

Development of human papillomavirus and its detection methods (Review)

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Abstract. Human papillomavirus (HPV) infection plays an important role in cervical cancer. HPV is classified within the *Papillomaviridae* family and is a non-enveloped, small DNA virus. HPV infection can be classified into two distinct scenarios: i) With or without integration into the host chromosomes. Detection of its infection can be useful in the study of cervical lesions. In the present review, the structural and functional features of HPV, HPV typing, infection and transmission mode, the risk factors for cervical susceptibility to infection and HPV detection methods are described in detail. The development of HPV detection methods may have far-reaching significance in the prevention and treatment of cervical disease. This review summarizes the advantages and limitations of each HPV detection method.

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1. Introduction

In 1907, papillomavirus was discovered to be responsible for the manifestation of skin warts (1). In 1933, Shope successfully identified papillomavirus in the cotton tail rabbit, marking the first instance of its detection in this species (2). Subsequently, papillomavirus was identified in humans and other animals (3). In 1974, zur Hausen *et al* (4) proposed a close association between human papillomavirus (HPV) infection and the occurrence of atypical hyperplasia of the reproductive epithelium and cervical cancer. At present, HPV infection has been established as the primary instigator of cervical intraepithelial neoplasia and cervical cancer, and this is substantiated by epidemiological and fundamental scientific research (5). The escalating prevalence of condyloma acuminatum, more commonly known as genital warts, within sexually transmitted infections, coupled with the rising rates of cervical cancer and anal cancer, has resulted in increasing attention being paid to HPV infection (6,7). HPV detection has been proposed as a useful tool for the management of HPV-related conditions, such as prompt treatment, symptom relief and the reduction of transmission. Early detection allows for timely intervention, reducing the risk of cancer development and lessening the burden of HPV-related diseases.

2. The structural and functional features of HPV

HPV is classified within the *Papillomaviridae* family and is a non-enveloped small DNA virus. Its external viral capsid is comprised of 72 shell particles, while its internal layer is enveloped by double-stranded closed-loop viral genomic DNA (8). The virus has a diameter of 50-55 nm, and possesses a molecular structure that is double-stranded and icosahedral in shape (9). The viral genome spans ~8,000 bp, encompassing both coding and non-coding regions (10). The coding region is further subdivided into the early (E) and late (L) coding regions, while the non-coding region is

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referred to as the upstream regulatory region (URR) or long control region (11-13). The E region spans ~4,500 bp, which contains six open reading frames that encode six functional viral proteins: E1, E2, E4, E5, E6 and E7. Notably, the E1 protein exhibits DNA helicase activity, thereby facilitating the initiation of HPV DNA replication (14). The E2 protein homodimer can bind to the virus control region's specific sequence, thus it can impede the transactivation of viral DNA transcription (15,16). Furthermore, the E2 protein can hinder cell proliferation (17,18). The E4 protein has the ability to impede the entry of Cyclin B1/CDK1 into the nucleus, resulting in cell cycle arrest at the G2 phase (19,20). Additionally, the E4 protein can form a complex with the E1 protein, resulting in the formation of the E1^{E4} splicing protein, which acts on the intracellular keratin network and degrades the cytoskeleton (21,22). The E5 protein can activate epidermal growth factor receptors through binding, and can also influence endocytosis and Golgi apparatus acidification, growth factor receptor metabolism and intercellular communication (23,24). E6-AP, a related protein of the E6 protein, has the ability to degrade the p53 protein, leading to the loss of its cancer-suppressing function (25). Additionally, the E6 protein can activate telomerase, resulting in chromosomal abnormalities (26). Notably, the E7 protein can degrade the pRb protein, allowing the transcription factor E2F to enter the nucleus and initiate the S phase (27,28). The L coding region is ~2,500 bp in length and encodes the L1 major capsid protein and the L2 minor capsid protein (29). L1 is highly conserved, while L2 is highly variable, reflecting the polymorphism of the region (30). The non-coding region, also known as the URR, spans ~1,000 bp and is situated between the L1 and E6 genes; this region harbors corresponding sequences that regulate gene expression (31,32).

Each region interacts with specific proteins. For example, specific URR sequences can specifically bind to E1 and E2 proteins, thereby regulating gene transcription. The upregulation of E6 and E7 proteins is attributed to the inactivation of the E2 protein (26,33). Additionally, the E5 protein synergistically interacts with E6 and E7 proteins to promote the degradation of p53 and pRb proteins, thereby facilitating the carcinogenic potential of HPV (34).

