The Role of Human Papillomavirus in Oral Carcinogenesis

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ABSTRACT: Human papillomavirus (HPV) infection with high-risk types 16 and 18 has widely been reported as one of the prominent mechanisms behind the development of cervical squamous cell carcinoma. Links between HPV and oral cavity cancer have been suggested as well, based on epidemiologic and molecular means, though the association is less well-established. It is likely that HPV plays a role in oral cavity carcinogenesis, though only in a small subset of cases. The difficulty in providing true causal evidence of HPV's role in oral cancer lies in our lack of understanding of the significance of mechanisms by which HPV leads to oral carcinogenesis, as well as limitations in the molecular analysis of HPV. Further studies are necessary for the contribution of HPV in oral cavity malignancy to be better demonstrated.

Key words. Head and neck cancer, pre-malignant.

(I) The HPV Genome and Its Contribution to Malignancy

Human papillomavirus (HPV) is a ~ 7.9-kb, non-enveloped, double-stranded, circular DNA virus that has been implicated in a variety of anogenital and aerodigestive diseases, ranging from common warts to laryngeal papilloma to cervical cancer. The first isolation of these virus particles was performed in 1933 in rabbit papillomatosis (Shope, 1933). The extract from these lesions was found to contain infectious particles, and many of the benign papillomas in rabbits were observed to progress to malignancy. Currently, sequences for over 81 different types of HPV have been identified, with several additional poorly characterized types described. These viruses infect cells in the basal layer of squamous epithelium, and the different types have been traditionally separated based on tropism for cutaneous and mucosal sites, as well as high, intermediate, and low risk, depending on their association with malignancy (zur Hausen, 1996). For the purpose of this review, we will focus on mucosal high-risk types, known to be significant in the head and neck, predominantly HPV 16 and HPV 18. Many other types have been implicated in head and neck cancer, including 31, 33, 39, 45, 52, 58, and 69 (zur Hausen, 2000), though these have not been found to be highly significant in the majority of studies. Furthermore, many of the consensus polymerase chain-reaction (PCR) primers developed to detect the presence of HPV DNA will encompass many of the aforementioned types.

The HPV genome typically consists of nine open-reading frame sequences, located on only one of the strands of DNA, and is divided into seven early-phase genes (E) and two late-phase genes (L). The early genes serve to regulate the transcription of DNA, while the late genes encode for proteins involved in viral spread, such as capsid proteins (Stoler *et al.*, 1989). The E1 and E2 gene products are more specifically involved in regulating the transcription and replication of viral proteins. These different gene regions and gene products provide the basis on which molecular detection methods have been created.

The mechanism of HPV carcinogenesis was first identified in cervical cancer. Worldwide, greater than 90% of cervical cancers are related to HPV infection, with types 16 and 18 being implicated in the majority of cases (Walboomers *et al.*, 1999). HPV DNA sequences found in cervical carcinoma cell lines were the first clue to the role that high-risk types 16 and 18 play in altered cell growth (Schwarz *et al.*, 1985; Schneider-Gadicke and Schwarz, 1986; von Knebel Doeberitz *et al.*, 1988). Further studies in cervical cancer cell lines have demonstrated many of the harmful effects of HPV in terms of cellular mutations (Havre *et al.*, 1995; Liu *et al.*, 1997) and genomic integrity (Hashida and Yasumoto, 1991; White *et al.*, 1994).

The E6 and E7 oncoproteins are normally under control of E2 and E1 inhibitory genes. These genes can be deleted or altered upon integration, leading to unchecked transcription of E6 and E7 (Baker *et al.*, 1987). These proteins are then able to disrupt the function of *Rb* and *p53*, known tumor suppressor genes (Werness *et al.*, 1990). *p53* has been implicated in a wide variety of cancers (Hollstein *et al.*, 1991) and is known to be the target of many different viral particles (Levine, 1990). *p53* and *Rb* are tumor suppressor genes in that they regulate cell-cycle checkpoints at the G1 phase. If inactivated, cells are more prone to push through division and replication, even in the setting of harmful gene mutations, which can lead to malignancy.

