

Review

Research Progress on Biomarkers Associated with Cervical Cancer Caused by HPV Infection

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ABSTRACT

Cervical cancer(CC), a major global health burden for women, is closely associated with persistent infection with high-risk human papillomavirus (HR-HPV). Biomarker research is crucial for the precise prevention, early diagnosis, risk stratification, and treatment monitoring of cervical cancer. This article systematically reviews virus-derived, protein, epigenetic, and tumor microenvironment-related biomarkers associated with HPV-induced cervical cancer. By evaluating their diagnostic and prognostic value, this review provides insights to further improve the early diagnosis of cervical lesions.

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women globally. According to the International Agency for Research on Cancer (IARC), there were approximately 662,000 new cases and 349,000 deaths in 2022, with over 94% of these cases occurring in low- and middle-income countries (1). Persistent infection with high-risk human papillomavirus (HR-HPV) is widely recognized as the primary factor driving the development of cervical precancerous lesions and CC (2). The progression from initial HPV infection to CC typically spans 10 to 15 years, advancing from low-grade cervical intraepithelial neoplasia (CIN1) to high-grade CIN (CIN2 and CIN3), and ultimately breaching the basement membrane to become invasive CC. Therefore, early screening remains the most critical and effective strategy for CC prevention. Accurately identifying high-risk individuals and implementing timely interventions can substantially reduce CC incidence and mortality rates.

Biomarkers are critical in CC screening, serving as objective indicators of structural or functional alterations at the systemic, organ, tissue, cellular, and subcellular levels (3). Driven by recent advancements

in molecular biology, bioinformatics, and artificial intelligence, numerous novel biomarkers have been discovered and validated, providing new avenues for early screening and diagnosis. However, given the multitude of available biomarkers, it is essential to clarify their applicability, limitations, and translational potential. Therefore, this review aims to systematically evaluate the biomarkers involved in the progression from HPV infection to CC, providing a clear framework for optimizing screening strategies and advancing diagnostic and therapeutic approaches.

LITERATURE SEARCH STRATEGY

This review systematically assesses research on CC biomarkers associated with HPV infection. To ensure comprehensiveness, a multi-keyword search strategy was employed across major academic databases, including PubMed, Web of Science, and the China National Knowledge Infrastructure (CNKI). Search terms included the following key concepts and their synonyms:

- 1) HPV-related terms: "Human Papillomavirus," "HPV," and "HPV infection";
- 2) CC-related terms: "Cervical Cancer" and "Cervical Carcinoma";
- 3) Biomarker-related terms: "Biomarkers," "Markers," "Protein Biomarkers," "Epigenetic Biomarkers," and "Tumor Microenvironment Biomarkers";
- 4) Other relevant terms: "HPV DNA," "HPV RNA," "HPV E6," "HPV E7," "p16," "Ki-67," "DNA methylation," and "Non-Coding RNA."

The search timeframe spanned from January 1, 2005, to December 31, 2025, to capture the latest research while maintaining coverage of key studies in the field.

Retrieved references were imported into reference management software (EndNote or Zotero) for organization and deduplication. Subsequently, records were screened according to the following inclusion

criteria: 1) the study explicitly focused on HPV infection, CC, and biomarkers; 2) the publication type was an original research article, meta-analysis, conference abstract, or commentary; and 3) the article was published in a peer-reviewed journal.

OVERVIEW of HPV

HPV is a common virus mainly transmitted through sexual and close skin-to-skin contact. Most sexually active individuals will be infected with at least one type of HPV during their lifetimes. Notably, most HPV infections are transient and clear within 1 to 2 years. Only a small proportion of persistent HR-HPV infections may progress to precancerous lesions or CC over several years or decades. Based on nucleotide sequence homology, over 200 HPV subtypes have been identified (4). These are classified into high-risk and low-risk types based on their carcinogenic potential. HR-HPV subtypes, such as HPV 16, 18, 31, 33, 45, 52, and 58, are associated with the development of several cancers, including CC, anal cancer, and head and neck cancer (5). Low-risk HPV (LR-HPV) types, such as HPV 6, 11, 42, 43, 44, and 81, primarily cause benign lesions, including genital warts and recurrent respiratory papillomatosis.

