REVIEW

Human Papilloma Virus (HPV) and cervical cancer

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Abstract : Epidemiological and experimental studies have clearly shown that high-risk HPV infection is the main etiologic factor for cervical cancer. Recent studies have indicated that the E6 and E7 gene products play a critical role in cervical carcinogenesis. The E6 and E7 products interfere with the p53 and pRB functions, respectively, and deregulate the cell cycle. The HPV DNA is integrated into the host's chromosomes with disruption of the E2 gene. This disruption promotes the expression of E6 and E7, leading to the accumulation of DNA damage and the development of cervical cancer.

The study of the immune response against HPV has been hampered by the lack of a cell culture system for the virus. A breakthrough was made by the discovery that a major capsid protein L1 self-assembles into virus-like particles (VLP) when expressed in eukaryotic systems. Clinical trials of VLP-based vaccines are in progress, and DNA vaccines for the HPV surface protein genes are under development.

The E7 and E6 oncoproteins are attractive targets for cancer immunotherapy because their expression is required to maintain the oncogenicity of cervical cancer cells. Cancer immunotherapy for cervical cancer with vaccinations of E7 peptides or dendritic cell-based immunotherapy is moving toward clinical trials. J. Med. Invest. 49 : 124-133, 2002

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Papilloma viruses (PVs) are small DNA viruses that are widespread in nature and infect a wide variety of species. PVs induce warts on the skin and internal squamous mucosae. The first papilloma virus was isolated from cottontail rabbits, and Rous PJB reported the progression to carcinoma of virus-induced rabbit papillomas (1). This was the first report of the oncogenic potential of PVs.

In humans, the association of coital and venereal factors with cervical cancer has been suggested over the past two decades. The incidence and mortality of cervical cancer is high among women who have more coital partners, or whose sexual activity begins at a younger age (2, 3). On the other hand, the rarity of cervical cancer among nuns has been confirmed by many investigators (4, 5). These facts implicate a venereally transmitted agent in human cervical cancer. In 1970s, Herpes Simplex Virus type 2 (HSV-2) was considered as a potential etiological factor (6). However, HSV DNA was not detected in any cervical cancer (7), and a prospective epidemiologic study failed to demonstrate any evidence to support the involvement of HSV-2 infection in cervical carcinogenesis (8).

The asscociation of HPV infection with cervical cancer was first recognized by Meisels A *et al*. (9). They reported that morphological abnormalities (koilocytosis), known as the cytopathic effect of HPV, were often accompanied with cervical cancer. Further support came from molecular virology which demonstrated that HPV DNA was present in approximately 70%-80% of cervical carcinomas (10). A recent study using PCR demonstrated that 93% of cervical cancers contained HPV DNA (11). How-

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ever, there is an argument that the high incidence of HPV in cervical cancer may result from the high susceptibility of tumor cells to viral infection.

This question can be addressed through cohort studies and experimental evidence. Prospective epidemiologic studies have shown that, in cytologically normal women, HPV infection precedes the development of precancerous lesions of the cervix. For example, in a study of 241 cytologically normal women recruited in a sexually transmitted disease clinic, the cumulative incidence of high-grade squamous intraepithelial lesions at two years was 28% in HPV-positive women compared with 3% in HPV-negative women (12-14).

Human keratinocytes may be immortalized by the transfection of HPV DNA and many studies have indicated that the E6 and E7 genes of HPV are transforming genes. Moreover, the E6 and E7 genes in high risk HPV can immortalize primary human keratinocytes, whereas these same genes in low risk HPV are incapable of immortalizing primary human keratinocytes (15-18). From this epidemiological and experimental evidence, it is now clear that high risk HPV infection is a major risk factor for the subsequent development of cervical cancer, although high risk HPV infection alone is not sufficient.

STRUCTURE AND CLASSIFICATION OF HPV

PVs are members of the papovavirus family, double-stranded DNA viruses that replicate in the nucleus. The PV virion is 55nm in diameter and has an icosahedral capsid made up of 72 capsomers. The outer protein coat consists of two different proteins, a major and a minor capsid protein. The 7900 base pair genome of HPV is divided into three groups: (1) early, (2) late, and (3) control (Fig. 1). The functions of the early and late genes are shown in Table 1 (19).

