Review

Human Papillomavirus and Cancers of the Upper Aerodigestive Tract: A Review of Epidemiological and Experimental Evidence

Silvia Franceschi, Nubia Muñoz, Xavier F. Bosch, Peter J. F. Snijders, and Jan M. M. Walboomers

Servizio di Epidemiologia. Centro di Riferimento Oncologico, Via Pedemontana Occidentale, 33081 Aviano PN, Italy [S. F.]; Unit of Field and Intervention Studies, IARC, 69372 Lyon, France [S. F. N. M.]; Serviè d’Epidemiologia y Registre del Cancer. Institut Català d’Oncologia, 08907 L’Hospitalet del Llobregat. Barcelona, Spain [X. F. B.]; Unit of Molecular Pathology, Department of Pathology, Free University Hospital, 1007 Amsterdam, the Netherlands [P. J. F. S.; J. M. M. W.]

Abstract
Human papillomaviruses (HPVs) are recognized as important causes of cancer of the anogenital tract and may be involved also in the etiology of cancers of the upper aerodigestive tract (UADT). Epidemiological and experimental evidence lend some support to this possibility. Increased risk of cancer of the oral cavity, pharynx, and larynx subsequent to the occurrence of cancer of the cervix has been found and suggests common etiological factors besides smoking. HPV has been found in a substantial proportion of benign UADT lesions, most notably laryngeal papillomas and oral verrucal-papillary lesions. Largest and most accurate case series (i.e., >15 UADT cancer cases, based on best HPV detection techniques) showed HPV DNA in 46% of cancers of the oral cavity and pharynx, 15% of cancers of the esophagus, and 24% of cancers of the larynx, with however, great discrepancies from one study to another. An additional 14 case series with a comparison group of noncancer patients revealed approximately a 4-fold higher HPV prevalence in UADT cancer tissues than in normal ones. The only two strictly designed case-control studies dealt with cancer of the oral cavity and provided inconclusive results, possibly because of interference of primary treatment with HPV detection in buccal exfoliated cells. An increasing bulk of experimental and in vitro evidence suggests that at least a proportion of UADT cancers harbor a relatively high copy number of HPV DNA, E6/E7 region transcripts and a clonal association with HPV have been demonstrated in these tumors. The combination of a good study design with reliable and noninvasive viral measurement will ultimately allow researchers to elucidate the role of HPV in the development of UADT cancer. Allowance for the strong effect of smoking, alcohol drinking, and betel quid chewing on UADT cancer and exclusion of noncausal associations will be the most difficult challenges of such studies.

Introduction
Certain HPV3 types are recognized as important human carcinogens (1). The role of some HPV types, most notably HPV 16 and 18, in the etiology of cancer of the cervix uteri is well established (2–4). HPV DNA was detected in over 90% of cervical tumors worldwide, with no significant variation in HPV positivity among countries (5). Elevated ORs for HPV in preinvasive and invasive cervical neoplasias emerged from the vast majority of nearly 100 case-control studies on the topic, most notably those in which the most accurate HPV DNA detection test (PCR) was used (i.e., several 10-fold risk increases, with attributable fractions above 70%; Refs. 2–4). Other, rarer anogenital cancers are also causally linked to HPV (1). It thus seems probable that the influence of such potent carcinogens as HPVs is not restricted to the genital tract, and cancers of the UADT (i.e., mouth, pharynx, esophagus, and larynx) are among the most likely candidates.

UADT cancers account for 15% of all cancer cases in men worldwide, with nearly 600,000 estimated cases per year (6). Approximately 270,000 additional cases of UADT cancer occur in women. In developing countries, the combination of these cancer sites (approximately 400,000 new cancer cases per year in men and 220,000 in women) would represent by far the most frequent cancer in males and the third most frequent in females (6). Tobacco smoking, alcohol drinking, and betel chewing are associated with very marked elevations of UADT cancer risk (80-fold in the most heavily exposed individuals), further aggravated by nutritional deficiencies (7–9). A role of HPV has been long suspected [see Scully et al. (10), for a review of early light- or ultramicroscopy findings]. The study of HPVs in the UADT, however, has been more difficult than in the genital tract because of the low HPV copy numbers found in many cancers at these sites (11), the uncertainty about which type(s) of HPV to search for, and the little knowledge on the natural history of UADT neoplastic lesions, as compared to cancer of the cervix.