The HPV life cycle is highly coupled with the differentiation cycle of host epithelial cells. The virus invades basal layer stem cells through minor cutaneous or mucosal abrasions. Its genome, in the form of circular double-stranded DNA, persists within the host cell nucleus as an episome (6). During early infection, HPV prioritizes the expression of early genes, such as *E1*, *E2*, and *E4–E7*. The *E6* protein forms a complex with *E6*-associated protein, targeting the tumor suppressor p53 for degradation; the *E7* protein competitively binds to the retinoblastoma (Rb) protein, inactivating Rb and releasing the transcription factor E2F (6). As infected cells differentiate toward the epithelial surface, the virus expresses the *L1* and *L2* capsid protein genes. The *L1* protein assembles into icosahedral viral particles, while *L2* assists in packaging the viral genome. During persistent infection, HR-HPV genomes may integrate into host chromosomes, often accompanied by the disruption or deletion of the *E2* gene. Because *E2* functions as a transcriptional repressor, its inactivation removes negative control over the *E6* and *E7* genes. The sustained expression of these oncogenic proteins exacerbates cell cycle dysregulation, the accumulation of DNA replication errors, and genomic instability, ultimately triggering malignant transformation (7). Furthermore, HPV infection

induces abnormal epigenetic modifications in the host genome. These modifications suppress tumor suppressor gene expression and promote the formation of a pro-tumorigenic microenvironment (8).

VIRAL-DERIVED BIOMARKERS

HPV DNA

HPV belongs to the genus *Papillomavirus* within the family *Papillomaviridae*. It is a non-enveloped, circular, double-stranded DNA virus with a genome size of approximately 8 kb (9). The viral genome consists of three primary regions: the early region (ER), the late region (LR), and the non-coding region (NCR). The LR encodes two open reading frames that produce the major and minor capsid proteins, *L1* and *L2*, respectively. The *L1* protein is highly conserved and immunogenic, making it the primary target for prophylactic HPV vaccines. The *L2* protein aids in viral genome entry into host cells and participates in viral particle assembly (10). As the most fundamental virus-derived biomarker, HPV DNA provides direct and critical information regarding infection status. Its detection allows for the confirmation of infection, the identification of specific viral genotypes, and the preliminary stratification of carcinogenic risk (11). Specifically, DNA testing can determine the presence and genotype of the virus, offer semi-quantitative data on viral load, and monitor infection persistence. Clinically, HPV DNA testing is primarily used for primary CC screening, the triage of cytologically abnormal results, and post-treatment follow-up of lesions; it also supports epidemiological studies and vaccine efficacy evaluations. It thus serves as a foundational metric for clinical decision-making and public health strategies related to HPV infection (12).

HPV RNA

HPV RNA expression levels serve as key molecular markers for assessing the transition from transient to persistent HR-HPV infection and the associated risk of carcinogenesis. Following HR-HPV infection of the cervical epithelium, the *E6* and *E7* oncogenes are expressed as oncoproteins. During the initial phase of infection, the virus predominantly remains in an episomal state without integrating into the host genome. At this stage, *E6* and *E7* mRNA expression levels are low or absent, and any corresponding cervical lesions are typically confined to Low-grade Squamous Intraepithelial Lesion (LSIL). If the HR-HPV infection

persists, high levels of *E6* and *E7* mRNA are transcribed, resulting in the overexpression of these oncoproteins. These oncoproteins inactivate the tumor suppressors p53 and retinoblastoma protein (Rb), allowing the virus to evade immune clearance while promoting the abnormal proliferation and malignant transformation of cervical cells (13). Therefore, quantifying HPV *E6/E7* mRNA expression provides valuable information for assessing the malignant potential of cervical lesions (14). HPV *E6/E7* mRNA testing serves as a critical secondary risk assessment tool following HPV DNA testing. This test not only confirms the presence of the virus but also specifically evaluates lesion progression driven by oncogene activity, effectively distinguishing transient from persistent infections. This approach substantially enhances specificity and positive predictive value in screening triage. It also allows for the more precise identification of patients with High-grade Squamous Intraepithelial Lesion (HSIL) (15).

