Chapter 3

Human Papillomavirus and Carcinogenesis in the Upper Aero-Digestive Tract

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Additional information is available at the end of the chapter

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1. Introduction

Infectious agents are suspected to play causal roles in a variety of human malignancies. The public health impact of the oncogenic effects of these infections is considerable. Infection is estimated to be responsible for about 17.8% of all incident cases of cancer worldwide, accounting for 26.3% of all malignancies in economically developing countries and 7.7% in developed countries [1].

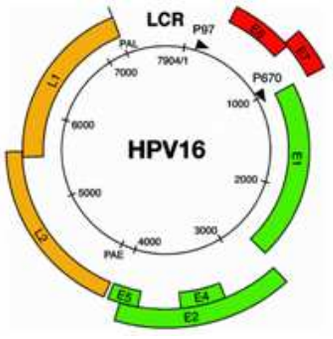
The evaluation of causality for these infectious agents as human carcinogens is difficult given their ubiquitous nature, the substantial length of time between infection and the cancer event, the nature of cofactors, and the rarity of malignancy among those infected. Thus, a central problem for the epidemiologist is to define the natural history of infection and to identify those factors that are related to the development of cancer. Hence, informative biomarkers of the agent (such as viral load), of the host (such as abnormal antibody pattern), and of other oncogenic exposures (such as tobacco use) are required for understanding the viral-human interactions and for developing interventions [2].

Case-control studies have now recognized that human papilloma virus (HPV) infection in the oral cavity is a strong risk factor for head and neck squamous cell carcinoma (HNSCC), and mostly for oropharyngeal cancer. The risk is increased for high-risk HPV-16 infection [3]. Therefore, HPV infection in the oral cavity has important health consequences, requiring more studies about these aspects to clarify the implications of a diagnosis of HPV in the oral cavity and HNSCC.

2. Human papillomavirus (HPV)

HPVs are nonenveloped icosahedral viruses with a diameter of 55 nm, belonging to the *papillomaviridae* family. This epitheliotropic virus has 72 capsomers enclosing an 8 kbp-long

circular DNA genome. Although its DNA is double-stranded, only one strand contains open reading frames (ORF) that are transcribed. The viral DNA has eight ORFs and an upstream regulatory region, also called the long control region (LCR), which contains an origin of replication and cis-acting transcriptional regulatory elements [4]. Figure 1 shows the genome organization of HPV-16, the HPV type most strongly related to cervical cancer. The early region of the HPV genome contains six ORFs corresponding to E1, E2, E4, E5, E6, and E7 genes, which encode proteins necessary for viral replication and cell transformation. The late region codes for the two proteins of the viral capside: L1, the major structural protein; and L2, the protein linking to encapsulated DNA [4].



PROTEIN	FUNCTIONS
E6	Destruction of p53 tumor supressor protein (Accumulation of mutation and Apoptosis inhibition)
E7	Inactivation of pRb tumor supressor protein (Cell cycle progression and accumulation of p16INK4a)
E1	Viral DNA replication
E2	Viral DNA replication; repression of E6/E7 genes
E4	Assembly and release of the viral particle
E5	Interaction with the Epidermal Growth factor (EGF)
L1	Major capsid protein in the viral particle
L2	Minor capsid protein in the viral particle

Figure 1. HPV genomic organization. The early region of the HPV genome contains six ORFs corresponding to E1, E2, E4, E5, E6, and E7 genes, which encode proteins necessary for viral replication and cell transformation. The late region codes for the two proteins of the viral capside: L1, the major structural protein; and L2, the protein linking to encapsulated DNA.

3. Classification of papillomaviruses

The L1 ORF is the most conserved gene in the papillomavirus (PV) genome and has therefore been used for identifying new PV types over the past 15 years. A new PV isolate is recognized as a new PV type if the complete genome has been cloned and the DNA sequence of L1 ORF differs by more than 10% from any known PV types. A difference between 2% and 10% homology defines a subtype, and less than 2%, a variant [5].

HPV has more than 100 types, of which approximately 90 have already been characterized and assigned with numbers, and has five genuses: alpha-papillomavirus, beta-papillomavirus, gamma-papillomavirus, mu-papillomavirus, and nu-papillomavirus [5]. HPV is also subdivided into two major groups, cutaneous and mucosal, based on data from clinical manifestations [6]. Most mucosal HPV types exist in the genital area, which can be divided into high-risk and low-risk HPV types [7]. HPV of high-risk types increases the risk of cervical cancer, which is almost always associated with HPV infection. To this date, approximately 20 HPV types have been identified as high-risk. Among them, HPV-16 and HPV-18 are considered to be associated with 70% of all cervical cancer. In contrast, low-risk type HPV, such as HPV-6 and HPV-11, causes genital warts but not cancer.

