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REVIEW

Strategies Against Human Papillomavirus Infection and Cervical Cancer

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Papillomaviruses infect a wide variety of animals, including humans. The human papillomavirus (HPV), in particular, is one of the most common causes of sexually transmitted disease. More than 200 types of HPV have been identified by DNA sequence data, and 85 HPV genotypes have been well characterized to date. HPV can infect the basal epithelial cells of the skin or inner tissue linings, and are, accordingly, categorized as either cutaneous or mucosal type. HPV is associated with a panoply of clinical conditions, ranging from innocuous lesions to cervical cancer. In the early 1980s, studies first reported a link between cervical cancer and genital HPV infection. Genital HPV infections are now recognized to be a major risk factor in at least 95% of cervical cancers. 30 different HPV genotypes have been identified as causative of sexually transmitted diseases, most of which induce lesions in the cervix, vagina, vulva, penis, and anus, as the result of sexual contact. There is also direct evidence demonstrating that at least four of these genotypes are prerequisite factors in cervical cancer. The main aim of this review was to evaluate the current literature regarding the pathovirology, diagnostics, vaccines, therapy, risk groups, and further therapeutic directions for HPV infections. In addition, we reviewed the current status of HPV infections in South Korean women, as evidenced by our data.

Key words: cervical cancer, human papillomavirus, risk group, therapy, vaccine

The human papillomavirus (HPV) is a member of the papovavirus family (Papovaviridae). Like other papovaviruses, HPV can induce lytic, chronic, latent, and transforming infections, depending on the host cell. The classification of HPV is predicated on DNA sequence homology. At least 200 types have been identified, and have been classified into 16 groups (zur Hausen, 1999). HPV can be distinguished further, as cutaneous HPV or mucosal HPV, according to the susceptible tissues. Cutaneous HPVs infect and replicate in the squamous epithelium of the skin (warts). Mucosal HPVs infect and replicate in the mucous membranes (genital, oral, and conjunctival papillomas), and induce epithelial proliferation (Orth and Favre, 1985). These HPV types are very tissue-specific, and result in distinctly different diseases. Several genotypes have been associated with sexually transmitted disease, which later culminate in cervical cancer. HPV has, in recent years, become one of the most common causes of sexually transmitted disease in both men and women worldwide, and is believed to be the most common sexually transmitted viral disease in the world.

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Cervical cancer is also one of the most globally common cancers in women. Worldwide, breast cancer is the only cancer which outstrips cervical cancer with regard to cancer deaths in females (Jin *et al.*, 1999). Cervical cancer constitutes up to 25% of all female cancers, and is the most common cancer in developing countries (Harro *et al.*, 2001). The correlation between genital HPV infections and cervical cancer was first documented in the early 1980s by Harold zur Hausen, a German virologist. Since that time, the link between HPV and cervical squamous cell carcinoma has been well documented. Interestingly, the association between HPV and cervical squamous cell carcinoma is far clearer than the association between smoking and lung cancer (Franco, 1995). Recently, scientists have identified approximately 30 HPV types transmitted by sexual contact, which infect mainly the cervix, vagina, vulva, penis, and anus (Burd, 2003). Among these, four HPV types are most often found within the malignant cells of cervical cancers, with type 16 accounting for approximately half of the cases, and types 18, 31, and 45 accounting for an additional 25 to 30 % of cases (Harro *et al.*, 2001). HPV has, in fact, been implicated in 99.7% of all cervical squamous cell cancer cases (Walboomers *et al.*, 1999).

In this review, the current literature regarding the pathovirology, diagnostics, risk groups, vaccines, therapy, and further therapeutic direction of HPV infection were evaluated. In addition, this study reviewed the current status of HPV infections in South Korean women, as evidenced by our data. Recently, a novel technique for the detection of HPV infection was developed. Over the last four years, a total of 14,264 samples were tested, and the HPV infections in South Korea were genotyped. The results of this study suggest that the targets for HPV protection in South Korea should be different than those in other countries. Therefore, future therapeutic strategies for HPV infection should consider the specific situation of South Koreans, and immuno-therapy will have to be coupled with the development of new vaccines in order to effectively combat the issue.

Pathovirology of HPV

HPV is a small (55 nm in diameter), nonenveloped virus. It consists of an icosahedral capsid which is composed of 72 capsomers, each of which contains at least two capsid proteins, L1 and L2 (Baker *et al.*, 1991). Each capsomer is a pentamer of the major capsid protein, L1. Each virion capsid consists of several copies of the minor capsid protein. Therefore, the virus is somewhat reminiscent of a golf ball when visualized by electron microscopy. The HPV genome is made from a single copy of circular double-stranded DNA, containing approximately 7,900 bp (Favre, 1975). Therefore, all the open-reading-frame (ORF) protein-coding sequences are linked in one strand. Three regions of the genome have been functionally classified to date (Fig. 1). The first region contains a noncoding upstream regulatory region of 400 to 1,000 bp. This region contains a p97 core promoter, along with enhancer and silencer sequences, which regulate DNA replication by controlling the transcription of the ORFs. This region exhibits the highest degree of variation in the HPV viral genome. The second region is referred to as an early region, and consists of ORFs E1, E2, E4, E5, E6, and E7. These ORFs are involved in viral replication and oncogenesis. The third region is referred to as a late region, and encodes for the L1 and L2 structural proteins for the viral capsid. Due to frequent genetic exchange, the DNA sequence homology of the E6, E7, and L1 ORFs between the related HPV types has been found to be 90% or less (Torrisi *et al.*, 2000).