PROTEIN BIOMARKERS

p16 Protein

The p16INK4a protein (p16) is a cell cycle suppressor belonging to the INK4 family. In normal cells, p16 inhibits the activity of cyclin-dependent kinases 4 and 6 (CDK4/6) by specifically binding to them, thereby preventing the G₁-to-S phase transition and maintaining normal cell proliferation. During HPV infection, however, this regulatory mechanism is disrupted. The E7 oncoprotein binds to and inactivates Rb, releasing the transcription factor E2F. This process abrogates the negative feedback inhibition normally exerted by the Rb-E2F complex on *p16* transcription, resulting in the characteristic overexpression of p16 in infected cells. Additionally, the E6 oncoprotein degrades the tumor suppressor p53, further disrupting cell cycle regulation and DNA repair capacity, which synergistically promotes p16 overexpression and abnormal cell proliferation (16). Clinically, the immunohistochemical detection of p16 has become a crucial adjunct for diagnosing and differentiating between CC and its precancerous lesions. The rate of p16 positivity increases substantially with the progression of cervical squamous intraepithelial lesions. In LSIL, p16 expression is predominantly focal and weakly positive, whereas in HSIL and CC tissues, it is diffuse and strongly positive. When combined with cytology or HR-HPV testing, p16

immunohistochemistry effectively improves screening accuracy, reduces unnecessary colposcopy referrals, and optimizes the early diagnosis and treatment of CC (17).

Ki-67 Protein

Ki-67 is a widely used cell proliferation marker. This protein is highly expressed during the G₁, S, G₂, and M phases of the cell cycle but is virtually absent in quiescent (G₀) cells. Its expression level correlates strongly with cellular proliferation activity, making it a key biomarker for assessing tissue proliferative status (18). During HPV-driven cervical carcinogenesis, Ki-67 expression patterns are characteristically altered. The HR-HPV E7 oncoprotein inactivates Rb, thereby derepressing the E2F transcription factor and directly activating *Ki-67* transcription, leading to uncontrolled cell proliferation (19). The clinical application of Ki-67 primarily involves its combined detection with p16. When cytology results indicate LSIL, atypical squamous cells of undetermined significance (ASC-US), or HSIL, dual p16/Ki-67 staining serves as an adjunct diagnostic tool. It assists pathologists in more accurately assessing lesion severity and determining the necessity for colposcopy and biopsy (20). Furthermore, for HPV-positive patients with normal cytology or ASC-US, p16/Ki-67 dual staining serves as an effective triage tool.

EPIGENETIC BIOMARKERS

DNA Methylation

DNA methylation is a critical step in HPV-induced carcinogenesis. This process is induced by the E6 and E7 oncoproteins through the stabilization of DNA methyltransferases (DNMTs) or the suppression of ten-eleven translocation (TET) family protein activity, ultimately leading to the epigenetic silencing of specific tumor suppressor genes. These tumor suppressors are involved in maintaining physiological functions such as cell cycle stability, DNA repair, and apoptosis regulation. Their inactivation contributes directly to cell cycle dysregulation, genomic instability, and apoptosis evasion, thereby forming a critical bridge from viral infection to malignant cellular transformation (21). *CDKN2A* and *RASSF1A* are core methylation markers for CC detection. A meta-analysis showed that the frequency of *CDKN2A* methylation was substantially higher in CC cases than in healthy controls. After excluding low-quality studies, no

heterogeneity was observed, and the association was strengthened, with consistent results across all subgroup analyses. Furthermore, *CDKN2A* methylation was strongly associated with precancerous risk, confirming its value as an early epigenetic biomarker for CC (22). Additionally, another meta-analysis suggested a potential interaction between HPV infection and *RASSF1A* promoter methylation in CC development (23).