In addition, there is a noncoding region refered as the Long Control Region (LCR) which regulates the expression of the ORFs.

PVs are classified by genotype. A HPV with less than 90% sequence homology in E6, E7 and L1 ORFs to any of the known HPV types is classified as a new type. There are over 90 genotypes at present and new types are discovered every few months.

Lorincz AT *et al*. compared the strengths of association between specific HPV types and different disease severities, and classified the HPV types into four clinical categories, "low risk" (HPV 6, 11, 42, 43, and 44), present in 20.2% of low-grade lesions but absent in all cancers, "intermediate risk" (HPV 31, 33, 35, 51, 52, and 58), detected in 23.8% of high-grade squamous intraepithelial lesions but only 10.5% of cancers, "high risk" (HPV 16) associated with 47.1% of both high-grade intraepithelial lesions and cancers, and "high risk" (HPV 18, 45, 56), found in 26.8% of invasive carcinomas but only 6.5% of high-grade intraepithelial lesions (20). HPVs are now classified into three categories according to their carcinogenic potential, low risk, intermediate risk, and high risk (Table 2).

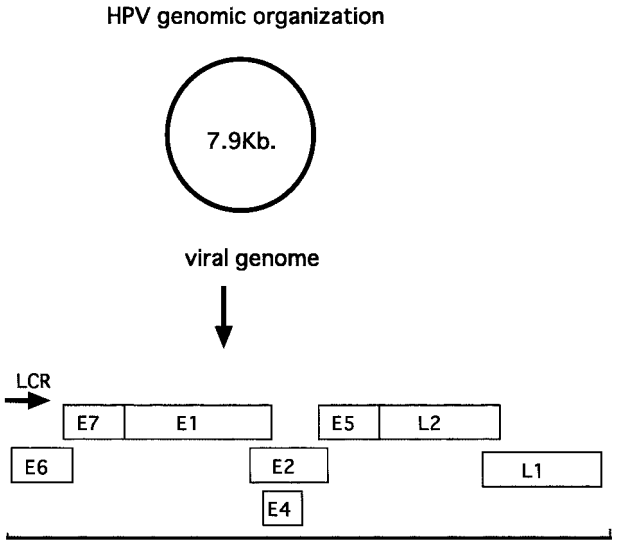

Fig. 1. Linear representation of the circular HPV genome. The HPV genome is a closed circular double-strand DNA containing 7.9Kb. Transcription begins at the promotor in the long control region (LCR). The early region genes are designated as E1-E6 and the late region genes as L1 and L2.

Table 1. Functions of HPV genes.

ORF	functions
E1	Viral DNA replication
E2	Regulation of viral transcription
E4	Unknown
E5	Transformation
E6	Transformation, p53 degradation
E7	Transformation, RB binding
L1	Major capsid protein
L2	Minor capsid protein

The genome is divided into late (L) and early (E) regions. The L region codes two capsid proteins, the major L1 and the minor L2. L1 forms more than 95% of the capsid. The E1 and E2 regions are involved in the early stages of viral replication. The E4 region is involved in virus maturation. The E6 and E7 regions encode the viral oncoproteins.

Table 2. Classification of HPVs.

classification of HPVs	
low risk :	60 11, 42, 43, 44
intermediate risk :	31, 33, 35, 51, 52, 58
high risk :	16, 18, 45, 56

The HPV types differ in their transforming potential and are subdivided into risk groups. Low risk types are found in benign lesions like condyloma and are not found in cervical cancers. High risk types are detected mainly in carcinomas and rarely in benign and low grade lesions. Intermediate risk types are detected in both invasive cancers and benign lesions.