The E6 gene is able to inactivate *p53* (Scheffner *et al.*, 1990) through association with E6 associated protein. This complex then interacts with *p53* and undergoes ubiquitin-dependent degradation of *p53* (Scheffner *et al.*, 1993). E7 is able to bind and interact with the *Rb* gene product (Dyson *et al.*, 1989). E7 has the ability to phosphorylate the *Rb* proteins, leading to degradation by ubiquitination (Boyer *et al.*, 1996). This subsequently leads to E2F activation, which produces a family of transcription factors leading to cell proliferation. Many other possible mechanisms

have been discovered by which these proteins can induce malignancy such that their role in carcinogenesis is ensured (zur Hausen, 2000). It has been shown that *p53* sequence alterations are decreased in the setting of HPV infection, since there is an alterative means of *p53* silencing with the production of E6 (Werness *et al.*, 1990; Kessis *et al.*, 1993a,b; Gillison *et al.*, 2000). Many of these pathways have also been implicated in head and neck cancer as well, which supports the possibility that HPV may play a significant role in head and neck cancer, and specifically in oral cancer, though other mechanisms are clearly involved.

The majority of studies involving oral lesions do not separate out specific subsites within the oral cavity, though the most common sites are the following: lip, anterior third of the tongue, floor of mouth, hard palate, gingiva, and buccal mucosa. This is an important, but often confused, distinction from the oropharynx, which includes the soft palate, base of tongue, tonsillar region, and posterior pharynx. Since there is general agreement that oropharyngeal carcinomas, most specifically tonsillar cancers, are frequently associated with HPV (Gillison *et al.*, 2000), we will focus our discussion on oral squamous cell carcinomas.

The link between oral squamous cell cancer and HPV seems logical, given the viral propensity for epithelial cell involvement. This connection was first proposed when cytopathic effects of HPV (koilocytosis) were noted on light microscopy (Syrjanen *et al.*, 1983) of oral lesions. *In situ* hybridization later confirmed the presence of HPV DNA in oral pre-malignancies (4/5 leukoplakias) and malignancies (3/6 carcinomas), thereby suggesting a causal association of HPV and carcinogenesis in oral lesions as well (Loning *et al.*, 1985), though we will further elaborate on the controversy behind these early findings. There have also been reports of altered cytologic features consistent with HPV infection, including a lack of keratin (Wilczynski *et al.*, 1998).

Researchers also discovered that human keratinocytes expressing E6 and E7 genes from HPV 16 become immortal (Munger *et al.*, 1989), as do oral epithelial cells (Park *et al.*, 1991; Oda *et al.*, 1996; Munoz, 2000). Analysis of these cell-line data supports the possibility that HPV infection was not specific to anogenital epithelium and could affect oral epithelium as well.

The majority of studies of head and neck lesions have focused on HPV 16 and 18, since these are known to be high-risk in cervical cancer. Other types—such as 6, 11, and 33—have not been identified in many oral malignancies (Mork *et al.*, 2001), though they are significant in other types of head and neck lesions, such as laryngeal papillomas. HPV 16 and, to a lesser extent, HPV 18 are the most widely implicated types in the oral literature and are therefore the focus of this review.

(II) Molecular Detection of HPV

While epidemiologic studies can draw an association between HPV seropositivity and oral cancer, it must also be demonstrated that HPV is present and functioning in these infected cells. There are many methods by which HPV can be detected, but every method has its strengths and weaknesses. Underlying all of the sensitive molecular assays is the problem of contamination. Miniscule amounts of RNA or DNA can theoretically be carried over from sample to sample by direct transmission on gloves or instruments, or could even be 'aerosolized'. Thus, even with compulsive isolation techniques, some contamination cannot always be ruled out.

Several studies have demonstrated the detection of antibodies to E6 and E7 in cervical cancer patients (Jochmus-Kudielka *et al.*, 1989), indicating an immune response to the virus. The frequency of seropositive individuals was higher in patients with HPV-associated genital lesions, but 18.1% of the control population had antibodies to E4, and 3.9% to E7 proteins. In a Colombian study, investigators found that 82% of patients with invasive cervical cancer had antibodies to HPV, while 56% of controls demonstrated seropositivity (Combita *et al.*, 2002). These findings suggest that there may be some biological role for HPV, given the formation of antibodies to these oncoproteins. However, in a small study of HNSC patients, only 11/92 (12%) had HPV antibodies to E6 or E7, while 10/288 (3.5%) of normal individuals had HPV seropositivity. None of the patients with oral tumors demonstrated seropositivity (Zumbach *et al.*, 2000). A more recent study on HPV 16 capsid antibody status noted a 2.3-fold higher risk of oral cancer development, though the authors conceded that the timing of serologic conversion could not be clearly linked to the acquisition of the oral carcinoma (Schwartz *et al.*, 1998). In other words, the presence of oral cancer may have preceded the development of HPV seroconversion. Furthermore, it is not known if antibody development to any region of the HPV genome is significant, or if there are particular antibodies that herald a worse prognosis.

