This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 24 February-3 March 2009.
Human papillomaviruses were considered by a previous IARC Working Group in 2005 (IARC, 2007). Since that time, new data have become available, these have been incorporated in the Monograph, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure, and biology

A concise overview of the taxonomy, structure, and biology of the human papillomavirus (HPV) is given below. For a more comprehensive description, the reader is referred to Volume 90 of the IARC Monographs (IARC, 2007).

1.1.1 Taxonomy

All papillomaviruses belong to the Papillomaviridae family, which includes 16 different genera. Of these, the alpha genus contains the viruses associated with the development of mucosal tumours in humans, and the beta genus contains those that are associated with the development of cutaneous tumours (Fig. 1.1).

1.1.2 Structure of the virion

Papillomaviruses are small non-enveloped icosahedral viruses of approximately 50–60 nm in diameter, containing a circular, double-stranded DNA genome (~7000–8000 bp) that exists in a chromatinized state.

1.1.3 Structure of the viral genome

The HPV genome is divided into three regions: the long control region (LCR), which regulates viral gene expression and replication; the early (E) region, which encodes proteins required for viral gene expression, replication and survival; and the late (L) region, which encodes the viral structural proteins. The designations E and L refer to the phase in the viral life cycle when these proteins are first expressed.

1.1.4 Host range and target cells

HPVs are restricted in their host range to humans, and primarily infect stratified epithelia at either cutaneous or mucosal sites. Mucosotropic HPVs can be further subdivided into high- and low-risk types depending upon their degree of association with human malignancy.

1.1.5 Function of the gene products

(a) E1

E1 is the only enzyme encoded by the virus possessing DNA helicase activity. Once bound to the viral origin of replication, this enzyme recruits the cellular DNA-replication machinery to drive viral DNA replication.
This protein serves three major functions in the viral life cycle. The first is to regulate the expression levels of the other viral gene products, and – depending upon the binding sites occupied in the LCR – to act as a transcriptional repressor or activator. Second, it recruits E1 to the viral origin, thereby enhancing viral DNA replication. Third, it has a critical role in the transfer of the viral genome to daughter cells during division of the host cell.

(b) **E4**

E4 is the most abundantly expressed viral protein, the function of which is still obscure. It has been linked to processes aiding viral DNA amplification and viral release.

(c) **E5**

E5 is one of three oncoproteins encoded by the virus (see Section 4.2). Its mode of action is still unclear, although it contributes quantitatively to the productive stage of the viral life cycle, and has been closely linked with the regulation of...
growth-factor signalling pathways and immune avoidance.

(e) **E6**

E6 is the second HPV-encoded oncoprotein (see section 4.2). It cooperates with E7 to provide an environment suitable for viral DNA replication, principally by overcoming cellular apoptotic processes. The most well characterized target of E6 from high-risk mucosotropic HPV types is the tumour-suppressor protein p53, which is directed by E6 towards degradation.

(f) **E7**

E7 is the third HPV-encoded oncoprotein (see section 4.2). By targeting cell-cycle regulatory pathways controlled by the tumour-suppressor protein pRb and the related proteins p107 and p130, it provides an environment favourable to viral DNA replication by maintaining an S-phase-like state in the differentiating keratinocytes.

(g) **L1 and L2**

L1 and L2 are the major and minor constituents, respectively, of the viral capsid. When overexpressed in various eukaryotic cells, L1 can self-assemble to form virus-like particles (VLPs). These VLPs are the basis for prophylactic vaccines against HPV, through induction of neutralizing antibodies.

1.1.6 **Life cycle**

HPVs are specifically epitheliotropic and their life cycle takes place within stratified squamous epithelia.

(a) **Entry**

It is assumed that HPVs initiate infection by penetrating through microtraumas in the epithelia to reach the basal cells, which are believed to be the target cells for HPV infection. The mechanism for virus entry into the basal cells is not entirely understood. Subsequent steps in the life cycle of the virus can be divided into three stages: establishment, maintenance, and production.

(b) **Establishment of the non-productive infectious state**

Once an HPV particle enters the host cell, it must rely primarily on the cellular machinery to replicate its DNA. In infected basal cells, the HPV genome becomes established as a low copy-number nuclear plasmid. Within these cells, only early viral gene products are expressed, and this is consequently referred to as the ‘non-productive’ stage of infection.

(c) **Maintenance of the non-productive infectious state**

A hallmark of HPV infection is its long-term persistence over many years, which, in the case of high-risk types, is a prerequisite for the development of cancer. This requires that the viral genome be maintained over multiple cell divisions; how this is achieved is still unclear.

(d) **Productive stage**

This begins when the daughter cells derived from the infected basal cells start to differentiate. The virus delays the terminal differentiation programme of the cell, and redirects the cell’s DNA replicative capacity. This then allows amplification of the viral genome and expression of the late viral genes necessary for the production of progeny virus, and subsequent viral release.

1.2 **Epidemiology of infection**

The epidemiology and natural history of HPV infection were extensively reviewed in the previous *IARC Monograph* (IARC, 2007).
1.2.1 Prevalence, geographic distribution

Most sexually active individuals will acquire at least one genotype of anogenital HPV infection at some time during their lifetime. The most comprehensive data on cervical HPV prevalence in women with normal cytology (the great majority of infections do not produce concurrently diagnosed cytological abnormalities) is provided by a meta-analysis including over 150,000 women (Castellsagué et al., 2007; de Sanjosé et al., 2007). After adjusting to the extent possible for study design, age, and HPV DNA detection assays, the estimated worldwide HPV DNA point prevalence was approximately 10%. The highest estimates were found in Africa and Latin America (20–30%), and the lowest in southern Europe and South East Asia (6–7%). Point prevalence estimates are highly dynamic because incidence and clearance rates are high; averaging across age groups can be particularly misleading.

Fig. 1.2 shows the eight most common HPV types (HPV 16, 18, 31, 33, 35, 45, 52, and 58) by geographic region. HPV 16 is the most common type in all regions with levels of prevalence ranging from ~3–4% in North America to 2% in Europe. HPV 18 is the second most common type worldwide.

Generally, similar results for the regional estimates of point prevalence of HPV DNA were observed in an IARC population-based prevalence survey conducted in 15,613 women aged 15–74 years from 11 countries around the world (Clifford et al., 2005a).

The age-specific prevalence curve showed a clear peak in women up to 25 years of age with subsequent decline until an age range of 35–44 years, and an increase again in all regions included in the meta-analysis except Asia (de Sanjosé et al., 2007). In the IARC population-based survey, a first peak was observed in women under 25 years of age, and a second peak after 45 years of age in most Latin American populations, but the HPV prevalence was high across all age groups in a few places in Asia and in Nigeria (Franceschi et al., 2006). In this survey, the prevalence of high-risk HPV correlates well with cervical cancer incidence, and the strength of the correlation steadily increases with age (Maucort-Boulch et al., 2008).

Data on HPV DNA prevalence and natural history of genital HPV infection in men is scant, and difficult to evaluate. There is great variation in the prevalence depending on anatomical sites sampled, sampling methods, and HPV DNA detection assays. In general, the overall HPV prevalence is over 50%, and the proportion of low-risk types is higher in men than in women (Giuliano et al., 2008). However, the biological or clinical meaning of the HPV DNA detected in the superficial layers of genital skin is not yet clear. Unlike what has been observed in women, no clear age pattern is detected in HPV prevalence rates in men (Giuliano et al. 2008).

HPV prevalence is lower in the oral cavity than in other anogenital sites. Among women who practiced prostitution, HPV DNA prevalences for specimens from the cervix, vagina, and oral cavity have been observed to be 27.8%, 26.1%, and 15%, respectively (Cañadas et al., 2004). HPV infections of the skin are extremely common, but the type distribution is different (beta and gamma genera predominating) than the mucosal types in the alpha genus that commonly infect the anogenital tract and the oral cavity.

1.2.2 Transmission and risk factors for infection

HPV infections are transmitted mainly through direct skin-to-skin or skin-to-mucosa contact. The viruses are easily transmitted and each genotype has its characteristic tissue tropism and characteristic age-specific peak transmission curve. In line with the unequivocal demonstration of sexual transmission of anogenital HPV, the number of sexual partners has been shown to be the main determinant of anogenital HPV infection both in women and men. The highest
Figure 1.2 HPV DNA crude prevalence and HR-HPV type-specific prevalence among women with normal cytology by world region: meta-analysis including 157,879 women from 36 countries

HPV DNA crude prevalence and the five most frequent HPV type-specific prevalence among women with normal cytology by world region: meta-analysis including 157,879 women from 36 countries.

Bosch FX et al, 2008 Vaccine
de Sanjosé et al, 2007 Lancet Infect Dis

'Other HR' includes the 6 most common HPV types in cervical cancer other than 16 and 18: HPV-31, 33, 35, 45, 52, 58

Art work: Laia Bruni adapted from Bosch et al. (2008) and de Sanjosé et al. (2007)
incidence of anogenital infection occurs in teens and young adults. Increasing age is linked to decreasing acquisition of anogenital HPV infection as a corollary of fewer new partners and, possibly, immunity to previously cleared infections (Burchell et al., 2006; Dunne et al., 2006).

HPV infection probably requires access to basal cells through micro-abrasions in the epithelium (Burchell et al., 2006). Circumcision and condom use have also been associated with a reduced risk of infection in men and their partners (Burchell et al., 2006; Dunne et al., 2006). Although it has been reported that smoking, use of oral contraceptives, parity, other sexually transmitted agents, age at first sexual intercourse, and host susceptibility may influence the risk of acquisition of HPV infection (Burchell et al., 2006; Moscicki et al., 2006), the epidemiological evidence is inconsistent.

Non-sexual routes account for a tiny minority of HPV infections, and include perinatal transmission and, possibly, transmission by medical procedures and fomites.

1.2.3 Persistency, latency, and natural history of infection

Most HPV infections clear within 1–2 years. However, estimates of duration of infection for individual types vary from study to study, and depend not only on the statistical methods used (definition of clearance, use of mean or median), but also on the accuracy of the HPV DNA detection methods. Although it has been reported that infections in older women last longer, suggesting greater risk of cancer (Castle et al., 2005a), this only pertains to detected infections found at the baseline of cross-sectional screening. There is no association between HPV incident infection duration and age, when infections detected during follow-up are followed in cohort studies (Trottier et al., 2008; Muñoz et al., 2009). Persistent HPV infection is a prerequisite for the development of high-grade precancerous lesions (cervical intraepithelial neoplasia [CIN]3) and cervical cancer, but for epidemiological purposes there is no consensus on the definition of persistent infection. Most investigators call persistent infections those in which the same HPV type or group of HPV types is detected during two consecutive visits, but these two visits could be 4 months up to 5–7 years apart, leading to serious conceptual problems (Woodman et al., 2007). A new definition of persistent HPV infection based on the duration of incident infection has been proposed (Muñoz et al., 2009). Moreover, there are many parameters of the natural history that are unknown (e.g., the precise time of HPV acquisition, and the probable existence of latent infections with possible reactivation as suggested by new detection among sexually inactive older women); overall, the distinction between transient and persistent infection is impossible to establish accurately.

Despite these limitations, persistence defined as HPV positivity at two or more visits has been associated with an increased risk of CIN2/3 lesions in most studies included in a meta-analysis (Koshiol et al., 2008). In particular, repeat detection of HPV 16 is associated with an extremely high cumulative risk of subsequent CIN3+ diagnosis, exceeding 30% in some cohorts (Wheeler et al., 2006; Rodríguez et al., 2008). Persistence is not sufficient for carcinogenicity because there are non-carcinogenic types, like HPV 61, that persist without carcinogenic risk (Schiffman et al., 2007).

Host susceptibility factors and immune responses are obviously important but poorly understood determinants of persistence and progression. Other cofactors are discussed under Section 2.6.

CIN3 can develop very quickly (within 2–3 years) following HPV exposure, especially in young women (Winer et al., 2005; Ault, 2007). Initially, CIN3 lesions are very small, and it takes a few years for them to grow and to be detectable by cytology and then colposcopy. In young,
Human papillomaviruses

intensively screened women, the median age of CIN3 diagnosis was around 23 years, while it was 38 years in a cohort of women from New Zealand where screening and treatment were inadequate (McCredie et al., 2008; Schiffman & Rodríguez, 2008).

A direct estimate of the rate of progression from CIN3 to invasive cervical cancer has been reported in the cohort of 1063 women from New Zealand for whom treatment for CIN3 was withheld or delayed in an unethical clinical study starting in the 1960s. Cumulative incidence of invasive cervical cancer was 31.3% at 30 years of follow-up among 143 women who had had only diagnostic biopsies, and it was 50.3% in the subset of 92 women who had persistent CIN3 during 24 months. Cancer risk at 30 years was 0.7% for women whose initial treatment for CIN3 was considered adequate (McCredie et al., 2008).

[The Working Group noted that McCredie et al. were not responsible for the unethical study but gathered data from that study, and did the final follow-up.] It is unknown which proportion of small early CIN3 lesions will eventually progress to invasive cancer.

1.2.4 Evaluation of HPV vaccination on precancerous lesions occurrence or decrease

Two prophylactic HPV vaccines are currently marketed. One is bivalent and contains VLP antigens for HPV 16 and 18, and the other is quadrivalent and contains VLP antigens for HPV 16, 18, 6, and 11. Both vaccines are designed to prevent HPV infection and HPV-related disease, and not to treat women with past or current HPV infection or disease. End-points of CIN2/3 or adenocarcinoma in situ (AIS) have been widely accepted as a proxy for cervical cancer that can be studied ethically in efficacy trials.

Both vaccines have efficacies of >90% against CIN2 or higher grade among women aged 15–26 years who had no evidence of past or current infection with HPV types related to type-specific VLP antigens. Efficacy estimates vary by vaccine, type of study, the population analysed, and duration of follow-up (Ault et al., 2007; WHO, 2009). In addition, the HPV vaccine trials with the quadrivalent vaccine have shown an efficacy close to 100% against high-grade vulvar (VIN2/3) or vaginal intraepithelial lesions (VaIN2/3) related to HPV 16 or 18, and against genital warts related to HPV 6 or 11 among the per-protocol susceptible population (Garland et al., 2007; Joura et al., 2007). Although protection with both vaccines has been shown to last 5–6 years, their long-term protection and their impact on the prevention of cervical cancer and of other genital and non-genital HPV-associated tumours remains to be determined.

2. Cancer in Humans

2.1 Cancer of the cervix

Epidemiological evidence for the carcinogenicity of HPV was originally presented in Volume 64 of the IARC Monographs (IARC, 1995), and was extensively updated in Volume 90 of the IARC Monographs (IARC, 2007), based on data available as of February 2005.

