Persistent human papillomavirus infection and cervical neoplasia

Alex Ferenczy and Eduardo Franco

The Lancet Oncol 2002; 3: 11–16

Cervical cancer is one of the most frequent diseases in women, and causes considerable morbidity. Indeed, after breast cancer, it is the most common cancer in women worldwide. Age-adjusted incidence rates vary from about 10 per 100 000 per year in many industrialised nations to more than 40 per 100 000 per year in developing countries. Four of five new cases and most deaths from cervical cancer occur in the developing world (Figure 1). The estimated global incidence of invasive cancer is about 371 000 cases per year, and 5-year survival ranges from 44% to 66% for all clinical stages. In recent years, strong pathological and cell and molecular evidence has been accumulated to implicate human papillomavirus (HPV) as the primary surrogate for the development of cervical neoplasia (Figure 2). In this review, we focus on advances in our understanding of the epidemiology and molecular biology of virus host–cell interactions, as related to HPV-mediated cervical carcinogenesis.

The epidemiological evidence

Epidemiological studies have shown that the number of sexual male partners a woman has is directly related to her risk of developing cervical cancer and its precursors, cervical neoplasia. The development of cervical cancer is preceded by precursor lesions (cervical intraepithelial neoplasia). Evidence-based epidemiological and molecular data suggest that persistent infections with human papillomavirus (HPV) types that carry a high oncogenic risk are the intermediate endpoints, leading to both intraepithelial and invasive cervical neoplasia. Integration of highly oncogenic HPVs into host-cell chromosomes is followed by binding of HPV E6 and E7 oncoproteins to tumour-suppressor genes p53 and RB, respectively. This process results in impaired tumour-suppressor-gene function, involving DNA repair, decreased apoptosis, and eventual cell immortalisation. Mutations causing chromosomal alterations, loss of heterozygosity, and proto-oncogene and telomerase activation in immunopermisive individuals have important roles in virus-induced cervical carcinogenesis. The so-called non-European variants of HPV 16 and 18 may increase the degradation potential of p53. HPV 16 is polymorphic and, although the evidence is controversial, the Arg/Arg genotype of p53 could have greater susceptibility to HPV-E6 degradation than the other genotypes. The coincident interplay between the non-European genomic variants of HPV 16/18 and p53 Arg/Arg may explain, at least in part, the persistence of HPV infection and tumour progression in women with cervical neoplasia. Further epidemiological and molecular research is needed, to gain insight into HPV-mediated cervical carcinogenesis.

AF is Professor of Pathology and Obstetrics & Gynecology at McGill University and the Sir Mortimer B Davis Jewish General Hospital, Montréal, Québec, Canada. EF is Professor of Epidemiology and Oncology and Director of the Division of Cancer Epidemiology, McGill University, Montréal, Québec, Canada

Correspondence: Alex Ferenczy, MD, Department of Pathology, SMBD-Jewish General Hospital, 3755 Côte Ste Catherine Road, Montréal, Québec, Canada, H3T 1E2. Tel: +1 514 340 7526. Fax: +1 514 340 7542. Email: aferen@po-box.mcgill.ca

Figure 1. Cervical carcinoma and hydronephrosis. (a) A large cancerous growth (viewed from the front) has essentially replaced the entire cervix. (b) The most frequent cause of death due to cervical carcinoma, ie, hydronephrosis due to tumour obstruction of ureters.
intraepithelial neoplasia (CIN) (Figure 3). There also appear to be variations in risk correlated with social class and male-partner factors. For example, cervical cancer is more common in women of lower social class and those with a history of early sexual intercourse and many partners. A woman’s risk can also be increased by her male partner’s sexual behaviour. Such high-risk ‘carcinogenic Charlies’ commonly carry HPV DNA or related lesions on their penises. Detection of HPV DNA from the man’s penis has been related to a substantially increased risk of cervical cancer in his female sexual partners. There is a strong relation between the male partner’s number of extramarital partners, particularly if they are prostitutes, and the woman’s risk. Circumstantial evidence for sexual transmission of an infectious agent also comes from studies which show that wives of patients with penile cancer are at increased risk of developing cervical cancer later in life (Figure 4). Such findings are confirmed by results from correlation studies, in which strong associations were found between cervical and penile cancer incidence and mortality.