Furthermore, HPVs have intratype variants. However, information on variants is limited to certain HPV types. Yamada *et al.* [8] showed five phylogenetic clusters with distinct

geographic distributions, analyzing the sequences of E6, L1, and LCR of HPV-16 isolated from cervical samples collected worldwide. The AA (Asian American) variant was isolated mainly from Central and South America and Spain. The African variants 1 and 2 (Af1 and Af2) and Asian (As) variant were present mainly from Africa and Southeast Asia, respectively. In all regions other than Africa, the European-350T (E-350T) prototype as well as the European-350G (E-350G) variant were detected.

4. HPV life cycle and its carcinogenesis

The life cycle of HPV is linked to the differentiation program of the infected host cell, epidermal or mucosal epithelial cell. Cells in the basal layer consist of stem cells, which persist indefinitely, and a much larger number of "transit amplifying cells", which arise from the stem cells and divide a finite number of times until they become differentiated, providing a reservoir of cells for subrabasal regions [9].

HPV initially infects the basal layer of epithelia via minor abrasions. Viral entry into a cell is not clearly understood. It is suspected that the heparin sulfate mediates the initial attachment of virions to cells [10] and that HPV enters a cell via interaction with certain receptors such as alfa-6 integrin for HPV-16 [11].

The first HPV genes to be expressed are E7 and E6 (Figure 2). The virus protein E7 promotes cell division by binding to pRb, a tumor suppressor protein that usually binds to and inactivates E2F, a transcription factor. E2F released from pRb causes transcription of genes involved in DNA replication and cell division. E6 virus protein binds to and inhibits p53 protein, which is active in repressing the cell cycle in the event of DNA damage, and in triggering apoptosis in the case of damage too severe to be repaired. E6 virus protein also activates cellular telomerase that synthesizes the telomere repeat sequences in eukaryotic cells and allows their immortal replication [12]. The transforming activities of high-risk HPV type represent a consequence of a viral replication strategy that is driven by the necessity to replicate the HPV genomes in suprabasal cells [12]. During the early phases of infection, the copy number of viral genome is between 50 and 100, and the viral genome exists as extrachromosomal plasmid or an episomal form that replicates as the host cell chromosomes replicate. As the infected cells differentiate, the rest of the early viral genes, such as E1, E2, E4, and E5 genes, become switched on [13]. E1 and E2 proteins, a helicase and a transcription factor binding to LCR viral region, respectively, support viral DNA replication so that the infected stem cells can be maintained in the lesion for a long period. E4 viral protein is thought to be involved in activating the productive phase of the HPV life cycle. E5, another viral protein, is involved in transformation, enhancing the activity of EGF. As infected daughter cells migrate to the upper layers of the epithelium, viral L1 and L2 late gene products and the major and minor viral capsid proteins are produced to initiate the vegetative phase of the HPV life cycle, resulting in high-level amplification of the viral genome. In the upper layers of stratified squamous epithelia, viral DNA is packaged into capsids and produced virions are freed through normal desquamation processes, triggering little inflammation [14]. In addition, E6 and E7 proteins inactivate interferon regulatory factor [15] so that HPV infection can remain persistent and asymptomatic.