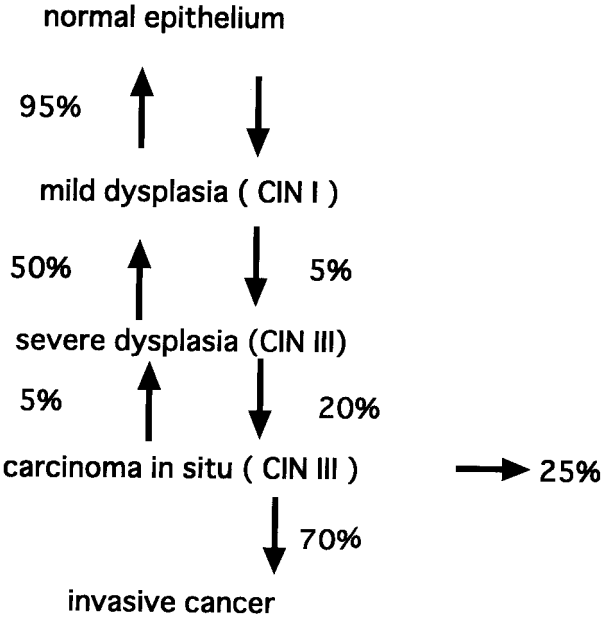

Fig. 2. The natural history of cervical intraepithelial lesions. Schematic illustration of the natural history of cervical intraepithelial lesions (CIN). Most mild dysplasia and 50% of severe dysplasia are estimated to regress spontaneously. However, the pathological criteria for dysplasia vary and the precise risk of CIN progressing to invasive cancer is less clear.

EPIDEMIOLOGY

Transient genital HPV infections are quite common in young sexually active women. Bauer HM *et al*. studied 467 healthy university students. Using PCR, they found that 46% of the study population was infected with HPV (21). In Germany, Villiers EM *et al*. determined the prevalence of HPV in 20,161 women to be 8.8% (22). In Japan, HPV prevalence is estimated to be around 10-15% (23). However, most infections are sub-clinical and resolve themselves (24). Most lesions of low-grade squamous intraepithelial lesions (LGSIL) are self-limiting and also resolve themselves. A minority of women develop persistent HPV infections, and some of these persistent high risk HPV infections progress to high-grade squamous intraepithelial lesions (HGSIL). Some HGSIL progress to invasive carcinoma (Fig. 2). Rozendaal L *et al*. studied a cohort of 1622 women who had normal Pap smears and no previous history of cervical dysplasia. The mean follow-up time was 40 months. Of the 86 high-risk HPV-positive women, 6 developed CIN III, whereas it developed in only 1 of the 1536 HPV-negative women. (25). Thus, the epidemiological studies clearly demonstrate that high risk HPV infection is a major risk factor for the development of cervical cancer, however, these studies also demonstrate that HPV infection alone is not sufficient and cervical cancer takes a long time to develop.

MECHANISMS

Recent experimental studies have indicated that the E6 and E7 gene products play a critical role in cervical carcinogenesis. The E6 of high-risk HPV interferes with the p53 function and deregulates the cell cycle. The E6 product binds to p53 to form a stable complex and this complex undergoes proteolysis (26). This process requires E6AP which, in combination with E6, acts as an E3 ubiquitin ligase (27). The E6 of high-risk HPV also down-regulates p53 acitivity by targetting the transcriptional coactivator CBP/p300, which has a role in the cell cycle and differentiation (28). Interactions between E6 and many molecules have been reported, such as src family kinase Blk (29), the mammalian homologue of the Drosophila disc large tumor suppressor protein (hDLG) (30), and paxillin (31). However, the roles of these protein-protein interactions with E6 is not yet clearly understood.

E7 protein shows similarities to the adenovirus E1A, and the SV40 large T-antigen, and the host cellular protein cyclin D1. The conserved regions of the amino acid sequence of these proteins form inactivating complexes with the retinoblastoma (pRB) antioncoprotein by competitive binding to the "retinoblastoma pocket". This binding releases a transcription factor, E2F. Free E2F accelerates DNA synthesis and cell-cycle progression (32-34) (Fig. 3).

HPV DNA exists in an extrachromosomal form in benign and premalignant lesions. On the other hand, HPV DNA is integrated into the host's chromosomes in cervical cancer (35, 36). This integration appears to be random because the virus genome