The association of HPV with cervical neoplasia is very strong, independent of other risk factors, and consistent in several countries. Certain types of HPVs are recognised today as human carcinogens. In 1995, the International Agency for Research on Cancer evaluated all relevant data on the carcinogenicity of HPV and concluded that there was sufficient evidence to categorise HPV types 16 and 18 as human carcinogens, but that the existing evidence was limited or inadequate for the other HPV types. The International Agency for Research on Cancer has carried out a multicentre case-control study on 2000 women with cervical cancers and 2000 control women in Spain, Colombia, Brazil, Paraguay, Morocco, Mali, the Philippines, and Thailand. The pooled analysis of the above studies revealed a strong association between cervical cancer and the presence of any HPV DNA, with age-adjusted odds ratios ranging from 17 in Colombia to 156 in the Philippines, and a pooled odds ratio of 60 (95%, CI 49–73). The association was equally strong for both squamous-cell carcinoma (odds ratio: 62) and adenocarcinoma of the endocervix (odds ratio: 51), and for HPV 16 (odds ratio: 129) and HPV 18 (odds ratio: 104), as well as for other, less common HPV types. Even the lowest odds ratio was greater than that for the association between smoking and lung cancer, and is similar to that of chronic hepatitis infection and liver cancer, causal relations that are universally accepted. Initially, HPV DNA was detected in 93% of more than 1000 biopsy specimens obtained from 22 countries around the world, by use of a PCR assay based on L1 consensus primers (MY09-MY11). On retesting of the 7% of the cancers that were negative for HPV DNA with the more sensitive GP5+ – GP6+ consensus primers and E7 primers, a prevalence of 99.7% was reached, indicating that HPV is indeed a cause of cervical cancer.

However, because only a small fraction of women with persistent HPV infection will eventually develop cervical cancer, the infection alone may not be a sufficient precursor condition for development of the disease. The results of case-control studies have suggested that hormonal factors associated with smoking, high parity, and long-term use of oral contraceptives, certain nutritional deficiencies, and infection with Chlamydia trachomatis, may be cofactors of cervical cancer. Many of these cofactors, although rather weak,
are related to increased host susceptibility to HPV infection as a result of genetic alterations of the immune response to HPV. The immune response seems to be particularly important for the persistence of HPV infection. Indeed, about 80% of young women who become infected with HPV have transient infections that clear within 12–18 months,\(^{12,15}\) and humoral neutralising antibodies clear the virus. Even the majority of those who develop low-grade precancer will clear HPV infection via a cytotoxic-T-cell response against HPV-infected keratinocytes.\(^{16}\) On the other hand, immunocompromised individuals such as organ transplant recipients and HIV-seropositive patients are at high risk of anogenital neoplasia.\(^{1,5,10}\) The HLA genes, particularly the class II HLA alleles, are the primary mediators of cell-mediated immune system responses to exogenous pathogens, including viruses. They are expressed in antigen-presenting cells such as macrophages and Langerhans cells (dendritic cells in the skin and genital mucosa). Although evidence on the precise role of HLA haplotypes has been mixed, some studies showed an increased risk of HPV infection and cervical disease in individuals with the putative DQB1*03 and DRB1*15 alleles and related haplotypes.\(^{17}\) On the other hand, DRB1*13 alleles appear to provide a protective effect against developing cervical disease. Similarly, an increased risk of cervical neoplasia was found in HLA-B7-positive as compared with HLA-B7-negative patients.\(^{18}\)