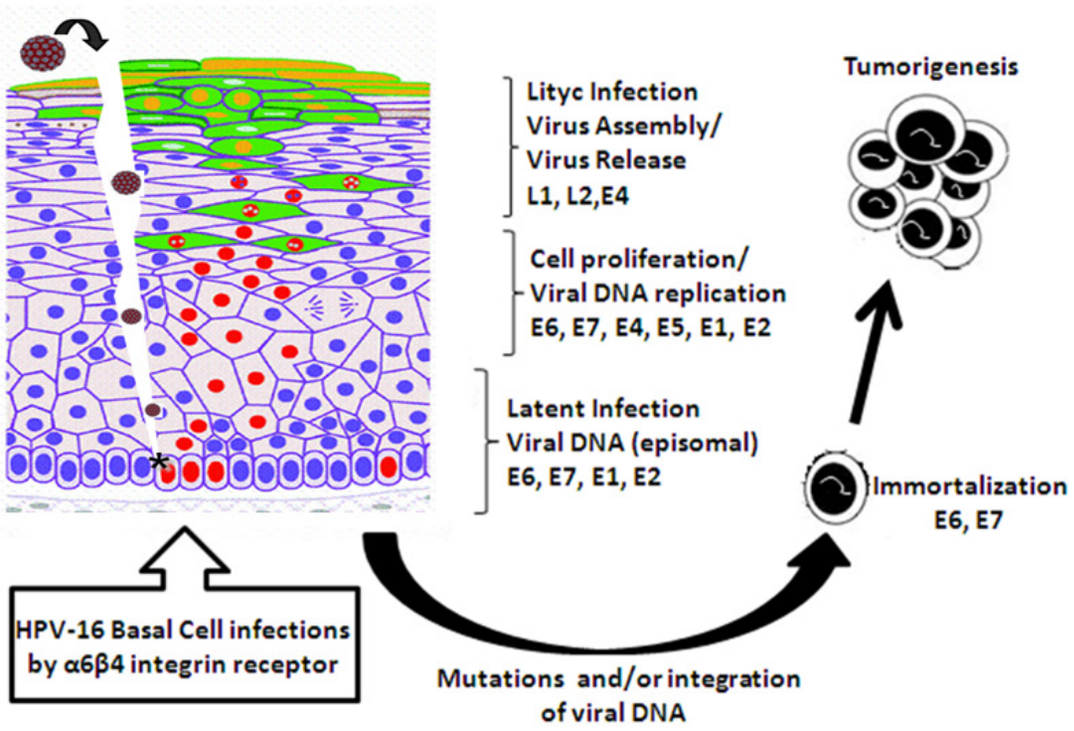


Figure 2. HPV life cycle. HPV establishes latent infection in the basal cells of the differentiating epithelium as episomal multicopy circular nuclear plasmids in order to support the viral life cycle via action of the viral replication proteins E1 and E2. Development of invasive cervical cancer is a stepwise process, which is associated with integration of the high risk HPV DNA into the host cell chromosome, upregulating the expression of viral oncoproteins E6 and E7.

The infection with high-risk HPV is associated with cervical dysplasia or cervical intraepithelial neoplasia (CIN). Long-term persistent HPV infection in these lesions is thought to give rise to cervical cancers. CIN I (mild dysplasia) and CIN II (moderate dysplasia) lesions, in which the viral genomes replicate episomally, show relatively low levels of E6 and E7 gene expression, and are, in most cases, resolved spontaneously by an effective immune response (Figure 3). In contrast, CIN III (severe dysplasia, carcinoma in situ) and invasive cancer lesions, where viral DNA is integrated into the host genome in most cases, often display high-level expression of E6 and E7 genes [16].

The integration of the viral genome into the host cell is a very rare event with a predilection for host chromosomal fragile sites [17], but after it has happened carcinogenic transformation progresses rapidly. HPV integration into the host genome induces the increased E6 and E7 protein expressions since integration results in disruption of HPV E2 gene, which is a negative regulator of HPV E6 and E7 transcription. In addition, once integrated, the E6 and E7 mRNA gains a longer half-life by using host genome poly (A) signals. However, the ultimate development of cervical cancer is rarely accompanied by high expression of E6 and E7 proteins [17]. High-risk HPV E6 and E7 oncoproteins can each independently induce genomic instability in normal human cells [18]. They cooperate to generate mitotic defects and aneuploidy through the induction of centrosome abnormalities

in normal human epithelial cells, and the characteristic multipolar mitoses in cervical lesions are caused by centrosome abnormalities [19]. HPV oncoproteins expressing cells also exhibit centrosome-independent manifestations of genomic instability. These manifestations include anaphase bridges that may be caused by double-strand DNA breaks as well as lagging chromosomal material [20]. To date, information on co-factors of HPV-related carcinogenesis in extra-genital organs is quite limited. Muñoz et al. [21] proposed the following three groups of potential cofactors in the cervical carcinogenesis: i) environmental or exogenous cofactors, including hormonal contraceptives, tobacco smoking, parity, and co-infection with other sexually transmitted agents; ii) Viral cofactors: such as HPV types, multiple HPV type infections, HPV integration, HPV viral load, and HPV variants; and iii) Host cofactors: including endogenous hormones, genetic factors, and other factors related to the immune response.