HPV carcinogenicity has been established most convincingly for cancer of the cervix. HPV behaviour is strongly correlated with phylogenetic (i.e. evolutionary or taxonomic) categories (Schiffman et al., 2005). All HPV genotypes that are known to be cervical carcinogens belong to the alpha genus, in an evolutionary branching or high-risk clade containing a few genetically related species (Table 2.1 and Fig. 2.1). HPV 16 (alpha-9) and HPV 18 (alpha-7) have been classified as cervical carcinogens since 1995. HPV 31 and HPV 33, in alpha-9, were categorized as probably carcinogenic. In 2005, the group of cervical carcinogens was expanded to include the following 13 types: alpha-5 genotype HPV 51,
alpha-6 genotypes HPV 56 and HPV 66, alpha-7 genotypes HPV 18, HPV 39, HPV 45, and HPV 59, and alpha-9 genotypes HPV 16, HPV 31, HPV 33, HPV 35, HPV 52, and HPV 58.

There is virtually no epidemiological evidence of cervical carcinogenicity for other species in the alpha genus or for other genera. To save considerable space presenting null evidence, this section will not include data related to HPV species alpha-1, -2, -3, -4, -8, -10 (other than HPV 6 or 11), -13, or -14/15. These species contain HPV types that cause skin or genital warts, minor cytological atypia, and often no apparent disease.

Since the previous IARC Monograph, new evidence has further supported that HPV types in the high-risk clade of the alpha genus cause virtually all cases of cervical cancer worldwide (Smith et al., 2007; Bosch et al., 2008). In case-control studies, the odds ratios (ORs) associating cervical cancer and its immediate precursor, CIN3, with HPV DNA positivity for these high-risk types in pooled probe tests consistently exceed 50. It is persistent infections that are associated with an extremely high absolute risk of CIN3 and cancer. In cohort studies, women who test negative for this group of HPV types as assayed by hybrid capture 2 (HC2, including a mix of the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and several cross-reacting genotypes in the high-risk clade) are at extremely low subsequent risk of cancer for at least 10 years (Khan et al., 2005).

Because persistent infection with HPV is a nearly necessary cause of cervical cancer, a reconsideration of HPV and cervical carcinogenicity based on the new (non-cohort) data must be made to decide whether any additional types within the high-risk clade are also carcinogenic, and whether any types that were previously categorized as carcinogenic should be downgraded. The types in the high-risk clade are listed below.

Given the existence of some HPV types that are very carcinogenic, notably HPV 16 and HPV 18, determining which less common and weaker types are also carcinogenic becomes, in epidemiology, an issue of confounding. The alpha genus types share a common route of transmission, and multiple infections are present in a large minority of women, both concurrently and sequentially. None of the traditional approaches to control confounding is entirely successful. Because HPV 16 causes over 50% of cases of cervical cancer (Clifford et al., 2003; Smith et al., 2007), logistic regression and similar approaches will parsimoniously attribute to HPV 16, cases associated with both HPV 16, and a less important type. HPV 18 is the second most important cervical carcinogen, responsible for approximately 15% of cervical cancer of all histological types combined (and a higher fraction of adeno-carcinomas) (Clifford et al., 2003; Smith et al., 2007). If a type co-occurs with either HPV 16 or HPV 18, its association with cervical cancer might be confounded by either of these powerful carcinogens. For types causing only a very small fraction of cervical cancer, confounding by any of the more important types is possible.

### Table 2.1 HPV types in the high-risk clade

<table>
<thead>
<tr>
<th>Alpha HPV species</th>
<th>Types classified as Group 1 carcinogens in Volume 90</th>
<th>Other Types in Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>51</td>
<td>26, 69, 82</td>
</tr>
<tr>
<td>6</td>
<td>56, 66</td>
<td>30, 53</td>
</tr>
<tr>
<td>7</td>
<td>18, 45, 39, 59</td>
<td>68, 70, 85, 97</td>
</tr>
<tr>
<td>9</td>
<td>16, 31, 33, 35, 52, 58</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>34, 73</td>
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</table>
Figure 2.1 Phylogenetic tree of 100 human papillomavirus types with an highlight of the high-risk alpha species

Phylogenetic tree of 100 human papillomavirus types inferred from the nucleotide sequences of 5 ORFs (E7, E1, E2, L2 and L1). The tree was constructed using the Markov chain Monte Carlo (MCMC) algorithm in BEAST v1.4.8 (Drummond & Rambaut, 2007). HPV species were generally classified according to the new classification system for PVs by de Villiers et al. (2004). All subtypes of the alpha PVs were included in the tree, followed as HPV 44 is a subtype of HPV 55, AE9 is a subtype of HPV 54, HPV 64 is a subtype of HPV 34, ME180 is a subtype of HPV 68, and AE2 is a subtype of HPV 82.

In red are highlighted the alpha HPV types previously classified as carcinogenic to humans (Group 1) in Volume 90, and the alpha species (high-risk species) to which they belong.

Adapted from an unpublished figure (courtesy of Robert D. Burk and Zigui Chen)
Dealing with confounding by exclusion, i.e. examining the possibility of carcinogenicity of a more minor type among cancer specimens that do not contain a more important type, becomes a problem of misclassification. The main epidemiological criterion used for the classification of an HPV type as a carcinogen, i.e. finding the HPV genotype as a single infection in a cervical scrape or biopsy specimen in a woman with cancer, might sometimes be too lax, and prone to error. Colposcopic biopsies and cytology specimens can be misdirected and fail to obtain the critical cells, whereas the contamination of scrapes and biopsies from lower-grade lesions that often surround cancers can lead to the detection of types other than the causal one. Studies relying on the testing of microdissected cervical malignancies will address these issues, but large-scale highly accurate data are not yet available.

Difficulty with control selection adds another level of complexity in assessing carcinogenicity. As discussed in the section on the HPV natural history of infection (Section 1.2), cervical cancer typically follows age of infection by decades. HPV DNA and RNA transmitted at young ages usually become undetectable and no sensitive serological assay exists to measure HPV exposure. Consequently, odds ratios based on a comparison of HPV DNA prevalence at the time of case diagnosis to age-matched HPV point prevalence in controls do not estimate true relative risks.

See Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.2.pdf, Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.3.pdf, and Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.4.pdf.

Only sparse type-specific prospective data is available on the carcinogenicity of the full range of individual HPV genotypes (Khan et al., 2005; Schiffman et al., 2005; Wheeler et al., 2006). Studies have categorically shown the unique carcinogenicity of HPV 16 (Khan et al., 2005; Bulkmans et al., 2007; Kjaer et al., 2009; Muñoz et al., 2009). HPV 18 causes a lower and more delayed absolute risk of CIN3+ diagnosis. Khan et al. (2005) observed that a 10-year cumulative risk of ≥ CIN3 for the women who were positive by the pooled probe HC2 assay, but negative for HPV 16 or HPV 18, was 3.0% (95%CI: 1.9–4.2) compared with the risk of 0.8% (95%CI: 0.6–1.1) among women who were HC2-negative at baseline. However, there is not convincing long-term prospective evidence for individual HPV types other than HPV 16 and HPV 18.

Finally, the accuracy of detection of HPV genotypes differs between the major polymerase-chain-reaction(PCR)-based systems used to generate most of the data. The epidemiological study of individual HPV genotypes is made more difficult with the variety of methods available for testing. In the past few years, the major HPV genotyping methods have converged towards a common, improved standard of analytical sensitivity and specificity, but none of the main methods is the reference standard (Gravitt et al., 2008). Sequencing of PCR products is also imperfect because multiple HPV types can infect tissues concurrently, and sequencing distinguishes multiple infections sub-optimally. The methods have evolved over time, producing additional testing variability that is difficult to appreciate as a reader. The residual error in HPV genotyping occurs mainly when multiple infections are present, and for the less important carcinogenic types. As a result, determining the major carcinogenic types can be done rather easily, but ruling out confounding in the context of multiple infections can be quite difficult.

With these caveats, the cervical carcinogenicity of the HPV types listed above varies in strength in a continuum without clear breakpoint, from extremely strong (i.e. HPV 16 and, to a lesser degree, HPV 18) to weak, but still may cause cervical cancer in rare instances (e.g. HPV 68, see below). Evaluators taking one extreme
position could rightfully claim that there is reasonable evidence for the carcinogenicity of virtually all the types in the species listed above, extending further the list established in the previous *IARC Monograph*. Strict interpreters of causal criteria could argue for a return to a more limited list. But based on current evidence, no clear cut-off between sufficient, limited, and inadequate evidence is entirely defensible.

The Working Group chose the following pragmatic approach to creating an imperfect cut-off between sufficient, limited, and inadequate epidemiological evidence for cervical carcinogenicity:

The Working Group considered only types in the high-risk clade because data are inadequate for all others. The Working Group evaluated the most recent and accumulated data on cervical cancers from very large single projects (e.g. *Bosch et al.*, 2008), and especially as summarized in meta-analyses from IARC (*Smith et al.*, 2007, updated as needed by the Working Group). The Working Group excluded from consideration high-grade precancerous lesions (CIN3 and the more equivocal CIN2 which occur in approximately 1% of screened women) often used as ethical surrogate end-points in prospective studies and clinical trials, because there are now enough data for invasive cancers, and because it appears that HPV types have different potential to progress from CIN2/3 to invasive cervical cancer (*Clifford et al.*, 2003). For comparisons with the background frequency of cervical HPV infection in the general female population, the Working Group noted the prevalence from a large meta-analysis of HPV genotypes found in women with normal cytology (*de Sanjosé et al.*, 2007).

About 10–30% of women with detectable HPV DNA exhibit definite cytological abnormalities, depending on the HPV type, cytological cut-off point and DNA test (*Kovacic et al.*, 2006). But low-grade or even equivocal lesions represent only a few percent of screening cytological tests; therefore, the population prevalence of an HPV genotype (in controls) can be approximated by its prevalence in cytologically normal women.

Comparing the prevalence in women with normal cytology (*de Sanjosé et al.*, 2007) to the prevalence in women with invasive cervical cancers compiled by *Smith et al.* (2007), one can see obvious “case–control” differences. The most clearly carcinogenic genotypes, HPV 16 and HPV 18 in particular, are more common among cervical squamous carcinomas than cytologically normal women or even in low-grade squamous intraepithelial lesions (*Clifford et al.*, 2005b). HPV 18 is especially common in adenocarcinomas (*Bosch et al.*, 2008), as are other members of the alpha-7 clade of which HPV 18 is a member (*Clifford & Franceschi, 2008*), lending additional support to the importance of genetic similarity in terms of the carcinogenicity of different HPV types. Almost all types of HPV in the high-risk clade – except for HPV 16 and HPV 18 – are (relatively) more common in low-grade lesions.

Including HPV 16 and HPV 18, eight HPV types (alpha-7, HPV types 18 and 45; alpha-9, HPV types 16, 31, 33, 35, 52, and 58) are the most common types found in cancers in both the Catalan Institute of Oncology (ICO) study and the IARC meta-analysis (*Bosch et al.*, 2008; *Clifford & Franceschi, 2008*) in all regions of the world providing data. These types are all much more common in cancer case specimens than in controls, providing sufficient epidemiological evidence of carcinogenicity.

To move beyond the most clearly carcinogenic eight HPV genotypes, the Working Group chose the presence of HPV 6 as a surrogate for estimating the percentage of cancers that might contain HPV DNA by accumulated and unknown measurement errors alone. The reasons being that HPV 6, the common cause of benign condyloma acuminata (external genital warts), is an archetype of a low-risk type, is not classified as a cervical carcinogen, and is very uncommonly detected in cervical cancer specimens. [When detected, even without detection...
### Table 2.5 Meta-analysis of type-specific HPV DNA prevalence in invasive cervical cancer

<table>
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<tr>
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<th>Invasive cervical cancer</th>
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<tr>
<td></td>
<td>N tested</td>
<td>% pos</td>
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<tr>
<td>HPV 16</td>
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</table>

Compiled by the Working Group during the meeting.

Data for women with normal cytology is from de Sanjosé et al. (2007), but for other HPV types, the data from Smith et al. (2007) was updated by the Working Group using the following 61 published studies: Andersson et al. (2005), Bardin et al. (2008), Beeren et al. (2005), Bertelsen et al. (2006), Bhatla et al. (2006), Bryan et al. (2006), Bulk et al. (2006), Bulkmans et al. (2005), Cambruzzi et al. (2005), Castellsagüé et al. (2008), Chan et al. (2006), Chen et al. (2006), Cioti et al. (2006), Dabč et al. (2008), Daponte et al. (2006), De Boer et al. (2005), de Cremoux et al. (2009), De Vuyts et al. (2008), Del Mistro, et al. (2006), Esmaeili et al. (2008), Fanta (2005), Gargiulo et al. (2007), Ghaffari et al. (2006), Gheit et al. (2009), Guo et al. (2007), Hadziiejdj et al. (2007), Hindryckx et al. (2006), Hong et al. (2008), Inoue et al. (2006), Khan et al. (2007), Kisaer et al. (2008), Klug et al. (2007), Kulmala et al. (2007), Lai et al. (2007b), Lee et al. (2007), Liu et al. (2005), Maehama (2005), Odida et al. (2008), Panotopoulou et al. (2007), Peedicayil et al. (2006), Piña-Sánchez et al. (2006), Pretet et al. (2008), Qi et al. (2007), Ressler et al. (2007), Sigurdsson et al. (2007), Sirianukul et al. (2008), Song et al. (2007), Sowjanya et al. (2005), Sriamporn et al. (2006), Stevens et al. (2006), Su et al. (2007), Tao et al. (2006), Tawfik El-Mansy et al. (2006), Tong et al. (2007), Tornesello et al. (2006), Wentzensen et al. (2009), Wu et al. (2008), Wu et al. (2006), Zhao et al. (2008), Zuna et al. (2007).
of a more likely causal type, the Working Group judged that misclassification of some kind was a more likely explanation than causality.] The best published estimate of percentage of detection of HPV 6 in cervical cancers (not necessarily as a single infection) was judged to be 0.5% (95%CI: 0.4–0.6), based on 15000 cases of cancer (Smith et al., 2007; estimated and confirmed by the Working Group update, see Table 2.5). The Working Group took the pragmatic approach once more and made the following rule—an individual HPV type in the high-risk alpha clade (i.e. with an elevated prior probability of being carcinogenic due to analogy to closely related viral types in the same or closely-related species) was considered to have sufficient epidemiological evidence of carcinogenicity if:

- its prevalence in cancers was significantly greater than that of HPV 6.
- its prevalence in cancer was significantly enriched in comparison to the background estimate for the general population, i.e. women with normal cytology.

By this logic, four more types were judged, as in the previous IARC Monograph, to have sufficient epidemiological evidence of cervical carcinogenicity: alpha-5 HPV 51, alpha-6 HPV 56, and alpha-7 HPV types 39 and 59.

