Abstract. Human papillomaviruses (HPVs) are a group of host-specific DNA viruses, with a remarkable epithelial cell specificity: they have been reported principally in the ano-genital tract, urethra, skin, larynx, tracheo-bronchial and oral mucosa. More than 100 different HPV types have been identified and classified as high (e.g. 16, 18, 31) or low (e.g. 11, 42, 36) -risk (HR and LR), based on their association with cervical carcinoma. The carcinogenic role of HR-HPV revolves mainly around two of its oncoproteins: HPV-E6 which promotes degradation of the p53 tumour suppressor gene product and HPV-E7 which modifies the pRb tumour suppressor gene product, inhibiting the activity of TGF-ß2. Since these viral oncoproteins are capable of transforming primary human keratinocytes from either genital or upper respiratory tract epithelia, they have been considered to play a role in disrupting cell-cycle regulatory pathways leading to a genetic progression to ano-genital cancer and, possibly, also to oral squamous cell carcinoma (OSCC). Recently, the oncogene HPV-E5 has also been found to transform cells by modulating growth factor receptors. On the basis of the high, although very variable, frequency of HR-HPV in OSCC, an oral malignant potential of HPV infection has been hypothesised but not definitively confirmed. Major aims of this review are to update the understanding of HPV activities with respect to oral oncology and to comment on the HPV DNA reported frequencies in OSCC and potentially malignant oral lesions. A computer database search was performed, through the use of MEDLINE (PubMED) and Cochrane Library, for the last three decades. Search key words used were: human papillomavirus, HPV and cancer, HPV and oral lesions, HPV and oral premalignant lesions, HPV and oral cancer, HPV and HNSCC, HPV and oral mucosa. The search was of all fields, all languages and all dates available.

Contents

1. Human papillomavirus: identikit of a virus
2. HPV and oral oncology
3. Conclusions

1. Human papillomavirus: identikit of a virus

Structural characteristics and natural history of infection. HPVs belong to the new Papillomaviridae family and are an heterogeneous group of viral agents which infect epithelia, with an intra-nuclear mode of replication (1). HPVs have a small diameter (50 μM) and a genome made of around 7200-8000 base pairs (5.2x10 dalton molecular weight), covered by an iso-exahedric capsid without envelope and consisting of 72 capsomeres (2-4).

Capsid proteins are represented by a major capsid protein, L1 (of ~54,000 daltons molecular weight), and a minor capsid protein, L2 (of ~76,000 daltons molecular weight). The latter, unlike the major capsid protein, appears to be highly type-specific and can therefore be used as a target in the immunohistochimical typing of HPV infection (5,6). The viral DNA guanine-cytosine content ranges between 42.6% and 50%, i.e. very similar to that of human host cells, where the guanine-cytosine base content ranges between 42% and 43% (7).

Molecular biology techniques have facilitated the characterization of the entire HPV genome, where three different functional regions are identified, as a profile of their gene expression. The first region (Early or ‘E’ region) extends for approximately 45% of the genome and codifies early-functional proteins. The second region (Late or ‘L’) extends for approximately 40% of the viral DNA and codifies late-structural proteins. The third region (Long Control Region or
‘LCR’), contains sequences regulating gene transcription, and performs exclusively regulatory functions (8) (Table I). The two first codifying regions contain nucleotide sequences defined as ‘Open Reading Frames’ (ORFs), with the potential for transcription of specific mRNA (9).

**HPV types.** From the phylogenetic point of view, HPVs are classified according to the level of homology existing among nucleotide sequences in the genomic regions (i.e. E6, E7 and L1). If the homology with respect to existing types is <90% the HPV is classified as a new type; if homology is 90-98% it is classified as a sub-type; if homology is ≥98% it is classified as a variant (6,10,11).

Thus far, more than 100 different genotypes of HPV have been isolated, by means of molecular cloning in plasmids or bacteriophages, sequenced and are available in the HPV database (http://hpv-lanl.web.gov).

**HPV infection.** HPVs are mostly transmitted by close contact, especially sexually but vertical spread (passage through the uterine canal during delivery) and self-inoculation are also recognised routes of infection (12-16).

After HPV inoculation, three mechanisms of infection can manifest: i) Plasmid replication, which occurs in the cells of lower epithelium and may in turn subdivide into two phases; a) amplification of viral DNA up to 50 to 400 couples/diploid genome, and b) maintenance of a constant number of couples for several cell generations. ii) Vegetative replication, which occurs in cells that differentiate from the epithelium and involves a link between cell differentiation and viral expression of the gene. iii) Productive replication, in which the virus is expelled from the epithelial cells when they undergo desquamation and is transmitted by direct contact (especially genital warts), or by indirect contact.