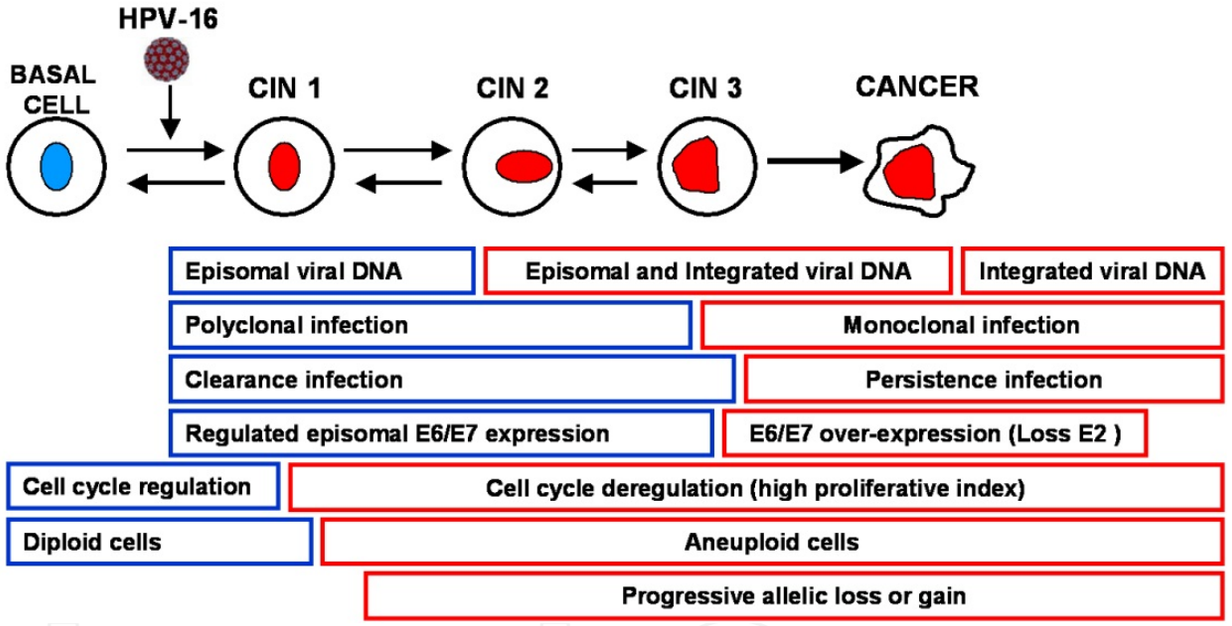


Figure 3. Cervical carcinogenesis. A long-term persistent HPV infection in cervical dysplasia or cervical intraepithelial neoplasia (CIN) could possibly lead to cervical cancer by integration of viral DNA into the host genome and overexpression of viral genes E6 and E7.

5. Squamous cell carcinomas (SCC) in the upper aero-digestive tract (UADT)

5.1. Epidemiology

Oral cancer is the 11th most common cancer in the world in terms of number of cases, while cancer of the pharynx ranks as 20th. Worldwide, about 389,000 new cases occurred in 2000, two-thirds of which were in economically developing countries, and these cancers are responsible for some 200,000 deaths each year [22]. The male-female ratio of its incidence varies from 2 to 15 depending on the anatomical sub-site. An extremely high ratio is a

characteristic of cancers of the tongue, floor of mouth, and pharyngeal. Cancers of the mouth and anterior two-thirds of the tongue are predominant in economically developing countries, whereas pharyngeal cancers are common in developed countries and in Central and Eastern Europe. In most countries, oral/pharyngeal cancer incidence and mortality rates have either been stable or increasing in the last four decades [23]. Cancers of the esophagus are the sixth most frequent cancers worldwide. In 2000, the number of deaths due to esophageal cancer amounted to some 337,500 out of a total of 6.2 million cancer deaths worldwide [24]. About 412,000 cases of cancer of the esophagus occur each year, of which over 80% are in economically developing countries. The incidence of esophageal cancer shows a distinct geographical difference, which is more evident than in any other cancers. In certain regions in Asia, the incidence rates of esophageal squamous cell carcinomas (ESCC) are as high as 200 per 100,000. Even within these high risk areas, there are striking local variations in ESCC risk

5.2. Genetic alterations

The genetic alterations observed in the cancers of the UADT include activation of protooncogenes such as cyclin D1, MYC, RAS, EGF receptor, HST-1, and HST-2, as well as inactivation of tumor suppressor genes (TSGs), such as those encoding p53 and p16INK4a [25]. Likewise, in cancer of the esophagus, the mutation of the p53 gene is detected in 35-70% of tumors, depending on geographic origin. Mutations in p53 have also been observed in dysplasia and in normal mucosa adjacent to cancer lesions, and considered as an early event. The p16INK4a gene is another TSG that plays an important role in the UADT development, and p16INK4a is often subject to hypermethylation of its promoter region, resulting in down-regulation of its expression [26].

5.3. Etiology

Consumption of tobacco and alcohol, associated with a low intake of fresh fruit, vegetables, and meat, is causally associated with SCCs of the UADT worldwide. However, the relative contribution of these risk factors varies from one geographic area to another [27]. Smoking is estimated to be responsible for about 41% of oral/pharyngeal cancers in men, and 15% in women worldwide. In more economically developed countries, it is estimated that 90% of ESCCs are attributable to tobacco and alcohol, with a multiplicative increase in risk when individuals are exposed to both factors. In addition, it has been reported that a genetic polymorphism of aldehyde dehydrogenase 2 (ALDH2), which plays a role in ethanol metabolism, is significantly associated with ESCC in the Japanese population [28].

Other environmental risk factors include nitrosamines, deficiency of vitamins A and C, copper, and zinc, poor nutrition, and ingestion of pickled and preserved foods contaminated with fungi such as *Aspergillus flavum, Geotrichum candidum* and *Fusarium sp*. Infectious agents, such as HPV and EBV, have also been suggested to be involved in the development of cancer of the UADT [22].

