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Current Status and Prospects of Diagnosis and Intervention for HR-HPV Persistent Infection

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Abstract: Persistent infection with high-risk human papillomavirus (HR-HPV) is the core pathogenic factor of cervical cancer (CC). Although HPV vaccination is an effective primary prevention method for CC, the global vaccination rate is generally insufficient (target population vaccination rate in China < 5%), far from meeting the requirements for herd immunity (80%) and the WHO target (90%). However, only about 10% of HR-HPV infections progress to persistent infections. Therefore, identifying and intervening in the "HR-HPV persistent infection" population can systematically narrow the scope of prevention and control, reduce prevention and control costs, and provide a new path for low-income countries to explore suitable prevention and control models for CC. Based on this understanding, the team has pioneered a systematic method for identifying HR-HPV persistent infections and a tiered intervention system based on drug classification, which has achieved good results in both basic research and clinical observations. This article will summarize the current research status of "HR-HPV persistent infection" in relation to CIN and CC, as well as the team's relevant concepts and research results, to provide a reference for the identification and intervention of "HR-HPV persistent infection."

Keywords: HR-HPV; Persistent infection; Diagnosis and intervention; Cervical cancer prevention

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1. Introduction

Human papillomavirus (HPV) infection has become a consensus in the academic community that, especially persistent infection with high-risk HPV (HR-HPV), leads to cervical cancer (CC). CC severely affects patients' quality of life and health, and is one of the major public health issues globally, as well as the primary burden of HPV-related diseases worldwide. The prevention and control model for CC is divided into a three-tier prevention system. Among them, the HPV vaccine, which has been available for many years, has not achieved an optimistic vaccination status. Taking China as an example, the vaccination rate among target populations is still less than 5%, far from meeting the requirements for achieving herd immunity (80%) and the WHO vaccination target (90%). This leaves the vast majority of unvaccinated individuals and some who have failed

to be protected at risk of HPV infection and developing CC, seriously affecting the actual effectiveness of the HPV vaccine in preventing CC.

The HPV infection rate among the general female population in China is approximately 17.70%, with an HR-HPV infection rate of about 13.12% [1]. Only about 10% of HR-HPV infections will progress to persistent infections, and "persistent HR-HPV infection" is the primary factor leading to cervical cancer (CC). Therefore, by focusing solely on the diagnosis and intervention of "persistent HR-HPV infection," it is possible to systematically narrow the intervention population, reduce prevention and control costs, and explore a prevention and control model for CC that is suitable for low-income countries.

In recent years, "persistent HR-HPV infection" has received increasing attention from the academic community. Relevant literature and academic conferences have demonstrated the correlation and research progress of persistent HR-HPV infection with cervical intraepithelial neoplasia (CIN) and cervical cancer (CC) at different levels and in different fields [2]. Our team has also been deeply involved in this field for many years, conducting extensive research on the diagnosis and intervention of persistent HR-HPV infection, ranging from basic to clinical levels. We have proposed a systematic diagnostic method, a drug classification system, and a hierarchical intervention concept based on lactic acid bacteria. This article provides a review of the current research status of "persistent HR-HPV infection" in relation to CIN and CC, as well as our team's relevant concepts and research results. The aim is to provide a reference for the diagnosis and intervention of "persistent HR-HPV infection," reduce or avoid the widespread over-intervention of HPV in clinical practice, alleviate the economic burden and psychological anxiety of infected individuals, and offer insights for low-income countries in exploring appropriate prevention and control models for CC.

2. HPV infection and cervical cancer

HPV is a small, circular, double-stranded DNA virus that prefers complex squamous epithelium, with a size of approximately 8kb and no envelope. It is widely present in nature. Currently, over 200 different subtypes have been identified, of which more than 60 subtypes are associated with infections of the reproductive tract system. The International Agency for Research on Cancer (IARC) classifies HPV subtypes and the risk of inducing cervical cancer (CC) into low-risk HPV (LR-HPV) subtypes and high-risk HPV (HR-HPV) subtypes. Low-risk subtypes mainly cause various warts, while high-risk subtypes can lead to various cancers, such as CC, vulvar cancer, vaginal cancer, oropharyngeal cancer, etc. Among them, CC is directly related to persistent HR-HPV infection and is the primary burden of HPV-related tumors globally.

