Role of human papillomavirus in oral squamous cell carcinoma and oral potentially malignant disorders: A review of the literature

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Abstract

Human papillomaviruses (HPVs) are epitheliotropic viruses with an affinity for keratinocytes and are principally found in the anogenital tract, urethra, skin, larynx, tracheobronchial and oral mucosa. On the basis of high, but variable frequency of HPV in oral squamous cell carcinoma (OSCC), malignant potential of HPV infection has been hypothesized but not definitely confirmed. The aim of this review was to highlight the genomic structure and possible mechanism of infection and carcinogenesis by HPV in the oral mucosa and to review the frequency of HPV prevalence in OSCC and oral potentially malignant disorders. A computer database search was performed through the use of PubMed from 1994 to 2014. Search keywords used were: HPV and oral cancer, HPV and oral leukoplakia, HPV and oral lichen planus, HPV and OSCC, HPV and verrucous carcinoma, HPV and proliferative verrucous leukoplakia, HPV and oral papilloma.

Key words: Human papillomavirus, oral squamous cell carcinoma, oral potentially malignant disorder

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide. Several risk factors are related to OSCC, with the main being tobacco use, alcohol consumption, and infection by high-risk genotypes of human papillomavirus (HPV). The specific role of HPV in the development of OSCC is still under debate despite its well-established role in the vast majority of squamous cell carcinoma of the cervix. The relationship between HPV and OSCC was first suggested in 1983, but the presence of viral DNA was only confirmed 2 years later, by means of in situ hybridization (ISH).

In this article, we are reviewing the genomic structure of HPV, its transmission, life cycle, mechanism of carcinogenesis with review of the studies on prevalence of HPV in OSCC and oral potentially malignant disorders (OPMDs) from 1994 to 2014.

HUMAN PAPILLOMAVIRUS GENOME

Human papillomavirus is a small, nonenveloped, double-stranded, circular DNA virus with a diameter of 52–55 nm. The genome contains a double-stranded DNA molecule that is, bound to cellular histones and contained in a protein capsid without envelope. The HPV-DNA genome encodes approximately eight open reading frames (ORFs). The ORF is divided into 3 functional parts: The early (E) region comprising 45% of genome, the late (L) region extending for 40% of genome and a long control region (LCR).

Early ORFs encode for E1, E2, E4, E5, E6, and E7 proteins which are necessary for replication, cellular transformation, and the control of viral transcription. E1 and E2 maintain viral DNA in an episomal form and facilitate the segregation of the viral genome during cell division. During productive infection, E6 and E7 stimulate cell cycle progression. E1, E2, E4, and E5 are required for and expressed during viral DNA amplification which occurs in differentiated cells in upper epithelial layers. Late region encodes...
the structural proteins or capsid proteins that take part in virion assembly. L1 ORF encodes for major capsid protein and L2 ORF for minor capsid protein. Noncoding upstream regulatory region encompassing the origin of replication, the E6/E7 gene promoter, and the enhancers and silencers, is located between the early and the late regions. LCR is necessary for viral DNA replication and transcription.\cite{2-4}

The different HPV types are characterized by genotypic variations in the DNA base sequences of E6 and E7. It is these genotypic differences that permit stratification of the virus oncogenic phenotype into high and low-risk type. High risk includes HPV-16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59 and low risk are HPV 6, 11, 42, 43, 44,\cite{2-4}

**TRANSMISSION**

Prevalence sites of HPVs include the epithelium of the vagina, vulva, penis, anal canal, cervix, perianal region, crypts of the tonsil, and oropharynx. The normal oral mucosa may act as a reservoir for new HPV infections and/or as a source of recurring HPV-associated lesions. The prevalence of HPV in normal oral mucosa range from 0.6% to 81%.\cite{5,6} Multiple pathways for HPV transmission to the oral cavity can exist. These include sexual transmission, autoinfection, and rarely through perinatal transmission of the neonate during its passage through an infected birth canal of the mother.\cite{5,7} Oral HPV acquisition was found to be more positively associated with number of recent oral sex and open mouth kissing partners than with the number of vaginal sex partners.\cite{5,6}

