Review

Relationship between human papillomavirus infection and cervical cancer progression

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Abstract

Cervical cancer is one of the most common malignant tumors that threaten women's life and health in both developed and developing countries. Diet and smoking are independent etiological factors of cervical cancer. Among many independent etiological factors, human papillomavirus (HPV) infection is the most important one. HPV infection is universal among women of appropriate ages, and only persistent HPV infection is considered necessary to cause cervical cancer.

HPV infection and cervical cancer

Previous studies tend to equal HPV infections with cervical cancer, which originates mainly from the misconception of the two HPV molecular epidemiologic data supported by Merck & Co. and GlaxoSmithKline. The study results of these companies showed that HPV nucleic acids are present in 99.7% or 99.8% of cervical cancer samples. Although a large number of studies have shown that high-risk HPV can be detected in more than 90% of cervical squamous cell carcinomas, the above molecular epidemiological data cannot ideally support the conclusion that HPV infection leads to cervical cancer due to limited understanding of the history of HPV infection. A considerably precise statement should be that HPV nucleic acids have been detected in the majority of cervical cancer samples. To date, some cell lines from cervical squamous cell carcinoma, such as C33A cell lines, are HPV-negative. However, combined results of cohort studies ascertained that HPV infection is a significant etiological factor of cervical cancer.

HPV infection is generally observed in women who had sex. Approximately 70%-80% of women suffer from at least one HPV infection in their lifetime. HPV infection rate declines with age and reaches the peak after 8-10 years of the first sexual intercourse. In the United States, among all 20-24-year-old females, HPV infection rate can exceed 40%, and the rate is 5%-15% among all women. Although HPV infection is common, the incidence rate of cervical cancer is not high in developed countries, thereby indicating that cervical cancer caused by HPV infection may be a considerably small probability event^[1]. For example, in

Europe and the United States, the incidence rate of cervical cancer per year is less than 10/100000. Considering 10% as the HPV infection rate in the whole population, about 10000 women of the 100000 people are HPV-infected. Nevertheless, according to this incidence rate, only less than 10 people from the 10000 women will progress to cervical cancer. Accordingly, 99.9% of HPV infection will not progress to cervical cancer. Therefore, transient infection is the main reason among HPV-infected patients. In addition, a large proportion of cervical intraepithelial neoplasia (CIN) patients will self-clear HPV.

In China, World Health Organization (WHO) figures have long been used for the incidence rate of cervical cancer per year, that is, 500000 new cervical cancer patients annually worldwide. On the basis of its population weight, 100000 new cervical cancer patients are estimated in China each year. For this estimated value, we adopted two more reliable figures to re-estimate. According to the two cancer screening projects in Beijing, the annual incidence rate of cervical cancer in Beijing is 18.5/100000. In addition, Sun *et al.* estimated that the annual incidence rate of cervical cancer in the suburbs of Wuhan is 40/100000. The census in December 31, 2005 showed that the rural and urban population proportions are 57.01% and 42.99%, respectively, where the total female population is 633190000. Considering 40/100000 as the annual incidence rate of cervical cancer among the women living in China's rural areas, 18.5/100000 as the number of women living in China's urban areas, and 400 million as the number of women of appropriate ages, we can speculate that over 120,000 women suffer from cervical cancer annually. These two rates represent the urban and rural areas with

improved disease control. Therefore, the above 120000 is an underestimated figure.

Natural history of HPV infection

The natural history of HPV infection is not yet clear. This ambiguity is reflected in the unclear definition of a number of commonly used virology terms in HPV, including self-clearing, latent infection, and persistent infection.

In the majority of cases, the virus will be cleared due to the establishment of the HPV immune mechanism. According to a congress report from the United States CDC in 2004, more than 90% of the women infected with HPV are negative in the follow-up HPV test within two years. The transition to being HPV-negative shows that HPV self-clearing occurs in the body, which takes an average time of six months. Therefore, HPV infection itself cannot be equal to tumor progression. The survey results of HPV molecular epidemiology of general patients also strongly supported this point. Ostor showed that HPV transition rates are 57%, 43%, and 32% among CIN 1, 2, and 3 patients, respectively. This result also showed that CIN3 patients also exhibit close to 1/3 possibility of HPV self-clearing. In summary, HPV self-clearing is the most frequent phenomenon that occurs in HPV-infected patients; it also occurs in a large proportion of CIN patients.

HPV self-clearing remains to be further studied mainly because we cannot distinguish HPV virus self-clearing from latent infection. Many viruses, such as hepatitis B virus and herpes simplex virus, also display an incubation period. During this period, detecting the replication of the virus and the expression of the viral coding genes often impossible. However, determining whether an incubation period also occurs for HPV is difficult due to our limited understanding of the natural history of its infection. Hence, distinguishing HPV's self-clearing from its latency is also difficult. Furthermore, the standard definition of self-clearing should be explored. For example, the HPV detection threshold set by QIAGEN's Hybrid Capture II (HC II) is relatively high. Thus, many reports indicated that HPV (including high-risk types) can be detected in HC II-negative samples. Consequently, some scholars are opposed to the use of HC II-negative results when referring to HPV self-clearing. Similar situation occurs in HPV detection using the PCR method. For example, when a system where only one reaction can detect at least 1,000 HPV copies is used, and the HPV is found negative, the HPV load in the sample often still exists, but the detection system cannot detect it. Accordingly, the

detection plan with high sensitivity should be preferred in the assessment of HPV self-clearing^[2].