The infection rate of HR-HPV among women in China is 13.12% [1]. Studies have shown that approximately 80% of women will be infected with at least one HPV subtype during their lifetime [3], which is particularly prevalent among sexually active individuals. Most HPV infections are transient, with about 90% of infections being cleared by the body's immune system within 1-2 years without any intervention [4]. However, a portion (about 10%) of infections, especially HR-HPV infections such as HPV16 and HPV18, persist, and persistent infection is closely associated with the development of various malignancies [5]. Persistent HR-HPV infection can lead to the development of normal cervical tissue into CIN and eventually progress to invasive cancer [6]. Although persistent HR-HPV infection is a necessary condition for the development of CC, it is not the only condition, suggesting that other factors, such as vaginal microbiota and immune status may also play a role in cancer susceptibility and progression [7].

3. Persistent HPV infection and cervical cancer

Persistent infection with HR-HPV is the primary pathogenic and initiating factor in the development of cervical cancer (CC). The main mechanism involves HPV interfering with host cell cycle regulation through oncogenic proteins E6 and E7. E6 protein induces the degradation of p53 tumor suppressor protein, while E7 protein leads to the inactivation of retinoblastoma (Rb) protein, collectively promoting cell immortalization [8]. During persistent infection, HPV also evolves sophisticated mechanisms to evade immune surveillance and evoke host immunity, thereby maintaining the stability of the viral genome. Furthermore, HPV infection often occurs in the transformation zone of cervical squamous epithelium and columnar epithelium, where the specific microenvironment provides favorable conditions for persistent infection. The complex interplay between HPV and the host constitutes the molecular basis for its persistent infection and further progression to malignant tumors.

It has become a consensus in academia that persistent HR-HPV infection leads to cervical cancer (CC). Accurate diagnosis is the prerequisite for intervention and treatment. Therefore, accurately determining the status of persistent HPV infection is crucial for risk stratification and clinical management of CC. However, to date, there is no unified standard for defining persistent HPV infection. Currently, commonly used time thresholds in clinical studies include 6 months, 12 months, and 24 months [9]. Studies have shown that the duration of HPV infection is positively correlated with the risk of CC transformation. When the infection persists for more than 12-24 months, the risk of carcinogenesis increases significantly. Additionally, HPV viral load is also considered an important indicator for assessing disease severity and clinical prognosis [9]. However, current guidelines do not require routine HPV testing for newly diagnosed CC cases, which may lead to insufficiently precise risk stratification and management for some cases.

Due to the lack of concepts and methods for judging “persistent infection” of HPV, there is a phenomenon of including “transient infection” in both clinical research and clinical treatment, which inevitably leads to widespread deviations in research results and excessive clinical intervention, affecting the rigor of research and increasing the economic burden and anxiety of patients [7]. Although current guidelines do not mandate HPV testing for newly diagnosed CC, a large body of evidence indicates that only a minority of HR-HPV infections (about 10%) will develop into persistent infections, which is the main cause of CC. Therefore, distinguishing between transient and persistent infections can significantly enhance the predictive value of cervical lesion progression. Especially in regions with limited medical resources, establishing a reliable basis for judging persistent infections is particularly important, as these areas often struggle to implement standardized CC screening. Secondly, from a public health perspective, intervening only on individuals identified as having persistent infections can avoid overtreatment of those with transient infections. Furthermore, new strategies for developing specific treatments targeting persistent infections, such as targeted therapies targeting E6/E7 oncoproteins, also require accurate judgments of persistent infections as a prerequisite. Therefore, establishing a unified and standardized diagnostic criterion for persistent infections is of great significance for optimizing CC prevention strategies and should become an important part of the CC prevention and control system.

4. Detection techniques and judgment methods for persistent HPV infection

Persistent HPV infection typically refers to the state where HR-HPV persists in the cervix (or other infected sites) for a duration exceeding a certain time threshold and is not naturally cleared by the human immune

system. Currently, it is mostly defined as the detection of the same type of HPV in cervical exfoliated cells from two consecutive tests, separated by 6-12 months, in the same individual[10]. Currently, commonly used HR-HPV detection techniques in clinical practice mainly include DNA-level PCR typing detection, RNA-level mRNA detection, and epigenetic marker methylation detection. PCR typing technology can accurately identify 14 high-risk HPV types through real-time fluorescent quantitative PCR. However, DNA detection cannot distinguish between active and latent infections, while HR-HPV E6/E7 mRNA detection can better differentiate between persistent and transient infections. Methylation detection shows good specificity (79.77%) in non-type 16/18 HR-HPV infections and is of great value in guiding clinical intervention. In addition, novel detection technologies such as the Xpert® HPV system achieve a sensitivity of 86.4% in detecting HR-HPV in urine samples, providing possibilities for non-invasive detection.