Non-coding RNA

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules approximately 19 to 25 nucleotides in length. They negatively regulate gene expression by binding to the 3' untranslated regions of target mRNAs, thereby inhibiting mRNA translation or promoting mRNA degradation (24). Abnormal miRNA expression is commonly observed in HPV-related cervical lesions (25–26). The HPV E6 and E7 oncoproteins regulate specific miRNAs: E6 upregulates *miR-21*, whereas E7 downregulates *miR-203* and *miR-125b* and upregulates *miR-155*. Elevated in both HPV-positive and HPV-negative cancers, *miR-21* promotes cellular proliferation via the Ki-67 and p53 pathways. Suppressive miRNAs (such as *miR-34a* and *miR-125b*) are downregulated, while oncogenic miRNAs (such as *miR-9* and *miR-155*) are upregulated, disrupting the cell cycle, apoptosis, immunity, and metastasis. These dysregulated miRNAs are promising non-invasive biomarkers for HPV-related CC. However, their clinical translation remains limited. Future studies need to validate miRNA panels to distinguish between transient and persistent HPV infections, assess miRNA signatures for predicting lesion progression or regression, and integrate miRNAs with established HPV DNA and methylation markers. Additionally, researchers should address HPV-negative cancer heterogeneity and standardize quantification methods (27).

Long non-coding RNAs (lncRNAs) regulate gene expression via chromatin remodeling, transcriptional and post-transcriptional control, and miRNA sponging. HPV alters lncRNA profiles; for example, *HOTAIR* in HPV16-positive cells promotes proliferation and inhibits apoptosis by sponging *miR-214-3p* (28). Specifically, lncRNAs act through competing endogenous RNA (ceRNA) mechanisms, and E6- and E7-mediated ceRNA networks remodel host gene expression related to the cell cycle, proliferation, and apoptosis (29). Dysregulated lncRNAs are also promising non-invasive biomarkers

for HPV-related CC, but their clinical translation remains limited. Future studies need to validate lncRNA panels to distinguish between transient and persistent HPV infections, assess signatures for predicting lesion progression, and integrate lncRNAs with established DNA and methylation markers. Standardizing quantification methods and addressing tumor heterogeneity are also critical requirements.

Circular RNAs (circRNAs) are a unique class of non-coding RNAs characterized by a covalently closed circular structure, which makes them resistant to degradation by nucleases. Consequently, they exhibit high stability and evolutionary conservation. Similar to lncRNAs, circRNAs modulate downstream gene expression through ceRNA activity, representing a key regulatory mechanism in HPV-associated CC (27). Among these, *circE7* has emerged as a potential biomarker in HPV-positive cancers. Furthermore, circRNAs can bind RNA-binding proteins or regulate the transcription and splicing of their parental genes, thereby influencing cellular functions. Dysregulated RNA editing and altered circRNA expression jointly contribute to CC development, highlighting their promise as diagnostic biomarkers and therapeutic targets (27).

TUMOR MICROENVIRONMENT-RELATED BIOMARKERS

Tumor-Infiltrating Immune Cells

As a core component of the adaptive immune system, T cells exhibit diverse functions in CC immune responses through their distinct subsets. CD8⁺ T cells can specifically recognize and kill HPV-infected target cells. By releasing cytotoxic proteins such as perforin and granzyme, they induce tumor cell apoptosis. The degree of their infiltration into tumor tissue correlates closely with CC patient prognosis, as high infiltration generally indicates better survival outcomes. CD4⁺ T cells exert immunoregulatory effects, aiding B cells in antibody production while activating other immune cells. The chronic inflammatory environment driven by HR-HPV induces a substantial local accumulation of Regulatory T cell (Treg) within tumor tissues. Treg cells suppress immune cell activity by secreting cytokines such as interleukin-10 (IL-10) and transforming growth factor- β (TGF- β), thereby facilitating tumor cell immune escape (30). Consequently, high intratumoral Treg infiltration has been established as an adverse

prognostic biomarker closely associated with tumor progression and poor prognosis. Furthermore, modulating Treg function has emerged as a critical strategy for restoring effective antitumor immunity and enhancing immunotherapy efficacy.

Natural killer (NK) cells serve as critical biomarkers for assessing the host's immune clearance capacity following HPV infection and the risk of CC progression. Mechanistically, they directly kill HPV-infected cells and cancer cells without prior sensitization by releasing cytotoxic molecules such as perforin, granzyme, and TNF-related apoptosis-inducing ligand (TRAIL), while simultaneously secreting key cytokines like Interferon gamma (IFN- γ) to activate and coordinate adaptive immune responses. However, within the CC microenvironment, NK cell numbers and functional activity are typically suppressed. This compromised immune state correlates closely with advanced tumor stage, increased metastasis risk, and poor patient prognosis (31). Thus, NK cell activity or abundance may serve as a valuable prognostic biomarker. Given their central role in antitumor immunity, current therapeutic strategies focus on adoptive NK cell infusion and immune checkpoint modulation.