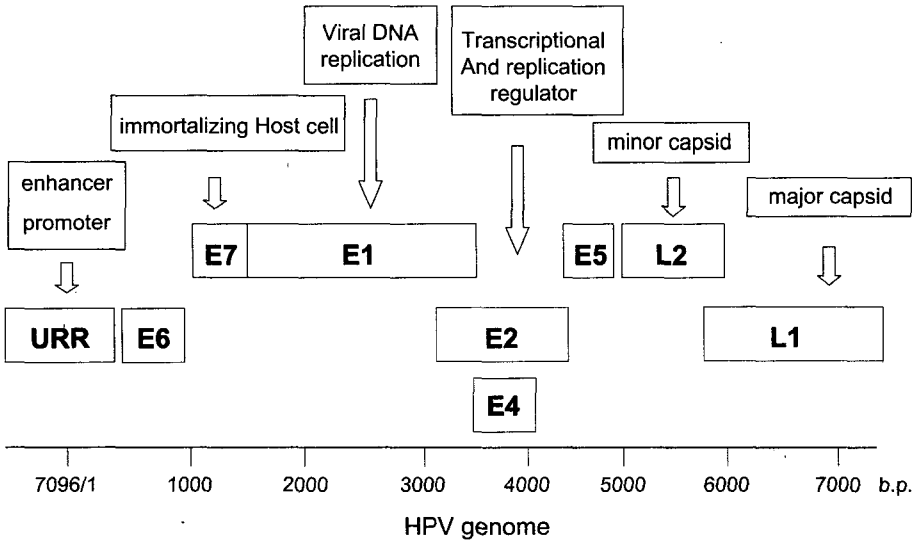

Fig. 1. Schematic presentation of the HPV-16 genome. L1 is the major capsid protein and L2 is the minor capsid protein. The E1 to E7 encoding proteins are involved in viral replication and oncogenesis. URR means upstream regulatory region (Favre, 1975; Murray *et al.*, 2002).

Normally, HPV infection occurs through breaks or tears in the basal level of the epithelial cells. Other cell types appear to be relatively resistant to HPV infection, as attempts to artificially culture HPV in other cell types have not been successful (Hummel *et al.*, 1992; Meyers *et al.*, 1992; Jeon *et al.*, 1995). The main reason for this may involve the differentiation process of keratinocytes when the viral DNA replicates (Jeon *et al.*, 1995). In an artificial HPV culture, epithelial cells containing latent HPV are grown in a collagen matrix, such as bovine tendon type 1 collagen. Occasionally, the matrix is prepared by mixing Human foreskin fibroblasts with collagen. Epithelial cells are pre-cultured on Swiss 3T3 fibroblast feeders, and plated on a collagen plug. After 4 days of incubation, the epithelial cells are placed on a cotton pad and fed with a specific medium containing 12-*O*-tetradecanoyl phorbol-13-acetate. This induces differentiation into supra-basal cells, representing the optimal condition for HPV replication (Meyers *et al.*, 1992).

The HPV replication cycle begins with the entry of the virus into the basal layer cells in the epithelium. HPV infection may also require additional mild epidermal abrasion. Integrin-α6 has been suggested as the epithelial cell receptor for HPV-6, but is not necessary for the attachment of the other types of HPV (Evander *et al.*, 1997; Joyce *et al.*, 1999; Giroglu *et al.*, 2001). Some HPVs, as with many other viruses, adhere to the host cells via cell-surface heparin sulfate (Giroglu *et al.*, 2001). Proteoglycans might also be involved in HPV attachment (Giroglu *et al.*, 2001). As HPVs encode for only 8 to 10 proteins, they must utilize the host cell factors in order to regulate viral transcription and replication. HPV replication begins with the interaction of host cell components and the LCR region of the HPV genome. This interaction induces the transcription of the viral E6 and E7 genes (Syrjänen *et al.*, 1999). The E6 and E7 proteins then down-regulate the mitotic activity of the host cell by binding and inactivating the p53 and pRB proteins (Fig. 2). The function of the E6 and E7 proteins during HPV invasion of the host cell is to interrupt the cell growth-regulatory pathways, and to modify the environment of the host cells, thereby facilitating viral replication (Syrjänen *et al.*, 1999). After the E6 protein targets p53, p53 is degraded rapidly by a cellular ubiquitin ligase. As a result, G1 arrest, apoptosis, and DNA repair are abrogated. The HPV E7 protein binds to the phosphorylated pRB. This binding disrupts the complex formed between pRB and the cellular transcription factor, E2F-1. Free E2F-1 then begins to transcribe the genes required for the host cells to enter the S phase. The E7 protein can also bind with cyclin E (Syrjänen *et al.*, 1999). Overall, the outcome of the E6 and E7 protein functions in the host cell is the stimulation of DNA synthesis, and cell proliferation.

In the later infection stage, the E2 protein functions as a DNA binding protein, which blocks the transcription of the E6 and E7 genes, and permits the E1 gene product to bind to the viral origin of replication, which is located within the LCR. This binding initiates the replication of