The remaining types in the high-risk alpha clade (see Table 2.1) were considered, as a group, to have limited evidence to support carcinogenicity. If phylogeny can be taken to predict behaviour, it is possible that most of these types can very rarely cause cancer. Indeed, many of the types have been detected, albeit uncommonly (no greater than HPV 6), in cancers. There are not enough data, even after testing of many thousands of specimens, to be sure which types are definitely carcinogenic or not. But, within this group, there are two types, alpha-7 HPV 68 and alpha-11 HPV 73, for which the data are slightly stronger than for the others despite methodological challenges. One of the major PCR-based testing methods (SPF10) cannot distinguish these two types because their amplicons using those primers are identical. Neither of these two types is optimally detected by MY09-MY11 dot blot (Gravitt et al., 2008). Nonetheless, the data supporting the carcinogenicity of HPV 68 and HPV 73 are suggestive.

This categorization scheme leads to the re-classification of HPV 66, for which the evidence of carcinogenicity was previously judged sufficient. In the assembly of much more testing data from cancer cases, HPV 66 has been found so rarely that its percentage of detection is less than the relative percentage of detection among the general population. In the Working Group review of each individual article, HPV 66 was found alone in cancers with extreme rarity, well below the possible threshold of confounding and misclassification.

2.1.1 Summary

The data accumulated supports:

- The unique carcinogenic strength of HPV 16.
- The importance of HPV 18 and genetically related types (Clifford & Franceschi, 2008) in causing adenocarcinoma compared with squamous cell carcinoma.
- The weaker but still clear carcinogenic potential of six additional types in alpha-7 (HPV 45) and alpha-9 (HPV 31, 33, 35, 52, and 58), with some regional variation in the etiological fractions of cancers due to each type. For example, HPV 52 and 58 are relatively more prevalent in Asia than in other regions, HPV 33 is most clearly prevalent in Europe, and HPV 45 has particular regions where it is prominent.
- The small, and less certain, incremental etiological contributions of another group of carcinogenic types from alpha-5 (HPV 51), alpha-6 (HPV 56), and alpha-7 (HPV 39 and HPV 59). Each causes a few percent at most of cervical cancer cases.
worldwide, although regional variability has been observed.

- Acknowledgement of an unresolved dividing line between the HPV types with the weakest evidence judged to be sufficient, and those with evidence judged highly suggestive yet limited (alpha-7 HPV 68 and alpha-11 HPV 73).
- A re-evaluation of the evidence for HPV 66. The data were re-evaluated and the evidence was judged to be very limited now that more cases have been studied showing that it is very rarely found in cancers despite being relatively common. HPV 53, also in alpha-6, shows the same pattern of relative common population prevalence with extremely rare cases of occurrence alone in cancer. The Working Group noted that for these types in particular, there could be harm to public health if the types are included as carcinogenic in screening assays, which would decrease the specificity and positive predictive value of the assays with virtually no gain in sensitivity and negative predictive value.
- The existence of a few types within the high-risk clade that have extremely sparse or no evidence of carcinogenicity. For some types there are anecdotal but very interesting cases that merit further study of additional carcinogenic types. For example, the carcinogenicity of alpha-5 HPV 26 has been supported by a recent report of multiple peri-ungual cancers in an immunosuppressed individual, containing high viral loads, and active transcription of HPV 26 alone (Handisurya et al., 2007). There have been reports of alpha-9 HPV 70 found as single infections in cervical cancer (Lai et al., 2007a), but the supportive data are sparse. There are only a few reports of HPV 67 in cancer (Gudleviciene et al., 2006; Wentzensen et al., 2009), which is intriguing because this is the only known type in the alpha-9 species that is not categorized as carcinogenic. For a few types in the high-risk clade, no reports of invasive cancers with single-type infections were found, but isolated reports might exist.

### 2.2 Cancer at other anogenital sites

#### 2.2.1 Cancer of the vulva

Cancer of the vulva is rare. The tumours are generally of epithelial origin and squamous cell carcinoma is the most common histological type. Tumours can be mainly categorized as keratinizing, non-keratinizing, basaloid, warty and verrucous vulvar tumours. Basaloid/warty types comprise about a third of cases, are more common in younger women, tend to harbour VIN lesions, and are often associated with HPV DNA detection. These tumours appear to share the epidemiological factors of cervical cancer. On the contrary, keratinizing types, with older average age at diagnosis, apparently arise from chronic vulvar dermatoses or from squamous metaplasia, and are more rarely associated with HPV. See Table 2.6 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.6.pdf](http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.6.pdf), Table 2.7 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.7.pdf](http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.7.pdf), and Table 2.8 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.8.pdf](http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.8.pdf).

(a) **Case series**

Tables 2.6 and 2.7 (on-line) present case series of more than ten cases of VIN3 or invasive cancer of the vulva.

A large proportion of VIN3 cases harbour HPV DNA with HPV 16 being the most common type detected in over 79% of positive samples (Table 2.6 on-line). More recent larger series confirm the presence also of HPV 33 and more
HPV 6 was slightly more frequent in vulvar (2.0%) and anal (2.9%) carcinoma than in cervical carcinoma (0.5%) (Smith et al., 2007; De Vuyst et al., 2009), but it was most often accompanied, among cases where this information was available, by multiple infections with high-risk types. De Vuyst et al. (2009) observed that HPV 6 and 11 were frequently detected in VIN1 and AIN1 (anal intraepithelial neoplasia, as in anogenital warts), but not in VAIN1 (vaginal intraepithelial neoplasia).

In Insinga et al. (2008), after multitype adjustment, HPV 6 was estimated to contribute to the largest fraction (29.2%) of VIN1 lesions, with the top two (HPV 6 and 11), four (HPV 6, 11, 68, and 16), and eight (HPV 6, 11, 68, 16, 58, 59, 31, and 66) reported HPV types accounting for 41.7%, 55.9%, and 67.8% of VIN1 lesions, respectively. The attribution of HPV 6 and 11 to VIN1 lesions (41.7%) was greater than that estimated for CIN1 (6.9%; P < 0.0001).

(b) Case–control studies

There are several case–control studies on invasive vulvar cancer using serology, but few have tested for HPV DNA (Sagerman et al., 1996; Madsen et al., 2008; see Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.9.pdf). Also, the design of these studies was considered inadequate (e.g. using endometrial cases as controls). Serological studies reported contradictory data on the association between type-specific epitopes and vulvar cancer. In Bjørgen et al. (1997), Madeleine et al. (1997), and Carter et al. (2001), statistically significant increased risks associated with antibodies against HPV 16 were observed but not against HPV 18. Results did not reach statistical significance in Hildesheim et al. (1997).

Overall, the data indicate that HPV plays a role in vulvar cancer, in particular in tumours with basaloid/warty features, with HPV 16 having a predominant role. Based on case series,
HPV 18 and HPV 33 may also be involved in a small fraction of these tumours.

2.2.2 Cancer of the vagina

Tumours of the vagina are very rare but varied. They can be of different cell origin: epithelial, mesenchymal, melanocytic, lymphoid, and secondary. Epithelial tumours are the most common and include keratinizing, non-keratinizing, basaloid, verrucous, and warty squamous cell carcinoma types, and are strongly related to HPV. In the previous IARC Monograph, HPV 16 was reported in more than 50% of the cases, and the evidence of an association with HPV 18 was reported to be weaker.

The epidemiology of vaginal cancer is not clearly understood as few analytical studies are available, and it is difficult to disentangle these tumours with those that originate in the cervix. Approximately 30% of all cases report treatment for a prior anogenital tumour compared to 2% in controls, most often of the cervix (Daling et al., 2002).

A recognized independent risk factor for vaginal cancer is the exposure to diethylstilbestrol during pregnancy. Tumours arising after this exposure are clear cell adenocarcinoma of the vagina. Recurrent clear cell adenocarcinoma has been observed as long as 20 years after primary therapy (Herbst & Anderson, 1990).

(a) Case series

In the two recent studies on VAIN3 tissues, over 90% harboured HPV DNA. The meta-analyses by De Vuyst et al. (2009), including 298 cases of VAIN and 136 cases of invasive cancer, reported detection of HPV DNA in 93.6% of VAIN3 lesions, and in 69.9% of vaginal carcinomas. In both VAIN3 and vaginal carcinomas, HPV 16 was by far the most common type detected followed by HPV 18, 31, 33, and 6.

The most recent series of invasive cases found HPV DNA in 17/21 women [80.9%] (Ferreira et al., 2008). HPV 16 was the predominant type but HPV 31 and HPV 33 were also relatively common. Madsen et al. (2008) detected HPV DNA in 24/27 [88.9%] squamous cell carcinomas of the vagina. HPV 16 was the most prevalent type followed by HPV 33. The meta-analysis by De Vuyst et al. (2008) estimated an overall HPV prevalence of 69.9% with a geographic range of 43.8% in Asia to 76.8% in Europe.

See Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.10.pdf and Table 2.11 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.11.pdf.

(b) Case–control studies

Since the previous IARC Monograph, only one case–control study of HPV and vaginal cancer has been published. Madsen et al. (2008) carried out a case–control study on cancer of the vagina in Denmark. [The Working Group considered this study as inadequate to provide analytical evidence of HPV and vaginal cancer due to problems with the selection of controls.]

In summary, recent data on HPV and vaginal cancer are few, and have not altered the evaluation of the role of genotypes other than HPV 16. The rarity of these tumours and their contiguity with cervical cancer remain difficulties for specific epidemiological research of this cancer.

See Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.12.pdf and Table 2.13 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.13.pdf.

2.2.3 Cancer of the penis

Cancer of the penis is a rare tumour whose incidence correlates with that of cervical cancer. Analogous to vulvar cancer, some risk factors vary by histology. The large majority of penile tumours are squamous cell carcinomas although making a histological subtype distinction can be
controversial. Preneoplastic intraepithelial lesions (PIN) are recognized precursor stages in which HPV is also generally identified. HPV-negative squamous carcinoma cases are suspected to relate to chronic inflammation with risk factors such as phimosis, lichen sclerosis, and lack of circumcision. Smoking and a history of genital warts have also been linked to cancer of the penis.

(a) Case series

The prevalence of HPV DNA in penile cancer varies by histological type. Case series tend to include a relatively small number of cases per histological subtype, ranging from one to over 170 cases (see Table 2.14 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.14.pdf).

Since the previous IARC Monograph, more than a dozen new case series on invasive penile cancer have been identified in which HPV DNA was assessed. No studies on PIN were identified. Among these studies, the largest series is that of Lont et al. (2006) with 171 paraffin-embedded penile tumours, in which the overall detection of HPV was 29.2%. The low prevalence of HPV among the warty or basaloid types (25% and 0%, respectively) in this study and in Guerrero et al. (2008) contrasts with some smaller series showing prevalence estimates over 60–70% (Rubin et al., 2001; Salazar et al., 2005; Pascual et al., 2007). Intermediate HPV prevalence estimates of < 50% were observed in another small serie of warty carcinoma of the penis (Bezerra et al., 2001).

Although there is a wide variation of HPV prevalence in penile cancer studies, two recent meta-analyses provide a summary estimate that may be more meaningful (Backes et al., 2009; Miralles-Guri et al., 2009). Taking into account over 1400 cases of penile cancer, HPV was detected in 47% of the cases with the highest HPV prevalence (76%) in penile tumours with a basaloid squamous cell carcinoma component, and the lowest (24.5%) in penile verrucous squamous cell carcinoma. Basaloid/warty tumours were overall 3.5 times more likely to be HPV-positive.

Contrary to that observed for vulvar cancer, Backes et al. (2009) reported a 2.7 times higher presence of HPV-positive tumours in Asia as compared to cases derived from North America (OR, 2.7; 95%CI: 1.8–4.0) without any clear explanation of why this should be. Recent studies were more likely to report HPV-positive cases. Consistent with the previous IARC Monograph, HPV 16 was the most common type detected (60.2%), irrespective of histology, followed by HPV 18 (13.3%) (Miralles-Guri et al., 2009). Other cervical carcinogenic HPV types such as HPV 35, 45, 51, 52, 56, and 59 were detected sporadically as were HPV 68, 70, and 74. Types not established to be carcinogenic were observed in close to 10% of cases, but it was not clarified how many of these were found in combination with carcinogenic infections.

HPV 6 was detected in 39 (6.7%) of 580 cases that were tested for HPV 6, with a notable presence of multiple co-infections with high-risk types.

(b) Case–control studies

The data from case–control studies in which HPV was evaluated using serological markers is summarized in Table 2.15 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.15.pdf). In these small studies, which were not included in the previous IARC Monograph, no detailed histological evaluation was provided. The risk for penile cancer, including in-situ cases, was associated with seropositivity to HPV 16 in particular.

In summary, studies of penile cancer suggest that HPV infection appears to play an important role in almost half of the cases. No additional validated information on the specific contribution of types other than HPV 16 or 18 can be derived from recent studies. HPV 6 and HPV 11 were detected in a small proportion of cases.
2.2.4 Cancer of the anus

Cancer of the anus is a relatively rare disease but increasing incidence is being reported in some countries with increases of >160% in men or 78% in women in the USA over a period of 20 years (Daling et al., 2004). Established risk factors for anal cancer include a high number of sexual partners, receptive anal sex, history of venereal diseases; smoking has also been proposed (Frisch et al., 1997; Daling et al., 2004). Anal cancer is more common among HIV-infected subjects (for anal cancer in HIV patient see Section 2.8.3b).

The anal canal consists of a segment of approximately 4 cm of squamous mucosa limited distally by the anal verge or margin (transitional zone between the skin and mucosa) and proximally by the dentate line (transitional zone between the squamous and glandular mucosa). Malignant tumours of the anal canal are largely carcinomas of squamous cell origin. Most adenocarcinomas arising in the anal mucosa represent a downward spread from an adenocarcinoma in the rectum or arise in colorectal-type mucosa above the dentate line, and generally are excluded in the studies specific to the anal canal.

(a) Case series

Case series of precursor lesions, AIN, published since the previous IARC Monograph have been included in Table 2.16 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.16.pdf). The more recent studies (Hampl et al., 2006; Varnai et al., 2006) demonstrate that the great majority of cases are HPV-positive, mainly with HPV 16. Case series of invasive lesions have been included in Table 2.17 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.17.pdf) (Kagawa et al., 2006; Varnai et al., 2006; Laytragoon-Lewin et al., 2007; Tachezy et al., 2007). All studies found high proportions of HPV in the tumours, predominantly HPV 16. Of interest is the lower proportion of HPV-related tumours in those cancers localized in the perianal skin as compared to other anal sites (Frisch et al., 1999).