There is still significant controversy regarding the criteria for determining the duration of HR-HPV infection. Existing studies have adopted various standards such as 6 months, 12 months, and 24 months. Among women aged 18-26, the incidence of persistent infection lasting for 6 months is 35.5 per 1000 person-years, which is significantly higher than that of the 27-45 age group (29.0 per 1000 person-years). Longitudinal studies have shown that 37 out of 72 individuals infected with HR-HPV developed into a persistent infection state. It is worth noting that persistent infection lasting for 6 months is significantly associated with the risk of cervical intraepithelial neoplasia grade 2 or higher (CIN2+) (HR=2.31), while follow-up data for 24 months indicates that bacterial vaginosis (BV) may affect the persistent infection state of HR-HPV. These differences in time standards reflect varying understandings among different studies regarding the threshold for cancer risk associated with persistent infection.

Currently, there is no unified standard for determining persistent HPV infection both domestically and internationally. In light of this, our team, combining relevant research findings and new technological advancements, has taken the lead in proposing seven methods for determining persistent HR-HPV infection, namely[11]: 1. Patients with clear records of HR-HPV infection who remain non-negative for the same HPV subtype after a one-year follow-up; 2. Patients who were initially screened positive for HR-HPV and remain positive for the same HPV subtype after a one-year follow-up; 3. HPV typing and integration detection technology, with positive HPV integration; 4. Cervical tissue P16/Ki67 detection technology, with single or dual positive results; 5. Cell/tissue immunohistochemical broad-spectrum HPV-L1 staining detection technology, with negative detection results; 6. Gene methylation detection technology, with positive detection results; 7. HPV E6/E7 gene/protein detection technology, with positive detection results. These methods provide reference and guidance for the current determination of persistent HR-HPV infection.

5. Current status of intervention for HPV infection

Currently, the prevention of HPV infection is primarily achieved through vaccination, which is also one of the most powerful weapons in the WHO's strategy to eliminate CC. However, to date, the global coverage rate of HPV vaccines remains unsatisfactory, with significant vaccination gaps particularly in low- and middle-income countries. Furthermore, existing vaccines do not cover all high-risk types, and preventive vaccines are ineffective for those who are already infected.

For individuals with persistent HPV infection, active intervention is an important approach to prevent the infection from leading to CIN/CC. Currently, there are many methods used clinically to treat HPV infection, such

as pharmacotherapy, surgery, physical therapy, immunotherapy, etc. For those without concurrent cervical lesions (CIN, cervicitis, etc.), pharmacotherapy remains the primary clinical treatment method. Based on the mechanism of action of commonly used anti-HPV drugs in clinical practice, the team categorizes them into three types [12]:

(1) The first type includes immunomodulators or enhancers, such as interferon, Paiteling, Baofukang Suppository, etc., which can enhance the anti-HPV ability of local tissues and cells through local immune regulation.

(2) The second type consists of ligand-receptor drugs, such as Ruilintang, which achieve the goal of completely eliminating the HPV virus through specific antigen-antibody binding, forming a "cocktail therapy" of humoral immunity + cellular immunity + phagocytic immunity. Additionally, the positive-negative charge adsorption type belongs to another category of ligand-receptor drugs, including almost all anti-HPV biological protein gels, which are dressings containing β-lactalbumin and carbomer components. Their main mechanism of action is to form a protective gel film on the vaginal wall, isolating bacteria and viruses. The β-lactalbumin in the gel utilizes the effect of positive and negative charges to destroy the protein structure of HPV, promoting the inactivation of HPV. Carbomer encapsulates the inactivated HPV and facilitates its smooth excretion from the body, thereby eliminating the HPV infection in the body. Studies have shown that the long-term negative conversion rate of anti-HPV biological protein dressings in the treatment of HPV infection is superior to that of recombinant human interferon alpha-2b gel.

(3) The third type includes gene therapy drugs, such as Ruibeisheng, which eliminate the invading HPV virus by removing adenine from the HPV virus DNA structure, hindering the binding of ribosomes and protein synthesis elongation factor 2 (EF-2). Clinical studies have reported that all types of drugs have certain anti-HPV effects, and some drugs also have the effect of treating CIN.

