually transmitted disease worldwide, with prevalence rates in sexually active women ranging from 10 to 50% [9]. The virus normally results in benign, self-limiting warts or tumors, characterized by abnormal maturation and differentiation of epithelial cells. After

initial infection, the virus can remain latent in the basal layer of the epithelium for months or even years before histologic change is detected [10].

HPV is extremely difficult to culture *in vitro*, so its presence is usually detected by polymerase chain reaction or hybridization techniques such as Southern blot. Despite this, epidemiologic studies have now established beyond reasonable doubt that it is the central cause of invasive cervical carcinoma, with HPV DNA being demonstrated in almost all (99.7%) tumor biopsies in a recent study coordinated by the International Agency for Research on Cancer [11]. There are approximately 100 different subtypes now recognized, with at least 14 of these being referred to as high risk because they are associated significantly with progression to invasive cervical cancer [12]. By far the most common subtype is HPV-16, which was found in 51% of cases in a recent meta-analysis [13].

Human papillomavirus in normal laryngeal epithelium

It has been estimated that the prevalence of HPV in the normal laryngeal mucosa could be as high as 20% [14]. However, this figure is based on a study performed in low numbers on postmortem specimens more than 10 years ago when polymerase chain reaction was in its infancy, and therefore may represent an overestimation [15]. Conversely, in the case–control studies looking at laryngeal carcinoma, the rate of infection in control samples is approximately 5% [16–18]. The unblinded nature of these studies would tend to underestimate the prevalence, so it is possible that the true figure is somewhere between the two. This raises the question of sampling, which is one of the inherent problems in looking for this virus in the upper aerodigestive tract. In the cervix, lesions arise from "the transformation zone," where columnar epithelium becomes squamous epithelium, and as such it is easier to sample the correct area even if it is normal macroscopically.

Human papillomavirus in benign lesions

It is clear from laryngeal papillomatosis that HPV is able to infect and replicate in the larynx. As in the cervix, benign lesions usually originate from the area of change between squamous epithelium and "respiratory"-type columnar epithelium, typically at the vocal cords. HPV-6 and HPV-11 are most often found in these papillomas, and although these subtypes are usually associated with benign lesions, malignant transformation can occur, albeit rarely [14]. In a series of 102 patients, including those with juvenile and adult forms of the disease, seven cases progressed to laryngeal carcinoma with a mean time of 24 years between onset of papilloma and diagnosis of carcinoma. This was 88 times more than expected when compared with the local population [19]. Although this report acknowledges that carcinoma may occur without any other exposure to known carcinogens, they found that the majority had cofactors such as smoking, irradiation, and chemotherapeutic agents such as bleomycin. This association is not supported in a more recent study by Go *et al*. [20•], who also found that there was no histologic progression from dysplasia through to carcinoma in the lesions, confirming that diagnosis early during the course of transformation is extremely difficult. Given the small numbers and the fact that there are no reliable predictors of future malignant change, one cannot justify more aggressive therapy to prevent malignancy in either juvenile or adult laryngeal papillomatosis. Patients and their families should, however, be counseled about the possible additional risks of smoking with this disease.

Human papillomavirus in laryngeal carcinoma

Although most laryngeal carcinomas are squamous cell in origin (>90%), the geographic variation in incidence and subsite suggests considerable heterogeneity in etiology. The overwhelming relative risks associated with tobacco smoking and alcohol consumption in practice makes the evaluation of other risk factors difficult.

A mathematic model estimating the number of genetic events required for the development of different types of cancer has suggested that 6 to 10 separate DNA-damaging incidents contribute to the development of squamous cell carcinoma in the head and neck region [21]. This is far more than is suggested for other solid tumors and explains the extensive period of carcinogen exposure before development of the disease. Using the cervix as a model for HPV carcinogenesis, it is quite feasible that persistent infection with a high-risk subtype in the larynx could be responsible for one or even several of these events. This initial link is supported in a study of the incidence of second primary cancers occurring after cervical and anal cancer. Women with primary invasive cervical cancer had a significantly increased RR of 3.4 for subsequent laryngeal carcinoma [22].

Epidemiologic studies and their limitations

Currently, there is no conclusive epidemiologic proof for the role of HPV in laryngeal carcinoma. One of the main reasons for this is the lack of any prospective cohort studies or adequately powered case–control studies looking only at the larynx. There is a huge variation in the reported prevalence, with HPV positivity varying from 3 to 47% in case–control studies [17,23] and 0 to 100% in case series [24••]. More important, as with cervical cancer, HPV-16 is the predominant subtype.

A recent systematic review of case–control studies by our group suggests an increase in the risk for laryngeal squamous cell carcinoma in those with evidence of HPV infection. Although only two of six studies individually showed a significant association [3,17], the combined

odds ratio associated with HPV-16 exposure was 2.6 (95% CI, 1.4 to 4.9; $P = 0.002$) [25]. Interestingly, one study showed an increased risk for glottic carcinoma compared with the supraglottis, if exposed to HPV-16 infection (odds ratio, 9.7; 95% CI, 1.5 to 64.0) [26].

