

The role of human papillomavirus infection in head and neck cancers

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The link between head and neck squamous cell cancer (HNSCC), especially oropharyngeal cancer, and HPV has become established. HPV16 is the most common genotype in these tumours but HPV6 and HPV11 can also be found in a minority of these cancers, implying that these low-risk HPV types are not entirely benign in the head and neck region. HPV status is also associated with p16 expression and HPV+ tumours are less likely to harbour p53 mutations. HPV DNA is closely associated with poorly differentiated cancers, positive lymph nodes and late-stage disease, which all indicate poor prognosis. Contradictory to this, patients with HPV+ HNSCC seem to have significantly improved response to chemotherapy and radiotherapy as compared with HPV-negative tumours. Interestingly, the risk factors of HNSCC are the same as for HPV, including the number of sexual partners, younger age at first sexual intercourse, practice of oral sex, history of genital warts and younger age.

Key words: head and neck cancer, HPV, mouth, oropharynx, risk factors, survival

Introduction

Head and neck squamous cell cancer (HNSCC) comprises tumours of diverse origin, but the present discussion is limited to squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, larynx, nasopharynx and sino-nasal tract. Annually, >650 000 patients worldwide are diagnosed with HNSCC and some 350 000 die of this disease every year. Thus, these global figures are higher than those for cervical cancer. Similarities in the morphological features between genital and oral HPV-associated lesions prompted us (in the early 1980s) to indicate that HPV might be involved in oral and laryngeal squamous cell carcinogenesis as well [1, 2]. Until recently, however, the role of HPV in the pathogenesis of HNSCC has been controversial, mainly because the detection rates of HPV DNA have been highly variable, ranging from 0% to 100% [3–12].

More recent data from case–control studies and meta-analyses indicate that HPV is an independent risk factor for oral and oropharyngeal carcinomas [13–20]. Oropharyngeal carcinomas, tonsillar cancers in particular, show the strongest association with HPV, with some 60% being ascribed to HPV. A recent systematic review showed an overall HPV prevalence of 25.9% in specimens obtained from 5046 patients with HNSCC, analysed in 60 separate studies [13]. The prevalence of HPV was significantly higher (36%) among patients with oropharyngeal cancer than in oral (23.5%) or laryngeal (24%) carcinomas. HPV16 was the most prevalent genotype, accounting for 87% of the oropharyngeal, 68% of oral and 69%

of laryngeal carcinomas [9, 13]. Data (first suggested by us) concerning the causal role of HPV in sino-nasal squamous cell carcinogenesis are also emerging. The overall detection rate of oncogenic HPV types was 21.7% in all tested sino-nasal carcinomas reviewed by us in 2002 [21]. HPV-positive HNSCCs seem to differ from HPV-negative HNSCCs. Patients with HPV-positive HNSCCs are often diagnosed at a late stage, with large cystic lymph nodes in the neck, and tend to be less differentiated on light microscopy. Despite this, HPV-positive HNSCCs have better outcome and survival than HPV-negative HNSCCs [13].

oropharyngeal cancer

The incidence of both tonsillar cancer and cancer of the base of tongue is increasing [22–24]. This increase has been attributed to an increase in HPV infection. In Sweden, an overall increase in the incidence of cancer of the base of the tongue from 0.15/100 000 person-years during 1970–74 to 0.47/100 000 during 2005–07 was reported [22]. In the meantime, the prevalence of HPV in cancer of the base of the tongue in Stockholm county increased from 58% during 1998–2001 to 84% during 2004–07 ($P < 0.05$). In HPV-positive tumours, HPV16 dominated (86%), but interestingly, HPV33 was detected in as many as 10% of cases. E6 and/or E7 RNA were found in 85% of the samples tested [22, 24]. This concomitant increase in incidence of cancer of the base of the tongue and proportion of HPV-positive tumours indicates that HPV may contribute to this increase [22]. According to SEER data (US National Cancer Institute), also in the USA the incidence of HNSCC at the sites that are potentially related to HPV infection (base of tongue, lingual and palatine tonsil, pharynx) significantly increased

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between 1973 and 2004, with an annual increase of 0.8% [23]. Thus, clinicians should be aware of this risk of oropharyngeal cancer among young people to avoid unnecessary delay in diagnosis and treatment.

the impact of method on HPV detection

The wide variation in HPV detection rates can be explained by several factors, such as (i) samples, i.e. whether frozen, formalin fixed or paraffin embedded, scrapings or oral rinses, (ii) sensitivity of the HPV testing method, as well as (iii) the coverage of HPV genotypes in the test panel. Currently, there is no consensus on the most appropriate method to detect HPV in HNSCC. The HPV testing methods are mostly based on detecting HPV DNA in cancer tissues either with *in situ* hybridization (ISH) or PCR or both. In a recent meta-analysis, the pooled prevalence of HPV DNA in the 4852 biopsy samples included in 62 studies was 34.5%; in oral squamous cell carcinomas 38.1%, and in non-site-specific HNSCC 24.1% [12]. With regard to the detection method, PCR-based studies reported a higher prevalence rate than ISH-based rates (34.8% compared with 32.9%) especially in the oral cancer subgroup (PCR based: 39.9%) [12]. Nested PCR will further increase the sensitivity compared with single PCR. In a comparative study, nested PCR using the novel PGMV/GP(+) primer set combination was found to be more type sensitive than nested PCR with the MY/GP(+) primer set, detecting (i) a wider range of HPV types, (ii) low-copy HPVs and (iii) better characterizing the samples infected with multiple strains of HPV [25]. Also the HPV detection rate is usually higher in frozen biopsy samples than in formalin-fixed samples. In addition, oral scrapings are not reliable for detecting HPV in HNSCCs [8, 14, 26, 27].

