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REVIEW ARTICLE

Evidence for a causal association for HPV in head and neck cancers

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Abstract Current data have now attributed a viral etiology and causality of Human papillomavirus (HPV). Epidemiological analysis of the last decade demonstrates a rapid increase of HPV-associated HNSCC. Genomic detection of HPV DNA in the nuclei of certain oro-pharyngeal cancer cells gives strong evidence of a viral etiology in HNSCC. Non-smokers, non-drinkers, and a sexual debut at a younger age and other sexual risk factors have an increased risk of HPV-positive oropharyngeal cancer. Sexual transmission is considered to play a causal role. In contrast to HPV-negative HNSCC most studies reveal a favorable prognosis for HPV-positive tumors. There is evidence of alterations in the p53 pathway through expression of E6 oncogene with subsequent induction of tumor cell proliferation. Synergies between viral oncogenes and other carcinogens are hypothesized. HPV alone appears to be insufficient as the sole cause of HNSCC; this may explain the long latency period between HPV infection and cancer development. There is now sufficient evidence for a causal role for HPV in HNSCC. As in cervical cancer, HPV requires oncogenes and co-factors for tumor development. Thus, inhibition or loss of such co-factors may lead to tumor regression. The vast amounts of epidemiological, molecular pathological and in vitro experimental data are consistent with the hypothesis that HPV does indeed have a causal role. We await final validation from animal experimentation in which regression of HPV-positive tumors will follow from loss or inhibition of E6 and E7.

Keywords HPV · Head and neck squamous cell carcinoma · Oropharyngeal cancer · Evidence

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Introduction

Squamous cell carcinoma represents the predominant group in head and neck tumors with a gradually increasing incidence comprising approximately 5% of all cancers in the western world [1]. Recent data have now attributed a viral etiology to a subset of head and neck squamous cell carcinoma (HNSCC), and there is sufficient evidence to support the hypothesis of a role for Human papillomavirus (HPV) in the development of HNSCC [2–4]. According to Robert Koch's postulates of germ theory an organism is the cause of disease if certain requirements are fulfilled (Table 1) [5]. In 1965 Austin Bradford Hill introduced the criteria of causation to demonstrate the causal link between disease and specific factors (Table 2) [6]. Although not always fully satisfactory, even today these two concepts of causality provide a valuable tool in the investigation of disease etiology. However, as in many cases of cancer, the long latent period between the initiation of the cause and overt illness represents a large obstacle to rapid

Table 1 Postulates of germ theory by Robert Koch

Koch's postulates of germ theory	
(1)	Found in all cases of the disease examined
(2)	Prepared and maintained in pure culture
(3)	Capable of producing the original infection, even after several generations in culture
(4)	Retrievable from an inoculated animal and cultured again

Table 2 Criteria of causation by Sir Austin Bradford Hill

Austin Bradford Hill criteria of causation			
(1)	Strength of the association	(6)	Plausibility
(2)	Consistency	(7)	Coherence
(3)	Specificity	(8)	Experiment
(4)	Temporality	(9)	Analogy
(5)	Biological gradient		

Table 3 Criteria for causality and evidence by the Cancer Aetiology Branch of the National Cancer Institute

Criteria to establish etiology	
(1)	Epidemiology
(2)	Molecular pathology
(3)	Tissue culture or in vitro experimental evidence
(4)	Experimental evidence from animal studies

classification of carcinogenicity. Today there is an important need for a more rapid identification of carcinogens (including viruses) to allow removal or prevention of the cause and the need for rapid establishment of preventive and therapeutic measures.

In this article we review the available evidence for the causality of HPV in head and neck cancers, and we determine the state of evidence with regard to the criteria for causality according to the Cancer Aetiology Branch of the National Cancer Institute (Table 3) [7].