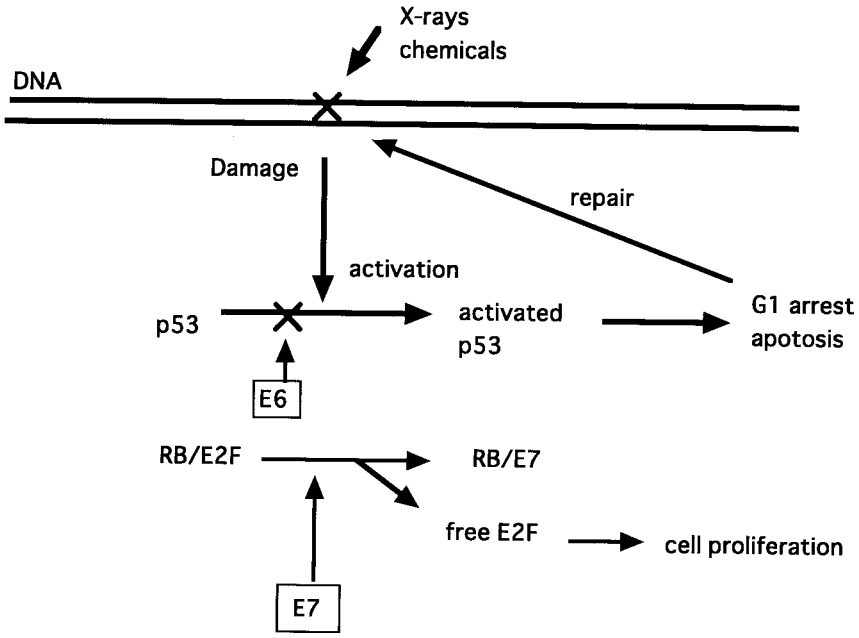

Fig. 3. Interactions between E7 and E6 oncoproteins and cell cycle regulators.
When DNA is damaged, the expression of p53 is elevated, leading to cell cycle arrest until the DNA damage is repaired. If the damage is severe, apoptotic genes are activated and cell death occurs. The E6 product of HPV interferes with this function. The cell carefully monitors cell cycle progress. The G1/S transition contains a major check-point regulated by pRB. The E7 product forms complexes with pRB, releasing free E2F, which allows the cell to pass through the check-point.

is integrated in different locations in different cancers. Integration occurs in the E1/E2 region, disrupting the E2 viral genome (37). In high-risk HPV, E2 represses the promoter from which the E6 and E7 genes are transcribed (38). Thus, after HPV DNA integration with disruption of the E2 gene, the expressions of the E6 and E7 genes are accelerated, leading to the accumulation of DNA damage and the development of cancer cells over an extended period of time (Fig. 4).

CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) AND HPV

Cervical intraepithelial neoplasia (CIN) is a good model for a mutlistage disease beginning with CIN I, progressing to CIN III (Fig. 5), and in some cases, developing invasive carcinoma. The risks of CIN progression are shown in Fig-2. Not all cases of CIN progress, and most CIN I regresses spontaneously. An effort has been made to identify the prognostic factors which govern the regression, persistence and progression. In 1986, Campion MJ *et al*. prospectively studied 100 women with CIN I, and 2 types of HPV, HPV16 and HPV 6 were. 22 of 39 HPV16-positive CIN I progressed to CIN III, whereas only 4 of 61 HPV16-negative CIN I progressed (39). The fact that persistent high-risk HPV infection is a major risk factor for CIN progression has been confirmed by many studies (40-44). The prevalence of HPV in CIN in our clinic is shown on Table 3. HPV18 is not common in Japan and the proportion of HPV16 increases with CIN progression. The prevalence of HPV is rather lower than reported because only 26 types of HPV were studied in our clinic. The prognosis of CIN in each HPV type is shown on Table 4. It should be noted that no CIN with HPV16 regresssed : On the contrary, 89% of CIN without HPV regressed. No clinical management of CIN has been established, for example, the management of CINII varies from "follow up" to "simple hysterectomy". However, these data suggest that CIN without HPV infection should be managed conservatively, whereas CIN with high-risk HPV infection may be treated with a shorter follow-up period.

HPV VACCINES

Genital HPV infection is common among young sexually active women, and in the majority of these women the virus resolves itself. The role of the immune system in viral clearance is unknown. The fact that HPV infection and HPV-related lesions are more common in immunosuppressed hosts such as those infected with HIV (45, 46) suggests that cell-mediated immunity plays an important role. The success of prototypic vaccines in animal models of PV infection suggests that prophylactic vaccines could be developed for clinical use (47, 48).