De Vuyst et al. (2009), in a meta-analysis of 1280 cases of AIN (671 AIN1 and 609 AIN2/3) of which 805 were HIV positive and 955 cases of invasive carcinomas, estimated that the large majority of AIN2/3 lesions (93.9%) and of invasive carcinomas (84.3%) were attributable to HPV. As seen in Fig. 2.2, the most frequent types detected for AIN2/3 were HPV 16 (59.8%), 18 (17.4%), 33 (13.6%), and 58 (13.1%). In anal carcinoma, the most frequent types were HPV 16 (73.4%), followed by HPV 18 (5.2%), and HPV 33 (4.8%). Although not studied extensively, HPV 45 was rarely identified, which probably reflects the squamous nature of these tumours. HPV 6 was detected in 2.9% of the anal carcinomas, but its presence could not be disentangled from concomitant infections with high-risk types.

(b) Case control studies

One case–control study has compared the presence of HPV DNA in anal tumours of 417 individuals (349 squamous cell carcinomas and 68 in-situ squamous cell carcinomas) to 534 individuals with rectal adenocarcinomas (Frisch et al., 1997). High-risk types of HPV were detected in 84% of the anal cancer specimens, but no HPV was identified in the rectal tumours. HPV 16 was the most commonly identified type, followed by HPV 33, 18, 6, and 31. HPV was more commonly identified in tumours of women with invasive or in-situ anal cancer (93%) as compared to men (69%). Table 2.18 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.18.pdf) and Table 2.19 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.19.pdf) summarize case–control studies of anal cancer that relied on HPV serology. The studies (Carter et al., 2001; Bjørge et al., 2002; Daling et al., 2004) resulted in odds ratios of 3–5 for the association between HPV 16 and HPV 18 seropositivity and
Figure 2.2 Prevalence of human papillomavirus types in case series of anal intraepithelial neoplasia (AIN) grades 2/3 and anal cancer

Adapted from De Vuyst et al. (2009)
anal cancer, for both men and women. As an indication of type specificity, Bjørge et al. (2002) found neither association for HPV 33 nor HPV 73.

Overall, HPV is detected in over 80% of anal cancer cases suggesting a causal relationship. The large majority of tumours are related to HPV 16, and although consistently identified in this tumour type HPV 18 and HPV 33 are detected in smaller proportions.

2.3 Cancer of the upper aerodigestive tract

The fraction of cancers of the oral cavity and pharynx associated with HPV infection varies between studies according to: 1) the accuracy in the distinction of cancer of the oropharynx and tonsil from other subsites; 2) the competing effect of tobacco smoking or chewing; and 3) the quality of tissue biopsies and HPV-testing protocols used.

As a consequence of the above, especially high proportions of HPV positivity have been recently found in the USA, where early cancer cases restricted to the oropharynx and detected in a substantial proportion among non-smokers have been very carefully evaluated (Andrews et al., 2009).

2.3.1 Cancer of the oral cavity

Cancers of the oral cavity, including the tongue, floor of the mouth, gum, palate, and other sites of the mouth have a clearly established association with smoking or chewing tobacco, and with alcohol drinking. However, there is a subset of cancers that occurs among subject not exposed to smoking or drinking. The previous IARC Monograph concluded that, in the oral cavity, there was sufficient evidence for the carcinogenicity of HPV 16, and limited evidence for the carcinogenicity of HPV 18.

Multiple case series have been reported with variable prevalence estimates of HPV – between 4–74% – including the series included in the previous IARC Monograph, and those reviewed for this current volume (see Table 2.20 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.20.pdf and Table 2.21 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.21.pdf).

The recent case series (Koppikar et al., 2005, India; Luo et al., 2007, Taiwan, China; da Silva et al., 2007, Brazil; Soares et al., 2007, Brazil; Liang et al., 2008, USA) confirmed the same pattern of variability. In Taiwan, China, and one of the Brazilian studies, the reported prevalence was about 25%, but the other Brazilian study that included only tongue cancers reported a prevalence of 74%. In contrast, the study in India only detected mucosal HPV types in 6% of the tumours, and in the US study, the reported prevalence was only 2% in tongue cancers. Three of the case series included non-cancer cases as comparison groups. In all studies, HPV positivity was much lower in these control biopsy specimen than in tumour specimens. HPV 16 was the most common type in all studies, followed or equalled by HPV 18, with the exception of one of the Brazilian studies, where this pattern was reversed (Soares et al., 2007).

In a meta-analysis that included 2642 oral cancers tested with PCR methods, the overall prevalence of HPV was 23.5%, with predominance of HPV 16, which was detected in 16% of the cases (68% of the positives), followed by HPV 18, detected in 8% (34.1% of the positives). About 3% had HPV 6 and 1.6% had HPV 11. No other type was detected in more than 1% of the cases (Kreimer et al., 2005). [The Working Group noted a tendency for the largest and more carefully conducted studies to yield lower prevalence estimates than the smaller studies, suggesting the possibility of publication bias.]

Evidence from case–control studies reported until the previous IARC Monograph was mainly
based on serological studies. Two studies that had used exfoliated cells from cases and controls failed to demonstrate an association (Schwartz et al., 1998; Herrero et al., 2003), and a third observed a doubling of the risk (Smith et al., 2004). A recent case–control study (Hansson et al., 2005, Sweden) demonstrated positive associations using exfoliated cells of the oral cavity. Another study conducted in Canada (Pintos et al., 2008) reported a non-significant odds ratio of 1.3.

In the study by Hansson et al. (2005), swabs of tumours and tonsillar fossa and mouthwash of cases and controls were collected. The odds ratios adjusted for tobacco and alcohol were 24 (95%CI: 3.2–180) for the tongue, 51 (95%CI: 3.2–810) for the floor of the mouth, and 22 (95%CI: 2.8–170) for the other oral sites.

In summary, there is epidemiological evidence for the role of HPV 16 and possibly HPV 18 in the etiology of cancers of the oral cavity.

2.3.2 Cancers of the oropharynx and tonsil

Cancers of the oropharynx and tonsil are also associated strongly with smoking and drinking, but extensive evidence has accumulated in recent years to support a causal role of HPV in a sizable fraction of those cancers, which have been increasing in incidence in some populations (Hammarstedt et al., 2006).

The fraction of cancers of the oropharynx that is HPV-related is larger than for the oral cavity, but as mentioned above, this fraction varies between studies. Many studies confirm this association, in particular for the tonsil. HPV 16 is present in about 90% of HPV-positive tumours (see Table 2.22 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.22.pdf and Table 2.23 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.23.pdf).

The original case series showed a high variability in the prevalence of HPV, although in general, the prevalence reported was higher than for the oral cavity, in particular for the tonsil. In recent case series (Tachezy et al., 2005, Czech Republic; Hammarstedt et al., 2006, Sweden; Ernster et al., 2007, USA; Kim et al., 2007a, Republic of Korea; Charfi et al., 2008, France), the prevalence of HPV in oropharyngeal tumours was close to 60% or more, with a clear predominance of HPV 16.

An interesting study from Sweden (Hammarstedt et al., 2006) retrieved tonsil cancer archival specimens from different time periods to determine if the observed increase in incidence of tonsil cancer in that area could be explained by increases in HPV-related tumours. They found that cases diagnosed in the 1970s had an HPV positivity of only 23.3%, and this increased to 68.0% in tumours diagnosed in 2000–02. HPV 16 was detected in 42% of cases (all years combined, 87.0% of the positives). Patients with HPV-positive cancers were younger at diagnosis.

In the meta-analysis by Kreimer et al. (2005), HPV positivity in cancer of the oropharynx was 35.6% with HPV 16 detected in 30.9% (86.8% of the positives) of oropharyngeal tumours. HPV 18 was detected in only 1.0% of the tumours, and HPV 6 in 2.5% of the tumours.

An important, well conducted case–control study was recently reported by D’Souza et al. (2007). They included 100 patients with newly diagnosed oropharyngeal cancer, and 200 gender- and age-matched controls. Sexual behaviours (vaginal and oral sex) were strongly associated with a risk of oropharyngeal cancer, an association that was stronger when the analysis was restricted to HPV-positive tumours. Strong associations with a risk of oropharyngeal cancer were detected for serological markers of HPV infection or progression (adjusted OR, 32.2; 95%CI: 14.6–71.3 for antibodies against HPV 16 L1 VLPs; adjusted OR, 58.4; 95%CI: 24.2–138.3 for antibodies against HPV 16 E6 or E7), and for detection of HPV DNA in oral exfoliated cells (OR, 12.3; 95%CI: 5.4–26.4). HPV 16 DNA was
detected in 72% (95%CI: 62–81) of tumour biopsies by in-situ hybridization.

Two other case–control studies (Pintos et al., 2008, Canada; Hansson et al., 2005, Sweden) also detected strong associations with risk.

In summary, there is strong epidemiological evidence for the causal role of HPV 16 in the etiology of cancers of the oropharynx and tonsil. HPV 18 is detected in 1% of the tumours.

2.3.3 Cancer of the oesophagus

Squamous cell carcinoma of the oesophagus is also associated with tobacco and alcohol consumption, but a potential role of HPV has been proposed (Kamangar et al., 2006; Lu et al., 2008).

There have been many studies of HPV and oesophageal cancer, and findings have been very inconsistent geographically, with studies showing extreme variations in HPV detection probably in relation to the lack of standardized testing methods. There are areas, particularly in Asia, where HPV is more commonly detected in oesophageal cancer.

Recent case series have been reported from Egypt (Bahnassy et al., 2005), Colombia and Chile (Castillo et al., 2006), Brazil (Souto Damin et al., 2006), Germany (Pantelis et al., 2007), the Republic of Korea (Koh et al., 2008), the Islamic Republic of Iran (Far et al., 2007), and China (Shuyama et al., 2007; Lu et al., 2008). HPV detection in these recent studies ranged from 0% (Republic of Korea) to 54% (Egypt). HPV 16 was the most common type in all studies, followed by HPV 18.

Several case–control studies based on serological measures of HPV have been reported, but the results have not been consistent. A recent study was reported from China (Kamangar et al., 2006) in which prediagnostic serum was tested by enzyme-linked immunosorbent assay (ELISA) for antibodies against HPV 16, 18, and 73 viral capsids; only HPV 16 was (weakly) associated. In Australia, another seroepidemiological study (Sitas et al., 2007) showed increasing risk of oesophageal cancer with increasing levels of antibodies against HPV 16.

See Table 2.24 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.24.pdf and Table 2.25 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.25.pdf.

In view of the inconsistency of results, the Working Group considered that the epidemiological evidence for a role of HPV in oesophageal cancer is inadequate.

2.3.4 Cancer of the larynx

HPVs (usually types 6 and 11) cause recurrent respiratory papillomatosis, and it has long been suggested that some of these viruses could be related to cancer of the larynx (IARC, 2007). Three recent case series (de Oliveira et al., 2006, Brazil; Gungor et al., 2007, Turkey; Koskinen et al., 2007, Finland, Norway and Sweden) reported HPV positivity of 37.3%, 7.4%, and 4.4%, respectively. In Brazil, HPV 18 was predominant, and in Turkey, HPV 11 was the most common type.

The meta-analysis by Kreimer et al. (2005) reported on 1435 cases of cancer of the larynx. Overall HPV positivity was 24.0%, with HPV 16 detected in 16.6% of laryngeal tumours (69.2% of the positives), followed by HPV 6 in 5.1%, and HPV 18 in 3.9% (see Table 2.26 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.26.pdf).

The Working Group considered that the epidemiological evidence is not conclusive to confirm the role of HPV 16 or 18 in cancer of the larynx. Similarly, as discussed in the previous IARC Monograph, there is some evidence for a role of HPV 6 and 11, which cause laryngeal papillomatosis that can occasionally become malignant.
2.4 Cancer of the skin

As summarized in the previous *IARC Monograph*, multiple case series have demonstrated that HPV DNA is frequently found both in skin lesions and in healthy skin. At present, it is known that cutaneous HPVs are found in five genera: alpha (species 2, 4, 8), beta, gamma, mu, and nu (Michael *et al.*, 2008). The cutaneous HPV types 1 (genus mu); 2, 27, 3, 10, 57 (genus alpha); and, HPV 4 (gamma) belong to different genera but are all associated with benign plantar, common, and flat skin warts. Certain HPV types in the beta-1 species (HPV 5 and 8 in particular) are found in the rare hereditary disease *epidermodysplasia verruciformis* (EV). It is these beta-1 HPV types, the other beta species and, more recently, the gamma genus HPVs, which have elicited the most interest with regard to risk of skin cancer. The other motivation for study has been the known increased prevalence of skin and genital warts and squamous cell cancers among individual with immunosuppressive treatments related to organ transplantation. These associations have suggested the importance of immune surveillance, and a possible direct role for HPV that has, nonetheless, proven difficult to demonstrate.

Improving test methods continue to detect an expanding variety of HPV types that infect the non-genital skin; these are largely types not found in the anogenital region or in the oral cavity. More types in addition to HPV 5 and 8 have now been found in the lesions of patients with EV, albeit at lower viral loads (Dell’Oste *et al.*, 2009). With an expanding number of characterized types, the taxonomy of cutaneous HPV types has been clarified; in this section, the current terminology will be used (de Villiers *et al.*, 2004; Forslund, 2007) (e.g., “beta papillomaviruses” replaces “EV types”). Fully-characterized types are shown in Fig. 2.1, but more are already known to exist.

Case–control studies of skin cancer have been complicated by methodological issues including: the heterogeneity of skin lesions, the multiplicity of HPV types including many novel types found in skin, evolving methods for sampling lesions and healthy control skin, variable and incompletely validated measurement techniques for DNA and serology, potential confounding by UV light exposure, and the possibility of “reverse causality” in which the development of a skin lesion could lead to increased HPV positivity thereby creating the false impression of a causal association.

Skin neoplasia comprises a diverse set of invasive and preinvasive lesions. Most recent studies have focused on non-melanoma skin cancers, especially squamous cell carcinoma rather than nodular or superficial focal basal cell carcinoma. The precursors to squamous cell carcinoma, called actinic keratoses or solar keratoses have also been included in several studies.

One measurement problem that is unique to skin cancer studies is the possible confounding role of UV light, which might enhance HPV replication, HPV detection and/or seropositivity through a local immune-modulating effect. The scant data are contradictory, and do not permit a conclusion. Because UV exposure and sunburn are strong risk factors for squamous cell carcinoma, it is possible that some of the association between HPV detection and squamous cell carcinoma could be due to confounding by the extent to which the skin under study is exposed to UV light. A fundamental question is whether the increased prevalence of HPV DNA in squamous cell carcinoma and actinic keratoses compared with that in healthy skin proves a causal role for HPV in these tumours (Pfister, 2003; Majewski & Jablonska, 2006; Bouwes Bavinck *et al.*, 2008; Feltkamp *et al.*, 2008; see Table 2.27 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.27.pdf).
2.4.1 Review of case–control studies measuring HPV DNA

To date, the studies have reported HPV DNA detection in lesions or healthy skin from persons with skin cancers compared with various sorts of controls. Only one very small study of RNA, not large enough to alter conclusions, was found in this review (Purdie et al., 2005).