5.4. HPV infection

The International Agency for Research on Cancer considers that there is convincing evidence that infection with HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -66 can lead to cervical cancer [29]. Regarding HPV-16, evidence supports its causal role in cancers of the vulva, vagina, penis, and anus. The association of HPV with cancers of the UADT is also suspected. The UADT consists of a complex mucosa-covered conduit for food and air that extends from the vermilion surface of the lips to the esophagus. Major malignancies observed in the UADT are cancers of the oral cavity, oropharynx, larynx, and esophagus. Among them, HPV-16 is strongly suspected to cause cancers of the oral cavity and oropharynx. Limited evidence is available for the association of HPV with cancers of the larynx and periungual skin, but there is insufficient evidence for roles of HPVs in cancer of the esophagus [30].

In our current research, we found HPV infections in 21 ESCC specimens (29%) [31]. Sequencing analysis of an amplified L1 fragment identified HPV-16 genotype in six Colombian cases (13%) and in five Chilean cases (19%). We also found that a large proportion of ESCC specimens harbour HPV-16 genotype in the integrated form in a certain geographical area with a high ESCC incidence in China [32]. In addition, studies on the association of HPV with cancers of the oral cavity, oropharynx, and esophagus, using cancer specimens from Japan, Pakistan, and Colombia found HPV DNA in around half of the cases in SCCs of the oral cavity and oropharynx, and in a smaller proportion of ESCCs. The high-risk type HPV-16 was the most prevalent type with a viral load in SCC of the tonsil similar to that of cervical cancer (Table 1). The HPV-16 genomes detected in SCCs of the oral cavity, oropharynx and esophagus were frequently integrated in the host genome [33].

The most recent systematic review that included 5,046 SCC of head and neck cancers cases from 60 studies employing PCR-based methods showed that the presence of HPV DNA was 25.9%, being significantly higher in oropharyngeal SCC (35.6%; range 11–100%) than in oral (23.5%; range 4–80%) or laryngeal SCC (24.0%; range 0–100%). HPV-16 accounted for a larger majority of HPV-positive oropharyngeal SCC (86.7%) than HPV-positive oral (68.2%) and laryngeal SCC (69.2%) [34]. Another meta-analysis of 4,680 samples from 94 reports published during the period between 1982 and 1997 showed that HPV was between 2 and 3 times more likely to be detected in precancerous oral mucosa and 4.7 times more likely to be detected in oral carcinoma than in normal mucosa [35]. Among the studies used in their meta-analysis, the largest-scale and most well designed study was the one by Maden *et al.* [36]. They examined 112 normal mucosa specimens and 118 oral carcinomas, and detected HPV-16 in six cases of oral carcinomas but only one sample of normal mucosa. On the other hand, HPV-6 was detected in 12 and 10 oral carcinomas and normal mucosa, respectively. A recent hospital-based case-control study of oropharyngeal cancer in the US detected HPV-16 DNA in 72% of 100 paraffin-embedded tumor specimens, and showed an association of oral HPV-16 infection with oropharyngeal cancer. The study also showed that 64% of patients with cancer were seropositive for the HPV-16 oncoproteins E6 or E7, or both [37]. HPV DNA in situ hybridization clearly showed its presence in the nuclei of cancer cells and not in

COUNTRY	Cancer site		HPV-16	HPV-16 viral load	HPV-16 physical status		
		N	Positive(%)	GM (95% C.I.)	Integrated	Mixed	Episomal
JAPAN							
	Tonsil	24	10(42)	17.76 (1.44 - 216.9)	6(60)	4(40)	-
	Tongue	4	1(25)	0.09	1(100)	-	-
	Others OC	13	6 (46)	0.04 (0.02 - 0.07)	6(100)	-	-
	Esophagus	75	9(12)	0.07 (0.02 - 0.22)	9(100)	-	-
			P* = 0.002	**P** < 0.001**			P*** = 0.083
PAKISTAN							
	Tongue	28	15(54)	0.01 (0.002 - 0.08)	13(87)	-	2(13)
	Others OC	20	12 (60)	0.16 (0.03 - 0.95)	11(92)	1(8)	-
	Esophagus	42	9(21)	0.12 (0.02 - 0.80)	5(56)	4(44)	-
			P* = 0.003	**P** = 0.031**			**P*** = 0.010**
COLOMBIA							
	Tongue	5	4(80)	2.1 (0.01 - 348.4)	2(50)	2(50)	-
	Others OC	1	1 (100)	1.29	1(100)	-	-
	Esophagus	49	6(12)	0.25 (0.04 - 1.73)	3(50)	1(17)	2(22)
			P* = 0.001	P** = 0.438			P*** = 0.844
Total							
	Tonsil	24	10(42)	17.76 (1.44 - 216.9)	6(60)	4(40)	-
	Tongue	37	20(54)	0.03 (0.01-0.23)	16(80)	2(10)	2(10)
	Others OC	34	19(56)	0.12 (0.04-0.37)	18(95)	1(5)	-
	Esophagus	166	24(14)	0.12 (0.05-0.27)	17(71)	5(21)	2(8)
			P* < 0.001	**P** < 0.001**			P*** = 0.141