Serologic studies have also been undertaken to detect the presence of HPV infection in an individual's lifetime. ELISA tests for serum antibody presence to HPV have been developed which correlate well with the presence of HPV DNA in cervical samples (Kirnbauer *et al.*, 1994; Carter *et al.*, 1996). However, without samples being tested directly for the presence of HPV, it is impossible for the anatomic site infected to be pinpointed, and there remains a variable rate of endemic HPV seropositivity among 'normal' individuals. Furthermore, antibody presence is not necessarily indicative of active infection, latent integration, or oncoprotein production that might be a clinically significant contributor to carcinogenesis. In addition, seropositivity may be a confounding factor associated with other risk factors for oral cancer, including tobacco and ethanol exposure.

Assays for the E6 mRNA as well as HPV DNA have been performed. Such studies involving RNA are less common, since they require fresh-frozen tissue, which is not as readily available as archived paraffin tissue. Recently, by polymerase chain-reaction (PCR), one study demonstrated HPV DNA in 20/84 HNSC and E6 mRNA transcript in only 9/20 of these samples (van Houten *et al.*, 2001). This study highlights our lack of understanding of the HPV life cycle, and that presence of DNA may not necessarily indicate active viral production. It is also possible that HPV can be a transient infection, that may or may not participate in the foundation of malignancy. In cervical cancer patients, it has been discovered that HPV DNA presence often declines with time (Hildesheim *et al.*, 1994; Evander *et al.*, 1995), indicating that there may be early effects on cellular function initiated by HPV that would lead to carcinogenesis, but would not be detected by traditional molecular biology techniques. In other words, HPV may initiate a genetic 'hit' and then disappear. There has been a suggestion that HPV in the oral cavity does not necessarily integrate into the host genome and may reside in an episomal form (Maitland *et al.*, 1987; Watts *et al.*, 1991; Yeudall and Campo, 1991). However, it is not clear that integration must occur for HPV to play a role in carcinogenesis. In cervical cancer, it is possible

that HPV exists in a dormant state and does not necessarily need to produce mRNA continuously to maintain a malignant state (Lehn *et al.*, 1985).

In situ hybridization (ISH) involves the use of type-specific radioactively labeled DNA probes complementary to HPV sequences for detection. It was the initial assay of choice for HPV DNA before more sensitive molecular techniques were invented. While these studies piqued the interest of investigators, the validity of the prevalence data provided by these studies is unproven (McKaig *et al.*, 1998). The sensitivity of this assay was found to be at least on the order of 20-50 copies *per* cell (Syrjanen *et al.*, 1988a). However, ISH depends on the consistency of the complementary sequence present in the sample, and it is known that the presence of HPV DNA in oral cavity samples is inconsistent. Furthermore, storage of samples and degradation of signal over time are also issues, as is intra-observer variability.

PCR is known to be a very sensitive assay for the detection of HPV DNA in any given sample (Shibata *et al.*, 1988). Universal primers to conserved DNA sequences in HPV have been designed to the L1 region (also known as MY09/MY11) (Snijders *et al.*, 1991), the E1 region (also known as CPI and CPII) (Gregoire *et al.*, 1989; Tieben *et al.*, 1993), the E6 region (Maitland *et al.*, 1989), and the E7 region (Evander and Wadell, 1991). Furthermore, there is a host of other primers utilized that can be type-specific. The use of consensus primers *vs.* type-specific primers would theoretically result in a higher detection rate, since many different types of HPV would be identified. However, one study compared the use of type-specific E6 and E7 primers to L1 consensus primers, and there was no difference in detection rates (Resnick *et al.*, 1990), even though there is a theoretical advantage to using E6 and E7, since these are the known oncogenic proteins with specific molecular downstream effects related to carcinogenesis. This finding is perhaps due to the overwhelming prevalence of HPV-16 and, secondarily, HPV-18, to the exclusion of other types of HPV in the head and neck. A different study in cervical carcinoma samples noted that using several primer sets spanning the different regions would provide a more accurate determination of HPV prevalence (Karlsen *et al.*, 1996).