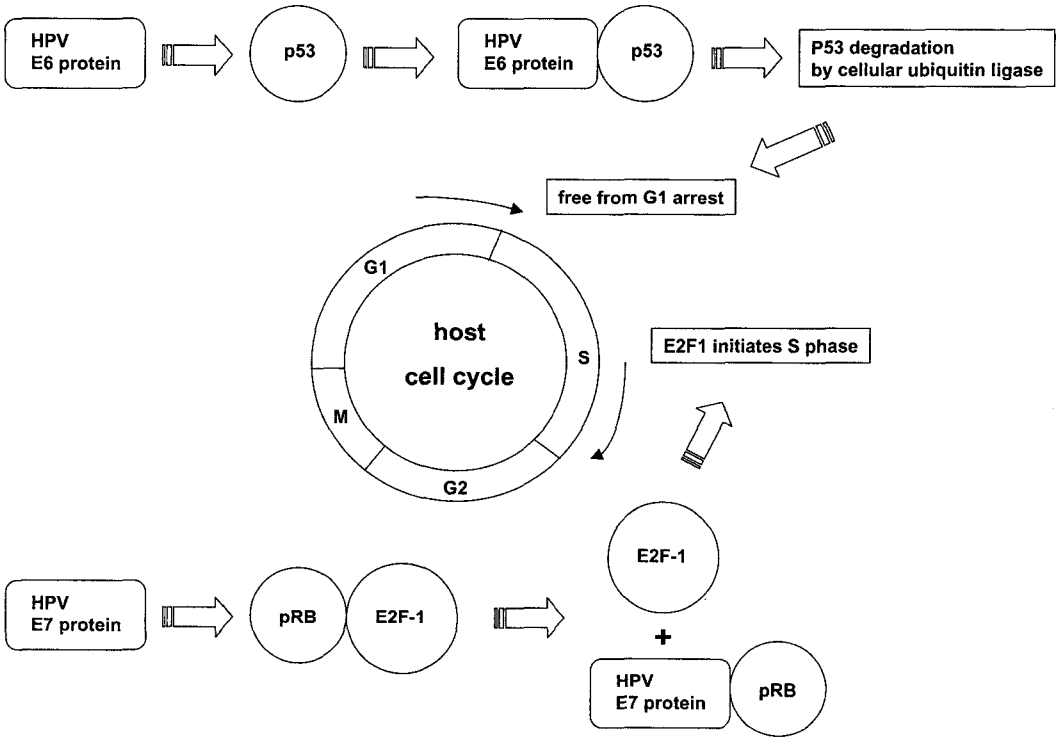

Fig. 2. Targets and actions of the oncogenic HPV E6 and E7 proteins. HPV E6 and E7 proteins bind to the cellular p53 and pRB proteins, and alter the host cell cycle, leading to tumorigenic cell transformation (Thomas *et al.*, 1999; Burd, 2003).

Table 1. HPV types which are associated with diseases (Bosch *et al.*, 1995; Bonnez and Reichman 2000; Burd, 2003)

Disease	HPV type
Cutaneous Disease	
Plantar warts	**1**[a], **2**, 4, 63
Common warts	**2, 1**, 7, 4, 26, 27, 29, 41, 57, 65, 77, 1, 3, 4, 10, 28
Flat warts	**3, 10**, 26, 27, 28, 38, 41, 49, 75, 76
Other cutaneous lesions	6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73
(e.g., epidermoid cysts, laryngeal carcinoma)	
Epidermodysplasia verruciformis	**2, 3, 10, 5, 8, 9, 12, 14, 15, 17**, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 50
Condyloma acuminata (genital warts)	**6, 11**, 30, 42, 43, 45, 51, 54, 55, 70
Mucosal Disease	
Recurrent respiratory papillomatosis	**6, 11**
Focal epithelial hyperplasia of Heck	**13, 32**
Conjunctival papillomas/carcinomas	**6, 11, 16**
Cervical intraepithelial neoplasia	
Unspecified	30, 34, 39, 40, 53, 57, 59, 61, 62, 64, 66, 67, 68, 69
Low risk	**6, 11**, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, 74
High risk	**16, 18**, 6, 11, 31, 34, 33, 35, 39, 42, 44, 45, 51, 52, 56, 58, 66
Cervical carcinoma	**16, 18**, 31, 45, 33, 35, 39, 51, 52, 56, 58, 66, 68, 70

[a]Bold letters are indicated as most frequent association with disease

the viral genomes in the S phase of the host cell. The E2-mediated down-regulation of E6 and E7 transcription releases p53 and pRB proteins, permitting the resumption of the normal cell cycle of the host cell. L1 and L2 then begin transcription, assembling the viral particles. Later, complete virions are released. In this stage, the E4 protein controls the maturation and release of the papillomavirus. This process does not appear to be cytolytic. Therefore, the massive proliferation of host cells occurs via rampant mitosis, which results in the formation of malignant cells.

HPV infection causes a variety of clinical conditions, ranging from innocuous lesions to cancer (Table 1). The benign conditions induced by HPV infection are normally limited to cutaneous warts (plantar warts, common warts, flat warts) on the hands and feet. Warts are areas of hypertrophied skin filled with keratin, which resolve spontaneously within 1 to 5 years. Some types of HPV infect the face, and these often result in skin cancer. Other HPV types, which grow mainly in the lining of the mouth, produce small elevated nodules, which can develop into fatal squamous cell cancer. Focal epithelial hyperplasia of the oral cavity (Hecks disease) is largely due to HPV-13 infection. Epidermodysplasia veruciformis, a rare genetic disease characterized by HPV-associated warts on the trunk and upper extremities, causes invasive squamous cell carcinomas in its later stages. Conjunctival papillomas and carcinomas have been correlated with HPV infection in numerous studies. Recurrent respiratory papillomatosis is reported to be a major disease of the larynx in young children, but can also occur in adults. HPV infection in young children appears to be acquired by passage through an infected birth canal, as a high percentage of mothers have a history of HPV.

Based on their association with cervical cancer and precursor lesions, HPVs are divided into two groups; high-risk and low-risk HPV (Table 1). The low-risk group includes types 6, 11, 42, 43, and 44. The high-risk group includes types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Some HPV types in the high-risk group are only infrequently associated with cancers, but are often found in squamous intraepithelial lesions (SILs). Therefore, some clinicians or scientists refer to these HPV types as intermediate-risk. Even low-risk subtypes, however, are occasionally found in cervical carcinomas.