In the previous IARC Monograph, a few case–control studies were summarized that in aggregate supported a possible association between the detection of DNA from beta-species papillomaviruses and a risk of squamous cell carcinoma. Additional studies reported since then have not provided consistent evidence of an etiological role for any one viral type or groups of types including anogenital HPV types (Gustafsson et al., 2004), beta-1 species (Patel et al., 2008), beta-2 species (Forslund, 2007), and novel types (Alotaibi et al., 2006).

Forslund et al. (2007) observed that heavily light-exposed areas of the skin were much more likely to test positive for HPV (OR, 4.4), raising the important possibility of confounding of the more modest positive associations found between HPV prevalence and squamous cell carcinoma (OR, 2.1), particularly with beta-species such as HPV 38.

2.4.2 Review of case–control studies measuring HPV seroreactivity

Two serological studies were reviewed in the previous IARC Monograph, with data on only a few beta-species papillomaviruses. Overall, the data from Feltkamp et al. (2003) and Masini et al. (2003) suggested an association of skin cancer with both beta-1 and beta-2 papillomaviruses, with the most consistent evidence for HPV 8.

Since, the advent of multiplex serology has permitted the assessment of many HPV types at once, although it is not known whether the assays are equally accurate for all of the types detected. In a population-based case–control study of 252 squamous cell carcinoma case patients, 525 basal cell carcinoma case patients, and 461 control subjects, Karagas et al. (2006) used multiplex serology to detect antibodies in plasma samples against HPV types from phylogenetic genera alpha, beta, and mu. They observed an association between squamous cell carcinoma and seropositivity to HPV types in genus beta as a group, and particularly the beta-1 HPV type 5, but no associations with basal cell carcinoma. Individuals with tumours on chronically sun-exposed sites were more likely to be seropositive for beta HPV types than individuals with squamous cell carcinoma at other anatomical sites. Waterboer et al. (2008) also used multiplex serology but observed associations with squamous cell carcinoma for other types, namely beta-2 species combined, and all gamma species combined (43 cases, 77 controls).

Casabonne et al. (2007) conducted a small but potentially revealing study of HPV seropositivity in stored plasma, comparing prevalent cases of squamous cell carcinoma and incident cases of squamous cell carcinoma to controls. This is the closest to a cohort study available to the Working Group in published form. Using a multiplex method, they assayed 38 HPV types. There were no differences between the 80 controls and 39 incident cases that developed squamous cell carcinoma subsequently to blood draw. However, the 15 cases with prevalent squamous cell carcinoma detected before blood draw tended to have non-significantly elevated seropositivity to multiple HPV types. Moreover, the incident cases diagnosed closest to blood draw were more likely than those diagnosed later to be seropositive (again non-significantly). Although too small to be more than suggestive, these data would indicate that antibodies could be produced during the process or as a consequence of tumour formation.

Casabonne et al. (2009) recently reported a null study among organ transplant recipients.
Human papillomaviruses

They studied 140 transplant recipients and 454 controls with multiplex serology, and found expected strong associations of HPV 16 antibodies in subjects with a history of abnormal Pap smears, and HPV 6 antibodies in those with a history of genital warts. However, they observed no associations between any HPVs in the beta genus and squamous cell carcinoma of the skin, by history of abnormal Pap smears or genital warts, or by examination.

2.4.3 Case–control studies combining both HPV DNA measurements and serology

Since the previous IARC Monograph, Termorshuizen et al. (2004) investigated 156 patients with squamous cell carcinoma and 320 controls from outpatient ophthalmology clinics. They tested beta HPV types from plucked eyebrow hairs, tested sera using ELISA, and administered an epidemiological questionnaire focused on sunlight exposure and sunburn. The results were complex, which probably reflects accurately the possible role of HPV in skin cancer etiology. Both the DNA and serological measures of HPV were associated with case status, but the two measurements were not associated with each other. HPV DNA was decreased in controls reporting more lifetime sun exposure, but elevated in controls reporting painful sunburn at specific ages. The type-specific results appeared to show elevations for virtually all types tested.

Struijk et al. (2006) compared 64 squamous cell carcinoma cases, 126 actinic keratosis cases, and 57 tumour-free ophthalmology clinic patients from the same catchment area in Brisbane, Australia. They employed type-specific PCR to study EV-type DNA in plucked eyebrow hairs, and assessed seropositivity using both L1 VLP and E6 fusion protein ELISA. The E6 assays were not associated with DNA or risk. However, HPV DNA prevalence was elevated in the actinic keratosis group compared with either the squamous cell carcinoma group or the healthy controls, and the results for HPV 20 achieved statistical significance. In this study, seropositivity to L1 VLPs was significantly associated with HPV DNA positivity among the controls and the actinic keratosis group. L1 VLP seropositivity was non-significantly associated with actinic keratosis and squamous cell carcinoma for all types combined, and for squamous cell carcinoma, the odds ratios were elevated (often significantly) for all the individual HPV types.

Andersson et al. (2008) collected serum and biopsy samples from both lesions and healthy skin from 434 non-immunosuppressed patients (72 squamous cell carcinomas, 160 basal cell carcinomas, 81 actinic keratoses, and 121 benign lesions). The presence of HPV DNA and of antibodies to the same HPV type was not significantly correlated. However, seropositivity to any HPV type was significantly more common among patients positive for HPV DNA of any HPV type. The seroprevalence tended to be somewhat higher among squamous cell carcinoma patients than among basal cell carcinoma patients.

2.4.4 Conclusion

Epidemiological data do not yet support that any single HPV type causes skin cancer in the general population owing to a lack of consistency of the associations described. At present, a positive association has been observed between HPV infection and both squamous cell carcinoma and its precursor actinic keratosis. The evidence is derived from a tendency, not type-specific, of increased odds ratios of DNA detection and seroreactivity compared to control study participants and/or paired healthy skin. The role of HPV in skin cancer may be complex, non-causal, and/or linked to other factors like UV damage, immunosuppression, and genetics.
2.5 Cancer at other sites

The evidence for the carcinogenicity of HPV in the following cancers was evaluated in the previous IARC Monograph: cancers of the nose and nasal sinuses, cancer of the lung, cancer of the colon and rectum, cancer of the breast, cancer of the ovary, cancer of the prostate, and cancer of the urinary bladder and urethra.

2.5.1 Cancer of the nose and nasal sinuses

Inverted papillomas of the nasal cavity and paranasal sinuses are frequently positive for HPV 6, 11 and 57, and a small percentage of rare carcinomas arising at those sites sometimes have HPV 16, 18, 11, 6 and 57 in decreasing frequency (IARC, 2007). The rarity of these tumours makes it difficult to evaluate the role of HPV, and no case–control studies have been reported.

A recent study by El Mofty & Lu (2005) conducted in the USA correlated HPV detection by PCR with histological subtypes of carcinoma of the sinonasal tract. They studied paraffin blocks of a small series (n = 39) of keratinizing, non-keratinizing, and undifferentiated tumours. HPV was detected more often in the small number of keratinizing tumours, with a predominance of HPV 16. On the other hand, another recent study (Kim et al., 2007b) studied 57 paraffin-embedded biopsies from inverted papillomas of different grades (I – IV, with grade IV being carcinoma originating in inverted papilloma). Only 12.3% of cases (restricted to grade I and II) had detectable HPV DNA, mainly of the mucosal carcinogenic type. Alos et al. (2009) recently reported a prevalence of HPV in 20% of a series of 60 sinonasal tumours, with HPV 16 detected in 11/12 cancers.

2.5.2 Cancer of the lung

Multiple groups have tested for HPV in lung cancer samples, with variable results. The geographic areas where the highest prevalence has been reported are predominantly in Asia. The rare occurrence of lung cancer in patients with recurrent laryngeal papillomatosis has been documented, and some studies have reported HPV-associated lung cancers among women with a history of CIN3.

Since the last IARC Monograph, there have been seven new case series reported including at least 40 cases of lung cancer, from various locations including France (Coissard et al., 2005), China (Fei et al., 2006), the Islamic Republic of Iran (Nadjji et al., 2007), the Republic of Korea (Park et al., 2007), Chile (Aguayo et al., 2007), Taiwan, China (Cheng et al., 2007), and India (Jain et al., 2005). HPV detection was variable, from 2% in France to 46% in Taiwan, China, with a predominance of HPV 16 in all studies. In the study from China, a non-cancer group was included without detection of HPV 16. In the Korean study, HPV 16 was more common in biopsies of younger subjects.

See Table 2.28 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.28.pdf.

2.5.3 Cancer of the colon and rectum

The discussion of the association of HPV and cancer of the colon and rectum (usually adenocarcinoma) requires a clear distinction of the histology and anatomical site, to distinguish cases from cancers of the anus (Section 2.2.4), which are clearly HPV-related. Several small case series have investigated the prevalence of HPV in biopsies of colorectal cancer, with variable results. Many of the studies were completely negative. However, one study (Bodaghi et al., 2005) reported 42% HPV positivity in cases, 29% in biopsies from adjacent tissues, and no HPV in control subjects. HPV 16 was the predominant type in tumours.

A recent case series from Brazil (Damin et al., 2007) investigated 72 cases of primary colorectal adenocarcinoma. For each patient, two specimens were collected: one from the tumour and
one from the normal colorectal mucosa at least 15 cm apart from the tumour. In addition, biopsies from 30 individuals without cancer were also evaluated. HPV DNA was detected in 83.3% of cancer patients but in none of the tissues from the non-malignant control group ($P < 0.001$). HPV was present only in the tumour of 32% of the cases, in the tumour and adjacent tissue of 32% of cases, and only in adjacent tissue in 19.5% of cases. HPV 16 was the predominant type (68.3% of positive cases), followed by HPV 18 (50% of positives). More than 30% of cancer patients were infected by multiple HPV types. HPV genotyping was confirmed by sequencing; there were no epidemiological differences between positive and negative cases.

2.5.4 Cancer of the breast

There is contradictory evidence for the role of HPV in breast cancer. Several small negative studies were reported in the previous IARC Monograph, as well as a few studies in which HPV was detected in different fractions of breast tumours. HPV 16 and 18 were the most common types. One of the studies (Hennig et al., 1999) reported HPV-type concordance between CIN3 cases and subsequent breast cancers.

A recent study from Switzerland (Lindel et al., 2007) analysed paraffin-embedded sections of 81 patients with breast cancer using the well validated SPF10 PCR system; all samples were negative. Another study conducted in the Syrian Arab Republic (Akil et al., 2008) found extremely high HPV positivity in 113 blocks. Cazzaniga et al. (2009) investigated the presence of cutaneous and cervical carcinogenic HPV types in ductal lavages, colostrum, and milk of 90 women at risk of breast cancer. A total of 14% (10/70 analysed) of the specimens contained cutaneous types, and only one had a mucosal type (HPV 16). Removal of the superficial epidermal cells significantly reduced prevalence, with HPV detection in only 2/45 specimens (beta HPV types).

2.5.5 Cancer of the ovary

There is limited information about the potential role of HPV in cancer of the ovary, and several small negative case series have been reported as well as a few studies with positive findings. The presence of HPV in the ovary may be related to HPV-associated disease ascending from the cervix.

A study by Quirk et al. (2006) from the USA reported the analysis of fresh frozen biopsies from 20 women with ovarian carcinomas. Using commercial PCR amplification kits, no HPV DNA was detected in any of the tumours. Another study in Turkey (Atalay et al., 2007) observed HPV in 8.5% of 94 patients. All HPV-positive patients had serous papillary tumours and advanced stage disease. A third study reported from Italy (Giordano et al., 2008) included 71 women with borderline ($n$, 21) and malignant ($n$, 50) ovarian tumours. Three cases (4.2%) of epithelial ovarian neoplasm had detectable HPV DNA.

2.5.6 Cancer of the prostate

Prostate cancer is associated with sexual behaviour in some studies (Damber & Aus, 2008), suggesting a possible role for HPV. A variety of studies have been conducted, including case series and case–control studies mainly based on serological measures of exposure. The results are inconclusive although most studies are negative.

Two case series of cancer of the prostate (Leiros et al., 2005, Argentina; Balis et al., 2007, Greece) have been reported recently. The study from Argentina detected HPV in 41.5% of tumours. Most of the tumours where HPV was identified harboured HPV 16. All prostatic hyperplasias were negative. In contrast, the study in Greece only found HPV DNA in 2/42 cases of prostatic carcinoma (4.8%).

Several case–control studies, some of them nested in prospective cohorts and using serological measures, have been reported on the
2.5.7 Cancer of the urinary bladder and urethra

Bladder cancers are predominantly transitional cell carcinomas, with the exception of areas where schistosomiasis is highly prevalent where a predominance of squamous cell carcinomas is observed. Similarly to other cancer sites, case series have reported a variable prevalence of HPV across studies.

Since the previous IARC Monograph, a case series was reported from the Islamic Republic of Iran (Barghi et al., 2005), which included paraffin-embedded biopsies of 59 transitional cell carcinomas, and detected HPV in 35.6% of the tumours. The most common type was HPV 18 but HPV 16 was not detected. Moonen et al. (2007) reported on 107 cases from the Netherlands, testing bladder wash samples. Overall, the prevalence of any HPV type was 13.7%. HPV 18 was the most common type detected, followed by HPV 16.

2.6 Cofactors of HPV in cervical cancer

HPV is a necessary cause of cervical cancer. However, only a small fraction of women infected develops cervical cancer or its high-grade precursors, indicating that there must be additional viral, host or environmental factors conditioning viral persistence and progression. Cofactors were reviewed extensively in the previous IARC Monograph.

The study of cofactors has been difficult because many of the exposures involved are correlated with sexual behaviour and therefore with HPV acquisition. Analyses restricted to HPV-positive subjects have been considered to be the best analytical alternative for control of this type of confounding. However, in the context of almost universal exposure, women with current infection are not necessarily the most appropriate control group, because they are a combination of both persistent and recently acquired infections that vary with the population and age of the control group, and there is currently no adequate measure of past exposure to HPV. Other exposures have the analytical difficulty of being associated with screening behaviour, and most suffer some degree of measurement error.