To add to the complexity, one study suggested that the use of consensus primers to the L1 and L2 regions would yield false-negative results, since these areas are disrupted upon viral integration into the host genome (Cruz *et al.*, 1996). Universal primers to the E1 or E2 region may also underestimate the true prevalence of HPV, since there is the possibility that these early-phase regions are disrupted upon integration (Resnick *et al.*, 1990). The majority of studies have settled on the use of MY09/11 primers for detection, which yields a product size of ~ 450 base pairs. In addition to the possibility of false-negatives due to primer selection, there is also a chance of sample contamination, even with the most careful of methods of tissue handling and processing (Paz *et al.*, 1997). In such a situation where contamination can be problematic, PCR does not offer as many possible means to control for this error, since it provides simply a binary finding. Those studies utilizing Southern blot or quantitative PCR techniques with fluorescent probes offer a means of quantification to differentiate low-level positivity from contamination.

Southern blot has long been one of the gold standard assays for the detection of HPV DNA. It offers the ability to distinguish between episomal and integrated DNA, and it can detect up to 0.1 copy *per* cell (Syrjanen, 1990). While it does have some technical variability (Brandsma *et al.*, 1989) and requires a significant amount of DNA, it is not as prone to contamination error. While Southern blot may boast a theoretically higher specificity, it is clearly less sensitive than PCR (Schiffman, 1992; Frazer *et al.*, 1993). One study utilizing both Southern blot and type-specific PCR for HPV 16/18 discovered that there was a marked difference in prevalence when these different methods were used (Yeudall and Campo, 1991). Two of 39 oral carcinoma samples were positive for either HPV 16/18 by Southern blot, whereas 18/39 were positive by type-specific PCR for HPV 16/18. The authors additionally sampled adjacent dysplasia and normal mucosa from these same patients, and by Southern blot, all samples were negative, whereas only 2 samples did not demonstrate HPV by PCR in these adjacent samples. This study clearly demonstrates the difference in sensitivity of these two assays and raises the further question of what threshold of HPV infection is adequate for carcinogenesis. In addition, Gillison *et al.* (2000) utilized consensus PCR and Southern blot and discovered that, in non-oropharyngeal tumors, Southern blot was rarely positive when compared with PCR.

A recent study used the advantages of quantitative PCR and analyzed oral tumor samples previously found to be HPV-positive by other molecular means (Ha *et al.*, 2002) as well as pre-malignant oral cavity lesions. Quantitative PCR utilizes a fluorescent probe that is cleaved upon each round of amplification by the DNA polymerase, and the degree of fluorescence in the reaction mixture is then measured. The ability to quantify the amount of HPV present allows one to set a threshold for a significant infection. The theory of clonal expansion would suggest that at least one viral copy is needed *per* cell. In this study, it was discovered that samples found to be positive by Southern blot were also uniformly positive by quantitative PCR, but those found to be positive by traditional PCR alone were below the threshold of detection by quantitative PCR. Thus, this technique combines the sensitivity of PCR with additional specificity as a result of one's ability to quantify viral particles *per* cell.

As the field of HPV has developed, researchers have utilized techniques for detection that have taken advantage of the most recent technology both directly for DNA or for other surrogate markers of infection. However, no method is without flaws, and it remains unclear what the molecular significance of HPV DNA detection is with regard to carcinogenesis. Critical evaluation of data based on the types of detection methods used as well as determination of what the data mean in a clinical context is necessary for appropriate analysis.

(III) HPV in Normal Individuals

There is a considerable body of literature on the prevalence of HPV in normal hosts. Detection of HPV in normal oral mucosa would suggest that not all HPV infections necessarily lead to carcinogenesis, and it would be important to identify the factors that lead to its ability to induce malignant transformation. However, due to the plethora of molecular techniques used for detection, a wide range of values in normal individuals has been reported, from 0% (Eike *et al.*, 1995; Cruz *et al.*, 1996; Mao *et al.*, 1996; Nielsen *et al.*, 1996; Bouda *et al.*, 2000; Sand *et al.*, 2000) up to 70% (Terai *et al.*, 1999) (see Table 1). It appears that even the technique one uses to sample oral mucosa affects the sensitivity of detection. In one study, up to 60% of normal vol-

unteers had some form of HPV in their oral mucosa (43% with either type 16 or 18), though the detection rate varied depending on whether buccal scrapings, biopsy, or mouthwash was collected (Lawton *et al.*, 1992). A similar prevalence (43%) of HPV-16 was found in buccal swabs of a healthy population, though these individuals did not demonstrate HPV DNA in their peripheral lymphocytes (Jalal *et al.*, 1992). The finding of high-risk HPV in presumably normal individuals' mucosa implies that these individuals may have a dormant infection that could contribute to the development of oral cancer in the future (Sugerman and Shillitoe, 1997).

