REVIEW

Human Papilloma Virus (HPV) in head and neck region: review of literature

L'infezione da papilloma virus umano (HPV) nel distretto cervico-facciale: revisione della letteratura

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SUMMARY

The evidence that human papillomavirus infection is related to head and neck squamous cell carcinoma is supported by molecular and epidemiological data. The definition of a distinct subset of head and neck squamous cell carcinoma, independent of the traditional risk factors and with different clinical presentation and outcome, has led to increasing interest in human papillomavirus infection. This review summarizes current knowledge regarding human papillomavirus biology, oncogenic mechanisms, risk factors for transmission, clinical significance and prophylactic strategies.

KEY WORDS: Human papillomavirus • Head and neck • Squamous cell carcinoma • Oropharynx • Oral cavity

RIASSUNTO

Dati molecolari ed epidemiologici sostengono una correlazione tra infezione da papilloma virus umano (HPV) e carcinomi squamocellulari del distretto cervico facciale (HNSCC). La definizione di un sottogruppo di HNSCC, indipendente dai tradizionali fattori di rischio e con caratteristiche cliniche e prognostiche differenti, ha portato ad avere un interesse sempre maggiore nell'infezione da HPV. Questa review riassume le conoscenze attuali sulle caratteristiche biologiche, i meccanismi oncogenetici, i fattori di rischio per la trasmissione ed il significato clinico e terapeutico dell'infezione da HPV.

PAROLE CHIAVE: *Papilloma virus umano • Testa e collo • Carcinoma squamocellulare • Orofaringe • Cavità orale*

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Introduction

Squamous cell carcinomas (SCCs) represent the most frequent malignancy in the head and neck region. They originate from the pluristratified squamous epithelium which lines the upper aerodigestive tract and are characterised by a multi-phasic and multi-factorial aetiopathogenesis [1-10].

Common risk factors in head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol abuse, however, in an increasing proportion of cases, no significant smoking or drinking history has been reported.

Approximately 35 years ago, a role of human papillomavirus (HPV) in cervical cancer was postulated. Today, it is well established how this very heterogeneous virus family represents an important human carcinogen, causing not only the vast majority of cervical and ano-genital tumours, but also a variable number of cancers in other districts of the human body including the head and neck. In females, HPV infections, on a global scale, account for more than 50% of infection-linked cancers, in males it is responsible for barely 5% [11].

HPV positive HNSCC have been reported to share some epidemiological and biological characteristics [3].

This review will attempt to focus on relevant characteristics of HPV, analyse its role in oral and oro-pharyngeal cancer and discuss some emerging developments.

Human Papilloma Virus (HPV) infection

Papilloma viruses are members of the Papillomavirus family and together with Polyomaviruses form the species Papovaviridae. The HPV virion consists of a circular double DNA strand of about 7.9 kb, protected by a small capsid. The capsid is about 55 nm in diameter and consists of only two structural proteins. HPV genomes reveal a well-preserved general organisation. All putative open reading frames (ORFs) are restricted to one DNA strand.