Macrophages exhibit distinct polarization states (M1 and M2) that serve as important immune biomarkers for assessing the progression of HPV-associated CC. Mechanistically, IFN- γ induces M1 macrophages to exert antitumor effects via TNF- α and IL-6, whereas IL-4 and IL-13 drive M2 macrophages to promote tumor progression through TGF- β and VEGF (32). In HPV-driven CC, the tumor microenvironment promotes polarization toward the M2 phenotype. Clinically, high M2 infiltration or a low M1/M2 ratio correlates with tumor invasion, metastasis, and poor prognosis (33). Thus, assessing M2 infiltration or the M1/M2 ratio in tumor tissues helps evaluate prognosis, and reprogramming M2 macrophages into the M1 phenotype represents a novel immunotherapeutic strategy for CC.

Immune Checkpoint Molecules

Programmed cell death protein-1 (PD-1) is a co-inhibitory receptor on the surface of T cells, whose primary ligand (PD-L1) is expressed on tumor cells, antigen-presenting cells, and other immune cells (34). The PD-1/PD-L1 signaling pathway is a key mechanism for tumor immune escape (35). The binding of PD-1 to PD-L1 activates downstream signaling pathways, suppressing T-cell activation and

enhancing immune tolerance to tumor cells. Consequently, effector T cells lose their ability to recognize and kill tumor cells (36). The PD-1/PD-L1 axis plays a particularly prominent role in HPV-induced CC. PD-L1 and IFI16 are abnormally overexpressed in HPV-positive CC cells. IFI16 promotes PD-L1 upregulation in the immune microenvironment via the STING-TBK1-NF- κ B pathway, thereby driving CC progression (37). PD-1/PD-L1 inhibitors (anti-PD-1/PD-L1 antibodies) have demonstrated substantial clinical efficacy in treating multiple malignancies. These drugs block PD-1/PD-L1 interactions, thereby relieving T-cell suppression and restoring antitumor activity (38). However, PD-1/PD-L1 inhibitors are not effective in all patients, presenting ongoing challenges regarding primary and acquired resistance. Identifying patients who can benefit from PD-1/PD-L1 checkpoint blockade and overcoming resistance are current research priorities (39). HPV infection modulates PD-1/PD-L1 immune checkpoint molecules within the tumor microenvironment, representing a key mechanism for tumor immune escape. By elucidating these mechanisms and integrating precise biomarker screening, clinicians can utilize PD-1/PD-L1 inhibitors and combination therapies to offer new hope for patients with HPV-associated cancers.

Cytokines and Chemokines

IFN- γ , secreted by activated T cells and NK cells, exerts dual antiviral and immunoregulatory functions against HPV: it directly inhibits HPV replication and transcription via antiviral proteins (e.g., Mx and PKR) and enhances immune clearance by promoting antigen-presenting cell maturation and CD8⁺ T-cell and NK-cell activity (40). However, during persistent HPV infection and CC progression, the tumor microenvironment may limit IFN- γ production or disrupt its signaling pathways, causing this critical immune defense axis to malfunction. Therefore, monitoring IFN- γ levels effectively reflects the dynamic transition from effective immune clearance to immune exhaustion, providing crucial information for determining infection outcomes, assessing disease progression risk, and predicting immunotherapy efficacy.

Tumor necrosis factor- α (TNF- α), primarily produced by macrophages and T cells, exhibits dual pro-inflammatory and antitumor properties. In the inflammatory response triggered by HPV infection, TNF- α activates inflammatory cells, promotes the

release of inflammatory mediators, and enhances local immune responses, thereby facilitating the clearance of virus-infected cells. However, prolonged high concentrations of TNF- α may lead to the formation of a chronic inflammatory microenvironment, promoting tumor cell proliferation, migration, and invasion, and thereby contributing to CC development and progression. Measuring TNF- α levels in the tumor tissues and peripheral blood of patients with CC has revealed correlations with tumor stage, lymph node metastasis, and patient prognosis. Elevated TNF- α levels generally predict poorer clinical outcomes (41). Thus, TNF- α levels serve as an adverse serum or tissue biomarker for assessing tumor malignancy and predicting patient prognosis.