3. HPV typing

The primary basis for HPV typing is the comparison of the homology of the L1 gene sequence, which is determined by the conserved nature of the HPV genome. By assessing the homology of the L1 gene with other types, new 'types' are designated if their homology is <90%, 'subtypes' are designated if their homology falls between 90 and 98%, and 'variant strains' are designated if their homology exceeds 98% (35,36). Additionally, HPV is classified based on the structure of the L1 gene and the parent-source relationship within the phylogenetic tree (37,38); the benefit of this approach lies in its facilitation of virus classification. Nevertheless, similar to the first method, virus types sharing comparable biological traits and pathogenicity cannot be entirely categorized into a single class. Consequently, numerous scholarly works classify viruses based on the biological characteristics and pathogenicity of HPV (39).

In a previous study, HPV was segregated into skin and mucosal groups depending upon the distinct tissue sites invaded by HPV (40). Additionally, the skin groups can be further subdivided into low-risk skin type and high-risk skin type based on the variance in lesions caused by HPV infection (41,42). The mucosa group can also be divided into low-risk and high-risk types (43). Notably, >90% of cases of genital warts and cervical low-risk squamous epithelial hyperplasia have been shown to be closely related to infection with low-risk HPV6 and 11, while ~70% of cervical cancer cases were linked to high-risk HPV16 and 18 infections (44,45). Furthermore, the potential of certain HPV types to induce lesions, whether benign or malignant, remains unclear and necessitates further investigation. The differentiation between high-risk and low-risk HPV can be discerned by examining the literature, which suggests that the ability of HPV to infect cells and induce malignant tumors serves as a crucial distinguishing factor (43,46,47). This distinction holds significant potential for guiding future identification of high-risk and low-risk HPV strains.

4. HPV infection

HPV infection can be classified into two distinct scenarios (48,49). The first scenario involves the failure of HPV-infected cells to integrate with the host chromosomes, resulting in the existence of multiple extrachromosomal copies (50). The second scenario involves the integration of HPV DNA with the host chromosomes, resulting in the integration of single or multiple copies into the cell genes (51,52). The current understanding of the receptor for HPV invasion into cells remains unclear, since HPV not only binds to normal squamous epithelial cells but also to various other cell types (53,54). Consequently, the characteristics of HPV keratophilic epithelium appear to be independent of the cell receptor, suggesting that the underlying cells may lack specific receptors for HPV (55).

The first scenario of HPV infection involves a comprehensive HPV life cycle. This entails HPV infecting the basal layer cells of the epidermis through a minor skin or mucosal wound, subsequently entering the host cell via endocytosis (56,57). HPV then gradually eliminates its capsid. Upon entry of the HPV genome into the nucleus, the early region promoter is activated, leading to the expression of various proteins, such as E1, E2, E6 and E7 (58,59). These proteins fulfill their respective roles in facilitating the replication and transcription of the HPV genome. The HPV genome then exhibits a copy number ranging from 20 to 100 in each infected cell, which is transported to daughter cells through cell division (20); this phase is referred to as the proliferative infection stage of the HPV life cycle (60). Subsequently, the infected cells in the basal layer migrate towards the surface, leading to the activation of the promoter in the HPV L region. This activation facilitates the expression of the L coding region L1 and L2 proteins. At this juncture, the necessary components for the assembly of HPV have been prepared, and the encapsulation of the virus genome by the virus shell protein occurs concurrently with its self-assembly (61,62), forming a complete HPV particle (63,64). After the cuticle cells are shed, the next

Table I. Main detection methods for HPV nucleic acid molecules.

Detection methods	Characteristic	Application	(Refs.)
Southern blot	Obtaining high-quality HPV information	Laboratory method of assessment	(100-103)
Northern blot	Obtaining high-quality HPV information	Laboratory method of assessment	(102,103)
Reverse dot blot	Expeditionousness, simplicity, high sensitivity, and robust specificity	Genotyping, gene mutation, and pathogen detection	(100-101,105,106)
<i>in-situ</i> hybridization	A high level of sensitivity and is capable of detecting low abundance mRNA expression	Widely utilized for the detection of HPV nucleic acid molecules in tissues due	(104,107-112)
Hybrid Capture II	High sensitivity, good specificity, repeatability, and objectivity	Most commonly utilized approach for clinical detection	(100-101,113-117)
Polymerase Chain Reaction	Instrumental in assessing HPV DNA expression and ultimately determining clinical HPV infection	Due to the inherent instability of RNA molecules, their utilization in HPV detection is infrequent	(104,118-119)
Microarray technology	Capable of detecting and analyzing a vast number of specimens simultaneously, boasting high sensitivity, minimal sample requirements, rapid detection speed, high throughput, and reduced environmental contamination	The cost of gene chip technology remains relatively elevated	(100-101,121-122)

round of infection is carried out, which forms a complete HPV life cycle.