HPV16 is the most common type in HNSCC. In a recent meta-analysis, a total of 5681 patients were included. The prevalence of HPV+ tumours was 22%, with 86.7% of HPV16+ genotype [13]. However, not all HPV-positive head and neck tumours are transcriptionally active [20]. The odds ratio (OR) for HNSCC in HPV16+ patients was 4.44 [95% confidence interval (CI) 2.87–6.02] [13]. However, HPV6 and HPV11 can also be found in a minority of these cancers, implying that these low-risk HPV types are not entirely benign types while infecting the oral, oropharyngeal or upper respiratory sites [9]. It is still too early to confirm whether the eight most common HR-HPV types in cervical cancer (16, 18, 31, 33, 35, 45, 53, 58) are also the most prevalent types in oral, oropharyngeal and laryngeal cancers, because of the lack of studies using HPV testing methods covering most of the mucosal types, e.g. Luminex-based multiplex genotyping, which can detect 100 different HPV genotypes simultaneously [28].

risk factors

Patients with HPV-positive HNSCC tend to be younger and have a lower intake of tobacco and alcohol [14]. Distinct molecular profiles separate them from HPV-negative cancers and show many similarities with HPV-positive cervical squamous cell cancer. There is evidence that HPV-positive HNSCC is a sexually transmitted disease. According to the current literature, the risk factors of HNSCC are surprisingly similar to those of cervical cancer and CIN, including the number of sexual partners, younger age at first sexual intercourse, practice of oral sex, history of genital warts and younger age [7, 8, 11, 14, 29]. HPV status is also associated with p16 expression (adjusted OR 3.00; 95% CI 0.90–9.70), and HPV+ tumours are less likely to harbour p53 mutations (adjusted OR 0.21; 95% CI 0.04–0.38) [13, 30–32].

prognosis of HNSCC is related to HPV status

Several studies have reported that detection of HPV DNA is closely associated with poor differentiation of the tumour, positive lymph nodes and late-stage disease, which traditionally indicate poor prognosis [14, 15, 26, 27, 29]. Despite this, patients with HPV-positive HNSCC seem to have significantly better response to chemotherapy and radiotherapy as compared with HPV-negative HNSCC, and these patients also seem to have a lower risk of second primary cancers. A study reviewed all published reports and conducted a meta-analysis on the relationship between HPV and overall survival (OS) and disease-free survival (DFS) in HNSCC [30]. Patients with HPV-positive HNSCC had a lower risk of dying (meta HR 0.85; 95% CI 0.7–1.0), and a lower risk of recurrence (meta HR 0.62; 95% CI 0.5–0.8) than HPV-negative HNSCC patients [30]. Site-specific analyses showed that patients with HPV-positive oropharyngeal tumours had a 28% reduced risk of death (meta HR 0.72; 95% CI 0.5–1.0) in comparison with patients with HPV-negative tumours. Similar observations were obtained for DFS (meta HR 0.51; 95% CI 0.4–0.7). There was no difference in OS between HPV-positive and HPV-negative patients with cancers at non-oropharyngeal sites. Thus, this improved OS and DFS for HPV-positive HNSCC patients is specific to the oropharynx, implying that these tumours may have an etiology distinct from the tumours at non-oropharyngeal sites [14]. Recent studies have also shown that patients with HPV16-positive HNSCC that express wild-type *TP53* and/or p16 have an improved DFS [20, 31, 32], which supports the notion that the improved prognosis may in fact be attributed to HPV infection.

Although the exact mechanism is not fully understood, three possible explanations could be: (i) the genome of HPV-positive cancer cells is less unstable and/or (ii) HPV-positive cells suffer from hypoxia and can be more easily induced to apoptosis or (iii) treatment improves the local immunity favouring the eradication of HPV (and regression of the tumour) [13, 27, 30].

prophylactic HPV vaccines and HNSCC

At present, there are two prophylactic HPV vaccines commercially available: bivalent (HPV16/18) vaccine Cervarix® (GSK), and the quadrivalent (HPV6/11/16/18) Gardasil® (Merck). Licensed globally, these two vaccines have been loaded with great expectations in prevention of infections and tumours induced by the vaccine HPV types. Theoretically, there is no reason why these vaccines should not work against these same viruses at other anatomical sites as well. If proved to do so, this would represent a major conceptual breakthrough, not only in prevention of these diseases, but equally importantly, by providing the ‘missing link’ in the chain of evidence with the final proof of HPV etiology of these tumours.

conclusions

HPV-positive HNSCCs are emerging as a separate biological entity among all HNSCCs. Particularly in cancers of the

oropharynx, HPV has been associated with the highest prevalence in the tonsils. HPV infection has been associated with more favourable disease outcome, although the reason for this is not clear. Further studies are needed to dissect the HPV-positive HNSCC in more detail. In diagnosis, additional methods of HPV DNA testing are needed, such as p16, p53 epidermal growth factor receptor immunostaining or real-time PCR for HPV oncoproteins E6 and E7, to delineate which subgroup of HPV-associated HNSCC has the most favourable outcome after chemo- or radiotherapy. In addition, the identification of activated pathways will aid in developing new treatment modes for these cancers. More information is also needed on why HPV copy numbers are higher and HPV integration is more rare in tonsillar cancer than in other HPV-related HNSCCs [17]. Also more natural history studies on symptomatic HPV infections in the head and neck region [11] are urgently needed to identify those infections that have an increased risk of progression towards malignancy.

disclosures

The author has consulted for Sanofi–Pasteur MSD in subjects related to head and neck cancer and HPV.

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