To elucidate the involvement of HPV in UADT cancer, the vast majority of which involves squamous cell carcinomas, the present review will consider: (a) epidemiological evidence (including descriptive epidemiology findings), the presence of HPV in benign UADT lesions, case series of UADT cancers (with or without a comparison group), and the few case-control studies in which the presence of HPV DNA has been assessed; and (b) experimental evidence on the role of HPV, including

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1 The abbreviations used are: HPV, human papillomavirus; UADT, upper aerodigestive tract; OR, odds ratio; SB, Southern blot; ISH, in situ hybridization; CI, confidence interval.

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3 To whom requests for reprints should be addressed, at Servizio di Epidemiologia. Phone: 39-434-659354; Fax: 39-434-659222.

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different HPV types, HPV copy number and clonality, putative mechanisms of HPV-induced malignant transformation, and the interaction with chemical and physical agents.

Epidemiological Evidence

Descriptive Epidemiology Findings. The correlation between UADT cancers and cancers for which HPV etiology is demonstrated or suspected has been studied. Muñoz et al. (12) reported a significant correlation between incidence rates of cancer of the oral cavity and those of cancer of the cervix uteri and penis. Furthermore, several studies based on cancer registry data showed significantly increased risks of UADT cancers subsequent to a diagnosis of cancer of the cervix (13–16). Rabkin et al. (17) examined incidence of second cancer in over 25,000 women with cervical cancer (about 150,000 woman-years) from nine United States cancer registries. Significantly increased risks were found for cancers of the oral cavity (relative risk, 2.2) and larynx (relative risk, 3.4). In 37,000 women with history of carcinoma in situ of the cervix an approximate 2-fold increased risk of cancers of the oral cavity, pharynx, and esophagus emerged in Norway (18).

Finally, a systematic population-based assessment of cancer risk in first-degree relatives of cancer probands found significant associations between cancer of the lip, larynx, and lung and cancer of the cervix (19).

HPV in Benign UADT Lesions. The first indication that HPV was involved in the development of UADT tumors came from the observation that juvenile laryngeal papillomas originated from a perinatal infection of children from mothers with genital condylomatoses lesions (20). Subsequently, it emerged that HPV 6 and 11 were a cause of laryngeal papillomas at all ages (21). Other benign lesions of the UADT associated with HPV included inverted nasal papillomas, oral verrucal-papillary lesions, sinus papillomas, and squamous epithelial hyperplasia (Heck’s disease), which is common among Inuits and American Indians (10). Most of these lesions resemble benign HPV manifestations in the genital tract morphologically (e.g., tendency towards flat or inverted papillomas), histologically (i.e., they often originate where different types of keratinized and nonkeratinized epithelia are juxtaposed (22, 23)), and cytologically (i.e., presence of koliocytes and dyskeratinocytes (24)). As a consequence of these similarities with genital tract, HPV types involved in genital warts (6 and 11) were most often searched for and detected in benign lesions of the UADT (25). HPV 6 and 11 prevalence ranged from 50 to 84% in laryngeal papillomas (26) and oral condylomata acuminata (27) to 6% in oral leukoplakia (28).

HPV 13 seems to be specific for focal epithelial hyperplasia, whereas type 57 has been associated with benign and malignant lesions of the nasal cavity and paranasal sinuses (29). Biopsies of normal mucosa adjacent to laryngeal papillomas were generally HPV-positive (30). Nongenital HPV types (such as HPV 2, 4, and 7) have, however, been identified occasionally in the UADT; they are responsible for various types of cutaneous warts in epidermodysplasia verruciformis, as well as in normal subjects (1).

These earlier observations on benign UADT lesions, however, were carried out on small samples and sometimes by means of HPV DNA detection techniques that are now shown to have severe limitations. Most important, the relevance of some of these benign UADT lesions as cancer precursors is doubtful. Maden et al. (31), in a population-based case-control study, found history of oral warts in only 1 of 131 cases of cancer of the oral cavity and 1 of 136 controls. Common warts and genital warts were similarly unassociated with cancer risk.