The more recent studies involving larger sample sizes (> 100) have reported a lower (1-2%) prevalence in normal individuals (Lambropoulos *et al.*, 1997; Smith *et al.*, 1998). Both of these latter studies utilized PCR, theoretically one of the most sensitive assays for HPV detection. Using similar techniques, investigators conducting a recent study in India in betel nut users found a detection rate of 27% (Nagpal *et al.*, 2002), raising the possibility that there are geographic, exposure-related, or other behavioral influences at play in individuals with normal oral mucosa.

Thus, normal individuals appear to have a wide range of reported prevalence rates which are likely dependent upon the different assays used for detection. It is possible that there are some geographical biases and that HPV is endemic in certain parts of the world. However, more recent, large studies indicate that the prevalence of HPV in normal oral mucosa is quite low (Smith *et al.*, 1998; Lambropoulos *et al.*, 1997).

(IV) Epidemiologic Support

A reasonable mechanistic link between HPV infection and oral cavity carcinogenesis is suggested by epidemiologic evidence, with odds ratios ranging between 0.5 and 6.2. One of the first case-control studies identified HPV-16 in various head and neck squamous carcinomas (HNSC), whereas none of the control group in matched anatomic sites harbored the virus (Brandsma and Abramson, 1989). A more recent case-control study examined the difference between an oral cancer group and a control group and demonstrated a higher risk of HPV infection in the oral cancer group (OR 3.70) after adjustment for age, smoking, and alcohol use (Smith *et al.*, 2000). Other case-control studies have also identified HPV-16 as a risk for the development of oral cancer (OR 6.2) (Maden *et al.*, 1992) and HNSC overall (OR 4.32) (Nishioka *et al.*, 1999).

In a landmark study, researchers examined a cohort of 900,000 individuals from Norway, Finland, and Sweden for HPV and the development of head and neck squamous carcinoma, while adjusting for smoking status by serum cotinine levels (Mork *et al.*, 2001). In this study, HPV status was ascertained in all patients by seropositive antibody status, and 160 of the 292 samples of head and neck cancer were tested with consensus and type-specific PCR. Of note, the investigators discovered that the mean time between serologic conversion and a diagnosis of cancer was 9.4 years, implying that there is a cause-and-effect relationship between HPV and cancer development. Overall, the adjusted odds ratio for development of HNSC in the setting of HPV 16 seropositivity was 2.2. Other types of HPV did not demonstrate an increased risk of HNSC development. This number increased to 14.4 and 20.7 in oropharyngeal and base-of-tongue tumors. respectively. Oral cavity carcinomas were also separated out by subsite: The odds ratio for lip lesions was 0.5 (95% CI = 0.1-2.1); for the tongue, it was 2.8 (95% CI = 1.2-6.6); for the floor of mouth, it was 0; and for the other areas, it was 3.6 (95% CI = 0.5-26.3). These particular ratios are less impressive than the oropharyngeal values obtained, with only the tongue having a statistically significant elevation.

Therefore, a link between head and neck cancer and oropharyngeal HPV infection (specifically type 16) was drawn with the use of large-scale epidemiologic data. While these values may seem impressive, they should be put into the context that the odds ratio of the development of cervical squamous cell carcinoma in the setting of HPV infection is 74 (Munoz, 2000). Furthermore, while the population studied was quite large, the actual number of cases of head and neck malignancy in the cohort was quite low (309 cases identified, 228 specimens analyzed for HPV). It is also interesting to note that the odds ratios of true oral cavity malignancies were rather low.

Large-scale epidemiologic data were also reviewed in patients with known HPV-associated anogenital carcinoma and their risk of subsequent development of HNSC. Analysis of Surveillance, Epidemiology, and End Results (SEER) data indicated a relative risk of 2.3 for oral cavity cancer and a relative risk of 4.3 for the development of tonsillar cancer (Frisch and Biggar, 1999) in these patients. These findings support the role of HPV in oral cavity carcinoma, though the odds ratios are modest.