One of the most studied cofactors is tobacco smoking, a well established carcinogen that is known to exert its effect in organs not directly exposed to smoke. Most studies, using different methods to adjust for confounding including the restriction to HPV-positive cases and controls, have found an approximately 2-fold increase in risk of cervical cancer or high-grade precursors (Kjaer et al., 1996; Ho et al., 1998a; Krüger-Kjaer et al., 1998; Olsen et al., 1998a; Ylitalo et al., 1999; Kjellberg et al., 2000; Hildesheim et al., 2001; Plummer et al., 2003; Giuliano et al., 2004), some of them with dose responses for the amount of cigarettes smoked and duration of smoking (Ho et al., 1998a; Kjellberg et al., 2000; Hildesheim et al., 2001; Harris et al.,...
In a large meta-analysis including more than 13000 cases and 23000 controls from 23 epidemiological studies, smokers were at an increased risk (pooled OR, 1.6; 95%CI: 1.5–1.7) of squamous cell but not of adenocarcinoma of the cervix. The risk of squamous cell carcinoma increased in current smokers with the number of cigarettes smoked per day, and with younger age at starting smoking (Appleby et al., 2006a). Several cohort studies have clearly shown the association, particularly for CIN3 (Deacon et al., 2000; Castle et al., 2002). Cigarette smoking is an established cofactor of HPV for cervical cancer, although it is still unclear if its effect is mediated by genotoxicity or immunomodulation. The role of other forms of tobacco exposure, including chewing and passive smoking, as well as the role of tobacco as a cofactor of HPV in other organs requires further research.

Multiple studies have also suggested a potential role of hormonal contraceptives as HPV cofactors. The agents investigated most extensively are oral contraceptives, and several case-control studies (Ylitalo et al., 1999), but not all (Kjær et al., 1996; Lacey et al., 2000; Shapiro et al., 2003; Giuliano et al., 2004) detected increases in risk, particularly among users of oral contraceptives for more than 5 or 10 years (Berrington et al., 2002; Moreno et al., 2002). One of the studies (Lacey et al., 1999) reported an association restricted to glandular lesions. A very large pooled re-analysis confirmed the association between oral contraceptives and cervical cancer and its precursors, and the observed reduction in risk after cessation of use (Appleby et al., 2007). These findings were not replicated in cohort studies of precursor lesions (Deacon et al., 2000; Castle et al., 2002), possibly reflecting the protective effect of screening among oral contraceptive users. Injectable contraceptives have also been shown to increase the risk of cervical cancer (Herrero et al., 1990, Appleby et al., 2007), and also to increase CIN3 among HPV-positive women (Castle et al., 2005b). An important consideration when interpreting the role of hormonal contraceptives is that most studies and systematic reviews evaluated the risk associated with the contraceptive products used in previous decades. Information is needed about the potential association of the newer contraceptive agents that have different hormonal formulations.

The number of pregnancies is also an established cofactor of HPV in cervical cancer, with most studies showing increases in risk associated with increasing number of pregnancies (Kjær et al., 1996; Hildesheim et al., 2001; Muñoz et al., 2002; Appleby et al., 2006b). A few prospective studies of precursor lesions have not confirmed this association (Deacon et al., 2000; Castle et al., 2002), possibly because they had limited numbers of highly multiparous women. The possible role of a hormonal, nutritional or immune status change during pregnancy or potential cervical trauma during delivery as a cofactor of HPV remains to be determined. It is possible that part of the decline in cervical cancer incidence and mortality observed in some populations is related to declines in fertility rates.

The likely role of nutrients as preventive agents in several epithelial cancers is generally accepted. However, despite numerous studies using different methods for ascertaining the impact of dietary exposures, the investigation of the role of nutrients as cofactors of HPV in cervical cancer and its precursors has yielded inconsistent results, with some studies suggesting potential protective effects of fruits and vegetables (Rajkumar et al., 2003), folate (Weinstein et al., 2001), vitamin C (Giuliano et al., 2003), carotenoids (Giuliano et al., 1997), tocopherols (Giuliano et al., 1997), and vitamin B12 (Sedjo et al., 2002), but other studies have shown no significant associations (Ho et al., 1998b; Wideroff et al., 1998). Notably, a recent study (Piyathilake et al., 2007) suggested that an increase in risk of CIN2+ was associated with a strong interaction between HPV 16 and low red-blood cell folate levels. The assessment of dietary exposures is difficult, and subject to
variation in different populations. In addition, it is correlated with sociodemographic and behavioural variables, requiring the consideration of multiple confounders. However, further studies are needed, particularly for nutrients in the one-carbon metabolic pathways like folate, given the potential for intervention.

There is some evidence of familial aggregation in cervical cancer (Amundadottir et al., 2004; Zelmanowicz et al., 2005; Couto & Hemminki, 2006), suggesting the possibility of genetic factors that could act as cofactors of HPV infection, although additional studies are needed to confirm these associations, to define possible mechanisms, and to rule out confounding by environmental exposures shared by families.

Immunosuppression, both in transplant recipients and in HIV-infected subjects, is an established cofactor of HPV infection in the development of precursor lesions and invasive cancer at most anatomical sites investigated (see HIV-Monograph in this volume). Immunosuppression was reviewed extensively in the previous IARC Monograph. In this context, the genetics of the immune response is also likely to play an important role in the fate of HPV infections. Some HLA (human leukocyte antigen) polymorphisms, related with antigen recognition, have been proposed as determinants of risk of progression, while others have been associated with protection. Many studies conducted in different populations have reported associations in both directions, particularly for DR and DQ class II genes (Apple et al., 1994, 1995; Duggan-Keen et al., 1996; Cuzick et al., 2000; Madeleine et al., 2002). Some of the difficulties of these analyses include variable ethnic compositions of the populations and multiple comparisons.

Other infectious agents, including Chlamydia trachomatis (Koutsky et al., 1992; Iha et al., 1993; de Sanjosé et al., 1994; Bosch et al., 1996; Giuliano et al., 2001; Smith et al., 2002a; da Silva et al., 2004) and herpes simplex virus (Hildesheim et al., 1991; Peng et al., 1991; Koutsky et al., 1992; Iha et al., 1993; Becker et al., 1994; Olsen et al., 1998b; Smith et al., 2002b), as well as inflammation associated with various infections have been shown in some studies to be associated with a risk of cervical cancer or its precursors. Most studies of these agents are seroepidemiological, and some have used markers of bacterial or viral DNA. The most abundant and consistent evidence is for C. trachomatis, but the correlation is so high between sexually transmitted agents that it is difficult to completely rule out residual confounding, and the mechanism for its interaction with HPV, if there is one, remains unknown. Cervical inflammation, as ascertained in cytological specimens has been associated with an increase in risk (Schiff et al., 2000; Castle et al., 2001), and genotoxic damage associated with the inflammatory response is a likely mechanism for the interaction between HPV and these infectious agents.

Most cofactors appear to act as determinants of persistence and progression to advanced precursors, but no risk factor has been established for progression to invasion, except probably age.

3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the Monographs on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.
4. Other Relevant Data

4.1 Mechanisms of HPV-associated carcinogenesis

There is compelling evidence that certain HPVs encode oncoproteins that directly contribute to the development and maintenance of cervical cancer. This is supported by extensive biochemical and biological studies performed with animal cells, primary human cells, and with cells derived from the cervical cancers.

4.1.1 Rodent cells

The earliest assays to confirm that HPV encodes proteins with cell-transforming capacity were carried out with established cell lines of murine origin, such as NIH3T3, Rat-1, and Rat3Y1. These tests can be considered to be the simplest and least rigorous for establishing the oncogenic potential of a given agent, in that the cells used in these assays are already immortal. The cells are transfected with entire HPV genomes or individual gene products, and their transforming capacity is assessed on the basis of either growth in an anchorage-independent manner, or tumour formation in nude mice. These early studies established that HPV 16, in particular, had transforming potential in established rodent cells (Tsunokawa et al., 1986; Yasumoto et al., 1986) and that the principal activity resided in the E7 oncoprotein (Kanda et al., 1988; Vousden et al., 1988), although transforming potential in such assays has also been reported for the E5 and E6 proteins of both HPV 16 and HPV 18 (Bedell et al., 1989; Sedman et al., 1991; Pim et al., 1992).

Oncogene-cooperation assays were next used to demonstrate the transforming potential of different HPV types. These tests most commonly involved primary baby rat kidney cells or primary baby mouse kidney cells. The cooperating oncoprotein was typically activated Ras (Matlashewski et al., 1987), although co-transforming activity has also been reported with activated Fos (Crook et al., 1988). In all of these cases, transformation was assessed on the basis of the appearance of morphologically transformed immortalized cells, or the ability of these cells to form immortalized tumours in syngeneic animals. In baby rat kidney cells, the majority of the transforming activity of HPV 16 appears to be carried by the E7 oncoprotein (Storey et al., 1988), but some transforming activity is also detectable from the E6 oncoprotein in baby mouse kidney cells (Storey & Banks, 1993).

4.1.2 Human cells

Human keratinocytes are the cells of choice to study HPV oncoproteins, as these cells are the natural target for the virus in vivo. In these cells, some mucosal and cutaneous HPVs can readily induce immortalization. This property has been demonstrated in cervical, foreskin and oral keratinocytes as well as tonsil epithelial cells (Dürst et al., 1987; Pirisi et al., 1987; Woodworth et al., 1989; Klingelhutz et al., 1996; Caldeira et al., 2003; Spanos et al., 2008). In the majority of cases, these activities required both E6 and E7 oncoproteins (Barbosa & Schlegel, 1989; Hawley-Nelson et al., 1989).

Although these assays measure only immortalizing capacity, full transformation, i.e. tumour growth, can be attained by prolonged passage in tissue culture or by transfection of additional activated oncogenes, such as RAS (Dürst et al., 1989; Spanos et al., 2008).

Other primary human cells that are susceptible to the transforming potential of HPVs include mammary epithelial cells, which appear to be particularly susceptible to the effects of E6 (Band et al., 1991).
4.1.3 Cells derived from cervical tumours

The clearest demonstration that E6 and E7 are necessary for the development of cervical cancer comes from studies where expression of either protein is blocked or inhibited in cells derived from cervical tumours. The expression of both viral proteins continues many years after the establishment of these cell lines in vitro (Schwarz et al., 1985; Smotkin & Wettstein, 1986; Androphy et al., 1987; Banks et al., 1987), suggesting a requirement for their continued expression for the maintenance of cell proliferation. This has in fact been confirmed in many studies using a range of techniques to ablate the expression or activity of E6 and E7. This includes selective alteration of viral gene expression (von Knebel Doeberitz et al., 1988; Goodwin & DiMaio, 2000), anti-sense RNA (Steele et al., 1992; von Knebel Doeberitz et al., 1992), RNAi (Butz et al., 2003; Yoshinouchi et al., 2003), and blocking peptides (Butz et al., 2000). In all of these examples, cervical cancer cells cease to proliferate, and enter into either senescent or apoptotic states. This is perhaps the single most important evidence of the role of HPV in the maintenance of the malignant phenotype of cervical cancer.

4.1.4 Transforming capacity of HPVs

HPV 16 and HPV 18 display transforming potential in all of the assays listed above. The other mucosal types that have been shown to possess transforming activity in keratinocyte immortalization assays are HPV types 31, 33, 45, 53, 56, 58, 66, and 82 (Woodworth et al., 1989; Hiller et al., 2006; Morandell et al., 2008). It should be stressed, however, that except for HPV types 31 and 33, this has only been reported once.

Cutaneous beta HPV types also exhibit transforming activity under the conditions of some of the above assays. HPV 5, 8, and 47 are active in established rodent cell transformation assays (Kiyono et al., 1992; Schmitt et al., 1994); HPV types 12, 14, 15, 24, 36, and 49 are active in primary rodent cell co-transformation assays (oncogene cooperation assays with activated Ras) (Massimi et al., 2008); HPV 38 is active in primary human keratinocyte assays (Caldeira et al., 2003), and a weak keratinocyte immortalizing activity has been reported for HPV 8 (Schmitt et al., 1994).

4.2 Biochemical properties of HPV proteins

4.2.1 The E5 protein

The E5 protein is a small hydrophobic polypeptide (10 kD) whose localization is believed to be in membrane compartments including the Golgi apparatus, and the endoplasmatic reticulum (Conrad et al., 1993). A possible role for the E5 protein of the mucosal high-risk HPV types may be at an early stage of carcinogenesis given that E5 is not expressed in many invasive cancers due to the integration of the viral DNA into the host genome, which frequently leads to the loss of the E5 ORF or at least loss of its expression. Surprisingly, there is still very little known about the biochemical mode of action of the E5 protein. There is some evidence to suggest that it can alter the processing of epidermal growth factor receptors (Straight et al., 1993), most likely through interaction with the 16-kD component of the vacuolar proton-ATPase, and endosomal processing (Conrad et al., 1993; Straight et al., 1995). However, there is also evidence that E5 can affect antigen presentation by interaction with, and downregulation of, class 1 HLA (Ashrafi et al., 2005, 2006), thereby contributing to immune evasion. It should be stressed that all the information on biochemical properties of the E5 proteins are derived from studies of HPV 16 and the low-risk HPV type 6. Finally, an anti-apoptotic function for E5 has also been described in keratinocytes, although the molecular basis
behind this activity is unknown (Kabsch & Alonso, 2002).

It should be noted that many beta HPV types do not possess an E5 ORF, so this protein would not seem to be relevant in these virus types. Whether other viral gene products have evolved to carry out some of the functions of the E5 protein described above, remains to be determined.

4.2.2 The E6 protein

The E6 protein is approximately 158 amino acids in length, and is one of the most extensively studied HPV proteins. A major feature of its activity is directed at the degradation of cellular binding partners through the combined activity of a ubiquitin ligase, E6AP (E6-associated protein), and the cellular ubiquitin proteasome pathway (Scheffner et al., 1993). More than a dozen cellular binding partners of the E6 protein have been described (Mantovani & Banks, 2001; Thomas et al., 2008). Because of the complex nature of the E6 structure, it has been extremely difficult to determine which particular binding partner is responsible for any given function (Nominé et al., 2006). Clearly, interaction with the tumour-suppressor protein p53 is fundamental to the activity of the E6 protein of transforming mucosal HPV types. Indeed, E6 proteins from multiple mucosal types (HPV 16, 18, 33, 35, 39, 45, 51, 52, 53, 56, 58, 66, 70, and 82) target p53 for degradation at the 26S proteasome (Hiller et al., 2006; see also Scheffner et al., 1990). Similarly, the ability to induce telomerase activity is a common theme in HPV-induced malignancy, although the precise mechanism by which this occurs is subject to considerable debate (James et al., 2006; Liu et al., 2008; Sekaric et al., 2008). An important feature of these E6 proteins is the presence of a PDZ-binding motif at the C-terminal. At present, multiple binding partners have been described, all of which are degraded by proteasomes. It remains to be determined, however, which of these are functionally important in vivo (Thomas et al., 2008).