Tobacco is a well-known risk factor for cervical cancer. Cigarette smoking may have a direct carcinogenic action on the cervical epithelium, because nitrosamines are carcinogenic in squamous mucous-membrane-type epithelium, and nicotine metabolites can be found in the cervical mucus of smokers.\(^{1,4}\) Furthermore, nicotine inhibits apoptosis in epithelial systems \textit{in vitro}.\(^{19}\) However, smoking is associated with sexual activity, which underscores the importance of avoiding epidemiological confounding in the analysis of cervical-neoplasia risk. Population-based studies showed an excess risk of cervical cancer in long-term (>12 years) users of oral contraceptives.\(^{14}\) The association is somewhat stronger for adenocarcinomas than for squamous-cell carcinomas. \textit{In vitro} and \textit{in vivo}, both oestradiol and progesterone stimulate growth of HPV-positive cells by promoting the long control region of HPV and, as a result, increasing expression of HPV E6/E7.\(^{20}\) Whether these animal experiments can be extrapolated to human beings has not been clearly established. Indeed, the association with oral-contraceptive use may simply be due to unprotected sexual activity and detection bias, because users of oral contraceptives have more frequent screening examinations than non-users. This results in earlier detection of disease. High intake of foods rich in β-carotene, vitamin C, and, to a lesser extent, vitamin A, reduces the risk of cervical cancer.\(^{14}\) The association may be confounded by the increased sexual activity and vitamin-poor diet seen in populations of low socioeconomic status.

**The molecular viral evidence**

Genital HPV types (each type has less than 90% homology with the genome of other known HPV types) are divided into those of low and high oncogenic risk. The most representative of these low-risk types is 6/11 and the most representative of the high-risk types are 16/18, 31, and 45.\(^{21}\) Low-risk HPVs cause benign genital warts and have no oncogenic potential. By contrast, high-risk HPVs are the causative agents of cervical cancer and its intraepithelial precursors. HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 account for about 93% of infections in high-grade CINs and cancer. At the molecular level, the difference between HPV types associated with low and high oncogenic risk is that the latter can integrate into the chromosomes of host cells (genome), whereas this integration does not seem to occur with the former. Results have shown integrated HPV 16 and 18 in 72% and 100% of invasive carcinomas of the cervix, respectively. Similarly, integrated DNA sequences are found in 5% to 50% of CINs, mainly of the high-grade variants.\(^{21-25}\)

Viral integration is central for cell transformation and the development of cervical neoplasia.\(^{21,24}\) When viral integration occurs, the HPV genome breaks in the E2 region, resulting in physical disruption of the E2 gene and loss of its suppressive functions on E6/E7. The E6 and E7 gene products of high-risk HPVs, but not of low-risk HPVs, functionally inactivate the products of two important tumour-suppressor genes, the p53 and the RB proteins, respectively. E6 and E7 therefore act...
High oncogenic HPV types

Integration

Loss of E2 function

E6/p53–E7/RB binding

Immortalisation

Chromosome rearrangement
Loss of heterozygosity
Proto-oncogene activation

CIN—Invasive carcinoma

Figure 5. Pathogenetic cascade of HPV-related cervical cancer. Persistent infection by high-oncogenic-risk HPV types, preferentially by the non-European molecular variants of HPV 16 and 18, results in viral integration into host-cell chromosomes. This results in break of HPV/E2 and loss of control of E6/E7 oncoproteins. These form complexes with p53 and RB protein, respectively, resulting in their inactivation. E6 may preferentially target and degrade Arg/Arg type versus Arg/Prol type p53. Loss of cell-growth control leads to immortalisation with impaired DNA-repair gene function, including inhibition of apoptosis. Immortalised cells show chromosomal deletions, loss of heterozygosity, proto-oncogene activation, and aneuploidy. In the susceptible individual with impaired cell-mediated immunity (HLA defect), the precancer CIN may progress to invasive carcinoma.

as oncogenes. This occurs by transbinding between E6 and p53 and E7 and RB protein. Only the E7 gene can immortalise primary human squamous-epithelial cells (keratinocytes), whereas E6 enhances the effectiveness of immortalisation by E7. The morphological features of HPV-16-immortalised keratinocytes are similar to those observed in routine biopsy samples of cervical-cancer precursors. The transcription of these viral oncogenes is enhanced by the long control region. The enhancer is cell-specific, activating viral transcription only in the natural target cells of the virus, i.e. squamous-epithelial cells.