**MECHANISM OF INFECTION BY HUMAN PAPILLOMAVIRUS**

Human papillomaviruses are characterized by a special tropism for squamous epithelial cells, keratinocytes. The synthesis of viral DNA and the expression of viral genes are linked to the keratinocyte level of differentiation.\cite{8,9}

During the initial phase of infection, the viral genome undergoes episomal replication, and few copies of viral DNA per host cell are present. The episomal form acts as a reservoir of infected cells and is responsible for the latent state of infection.\cite{18}

When the infection becomes productive, the viral genes are expressed sequentially from early genes to late genes, following the epithelial squamous differentiation, starting from basal and parabasal cells, where early portions of the viral genome are more active and proceeding to higher epithelial layers along with the formation of complete virion.\cite{8,10} In HPV-infected basal cells, E1 and E2 proteins are expressed, and they regulate early viral DNA transcription. When expression of E2 is more pronounced, E2 represses viral DNA replication by blocking cellular transcription factors, thus controlling the number of HPV DNA copies in the basal cell by a process analogous to negative feedback.\cite{11,12} In cases of high-risk HPV infection, E6 and E7 may also be expressed in HPV-infected basal cells, and the epithelium may then enter a proliferative phase characterized by an increasing number of HPV-infected basal cells, culminating in intraepithelial or invasive neoplasia.\cite{11,13} As the basal cells divide, E2 mediates distribution of some HPV DNA copies to daughter cells, while some copies remain in the progenitor cells, in both cases, as episomes.\cite{11,14} As the epithelial cells mature, HPV cycle progresses to productive replication.\cite{11,13} In HPV-infected epithelium, the matured epithelial cells express HPV E6 and E7 proteins in the suprabasal layers. E6 prevents apoptosis and E7 activates the cellular DNA replication mechanism allowing matured epithelial cells to re-enter the S-phase of the cell cycle, and makes the cellular replication machinery available for viral DNA replication.\cite{11,12,14,15} Specific cellular factors associated with epithelial cell maturation activate late viral promoter located within E7 ORF, which activate late viral gene expression. Eventually, mediated by L1 and L2 proteins, the virus escapes from the shedding epithelial cells.\cite{11,13}

**MECHANISM OF HUMAN PAPILLOMAVIRUS INDUCED CARCINOGENESIS**

The possibility of evolving into direction of malignancy depends on the type of virus, the synergic action with different physical, chemical, and biological agents, the genetic constitution, and the immune defense mechanisms of the host, all of which are able to modify the course of HPV infection. In the case of high-risk HPV infection and under favorable conditions, the viral genome is integrated into the host genome, which is the necessary event for the keratinocytes immortality.\cite{13}

During this process of integration, the circular form of viral genome breaks at the level of the E1 and E2 regions. The loss of E2 during this process of integration produces the loss of E6 and E7 control.\cite{12-4} Therefore, the sequences E6 and E7 are directly involved in the cellular cycle by inhibiting the normal functions of p53 and pRb, respectively.

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The most manifest function of the E6 protein is to promote the degradation of p53 through its interaction with a cellular protein, E6 associated protein (E6AP). The p53 tumor suppressor gene itself regulates growth arrest and apoptosis after DNA damage. In addition, E6 interferes with other pro-apoptotic proteins, Bak, and procaspase 8, to comprehensively prevent apoptosis.\textsuperscript{16-18} Recently, the product of the notch1 gene has been identified as a novel target of p53.\textsuperscript{16,19,20}

Over the past dozen years or so, an increasing number of other proteins have also been revealed to be target proteins of E6 that might contribute to cellular transformation, with telomerase as one probable important example.\textsuperscript{16}

E7 is known to bind to the retinoblastoma tumor suppressor gene product, pRb, and its family members, p107 and p130. In the hypophosphorylated state, pRb family proteins can bind to transcription factors such as E2F family members and repress the transcription of particular genes involved in DNA synthesis and cell cycle progression.\textsuperscript{16,21} Because E7 is able to bind to unphosphorylated pRb, it may prematurely induce cells to enter the S phase by disrupting pRb–E2F complexes. The E7 protein function enables HPV replication in the upper layers of the epithelium where uninfected daughter cells normally differentiate and completely exit the cell cycle. P16\textsuperscript{INK4a}, which prevents the phosphorylation of pRb family members, is overexpressed when pRb is inactivated by HPV E7. Thus, overexpression of p16\textsuperscript{INK4a} is suggested to be a useful biomarker for evaluating HPV pathogenic activity.\textsuperscript{16,22}