HPVs are characterized by a special tropism for squamous epithelial cells, keratinocytes. The synthesis of viral DNA and the expression of viral genes (especially for those codifying capsid proteins) are linked to the keratinocyte level of differentiation (Fig. 1). The normal viral replication cycle is a highly regulated process, depending both on some viral proteins codified by the viral genome and the degree of differentiation of the infected cell. Infection usually starts in the basal and

---

**Table I. HPV genoma and functions of codified proteins.**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>pE1</td>
<td>Initiating DNA replication and transcription</td>
</tr>
<tr>
<td>pE2</td>
<td>Controlling DNA replication and transcription (ORFs E6-E7)</td>
</tr>
<tr>
<td>pE3-pE8</td>
<td>Not still clear</td>
</tr>
<tr>
<td>pE4</td>
<td>Disrupting the cytoskeleton</td>
</tr>
<tr>
<td>pE5</td>
<td>Interacting with cellular proteins (EGFR)</td>
</tr>
<tr>
<td>pE6</td>
<td>Degrading p53</td>
</tr>
<tr>
<td>pE7</td>
<td>Binding Rb proteins</td>
</tr>
<tr>
<td>pL1</td>
<td>Capsid major structural protein</td>
</tr>
<tr>
<td>pL2</td>
<td>Capsid minor structural protein</td>
</tr>
</tbody>
</table>

---

**Figure 1. HPV infection in relation to keratinocyte level of differentiation (modified by Longworth).**
para-basal cells of squamous epithelium. The changes in keratinocytes from the basal layer to the surface of the epithelium, provide a suitable micro-environment for productive cell replication, responsible for the transformation of the keratinocyte into a permissive cell (17).

To activate infection, the virus must have access to the ‘generative’ compartment of the epithelium through the exposure of superficial layers to reach the basal layer where the specific $\alpha$6 integrin receptor is present (18). Then, during the initial phase of infection, when the virus colonizes basal and para-basal cells of the epithelium, the viral genome undergoes episomal replication, since it is present as an extra-chromosomal fragment of circular DNA. At this stage of episomal or early replication, relatively few copies of viral DNA (20-200) per host cell are present. The episomal form acts as a reservoir of infected cells, which are morphologically indistinguishable from non-infected cells, and is responsible for the latent status of infection.

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 para-basal cells, where early portions of the viral genome are more active and proceeding to higher epithelial layers (both the intermediate and the superficial) along with the formation of the complete virion (i.e. the infecting viral particle) (19). The classic viral cytopathic effects may then appear: koilocytosis in particular, is considered as the evident expression of a viral cytopathic effect. The koilocytic cell shows a thicker cytoplasm at the level of the cell internal membrane wall and an atypical morphologically collapsed and stellate nucleus (20,21). Histologically, HPV infection may then appear as acanthosis, dyskeratosis, keratinocyte multinucleation and koilocytosis.

Immune responses play an important role in HPV infections. It is well documented that, in immunocompetent subjects, skin warts often regress spontaneously whereas, in immune deficiency, a higher incidence and persistence of skin and mucosal infections induced by HPV are found (22). Nevertheless, in HPV-related diseases in immunocompetent hosts, immune responses are generally poorly expressed, as compared to those seen in other viral infections, presumably because the HPV replication cycle takes place inside maturing keratinocytes which are able to continuously remove mature virions, and HPV thus tends to cause cell proliferation rather than cell lysis (23). As a result, during HPV infection, the local or systemic viral antigen presentation to the immune system by antigen-presenting cells (APC), is minimal and thus infection often persists for months or even years (24), albeit without necessarily being recognised clinically. Absence of clinically detectable lesions is not, therefore, synonymous with absence of HPV infection.

Molecular and oncological aspects. Based on their relationship with cervical cancer, HPVs have been divided into two groups: those at ‘high risk’ (HR-HPV: 16-18-31-33-35) associated with potentially and overtly malignant lesions and those at ‘low risk’ (LR-HPV: 6-11-13-32), more commonly associated with benign diseases (18,25,26).

HPV genes. The HPV genome contains 10 genes, 3 of which have potential transforming action (E5, E6, E7). In synthesis, the initial viral infection factor is pE1 which binds to a genomic region rich in adenine and thymine and codifies for factors directly involved in plasmid replication. The other protein needed for replication is pE2, a polypeptide with trans-activating function, that binds DNA in the ACCNGGTT sequence, common to all papilloma viruses. The E5 region codifies for a 7-kD protein that, alone, can cause stable alterations in cell growth and morphology. Expression of the E6 and E7 genes mediates the blockade of cell differentiation and cell immortalization respectively, by codifying oncoproteins pE6 and pE7, forming complexes with the p53 and p105Rb tumor suppressor gene products respectively, inhibiting their function as down-regulators in cell replication (27). HPV integration occurs through the viral episomal DNA rupture, in correspondence with E2 and E1 ORFs, with preservation of E6 and E7 segments that may therefore undergo transcription (28), causing a disturbance in cell control mechanisms and an increased proliferation of infected cells.