Case Series. This review is restricted to case series that included at least 15 UADT cancer cases and in which the most accurate detection techniques (i.e., SB and PCR) were used. Fourteen studies have been reported on cancer of the oral cavity and pharynx (32–45), 5 on cancer of the esophagus (46–50), and 10 on cancer of the larynx (39, 51–59). Overall, the prevalence of HPV was 319 of 1027 (31%). The highest prevalence (205 of 446; 46%) was found for cancers of the oral cavity and pharynx, with values, however, ranging from 0 (39) to 100% (35). Corresponding figures were 38 of 260 (15%) for cancer of the esophagus (range, 0–5%) and 76 of 322 (24%) for cancer of the larynx (range, 2–85%). Studies in which PCR methods were used tended, on average, to show higher prevalence than those based on SB. In a large survey of 363 patients with invasive squamous cell carcinomas of the esophagus in Lixian, China, ISH under low-stringency conditions revealed 85 (23%) HPV-containing tumors (60).

The high prevalence (74%) of HPV reported by Balaram et al. (43) in oral cancer is of special interest because it used consensus primers on 91 oral cancer cases and because the identity of every positive sample was confirmed by direct sequencing of the PCR product. This was crucial to exclude false positives. Conversely, recent work did not lend much support to a role of HPV in the onset of cancer of the esophagus at least in developed countries (47, 49). A few investigations (43) looked for nongenital HPV types (e.g., 1, 2, 3, and novel types); they showed, however, very few positive cancer specimens or none.

Case Series with a Comparison Group. At least 14 studies showed a comparison of HPV DNA presence in UADT cancers and corresponding tissues from noncancer controls. Conversely, some studies in which a comparison was made between the presence of HPV DNA in UADT cancer and normal tissue of the same patients (e.g., Ref. 59) were dealt with in the previous section.

Table 1 shows five studies of UADT cancers in which hybridization techniques without DNA amplification were used, for a total of 173 cancer cases and 199 controls subjects (61–65). A higher proportion of cancer cases (23%) than control subjects (5%) were HPV-positive, the difference being statistically significant in three investigations. Among specific sites, the difference in HPV prevalence between cancer cases and control subjects was especially marked for the tonsil (63, 64).

Although ISH and SB methods had some strengths (e.g., ISH permitted the localization of HPV within the cells, whereas SB provided information about viral physical state), they also had important drawbacks (e.g., they are time-consuming and require a considerable amount of HPV DNA to achieve positive results). These drawbacks were largely overcome by the introduction of PCR-based methods.

PCR is a primer-directed in vivo DNA amplification reaction, allowing an exponential amplification of a target DNA fragment spanned by two oligonucleotide primers. Theoretically, more than a million-fold amplification can be obtained, yielding a sensitivity of down to one HPV copy per sample. The introduction of general/consensus PCR methods, using primers selected from conserved regions within the viral genome, has particularly revolutionized HPV detection strategies because these methods enable the sensitive detection of a broad spectrum of HPV genotypes in a single reaction. The original HPV consensus PCR methods are based on different principles (66).
Among the most commonly applied methods, the MY11/09 primer PCR uses degenerate primers comprising primer mixtures to fit as many as possible different HPV genotypes. In contrast, the GP5/6 primer PCR and derivatives are based on low-stringency conditions of primer annealing, allowing a certain degree of mismatch acceptance. Apart from its high sensitivity, major advantages of the PCR method are the requirement of relatively low amounts of input DNA and the fact that it can be applied to crude cell suspensions (i.e., cervical scrapes and formalin-fixed, paraffin-embedded tissue). However, its extraordinary sensitivity makes the PCR highly susceptible to contamination, which may lead to false positive results. Consequently, effective anticontamination measures are necessary for a proper application; these measures include the use of disposable materials and spatially separated rooms for the different pre- and post-PCR handlings.

Major characteristics and results of nine studies that used PCR methods can be found in Table 2, for a total of 254 UADT cancer patients and 235 control subjects (67–75). Again, HPV presence in cancer patients varied widely from one study to another, but was approximately 2-fold higher in PCR studies than in those without amplification. A statistically significant difference in HPV DNA prevalence emerged in three of these investigations (67, 69, 71). An especially strong association was found for cancers of the oral cavity (69) and tonsil [10 of 10 HPV positive in Snijders et al. (71)].