The second, presumably non-coding strand, contains only short ORFs which are conserved regardless of localization and composition. The individual frames are classified as "early" (E) or "late" (L) genes not unlike other DNA viruses, where genes are turned on according to a specific time schedule in the course of a productive infection. The so-called early genes (E1-E8) are expressed shortly after infection and prior to the onset of DNA replication. Products of these genes mediate specific functions controlling replication and expression of viral DNA. In the case of oncogenic viruses, early gene products are also involved in the transformation of the host cell. The late genes (L1-L2) code for the structural proteins of viral capsid and are activated during the final stages of the viral cycle. Up to 6 early genes and 2 late genes can be detected in HPV [12-20].
HPVs are epitheliotropic viruses that, in the majority of cases, produce a benign epithelial proliferation. However, some viral types can be associated with malignant transformation. The genomes of many HPV types have been re-isolated, sequenced and compared to reference "prototypes", countless times, by laboratories throughout the world. It was found that each HPV type occurs in the form of "variants", identified by about 2% nucleotide differences in most genes and 5% in less conserved regions. Less than 100 variants of any HPV type have been detected, a scenario that is very different from the quasi-species formed by many RNA viruses. The variants of each HPV type form phylogenetic trees, and variants from specific branches are often unique to specific ethnic groups. Immigrant populations contain, depending on their respective ethnic origins, mixtures of variants. Currently, more than 200 types of HPV are known. The absence of HPV genomes, intermediate to specific types, show that all HPV types existed already when humans became a species. A growing number of epidemiological, aetiological and molecular data suggest that variants of the same HPV type are biologically distinct and may confer differential pathogenic risks [21].
According to the oncogenic potential, they are classified as low-risk and high-risk [22]. Both high-risk and low-risk types of HPV can cause the growth of abnormal cells, but only the high-risk types lead to cancer because only the E7 protein encoded by high-risk HPVs can immortalize human epithelial cells. Sexually transmitted, high-risk HPVs include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73 [23]. It is important to note, however, that in the genital tract the large majority of high-risk HPV infections regress on their own and do not cause cancer [24].
The association between HPV and squamous cell lesions at various sites of the body, including the oral cavity, was first described by Syrjänen et al. [25], in 1983. The results suggested that HPV might be the agent involved in the development of at least certain special types of oral SCCs (OSCC) [25]. In the next few years, the literature provided further quantitative evidence that oral infection with HPV, particularly with high-risk genotypes, is a significant independent risk factor for OSCC [21 26 27].

Oncogenetic mechanism of HPV infection

On the basis of epidemiological and molecular evidence, in 1995, the International Agency for Research on Cancer recognized that high-risk HPV types 16 and 18 are carcinogenic in humans [28].
It is widely accepted that cancer of the uterine cervix is related to HPV infection. These two HPV types are responsible for approximately 70% of cervical cancer cases [29].
In addition, high-risk HPVs are associated with other ano-genital carcinomas, including vulvar, anal, and penile cancers [30 31] and with some HNSCCs [3].
HPV infection was first held responsible for the development of head-and-neck cancer in certain individuals lacking the classical risk factors for this disease (tobacco and alcohol abuse).
The majority of HPV-related cancers contain HPV DNA integrated into the host cell genome and express only two viral genes, E6 and E7, both of which encode oncoproteins [32].
Through wounds or abrasions, the papilloma viruses infect the basal epithelial cells that are the only actively dividing cells in the epithelium. Virus maturation is closely related to the degree of epithelial differentiation. Expression of early viral antigens is found in cells of the basal layer whereas late viral antigens are formed in the superficial keratinizing epithelial layer [32-36].
The E6 protein of the high-risk HPVs binds and induces the degradation of the p53 tumour suppressor protein via an ubiquitin-mediated process, while the high risk E7 may play a role in the HPV life cycle by disrupting pRB family member-mediated transcriptional repression of certain genes involved in cell cycling. The low-risk HPV-6 E7 protein binds to pRB family members with lower affinity than that of high-risk HPV-16 and it is not able to immortalize the cells [37-41].
Loss of cell cycle and apoptosis control, therefore, constitutes an early and central event in HPV-mediated carcinogenesis and the integration of HPV DNA into the host genome is believed to be the key event [42 43]. However, recent studies suggest that transcription of HPV-16 E6/E7 mRNA in tonsillar carcinomas is not necessarily dependent on viral DNA integration and that the viral DNA is predominately in episomal form [44] and, in this form, also takes part in the carcinogenesis process [45].
Gene expression profiles of HPV-positive and negative HN and cervical cancers show substantial differences. A distinct and larger subset of cell cycle genes is up-regulated in HPV-positive tumours. Moreover, HPV-positive cancers have been shown to over-express testis-specific genes that are normally expressed only in meiotic cells. HPV-