Under normal conditions, the tumour suppressor genes p53 and RB are produced whenever cells sustain DNA damage or when oncogenes force the cells into a replicative state. If the damage is minor, an increase in p53 concentrations leads to arrest of mitosis and repair of damaged DNA. If the damage is major, the cell is subjected to nuclear death or apoptosis. These ‘molecular police’ are critical for normal growth control, because they prevent the formation of abnormal cells and their entry into the cell cycle. Binding of RB by HPV/E7 results in activation of protein 16 and cyclin E, both of which push the infected cell entering from G1 to the DNA S phase of the cell cycle. In experiments in vitro, where E6 binds to p53, the latter is degraded through a selective ubiquitin-dependent proteolytic pathway. This degradation results in reduced apoptosis and activation of endogenous c-myc and myc c-Ha-ras proto-oncogenes. These events, in turn, lead to uncontrolled cell proliferation and the eventual development of cervical neoplasia. Indeed, such cell populations are likely to be subjected to mutational events such as chromosomal rearrangement, microsatellite instability, and proto-oncogene activation, all presumed requisites for cervical carcinogenesis (Figure 5).

Chromosomal alterations, reviewed by Park and colleagues, have been observed in 95% of cervical cancers; chromosome 1 is most commonly involved, with deletions and translocations in the 1p11-p13 and 1q21-q32 regions. No chromosomal break points specific for cervical cancer have so far been identified, and chromosomal alterations are not confined to chromosome 1 in cytogenetic studies of cancers. Allelotyping showed allelic loss in chromosome 5 (5p) in 56% of invasive cancers and 21% of precursor lesions; in one study, microsatellite instability at the locus D5S406 was observed in 67% of 12 cancers and precursors. Alterations of chromosome 11q are associated with impaired tumour-suppressor-gene functions and deletions, as well as mutations of the proto-oncogenes c-Ha-ras in cervical cancers, and some researchers have observed a relation with the risk of early recurrence of tumour.

Telomerase is essential for elongation of chromosomes and cell proliferation. Promotion of proliferation may occur by inhibition of apoptosis by telomerase. The relation between telomerase activity and HPV infections has been investigated in cervical epithelium in both health and disease. In most tracing studies, telomerase activity was absent or weak in normal epithelium and low-grade CIN 1 (0–30%), was much higher in high-grade CIN 3 (60%), and was found in over 90% of invasive squamous-cell carcinomas. In addition, telomerase activity was detected mainly in the lower suprabasal layers of normal epithelium, whereas intra-epithelial lesions and invasive carcinoma displayed full-thickness involvement. Furthermore, telomerase expression was related to lesions that contained HPV types associated with high oncogenic risk. Telomerase activity may be involved in the CIN-invasive carcinoma sequence, and possibly in the progression of high-grade CIN to invasion.

Another molecular system that promotes proliferation and inhibits apoptosis involves the insulin-like growth factor (IGF), and particularly IGF BP-3. Cervical-cancer cells overexpress IGF-1 receptor, and such overexpression is associated with the potential for neoplastic transformation, possibly because of a loss of p53 function. Interestingly, the prerequisite for the oncogenic function of SV-40 (a virus from the same family as HPV) is overexpression of IGF receptors.

Tumour-suppressor genes have been localised in chromosomal regions 3p, 4p, 4q and 11q. In one study of 42 cervical-cancer precursors of varying grades, allelic losses

Figure 5. Pathogenetic cascade of HPV-related cervical cancer. Persistent infection by high-oncogenic-risk HPV types, preferentially by the non-European molecular variants of HPV 16 and 18, results in viral integration into host-cell chromosomes. This results in break of HPV/E2 and loss of control of E6/E7 oncoproteins. These form complexes with p53 and RB protein, respectively, resulting in their inactivation. E6 may preferentially target and degrade Arg/Arg type versus Arg/Prol type p53. Loss of cell-growth control leads to immortalisation with impaired DNA-repair gene function, including inhibition of apoptosis. Immortalised cells show chromosomal deletions, loss of heterozygosity, proto-oncogene activation, and aneuploidy. In the susceptible individual with impaired cell-mediated immunity (HLA defect), the precancer CIN may progress to invasive carcinoma.

as oncogenes. This occurs by transbinding between E6 and p53 and E7 and RB protein. Only the E7 gene can immortalise primary human squamous-epithelial cells (keratinocytes), whereas E6 enhances the effectiveness of immortalisation by E7. The morphological features of HPV-16-immortalised keratinocytes are similar to those observed in routine biopsy samples of cervical-cancer precursors. The transcription of these viral oncogenes is enhanced by the long control region. The enhancer is cell-specific, activating viral transcription only in the natural target cells of the virus, i.e. squamous-epithelial cells.