As far as type of material is concerned, frozen biopsies were used in all but four studies. Fixed tissue was examined in the remaining ones, except Ostwald et al. (69), who reported results from exfoliated cells for control subjects and biopsies for cancer cases. With respect to specific types, "mucosotropic" HPVs (76) have been searched for in the majority of presented investigations (Tables 1 and 2). As in case series, HPV 16 was by far the type most often searched for and identified in UADT cancers. An important prerogative of later PCR-based methods [i.e., consensus, universal, or general primer-mediated PCR (66)] is, however, the ability to amplify a wide range of different HPV types in a single test. Different HPV consensus or general primers have been developed, based on different amplimer length and mechanisms (e.g., degenerate primers, mismatch acceptance, and so forth; Ref. 66) and have been used in four out of nine studies (Table 2). They led to especially strong associations between UADT and HPV in three instances (67, 69, 71). Finally, most of the investigations included very few details about study design (Tables 1 and 2). Virtually no information on the source and major characteristics (e.g., sex and age) of cases and controls were provided by six studies. In five studies, however, it was clear that the control group (generally represented by UADT benign diseases or volunteers), was, on average, 7 or more years younger than cancer subjects (up to 37 years younger in one study). Also, men tended to be overrepresented among cancer patients. Reasons that led to the choice of a certain study size or to the interruption of data collection were never discussed. An attempt at stratification and/or allowance for potential confounding factors was performed in only two investigations, Chang et al. (62) and Benamouzig et al. (65), who put emphasis on the frequent combination of HPV and other risk factors in UADT cancer patients, most notably smoking and betel chewing.

**Case-Control Studies.** Only two formal case-control studies examined the association between HPV DNA and cancers of the UADT (Refs. 31 and 77; Table 3). Both dealt with cancer of the oral cavity and oropharynx, relied on search for HPV DNA by means of PCR in exfoliated cells, collected with a toothbrush and included population controls. In the study by Maden et al. (31), 22 of 118 cases and 10 of 112 controls showed an oral HPV 6 infection (OR, 2.9; 95% CI, 1.1–7.3).

<table>
<thead>
<tr>
<th>Author, year, study area</th>
<th>Site</th>
<th>Type of material</th>
<th>Method</th>
<th>HPV types</th>
<th>HPV-positive carcinomas</th>
<th>HPV-positive normal tissues</th>
<th>Source of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maitland et al., 1987 (61), United Kingdom</td>
<td>Oral cavity</td>
<td>Frozen biopsies</td>
<td>SB, low stringency</td>
<td>16</td>
<td>7/15</td>
<td>47</td>
<td>5/12</td>
</tr>
<tr>
<td>Chang et al., 1989 (62), Taiwan</td>
<td>Oral cavity</td>
<td>Frozen biopsies</td>
<td>SB, high stringency</td>
<td>6, 11, 16, and 18</td>
<td>13/17</td>
<td>76</td>
<td>1/17</td>
</tr>
<tr>
<td>Niedobitek et al., 1990 (63), Germany</td>
<td>Tonsil</td>
<td>Fixed biopsies</td>
<td>ISH, high stringency</td>
<td>6, 11, 16</td>
<td>6/28</td>
<td>21</td>
<td>0/30</td>
</tr>
<tr>
<td>Brandsma et al., 1989 (64), United States</td>
<td>Various UADT sites</td>
<td>Frozen biopsies</td>
<td>SB, low stringency</td>
<td>11, 16, 18</td>
<td>8/101</td>
<td>8</td>
<td>2/116</td>
</tr>
<tr>
<td>Benamouzig et al., 1992 (65), France</td>
<td>Esophagus</td>
<td>Fixed biopsies</td>
<td>ISH, SB, high stringency</td>
<td>6/11</td>
<td>5/12*</td>
<td>42</td>
<td>1/24</td>
</tr>
</tbody>
</table>

Total | 39/173 | 23 | 9/199 | 5 |

*χ² = 3.87; p < 0.05.  
*Anticontamination PCR techniques during the course of the study led to the discovery of three falsey positive results among cancer patients (47).
### Table 2  Case series of cancer of the UADT with a comparison group; HPV hybridization assays with amplification (PCR) methods