Diagnostics of HPV infection

Cervical cancer progresses gradually, from a mild cervical intraepithelial neoplasia (CIN1) to more severe degrees of neoplasia and micro-invasive lesions (CIN2 or CIN3), finally becoming an invasive disease, in the form of

malignant cancer (Holowaty *et al.*, 1999). This means that the progression of cervical cancer may be the result of a high-risk HPV infection occurring early in life, and may lead to the gradual progression to a more severe disease, in association with other factors which promote cell transformation. Therefore, the early detection and subsequent early treatment of HPV in pre-cancerous lesions is crucial in the prevention of this progression to cancer.

Since the early 1950s, our primary diagnostic tools have been cytology and histology. Recently, though, molecular biological techniques for detecting the DNA sequences of HPV in clinical specimens have been introduced. However, the most common method for detecting high-risk HPV remains the Papanicolaou-stained (Pap) smear. This method was named after pathologist George Papanicolaou, who pioneered this method in 1949, before HPV was implicated in cervical cancer (Papanicolaou, 1949). In the last few decades, the Pap smear has helped reduce the incidence and mortality rates of cervical cancer. The Pap smear is a screening tool for morphological changes in cervical cells. The reporting system of a Pap smear has evolved gradually, and the current reporting system is the Bethesda System, which was introduced in 1988 and updated in 1999. The CIN System, another Pap smear reporting system, is based on tissue architecture, and was introduced in 1973. Both of these reporting systems were developed to score the severity of the cervical neoplasia, and to set up a gold standard of diagnostic terminology. Based on the Pap smear reporting system, the progression of cervical cancer is classified into one of four categories, based on the histopathology: (i) ASC (atypical squamous cells), (ii) LSIL (low-grade squamous intraepithelial lesions), (iii) HSIL (high-grade squamous intraepithelial lesions), and (iv) squamous cell carcinoma (Kiviat *et al.*, 1993; Solomon *et al.*, 2002).

The combination of immunochemical staining with a Pap smear can be used to detect HPV-specific antigens. Monoclonal and polyclonal antibodies have also been developed to detect a particular HPV specific antigen, which is derived from the major capsid protein expressed in almost all HPV types. Using the peroxidase-substrate system, the presence of an HPV-specific antigen can be easily scored.

However, the Pap smear procedure has some limitations. The false-negative rate has been reported to be as high as 20 to 30%. False-negative results can occur due to a misreading of slides, especially when the cells are not spread evenly or uniformly on the microscope slide. In addition, contaminants such as bacteria or yeast prevent the detection of abnormal cells in the specimens. When the specimens are exposed to air too long before their fixation on the slides, the cervical cells can become distorted and unreadable. Human error is probably the main cause of false interpretation from a Pap smear. The average Pap smear slide contains 50,000 to 300,000 cells to be examined. Abnormal cells can easily be missed if the sample contains only a few abnormal cells within a crowded background of healthy cells, particularly by overworked readers.

Several more accurate methods have been developed for the detection of HPV infection by molecular techniques. *In situ* hybridization can be used to detect HPV DNA or RNA in biopsy tissues, using DNA probes labeled with either radioisotopes or chemi-fluorescence ligands (Lizard *et al.*, 2001). The main advantage of *in situ* hybridization is that the location of HPV-infected cells can be specifically localized. Therefore, *in situ* hybridization with tissue morphology is a more accurate tool, as it allows for the simultaneous assessment of the morphological alterations associated with HPV infection.

Type-specific PCR assays have also been developed, which are based on the sequence variations present in the E6 and E7 genes of the different HPV types. Fourteen type-specific PCRs for high-risk HPV types have been developed, which target approximately 100 bp of the E7 ORF (Walboomers *et al.*, 1999). The analytical limitations of these methods range from 10 and 200 HPV copies per specimen, depending on HPV type. Type-specific PCRs require more development and testing, because they are limited by the necessity for different primer sets to identify different types of HPV.

The Hybrid Capture assay system (Digene, USA) is the only kit currently approved by the Food and Drug Administration (FDA, USA) for the detection of HPV DNA in cervical specimens. The Hybrid Capture assay is based on DNA/RNA hybridization as recognized by an antibody, which is visualized by chemiluminescence detection in order to qualitatively measure the presence of HPV. In this assay, the DNA from a specimen is denatured, and then mixed with an RNA probe pool in a tube containing buffered solution. The RNA probe pool consists of two sets and the assay can be performed using both probe sets either together or separately. The first probe pool contains the RNAs from low-risk HPVs and the second probe pool contains the RNAs from high-risk HPVs. After hybridization, the DNA-RNA complexes are immobilized onto the wells of a microtiter plate, which has been coated with an antibody against the DNA-RNA hybrids, conjugated with alkaline phosphatase. After mixing with a substrate, the presence of the HPVs can be detected as a form of chemiluminescence. This assay is widely performed in many clinical diagnostic laboratories, but its main disadvantage is that it cannot distinguish the specific types of HPVs within the low-risk or high-risk groups. In addition, cross-activity between the HPV probes and the plasmid pBR322 is sometimes observed, resulting in false-positive results with the high-risk probe pool (Burd, 2003). The estimated false-negative rate ranges from 1.1 to 7.5%.