TABLE 1
HPV Prevalence in Normal Oral Cavity Mucosa

Study	Mode of Detection	HPV+*	%	Tumor Type
Maitland *et al.*, 1987	Southern blot	5/ 12	41.6	Normal control mucosa
Jalal *et al.*, 1992	HPV16-specific primers	21/ 48	43.8	Normal oral mucosa
Holladay and Gerald, 1993	E1 PCR	1/ 6	16.7	Normal control mucosa
Ostwald *et al.*, 1994	Consensus PCR	1/ 97	1	Normal control mucosa
Eike *et al.*, 1995	L1 consensus PCR	0/ 61	0	Normal oral mucosa
Cruz *et al.*, 1996	Consensus PCR	0/ 12	0	Normal control mucosa
Mao *et al.*, 1996	L1 consensus and E6/7 PCR	0/ 6	0	Normal control mucosa
Nielsen *et al.*, 1996	ISH/HPV 16 PCR	0/ 20	0	Normal control mucosa
Lambropoulos *et al.*, 1997	HPV16-specific primers	4/169	2.4	Normal oral mucosa
Smith *et al.*, 1998	L1 consensus PCR	2/205	1	Normal control mucosa
Terai *et al.*, 1999	L1 consensus PCR	26/ 37	70.3	Normal oral mucosa in individuals with cutaneous warts
Bouda *et al.*, 2000	Nested consensus PCR	0/ 16	0	Normal control mucosa
Sand *et al.*, 2000	L1 consensus type specific	0/ 12	0	Normal control mucosa
Nagpal *et al.*, 2002	Consensus PCR	7/ 26	26.9	Normal control mucosa

* These values were taken specifically for HPV 16 and/or HPV 18 when possible.

(V) HPV in Pre-malignant Lesions

It has been well-established that head and neck cancer follows a genetic progression from normal to invasive disease. Early lesions begin with dysplasia and subsequently undergo an accumulation of genetic alterations leading to the development of malignancy (Califano *et al.*, 1996). Given these findings, many investigators have studied the prevalence of HPV in these early lesions, hoping to find a similar progression of HPV prevalence with malignant disease. An increasing prevalence of HPV in pre-malignant lesions would suggest that it does play a role in malignant transformation. Again, the studies have reported varied results due to the differences in samples and molecular assays utilized, from 0% (Zeuss *et al.*, 1991; Fouret *et al.*, 1995) to 85% (Bouda *et al.*, 2000) (see Table 2). Pre-malignant lesions offer an additional level of complexity in cross-comparison of studies, since many of the terms used to describe these lesions have changed over the years. Currently, dysplasia (mild, moderate, and severe) and carcinoma *in situ* are recognized as pre-cancerous, whereas many of the other terms commonly used—such as leukoplakias, erythroplakia, lichen planus, etc.—describe a gross morphology, not necessarily a histologic alteration. Therefore, we have attempted to focus on those studies that have defined lesions by histopathologic diagnosis.

A recent study using quantitative PCR examined over 100 pre-malignant oral cavity lesions and found a prevalence of 1.0% (Ha *et al.*, 2002). This particular study is of interest in that the quantitative assay allowed the authors to evaluate the amount of HPV 16 or 18 DNA present in any given sample. The authors proposed that clonal, neoplastic proliferations should have at least one HPV copy *per* genome to be consistent with a role as an etiologic agent in malignant progression. Therefore, small quantities or contamination that would otherwise be called positive on routine PCR could be excluded on this basis. New techniques such as quantitative PCR have found that HPV is present in only a very small minority of oral pre-malignant lesions.

(VI) HPV in Oral Cavity Malignancies

The majority of literature on oral cavity lesions and HPV has focused on squamous cell carcinomas. Many studies have been performed with a wide array of molecular assays described earlier. Once again, the data range from 0% (Zeuss *et al.*, 1991; Matzow *et al.*, 1998; Miguel *et al.*, 1998) to 100% (Uobe *et al.*, 2001) (see Table 3). Many other reviews have looked at these trends to 'tease out' factors that account for the differences between and among studies.

In a large review of the literature examining the role of HPV in oral lesions, HPV was detected in 13.5% of normal mucosa and 26.2% of squamous carcinoma (Miller and White, 1996). The authors noted that DNA was more likely to be detected in fresh-frozen than in paraffin-embedded samples, and that the mode of detection was a significant factor in the prevalence reported in various studies (Miller and White, 1996). Another large review of head and neck samples noted that the HPV prevalence in HNSC as detected by PCR was 34.5%, by ISH 15.8%, and by Southern blot 24.5% (McKaig *et al.*, 1998). Thus, it is no surprise that, overall, PCR exhibits a higher sensitivity and ability to detect the presence of HPV. However, PCR-positive lesions may be a result of minute contamination or subclonal infection that does not necessarily indicate a real contribution to carcinogenesis.

