Carcinomas of the anogenital tract — particularly cancer of the cervix — account for almost 12% of all cancers in women, and so represent the second most frequent gynaecological malignancy in the world. Cervical cancer is caused by specific human papillomavirus (HPV) infections (reviewed in Ref. 2). Even ~25% of oral cancers contain anogenital HPV DNA. These HPV types have therefore emerged as one of the most important identified risk factors for widespread human cancers.

Here, cervical cancer will be used as a 'test case' to reveal some of the properties of specific HPV types in human carcinogenesis, and to show the development of clinical approaches that interfere with these infections. Between 1974 and 1976, researchers started to postulate and analyse a possible role of HPV in cervical cancer. In 1976, Meisels and Fortin published two reports outlining that the appearance of koilocytes in cervical smears indicates the presence of a papillomavirus infection. They also suggested that it might be possible to differentiate between 'benign, warty' lesions that do not progress to cervical cancer and — as they suspected initially — 'non-viral' precursor lesions that do progress to cervical cancer. This idea was supported by the identification of typical papillomavirus particles in mild dysplastic lesions of the cervix.

The demonstration of heterogeneity within the papillomavirus family, and the subsequent isolation of specific types from genital warts and laryngeal papillomas, as well as the application of nucleic-acid hybridization procedures at reduced stringency to screen for related but not identical HPV types, allowed a fresh look into the possible involvement of papillomavirus infections in anogenital cancers. The first HPV types were isolated directly from cancer biopsies of the cervix — HPV16 and HPV18 were cloned in 1983 and 1984, respectively — and this initiated a rapid expansion of the field. Within four years, the basic experimental details that explained the role of HPVs in cervical cancer aetiology had been described: the expression of specific viral genes (such as E6 and E7) was shown in cervical cancer cell lines and cancer biopsies; a specific opening within the viral ring molecule was shown for integrated genome copies; and the immortalization property of viral DNA and the encoded viral oncogenes supported the initial suspicions. An early epidemiological study pointed to a high rate of infection in younger women and to a decreasing rate of infection with age, although persisting infections still represent a high risk factor for cervical cancer development in older women. Shortly after, the requirement for viral oncogene expression...
Summary

- High-risk human papillomavirus (HPV) genotypes cause cervical cancer. The same types also seem to be responsible for other anogenital, and a subset of head and neck, cancers.
- Viral oncogene transcription and oncoprotein expression are controlled by cellular signalling cascades.
- Expression of specific viral oncoproteins, E6 and E7, is required for maintaining the malignant growth of cervical cancer cells, specifically by inhibiting the tumour suppressors p53 and RB.
- The detection of viral DNA and cellular proteins that are induced by high-risk HPV allows new approaches to cervical cancer screening to be taken.
- Preventive and therapeutic vaccines against HPV infections are, at present, in clinical trials. The prospects for efficient prevention are excellent.
- Global application of such vaccines could contribute significantly to reducing the human cancer burden.

Figure 1 | The human papillomavirus life cycle. Infection requires the availability of a basal layer cell. This usually occurs in microlesions of skin or mucosa. The infected cell divides and the population spreads laterally. Some of the progeny migrate into the suprabasal differentiating cell layers, where viral genes are activated, viral DNA is replicated and capsid proteins are formed. Viral particle formation ensues. Particles are released at the surface and might then infect additional tissues.