The consequence is unregulated cellular replication with an increased risk of chromosomal aberrations and excessive production of viral proteins interacting with cellular proteins (29,30) (Fig. 2).

HPV identification. HPV is not cultivable and, since direct investigation by electronmicroscopy or immunological analysis are not especially useful (antigenic viral components are not always present in the infected tissues), and histopathology is only suggestive, definitive diagnosis of HPV infection is now by molecular biology methods (e.g. techniques of amplification and/or hybridisation). The techniques of Southern blotting (SB) and In situ hybridization (ISH) have been used extensively in the past to identify viral sequences in tissues. SB requires the isolation and purification of cellular DNA from a specimen, and the DNA is then digested into fragments by restriction enzymes; the fragments are separated by size; immobilized on filters; and then probed with radiolabeled or chemiluminescent probes specific for a given HPV type. ISH does not require DNA isolation from tissue but rather the tissue or smear is probed directly for the presence of viral sequences (8).
More recently, polymerase chain reaction (PCR) has been recognised as the most appropriate method to identify and type the HPV genome (31) because of higher sensitivity and specificity. For example, studies on head and neck squamous cell carcinoma (HNSCC) using PCR show an average HPV positivity of 34.5% compared with 24.5% by SB (8). In a PCR reaction, the target sequence of viral DNA is denatured and hybridised with two small complementary genomic sequences (primers) which permit Taq polymerase-enzyme to bind to it and to duplicate the target DNA sequence. This series of reactions (denaturation, hybridisation of primers and polymerization of Taq) is then reproduced 30-40 times to hugely amplify the target DNA sequence that is then identified by other methods (e.g. migration on agarose-gel, hybridisation by specific probes).

2. HPV and oral oncology

LR-HPV (i.e. HPV types 2, 4, 11, 13, 32) are responsible for benign skin and mucosal lesions (ordinary warts, condylomas, focal epithelial hyperplasia, squamous cell papillomas, Bowen's papillomatosis), whilst HR-HPV (i.e. 16, 18, 31, 33, 35, 58) are related to potentially or explicitly malignant lesions (cervical intra-epithelial neoplasms, cervical, penile and vulvar carcinomas, giant condylomas of Bruschke and Lowenstein) (26,32-36). However, both kinds of HPV are sometimes recognised in clinically apparently healthy oral mucosa (37-43) and the significance of this, if any, is unclear.

The hypothesis of a role for HPV in HNSCC and in the onset of oral squamous cell carcinomas (OSCC) (54), is supported by the observation that oral mucosa has similar histological features and properties as vaginal mucosa (55) and that the virus is able to immortalize human keratinocytes in vitro (45,56-58).

HPV and potentially malignant oral lesions. HPV DNA prevalence, in oral potentially malignant lesions (PML) ranges from 0% to 85% (59,60) with a higher prevalence of HPV 16 or 18 genotypes (61-65). Such a wide variation is probably due to demographic variables, different categorization standards of lesions investigated (sometimes not supported by histology) and, above all, to different sampling techniques (biopsies, mouth rinsing or brushing) and different imaging techniques, from the less sensitive ISH to the highly sensitive 'nested' PCR (34) (Table II).

In order to understand the more recent findings on HPV infection in PML, we therefore analysed only the results from those studies investigating HPV infection in lesions clearly described from both clinical and histological viewpoints. Following data abstraction, the most well-defined PML that have been studied in relation to HPV infection are: oral leucoplakia (OL) and oral lichen planus (OLP) (66,67). Oral leucoplakia was defined as a white patch or plaque, clinically and pathologically unrelated to any other disease (Fig. 3a). From the viewpoint of aetiology, OL can be distinguished into OL correlated with the use of tobacco, and idiopathic OL, as showing a higher risk to progress to malignancies (68).