Clinically, almost all drugs for treating HPV infection are administered vaginally. Prolonged vaginal administration may alter the vaginal microenvironment, leading to "vaginal microecological imbalance" and resulting in various manifestations of vaginitis, thereby reducing patients' treatment experience, even disrupting treatment and affecting the continuity and ultimate effectiveness of therapy. Therefore, in prolonged vaginal administration therapy, maintaining or correcting vaginal microecological imbalance is a crucial clinical consideration, which requires the intervention of exogenous lactic acid bacteria (bacilli/cocci). On the other hand, numerous studies have shown that a high proportion of women with HPV infection, especially those with persistent HR-HPV infection, exhibit vaginal microecological imbalance. This condition is observed in almost all types of vaginal microecological imbalance, except for vaginal candidiasis, indicating a clear correlation between vaginal microecological imbalance characterized by reduced lactic acid bacteria and persistent HPV infection. This suggests that the combined use of lactic acid bacteria preparations in the treatment of HPV infection through vaginal administration can not only exert its anti-HPV effect but also correct or maintain vaginal microecological imbalance, achieving a dual purpose. Based on this understanding, our team proposed the "Lactic Acid Bacteria Three-Pronged Approach" several years ago as a methodological system for graded intervention in persistent HPV infection.

6. Lactic acid bacteria and their functions

Lactic acid bacteria (LAB) are a general term for a group of bacteria capable of utilizing fermentable carbohydrates

to produce large amounts of lactic acid. They are widely distributed in nature and exhibit rich species diversity. LAB belongs to the Gram-positive bacteria category, typically does not form spores, and reproduces through binary fission. They are primarily divided into the genera Lactobacillus and Coccus based on morphology.

Except for a very small minority, the vast majority of LAB are essential and have important physiological functions in the human body. LAB are widely present in the human oral cavity, intestines, and vagina. Lactic acid bacteria in the vagina play a role in maintaining and repairing the vaginal microecology. Lactobacillus capsules for vaginal use made from live lactobacilli (brand name: Dingjunsheng) and lactobacillus vaginal capsules made from live enterococcus (brand name: Yanhua) have been widely used clinically for the treatment and repair of vaginal microecological disorders.

In addition to its role in maintaining and improving vaginal microecology, LAB also exhibits a certain anti-HPV effect. In recent years, the role of LAB in anti-HPV infection has received increasing attention. Relevant studies have shown that LAB forms a multi-level anti-HPV defense network through mechanisms such as microecological remodeling, immune activation, and epigenetic regulation, playing a preventive and adjuvant therapeutic role in HPV infection. The main mechanisms are as follows [13,14]:

(1) Acidic and antibacterial substances synergistic defense: LAB-metabolized lactic acid and acetic acid can stabilize the vaginal pH at 3.8-4.5, creating an acidic environment that is unfavorable for HPV survival. At the same time, the secreted hydrogen peroxide (H2O2) and bacteriocins (such as Lactocin 160, Reuterin) can directly destroy the HPV capsid protein structure and inhibit the fusion of the virus with host cells. Studies have shown that the bacteriocin produced by Lactobacillus crispatus can reduce the viral load of HPV16 by up to 67%.

(2) Immune regulation: LAB stimulates vaginal epithelial cells to secrete β-defensins, LL-37, and other antimicrobial peptides through the TLR2/4 signaling pathway, directly neutralizing HPV particles.

(3) Adaptive immune enhancement: LAB metabolites (such as lipoteichoic acid) can activate dendritic cells (DCs), promote the secretion of IL-12 and IFN-γ, and drive the differentiation of HPV-specific CD8+ T cells. LAB can enhance systemic mucosal immune responses by regulating the gut-vaginal axis.

(4) Competitive occupancy: LAB preferentially binds to the heparan sulfate proteoglycan (HSPG) receptors on vaginal epithelial cells through surface adhesins (such as Mub protein, S-layer protein), occupying and blocking the interaction between HPV L1 protein and host cells. Animal experiments have shown that colonization with L. crispatus can reduce the HPV infection rate in mice by 52%.

(5) Epigenetic regulation: LAB metabolites butyrate and propionate can upregulate the expression of host cell tumor suppressor genes (such as p53, p21) by inhibiting histone deacetylase (HDAC), while silencing HPV E6/E7 oncogenes, blocking the progression of cervical intraepithelial neoplasia.

(6) Biofilm protection: LAB forms a biofilm on the vaginal wall to resist the invasion of external pathogens. Nanoscale imaging technology research has confirmed that LAB biofilms can physically block HPV viruses from contacting epithelial cells and continuously release antimicrobial substances.

The aforementioned characteristics of LAB provide an evidence-based basis for the treatment of HPV infection by combining LAB with other anti-HPV drugs.