Figure 2 | The organization of circular HPV DNA and its integration into host-cell DNA. The human papillomavirus (HPV) genome contains between 6800 and 8000 base pairs and is divided into eight open reading frames — E6, E7, E1, E2, E4, E5, and L1 — coding for 'early' (E) or 'late' (L) functions. In the course of cancer development, the viral molecule frequently becomes integrated into host-cell DNA. The ring molecule is most often opened within the E2 open reading frame, disrupting the continuity of that gene. Part of E2 and open reading frames that are adjacent to E2 — E4, E5 and part of L2 — are deleted after integration (partial genes are represented by an asterisk). Viral transcripts, which uniformly span the E6 and E7 region, and are often linked to flanking cellular sequences, are present and transcription might be modulated (enhanced) by flanking host-cell promoters. LCR, long control region.
Several functions have been described for E6 and E7 (FIG. 3). Initial observations revealed that E6 interacts with p53 (REF. 32), and E7 interacts with RB33 to block the activity of these tumour suppressors. Indeed, some of the prominent functions of the E6 protein originate from its interaction with, followed by degradation of, p53 and the pro-apoptotic protein BAK38, which results in resistance to apoptosis and an increase in chromosomal instability. In addition, the activation of telomerase35 and the postulated inhibition of degradation of SRC-family kinases could contribute to the HPV-transformed phenotype39. The cyclin-dependent kinase inhibitor INK4A (also known as p16) seems to counteract these functions.

E7, however, interacts with and degrades RB, which releases the transcription factor E2F from RB inhibition and upregulates INK4A33,37. The resulting high E2F activity might lead to apoptosis in E7-expressing cells. Moreover, E7 stimulates the S-phase genes cyclin A and cyclin E38, and seems to block the function of the cyclin-dependent kinase inhibitors WAF1 (also known as CIP1 and p21) and KIP1 (also known as p27)39-41. By inducing centriole amplification, E7 also induces aneuploidy of the E7-expressing cells, which contributes to tumorigenesis41.

E6 and E7 can independently immortalize human cells, but at reduced efficiency34,44; their joint function results in a marked increase in transforming activity. This seems to be due to an interesting complementary and synergistic effect. As mentioned previously, E6 seems to be impaired by INK4A, whereas E7 bypasses this inhibition by directly activating cyclins A and E. E6, in turn, prevents E7-induced apoptosis by degrading the apoptosis-inducing proteins p53 and BAK38,45.

At present, it is difficult to assign a role for other HPV early proteins (such as E1, E2 and E4) in the process of malignant conversion. The two structural proteins L1 and L2 are not expressed in precancerous and malignant cells, but they are important for vaccine development (see below).

High- and low-risk HPV infections

HPV types that are found preferentially in cervical and other anogenital cancers have been designated as ‘high-risk’ types42. Conversely, those found primarily in genital warts and non-malignant lesions were labelled as ‘low-risk’ types. Subsequently, it was shown that only the E6 and E7 genes of high-risk types were able to immortalize human cells in tissue culture22,47.

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High-risk HPV types (TABLE 1), particularly HPV 16, are widespread within all human populations. Infection is commonly transmitted by sexual contact and results...
Initially in inconspicuous squamous intraepithelial lesions (SIL) in women (reviewed in Ref. 48). Most of these lesions are cleared 6–12 months after appearance, probably due to immunological intervention. A small percentage, however, persists, progresses to high-grade SIL, carcinoma in situ and, without surgical interference, to squamous-cell carcinoma or adenocarcinoma of the cervix. The individual stages are outlined in FIG. 4.

**Host control of HPV infection**

The immune system is important in the control of HPV infections. This can be indirectly deduced from the increased incidence and prolonged persistence of SIL in immunosuppressed women49. There is also evidence for T-helper-cell involvement in regressing lesions, and a concomitant detectable humoral and cellular immune response against HPV antigens during the course of regression16,51. The escape of high-grade SIL and carcinoma in situ from immunological control seems to be based on different modifications of the cellular antigen-presenting system, which might involve the proteasome transportation system, the HLA receptors and the cellular recognition system for presented oligopeptides52–54. The escape from immune-surveillance mechanisms emerges as one important step in the progression of HPV-linked tumours (FIG. 4).