<table>
<thead>
<tr>
<th>Author, year, study area</th>
<th>Site</th>
<th>Type of material</th>
<th>Method</th>
<th>HPV types</th>
<th>HPV-positive carcinomas</th>
<th>HPV-positive normal tissues</th>
<th>Source of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostwald et al., 1994 (69), Germany</td>
<td>Oral cavity</td>
<td>Frozen and fixed biopsies (cases), exfoliated cells (controls)</td>
<td>PCR, consensus, degenerate</td>
<td>Broad spectrum</td>
<td>16/26 62</td>
<td>1/97 1</td>
<td>Hospital. Cases were 21 men and 5 women, ages 39–84 years (mean, 61). Controls were volunteers, 45 men and 52 women, ages 5–64 (mean, 24).</td>
</tr>
<tr>
<td>Bryan et al., 1990 (70), United Kingdom</td>
<td>Pharynx, larynx</td>
<td>Fixed biopsies</td>
<td>PCR, type specific</td>
<td>6, 11</td>
<td>11/13 85</td>
<td>9/14 64</td>
<td>Hospital. No information on sex and age.</td>
</tr>
<tr>
<td>Snijders et al., 1992 (71), the Netherlands</td>
<td>Tonsil</td>
<td>Frozen biopsies</td>
<td>PCR, consensus, not degenerate</td>
<td>Broad spectrum</td>
<td>10/10 100</td>
<td>0/7 0</td>
<td>Hospital. Age of cases: 46–79 (mean, 64). No information on sex and age of controls.</td>
</tr>
<tr>
<td>Tyan et al., 1993 (72), Taiwan</td>
<td>Oral cavity, pharynx, larynx</td>
<td>Frozen biopsies</td>
<td>PCR, type specific</td>
<td>6, 11, 16, 18, 33</td>
<td>11/50 22</td>
<td>1/11 1</td>
<td>Hospital. No information on sex and age.</td>
</tr>
<tr>
<td>Williamson et al., 1991 (74), South Africa</td>
<td>Esophagus</td>
<td>Frozen biopsies</td>
<td>PCR, consensus, degenerate</td>
<td>6, 11, 16, 18, 31, 33</td>
<td>6/14 43</td>
<td>6/41 15</td>
<td>Cases: ages 43–87 (mean, 56). Controls: endoscopy patients; mean age, 45. All black, sex not given.</td>
</tr>
<tr>
<td>Perez-Ayala et al., 1990 (75), Spain</td>
<td>Larynx</td>
<td>Frozen biopsies</td>
<td>PCR, type specific</td>
<td>11, 16</td>
<td>29/51 57</td>
<td>3/6 50</td>
<td>Hospital. No information on sex and age.</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>112/254 44</strong></td>
<td><strong>27/235 11</strong></td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2 > 3.87; P < 0.05. \]

### Table 3  Case-control studies on cancer of the UADT

<table>
<thead>
<tr>
<th>Author, year, study area</th>
<th>Site</th>
<th>Type of material</th>
<th>Method</th>
<th>HPV types</th>
<th>HPV-positive carcinomas</th>
<th>HPV-positive normal tissues</th>
<th>OR (95% CI)</th>
<th>Source of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maden et al., 1992 (31), United States</td>
<td>Oral cavity</td>
<td>Exfoliated cells</td>
<td>PCR, type specific</td>
<td>6, 16</td>
<td>22/118 19</td>
<td>6/108 6</td>
<td>2.3 (1.1–5.2)</td>
<td>6.8 (0.8–57.1)</td>
</tr>
<tr>
<td>Schwartz et al., 1995 (77), United States</td>
<td>Oral cavity</td>
<td>Exfoliated cells (cases and controls), biopsies (cases)</td>
<td>PCR</td>
<td>6, 11, 16, 18, 31, 33</td>
<td>17/206 8</td>
<td>20/206 10</td>
<td>0.6 (0.3–1.3)</td>
<td>Population-based case-control study. OR was 8 when results from biopsies were considered (cases only).</td>
</tr>
</tbody>
</table>

For HPV 16, corresponding figures were 6 of 108 and 1 of 116 (OR, 6.2; 95% CI, 0.7–52.2). OR for HPV 6 was not modified by adjustment for age, smoking, and alcohol consumption. More than one-third of eligible subjects refused to participate, but refusals were equally common in cases and controls. Schwartz et al. (Ref. 77; 206 cases and 206 controls) did not find any association with HPV infection. HPV was detected in 17 of 206 cases and 20 of 206 controls with an OR for any type of HPV of 0.6 (95% CI, 0.3–1.3). The presence of HPV DNA did not seem to add anything to the risk associated with smoking.
Among cancer cases in both studies, exfoliated oral cells were collected after treatment, possibly hampering HPV detection. Indeed, in Schwartz et al. (77), DNA of any HPV type was detected, among cancer cases, four times more frequently in histological specimens than in exfoliated cells. Finally, no significant relationships between oral cancer risk and either sexual practices (including oral sex) or sexually transmitted diseases emerged in these two studies.