positive tumours are characterised by the loss of pRb and Cyclin D1 expression and by over-expression of p16. On the contrary, HPV-negative over-express pRB and Cyclin D1, and under-express p16. These findings emphasize the potential value of targeting E6 and E7 protein. The product of the E6 gene binds wild-type p53 and induces its degradation. The loss of functional p53 impairs apoptosis and induces genetic instability. Moreover, E6 inactivates telomerase, an enzyme that maintains telomeric DNA stability. Protein E7 binds retinoblastoma protein (pRb) and other related proteins. In this way, it causes the release of transcriptional factors that activate genes regulating cell proliferation. pRb down-regulation by HPV E7 results in p16 up-regulation[46-54].

At least in SCCHN, HPV-induced genomic instability does not necessarily lead to cancer. There are many factors involved in carcinogenesis, HPV infection being only one of them. However, HPV high risk serotypes are detected 3-fold more frequently in patients with malignant lesions than in those with benign or pre-cancerous lesions[54].

Risk factors for HPV infection and cancer

Epidemiological studies on cervical cancer have clearly demonstrated that high-risk mucosatropic HPVs are transmitted by sexual contact [28]. The means by which HPV is transmitted to the upper airways is unclear. Oral HPV infection is rare in newborn babies of infected mothers [55] and in children prior to sexual activity [56]; infections increase after onset of sexual activity [57].

It is generally assumed that HPV infection precedes the development of HPV-positive HNSCC: the presence of high risk HPV infection in the oral mucosa and seropositivity significantly increase the risk of development of OSCC [58-61]. Therefore, risk factors for HPV oral infection are likely, by extension, to be risk factors for HPV positive HNSCC.

Patients with HPV-positive tumours appear to be distinct from HPV-negative patients. While tobacco-associated OSCCs are more frequent in men, men and women are at equal risk of HPV-associated OSCC. In addition, patients with HPV-associated OSCC are often non-smokers and non-drinkers [62-64] and younger than patients with HPV-negative tumours [65].

Although evidence suggests that HPV is associated with cancer in non-smokers and non-drinkers, the degree to which oral HPV infection may combine with tobacco and/or alcohol use to increase the risk of cancer is unclear. Controversial data exist, suggesting a concurrent action, either as a synergistic (multiplicative) [66] or as an additional effect [2].

Several case-control studies have reported that certain types of sexual behaviour increase the risk of OSCC. Risk factors, among men, include young age at first intercourse, number of sexual partners and history of genital warts. Risk is increased in women with a large number of sexual partners [66-69].

Furthermore, some investigations have demonstrated that certain types of sexual behaviour are strongly associated with the risk of HPV-positive tumour, including history of oral sex and oral-anal contact [70 71]. An increased risk of HPV-associated OSCC in individuals with a history of HPV-associated ano-genital cancers and in husbands of women with *in situ* carcinoma and invasive cervical cancer confirms oral-genital transmission, although direct mouth-to-mouth contact could not be excluded as a means of transmission [72].

The role of marijuana use in HPV-associated OSCC is still unclear, as well as the relationship with poor oral hygiene; a recent study reported that HPV-positive cancers were independently associated with sexual behaviour and exposure to marijuana but not related to tobacco smoking, alcohol drinking, and poor oral hygiene [73].

Increased risk for OSCC seems to be related also to immuno-suppression. Recent evidence has indicated that HPV-related diseases are increased in the oral cavity of human immunodeficiency virus (HIV)-positive individuals [74] and new data imply that the problem of HPV-related cancers do not decrease in HIV-positive men and women in the anti-retroviral therapy era [75]; genetic susceptibility such as that in patients with Fanconi anaemia also increase the risk of HPV-mediated tumourigenesis [61].