The complete clearing of HPV is more common than the persistent HPV infection, which is less common and should be accounted for less than 10% of all HPV infections. Persistent HPV infection refers to the long-term HPV infection that can be detected. In addition, persistent HPV infection cannot be easily delineated. According to the most recent field epidemiological and clinical research, persistent HPV infection refers to the type of HPV that is detected the same twice or more during a certain period of time. Some scholars also emphasized the variant of the same HPV type, for example, a continuous observation of HPV16 Asian-American type (Asian-American variant, referred to as AA type) variant. Such detection poses a significant challenge to clinical testing because the HPV nucleic acid detection protocol captured by the traditional hybrid cannot cope with the detection, and the current HPV typing test is not sufficient to satisfy the test requirements. Only the laboratory protocol based on sequence analysis can cope with the monitoring of persistent HPV infection. Many controversies exist about the interval between two adjacent HPV detections, which range from 2 months to 7 years in practice; nevertheless, most scholars adopt 6-12 months as the interval between two HPV detections. However, the adoption of this time interval still lacks support from evidence-based medicine. Evidently, the short detection interval results in the large number of tests and easy determination of persistent HPV infection. The experimental data showed that the infection duration of different HPV types plays a different role in promoting tumor progression. Furthermore, variants of the same type, such as the HPV16, show completely different effects. Tumor progression requires eight years of persistent infection for HPV16 European prototype. The AA type, which also belongs to HPV16, only needs 2-3 years. Given that tumor progression requires different persistent infection periods for different HPV types, the shortest duration of HPV infection for different types should be defined by actual on-site epidemiological work. In addition, Schlecht *et al.* found in their persistent HPV infection and persistent SIL study in São Paulo, Brazil that when high-risk HPV is detected twice in a row but the types are different, the probability of persistent SIL occurrence is lower than that of the low-risk HPV infection of the same type. Moreover, high-risk persistent HPV infection of the same type for three consecutive times is more dangerous to persistent SIL than that of persistent HPV infection with different types. This result strongly supported the importance of HPV typing

protocol in screening.

In the assessment of persistent HPV infection, the HPV type should be identified, and the HPV viral load and integration status of HPV virus genome in the host chromosome should be detected.

Viral load and persistent HPV infection

The determination of viral load was once considered an effective means of predicting cervical intraepithelial neoplasia and cervical cancer progression mainly because early scholars believed that high viral load can increase the likelihood of persistent HPV infection. However, recent studies showed that simple determination of viral load often misleads the predictions of the above progression^[3]. When HPV is tested, the viral load of HPV is tested intentionally or unintentionally by means of the previously mentioned HC II, typing detection, and non-typing fluorescence quantitative PCR detection. Remarkably, the determination of viral load is important to the prediction of most disease progression.

For example, the detection threshold of the HC II is based on the detection results of a large number of clinical samples; the threshold is also set at the sample detection value above CIN2. Thus, the high RI/CO value often results in the high HC II level. Nevertheless, this consistency is limited to statistical significance. For some specific cases, HCII measurements may not predict disease progression and may lead to opposite predictive results. For example, the virus load detection value of HPV-integrated samples is often considerably low, but the possibility of persistent HPV infection for such patients and the progression to cervical cancer is significantly high.

The relationship between viral load and HPV infection and cervical cancer progression is considerably complex. Previous longitudinal studies often used a certain HPV viral load as the baseline for the population's HPV load. Recent studies showed that the acquisition of new infection HPV type is associated with both viral load and new CIN damage. In addition, different types of HPV exhibit different virus load baselines in the population. For example, HPV16 and HPV18 both statistically possess a viral load baseline, clinical samples above this baseline can be predicted as above CIN2, and those below this baseline are not likely to progress above CIN2. Accordingly, the viral load baselines of those above 16 and 18 types may differ remarkably. Therefore, the viral load baseline of a certain type of HPV that presents statistical significance from the perspective of evidence-based medicine

must be obtained.

Furthermore, different HPV types are not related with the severity of cervical diseases. Most studies also often carry out viral load on a range of high-risk HPVs without screening the types. The HPV16 load relates with the progression of the disease, but HPV18 shows no similar pattern. The viral load detection method for different HPV types will result in an underestimated HPV18-related cervical intraepithelial neoplasia level, which explains why some countries can control squamous cell carcinoma but display a poor control of adenocarcinoma. Hence, the viral load study must be based on typing and integration detections. Only this manner can provide effective information about the natural history of HPV infection and explain the relevance of HPV load to diseases.