In addition, two other modes of control exist in proliferating cells that are infected by HPV to protect them against malignant transformation: one involves inhibition of viral oncoprotein function, the other involves transcriptional control (FIG. 4). The evidence that host cells can impair the function of viral oncoproteins is still indirect and, so far, exists only for the E6 oncoprotein in cells that have been immortalized by E6 alone56. In cell lines that have been transfected with E6 DNA, the CDKN2A gene — which encodes INK4A — is commonly inactivated by methylation, mutation or deletion. INK4A inactivates cyclin D1–CDK4 or cyclin D1–CDK6 complexes, which prevents expression of cyclin E and, therefore, progression through the cell cycle. The consistent interruption of CDKN2A gene function in E6-immortalized cells indicates that INK4A can functionally interfere with the transforming activity of E6 (Ref. 55). As outlined in FIG. 3, expression of E7 can overcome this block, as it directly stimulates cyclins A and E through its interaction with RB38. In cells that are immortalized by both E6 and E7, the CDKN2A gene remains intact. It is still highly overexpressed, but seems to be functionally sequestered from its interference with the cell cycle58. No firm evidence exists at present for a negative interference of other cyclin-dependent kinase inhibitors, such as WAF1 and KIP1, with the E7 protein. This could be relevant when E7 is expressed at low levels in the early stages of infection.

Another pathway — which involves blocking the transcription of HPV DNA and is known as the cellular interference factor (CIF) concept — has been analysed in more detail. It is triggered by paracrine stimulation of cervical epithelial cells by macrophages and tumour necrosis factor-α (TNF-α), and causes several effects in HPV-immortalized cells. These include a modification of the transcription factor AP1, which is essential for HPV gene expression, and the induction of endogenous synthesis of the antiviral interferon-β57. It is suspected that the change in AP1 composition mediates the suppression of high-risk HPV transcription58. These pathways do not function in cervical carcinoma cells, indicating that the TNF-α-mediated signalling cascade is interrupted during malignant transformation59.

**Factors that affect HPV malignancy**

High-risk HPV infections result in progression to cervical cancer in only a small percentage of infected women, after a long latency period (reviewed in Ref. 2). A high percentage of infected women clear the infection by immunological mechanisms. Lasting immunosuppression represents a risk factor for viral DNA persistence and lesion progression50, but the factors that determine
Viral and non-viral risk factors that influence the progression of infected cells are listed on the left side. Hormonal factors (oestrogen and its derivatives) activate the human papillomavirus (HPV) promoter and facilitate immortalization of HPV-infected cells. Mutagenic agents amplify persisting HPV DNA. They enhance progression by modifying cellular signalling cascades that control HPV persistence, or lead to increased viral oncogene expression by a gene-dosage effect. A rising level of E6 and E7 oncogene expression, in turn, results in increasing genomic instability, which further facilitates progression of the infected cells towards invasive growth.

Figure 5 | HPV and non-HPV factors that contribute to HPV-induced malignant progression. Viral and non-viral risk factors that influence the progression of infected cells are listed on the left side. Hormonal factors (oestrogen and its derivatives) activate the human papillomavirus (HPV) promoter and facilitate immortalization of HPV-infected cells. Mutagenic agents amplify persisting HPV DNA. They enhance progression by modifying cellular signalling cascades that control HPV persistence, or lead to increased viral oncogene expression by a gene-dosage effect. A rising level of E6 and E7 oncogene expression, in turn, results in increasing genomic instability, which further facilitates progression of the infected cells towards invasive growth.

Cancer screening
Cervical cancer screening is commonly based on cytological and colposcopic analyses. Screening performed by experienced cytologists and gynaecologists has resulted in a dramatic reduction of cervical cancer incidence. In countries with less experienced cytologists, cytology nurses or gynaecologists; however, these tests run a high risk for false-negative or false-positive results. Simple, reliable and cheap tests for effective large-scale screening programmes are therefore required — particularly in tropical countries that do not have effective screening programmes, and which therefore have the highest rates of cervical cancer.

The identification of specific papillomavirus types as causative agents for cancer of the cervix and its precursor lesions allowed the development of a new method for cancer screening and early diagnosis. HPV genomes and viral oncoproteins, which are present in all affected cells, should represent convenient markers for a transient or persistent infection. Similarly, cellular proteins that are induced in response to these infections could have an analogous role. The presence of high-risk HPV markers per se should not, in itself, be cause for alarm, but rather should lead to the instigation of a careful clinical investigation and repeated testing for HPV persistence, which is a significant risk factor for the development of proliferative lesions and their progression.