As for the second scenario of HPV infection, the E2 gene fragment is missing, and the expression of the E6 and E7 genes is also dysregulated. At this point, the L1 shell protein ceases to be expressed (65), frequently leading to the development of invasive cancer.

5. HPV transmission mode and risk factors for cervical susceptibility to infection

The primary modes of HPV transmission include: Firstly, sexual transmission, wherein individuals are susceptible to HPV infection during sexual contact with individuals carrying HPV (66,67). Secondly, vertical transmission between a mother and child may occur; during birth when the baby comes into close contact with the birth canal of the pregnant mother infected with HPV, thereby leading to HPV infection (68-70). Thirdly, close contact with HPV-infected individuals may result in infection. Fourthly, indirect contact, primarily through contact with the daily necessities and clothing of infected individuals, may lead to HPV transmission (68). Finally, infection during medical procedures, which refers to the transmission of HPV between medical personnel and patients due to inadequate protective measures during the course of treatment (20,71).

The risk factors associated with susceptibility to HPV infection in the cervix primarily include: Firstly, sexual activity. Early initiation of sexual intercourse, a high frequency of sexual encounters, multiple sexual partners and a lack of protective measures during sexual intercourse may contribute to the acquisition of HPV infection in the cervix (72,73). Additionally, the relatively young age of individuals, coupled

with the immaturity of the human cervical epithelial tissue, renders the cervical epithelium susceptible to repeated HPV infections and other influencing factors. Consequently, the potential for cellular variation arises, which, if left unchecked, may progress to the development of cancer (74). A relatively active sexual life can also increase the susceptibility to HPV infection, if accompanied by the lack of good sexual hygiene habits (54,75,76).

Secondly, individuals with low immune function experience a decline in their ability to clear the virus, leading to persistent infection and an increased occurrence of cervical lesions (77). Thirdly, studies have indicated a correlation between sex hormone level disorders and higher HPV detection rates (78,79), with some research suggesting that the use of sex hormone drugs by menopausal women may also contribute to an increased risk of cervical HPV infection (80-82). Fourthly, genetic factors (9,83). Cervical cancer has been observed to exhibit familial clustering, with a higher susceptibility to HPV infection observed among individuals within particular families (75,84).

Fifthly, age, education level and socioeconomic status all play a role in the susceptibility to HPV infection (85,86). Individuals with higher education levels and socioeconomic status tend to have access to abundant medical and health resources, possess a greater understanding and command of relevant medical and health knowledge, and adhere to regular medical and healthcare measures (72). Consequently, this particular group exhibits a lower susceptibility to HPV infection compared with other groups. Moreover, studies have indicated that over 85% of cervical cancer cases worldwide occur in developing countries, thereby underscoring this phenomenon (87,88). Finally, other risk factors for cervical susceptibility to HPV infection, such as smoking, pregnancy and age, remain to be explored.

6. Methods of HPV detection

Due to its strict tissue and species restrictions, the complete virus cycle and infectivity of HPV are limited to human epithelial tissue, and cannot be cultured *in vitro*. Consequently, the detection of HPV has been primarily reliant on serological, histological and molecular biological methods (89,90). However, the serological test for HPV lacks the ability to differentiate between recent and late infections, and its accuracy is suboptimal (91,92). Regarding the histological identification of HPV, the identification of vacuolated or keratinized cells in the spinous layer serves as the primary indicator of HPV infection (93,94). The conventional Pap smear, which is commonly employed for HPV detection, possesses a high false-negative rate despite its effectiveness in early-stage cervical cancer screening and its role in the significant reduction in the incidence of cervical invasive cancer (95,96). Although thin layer liquid-based cytology has replaced the Pap smear, its specificity and sensitivity remain suboptimal (97,98). Consequently, the accurate diagnosis of HPV relies on molecular biological techniques.

In the molecular biological detection of HPV, HPV nucleic acid molecules (DNA and RNA) and viral proteins are commonly utilized as indicators for detection. The detection methods for HPV virus proteins include immunohistochemistry, electron microscopy, western blotting and others. These methods are not only complex, but also require a significant amount of time (99). Notably, HPV nucleic acid molecular detection methods include Southern blotting, northern blotting, reverse dot blot hybridization, *in-situ* hybridization, hybrid capture (HC)II, polymerase chain reaction (PCR) and microarray technology (100,101) (Table I).

Southern blotting is utilized to detect DNA molecules, while northern blotting detects RNA molecules, with the aim of identifying HPV through gel electrophoresis, which facilitates HPV typing (102,103). Despite the potential for obtaining high-quality HPV information, the utilization of these two methods necessitates a considerable quantity of highly purified nucleic acid molecules, resulting in a time-consuming and cumbersome process. Additionally, hybridization is conducted in the solid phase, necessitating strict preservation conditions for nucleic acid molecules to maintain their ideal state, rendering it unsuitable for clinical detection, and limiting its use to a traditional laboratory method of assessment (15).