* Fisher's exact test value for difference of HPV-16 detection rate among cancer sites
** Kruskal-Wallins test value for difference of HPV-16 geometric mean copies per cell
*** Fisher's exact test value for difference of HPV-16 genomic status

Table 1. HPV-16 genomes detected in SCCs of the oral cavity, oropharynx and esophagus.

surrounding normal cells. Another case-control study in the US found high-risk HPV, mainly HPV-16, more frequently in exfoliated oral cells from cancer patients than in those specimens from controls, suggesting an association of oral HPV infection with an increased risk of SCC in the head and neck [38]. In addition, elevated antibodies against L1 and/or E6/E7 were shown in an international study [39].

A review also showed that 15.2% of the 2,020 Esophageal SCC cases tested by PCR until the year 2002 were HPV positive [40]. However, the role of HPV in esophageal carcinomas remains unclear and controversial. European prospective serologic studies that used stored serum specimens [41] as well as a Chinese case-control study [42] found a strong association between the risk of ESCCs and seropositivity to HPV-16. In contrast, other retrospective studies conducted in Europe [43] and a large prospective serologic study in China [44] found no significant association of HPV-16 or HPV-18 with SCCs or adenocarcinomas of the esophagus.

Recently, in United State, Gillison [45] showed that HPV is a causal factor for a distinct group of UADT cancers particularly in oropharyngeal cancers that occur more frequently in men than women, where oral sex appear to be the principal risk factor for HPV-associated oral cancers in adolescents [46]. Also, the tumor HPV status may be a strong and independent prognostic factor for survival among patients with oropharyngeal cancer [47].

One important question is the route of HPV infection in the oral cavity, oropharynx, and esophagus tract. HPV is known to be sexually transmitted in the case of the anogenital organs. However, limited available data suggest that HPV infection in oral cavity is possibly sexually acquired: a history of sexually transmitted disease and number of oral sexual partners are associated with both oral HPV infection [48] and HPV-positive oropharyngeal cancer. Some data suggest that the presence and persistence of an oral high-risk HPV infection is associated with a persistent oral infection in a spouse [49] as well as with an increased risk for oral cancer among women with a history of cervical cancer and their husbands [50]. In addition, other several conceivable ways have been proposed for acquiring HPV infection in oral and pharyngeal cavities. These include intrapartum infection during passage through the infected birth canal, transplacental infection in uterus prior to birth, and postnatal infection by contact. For instance, HPV can be transmitted from a mother to her newborn baby during vaginal delivery resulting in recurrent respiratory papillomatosis. In addition, HPV DNA has been detected in the foreskin of normal newborn and in a high percentage of neonates vaginally delivered by HPV-infected mothers as well as in the amniotic fluid [51].

5.5. Etiological role of HPV

Studies on prevalence of HPV infections in premalignant and malignant lesions of the oral cavity suggested the implication of HPV during the early stages of oral neoplasia and a role in malignant progression [52].Studies in oral keratinocytes immortalized by HPV-16 have

demonstrated an accumulated progression of chromosomal aberrations as well as high levels of cellular differentiation [53], however, in nude mice model, the tumorigenic activity is possible if there is a chronic exposure to the carcinogen benzopyrene of tabacco [54]. Both benzopyrene stimulation and the HPV-16 infection in cultured oral epithelial cells have been shown to confer anti-apoptotic characteristics, such as downregulation in the expression of the Fas and Bax proteins, as well as overexpression of Bcl2 via p53 deregulation. Consequently, HPV alone is not sufficiently to induce malignant transformation in several oral anatomic locations. Therefore further studies are needed.