The detection of high-risk HPV DNA by hybridization procedures or by polymerase chain reaction (PCR) technology has been developed to some maturity, and enables a risk assessment to be made for low-grade squamous intraepithelial lesions (LSIL). This strategy — for which molecular probes are already available as kits — has also been recommended to replace or be combined with conventional cytological screening procedures. If competently performed, these techniques clearly improve the detection rate of high-risk HPV DNA in women, and also avoid some false-negative diagnoses; however, they are relatively expensive and therefore prohibitive for a large part of the world where cervical cancer is still a major cause of death.

Another consequence of HPV infection might also be capitalized on to improve screening procedures. Papillomavirus infection induces cell proliferation within the differentiating layers of the epithelium that are otherwise devoid of replicating cells. Theoretically, the abnormal expression, within these cells, of a set of proteins that are involved in progression through the cell cycle might serve as a marker for HPV infections. In particular, INK4A emerges as a particularly powerful candidate for such a marker. In high-risk HPV infection, E7 binds to and degrades RB (seen above and Fig. 3), which results in substantial upregulation of INK4A synthesis — readily detectable in high-risk HPV-infected cells and tissues. In spite of the high level of INK4A synthesis, this protein remains functionally inactive, as E7 induces cyclin A and cyclin E, thereby functionally bypassing its interference with the cell cycle. Antibodies that are directed against INK4A allow selective staining of high-risk HPV-infected histological sections or of cytological smears. It is anticipated that this procedure, if affordable, could be used in cervical screening and might significantly reduce the number of false-negative diagnoses.
Tests for HPV DNA originate as a direct consequence of HPV identification; the presence of INK4A allows, by contrast, an indirect approach for detecting high-risk HPV.

Cancer prevention

Hygiene. The most visible benefit from HPV research is prevention of cervical cancer. A very early benefit originated from the recognition by clinicians that cervical, vaginal and perianal squamous intraepithelial lesions are of infectious origin and produce large quantities of infectious virus (reviewed in REF. 2). Specific standards of hygiene were therefore enforced in order to prevent iatrogenic transmissions among gynaecological patients.

Conversely, the mechanical prevention of anogenital HPV infections that are transmitted by sexual contact is impractical. HPV infections are highly prevalent within the sexually active population and the use of condoms offers only limited protection in view of the common presence of virus-infected cells at external genital sites.

Vaccines. One of the main benefits of HIV research, however, would be vaccination against high-risk HPV types. It is widely accepted that in most cases, HPV infection is cleared by immunological intervention. Spontaneous regression of LSILs, or even HSILs, is commonly accompanied by humoral and cellular immune responses against virus-specific antigens59–61. Although the surface localization of most viral antigens does not sufficiently stimulate the immune system, vaccination with viral structural proteins in animal systems and in humans regularly leads to the induction of an effective immune response66–69.

Initial experiments performed with purified papillomavirus structural proteins — that spontaneously assemble into 'virus-like particles' (VLPs) — of animal papillomaviruses (such as canine oral papillomavirus and cottontail rabbit papillomavirus) resulted in effective protection against the primary infection of dogs and rabbits, respectively66,67. These results provided the background for attempts to develop vaccines against human high-risk HPV types.

Several companies, but also academic research laboratories, are engaged, at present, in preclinical and clinical trials of vaccines against high-risk HPV. Most approaches are based on the use of VLPs that are derived from the structural proteins L1, or L1 and L2. Frazer and colleagues initially used L1 and L2 VLPs68 and applied them to HPV-infected patients and patients with genital warts69. The conclusion of a Phase I safety and immunogenicity trial in adult volunteers of an HPV16L1 VLP vaccine was recently reported70. This vaccine was highly immunogenic and well tolerated, even without adjuvant, as was previously shown for an HPV6b VLP vaccine68. The antibody titres seem to be, on average, at least 10 times higher than after clearing of a natural infection and persist for prolonged periods. Although the available data do not yet allow firm conclusions to be made about protective effects, animal papillomavirus vaccination studies do point in this direction. So, the prospects for this vaccination are remarkably promising. On the basis of previously mentioned studies in dogs and rabbits, an effective prevention can also be expected for the human vaccine. If this turns out to be true and if the vaccine was globally applied, prevention of infection by the most prevalent high-risk HPV types could theoretically prevent more than 300,000 cervical cancer cases per year on a global scale.