Serological assays for antibodies to HPV antigens have seldom been used to evaluate the association between HPV and UADT cancer. Dillner et al. (78) took advantage of a serum bank of 39,268 healthy Finnish adults to perform a nested case-control study on 39 cases of esophageal cancer diagnosed between 1984 and 1986 and 35 cases of UADT cancer cases and 78 sex-, age-, and municipality-matched controls. IgG antibodies against HPV type 16 capsids were found in 20% of cancer patients. The OR was 14.6 (95% CI, 1.8-117) and was not altered by allowance for smoking habits. Results, however, were not adjusted for alcohol drinking habits. Other UADT cancer sites were rarer in this Finnish cohort and showed very low prevalence of HPV 16 IgG (78).

HPV in Immunodepressed Patients. Immunosuppression may affect the natural history of HPV and related neoplasms (e.g., cancer of the cervix and anus) in different ways (e.g., reactivating latent HPV infection, inducing higher HPV replication or progression from a premalignant to a malignant stage; Ref. 11). Still, the cases of UADT cancer reported in transplant recipients (79) and HIV-positive subjects (see Ref. 80 for a review) are few.

Route of HPV Transmission. The transmission route of HPV to the UADT is still poorly understood. Sexual transmission from the anogenital tract is conceivable (81). From the scant available data, however, promiscuity does not seem to be an important risk factor for UADT cancers (31), and a clear correlation has not been found between HPV types in the oral cavity and in the anogenital tract in women and their male partners (82). Vertical transmission at delivery (implicated in the early onset of recurrent respiratory papillomatosis), digital transmission from periungual infections (known to harbor HPV 16 [83]), and transmission from HPV-contaminated fomites are also possible, especially in the presence of macerated or abraded epithelial surfaces (81).

Experimental and in Vitro Evidence
As discussed already, progress in molecular biology is allowing increasingly good measurement of exposure to HPV at the DNA level and thus more accurate estimation of the association between HPV infection and cancer. These can also contribute to ruling out the possibility of noncausal associations by means of quantitative as well as qualitative information on HPV presence in UADT cancers. The majority of such information refers, for the time being, to cancer of the oral cavity and pharynx.

Different HPV Types. A major difficulty in the study of the role of HPV in the UADT has stemmed from the high number of HPV types [more than 70 identified thus far (84)]. They have been grouped, according to the tropism for certain tissues, into mucosotropic (including genital HPVs, such as types 6, 11, 16, 18, 31, 33, and 35) and cutaneous ones (associated with benign and malignant skin lesions in epidermodysplasia verruciformis patients). Furthermore, according to differences in the distribution in benign and malignant lesions and functional properties, HPV types have been distinguished as low-risk (mainly HPV 6 and 11) and high-risk (mainly HPV 16 and 18) types. In analogy to several other DNA tumor virus systems, oncoproteins of high-risk types (i.e., E6 and E7) showed specific interactions with cellular proteins engaged in the regulation of proliferation.

Some early findings in UADT neoplasias pointed to the existence of novel HPV types specific for UADT mucosa (51, 71). However, after more extensive HPV sequence information became available, some putatively novel HPV types could be identified as known HPVs (71). Furthermore, in the most recent studies that used different PCR primer combinations tailored to all major branches of HPV types, HPV 16 appeared by far the predominant type (11, 43-45), in analogy to what has been seen for cancer of the cervix uteri (5). Also similarly to cervical cancer in Asian countries (5), an especially high prevalence (47%) of HPV 18 was disclosed among cases of oral cancer in India (43).