The discussion above relates primarily to E6 proteins of the mucosal HPV types. Much less is known about the biochemical activity of the beta HPV E6 proteins. They all lack the capacity to degrade p53 and none of them, whether they are associated with cancer or not, possesses PDZ-binding motifs. This suggests major differences in how the beta HPV types interact with p53, and in the pathways that involve PDZ-domain-containing substrates. Intriguingly, in the case of HPV 38, p53-responsive pathways appear to be abrogated through the induction of the p53 repressor, ΔNp73, although it remains to be determined whether this is due to E6 or E7 (Accardi et al., 2006). The E6 protein of HPV 20 displays a unique characteristic in that it is actually targeted for degradation by p53 in a caspase-dependent manner. However, this does not occur if p53 is mutated as is frequently the case in non-melanoma skin cancer (Fei & de Villiers, 2008). A common feature of E6 proteins from cutaneous and mucosal HPV types is their ability to interact with and inhibit the activity of the pro-apoptotic protein Bak. This has now been reported for E6 proteins of HPV 16, 18, 5, 8, 11, 20, 22, 38, 76, 92, and 96 (Thomas & Banks, 1998, 1999; Jackson et al., 2000; Dong et al., 2005; Underbrink et al., 2008). This appears to be an important evolutionarily conserved activity that may also play a role in the development of HPV-induced malignancy. Likewise, telomerase activation has also been described for the oncoproteins E6 and E7 of several beta HPV types, in particular HPV 38 (Bedard et al., 2008; Gabet et al., 2008), with other types such as HPV 5, 8, 20 and 22 exhibiting only low-to-background levels of telomerase activation (Bedard et al., 2008).
4.2.3 The E7 protein

The E7 protein is approximately 100 amino acids in length and, like E6, targets many of its cellular substrates for proteasome-mediated degradation. To achieve this, E7 makes use of a multiprotein cullin-2 ubiquitin-ligase complex (Huh et al., 2007). Again, numerous cellular targets have been described for E7 (Münger et al., 2001). As for E6, a subset of these targets has been clearly shown to be important for the biological activity of E7. Perhaps the most prominent are pRb and the related pocket proteins, p107 and p130, all of which are degraded by E7, thereby allowing the induction of S-phase progression. Paradoxically, E7 can also cause an increase in p53 protein levels by a mechanism that is not yet understood (Blanton et al., 1992; Seavey et al., 1999). E6 can overcome E7’s induction of p53, which can be at multiple levels, both by directly targeting p53 for degradation, and by inhibiting p53 acetylation (Thomas & Chiang, 2005; Shamanin et al., 2008). Other key regulators of the cell cycle that are known interacting partners of E7 include the p21/p27 cdk inhibitors, and a subset of cyclins (Münger et al., 2001).

E7 also has a profound impact on transcriptional regulation, being found in association with AP1 transcription factors (Antinore et al., 1996), histone deacetylases (Longworth et al., 2005), and MPP2 (Lüscher-Firzlaff et al., 1999). Interestingly, one of the consequences of E7 activity is the strong upregulation of p16INK4a in cervical cancers, a feature that can be used as a surrogate marker for the presence of cancer-associated HPV types (Vinokurova et al., 2005).

As is the case with the E6 protein, the function of the beta HPV E7 protein is also poorly understood at the biochemical level. However, the ability to target pRb is a feature of the E7 protein of some beta HPV types, as has been shown for HPV 38 (Caldeira et al., 2003).

Certain biomarkers that reflect the expression and function of E6 and E7, such as the overexpression of p16 induced by E7, and the lack of mutation in p53 due to its inactivation by E6, have been useful indicators of the involvement of HPV in cervical cancer. In several studies, the correlation between the presence of HPV, an increased level of p16, and the absence of p53 mutation has been investigated in cancers other than cervical cancer. The results are summarized in Table 4.1.

4.3 Biological Properties of HPV

4.3.1 Immortalization

As discussed above, for many different HPV types, effective immortalization of keratinocytes requires the combined presence of the viral proteins E6 and E7, although there are reports of a weak immortalizing activity with E7 alone.

4.3.2 DNA-damage responses

HPVs have the capacity to abrogate normal DNA-damage responses, which is likely to contribute to the accumulation of genetic alterations in HPV-positive cells, including those that contribute to HPV-associated cancers (see Section 4.3.3).

Because E6 can inactivate p53 by stimulating its proteasome-mediated degradation (Scheffner et al., 1990), it is not surprising that many studies have confirmed that E6 can abrogate cell-cycle arrest induced by a variety of DNA-damaging agents (Kessis et al., 1993; Foster et al., 1994). In vivo, this activity of E6 correlates at least in part with its ability to inactivate p53, as was shown in mice (Song et al., 1998).

Cell-cycle arrest induced by p53 may arise through the induction of p21, and the consequent inhibition of cdk-mediated phosphorylation of pRb and the related proteins p107 and p130 (el-Deiry et al., 1993; Harper et al., 1993; Slebos et al., 1994). E7 is able to inhibit the activity of p21 (Funk et al., 1997; Jones et al., 1997) as well as that of the pRb family of proteins (Münger et al., 1993).
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1989; Slebos et al., 1994). The inactivation of pRb and p107 by E7 is mediated in part by its ability to induce their proteasome-mediated degradation (Münger et al., 2001). Given the properties of E7, and the role of these targets in mediating the cellular response to DNA damage, it is not surprising that E7 was found to abrogate these responses both in tissue culture (Slebos et al., 1994), and in vivo (Song et al., 1998).

4.3.3 Genomic instability

As noted above, HPV E6 and E7 proteins can abrogate the cellular response to DNA damage, which under normal conditions would help prevent the accumulation of genetic changes in host chromosomes. It is, therefore, not unexpected that a hallmark of E6- and E7-expressing keratinocytes is genomic instability (Smith et al., 1989; Popescu & DiPaolo, 1990; Hashida & Yasumoto, 1991; Steenbergen et al., 1998). Abnormalities observed include monosomies, chromatin gaps and breaks, double minutes, and aberrant chromosomes. Structural changes are most commonly detected in chromosomes 1, 3 and 5, and less frequently in chromosomes 6, 7, 8, 10, 12, 13, 16 and 22. Some of the allelic losses have been associated with particular genes that could be involved in malignant conversion and/or progression. Allelic imbalance on 6q has been associated with telomerase activation (van Duin et al., 2003), which is a crucial step for cell immortalization mediated by high-risk HPVs (Klingelhutz et al., 1996; Anderson et al., 1997). Mitotic abnormalities can also be induced by E6 and E7 through direct subversion of the mitotic spindle checkpoint (Thomas & Laimins, 1998; Duensing et al., 2000).

The ability of E6 to induce genomic instability most likely reflects its capacity to inhibit the function of p53 (see above), leading to the disruption of normal DNA-repair processes and the consequent accumulation of genetic damage.

Table 4.1 Assessment of the correlations between p16 positivity and HPV status

<table>
<thead>
<tr>
<th>Site</th>
<th>Reference</th>
<th>p16/p53 status</th>
<th>HPV status</th>
<th>p16+:HPV+ correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Carlson et al. (2007)</td>
<td>All p16⁺</td>
<td>All negative</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Aguayo et al. (2007)</td>
<td>Most p16⁺</td>
<td>Some positive</td>
<td>None</td>
</tr>
<tr>
<td>Colorectum</td>
<td>Kong et al. (2007)</td>
<td>All p16⁺</td>
<td>All positive</td>
<td>Yes but no HPV- samples</td>
</tr>
<tr>
<td></td>
<td>Lu et al. (2003)</td>
<td>All p16⁺</td>
<td>All positive</td>
<td>Yes but no HPV</td>
</tr>
<tr>
<td>Sinus</td>
<td>El-Mofty &amp; Lu (2005)</td>
<td>9/39 p16⁺</td>
<td>9/39 positive, all 9 were p16⁻ and p53⁻</td>
<td>Yes</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>Castillo et al. (2006)</td>
<td>Some p16⁺</td>
<td>Some positive</td>
<td>Incomplete: some p16⁻ were HPV⁺, some p16⁺ were HPV⁻</td>
</tr>
<tr>
<td>Tonsil</td>
<td>Klussmann et al. (2003)</td>
<td>16/34 p16⁺</td>
<td>18/34 HPV⁺</td>
<td>Near complete (one HPV cancer was p16⁺)</td>
</tr>
<tr>
<td>Larynx</td>
<td>Laco et al. (2008)</td>
<td>14/24 p16⁺</td>
<td>14/24 HPV16⁺ same 14 that were p16⁺</td>
<td>Yes⁺</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>O’Regan et al. (2008)</td>
<td>Some p16⁺</td>
<td>Some positive (10/24)</td>
<td>Incomplete Some p16⁻ were HPV⁺, some p16⁺ were HPV⁻</td>
</tr>
<tr>
<td></td>
<td>Shuyama et al. (2007)</td>
<td>Some positive</td>
<td>Some positive (19/59)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Reimers et al. (2007)</td>
<td>25/29 p16⁺ and HPV⁻</td>
<td>27/96 HPV⁺ in total</td>
<td>High⁺: 4 HPV⁻ p16⁺; 2 p16⁻ HPV⁺</td>
</tr>
</tbody>
</table>

a Data for laryngeal carcinoma
b Data for tonsil/base of tongue
Compiled by the Working Group
In the case of E7, genomic instability may also reflect its effect on centrosome biogenesis, and the consequent defects in segregation of daughter chromosomes during cell division (Duensing & Münger, 2002). How E7 induces genomic instability remains unclear. Although studies in mice suggest that the inactivation of pRb in tissues that express E7 is sufficient to induce centrosome abnormalities (Balsitis et al., 2003), another study demonstrates that E7 can induce such abnormalities through a pRb-independent mechanism (Duensing & Münger, 2003). Furthermore, this may occur through cell-cycle perturbations that involve cyclin/CDK2 activity (Duensing et al., 2004).

Chromosomal instability has been associated with the integration of the viral genome upon prolonged culture of the keratinocyte cell line W12 harbouring episomal HPV, when grown as monolayers (Pett et al., 2004). However, the contrary has been shown in raft cultures of keratinocytes, in which genomic instability was observed in the absence of viral DNA integration (Duensing et al., 2001). Clearly more work is required in this area to fully understand the possible effects of viral integration upon genomic integrity of the host cell.

4.3.4 Cell proliferation and differentiation

The most pronounced effects on cell proliferation and differentiation are exerted by the E7 oncoprotein. When expressed in immortalized rodent cells, HPV 16 E7 can stimulate DNA-synthesis and cell proliferation (Sato et al., 1989). As E7 enhances proliferation of keratinocytes, it reactivates the host DNA-replication machinery in suprabasal differentiated, non-cycling cells (Blanton et al., 1992; Cheng et al., 1995). Thus, while differentiation still takes place, E7 also induces cell proliferation in cells that have already migrated away from the basal cell layer, suggesting that differentiation occurs, but in a modified state (Blanton et al., 1992; Cheng et al., 1995; Jones et al., 1997).

4.3.5 Inhibition of apoptosis

E6 has long been known to be a potent inhibitor of the apoptotic response. This has a major impact on the survival of cells that are being driven to proliferation by E7. This activity of E6 can occur in both a p53-dependent and -independent manner (Pan & Griep, 1994, 1995), the latter probably being related to the ability of E6 to induce the degradation of the pro-apoptotic factor Bak (Thomas & Banks, 1998), which is a common feature of both cutaneous and mucosal HPV E6 proteins. Recent studies also suggest the ability of HPV 16 E6 to inhibit receptor-mediated pathways of apoptosis, such as the TNF- and Fas-induced pathways (Filippova et al., 2002, 2004).

4.4 Role of HPVs in malignant conversion

4.4.1 Requirement of HPV genome expression for cell growth

As previously described for HPV 16 and HPV 18, E6 and E7 need to be continually expressed in cervical-cancer-derived cell lines to maintain cell proliferation (von Knebel Doeberitz et al., 1988, 1992; Butz et al., 2003; Yoshinouchi et al., 2003). Without either protein, the cells cease to proliferate and, depending on the experimental conditions, will either enter apoptosis or senesce.

4.4.2 Integration of the HPV genome

The integration of the viral genome into the genome of the host cell is not a pre-requisite for the development of malignancy. Although integrated HPV genomes are still the most prevalent form of the viral DNA in tumours, it is apparent that cancers can arise, in a significant number of cases, before genome integration. It is worth noting that there are differences depending upon the genotype involved, with HPV 16, 18 and 45
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being more frequently found in an integrated state than HPV 31 and 33 (Vinokurova et al., 2008). Integration has not been found to be a common characteristic of beta HPV types.

4.4.3 Alteration of specific cellular proto-oncogenes

The etiological association of high-risk HPVs with anogenital cancers and a subset of head and neck cancers is well established. It is also clear, however, that other genetic or epigenetic changes are required for these HPV-associated cancers to develop. This conclusion is based upon the long latency period between infection and the onset of cancer, and the fact that only a small percentage of infected individuals will develop cancer. Several studies have looked for alterations in cellular proto-oncogenes, particularly the MYC and RAS gene families, as possible cofactors for the development of cervical cancer, but the results are inconsistent. Some investigators have reported that MYC is amplified at the gene level or increased in its expression in cervical cancers and sometimes in CIN lesions, but others have not observed this. Likewise, some studies have reported upregulation of, and mutation in, genes of the RAS family, while others have not. At this stage, the overall conclusion is that the activation of proto-oncogenes can occur in cervical cancers and precursor lesions, but none of those reported so far is an absolute pre-requisite for the development of the cancer, nor is there evidence that any of these alterations have prognostic value (for reviews, see Whang & Lee, 1997; Spandidos et al., 2000).

4.4.4 Effect on the host immune response

Evasion of the host immune system by the virus is a likely key event in HPV-mediated carcinogenesis. Persistence of the viral infection is a necessary condition for the development of cancer. Innate immunity is considered as a first line of cellular defence against pathogens. HPVs activate Toll-Like Receptors (TLRs) in antigen-presenting cells (Fausch et al., 2003; Yang et al., 2004; Yan et al., 2005). The high-risk HPV oncoproteins interfere with the innate immune responses at multiple levels. Both high-risk HPV E6 and E7 can interfere with factors induced by innate immune responses including those mediated by interferon (Ronco et al., 1998; Li et al., 1999; Park et al., 2000; Perea et al., 2000; Nees et al., 2001). HPV 16 E6 and E7 together can cause a reduction in the expression of TLR9 (Hasan et al., 2007).

Effects of HPV genes on acquired immune responses have also been noted. The E6 and E7 proteins of high-risk HPV can inactivate components of the biosynthetic pathway of the major histocompatibility class I (MHC I) complex such as class I heavy chain, transporter associated with antigen processing subunit 1 (TAP1), and low molecular weight protein 2 (LMP2) (Georgopoulos et al., 2000). HPV 16 E5 downregulates surface MHC class I (Ashrafi et al., 2005, 2006) as well as class II (Zhang et al., 2003) expression. There is also evidence that beta HPV types, specifically HPV 38, can inhibit innate immune responses (Cordano et al., 2008). These findings may have important implications in predicting the overall efficacy of immunotherapeutic strategies for treating HPV infections.