Recently, a new kit has been developed, which utilizes DNA chip technology in combination with DNA hybrid-

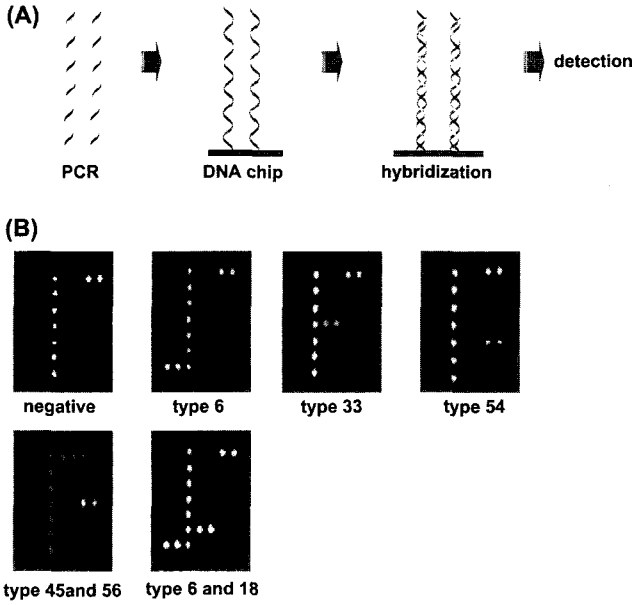

Fig. 3. The principle and the example of a specific type of HPV detection using a MyHPV chip kit (Mygene, Republic of Korea). (A) Twenty four different HPV DNA sequences were implanted into the DNA chip and hybridized with PCR amplification from the patients samples. Each PCR primer was anchored with fluorescent dye, and this signal was monitored using a fluorescent detection monitor. (B) The example of screening for a specific type of HPV infection. The MyHPV chip kit can distinguish between each different type of HPV or co-infection.

ization. The basic concept of the MyHPV chip kit (Mygene, Republic of Korea) is similar to that of the Hybrid Capture system, but is more advanced, and uses DNA chip technology rather than simple DNA-RNA hybridization followed by visualization. Twenty four different HPV DNA sequences were implanted into a DNA chip and hybridized with a single strand of PCR amplification from the patients samples. Each PCR primer is anchored by fluorescent dye, and this signal is monitored by a fluorescent detection monitor (Fig. 3). Using this method, each individual HPV genotype can be screened. This system is also able to discern HPV co-infections (Fig. 3). Therefore, the MyHPV chip kit is more advanced and accurate than the Hybrid Capture kit, due to its ability to distinguish between each type of HPV, and to detect co-infection, as opposed to the simpler delineation of high- versus low-risk HPV (Table 2). The detection of HPV co-infection is quite therapeutically relevant, as various studies have demonstrated that infection with multiple HPV types tends to increase the severity of cervical cancer (Jacobs *et al.*, 1997; Kleter *et al.*, 1999; Quint *et al.*, 2001). Using this kit, approximately 15,000 specimens were screened over the last four years, and the false-positive and estimated false-negative rates are both estimated to be < 1%. Therefore, this kit should prove quite useful in both diagnosis and the development of strategies for new vaccines in the future.

Risk groups of HPV infection

HPV transmission occurs mainly by physical contact through the skin. Epidemiological studies clearly demonstrate that the risk of a genital HPV infection and, thus, cervical cancer is related to sexual activity. Sometimes, nonsexual transmission, such as in prolonged exposure to shared contaminated clothing via fomites, can also occur, as HPV is quite resistant to heat and desiccation (Roden *et al.*, 1997). An individual with multiple sexual partners, or the partner of such an individual, runs the highest risk of becoming infected with HPV. The risk of an HPV infection also increases in individuals who engage in sexual activity at an early age. Condom use may not fully protect individuals from exposure to HPV, as HPV can be transmitted by contact with other infected parts of the body.

In addition to sexual activity, age is an important risk factor in HPV infection (Burk *et al.*, 1996; Adam *et al.*, 2000). Most cervical cancers occur at the squamocolumnar junction, between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. This is due to the fact that the squamocolumnar junction is an active metaplastic changing site. Metaplastic activity occurs most profoundly at puberty and first pregnancy, and declines after menopause. HPV infections most commonly occur in sexually active young women, 18 to 30 years of age, with a sharp decrease in incidence after 30 years of age. However, cervical cancer is more often found in women over 35, indicating that HPV infection at younger ages can result in a slow progression to cancer. Frequent infection or co-infection with high-risk HPV is more likely to cause cervical cancer. Immunocompromised individuals, such as those who have undergone a renal transplant, or those with human immunodeficiency virus disease, run increased risks for the acquisition and progression of HPV,

Table 2. Comparison between PCR detection, hybrid capture and MyHPV chip kit

	MyHPV chip	Hybrid capture	PCR detection
Technology	DNA chip	DNA-RNA hybridization	PCR with primers
Detection	fluorescence dye	chemiluminescence	electrophoresis
HPV typing	specific HPV type	high risk vs. low risk group	only detect presence of HPV infection
Co-infection detection	possible	impossible	impossible

as the primary immune response to HPV infection is cell-mediated (Cubie *et al.*, 2000; Torrisi *et al.*, 2000; Calore *et al.*, 2001).

The risk for cervical cancer also tends to be influenced by other lifestyle factors, including current smoking and parity (Adam *et al.*, 2000). The regional immune suppression induced by smoking, in addition to the mutagenic effects of cigarette ingredients, have been shown to favor the persistence of HPV or to malignant transformation, in a fashion similar to that observed in the lung (Philips and NiShé, 1993; Yang *et al.*, 1996; Villa, 1997). Parity is also an important risk factor in women infected with HPV (Adam *et al.*, 2000). Other factors, such as alcohol consumption and diet, have not been well researched at this time.