Aetiological role for HPV in OL was initially suggested from light microscopy studies (69), and supported by the
identification of HPV antigens (70) and then HPV-DNA (37,71). However, data are controversial with respect to the prevalence of HPV infection. Miller and White (39), in a review of studies using ISH to identify HPV in benign OL (i.e. without dysplasia), reported a prevalence ranging from 0% to 80%, significantly greater in fresh and frozen specimens (43.1%) than in paraffin-embedded tissues (12.2%). However, in OL with dysplasia and using PCR for HPV-DNA detection, the prevalence ranged from 17% (72) up to 68.6% (42). Moreover, if Uppsala criteria are considered (73) and the proliferative verrucous form of leucoplakia (proliferative verrucous leucoplakia or PVL) is excluded from the analysis, the overall HPV prevalence in other OL dropped to 17.6% (in paraffin-embedded biopsies from non-dysplastic and dysplastic lesions) (33).

Campisi et al, examined the relationship in OL between HPV and some molecular predictors of malignant progression such as apoptosis markers (bcl-2 and survivin) and proliferation markers (proliferating cell nuclear antigen; PCNA). The risk of HR-HPV infection was found to be independently associated with survivin and PCNA expression, suggesting that these markers could be involved in HPV-mediated disorders of epithelial maturation (74).

PVL is the form of OL thought to have the strongest relationship to HPV infection. This clinical disorder is a rare and particularly aggressive form of exophytic leucoplakia, with a high potential for malignant transformation (Fig. 3b and c) (75), with a 90% malignant evolution to carcinoma in situ (65), regional lymph-nodal involvement and distant metastases (76), and is highly resistant to treatment. Malignant transformation of PVL has been primarily found in elderly non-smoking women with a history of successive biopsies in long-standing leucoplakia (77). The aetiology of PVL does not seem to involve cigarette smoking or Candida spp infection, as suggested by Silverman and Gorsky (76). The association of PVL and HPV (especially genotype 16) has been supported by several reports (65,76,78), although with a wide range from 10% to 85%, of HPV infection (65,78) (Table III). However, a multi-centre study recently reported no statistically significant difference, in terms of HPV-DNA detection,
between PVL (24.1%) and common OL (25.5%), nor any special role for HPV in PVL onset (33). This controversy remains to be resolved.

Oral lichen planus is a chronic inflammatory disease of unknown aetiology and immune pathogenesis, with a small malignant potential (Fig. 3d). Information on HPV infection in OLP is scanty, but the few studies published report a high frequency (37.79%) from 27.3% (80), to 42.0% (81) or even 65.0% (82). Both Gonzales-Moles (83) and Jontell et al (82) observed that only the erosive variant of OLP was found to be HPV positive but this was not confirmed in another study (33). There is agreement between several authors (34,84) on the prevalence of HPV sub-type 18, as indicative of a peculiar geographic genotypic pattern of HPV (33).

Oral cancer and HPV. Extrinsic factors primarily responsible for the onset and evolution of OSCC include UV rays, tobacco and alcohol, especially if coexistent (85-89,90). In some
cases, trauma (86,91), poor oral hygiene (92-95), or Vitamin A, B and C deficits (96,97), may be implicated. However, many cases have none of these identifiable risk factors.

Miller and Johnstone (18), in a meta-analysis of OSCC, observed that HPV may be a significant and independent risk factor. The prevalence of HPV in HNSCC and OSCC varies, depending on variations in several parameters considered: ethnic and geographic differences in population, type of specimen (biopsies, scrapes), selection of preparation method (fresh frozen or fixed), use of HPV detection methods with different sensitivity levels (SB, ISH, PCR) and, especially, the different type of HNSCC examined. Although early data reported an HPV prevalence in OSCC ranging between 20% and 30% (39,98), numerous other studies have reported HPV-DNA in 50% of cases (44.64,99,100). In agreement with Ha and Califano (101), we have suspected a misestimating of these latter data of HPV prevalence, attributed mostly to the different identification methods used (Table IV).

It is also clear that HPV is more frequently detected in cancer of Waldeyer's tonsillar ring (102,103), thanks to the easier viral access to basal mucosal cells of the tonsillar crypts (104) than in cancer elsewhere in the mouth. However, few studies have been based on a correct distinction between cancers at oral and oropharyngeal sites, as recommended by the American Joint Committee on Cancer (AJCC) (105). For this reason, Herrero et al (103) and, later, Kreimer et al (106) hypothesized that, although there is a proven association between HPV and oropharyngeal tumours, the misclassification of some HPV-positive oropharyngeal cancers as OSCC, could partly explain the HPV-posivity of some 'oral' cancers.