This study also identifies two major problems. First, there is much heterogeneity in the methods used to collect specimens and the sites from which they are isolated. For example, because HPV infection is focal and only occurs in differentiating squamous epithelium, random biopsies have a much lower chance of detecting HPV than exfoliated epithelial cells. Second, the techniques used to isolate viral DNA vary considerably, both in sensitivity and the ability to identify viral genome integration. This means that it is quite difficult to compare studies and correlate significant associations. In particular, it is impossible to know whether the extent of variation in HPV prevalence between studies represents a fundamental diversity between different populations or whether it simply reflects the discrepancy in methods [25]. To confirm the association in epidemiologic terms, a suitably powered, multicenter case–control study is needed, with the ability to examine the differences in laryngeal subsite and the relation with smoking and other risk factors.

Molecular evidence

The molecular biology underlying the development of head and neck carcinoma is an important area of research, not only because of possible advances in screening and surveillance, but because it may identify a whole new area of therapy that targets molecular pathways. Furthermore, there is evidence to support an oncogenic role for HPV.

The HPV genome consists of three regions: an upstream regulatory protein and two regions named according to the phase of infection in which they are expressed, the early (E) and the late (L) regions. The most potent oncogenes, E6 and E7, code for proteins that facilitate the proliferation of infected cells by disrupting the function of the tumor suppressor proteins p53 and pRb (retinoblastoma protein) respectively [27•]. This can lead to immortalization of the cell and eventually to carcinogenesis. Protein p53 has wide-ranging roles in the control of genomic integrity, proliferation, apoptosis, and DNA repair [28], and it represents one of the most frequent genetic alterations in human cancer, with 60 to 80% of laryngeal carcinomas showing mutations [27•,29•]. The relation between this and HPV is that HPV-positive cancers are more likely to express wild-type p53 than those lacking HPV DNA [30], although there are small numbers of tumors with both the mutation and the virus. It is therefore quite likely that inactivation of p53, either through mutation or degradation by the HPV E6 transcript, is an obligatory event in the multistep process of head and neck carcinogenesis [29•]. A further study has shown that p53 mutation may be an independent prognostic factor associated with shorter survival [31]. This adds weight to the suggestion that HPV-positive head and neck squamous cell carcinomas have a better prognosis than HPV-negative cases [30].

Current interest in the proinflammatory transcription factor nuclear factor κB, which has the ability to inhibit apoptosis and to promote and maintain the growth of cells, suggests that it has a role in several human cancers. Recent research by Du *et al.* [32] has found that nuclear expression of p65 (a nuclear factor κB subunit) correlated significantly to HPV-16 E7 expression in late squamous cell carcinoma . They speculate that nuclear factor κB may serve as a signal to relay the effect of the E7 oncogene to other tumor suppressors such as the retinoblastoma gene.

High-risk HPV DNA should be present in the tumor cell at a minimum of one copy per cell genome and it should be transcriptionally active [33]. It has also been suggested that HPV DNA needs to be incorporated into the cellular genome to initiate transformation [34]. However, even in cervical carcinoma, a proportion has been found with only episomal HPV DNA, and in skin cancers associated with epidermodysplasia verruciformis, the HPV genome remains episomal [33]. Viral integration into the host genome has been identified in laryngeal carcinoma, but not consistently (43% of cases that were HPV-16 positive) [35].

The role of mucosal immunology

Little is known about the immunologic mechanisms involved in HPV infection, but it is generally thought that cellular immunity is more important than humoral immunity. This is partly because individuals who have a defect in their cell-mediated immunity, either in HIV infection or iatrogenic after organ transplantation, have been found to be at greater risk for cervical intraepithelial neoplasia [33].

Why certain anatomic sites and subsites in the head and neck region are more prone to HPV-mediated malignant change, and why some persons should be more susceptible to the infection–transformation process remains unclear. Cervical cancer is known to be associated with several major histocompatability complex class II alleles [36], and a recent report by Gelder *et al.* [37•] also identifies a genetic association between the presence of human leukocyte antigen DRB1*0301 and susceptibility to laryngeal papillomatosis. This group also measured T-cell proliferation to HPV-11 viruslike particles in individuals with juvenile-onset disease and found a range of responses, the magnitude of which correlated negatively with a higher clinical staging score ($P = 0.012$) [37]. We

would hypothesize that similar defects will be identified in HPV-related laryngeal carcinoma.

Conclusion

With the recent advent of HPV vaccination, it is important that HPV as a potential risk factor for carcinoma of the head and neck be fully elucidated. Although the evidence for HPV in the larynx is not as compelling as that for the oropharynx, epidemiologic studies suggest that it may have a role in at least a proportion of carcinomas. The wide variation in HPV prevalence in laryngeal carcinoma reflects not only the heterogeneity of this malignancy but also the variation in methods used to collect and analyze samples. Although the relation between HPV-induced cell changes and those produced by the more established carcinogens such as tobacco is uncertain, these cofactors may interact in a complex manner. So far, there is no obvious subset of laryngeal carcinomas that have the features normally associated with an HPV malignancy. Better quality epidemiologic studies are needed to define this group properly before future HPV vaccination for prevention and treatment of laryngeal squamous cancer can be considered.

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