Under normal conditions, the tumour suppressor genes p53 and RB are produced whenever cells sustain DNA damage or when oncogenes force the cells into a replicative state. If the damage is minor, an increase in p53 concentrations leads to arrest of mitosis and repair of damaged DNA. If the damage is major, the cell is subjected to nuclear death or apoptosis. These ‘molecular police’ are critical for normal growth control, because they prevent the formation of abnormal cells and their entry into the cell cycle. Binding of RB by HPV/E7 results in activation of protein 16 and cyclin E, both of which push the infected cell entering from G1 to the DNA S phase of the cell cycle. In experiments in vitro, where E6 binds to p53, the latter is degraded through a selective ubiquitin-dependent proteolytic pathway. This degradation results in reduced apoptosis and activation of endogenous c-myc and myc c-Ha-ras proto-oncogenes. These events, in turn, lead to uncontrolled cell proliferation and the eventual development of cervical neoplasia. Indeed, such cell populations are likely to be subjected to mutational events such as chromosomal rearrangement, microsatellite instability, and proto-oncogene activation, all presumed requisites for cervical carcinogenesis (Figure 5).

Chromosomal alterations, reviewed by Park and colleagues, have been observed in 95% of cervical cancers; chromosome 1 is most commonly involved, with deletions and translocations in the 1p11-p13 and 1q21-q32 regions. No chromosomal break points specific for cervical cancer have so far been identified, and chromosomal alterations are not confined to chromosome 1 in cytogenetic studies of cancers. Allelotyping showed allelic loss in chromosome 5 (5p) in 56% of invasive cancers and 21% of precursor lesions; in one study, microsatellite instability at the locus D5S406 was observed in 67% of 12 cancers and precursors. Alterations of chromosome 11q are associated with impaired tumour-suppressor-gene functions and deletions, as well as mutations of the proto-oncogenes c-Ha-ras in cervical cancers, and some researchers have observed a relation with the risk of early recurrence of tumour.

Telomerase is essential for elongation of chromosomes and cell proliferation. Promotion of proliferation may occur by inhibition of apoptosis by telomerase. The relation between telomerase activity and HPV infections has been investigated in cervical epithelium in both health and disease. In most tracing studies, telomerase activity was absent or weak in normal epithelium and low-grade CIN 1 (0–30%), was much higher in high-grade CIN 3 (60%), and was found in over 90% of invasive squamous-cell carcinomas. In addition, telomerase activity was detected mainly in the lower suprabasal layers of normal epithelium, whereas intra-epithelial lesions and invasive carcinoma displayed full-thickness involvement. Furthermore, telomerase expression was related to lesions that contained HPV types associated with high oncogenic risk. Telomerase activity may be involved in the CIN-invasive carcinoma sequence, and possibly in the progression of high-grade CIN to invasion.

Another molecular system that promotes proliferation and inhibits apoptosis involves the insulin-like growth factor (IGF), and particularly IGF BP-3. Cervical-cancer cells overexpress IGF-1 receptor, and such overexpression is associated with the potential for neoplastic transformation, possibly because of a loss of p53 function. Interestingly, the prerequisite for the oncogenic function of SV-40 (a virus from the same family as HPV) is overexpression of IGF receptors.

Tumour-suppressor genes have been localised in chromosomal regions 3p, 4p, 4q and 11q. In one study of 42 cervical-cancer precursors of varying grades, allelic losses
were assessed for loss of heterozygosity in these chromosomal regions.\textsuperscript{13} Functionally, inactivated tumour-suppressor-gene loci were found in 88\% of grade 3 CIN and in none of the grade 1 CIN. These findings corroborate the many clinical observations that CIN 3 is the true precursor of cervical carcinoma and is characterised by viral integration, nuclear aneuploidy, and genomic instability, all mediated through inactivation of p53 gene function. The results of one study suggested that the p53 variant with an arginine instead of a proline residue on codon 72 is more susceptible to HPV-E6-mediated degradation.\textsuperscript{29} The importance of this finding was underlined by the observation that the p53 Arg/Arg genotype was associated with increased cervical-cancer risk in some studies.\textsuperscript{30,31} However, many more studies have failed to identify an association between codon 72 p53 polymorphism and cervical-cancer risk.\textsuperscript{31} Although some of the disparate results could be partly explained by methodological differences between studies, viral factors may also contribute to making this association difficult to identify by epidemiological studies. For instance, results from another study on this issue found no association between the Arg/Arg genotype and risk of cervical cancer.\textsuperscript{32} However, among women who had the Arg/Arg genotype, the risk of cervical neoplasia was substantially increased after exposure to a specific molecular variant of HPV 16 (in codon 350 of E6 viral gene). This finding adds further to the controversy, by suggesting that viral factors may interact with genetic susceptibility to HPV-induced cervical carcinogenesis.