Finally, the detection of the expression of HPV oncogenes is another issue. Available data showed that the expression of two HPV oncogenes, namely, E6 and E7, is the key to maintaining persistent infection. Commonly, the high expression of these two oncogenes negatively regulates virus replication. As an effective means of monitoring viral replication, viral load detection still exhibits several shortcomings in predicting persistent infection.

HPV integration and persistent infection

HPV integration refers to the HPV genome embedded in a specific place on the host chromosome. In general, only one chromosomal integration site exists in the tumor cells; this feature is consistent with the fact that cervical cancer is a clonal disease. The majority of cervical lesion is HPV in the form of appendages, and the minority is integrated and mixed (both in the form of appendages and integrated) HPV. HPV integration often occurs in the E1 and E2 genes at the downstream of the carcinogenic genes E6 and E7 (both genes belong to the same operon). This feature determines the detection method of HPV integration in recent years. Generally, the transcription products of integrated HPV gene are more stable than that in the form of appendages. A large number of research results also confirmed that HPV16 integration favors the growth of selective cells in the host.

The determination of the early integration position is primarily performed by DNA blotting hybridization (Southern blots), which is also considered the most accurate method. However, given that the method uses conventional hybridization techniques, detecting integration is difficult when the mean viral load is low. In recent years, DNA-

based detection of integrated papilloma virus sequences by ligation-mediated PCR assay and the amplification of papilloma virus on cogene transcript test are often used and applied in commercial kits.

HPV integration can occur on all chromosomes, including sex chromosomes. Additionally, the integration sites show no regularity. HPV integrations often occur at fragile sites, which are sites on the chromosome that can easily break. In addition, about 10% of the integration occurs in the 8q24. The collected integration sites are from cervical cancer cells. Therefore, the correlation between HPV integration site and tumor progression cannot be inferred. In recent years, many scholars compared the number of E2 and E6 copies in the sample by applying real-time fluorescent PCR to determine the integration of HPV. As in most cases, HPV integration will cause the lack of E2. Accordingly, zero number of E2 copies indicates that only integrated HPV is present in the sample. When the numbers of E2 and E6 copies are equal, only HPV in the form of appendages is present in the sample. If the number of E2 copies is not zero and is smaller than that of E6 copies, then mixed types of HPV exist in the form of mixed types in the sample. Although this method presents errors due to the amplification efficiency of E2 and E6, majority of scholars have accepted the reliability of such data.

HPV integration can also be observed in the sample of patients who do not have cervical disease. Although this is a rare case, this observation shows that using HPV integration as a predictor of CIN or cervical cancer cannot be fully established. In addition, two problems exist when HPV integration is used as an indicator of disease progression: (1) in the mixed type, the existing detection method cannot confirm whether the sample contains the integrated HPV when the proportion of the integrated type is low. The traditional sequence analysis method can determine integration only when the ration of integrated type to appendages type is 1:10. The resequence analysis method can only detect the 1:100 relationship. Therefore, the detection method also restricts the detection of the integrated type. (2) In the sample of CIN3 patients with integrated HPV, transcriptional products activated by integration are detected only in a small proportion of the sample. This observation showed that the detection method based on oncogene transcription presents only limited prognostic use; it cannot provide accurate predictions of disease progression for each individual.

Regarding the role of integration, many scholars supported that integration is related with disease progression

mainly because, from the perspective of molecular mechanism, integrated HPV can easily escape the self-cleaning mechanism of the immune system, which is the most effective means of providing persistent HPV infection.

Other factors and persistent HPV infection

HPV, as a small DNA virus, possesses structural proteins L1 and L2 in its genome encoding genes and other genes that only function when interacting with the host. Among these genes, only E1 and E2 are involved in virus replication; these genes also both function through trans-acting on the viral genome itself, thereby inhibiting the replication of the viral genome. Thus, HPV virus replication should take over the replication mechanism of the host. The gene expression of E6 and E7 is affected by the LCR region, which shows promoters and the E1 and E2 proteins, and the methylation of the E6 and E7 genes. Hence, in the mechanism of maintaining persistent infection, these mutant sections are associated with methylation^[4].

In addition, recent study on the correlation between HPV and high-grade CIN, in particular HIV-HPV co-infection, showed that the prognosis of high-grade CIN and its progression to cancer are independent of immune status. Therefore, in the event of a high-grade CIN, other similar host genetics changes may play a remarkably direct role in the progression of cancer. According to this perspective, the host genetic changes should exert certain significance to maintain persistent HPV infection.

In summary, many aspects regarding the persistent HPV infection and its clinical significance are still unclear, and they need further study. We believe that with the gradual establishment of HPV monitoring around the country by the Chinese Center for Disease Control and Prevention, we can establish a comprehensive triage program based on HPV persistent infection in a relatively short period.

Declarations

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No.

Competing interests

The author declare that they have no competing interest.

Authors' contributions

MC Ai made the literature analysis and wrote, discussed and

revised the manuscript of this review.

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