The conventional method of dot hybridization involves immobilizing target DNA and subsequently hybridizing it with a labeled probe to facilitate color development (104). Conversely, the reverse dot hybridization approach entails immobilizing a labeled probe and subsequently hybridizing it with the target DNA to achieve color development (105). In comparison to forward dot hybridization and gel electrophoresis, reverse dot hybridization exhibits certain benefits, such as speed, simplicity, high sensitivity and robust specificity, particularly regarding genotyping, and gene mutation and pathogen detection (106).

The application principle of *in-situ* hybridization involves the pairing of radioactive or non-radioactive foreign nucleic acid, referred to as a probe, with the DNA or RNA to be examined on tissues, cells or chromosomes (107). This technique

is utilized to determine the presence of HPV infection in the body and to perform HPV typing (108). Notably, fluorescence *in-situ* hybridization enables the mapping of the precise location of HPV DNA in the cell chromosome, which is crucial for predicting the integration of HPV DNA with the host cell chromosomes (109). The detection of HPV nucleic acid molecules through *in-situ* hybridization exhibits a high level of sensitivity and is capable of detecting low abundance mRNA expression. Despite the potential for genotyping errors, this method remains widely utilized for the detection of HPV nucleic acid molecules in tissues due to its high sensitivity and specificity (110-112).

The HC method operates on the fundamental principle of utilizing a sensitive chemiluminescence signal amplification system and an efficient liquid phase technique for the typing and quantitative analysis of HPV DNA (113,114). Currently, there are three hybridization capture methods: HCI, HCII and HCIII (115). The HCII method is the most commonly utilized approach for the clinical detection of HPV due to its high sensitivity, good specificity, repeatability and objectivity (116). By contrast, the HCI method can detect nine high-risk HPV types with limited sensitivity, but strong specificity and positive prediction. HCIII, on the other hand, is incapable of typing HPV DNA (117). Notably, HCII has been approved by the US Food and Drug Administration and offers several advantages, including suitability for automated operation, no requirement for a strict laboratory environment or complex laboratory skills and no gene amplification, thereby eliminating the possibility of cross-contamination (116).

The fundamental concept of PCR involves the amplification of a specific DNA segment to identify HPV. PCR methodologies include conventional PCR, real-time fluorescent quantitative PCR, reverse transcription PCR (RT-PCR) and the amalgamation of PCR and *in-situ* hybridization (104). Despite the heightened sensitivity of conventional PCR detection, the potential for sample or reagent cross-contamination may result in false positives and other problems (118,119). Real-time fluorescent quantitative PCR technology represents an improvement over conventional PCR technology, offering superior sensitivity, accuracy, reduced contamination and quantitative detection capabilities (120). Consequently, the utilization of this technology in HPV detection is gaining traction. RT-PCR is a viable method for detecting HPV RNA, and is instrumental in assessing HPV DNA expression and ultimately identifying clinical HPV infection. However, due to the inherent instability of RNA molecules, they are infrequently used in HPV detection.

Microarray technology involves the immobilization of numerous probe molecules onto a solid phase support, allowing for typing and quantitative detection of HPV DNA samples through nucleic acid molecular hybridization pairing (121,122). In accordance with the principles of Southern and northern blotting, these techniques employ established nucleic acid sequences and complementary target sequences to conduct qualitative and quantitative analyses based on hybridization signals (123). Gene chip technology, on the other hand, is capable of detecting and analyzing a vast number of specimens simultaneously, boasting high sensitivity, minimal sample requirements, rapid detection speed, high throughput

and reduced environmental contamination (124). However, the cost of gene chip technology remains relatively high.

7. Conclusion

HPV infection is a major risk factor for the development of cervical cancer. The clinical surveillance of HPV plays a crucial role in the prevention and diagnosis of cervical cancer. Molecular assays are the gold standard for HPV identification. The aim of the present review and the clinical material available is to determine the optimal method for HPV detection. The present review aims to provide a comprehensive overview of the existing HPV detection methods, including their principles, application environments and clinical value, to provide a reference for the detection of HPV in clinical practice, with the ultimate aim of reducing the incidence and mortality of HPV-related diseases. Further investigation is required to clarify the role of molecular HPV testing in current primary cervical screening programs. With the continued development of detection technology, low-cost methods with high versatility, operability, and improved sensitivity and specificity will be needed for the early diagnosis of cervical HPV infection.

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Competing interests

The authors declare that they have no competing interests.

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