It has not been possible to produce a large amount of PV virions in culture cells. PV virions are possibly

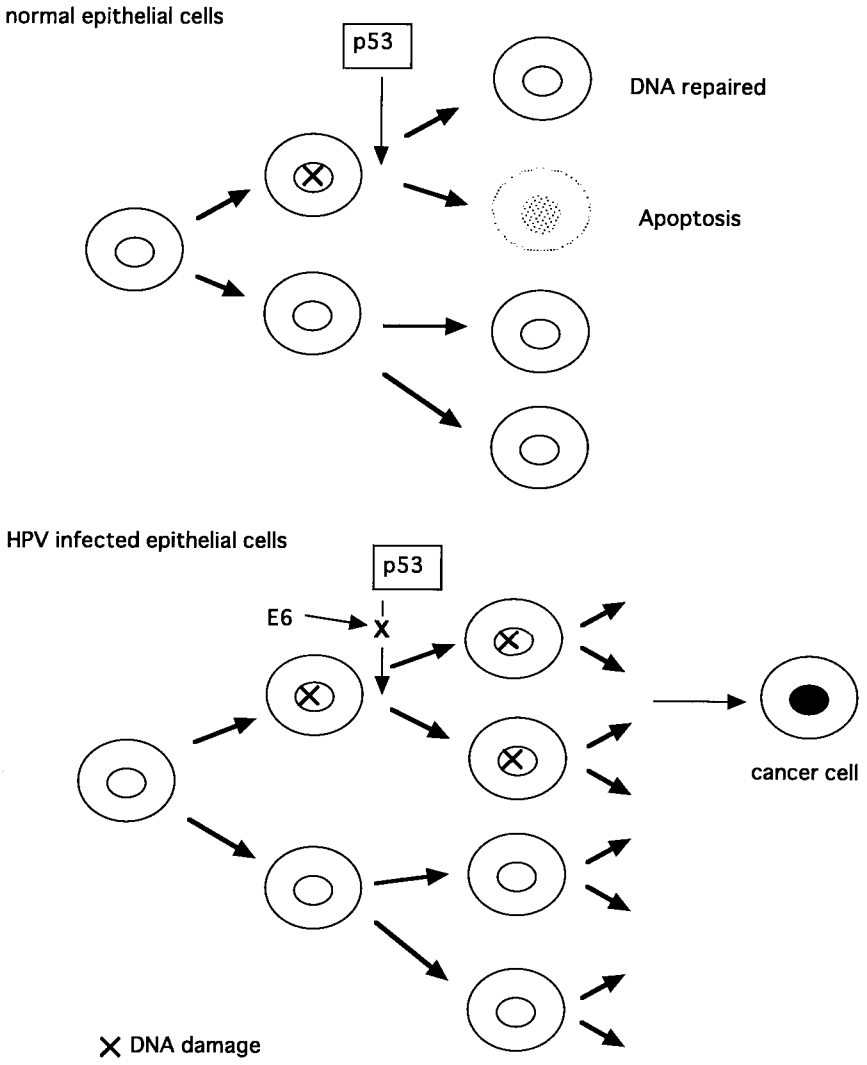

Fig. 4. Dysfunction of p53 and pRB results in the accumulation of DNA damage.
Without HPV infection, DNA damage is repaired by p53 and pRB. When DNA damage can not be repaired, the cell apoptosis is induced. With HPV infection, this function of p53 and pRB is disrupted, leading to the accumulation DNA damage and the development of cervical cancer cells over a long period of time.