The larger studies using PCR detected HPV infection rates of approximately 10-15%. Thus, even when the most sensitive of techniques is used, there is still a low rate of detection of HPV in oral cavity malignancies. Moreover, the significance of

TABLE 2

HPV Prevalence in Oral Cavity Pre-malignant Lesions

Study	Mode of Detection	HPV+*	%	Lesion Type
Maitland *et al.*, 1987	SB using HPV 16 probe	16/ 21	28.6	Dysplasia keratosis hyperplasia lichen planus
Gassenmaier and Hornstein, 1988	ISH	19/103	18.4	Dysplasia
Syrjanen *et al.*, 1988a	ISH 6, 11, 13, 16, 18, 30	6/ 22	27.3	Dysplasia CIS
Greer *et al.*, 1990	ISH 6, 11, 16, 18, 31, 33, 35	5/190	2.6	Leukoplakia dysplasia
Shroyer and Greer, 1991	E6 HPV-16 PCR/ISH	7/ 48	14.6	Dysplasia hyperplasia keratosis
Zeuss *et al.*, 1991	ISH 6/11, 16/18, 31/33/35	0/ 20	0	Dysplasia CIS
Holladay and Gerald, 1993	E1 PCR	13/ 45	28.9	CIS dysplasia inflammation hyperplasia
Fouret *et al.*, 1995	E6 consensus PCR	0/ 3	0	Dysplasia
Mao *et al.*, 1996	L1 consensus and E6/7 PCR	8/ 23	34.8	Dysplasia CIS
Nielsen *et al.*, 1996	ISH/ HPV 16 PCR, SB PCR	20/ 49	40.8	Dyplasia leukoplakias
Wen *et al.*, 1997	E6 HPV 16/18 PCR	5/ 17	29.4	Papilloma leukoplakias lichen planus
D'Costa *et al.*, 1998	L1 consensus PCR	27/ 80	33.8	Leukoplakias lichen planus submucous fibrosis melanoplakia
Elamin *et al.*, 1998	Nested L1 PCR	4/ 12	33.3	Dysplasia
Matzow *et al.*, 1998	Consensus PCR	1/ 5	20	CIS hyperplasia
Bouda *et al.*, 2000	Nested consensus PCR	29/ 34	85.2	Hyperplasia dysplasia
Sand *et al.*, 2000	L1 consensus type-specific	8/ 29	27.6	Lichen planus leukoplakias
Ha *et al.*, 2002	Quantitative PCR	1/102	1.0	Dysplasia CIS

* These values were taken specifically for HPV 16 and/or HPV 18 when possible.

HPV DNA presence in the progression to malignancy is still unclear. It *is* clear, however, that oral carcinoma is different from cervical cancer, where HPV infection is necessary for disease development.

This current review highlights the same challenges identified in previous review articles: diverse patient populations with likely different rates of endemic infection, different molecular assays used by a variety of authors, a lack of understanding of the link between HPV and carcinogenesis in the integrated *vs.* the non-integrated state, and an unknown link between HPV DNA presence and activity. While many new studies have proposed epidemiologic, serologic, molecular, and mechanistic roles of HPV and its contribution to oral cancer, there continue to be debate and a wide range of reported prevalence in normal individuals and those with pre-malignant and malignant lesions.

In addition to the technical aspects of HPV detection, the simple nomenclature regarding anatomic locations of oral cavity *vs.* oropharyngeal lesions is often unclear. The literature clearly supports the idea that oropharyngeal cancers are more likely to have HPV than other head and neck tumors. Anatomically, the oral cavity and oropharyngeal border is the posterior 1/3 of the oral tongue, which is clinically difficult to delineate in many cases. Thus, there may be a significant portion of oropharyngeal tumors that are included in the oral cavity group, falsely elevating the number of HPV-positive samples, or *vice versa*.

Summary

HPV has been shown to be a significant carcinogen in cervical cancer, but the significance of human papillomavirus' contribution to oral squamous cell carcinoma has been studied for several decades and remains debated. Putative molecular mechanisms have been identified that clearly demonstrate its ability to disrupt key cellular elements responsible for the regulation of cell division and apoptosis. However, while epidemiologic and molecular data provide some evidence of high-risk HPV presence in oral pre-malignant and malignant lesions, it likely exists in only a small minority of cases. Thus, HPV may be a contributing factor in a subset of oral malignancies but is not a necessity in all cases, as it is in cervical cancer. Further studies using newer molecular techniques will shed light on this controversial topic and clarify the prevalence of HPV DNA in these samples and, more importantly, elucidate the significance of HPV infection in the oral cavity.

