

5. Summary of Data Reported and Evaluation

5.1 HPV infection

Papillomaviruses are small, non-enveloped, viruses that contain a double-stranded, circular 8 kb DNA genome. They are highly host-specific, cannot be propagated in tissue culture and, with the exception of some ungulate papillomaviruses, infect only epithelial cells. Of the types described, more than 70 are human papillomaviruses (HPV) and approximately 20 are animal papillomaviruses. The types are classified according to their nucleotide sequences and form five taxonomic groups. These groups are not phenotypically homogeneous. Two of these groups contain most of the HPVs.

The papillomavirus genome can be divided into three regions. The long control region contains *cis*-responsive elements, which are required for the regulation of gene expression and DNA replication. The early region codes for proteins involved in the regulation of viral transcription (E2), viral DNA replication (E1 and E2), cell proliferation (E5, E6 and E7) and, possibly, some late steps in the viral life cycle (E4). The late region contains two genes, which code for the capsid proteins L1 and L2.

Several advances have recently been made in understanding the immune response to HPVs. Seroreactive and T-cell epitopes of HPV proteins have been identified using a variety of techniques. In most instances, it is unclear if these epitopes are recognized in the course of natural infection. Using ELISA (enzyme-linked immunosorbent assay), based upon HPV virus-like particles synthesized by the expression of HPV late genes in recombinant vectors, antibodies reactive to the virus capsid have been found in a proportion of patients with HPV infections and HPV-related diseases. Antibodies to early HPV proteins have also been detected in patients with HPV-associated diseases as well as in healthy individuals. It remains to be seen whether accurate and reliable immunological assays that measure exposure to HPVs can be developed.

Over the last five years, there has been substantial improvement in the methods used to detect HPV DNA. Different assays are now available that can detect small amounts of HPV DNA, quantify the amount of viral DNA in clinical specimens, identify a broad spectrum of genital and cutaneous HPV types, test for selected HPV types and localize the viral genome and viral transcripts to individual cells.

Polymerase chain reaction (PCR) amplification has provided sensitive and specific assays for a broad spectrum of HPV DNAs. Using consensus or general primers, a large number of HPV DNAs can be amplified in a single reaction. Detection systems using oligonucleotide probes permit identification of individual HPV types in a fashion suitable for large-scale studies. Nucleotide sequences of PCR products can also be compared with databases of HPV genomic sequences for unequivocal identification of HPV types. Some commercially available non-PCR-

based assays provide standardized reagents, test separately for high-risk and low-risk HPV types, and quantify HPV DNA levels in clinical specimens. Future investigations will determine whether HPV DNA assays are valuable in screening for cervical cancer and its precursors and in the management of women with cervical cytological abnormalities.

Genital HPVs are transmitted primarily through sexual contact with infected cervical, vaginal, vulvar, penile or anal epithelium. Perinatal transmission, digital and oral transfer, and auto-inoculation of genital HPV types have also been documented. Transmission of all HPV types is probably more efficient in the presence of an abraded epithelial surface.

The prevalence of genital HPV infection is highest among sexually active young adults and is similar for men and women. Shortly after sexual debut, risk of infection with each new sexual contact is high. HPV infections are common throughout the world. Recent data suggest that there may be geographic differences in the prevalence of specific HPV types and variants.

Prevalent HPV infections have been studied extensively but little is known about the incidence and natural history of primary infections. Findings from a variety of studies suggest that after infection, most individuals do not develop clinical signs or symptoms. The natural history of specific viral types may be different. Only a small percentage of infected individuals will develop HPV-associated cancer.

Morphological changes occur in the epithelium of the lower genital tract and anus in response to infection with HPV. The cytological and histological alterations that occur as a result of a productive infection reflect the cytopathic effects of the virus on epithelial cells and include nuclear atypia, an increased mitotic rate and koilocytosis. The productive phase of HPV infection is generally pronounced in what is referred to as low-grade cervical intraepithelial neoplasia (CIN). Following HPV infection, a cancer precursor state may develop. This is accompanied by an increase in nuclear atypia and mitotic rate, as well as architectural disorganization. Such lesions are generally aneuploid and frequently contain abnormal mitotic figures. These precursor lesions are referred to as high-grade CIN.

The presence of CIN adjacent to areas of invasive cancer, the cytological and histological similarities between CIN and invasive cancer, and the observation of tongues of early invasion arising directly from CIN are compelling pathological evidence that CIN is a precursor to invasive cancer.

Genital HPVs are the etiological agents for condylomata, recurrent respiratory papillomas and some papillomas at other mucosal surfaces. Cutaneous HPVs cause skin warts. Benign HPV disease is treated by chemical and physical agents or by surgical removal. Excisional therapy is preferred for treatment of HPV-associated intraepithelial neoplasia, especially for treatment of high-grade lesions. Non-invasive treatment strategies of proven efficacy are not available for these lesions. However, immunization strategies are being devised and in the future these may help prevent or control HPV-associated diseases.