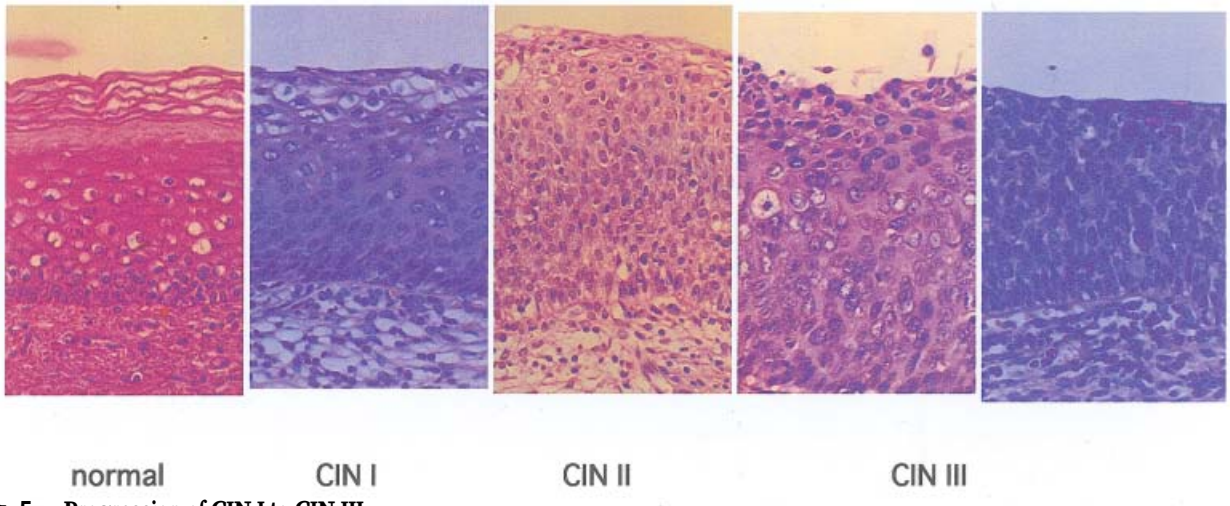

Fig. 5. Progression of CIN I to CIN III.
Hematoxylin and eosin-stained histology of CIN. CIN is divided into three categories according to the extent of parabasal hyperplasia.

Table 3. Prevalence of HPV types in CIN and invasive cervical cancer.

HPV	n	16	58	52	others	negative
CIN I	80	10%	15%	20%	34%	21%
CIN II	44	16%	23%	11%	39%	11%
CIN III	64	28%	16%	16%	27%	14%
invasive	29	38%	3%	14%	31%	14%

Types 16, 58, and 52 are common and type 18 is infrequent in Japan, whereas type 18 is common in Western countries. The prevalence of type 16 is elevated with the progression of CIN. This behavior is characteristic of high risk HPV and type 16 is the most important single type. Types 58 and 52 are classified as intermediate risk.

Table 4. Relationship between HPV types and the prognosis of CIN.

	n	progress	persistent	regress
HPV16	16	44%	56%	0%
HPV58	20	25%	40%	35%
HPV52	17	6%	47%	47%
others	41	12%	32%	56%
negative	27	0%	11%	89%

It is noted that no patient with HPV16 infection regressed. Conversely, 89% of HPV negative patients regressed. Infection with high-risk HPV is the major prognostic factor of CIN.

not suitable for vaccines because they contain the oncogenic genome. Thus, most studies have been carried out using a recombinant viral protein. However, recombinant subunit vaccines based on the major capsid protein L1 were of limited effectiveness in animal models. The breakthrough was made by Kirnbauer *et al*. who discovered that L1 self-assembles into virus-like particles (VLPs) when expressed at high levels in cultured insect cells, and VLPs induced the production of neutralizing antibodies to conformational epitopes (49). Moreover, vaccination with VLPs has been shown to protect against experimental infection in animal models (50, 51). These encouraging results in animal models have encouraged several commercial and public institutions to undertake clinical trials of VLP-based vaccines. Schiller JT *et al*. reported the preliminary results of the phase I trials conducted at Johns Hopkins University. Seventy-two men and women were enrolled, who had four or fewer sex partners. They were randomized into 10 μg or 50 μg VLPs and a placebo with or without adjuvant. The clinical grade VLPs were purified from HPV16 L1 recombinant baculovirus-infected Sf-9 insect cells. All the vaccinees receiving VLP were seroconverted for one month, as measured in a VLP-based IgG ELISA, whereas none of the placebo-vaccinated subjects was seroconverted during the course of the study. The problem is whether serum IgG antibodies alone are sufficient for protection (52).

Another approach is DNA vaccines. Recently, it was demonstrated that an intramuscular (i.m.) injection of DNA expression vectors in mice resulted in DNA uptake in the muscle cells and expression of the protein encoded by the DNA (53). Ulmer JB *et al*. reported that an injection of plasmid DNA encoding influenza A nucleoprotein resulted in the generation of nucleoprotein-specific cytotoxic T cells (CTLs) and protection from a subsequent challenge with a heterologous strain of influenza A virus (54). Naked DNA vaccines created by combining one or more of the HPV surface protein genes with plasmid DNA are under development.