It is anticipated that polyvalent vaccines will eventually reach the market. Vaccines that cover the four or five most prevalent high-risk HPV types should be able to prevent 80–90% of cervical cancer incidence. In view of some geographical differences in the prevalence of specific high-risk HPV types, polyvalent vaccines might need to be adapted slightly for use in different countries.

Attempts are, at present, being made to develop vaccines that do not require injections. Particularly in parts of the developing world, the repeated use of syringes without appropriate intervening sterilization bears the risk of transmitting other agents, such as the hepatitis B and C viruses, and human immunodeficiency virus (HIV). Some studies are testing the inhalation of VLPs or L1 genes in viral vectors71–74. These vectored constructs seem to be particularly suited to intranasal inhalation, and are capable of inducing an immune response at cervical sites75.

The discovery of infectious agents as causative factors for specific human cancers has already been shown to have important consequences for cancer prevention. The Taiwan vaccination programme of newborn children against hepatitis B virus (HBV) infections, introduced in 1986, not only drastically reduced the percentage of persistently HBV-infected children, but also resulted in the first measurable decrease in liver cancer incidence76. Provided that the vaccines against human high-risk HPV prove to be as effective as in animal experiments, it can be estimated that a global application of HPV vaccines could theoretically reduce the cancer risk in women by approximately 10–15%46.
**Cancer therapy**

**Immunotherapy.** Concepts similar to those developed for immunoprevention of HPV infections have also been adapted for immunotherapy. The development of chimeric vaccines seems to be an appropriate step in this direction. For example, antigenic epitopes from the E7 oncoprotein have been added to the L1 protein to generate deformed VLPs. In animal experiments, these VLPs exert both a preventive and a therapeutic effect, as an immune response is induced against the viral E7 oncoprotein; a Phase I trial was initiated in 2001 (L. Gissmann, personal communication). This type of combined immunopreventive and immunotherapeutic vaccine might be particularly useful in the treatment of early lesions (cervical intraepithelial neoplasia), but it is more questionable whether it will still be effective in invasive HPV-caused cancers that have a large tumour mass. A Phase I clinical trial that vaccinated cervical cancer patients with E6 and E7 proteins — with the aim of generating an immune response against these viral oncoproteins — has also been reported. Unfortunately, for the women vaccinated in this study — who had late-stage cervical cancer — the vaccine did not seem to be beneficial. Oligopeptide vaccines derived from the E7 oncoprotein might, however, show some clinical activity.

It remains to be seen whether vaccinations will be of benefit to those who have developed cervical cancer, as the cellular adaptations that take place during tumorigenesis regularly seem to result in the loss of appropriate viral antigen presentation. Immunotherapy might have a reasonable chance of success in patients with precursor lesions, but its effectiveness in invasive cancer is less likely.

**Immunomodulatory therapy.** Several cytokines have been shown to repress papillomavirus transcription, and this repression involves transforming growth factor-β (TGF-β) and interleukin-1 (IL-1). The repression by TNF-α is lost during malignant conversion. None of the cytokines have been used in clinical trials so far. The activation of retinoic-acid receptors by a tissue hormone, retinoic acid, can also suppress HPV gene activity, and has been shown to reveal some clinical effects in premalignant and malignant cervical lesions. Unfortunately, unpleasant side effects accompany its use.

An ACYLIC NUCLEOSIDE PHOSPHONATE, cidofovir, which has broad-spectrum activity against DNA viruses, also shows some activity against papillomaviruses, by inducing apoptosis, if locally applied in infected cells. In addition, the IMMUNOMODULATORY DRUG imiquimod has proved to be effective in HPV-infected cells (reviewed in Ref. 89) and seems to act by the stimulation of cytokines.