Genetic predisposition has been recognized and accepted to play a significant role in colorectal cancer, lung cancer, and melanoma. The genetic correlation with cervical cancer was found to be even more profound than with other cancers (Magnusson *et al.*, 2000). The inheritance of the disease factor for tumor development was estimated to be approximately 27%. It is, as yet, unclear as to whether one dominant gene or a polygenetic set of traits mediates the destiny of this disease. However, it is clear that the correlation between HLA haplotypes and cervical HPV diseases is low (Apple *et al.*, 1995; Allen *et al.*, 1996; Bontkes *et al.*, 1998).

Over the last four years, we screened total 14,264 specimens and genotyped HPV infection profile of Republic of Korea. Our results suggested that HPV infection correlated with the age at which South Korean women typically become sexually active (Fig. 4). Interestingly, conventional high-risk HPV genotypes, such as 16 and 18, were still dominant, but new HPV genotypes such as 33, 56, and 58 have appeared in the South Korean population during the past few years. The results for low-risk HPV genotypes revealed that HPV 6, 11, 53, 54 and 70 were dominant in South Korean women. After analyzing the regional incidence of HPV infection, the populations of Inchon and Busan were found to be more susceptible to HPV infection, which might indicate geological specificity, as Inchon and Busan are major international ports (Fig. 5).

The occurrence of high-risk HPV infections is somewhat different in Republic of Korea than in Europe and the USA. For example, types 16, 18, 31, and 45 of the

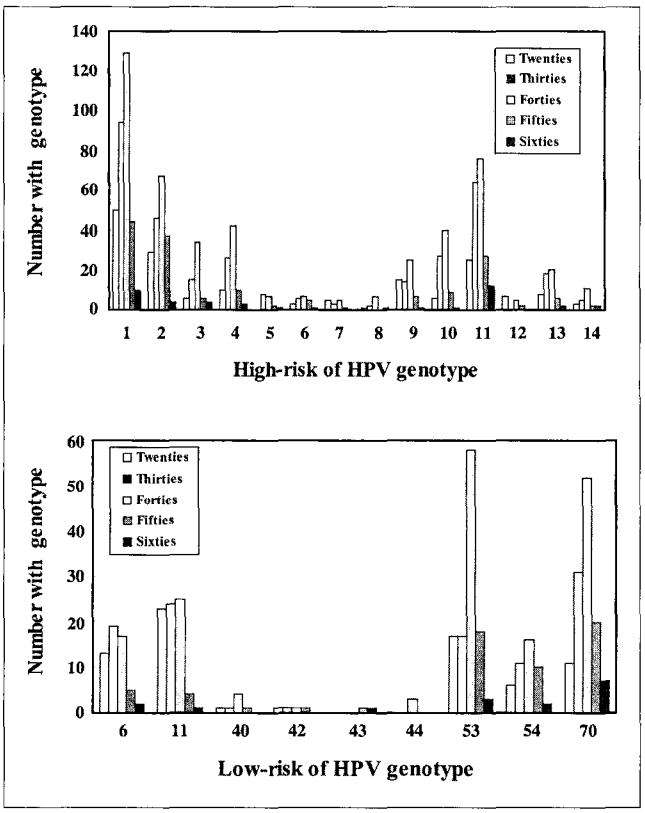

Fig. 4. High risk and low risk groups of HPV infection in South Korean women over the last four years. A total of 14,264 specimens were screened. The upper panel shows the result of a high-risk group of HPV infection in South Korean women, and the lower panel shows a result of a low-risk group of HPV infection.

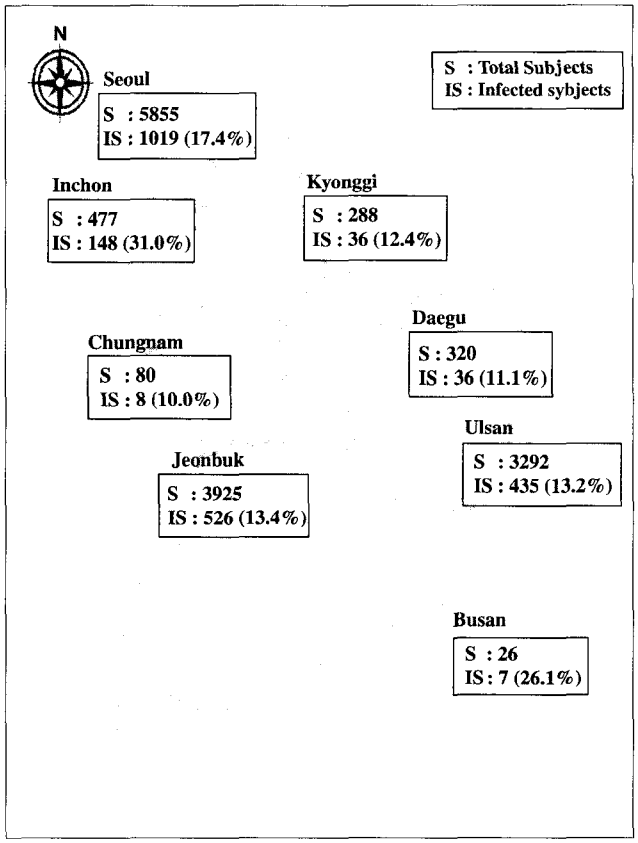

Fig. 5. Geological map of HPV infection in South Korean women over the last four years. The frequency of HPV infection was higher in Inchon and Busan than in any other cities, which might indicate geological specificity.