It is noted that HPV E7 is a tumor rejection antigen. Chen L *et al*. demonstrated that immunizing mice with syngeneic nontumorigenic fibroblast-like cells containing the HPV-16 E7 gene, conferred protection against transplanted cells from an HPV-16 E7-positive syngeneic tumor (55). The E6 and E7 oncoproteins of HPV are constitutively expressed in cervical cancer because they are required to maintain the cells in a transformed state. Thus, E6 and E7 oncoproteins are attractive targets for the immune response and are candidates for active immunotherapy. CTL responses are an important defense mechanism against viral infection and tumors. CD8+ CTL recognizes peptides derived from HPV-related proteins presented on the MHC class I molecule at the cell surface. These peptides usually have a length of 8 to 11 amino acids. Ressing ME *et al*. studied the immunogenicity of 9 HLA-A0201 binding peptides encoded by HPV16 E6 and E7, and identified three peptides which were highly immunogenic in CTL induction in the peripheral blood mononuclear cells (PBMC) of HLA-A0201 healthy donors. Human CTL clones specific for these three peptides were capable of lysing the HPV16 E7-containing HLA-A0201 cervical carcinoma cell line CaSki (56). A HPV-specific CTL response was also detected in patients with CINIII (57), and CD4+ tumor infiltrating lymphocytes (TIL) in cervical cancer recognize HLA-DR-restricted peptides provided by human HPV-E7 (58).

A phase I-II clinical trial was performed involving vaccination with the HPV16 E7 peptides of patients (HLA-A0201) suffering from HPV16-positive cervical carcinoma which was refractory to conventional treatment. The vaccine consisted of two HPV E7 peptides and one helper peptide emulsified in

adjuvant. No adverse side-effects were observed. Of 19 patients enrolled, 2 were stable for one year after the vaccination, 15 showed progressive disease, and 2 showed tumor-regression after chemotherapy following the vaccination (59). Another phase I trial of peptide vaccine was conducted by Munderspach L, *et al*. Eighteen women with high grade cervical and vulvar intraepithelial neoplasia, who were HPV16 and HLA-A2 positive, were treated with a vaccine consisting of a 9-amino acid peptide from amino acids 12-20 encoded by the E7 gene emulsified with incomplete Freund's adjuvant. Only 3 of 18 patients cleared their dysplasia, but increased dendritic cell infiltrate was observed in 6 of 6 patients tested (60). Adams M *et al*. reported that the intradermal administration of live vaccinia virus HPV16 and 18 E6/E7 construct induced a clinical response in 1/3 advanced cervical cancer and 3/12 CIN III (61). These results were preliminary but promising.

Dendritic cells (DC) are believed to be critical for the induction of CTL responses. Numerous studies have been conducted using peptide-, tumor lysate-pulsed, and genetically engineered DC for the induction of antitumor immunity (62-65). Tuting *et al*. genetically modified DC by particle-mediated transfer of the HPV16 E7 gene. The i.v. injection of these genetically modified DC induced antigen-specific CD8+ CTL *in vivo* and promoted the rejection of a subsequent, normally lethal challenge with an HPV16-transformed tumor cell line (66). The DC-based immunotherapy was successful in animal models (67), but only a few preliminary studies have been reported in humans. Schoell WMJ *et al*. demonstrated that CTL activity could be induced by the co-culture of PBMC and HPV16 E711-20 peptide-pulsed DC *in vitro* (68). CTL activity could also be induced by co-culture with DC transfected with the HPV16 E7 gene by an adeno-associated virus (AAV) vector (69). Santin AD *et al*. reported a case with multiple lung metastasis secondary to recurrent HPV 18-associated cervical adenocarcinma. DC pulsed with HPV18 E7 oncoprotein were administered subcutaneously. She received 14 vaccinations with low-dose interleukin-2, and CT scans showed no evidence of tumor progression during 13 months of therapy. (70).

The studies on DC-based immunotherapy in humans are preliminary and unsatisfactory, however, future refinements of this strategy to boost antigen-specific immunity should be explored.

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