5.2 Studies of cancer in humans

There is compelling epidemiological evidence that some HPV types are human carcinogens. In methodologically sound studies, HPVs are found in over 90% of all invasive cervical cancers and in a high proportion of certain other anogenital cancers. Carcinogenicity in humans

has been most firmly established for HPV-16, but strong evidence of carcinogenicity also exists for certain other HPV types.

Case series from many areas of the world have established that a high proportion (~50%) of cervical cancers and high-grade CIN lesions contain HPV-16 DNA. Almost 100 case-control studies have been reported that examine the relationship between HPV and cervical neoplasia. Almost all have found positive associations. Among the most informative studies, strong associations (prevalence odds ratios > 20) with HPV-16 DNA have been observed with remarkable consistency for invasive cancer and high-grade CIN, ruling out the possibility that this association can be explained by chance, bias or confounding.

Currently available prospective data indicate that HPV-16 infection precedes high-grade CIN and predicts an elevated risk of developing it, although the relative risks observed are lower than the prevalence odds ratios generated by case-control studies. The epidemiological data regarding HPV-16 and cervical neoplasia are consistent with the established epidemiological risk factors (e.g. sexual behaviour) and with the biological data cited below.

On the basis of strong and consistent case-control study results, a causal role for HPV-16 in anal cancer is highly likely. The lack of prospective data is partially mitigated by similarities between cervical and anal anatomy, pathology, and risk factors.

Based on more-limited case series and case-control data, HPV-16 infection is likely to have a causal role in the etiology of poorly keratinized squamous-cell cancers of the vulva associated with adjacent vulvar intraepithelial neoplasia (VIN), and in carcinoma of the penis. The epidemiological data linking HPV-16 to the risk of other cancers are currently inadequate.

After HPV-16, HPV-18 is the type most clearly shown by epidemiological data to be a human carcinogen. The evidence is limited to the cervix, in which HPV-18 appears to be strongly linked to a substantial minority of squamous cancers and approximately half of the adenocarcinomas.

Additional anogenital HPV types are implicated as human carcinogens of the cervix (including, at least, HPV types 31, 33, 35, 39, 45, 51, 52, 56 and 58). Among these types, the epidemiological evidence is strongest for HPV-31 and -33. However, full evaluation is hampered by the low prevalence of individual types.

Although there are rare case reports of tumours containing HPV-6 or -11 DNA, the epidemiological data suggest that HPV-6 and -11 are not human carcinogens for the cervix. The epidemiological evidence for carcinogenicity of HPV-6 and -11 at non-cervical sites is judged to be inadequate. There are no adequate epidemiological studies of other anogenital HPV types.

In addition to the epidemiological studies conducted in the general population, some studies of HPV and cancer have been conducted in special populations. An increasing number of studies have shown women immunosuppressed by human immunodeficiency virus (HIV) infection to be at higher risk of HPV-associated cervical intraepithelial neoplasia, but, so far, an increased risk of invasive cervical cancer has not been established. Many studies have shown a high prevalence and viral load of anal HPV in immunosuppressed HIV-infected individuals. There is increasing evidence for a direct effect of immunosuppression on the development of HPV-associated AIN (anal intraepithelial neoplasia) and anal cancer.

To date, case series have not shown a high prevalence of HPV infection in skin cancer patients in the general population, with the exception of two rare types of skin neoplasms (periungual squamous-cell carcinoma and periungual and palmoplantar Bowen's disease) in which HPV-16 and/or -18 have been consistently found. In contrast, HPVs are recovered frequently from skin cancers in immunocompromised individuals. In a total of nine studies, HPV DNA was found in a high proportion (~60%) of squamous-cell carcinomas of the skin in epidermodysplasia verruciformis (EV) patients; HPV-5 was the predominant type. The prevalence of HPV infection varies widely in case series of skin cancer among transplant patients. A broad range of HPV types has been found, with no particular HPV type predominating.

5.3 Molecular mechanisms of carcinogenesis

Molecular studies have identified mechanisms utilized by high-risk HPV types that contribute to carcinogenesis. DNA and transcripts of specific HPV types are regularly detected in biopsies from cervical cancer and in its precursor lesions. Specific viral oncogenes have been identified that differ functionally between cancer-linked HPVs and those preferentially found in genital warts, leading to their classification as high-risk and low-risk HPVs, respectively. Besides growth promotion, high-risk HPVs induce chromosomal instability and could therefore act as solitary carcinogens. High-risk HPVs immortalize human and rodent cells *in vitro*, which can subsequently convert to malignant growth either spontaneously or after exposure to other carcinogens. In several HPV-positive cervical carcinoma cell lines of human origin, the malignant phenotype has been shown to depend on the activity of viral oncogenes. The E6 and E7 oncoproteins of the high-risk HPVs interfere with the functions of negative cellular regulators, including the tumour-suppressor proteins p53 and pRB, respectively. The activity of these viral oncogenes in transgenic animals supports the importance of the viral-host protein interactions in tumorigenesis.