**Molecular and chemotherapy.** Several interesting in vitro studies might have an impact on future developments in chemotherapeutic approaches to HPV infections and HPV-linked human cancers. Because the malignant growth of cervical cancer cells depends on the presence and expression of the E6/E7 oncogenes, antisense constructs were generated against both the E6 and E7 message. This was shown to inhibit the tumorigenicity of cervical cancer cells in nude mice.

Another report revealed that transcription of high-risk HPV could be selectively inhibited by the antioxidant pyrrolidine-dithiocarbamate (PDT) in HPV-immortalized, but not in malignant, cells. 2-Deoxyglucose also suppresses HPV transcription — in both immortalized and malignant cells. More recently, HPV 16 E6-binding peptide aptamers have been shown to eliminate HPV 16-positive cancer cells by inducing apoptosis. In addition, histone deacetylase inhibitors, such as sodium butyrate and trichostatin A, arrest HPV-positive cells and induce apoptosis without affecting the expression of HPV oncoproteins. None of these studies has yet been clinically applied. It remains to be seen whether some of them turn out to be useful for treating patients.

**Future directions**

The present developments in preventive HPV vaccination look particularly promising. The next few years will reveal the protective and therapeutic potential of those preparations that are presently being tested in clinical studies. Although all available results seem to support the idea that interference with infections by a number of HPV types will be achieved, some important questions remain unanswered. Which vaccine formula will provide optimal results? Do we need different combinations of HPV types for different regions of the world? Will it be possible to develop a cost-effective vaccine that is suitable for global application? How do we reach people in those tropical and subtropical regions where cervical cancer is most prevalent? Appropriate answers to these questions and the respective actions have the potential to save the lives of more than 200,000 women per year.

**Conclusions**

Research in high-risk HPVs started actively in the second half of the 1970s. The concept of HPV heterogeneity and its proof, as well as the demonstration of specific HPV types in cervical and other anogenital cancers, substantially boosted this research. After partial elucidation of the viral functions that contribute to malignant conversion, clinical application of these results started to emerge. An important practical impact originates from the development of prophylactic and therapeutic vaccines. Theoretically, cervical cancer, which contributes to approximately 12% of the global cancer burden in women, should be preventable. In addition, however, new screening procedures that are based on the molecular understanding of high-risk HPV–host-cell interactions and targeted therapeutic approaches are emerging. These will, in all likelihood, contribute in the future to the control of this, as well as of other, HPV-linked human cancers. HPV research exemplifies the value of basic research for cancer prevention and shows the importance of infectious events in human carcinogenesis.

First experiments to search for papillomavirus DNA in cervical cancer.


Provides a new approach for the screening of high-risk HPV infections.


First evidence for a genetic component in cervical cancer development.


First report on preventive large-scale papillomavirus vaccination in dogs.


First report on preventive large-scale papillomavirus vaccination in dogs.


In a similar way to that described in reference 66, preventive vaccine against CRPV type L2 and L12 CRPV is sufficient for the development of cervical lesions in non-immunized experimental animals. Virology 185, 251–257 (1991).
Expression of HPV structural proteins leads to spontaneous virus-like particle formation.


The first report on immunogenicity of VLP vaccines in humans.


The first report of an effective trend for a human tumour virus vaccine in preventing cancer.


Online links

DATABASES
The following terms in this article are linked online to:
Cervical cancer | Her2 cancer | oral cancer
BAK | CDK4 | CDK6 | colony-stimulating factor-1 receptor | cyclin A | cyclin D1 | cyclin E | E2F | epidermal growth factor receptor | INK4A | interferon-β | interleukin-1 | KIP1 | p53 | p21 | p27 | RB | SRC | telomerase | TGF-β | TNP-1 | WAF1
Cidofovir | Imiquimod

FURTHER INFORMATION
Swedish Cancer Registry: http://www.sos.se/epc/cancereng.htm
Access to this interactive links box is free online.