6. Methods for HPV detection

As HPVs cannot be cultured easily, HPV detection and genotype assays are based on the detection of viral nucleic acids, usually viral DNA. HPV-DNA is detected by target amplification methods and/or signal amplification methods. The most used target amplification-based method is the polymerase chain reaction (PCR) using conserved sequences of the HPV genome, almost exclusively within the L1 open reading frame (ORF). In our studies in UADT using formalin-fixed and paraffin-embedded specimens [31], we detected HPV-DNA by GP5+/GP6+ primer pair for PCR [55] that amplified 150 base pair regions within L1, and the results were confirmed by southern blot analysis. Also, sequencing of an amplified L1 gene fragment was used to identify HPV genotype. In other studies in the UADT using formalin-fixed and paraffin-embedded specimens [32,33], we used an ultrasensitive short-fragment PCR assay, the SPF10, which amplifies a 65 base pair region within L1 [56]. The HPV types were determined using the INNO-LiPA HPV genotyping v2 kit (Innogenetics NV, Belgium), which is based on the reverse hybridization principle. In brief, part of the L1 gene region of the HPV genome is amplified using SPF10 primers tagged with a biotin at the 5′ and denatured. Biotinylated amplicons are hybridized with specific oligonucleotides probes immobilized on the strip. In total 25 genotypes (HPV-6, -11, -16, -18, -31 -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -66, -68, -70, -73 and -74) were examined. Recently, real-time PCR assays have been used to determine the number of viral copies of HPV as well as to determine its integration status [57]. HPV-16 physical status is determined on the assumption that the E2 gene is disrupted in the integrated viral genome. On the other hand, episomal viral genome has equivalent copy numbers of the E2 and the E6 genes. In addition, mixed viral genome for HPV-16 shows both integrated form and episomal forms (figure 4).

Other target amplification-based methods such as reverse-transcriptase (RT-) PCR assays can be applied to detect HPV mRNAs in fresh-frozen specimens or samples in which RNA is well preserved (i.e. liquid-based cytology samples of cervical scrapings). Signal amplification methods are based on an initial hybridization step of nucleic acids in the specimen with target-specific probes in liquid phase or in situ on cells or tissue slides, after which the signal (i.e. the hybridization event) is amplified and ultimately visualized with one of the various available methodologies. The liquid-phase signal amplification method the Digene Hybrid Capture 2 (HC2) assay [58] (Qiagen, Gaithersburg, MD, USA), is the first

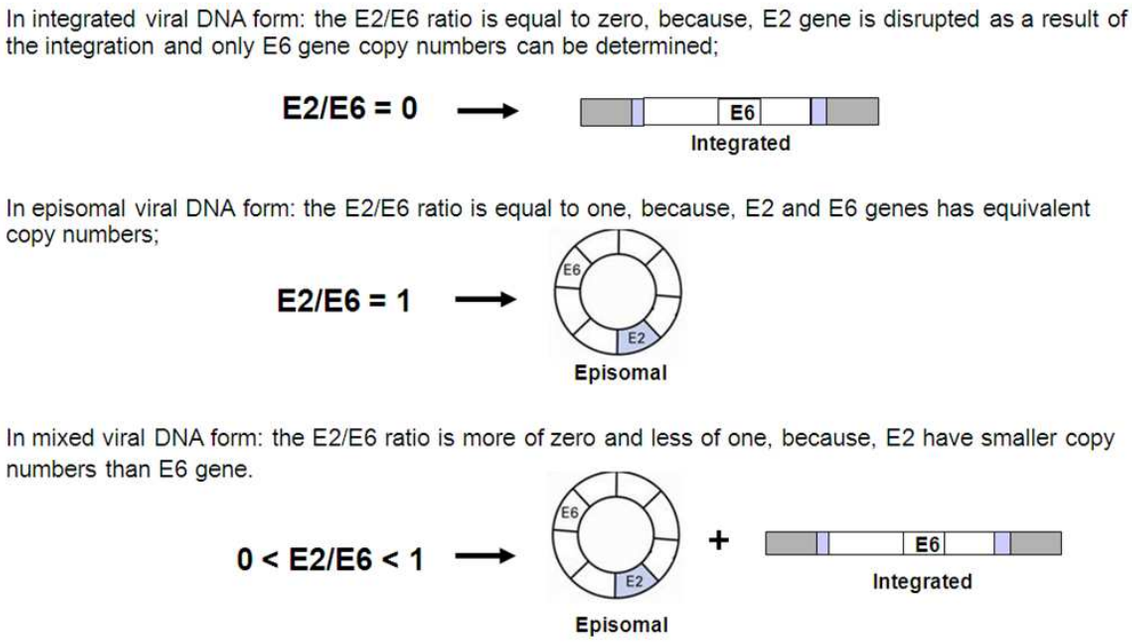


Figure 4. HPV Physical status by real-time PCR assay.

Food and Drug Administration (FDA) approved test that screens for the presence or absence of oncogenic HPV types. The HC2 assay uses a mixture of RNA probes representing 13 HPV genotypes (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68) to hybridize to HPV-DNA positive samples. DNA–RNA hybrids are subsequently captured in microplate wells coated with antibodies that specifically recognize DNA–RNA hybrids. For clinical validation of a new HPV tests a clinical equivalence analysis is necessary that compare the new test with a clinically validated reference for HPV using samples that originate from a population-based screening cohort.