The importance of deregulation of HPV oncogene expression to the malignant progression of HPV-infected cells is well supported. This commonly occurs following integration of the viral genome, but other mechanisms, such as alterations within the host cell genome and in the viral promoter, have been identified. Chromosomal abnormalities occur during tumour progression, indicating that alterations in cellular genes contribute to this process. The cellular genes that are affected by these chromosomal abnormalities have not been identified. The status of the *p53* tumour suppressor gene has been most extensively studied; somatic mutations within this gene occur only very rarely in HPV-positive cancers. The incidence of *p53* mutations in apparently HPV-negative cancers appears to be somewhat higher, although many studies fail to find such alterations in either HPV-positive or -negative cancers.

Cofactors may play a role in HPV-linked carcinogenesis. Herpes-group viruses were initially implicated. There exist no convincing epidemiological data in support of this hypothesis, although some experimental findings show that HPV-immortalized cell lines can be transformed by segments of the herpes simplex virus (HSV) type 2 or the human herpesvirus type 6 genome. HSV infections lead to efficient amplification of latent papovavirus genomes. Glucocorticoid hormones and progesterone may co-operate with HPV infections by activating early gene

transcription. The effects of chemical or physical carcinogens on progression of papillomavirus-induced lesions have been documented in a number of studies.

Regression of HPV-induced warts is accompanied histologically by a response characteristic of a CD4⁺-cell-dependent delayed-type hypersensitivity reaction; animal models support this observation. The increased prevalence of HPV infections in immunosuppressed individuals (such as those undergoing organ transplantation, those with HIV infection or those with inherited T-cell immunodeficiencies) further supports a role for CD4⁺ cells in the control of HPV infection. The association between the duration and degree of immunosuppression and the increased risk for HPV-associated neoplasia supports the notion that viral DNA persistence is important for neoplastic transformation and progression. Preliminary evidence suggests that some HPV-infected individuals make a serum-neutralizing antibody in response to viral proteins.

The oncoproteins of HPV-16 can act as tumour rejection antigens in animal models and induce HPV-16 specific cytotoxic T cells. Conventional immunization strategies can induce such cytotoxic T cells. Although there is no evidence for HPV-16-specific cytotoxic T cells in humans with HPV-associated disease, the animal models suggest that effective presentation of HPV oncoproteins to the immune system is potentially immunotherapeutic. However, in HPV-associated cervical neoplasia, there is an overall downregulation of HLA class I molecules, raising doubts about the potential effectiveness of such therapies. The presentation by HLA-B7 of an HPV-16 E6 variant peptide may be a biological mechanism for immune escape by the virus. A role for major histocompatibility complex (MHC) haplotypes in susceptibility to, or protection from papillomavirus-associated neoplasia is supported by data from rabbits and from humans.

5.4 Studies of cancer in animals

Although there is no established animal model for any of the HPVs, several of the animal papillomaviruses are associated with the development of malignant lesions both in the natural and heterologous hosts. Experimental studies of cottontail rabbit papillomavirus (CRPV) in domestic rabbits clearly demonstrate a direct causal relationship between infection by this virus and development of cancer. There is also strong evidence supporting a causal role for bovine papillomavirus (BPV)-2 in the development of bladder cancer and BPV-4 in alimentary tract cancer in cattle, although malignant progression has not been documented in the absence of additional carcinogens. Synergy in carcinogenesis between papillomaviruses and other factors is evident in the rabbit (experimentally applied chemical carcinogens) and cattle models (naturally occurring immunosuppressants and mutagens). Carcinogenic activity of other animal papillomaviruses has not been established, although malignant progression of virus-associated lesions has been reported in several cases.

The rhesus monkey model provides evidence for the sexual transmission of RhPV. The rabbit and the cattle systems are of value in the study of viral latency.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of human papillomavirus (HPV) types 16 and 18.

There is *evidence suggesting lack of carcinogenicity* to the cervix in humans of HPV types 6 and 11.

There is *limited evidence* in humans for the carcinogenicity of some other HPV types.

HPVs cannot infect animals. Some animal papillomaviruses cause cancer in their natural hosts.

Overall evaluation

HPV types 16 and 18 are *carcinogenic to humans (Group 1)*.

HPV types 31 and 33 are *probably carcinogenic to humans (Group 2A)*.

Some HPV types other than 16, 18, 31 and 33 are *possibly carcinogenic to humans (Group 2B)*.

The carcinogenicity of HPV types 16 and 18 is supported by experimental evidence that proteins of these viruses interfere with the functions of cellular regulatory pathways.