Recent research has focused on the nucleic acid sequence variation within HPV 16 long control region, E6 and L1 regions. Two main groups of HPV 16 variants have been identified, on the basis of their geographical distribution, namely the prototype European (to which the HPV 16 prototype belongs) and non-European (eg African, Asian, Asian-American) variants.\textsuperscript{34,35} Several researchers have found HPV 16 non-European variants to be associated with cervical and anal lesions more often than the European variants. Also, infections caused by the European HPV 16 variants had a lower risk of progression to CIN than those caused by their non-European counterparts. Furthermore, infections with non-European branch variants of HPV 16 and 18 had a greater tendency to persist (in a latent form) and to be associated with high-grade precursor lesions, both cross-sectionally and prospectively, than the European branch variants. The putative non-European branch was found more frequently than the European branch variants in Brazilian black and mixed-race women. These findings may partly explain the fairly high cervical-cancer rates in South America and other regions of the world, such as Africa and India.\textsuperscript{36} Sequence variation in the E6/E7 oncogenes and in the long control region, which controls their transcription, has functional importance. Indeed, there may be differences in transcription in the oncogenic potential of different variants of the E6/E7 oncogenes and, by inference, differences in interactions with p53 and RB. In this respect, the long control region in the non-European HPV 16 variants has greater transcriptional activity than reference European isolates. Overall, these findings may explain why only a fraction of women infected by HPV 16 (by far the most common HPV type in women) develop cervical neoplasia. Intratype variations of HPV 16 may also be related to persistent infections, and may be a prerequisite for progression to precursor and invasive states. They also offer an alternative or partial explanation for the classic cervical-carcinoma cofactors, such as smoking, sexually transmitted diseases, parity, and oral-contraceptive use. HPV intratype genomic variability may have clinical and epidemiological implications for developing diagnostic tests and prophylactic vaccines specific for HPV molecular variants with high persistence or potential progression to cancer. Such efforts must be combined with research on the roles of both HLA and p53 polymorphism.\textsuperscript{37} In addition to the substantial advances in our understanding of the molecular pathogenesis of cervical cancer, it may be equally important to develop testing methods for high-oncogenic-risk HPV, to improve diagnosis and screening of women at risk of persistent HPV infection and its consequences, ie cervical cancer and its precursors.

Conclusions

On the basis of the epidemiological and molecular evidence implicating high-risk HPV types (acquired through sexual contact) in the development of cervical carcinoma and its precursors, the International Agency for Research on Cancer, part of the World Health Organization, has classified HPV 16 and 18 as carcinogens in human beings. The data accumulated so far underline the need for HPV-based diagnostic and screening tests, and the eventual development of prophylactic HPV vaccines.

Research on HPVs has progressed rapidly and now generates nearly 1000 publications in MEDLINE each year. The HPV–cervical cancer model has become an example of progress in cancer research and among neoplastic diseases based on infections. After 20 years, we have reached the point at which prevention of cervical cancer by vaccination against HPV infection will be possible in the foreseeable future. It would be disastrous, however, if countries relaxed their screening programmes for cervical cancer in anticipation of a successful HPV vaccine. Existing cytology-based screening programmes that seem to work need to be constantly assessed for quality and coverage. Continuing research on the effectiveness and cost-efficiency of HPV testing as a mass screening tool will help countries to decide on the best approach for secondary prevention of cervical cancer and will probably lead to reduced morbidity and mortality from this disease.
References