E6 and E7 can cooperate with cellular oncoproteins such as ras and myc, which enables the virus to act at the level of growth factors and cellular and nuclear metabolism producing oncogenic cells.\textsuperscript{16}

We carried out PubMed search for prevalence of HPV in oral lesions including Oral Lichen Planus, lichen planus, OSCC, oral papilloma, verrucous carcinoma (VC), and PVL during period of 1994–2014. The results are presented in Tables 1 and 2.

A total of 50 studies are included in this review including frequency of HPV in OSCC, OL, oral lichen planus, VC, PVL, and benign and malignant papillary lesions. Except for 8 studies which utilized \textit{In situ} hybridization (ISH) as the assay for detection, most of the authors quantified HPV-DNA using polymerase chain reaction (PCR). The frequency of HPV in OSCC varied from 0% to 80%. The HPV type most commonly detected in OSCC and OPMDs was HPV-16, 18 with HPV-6, 11 found in only a few studies. Whereas, HPV type found in oral benign lesions and papilloma was HPV-6 and 11.

Miller and Johnston in a meta-analysis of OSCC observed that HPV may be a significant and independent risk factor. The prevalence of HPV in OSCC varies depending on several parameters such as geographic differences in population, type of specimen, selection of preparation method, and use of HPV detection method.\textsuperscript{71} A brief review of the association of various parameters and OSCC based on these studies is presented as follows:

Molecular factors
Human papillomavirus-positive OSCCs seem to have a different molecular profile compared with that of HPV-negative cancer. In addition, HPV-positive cancers share some similarities with cervical carcinoma. By immunohistochemistry (IHC), most HPV-positive tumors show p16 overexpression. The expression of p53 and bcl-2 is not associated with HPV-positive OSCC and mutations in p53 are rarely seen in HPV-positive tumors compared with HPV-negative tumors.\textsuperscript{72} Genetic signatures of HPV-positive OSCC have been shown to be different from those of HPV-negative OSCC.\textsuperscript{72}

Influence of method used for analysis
There is a wide array of assays used for detection of HPV in sample including PCR, ISH, IHC, and
Western blot analysis with PCR being the most widely used to estimate the HPV-DNA in samples. Besides the method used, different results are obtained when using fresh frozen or formalin-fixed paraffin-embedded material. HPV in saliva and oral exfoliated cells has been detected in some recent studies, but the sensitivity and specificity are too low, and the role of HPV detection in saliva is still uncertain.

**Other risk factors and human papillomavirus**

No correlation between HPV-positive OSCC and tobacco or alcohol consumption has been found. A strong association has been found between sexual behavior and risk of HPV infection. In India, HPV-DNA was detected less frequently in tumor specimens from tobacco chewers than in those from nonchewers.

**Patient factors**

Patients with HPV-positive OSCC usually are younger and more often present at a higher stage and with large metastatic lymph nodes.

**Prognostic factors**

Many studies have now confirmed that HPV-positive tumors in head and neck area have a better prognosis compared with those that are HPV-negative. The better prognosis is independent of the treatment given. The positive prognosis is also more pronounced in HPV-positive patients who are also p16 positive.

**CONCLUSION**

Risk factors mainly responsible for OSCC include tobacco, alcohol, ultraviolet rays but many cases...
have none of these identifiable risk factors. On the basis of high frequency of HPV in some types of OSCC and OPMDs, an oral malignant potential of HPV infection in oropharyngeal carcinoma is likely. Of particular significance is the association of high frequency of HPV in oral cancers involving base of the tongue, in those occurring in younger patients and without the prior history of exposure to the usual risk factors. Still further research is needed in order to standardize a particular protocol for screening of patients with OSCC and OMPDs for HPV as well as to determine a specific and universal method/assay for analysis.

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