Epidemiological evidence

Detection and demographics

The first suggestion that HPV was involved in head and neck cancers must be credited to Stina Syrjanen in 1983. Her team discovered that 40% (16/40) of the laryngeal and oral cancers analyzed in her series contained histological and morphological similarities with HPV-infected lesions, and 8 of those 16 samples demonstrated HPV structural proteins by immunohistochemistry [8]. Seven years later (1990), the same group published data on HPV detection on those same samples—they had found that 12/40 samples were positive for HPV 6, 11, 16 and 18 DNA by ISH and PCR [9]. Since then, a wealth of epidemiological data has established that those initial observations were correct, i.e., that differing percentages of head and neck cancers are indeed positive for HPV DNA (by different methods) in different studies. The two largest reviews/meta-analyses of recent times documenting HPV detection in HNSCC are those of Kreimer et al. [10] which gave an overall worldwide prevalence of 26% and Termine et al. [11] which estimated a worldwide prevalence of 34.5%. However, estimates of DNA detection range from 0 to 100% in different studies, with multiple different techniques, fresh frozen or formalin-fixed paraffin embedded samples (FFPE) and the study of multiply classified cohorts of cancer samples, all confounding variables with little standardization. In developed countries, analysis of detection of HPV in HNSCC shows that the trendline for HPV-associated HNSCC is increasing rapidly while non-HPV-associated HNSCC is decreasing gradually [11–13]. Data from the USA [14], Sweden [15] and Finland [16] support this increase. Recently, Marur et al. described HPV-associated head and neck cancers as a virus-related cancer epidemic [17]. Demographic analysis demonstrates that HNSCC patients with HPV-positive tumors tend to be younger than HPV-negative tumor patients by a mean of 5 years. Furthermore, men are at the same risk as women of having HPV-positive HNSCC [18–20]. Clinical studies revealed that an excess of cancers developing from the lingual and palatine tonsils is HPV-positive compared to other sites [21–23]. Although alcohol [24] and tobacco [25] have been considered for decades as the most common risk factors for head and neck cancer in the United States, today non-smokers have a 15-fold increased likelihood to have an HPV-positive oro-pharyngeal cancer than smokers [26]; similarly non-drinkers have an increased risk of HPV-positive cancers [20, 21, 26]. Several studies indicate that oral HPV infection is likely to be sexually acquired [27, 28]. Sexual transmission of HPV is thought to be the dominant mode of transmission [29]. Consistent with this view, sexual risk factors such as younger age at sexual debut, an increased number of partners, a history of genital warts, oral–genital and oral–anal contact are all associated with an increased risk of developing HNSCC [23, 30–32]. Consistent with the view that HPV is causative in a subset of HNSCC, immunodeficient or immunosuppressed patients such as HIV-positive or transplant patients are at a higher risk of developing these cancers [33, 34]. HIV-seropositive individuals demonstrate a markedly increased

rate of oral HPV infection and associated HNSCC incidence compared to HIV-seronegative individuals [35].

Prognosis and favorable outcome

Several lines of evidence suggest that HPV-positive and HPV-negative HNSCC represent distinct subgroups with different biological, epidemiological and prognostic profiles [36, 37]. Recent data suggest that a positive HPV status represents an important prognostic factor and is associated with a favorable outcome in head and neck cancer [38–41]. In HPV-positive tumor patients, the majority of studies show a large increase in survival, lower recurrence rates and a better response to standard therapy [18, 21–23, 40–44]. It should be noted that two prospective studies support the evidence that HPV-positive tumor status is associated with a better treatment response and with an improved survival rate compared to HPV-negative tumor status. There is an approximate 30% absolute survival difference at 5 years (HPV-positive = 60% vs. HPV-negative = 30%) [45]. Recently, Ang et al. [46] confirmed the favourable prognosis of HPV-positive oropharyngeal SCC in a randomized trial. However, there appears to be a subgroup of HPV-positive patients whose clinical prognosis is worse than the typical HPV-positive patient. This subgroup has higher smoking rates, higher rates of p53 mutations and higher expression of EGFR and Bcl-xL (low rates which are associated with HPV-positive status, i.e., the typical HPV-positive HNSCC patient). In short, this subgroup may be an intermediate group with positive HPV status defined by ISH or DNA detection, but in fact "HPV inactivity" defined by p16 detection by immunohistochemistry [47]. Weinberger et al. [40] grouped 77 oropharyngeal cancers into a three class model: HPV-negative and p16low (class I); HPV-positive and p16low (class II), and HPV-positive and p16high (class III). They found that only class III had significantly higher overall survival (79%) compared to class II and I (20 and 18% respectively, $P = 0.0095$). A more recent publication by the same group has confirmed that the molecular phenotype in terms of tumor progression proteins among the three classes was different, thus validating the hypothesis. Specifically, class III cancers displayed high expression levels of beta-catenin, similar to cervical cancers [48].

Molecular pathological evidence

Molecular pathology

Viral DNA has been specifically localized to the tumor cell nuclei of certain oro-pharyngeal cancers [21, 49]. The presence of HPV in the nucleus makes it more likely that HPV is associated with genomic DNA activity. Many oropharyngeal cancers have integrated HPV DNA within their genomic DNA. This is another proof of evidence similar to the above [50–52]. Even more importantly, HPV DNA sequences are transcriptionally active, thus strongly suggesting that the viral transcripts are being translated into active proteins, and therefore are performing functions within that cancer cell [50, 53–56]. Nearly all the above studies have focused on the transcripts of E6 and E7. The manifold functions of the two major HPV 16 oncogenes E6 and E7 are well known but additional functional pathways are being worked out. Their most important functions are to bind p53 and pRb tumor suppressor proteins, respectively, amongst others [57, 58]. HPV E5 oncogene is relatively minor, and thought not to play a role in late stage carcinogenesis because it tends to be deleted during integration. It binds to EGFR, platelet-derived growth factor beta receptor and colony stimulating factor 1 receptor to promote cell proliferation [59]. The importance of continuous high-level expression of E6 and E7 in cervical cancer is also the model for all HPV-associated cancers. Strong experimental evidence for E6 and E7 expression exists for cervical carcinoma. Anti-sense RNA strategies to inhibit E6 and E7 expression in cervical cancer cell lines have led to decreased cellular proliferation [60, 61]. E2 mediated repression of E6 and E7 has led to activation of the p53 and pRb pathways, cellular growth arrest and inhibition of telomerase activity [62, 63]. These are necessarily only a selection of the in vitro evidence.