7. Lactic acid bacteria three-pronged approach

Based on the fact that LAB has dual effects of maintaining/repairing vaginal microecology and anti-HPV, the

team, after long-term non-systematic clinical observation and exploration, has proposed the concept and method system of "Lactobacillus Three-Pronged Approach" for the graded treatment of HR-HPV persistent infection, which is based on lactic acid bacteria and combined with other anti-HPV drugs with different mechanisms of action. Using Hela cells as a carrier, experimental studies were conducted on HPV18 E6/E7 gene expression and cell viability, and the following main research results were obtained [15,16,17]:

(1) LAB fermentation broth has a significant effect on down-regulating HPV18 E6/E7 gene expression in HeLa cells and inhibiting cell proliferation activity.

(2) Meifukang, Ruilintaka, and Ruibeisheng all have effects on down-regulating HPV18 E6/E7 gene expression in HeLa cells and inhibiting cell proliferation activity.

(3) The effect of LAB fermentation broth combined with three anti-HPV drugs is superior to that of single-drug treatment. This indicates that the idea of using LAB as a basis, combined with other anti-HPV drugs to treat persistent HPV infection, is feasible.

The "Lactobacillus Three-Pronged Approach" consists of three schemes:

(1) First-line scheme: LAB combined with immunomodulatory drugs;

(2) Second-line scheme: LAB combined with ligand-receptor drugs;

(3) Third-line scheme: LAB combined with gene therapy drugs. Currently, the team is conducting a regional multicenter study, hoping to objectively evaluate the effect of the Lactobacillus Three-Pronged Approach in treating HR-HPV persistent infection in the real world through multicenter and large sample data.

8. Conclusion and outlook

Cervical cancer (CC) prevention and control is a major public health issue of global concern. Persistent human papillomavirus (HPV) infection is the primary factor leading to CC. Therefore, blocking HPV infection is the first step in preventing CC. According to the early prevention strategy of the World Health Organization (WHO), HPV vaccination is one of the simplest and most effective means to prevent HPV infection, and it should be vigorously promoted, advocated, and implemented. However, to date, no vaccine has achieved 100% protection efficacy, and existing HPV vaccines cannot prevent all HPV infections. Even with vaccination, regular cervical screening is still necessary. In addition, according to data from the National Immunization Program Information Management System, from 2018 to 2020, the cumulative full-course vaccination rate of HPV vaccine among women aged 9 to 45 in China was only 2.24% [18]. The coverage rate in rural areas may be even lower due to economic and educational resource constraints, and the first-dose vaccination rate among girls aged 9 to 14 is only 4%, far below the WHO target (90%), hindering the achievement of herd immunity against HPV. Therefore, in the process of implementing primary prevention, scientific concepts should be upheld, and the protective effect of HPV vaccines should not be exaggerated. Overemphasizing the protective effect and vaccination compliance rate of HPV vaccines may, on the one hand, deviate from the scientific spirit; on the other hand, it may lead to a huge financial burden and commitment trap. Most HPV infections are transient (90%), and only a small percentage (10%) of HR-HPV can develop into a persistent infection. Intervening only in a small number of HR-HPV persistent infections may systematically reduce the intervention population and lower intervention costs. Therefore, in the absence of vaccine protection (non-vaccination) or in cases of protection failure, paying attention to the diagnosis and intervention of HR-HPV persistent infection is undoubtedly one of the effective measures to prevent cervical cancer. This should become an important

component of CC prevention and a distinctive feature of China's prevention and control efforts.

The "Guidelines for Cervical Cancer Screening in China (Part I)" has recommended HR-HPV nucleic acid testing as the primary screening method for cervical cancer (CC) [19], indicating that HPV is the preferred item for CC screening. Since persistent HR-HPV infection is the primary cause of CC, it is logically and from a preventive medicine perspective, completely correct and necessary to diagnose and intervene in persistent HR-HPV infection. Given that persistent HR-HPV infection accounts for only a small proportion, intervening solely on this may systematically reduce prevention and control costs, achieving optimal cost-effectiveness.

China is a populous country with relatively limited public health resources. With less than six years remaining before the WHO's global goal to eliminate cervical cancer (CC) is reached, there are obvious difficulties in achieving the "90-70-90" strategic targets, whether in terms of funding, awareness, or operational aspects. Therefore, if we can correctly examine the HPV vaccine from a scientific and practical perspective, and focus our limited funds, manpower, and financial resources on the diagnosis and intervention of HR-HPV persistent infection, three-tier screening for CC, and CIN management, it will be possible to systematically narrow the target population, reduce prevention and control costs, improve prevention and control effectiveness, and embark on a path of CC prevention and control that is suitable for China's national conditions.

CC prevention should have Chinese characteristics, wisdom, and solutions!

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