HPV high-risk group are dominant in Europe and the USA, whereas types 16, 18, 33, 56 and 58 are dominant in Republic of Korea. This indicates that genotypes 16 and 18 might be the most globally typical types. Types such as 33, 56, and 58 of the HPV high-risk group appear to be unique to South Koreans. This suggests that the targets for HPV protection in Republic of Korea should be different than those of other countries. Future strategies against HPV infection should take into account the specific situation of Republic of Korea.

HPV vaccines

When producing HPV vaccines, scientists normally employ virus-like particles (VLPs). These VLPs contain empty virus capsids containing the major HPV capsid antigen and, possibly, the minor capsid antigens, but lack viral DNA. In order to obtain the vaccines, DNA constructs encoding for L1 or L1 and L2 ORFs are transfected into eukaryotic cells, which then produce these proteins. These proteins then self-assemble into VLPs, and a further purification step is undertaken in order to confirm the existence of the VLPs, which are highly immunogenic. The proper folding of the L1 or L2 proteins is essential for vaccine development, as the neutralization epitopes have been shown to be sensitive to the conformation. Due to the high level of antigenic specificity exhibited by the HPV capsid antigens, no cross-protection among the HPV types has been observed. Therefore, vaccines need to be developed for each HPV type. HPV vaccines using the proper VLPs have convincingly shown that the neutralizing antibodies can block a new infection in several animal models of papillomavirus infection (Campo *et al.*, 1993; Brieburd *et al.*, 1995; Suzich *et al.*, 1995).

Currently, vaccines directed against HPV types 6, 11, and 16 are currently in phase I and phase II clinical trials. Early studies have shown that vaccination with the VLPs is well tolerated, and induces a 50-fold increase in titer, in terms of both binding and neutralizing antibodies (Evans *et al.*, 2001; Emeny *et al.*, 2002). These vaccines were also able to boost T-cell responses.

In 2002, investigators from the University of Washington reported remarkable clinical findings using the VLP vaccine against HPV 16 (Koutsky *et al.*, 2002). In order to test whether vaccination with the HPV16 VLPs could prevent HPV16 infection, they vaccinated young female volunteers, aged between 16 and 23, with HPV16 VLPs. Three vaccinations were performed over 6 months, on a total of 1533 individuals. The volunteers were examined at 6-month intervals for 48 months after vaccination by a PCR reaction, to determine whether there was any evidence of persistent HPV 16 infections. The results of the HPV VLP vaccine trials were quite promising. No incidences of HPV infection were found in the vaccinated group (n=768), whereas 29 cases of persistent HPV16 infection were found in the placebo group (n=765). These results (100% efficacy, 95% CI 90-100; $p<0.001$) provide strong evidence that type-specific VLPs can offer protection from HPV infection and, thus, from cervical cancer.

In order to obtain more effective HPV VLP vaccines, scientists have attempted to construct chimeric VLPs. Several additional approaches for inducing cellular immunity, besides humoral immunity, were attempted using chimeric VLPs. For example, investigators tried to link a segment of the E7 gene to the carboxy terminus of L1 or L2 (Muller *et al.*, 1997; Greenstone *et al.*, 1998). These chimeric VLP vaccines were found to boost both humoral immunity to the VLP, and cellular immunity to L1 and E7.

The major limitation of the VLP vaccines is that they are restricted to specific HPV types. Therefore, scientists have attempted to construct vaccines which are cross reactive with other types of HPV. One approach employs a denatured L1 protein, but the results indicated that denatured VLPs are insufficient to generate neutralizing antibodies (Christensen *et al.*, 1990; Hines *et al.*, 1994). Interestingly, recent studies have reported that denatured L2 protein can generate antibodies which neutralize both homologous and heterologous HPV types (Roden *et al.*, 2000), However, it is unclear as to whether the neutralizing antibodies against the denatured L2 protein are effective in preventing natural infections, as most neutralizing antibodies appear to recognize the L1 protein.

Finally, various attempts to develop DNA vaccines are currently in progress. DNA vaccines have several advantages. The production of a DNA vaccine is relatively easy, and tends to be more effective in the generation of both humoral and cellular protective immunity responses. However, a previous trial, using a L1 DNA vaccine, evidenced only weak immune responses to HPV, due to the different codons of the vial L1 gene used (Dupuy *et al.*, 1999). Recently, a codon-modified version DNA vaccine targeting HPV6 L1 and L1-E7 was constructed and tested, to determine if this DNA vaccine would elicit an immune response in mice (Liu *et al.*, 2001).

Overall, continuous attempts are being made to produce more effective vaccines against HPV, in the hopes of preventing HPV infection and disease. Although the results of several new vaccine trials have been extremely encouraging, several issues must be solved before more effective vaccines can be developed. First, larger clinical trials are necessary in order to confirm that the vaccine can prevent HPV-associated disease. Second, the development of other HPV VLP vaccines is required, in order to provide broad coverage. Third, the durability or efficacy of protection needs to be well understood.

Therapy for HPV infection

In healthy individuals, the immune system can eliminate most HPV-infections, and if a further HPV infection does not occur, 90% regress spontaneously within 12 to 36

months (Moscicki *et al.*, 1993; Chua and Hjerpe, 1996; Ho *et al.*, 1998). The initial immune response to a HPV infection is normally mediated by antigen-presenting cells, which infiltrate local lymph nodes. The subsequent activation of T-lymphocytes can cause the B cells to secrete antibodies, but the levels of HPV-specific immunoglobulin G (IgG) and IgA in the local infected tissue tend to be insufficient for the clearance of viral infections (Bontkes *et al.*, 1999). Successful humoral immunity in healthy individuals plays the key role in terms of the degree of symptoms or the progression of disease. Factors affecting humoral immunity, such as genetic predisposition, frequency of re-infection, genetic variations of the HPV type, co-infection with more than one HPV type, and hormone levels can influence the bodys ability to clear an HPV infection.