Serological evidence

Serological evidence is circumstantial since it provides only data on prior exposure to HPV. Mork et al. [64] were first to demonstrate the important relation of seroconversion prior to HNSCC in 2001. However, as it has been shown that only approximately 50–60% of patients with natural exposure to HPV cervical infection will seroconvert, it is likely to provide an underestimate [65]. This is probably also the case for oro-pharyngeal mucosal infection. However, detectable HPV 16 antibody in sera is associated with an increased risk for HNSCC. The most important case–control study detailing prior HPV exposure in oro-pharyngeal cancer is that of D'Souza et al. [30]. The authors showed that oro-pharyngeal cancer was significantly associated with oral HPV 16 infection (odds ratio (OR) 14.6, 95% confidence interval (CI) 6.3–36.6), oral infection with any of 37 types of HPV (OR 12.3, 95% CI 5.4–26.4), and seropositivity for *HPV 16 L1* (OR 32.2, 95% CI 14.6–71.3). Thus, these are important data regarding the strength of association. Recently, the authors confirmed their findings reporting a predictive value of demographic and behavioral characteristics in the

diagnosis of HPV associated head and neck cancer [66]. Interestingly, Furniss et al. [67] report that HPV 6 seropositivity is also associated with HNSCC, independent of tobacco, alcohol use and HPV 16 seropositivity. Whether this is a causal relationship or merely a marker of HPV infection is less clear.

Experimental evidence

Experimental studies and animal experimentation

In HNSCC, the experimental evidence is much less, both in vitro and in animal experimentation. Transformation studies utilizing oral keratinocytes argue for some synergy between viral oncogenes and other carcinogens. Transfection of normal human oral keratinocytes with cloned HPV 16 or 18 genomes results in immortalization of these cells [68, 69]. However, organotypic raft cultures generated using these immortalized cells require further exposure to other carcinogens to form tumors in nude mice [70, 71]. These seminal studies originated from the laboratory of No-Hee Park. They established that oral keratinocytes (similar to cervical keratinocytes) could not be transformed by HPV alone but required further mutations in other oncogenes. This is consistent with the long latency period seen between HPV infection and the development of cancer. What was not established was whether cell lines from HPV-positive HNSCC relied upon continued expression of E6 and E7, again similar to cervical cancer cell lines. A report from the Psyrri laboratory last year has now addressed this problem. They used shRNA-mediated inhibition of E6 and E7 and found that E6 and E7 suppression in HNSCC led to the apoptosis of oro-pharyngeal cell lines 147T and 090. The experiments induced substantial apoptosis (13.4% in control vs 84.3% in 147T, 3.3% in control vs 71.2% in 090 cell lines) via restoration of the p53 and pRB suppressor pathways [72]. This must be considered an important area of further research. The lack of suitable experimental animal models has hindered research into HPV cancers for many years. Most animal models available such as the COPV in rabbits, BOPV in cattle, etc., do not cause cancer and are used to model the life cycle of papillomaviruses. However, the Lambert group has published on their in vivo mouse model of HNSCC, established using their K14E6 and K14E7 transgenic mice. These mice have a human keratin 14 expression construct cloned with the E6 and E7 open reading frames (ORFs), and these are expressed in the basal layer of stratified squamous epithelia (to mimic HPV infection of the basal layers in the human) [73]. They have found that K14E6/K14E7 doubly transgenic mice develop oral cavity cancers when treated with the tobacco carcinogen, 4-nitroquinoline 1-oxide (4NQO). They showed that these tumors mirror the molecular and histopathological features of human HNSCC, including basaloid tumors and overexpression of p16 and MCM7.

Conclusion

The weight of evidence is now overwhelmingly in favor of a causal role for HPV in a certain subgroup of HNSCC. As seen in cervical cancer, HPV alone appears to be insufficient as the cause of HNSCC but requires other co-factors. The vast amounts of epidemiological, molecular pathological and in vitro experimental data are consistent with the hypothesis that HPV does indeed have a causal role. We await final validation from animal experimentation in which regression of HPV-positive tumors will follow from loss or inhibition of E6 and E7.

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