Our studies showed that the HPV genome was detected in 56% and 19% of SCCs of the oral cavity and esophagus, respectively, in cases collected from Japan, Pakistan and Colombia and the HPV prevalence in both oral cancers (OCs) and esophageal cancers (ECs) did not significantly differ by country [33]. HPV16 was frequently integrated into the host genome in patients with OCs and ECs, however, the viral loads in these malignancies were much lower than those found in cancer of the cervix [33]. It should be noted, however, that human cancer is often regarded as a stem cell-like disease originating from a small fraction of cancer cells that show self-renewal and pluripotency and are capable of initiating and sustaining tumor growth [59]. This means that HPV may be present in only a small fraction of cancer cells with a stem cell-like nature present even in advanced tumors.

On the other hand, there was a significant geographical difference in the distribution of HPV16 E6 variants, which was also related to the viral load. HPV-16-positive OC cases with the E-350G variant showed a higher viral load than those with non-E-350G variants. Similar trends were observed in ECs although the difference was not statistically significant. Among HPV-16 intratypes, there is one polymorphism in the sequence of the E6 probe at nucleotide 145, and the Asian-American variant harbors this nucleotide substitution (C to T) [33]. However, this polymorphism is unlikely to cause a difference in viral load because the copy

number of HPV-16 in the Asian-American variant was similar to other intratypes except E-350G. The HPV-16 E-350G variant contains a polymorphism at residue 83, leucine to valine (L83V), which is associated with the risk of invasive cancers of the cervix in European studies [60,61]. Only the E-350T prototype and the E-350G variant were detected in Japan. On the other hand, in Pakistan, E-350G was the predominant HPV16 variant. In Colombia, the Asian-American variant was the most commonly found type, but this variant was not found at all in Japan and Pakistan. Our findings are similar to those of Yamada et al [25], who detected the E-350G, E-350T, and Asian-American variants in 52%, 25%, and 20%, respectively, of 228 HPV16-positive cervical cancer specimens from Central and South America. No particular HPV16 E6 variant predisposed those infected to OCs or ECs.

7. HPV vaccine

Preventive HPV vaccines are based on empty virus-like particles (VLPs) assembled from recombinant HPV coat proteins. Two prophylactic HPV vaccines are currently on the market: Gardasil and Cervarix. The vaccine works by making recipient immune to two key strains of the HPV (HPV-16 and -18). Together, the two strains are known to cause approximately 70% of all cervical cancer cases worldwide and some other genital cancers. Gardasil also protects against the two HPV types (HPV-6 and HPV-11) that cause 90% of genital warts [62]. In UK, the Joint Committee on Vaccination and Immunization recommended routine vaccination for 11 to 12-year olds, including the possibility of a catch-up campaign but only up to the age of 16. Similar recommendations on vaccination of young women against HPV to prevent cervical cancer were made by Public health officials in Australia, Canada, Europe, and the United States [63]. It is suggested that vaccinating most teenage girls could save hundreds of lives a year, although, the benefits would not be seen until those receiving the vaccine enter middle age.

In 2009, the Food and Drug Administration (FDA) licensed to Gardasil for use in males aged 9 through 26 years for prevention of genital warts caused by HPV-6 and -11. HPV-6 and -11 cause approximately 90% of genital warts and most cases of recurrent respiratory papillomatosis [63]. HPV-associated cancers in males include certain anal, penile, and oropharyngeal and oral cavity cancers caused primarily by HPV 16 [45, 64, 65]. Mathematical modeling suggests that adding male HPV vaccination to a female-only HPV vaccination program is not the most cost-effective vaccination strategy for reducing the overall burden of HPV-associated conditions in males and females when vaccination coverage of females is high (>80%) [66]. Since the health burden is greater in females than males, and numerous models have shown vaccination of adolescent girls to be a cost-effective use of public health resources, improving coverage in females aged 11 and 12 years could potentially be a more effective and cost-effective strategy than adding male vaccination.

Men who have sex with men (MSM) are particularly at risk for conditions associated with HPV-6, -11, -16, and -18; diseases and cancers that have a higher incidence among MSM include anal intraepithelial neoplasias, anal cancers, and genital warts [67]. Gardasil has high efficacy for prevention of anal intraepithelial neoplasias in MSM [68]. The 3-dose series

of gardasil may be given to fenales and males aged 9 through 26 years to reduce their likelihood of acquiring genital warts and gardasil would be most effective when given before exposure to HPV through sexual contact.

On the other hand, laboratory research and clinical trials are focused on the development of therapeutic vaccines against HPV oncogenes, such as E6 and E7. It is hoped that immune responses against the two oncogenes might eradicate established tumors [69, 70].

8. Conclusions

Currently, the HPV vaccines potentially hold promise for the prevention of a greater majority of HPV-positive cervical cancers in woman. Thus, studies that attempt to clarify the association between HPV with cancers of the oral cavity and esophagus are important. They give us reason to be optimistic that HPV vaccines may be protective against UADT HPV infection, and consequently, effective in preventing HPV-associated UADT cancers in both men and women.

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