TABLE 3
HPV Prevalence in Oral Cavity Carcinoma Studies

Study*	Mode of Detection	HPV+**	%	Tumor Type
de Villiers *et al.*, 1985	Southern blot	2/ 7	28.5	SCC
Maitland *et al.*, 1987	Southern blot	7/ 15	46.7	SCC
Gassenmaier and Hornstein, 1988	ISH	16/ 68	23.5	SCC
Syrjanen *et al.*, 1988b	ISH 6, 11, 13, 16, 18, 30	6/ 51	11.8	SCC
Greer *et al.*, 1990)	ISH 6, 11, 16, 18, 31, 33, 35	6/ 70	8.6	SCC
Shroyer and Greer, 1991	E6 HPV-16 PCR/ISH	1/ 13	7.7	SCC
Watts *et al.*, 1991	Southern blot	11/ 23 by SB	47.8	SCC
	E6 type-specific PCR	11/ 14 by PCR	78.6	
Yeudall and Campo, 1991	Southern blot (16&18),	2/ 39 by SB	5.1	SCC
	type-specific PCR (16/18)	18/ 39 by PCR	46.2	
Zeuss *et al.*, 1991	ISH 6/11, 16/18, 31/33/35	0/ 15	0	SCC
Shindoh *et al.*, 1992	PCR and dot-blot	8/ 24	33.3	SCC
Holladay and Gerald, 1993	E1 PCR	7/ 39	17.9	SCC
Ostwald *et al.*, 1994	Consensus PCR	16/ 26	61.5	SCC
Balaram *et al.*, 1995	Consensus PCR	67/ 91	73.6	SCC
Fouret *et al.*, 1995	E6 consensus PCR	2/ 21	9.5	SCC
Cruz *et al.*, 1996	Consensus PCR	19/ 35	54.3	SCC
Mao *et al.*, 1996	L1 consensus and E6/7 PCR	12/ 41	29.3	SCC
Paz *et al.*, 1997	L1 and E1 consensus	10/ 71	14.1	SCC
Wen *et al.*, 1997	E6 HPV16/18 PCR	14/ 45	31.1	SCC
D'Costa *et al.*, 1998	L1 consensus PCR	15/100	15	SCC
Elamin *et al.*, 1998	Nested L1 PCR	14/ 28	50	SCC
Matzow *et al.*, 1998	Consensus PCR	0/ 30	0	SCC
Miguel *et al.*, 1998	L1 consensus PCR	0/ 16	0	SCC
Mineta *et al.*, 1998	PCR	3/ 14	7.1	SCC
Schwartz *et al.*, 1998	L1 consensus and E6 PCR	22/193	11.4	SCC
Smith *et al.*, 1998	L1 consensus PCR	8/ 93	8.6	SCC
Pintos *et al.*, 1999	L1 consensus PCR	3/ 29	10.3	SCC
Bouda *et al.*, 2000	Nested consensus PCR	17/ 19	89.5	SCC
Gillison *et al.*, 2000	L1 consensus, type-specific	10/ 84	11.9	SCC
	PCR 16/18, Southern blot, ISH			
Sand *et al.*, 2000	L1 consensus, type-specific	3/ 24	12.5	SCC
Shima *et al.*, 2000	E6/7 consensus PCR	34/ 46	73.9	SCC
Klussmann *et al.*, 2001	Nested PCR	4/ 22	18.2	SCC
Uobe *et al.*, 2001	L1 *in situ* PCR	20/ 20 by ISPCR	100	SCC
	ISH	0/ 20 by ISH	0	
Ha *et al.*, 2002	Quantitative PCR	1/ 34	2.9	SCC
Nagpal *et al.*, 2002	Consensus PCR	37/110	33.6	SCC
Ringstrom *et al.*, 2002	Consensus PCR	2/ 41	4.9	SCC
Ritchie *et al.*, 2003	Consensus PCR	10/ 94	10.6	92% SCC

* These studies cited have samples solely from primary tumor, not cell line, DNA.
** These values were taken specifically for HPV 16 and/or HPV 18 when possible.

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