Oral and oro-pharyngeal squamous cell carcinomas related to HPV infection and clinical meaning

Oral and oro-pharyngeal squamous cell carcinoma is a disease usually related to environmental exposures, primarily tobacco and alcohol abuse. However, 15-20% of these cancers occur in patients without traditional risk factors [3 61-66 76].

Data appearing in the literature have provided strong evidence that HPVs may be the cause of a defined subset of head and neck cancers, particularly those of the oro-pharynx (tonsils and throat) and also an indicator of improved survival. The International Agency for Research on Cancer conducted a multicentre case-control study on oral cavity and oro-pharynx carcinomas, in 9 countries [61]. Of these tumours, 70% harboured HPV DNA. HPV16, most commonly observed in genital cancers, was also the most common type found in these tumours. The study concluded that HPV appears to play an aetiologic role in many cancers of the oro-pharynx and possibly a small subgroup of cancers of the oral cavity [61].

Some studies aimed at defining the molecular profile of HPV-positive OSCC. In a recent study, OSCC HPV-positive cancers with a high p16 expression showed significantly better response, independently of the treatment (surgery vs organ-sparing) [62]. However, at present, data on

immunohistochemical markers of radiochemiosensitivity are controversial. An organ-sparing trial on advanced oropharyngeal cancer demonstrated that low EGFR and high p16 (or higher HPV titre) expression were favourable predictive markers in outcome [77] whereas a retrospective study concerning surgically treated OSCC, characterized by the same immuno-histochemical profile (HPV positive/high p16 expression) showed that p16 over-expression is not a prognostic marker of relapse and second tumours [78].

The presence of antibodies against HPV, in the serum, should be an easily accessible marker of virus detection [79]. The human body produces antibodies against viral capside proteins, against early viral proteins, including oncoproteins E6 and E7. The relationship between the levels of antibodies and presence of HPV positive tumour cells could be used in early diagnosis [60], monitoring the course of the disease and early detection of recurrence of oral and oro-pharyngeal cancer [59 78 80]. Approximately 10% of healthy individuals develop a persistent infection and it is this cohort that should be monitored as being at risk of cancer progression [81].

In the majority of the studies, OSCC related to HPV infection shows a better outcome and a reduced risk of relapse and second tumours compared with HPV-negative tumours [2 59-61 82 83]. The concept of better treatment results in HPV-positive tumours has been confirmed both in patients submitted to organ-preservation treatment as well as in those submitted to_surgery [82-84]. The reason for the improved survival is unclear. However, better outcomes seem to be attributed to the ability of HPV-positive cancer cells to induce apoptotic cell death in response to DNA damage [85]. Another reason appears to be attributed to the absence of carcinogen-induced early genetic changes in the epithelium and the development of multifocal tumours ("field cancerization concept") [86].

The enhanced radiochemiosensitivity reported in the literature highlights the need of meticulous clinical trials to determine optimum management of HPV-associated OSCC, compared with HPV-independent OSCC [87 88].

A better understanding of HPV-associated carcinogenesis is necessary for the development of HPV-targeted strategies. In the absence of cell mutations, classically associated with carcinogenesis in tobacco-associated oropharyngeal cancers, the repression of E6 and E7 expression, in HPV-positive carcinomas, results in restoration of Rb and p53 tumour-suppressor pathways and is sufficient to arrest cell growth or cause apoptosis [89 90]. In fact, HPV-associated cancers constantly express the HPV E6 and E7 viral oncogenes, even in the late stages of the disease, and repression of viral oncogene expression by drugs that interfere with the expression or function of viral proteins can induce senescence or apoptosis of cancer cells. Moreover, therapeutic vaccines that elicit a cytolytic immune response to cells expressing viral proteins could be curative, possibly even in the advanced stages of the disease [89 90].

As far as diagnostic implications are concerned, it has been postulated that the identification of HPV in metastatic cervical lymph nodes could be used to assess the tonsillar origin of metastatic carcinomas with an unknown primary tumour. HPV-positive metastases have been identified not only by ISH for HR-HPV and p16 immuno-reactivity [91 92] but also by the strong correlation with a distinct non-keratinizing morphology [93 94].