4.5 Transgenic models for HPV-associated cancers

The advent of the technology to generate germline-transgenic mice has provided investigators with the ability to assess the in vivo properties of HPV oncoproteins implicated in human cancers. This section summarizes advances made in the assessment of the role of HPV oncogenes in anogenital and head and neck cancers in the case of high-risk anogenital HPVs, and skin cancers in the case of beta HPVs (i.e. EV-associated HPVs).
4.5.1 Cancer of the cervix

Studies on the role of HPV oncogenes in anogenital cancers were greatly facilitated by the use of the human keratin 14 (K14) transcriptional promoter, which directs the expression of HPV genes to the stratified epithelium of the lower female reproductive tract and of the oral cavity. The first germline-transgenic mouse model for HPV-associated cervical cancer was developed by the use of a K14HPV 16 transgenic mouse line in which the early genes of HPV 16 were placed under the control of the K14 promoter (Arbeit et al., 1994). Although these mice did not develop cervical cancers spontaneously, treatment with exogenous estrogen, sufficient to induce continuous estrus, led to a highly penetrant cervical cancer phenotype (Arbeit et al., 1996). The cervical cancers occurred in the context of a progressive disease much like that seen in women, being preceded by the onset of CIN grades 1–3. As in women, the cancers preferentially arose in the transformation zone (Arbeit et al., 1996). The individual contributions of the two HPV 16 oncogenes E6 and E7 have been assessed (Riley et al., 2003). From these studies, it has become clear that HPV 16 E7 is the dominant oncogene in the context of cervical carcinogenesis in the mouse. Whereas 100% of K14E7 mice treated with exogenous estrogen for 6 months developed high-grade dysplasia and/or cervical cancers, K14E6 mice only developed cervical cancer when estrogen treatment was extended to 9 months (Shai et al., 2007). Synergy between E6 and E7 was also observed (Riley et al., 2003; Shai et al., 2007, 2008).

E6 is also known to bind to several cellular proteins that contain PDZ domains (see above). Transgenic mice expressing a mutant form of HPV 16 E6 unable to inactivate p53, displayed a reduced tumorigenic phenotype in the cervix when treated with estrogen, compared with mice expressing the wild-type HPV 16 E6; however, this mutant E6-expressing strain also displayed an enhanced susceptibility to cervical carcinogenesis compared with estrogen-treated non-transgenic mice (Shai et al., 2007). These results are consistent with the hypothesis that the inactivation of p53 partially contributes to the oncogenic potential of the E6 protein. Consistent with this observation, mice conditionally null for p53 displayed an increased susceptibility to cervical cancers when treated with estrogen compared with treated p53-proficient mice (Shai et al., 2008).

E6 is also known to bind to several cellular proteins that contain PDZ domains (see above). Transgenic mice expressing a mutant form of HPV 16 E6 unable to bind to PDZ-domain proteins (Nguyen et al., 2003) had a reduced susceptibility to cervical cancers compared with mice expressing the wild-type E6 protein (Shai et al., 2007). It remains unclear which of the interactions with PDZ-domain proteins contribute to E6-mediated carcinogenesis in vivo, and how E7 contributes to this carcinogenic process (Simonson et al., 2005; Shai et al., 2007).

The mechanism by which E5 may contribute to cervical carcinogenesis in vivo has yet to be determined. In mouse skin, E5 can induce epithelial hyperplasia, and this is dependent upon the presence of a functional epidermal growth-factor receptor (EGFR) (Genther Williams et al., 2005). E5 has been implicated in two steps in skin carcinogenesis, promotion and progression (Maufort et al., 2007). It remains to be seen whether the activation of EGFR contributes to
the oncogenic potential of E5 in vivo, in the skin or the cervix.

The importance of the continued expression of E6 and E7 for maintenance of the tumour phenotype in vitro is well documented (see above). This has now been evaluated in HPV-transgenic mice through the use of a tet-regulated HPV 16 E7 transgene. In these mice, the continued expression of E7 was found to be critical for the maintenance not only of cervical cancers but also the dysplastic neoplasia that is recognized as the precursor lesion to cervical cancer (Jabbar et al., 2009). These data support the development of therapies aimed at inhibiting the expression or function of HPV oncogenes implicated in cervical cancers.

Estrogen is an important cofactor in the development of cervical cancers in HPV-transgenic mouse models (Arbeit et al., 1996). Estrogen was found to be necessary not only for the development of cervical cancers in HPV-transgenic mice, but also for their persistence (Brake & Lambert, 2005). Estrogen receptor α (ERα) was found to be necessary for cervical carcinogenesis in K14E7-transgenic mice (Chung et al., 2008). Indirect evidence potentially supporting a role of estrogen in human cervical cancers has come from studies demonstrating that women who have used oral contraceptives for at least 5 years or have had multiple pregnancies are at increased risk for cervical cancer, although there are also conflicting reports (Beral et al., 1999; Hannaford et al., 2007; IARC, 2011). Furthermore, a subset of human cervical cancers has been found to overexpress aromatase, a key enzyme in estrogen biosynthesis, suggesting a role of estrogen in these cancers (Nair et al., 2005). However, women on estrogen replacement therapy have not been found to be at increased risk for cervical cancer (Archer, 2004). [The Working Group noted that this study did not control for HPV status.] The fact that tamoxifen treatment did not reduce cervical disease in women (Bigler et al., 2004) could be explained by the fact that tamoxifen acts as an ER-agonist, not antagonist, in the human cervix (Senkus-Konefka et al., 2004). It remains to be determined whether anti-estrogens (specifically, drugs that act as ER-antagonists in the human cervix, e.g., fulvestrant) and/or aromatase inhibitors will be effective in treating women with HPV-associated cervical disease.

4.5.2 High-risk HPVs and cancers of the head and neck

There is a growing appreciation that the same high-risk HPV types etiologically associated with anogenital cancers, particularly HPV 16, are also associated with a subset of human head and neck squamous cell carcinomas (HNSCC), most notably of the oropharynx (e.g., tonsils) and the base of the tongue. The role of HPV 16 E6 and E7 oncogenes in HNSCC has been evaluated in HPV-transgenic mice that express these viral oncogenes in the relevant tissues. These mice do not spontaneously develop HNSCC, but when treated with the synthetic carcinogen 4-nitroquinoline-N-oxide (4-NQO), they become more susceptible to head and neck cancers (Strati et al., 2006). The progressive disease observed in the 4-NQO-treated HPV-transgenic mice was similar to that seen in humans. Also, the cancers that occurred were primarily high-grade HNSCC as observed in HPV-positive HNSCC in humans. As in cervical cancer, E7 proved to be the more potent oncoprotein and the inactivation of pRb could not fully account for the role of the E7 protein in HNSCC (Strati & Lambert, 2007).

4.5.3 Carcinogenic potential of EV-associated beta HPVs in the skin

HPVs of the genus beta are associated with a rare, familial benign disease termed epidermodysplasia verruciformis (EV). EV patients are at an increased risk for squamous cell carcinomas of their skin at sites exposed to the sun. Transgenic mouse models have been developed to investigate...
the role of EV-associated HPVs (specifically HPV 8 and HPV 38) in skin carcinogenesis. K14HPV-8-transgenic mice expressing the early genes of HPV 8 in the epidermis were found to be susceptible to the spontaneous development of both benign and malignant skin cancers (Schaper et al., 2005). Likewise, K10-HPV38 E6/E7 transgenic mice were highly susceptible to multistage skin carcinogenesis, specifically when treated with ultraviolet light (UVB) or chemical carcinogens (Dong et al., 2005). Interestingly, these mice express high levels of ΔNp73, which appears to be a primary mechanism for these mice to overcome the growth-suppressive activities of p53 (Accardi et al., 2006; Dong et al., 2008).

The synergy between cutaneous HPVs and UV in the development of squamous cell carcinomas in the skin has also been studied in transgenic mice expressing in their epidermis the E6 and E7 genes of HPV 20, which is commonly associated with squamous cell carcinoma arising in renal transplant patients, or HPV 27, which is only associated with benign papillomas. Upon UV irradiation, both HPV-20- and HPV-27-transgenic mice are more susceptible to tumours than non-transgenic mice, however, the HPV-20-transgenic mice displayed an increased incidence of malignant tumours. Alterations in the expression of both p53 and p63 were noted in the UV-exposed transgenic mice (Michel et al., 2006).

4.6 Synthesis

The characterization of the mechanisms of action of the HPV oncoproteins in assays both in vitro and in vivo provides compelling evidence for a direct role of high-risk mucosotropic HPVs in the development of cervical cancer. The mechanisms involve immortalization, transformation, inhibition of apoptosis, induction of genomic instability, and deregulation of the immune response. The mechanistic evidence for individual mucosotropic HPV types found in cancers is as follows:

- A common feature of mucosotropic HPV-associated cancers is the expression of the viral genes E6 and E7.
- The E6 and E7 genes of HPV 16 and HPV 18 have been the most extensively studied and were found to confer a similar set of biological phenotypes (e.g., immortalization, inhibition of DNA-damage response, genomic instability, inhibition of differentiation) on epithelial cells from multiple human tissues in which HPV-associated cancers are found, including the cervix, penis, and tonsil.
- The E6 and E7 proteins of the same HPVs (16 and 18) share similar sets of biochemical properties (e.g., for E6: inactivation of p53, induction of hTERT, binding to PDZ; for E7: inactivation of pRb and related pocket proteins, activation of E2Fs) that correlate with their transforming, immortalizing and tumorigenic properties both in vitro and in vivo.
- Suppression of HPV 16/18 E6 and E7 gene expression in cell lines derived from human cervical cancers leads to senescence or apoptosis.

Given these observations, there is strong mechanistic evidence that HPV 16 and HPV 18 act directly to cause cancers in those tissues in which they are found.

For other mucosotropic HPVs (31, 33) that are found in human cancers, there is moderate mechanistic evidence from available experimental data (biochemical and/or biological) that they are directly acting in causing cancers in those tissues in which they are found.

For a third set of mucosotropic HPVs (35, 39, 45, 51, 52, 53, 56, 58, 66, 68, 82) found in human cancers, there is positive mechanistic evidence from one or a few studies (biochemical and/or biological) that they directly act to cause cancers in those tissues in which they are found.
For HPV 6 and 11, there is little to no mechanistic evidence that they can contribute to carcinogenesis (weak inactivation of p53, no activation of hTERT, no binding to PDZ, weak inactivation of pRB, no immortalization, no transformation).

With respect to most of the cutaneous HPV types of the genus beta, there is a general paucity of experimental studies assessing their role in cancer development. For a subset, HPV 8, and 38, which have been analysed in both in vitro and in vivo models, there is strong evidence of their potential capacity to cause cancer. However, unlike what has been found for mucosotrophic HPVs associated with human cancers, the limited data available indicate that the majority of, if not all, cells within skin cancers potentially associated with beta HPV types do not contain the viral genome. Thus, novel mechanisms for HPV-induced carcinogenesis would need to be invoked, for which there is currently little evidence.

5. Evaluation

There is sufficient evidence in humans for the carcinogenicity of HPV 16. HPV 16 causes cancer of the cervix, vulva, vagina, penis, anus, oral cavity, oropharynx, and tonsil. Also, a positive association has been observed between infection with HPV 16 and cancer of the larynx.

There is sufficient evidence in humans for the carcinogenicity of HPV 18. HPV 18 causes cancer of the cervix. Also, a positive association has been observed between infection with HPV 18 and cancer of the vulva, penis, anus, oral cavity, and larynx.

There is sufficient evidence in humans for the carcinogenicity of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 cause cancer of the cervix. Also, a positive association has been observed between infection with HPV 33 and cancer of the vulva and of the anus.

There is limited evidence in humans for the carcinogenicity of HPV types 26, 53, 66, 67, 68, 70, 73, and 82. A positive association has been observed between infection with HPV types 26, 53, 66, 67, 68, 70, 73, and 82 and cancer of the cervix.

There is inadequate evidence in humans for the carcinogenicity of HPV types 30, 34, 69, 85 and 97. Nonetheless, their phylogenetic analogy to HPV types with sufficient or limited evidence in humans suggests that these types may be potentially carcinogenic.

There is inadequate evidence in humans for the carcinogenicity of HPV 6 and HPV 11 in the larynx.

There is inadequate evidence in humans for the carcinogenicity of HPV genera beta and gamma types in the skin. In the rare case of patients with epidermodysplasia verruciformis, there is limited evidence for the carcinogenicity of HPV genus-beta types 5 and 8 in the skin.

HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are carcinogenic to humans (Group 1).

HPV 68 is probably carcinogenic to humans (Group 2A).

HPV types 26, 53, 66, 67, 70, 73, 82 are possibly carcinogenic to humans (Group 2B).

HPV types 30, 34, 69, 85 and 97 are possibly carcinogenic to humans (Group 2B) based on their phylogenetic analogy to HPV types with sufficient or limited evidence in humans.

HPV types 6 and 11 are not classifiable as to their carcinogenicity to humans (Group 3).

Some types of HPV genera beta and gamma are not classifiable as to their carcinogenicity to humans (Group 3), with the notable exception that HPV 5 and HPV 8 are possibly carcinogenic to patients with epidermodysplasia verruciformis (Group 2B).
References


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Bjørge T, Dillner J, Anttila T et al. (1997). Prospective serepohidemological study of role of human papillo-


Bodaghi S, Yamanegi K, Xiao SY et al. (2005). Colorectal papillomavirus infection in patients with colore-

Bosch FX, Burchell AN, Schiffman M et al. (2008). Epidemiology and natural history of human papil-


Bryan JT, Taddeo F, Skulsky D et al. (2006). Detection of specific human papillomavirus types in paraffin-


Cambruzzi E, Zettler CG, Alexandre CO (2005). Expression of Ki-67 and squamous intraepithelial lesions are related with HPV in endocervical adeno-


Casabonne D, Lally A, Mitchell L et al. (2009). A case-

Casabonne D, Michael KM, Waterboer T et al. (2007). A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carci-


Dong W, Kloz U, Accardi R *et al.* (2005). Skin hyperplasia and susceptibility to chemical carcinogenesis in transgenic mice expressing E6 and E7 of...


Dürst M, Dzarlieva-Petrusevska RT, Boukamp P et al. (1987). Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. Oncogene, 1: 251–256. PMID:2838778


el-Deiry WS, Tokino T, Velculescu VE et al. (1993). WAF1, a potential mediator of p53 tumor suppression.


Georgopoulos NT, Proffitt JL, Blair GE (2000). Transcriptional regulation of the major histocompatibility complex (MHC) class I heavy chain, TAP1 and LMP2 genes by the human papillomavirus (HPV) type 6b, 16 and 18 E7 oncoproteins. Oncogene, 19: 4930–4935. doi:10.1038/sj.onc.1203860 PMID:11039910


IARC (2011). Pharmaceuticals. IARC Monogr Eval Carcinog Risks Hum, 100A:


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