Low-risk HPV types were found in 15-40% of UADT cancer, sometimes in the absence of high-risk HPV types (31, 43, 59). Whereas multiple HPV type infection is difficult to reconcile with the monoclonality of cancer lesions, involvement of HPV types with low carcinogenic potential may be conceivable in the UADT, most notably in the oral cavity and pharynx, on account of the heavy exposure of these sites to chemical and physical traumas.

HPV Copy Number and Clonality. Low HPV DNA copy numbers in UADT cancers has, thus far, hampered the search for and the interpretation of the presence of HPV at these sites. Relatively high copy numbers of HPV DNA, however, have been found in a minority of HPV-positive UADT cancers (11).

In this subgroup of tumors, the majority of which originated from the oropharynx, E6/E7 gene region transcripts could be demonstrated, and ISH analysis pointed to a clonal association of HPV with the neoplastic cells (11, 85). These findings support a viral etiology for these malignancies.

Conversely, a nonclonal HPV association, as suggested by weak PCR signals and focal ISH positiveness, makes a causal association unlikely, because a viral role in the maintenance of the malignant state can be excluded. A “hit and run” mechanism has been proposed, in analogy to observations in bovine papillomavirus-induced neoplasms in cattle, to explain focal presence or even absence of HPV in cancer tissues (86). There is, however, no experimental evidence for the existence of such a mechanism.

Mechanisms of HPV-induced Malignant Transformation of UADT Epithelial Cells. In vitro studies disclosed that high-risk HPV types can immortalize primary human oral (87, 88) and tonsillar (11) epithelial cells. This suggests, similarly to the anogenital epithelium, an important role for these viruses as initiating factors in malignant transformation. The immortalization property of high-risk HPVs has been linked to their E6 and E7 proteins being able to bind and inactivate p53 and pRb tumor suppressor gene products, respectively (89). Therefore, it has been suggested that HPV presence obviates the need for mutational p53 and Rb inactivation in carcinogenesis. However, UADT cancers are characterized by an altered p53 gene and/or expression in nearly half of the cases (90). The mutation profile may vary according to the specific exposure (e.g., smoking or betel) and/or geographic area involved (42, 90). This is in contrast to cervical cancers, in which p53 alterations are generally rare (91-93). After relating HPV status with p53 status, it appeared that altered p53 and HPV presence are not mutually exclusive in UADT cancers (94, 95), and recent

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analysis of a HPV 16-containing oral carcinoma and its derivative cell line points to the presence of HPV and altered p53 gene in the same neoplastic cells (85). Consequently, although currently unclear, the relationship between p53 and HPV in the pathogenesis of UADT cancers might be different from cervical cancers.

Moreover, it has been widely accepted that high-risk HPVs, despite their immortalization capacity, do not directly induce malignant transformation and that additional genetic changes are required for progression to overt malignant cells. It has been proposed that viral oncoproteins introduce an element of continuous chromosomal instability in their host cells, making a persistently infected cell amenable to the accumulation of additional mutations leading to progression (89). Although the nature of genetic changes supplementing HPV functions in carcinogenesis is unknown, recent in vitro studies have suggested that recessive changes (i.e., inactivation of tumor suppressor genes) play a pivotal role (96, 97).

In UADT cancers, very few studies have related HPV status with genetic changes. A HPV 16-containing oral squamous cell carcinoma and its derivative cell line were subjected to molecular genetic analysis using polymorphic microsatellite markers (85). Both cell line and primary tumor displayed losses of alleles at 1q and 1q, suggesting that loss of function of tumor suppressor genes at these loci represent important hits in the multistep pathway of carcinogenesis. The strong interrelationship with HPV in this tumor is further emphasized by continuous expression of HPV 16 E6/E7 region mRNA in its derivative cell line, pointing to a role for the encoded proteins in the maintenance of the malignant phenotype. Consequently, the study of HPV-containing UADT carcinoma cell lines can not only strengthen the evidence of a causal role for this virus but may also, in turn, help identify new tumor suppressor genes (85).

In cancer of the UADT, the presence of extrachromosomal (i.e., episomal) HPV DNA integration seems more common than in cancer of the cervix uteri (61, 67, 98). It has been suggested that HPV DNA integration, by disrupting the E2 region encoding transcriptional modulators, is essential for driving the expression of the transforming genes E6 and E7. However, studies on tonsillar carcinomas have revealed that E6/E7 transcription is not necessarily dependent on viral DNA integration (98). Therefore, if integration were important in carcinogenesis (e.g., by inactivating E2 transregulation) it is likely that viral intragenomic modification or changes in the expression of host cell genes could be alternative events to gain an equivalent effect from viral episomes. In this aspect, it is interesting that some studies have revealed gross intragenomic alterations within episomal HPV 16 DNA in UADT cancers (25, 61, 67).