Vaccination

Vaccines designed strictly for prevention of cervical cancer and vulvar genital warts have been introduced over the last few years. To date, there are two commercial vaccines, quadrivalent Gardasil® (registered trademark of the Merck & Co., Inc., USA) protects against HPV types 6, 11, 16, 18 and bivalent vaccine, Cervarix® (registered trademark of the GlaxoSmithKline Group of Companies, Australia) which targets HPV types 16 and 18. Experimental studies with L1-VLP vaccines showed their ability to induce natural immunity. Antibodies are a primary defence against HPV infection. Both existing vaccines are able to create a robust humoral immune response [95 96] which is much more effective than the levels of antibodies that can be acquired after a natural infection, and persist for a period of at least 60 months [97]. A 5-year follow-up demonstrated 100% effectivity in the prevention of persisting infection and HPV-16 and HPV-18 CIN 2/3 lesions in young women.

Oral HPV infection seems to be the main risk factor for cancerogenesis of HPV-positive oro-pharyngeal cancer and HPV 16 (type contained in both above-mentioned vaccines) is found in the majority of cases of HPV positive oral cancer [98]. For this reason, it might be possible to prevent or even treat these cancers by means of vaccines designed to elicit appropriate virus-specific immune responses. It is tempting to suggest that if high-risk HPV infection is prevented, the subsequent development of invasive cancer caused by HPV will be abolished. The impact of current HPV vaccines on the incidence of persistent oral HPV infection remains to be identified. Clinical trials to evaluate the efficacy of the quadrivalent HPV vaccine in protecting against oral HPV infection are currently underway.

HPV infection is, generally, sexually acquired [99], and, therefore, vaccination should be performed prior to sexual debut in order to prevent serious HPV-related genital and oral diseases. All vaccine trials reported to date have been designed to investigate the ability of the vaccines to generate protection against the consequences of ano-genital HPV infection in women. However, there is reason to be optimistic that the existing vaccines may be protective against oral HPV infection, and, therefore, effective in preventing vaccine-type HPV-associated head and neck cancer both in men and women. Although vaccination

currently involves exclusively the female population, immunogenicity studies have demonstrated that the vaccines elicit a robust humoral immune response also in men, an important finding considering that the majority of HPV-associated head and neck cancers occur in men. Thus, it is possible that an HPV vaccine could have benefits beyond the target population [94-104].

Data on oral HPV infections, in the immunization setting, are limited to animal models, which have shown a protective effect and a reduction in the development of HPV-related oral lesions [105 106]. These data imply that therapeutic vaccines will likely be effective for low-volume disease. Therefore, these vaccines could be used as adjuvant treatment following surgery or radiotherapy to clear microscopic residual disease by generating an immune response.

Conclusion

In recent years, there has been an increase in the annual incidence of HPV-related HNSCC in the United States and Europe [107].

Approximately 75% of the adult population in Europe has been, or will become, infected by one or more HPV serotypes [108] and evidence is accumulating for the aetiological role of HPV in the pathogenesis of potentially malignant oral mucosal lesions and SCC [109].

OSCC is increasing in incidence in patients without the usual risk factors for the disease. Practitioners need to be aware that young, non-smoker patients are also at risk for certain types of head and neck cancer [110]. It has now become clear that this subset of HNSCC is a sexually transmitted disease with distinct pathogenesis and clinical/pathological features [107].

These findings implicate further research efforts in a screening project on a healthy population and scrupolous detection of HPV-related tumours.

In the majority of studies, HPV-associated HNSCCs have a better prognosis compared to the stage-matched non-HPV-related HNSCC. Clinical trials are now focusing on de-intensification of treatment to reduce treatment-associated morbidity and HPV-targeted therapies are under investigation [107].

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