Other authors hypothesise that the carcinogenic potential of HPV might be attributed to the concomitant action of several risk habits (i.e. tobacco smoking and alcohol drinking) and genetic factors (107). Consistently to this theory, Schwartz et al (111) reported a potential interaction effect with HPV expression only in current smokers. On the contrary, Hafcamp et al (108) showed a significant correlation between HPV integration and reduced or absent exposure to the known risk factors of HNSCC/OSCC. Smith et al (109) and Lo Muzio et al (110) demonstrated a statistically significant association between HPV infection and alcohol consumption but not with tobacco use, as being found to be even inversely related to HPV infection. In India, HPV DNA was detected less frequently in tumour specimens from tobacco chewers than in those from non-chewers (103). The theory that non-tobacco users are more likely than tobacco users to have HPV-related tumours could be explained by the evidence that alcohol acts as a permeability enhancer of oral mucosa altering the mucosal structure and thereby aiding penetration of HPV through the epithelial layers (110); whereas tobacco using promotes epithelial keratosis as inhibiting viral infiltration and maturation (103).

Some studies report, on an epidemiological and/or molecular basis, a significant association between HPV 16-18 infection and OSCC (42,43,113,114). Kreimer et al (106), in a recent systematic review of the literature, found HPV 16 and 18 to be the most common subtypes in HNSCC, respectively present in 16.0% and 3.9% of 2642 cases reviewed. These HPV subtypes are HR-HPV and able to transform both cervical and upper aero-digestive tract epithelia via expression of the viral oncoproteins E6 and E7 and the following deregulation of cell cycle and apoptotic pathways, by inhibiting respectively, the activity of the cellular p53 and Rb tumour suppressor proteins (115,116). HPV E6 and/or E7 transcripts and/or viral integration have been detected more frequently in OSCC containing HR-HPV (16, 18 and 33) (103).

In the light of these findings, it has been suggested that HPV detection, plus the expression of E6 and E7 are important prerequisites for HPV-dependent inactivation of p53 and Rb and important indicators of HPV involvement in oral carcinogenesis. In particular, Dai et al (115), in their recent AIRC multi-center study on the relationship between HPV 16 infection and TP53 mutation in oral cancer, conclude that it is not sufficient to find HPV-DNA in cancer specimens, but that some markers of E6 expression must also be identified, such as the presence of HPV 16 E6 antibodies or, as suggested by Akerman et al (117), increasing mRNA levels of epidermal growth factor receptor (EGFR).

One field recently investigated by Lo Muzio et al is the assessment of the type of relationship between HPV infection and cell cycle markers involved in the proliferation pathway and in the regulation of apoptosis. These authors found a negative association between HPV and PCNA, MIB-1 and survivin expression: indices of proliferative activity (PCNA and MIB-1 expression) were higher in HPV-negative cases (respectively 88.0% and 77.0%) than in HPV-positive cases (respectively 66.0% and 44.0%); similarly, survivin expression (an index of apoptosis blockage), was higher in HPV-negative cases (100.0%), than in HPV-positive cases (44.0%) with the maximum protective effect in HPV-positive smokers (110). Ringstrom et al reported a 5% mortality in HPV-16-positive OSCC vs 31% in an HPV-negative group (118), findings which, although suggesting a favourable prognostic value of infection, are yet to be explained.

Studies which have tried to define a carcinogenic role for HPV by examining viral infection in relation to conventional OSCC determination parameters, such as histological grading (G1-2-3) and TNM staging have found a significant correlation between HPV infection and poorly differentiated (G3) HNSCC, showing, at the same time, a better prognosis for those found to be positive to infection (42,114,119). Other authors, on the contrary, have failed to show any significant correlation between HPV-positive oropharyngeal carcinomas and their histological differentiation (43,118,120); whereas Hilga et al (121) observed such correlation in well-differentiated (G1) and Correnti et al (122) in moderately differentiated carcinomas (G2). As regards TNM staging, Schwartz and Yueth (123) have observed HPV more frequently in stage II and III OSCCs, while Smith et al (109) observed HPV in advanced stage HNSCC, characterized by lymph node involvement. Finally, Campisi et al, have recently shown that HPV infection, if simultaneously correlated with histological grading and TNM staging, seems to prevail in intermediate stage lesions (II/II), but without any specific histological degree. In other words, evidence relating HPV infection and histological grading of OSCC appears equivocal (124).

3. Conclusions

Risk factors primarily responsible for OSCC include UV rays, tobacco and alcohol, but many cases have none of these
identifiable risk factors. HR-HPV is able to transform epithelia via expression of the viral oncoproteins E6 and E7 causing deregulation of cell cycle and apoptotic pathways, by inhibiting respectively, the activity of the cellular p53 and Rb tumour suppressor proteins. On the basis of the high frequency of HR-HPV in some types of OSCC, an oral malignant potential of HPV infection in oropharyngeal carcinoma is likely. The precise role of HPV in oral PML and in other forms of OSCC remains to be elucidated.

References