The procedure and types of treatment for cervical cancer are determined by several complex factors, including the size, stage, or histological features of the tumor, the degree of lymphocyte infiltration, and the patients preference with regard to surgery or chemo-radio therapy. In general, when a mild progression of defective malignant cells in noninvasive intraepithelial lesions is identified, simple cryotherapy or laser therapy is the treatment of choice. These patients usually retain their fertility. Cryotherapy is usually performed with a supercooled probe, which induces necrosis around the abnormal tissue. Other cryotherapy techniques use a carbon dioxide laser beam. The tissue heals faster and with less distortion when the laser beam is used, but the procedure is more expensive. The clearance of suspicious lesions is important, because patients with uncleared regions run a higher risk of recurrence than do patients in whom these regions are cleared. Human immunodeficiency virus-infected individuals exhibit significantly higher recurrence rates than do immuno-competent individuals, indicating the importance of an effective immune system against HPV-associated disease (Calore *et al.*, 2000). After detection and therapy, the progression to invasive cancers is rare, comprising $< 2\%$ of patients. Therefore, the early detection of HPV infection is clearly important. Patients with locally progressive cancers are treated with radiotherapy on the primary developing site and potential spreading sites of a tumor.

Several antiviral and immunomodulatory agents have been evaluated as treatment modalities for HPV-associated cervical lesions, in addition to mechanical or chemo-radiotherapeutic techniques. Cidofovir, an acyclic nucleoside phosphonate derivative, is used clinically for the treatment of CMV infections. Cidofovir has broad-spectrum activity against DNA viruses, and inhibits abnormal cell proliferation (Andrei *et al.*, 1998). Podophyllin, a cytotoxic agent which arrests mitosis in metaphase, and vidarabine, a DNA polymerase inhibitor, are also used to treat patients with cervical cancer (Okamoto *et al.*, 1999). Some cytokines are also under consideration for their possible use in the treatment of cancer patients. IFNs, such as IFN-α, IFN-β, and IFN-γ, have been shown to suppress the E6 and E7 gene transcripts of HPVs (Kim *et al.*, 2000).

Further therapeutic direction of HPV infection

Many diagnostic tools and therapeutic agents have helped reduce the incidence of cervical cancer over the last few decades. However, although the incidence of HPV and cervical cancer has declined in developed countries, it has increased in developing countries. More efficient systems for the detection of HPV infection are required for the early detection of HPV infection in developing countries. This may be facilitated by several new detection systems, including the MyHPV chip kit (Mygene, Republic of Korea), which will allow us to detect specific HPV types in cervical specimens with a great degree of sensitivity and specificity. This test kit is available in clinical laboratories at many medical centers in Republic of Korea.

Currently, a great deal of effort has been expended in the development of more effective treatments, in addition to prophylactic vaccines. One example is a method for activating the cytotoxic T lymphocytes (CTLs), the primary effectors of tumor rejection. Several strategies exist for the generation of effective CTLs by modifying the capability of a tumor or viral antigen presentation in the antigen-presenting cells, and also modifying the adhesion and co-stimulatory molecules. Several approaches toward the generation of HPV-specific CTLs have yielded promising results in preclinical models (Da Silva *et al.*, 2001).

The other approach for boosting cellular immunity against HPV is to use dendritic cells as a carrier for a vaccine or therapeutic reagent. Preclinical studies have shown that dendritic cells (DCs) play crucial roles in antigen presentation *in vivo* (Santin *et al.*, 1999). Tumor-specific peptide-loaded DCs may constitute more powerful tools for the generation of anti-tumor CTLs than the peptides alone. Therefore, DC-based cell therapy has been carried out upon cervical cancer patients. Normally, the peripheral blood monocytes of cervical cancer patients are required for the differentiation of DCs in the presence of IL-4 and GM-CSF in the culture. The DCs, mixing with a peptide epitope, then autologously sensitize to the cancer patients. The disadvantage of this method lies in the restriction of its use due to the diversity of MHC haplotypes.

DNA has emerged as an attractive vaccine candidate, as it is relatively inexpensive and easy to prepare. The DNA encoding for viral antigens can be taken up by the APCs to induce both antibodies and CTLs. The other method for delivering antigenicity using a DNA vaccine involves the use of viral vectors as delivery carriers into the body. Recently, a recombinant vaccinia virus, expressing the HPV16 and 18 E6 plus E7 genes, was created without introducing the oncogenes, the E6 and E7 proteins (Gal-

loway, 2003). This is one example of how a recombinant vaccinia virus delivery system can be used as a vaccine, which has the large capacity of the viral genome, but has no harmful viral genes.

New therapeutic strategies are important in South Korea, as the incidence of HPV infection has increased every year in South Korea. Three criteria need to be met for the effective eradication of cervical cancer in South Korea. First, several unique types of HPV have been observed in HPV infections in South Korean women. These types are clearly distinct from the HPV types of other countries, particularly the USA and western countries. As HPV vaccines which are limited to a few strains can be adapted to the generation of already-developed vaccines, a South Korean-type vaccine needs to be developed. Second, efforts to support all immune reactions need to be carried out. The appropriate method, which may involve cell therapy, DNA vaccines, or recombinant DNA technology, needs to be developed if HPV infection is to be prevented. Not only humoral immunity, but also cellular immunity must be part of this process. Third, immunosuppression at the late stages of cervical cancer needs to be considered. Therefore, combinatorial therapies would be much more beneficial to South Korean women, with regard to boosting immune response with the development of South Korean-style HPV vaccines.

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