Interaction with Other Risk Factors. Data collected thus far point to a combined effect of exposure to HPV and other, better known risk factors for UADT cancers, rather than HPV infection substituting for other carcinogens. Recent studies on cancer of the oral cavity and pharynx did not show, in cancer patients, any inverse correlation between HPV presence and smoking, alcohol drinking, and/or betel quid chewing (43, 45).

In vitro studies also lent support to scant human data because they showed that HPV-immortalized primary oral epithelial cells require exposure to tobacco-related chemicals for progressing to a fully malignant phenotype, reflected by tumor formation in nude mice (99). In fact, interference of cell cycle control mechanisms by HPV functions may enhance the induction of genetic alterations by carcinogenic agents. Finally, with respect to the age of cancer patients, some (45), but not all (43, 44, 100), studies showed a higher prevalence of HPV in younger patients with oral cancer.

Much larger epidemiological studies are, however, warranted to assess the existence of any interaction or effect modification between HPV, age, tobacco, betel quid, and alcohol and to choose between several possible scenarios (i.e., no risk increase from HPV infection, risk increase independent of other risk factors, or predominance of HPV role in subjects heavily exposed or in those unexposed to other risk factors).

Summary and Conclusions

Overall, epidemiological and experimental evidence lend some support to the possibility of HPV playing a role in the onset of UADT cancers, most notably cancer of the oral cavity and pharynx. For these cancer sites, the association with HPV seemed somewhat stronger and more consistent than for cancer of the esophagus and larynx. Many uncertainties and weaknesses, however, hamper the interpretation of available data.

Increased risk of cancer of the oral cavity, pharynx, and larynx subsequent to the occurrence of cancer of the cervix suggests that these cancers may share HPV as a causal agent. Similar exceptions, however, were recorded for other cancer sites related to smoking (e.g., lung) or low socioeconomic class (e.g., stomach), thus making explanations other than HPV involvement also possible (17, 101).

HPV has been found in a substantial proportion of benign and malignant lesions of the UADT. In the present review of the largest and most accurate reports, 29% of 1778 examined UADT cancer revealed HPV DNA as compared to only 9% of 752 UADT samples from cancer-free controls. Available studies suffer, however, from important limitations, including small study size, lack of inadequacy of control group, and use of suboptimal HPV detection techniques. The effects of misclassification of HPV status and bias, especially publication bias, are difficult to estimate.

Cancer of the oral cavity and pharynx seem, however, to represent, within the UADT cancers, the most likely candidate for HPV-mediated carcinogenesis. High HPV prevalences have been disclosed, especially in the most recent studies on oral cancer (43, 45). The oral cavity also represents an area where, as in some HPV-related genital sites, transition from keratinized to nonkeratinized epithelium is present (22) and chronic chemical (e.g., tobacco, alcohol, betel) and physical (e.g., dental traumas) insults are especially frequent.

New knowledge on the etiology of cancer of the oral cavity and pharynx may also help to elucidate rising incidence and mortality rates for these sites in most areas of the world (102). In particular, more than 2-fold mortality increases have been seen in the last three decades among young adults in Central and Eastern Europe, perhaps not totally attributable to rising tobacco or alcohol consumption (103).

The elucidation of HPV transmission routes and mechanisms of putative HPV carcinogenesis in the UADT (e.g., copy number, clonality, and interaction with oncogenes and cancer suppressor genes) will greatly contribute to ruling out noncausal associations. Case-control studies enable researchers to combine a good study design with reliable and noninvasive measurement of HPV DNA (e.g., from buccal exfoliated cells (104)) and will, therefore, be necessary to further elucidate the etiology of cancer of the UADT. The
strong associations of these cancer sites with smoking, drinking, and betel chewing do not refute, per se, a viral hypothesis but oblige researchers to consider, at the study design stage, the need for an accurate lifetime history of these exposures and sufficient power to assess confounding and/or modifying effects.

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