Interleukin-6 (IL-6), a multifunctional cytokine, plays critical roles in immune regulation, acute phase responses, and tumor progression. Following HPV infection, IL-6 is secreted by local immune cells and tumor cells. It not only modulates immune cell differentiation and function — such as promoting B-cell proliferation and differentiation into antibody-producing plasma cells — but also activates signaling pathways, such as the signal transducer and activator of transcription 3 (STAT3) pathway, thereby enhancing tumor cell survival, proliferation, and drug resistance (42). IL-6 levels are substantially elevated in the serum and tumor tissues of patients with CC, a finding closely associated with a poor prognosis. This makes IL-6 a potential biomarker for monitoring disease activity and treatment response.

Chemokines are a class of small-molecule cytokines that primarily mediate the migration and recruitment of immune cells via specific chemokine receptors. In the context of HPV infection and CC, chemokines influence the immunosuppressive tumor microenvironment. HPV-infected keratinocytes and tumor cells secrete multiple chemokines that specifically recruit immune cells to sites of infection or tumorigenesis (43). However, chemokine recruitment can be detrimental: Tregs suppress CD8⁺ T cells and NK cells via IL-10 and TGF- β , and M2 Tumor-Associated Macrophages (TAMs) promote angiogenesis and tissue remodeling, aiding immune escape and tumor progression. Chemokines and their pathways therefore serve as prognostic biomarkers in CC. For instance, specific chemokine profiles influence the response to chemoradiotherapy, and patients with non-HPV16 genotypes often exhibit poorer post-treatment survival (44). Because chemokines act as key regulators of the immune microenvironment, alterations in their

expression levels or patterns may predict patient responses to immunotherapy or other targeted therapies.

CURRENT CHALLENGES

From a health economics perspective, existing screening methods face major challenges regarding cost-effectiveness and resource equity. Liquid-based cytology and HPV testing are costly, and their high false-positive rates lead to unnecessary diagnostic and treatment expenses, which simultaneously waste medical resources and increase patient financial burdens. In resource-limited settings, shortages of equipment and personnel drive severe disparities in screening coverage, while the lack of standardized sampling and testing protocols results in inaccurate diagnoses and redundant expenditures. From a clinical standpoint, cytological tests suffer from operational subjectivity that limits sensitivity; HPV DNA testing lacks sufficient specificity to distinguish between transient and persistent oncogenic infections; novel molecular markers require further validation in large-scale multicenter studies; and the lack of standardized workflows renders results incomparable across institutions. Additionally, the delayed development of robust digital health systems prevents the accumulation of the long-term risk data essential for risk-stratified decision-making. These challenges collectively compromise the accuracy, accessibility, and evidentiary quality of current screening systems (45).

FUTURE PROSPECTS

The development of CC is fundamentally driven by persistent infection with HR-HPV. Future research needs to focus on overcoming current diagnostic bottlenecks through the standardized integration of multidimensional data. Currently, emerging biomarkers — such as those derived from liquid biopsies or epigenetic profiling — lack unified testing standards, a deficit that severely limits their reproducibility and translational potential. Therefore, establishing rigorous testing protocols through large-scale clinical validation will form the basis for effectively integrating multi-omics data (e.g., viral E6/E7 oncoproteins, host methylation profiles, and non-coding RNAs) with artificial intelligence models (46). Moreover, the shift of the vaginal microbiota from a symbiotic balance to a dysbiotic state has been

confirmed as a key factor in CC development. Future research should also aim to clarify the microbiota-immune-metabolism axis to identify microbiological markers suitable for standardized testing, thereby providing a basis for microbiome-targeted interventions (47). Ultimately, by integrating rigorously standardized multi-omics markers with artificial intelligence, researchers can build more precise CC risk prediction, diagnosis, and prognosis management systems, successfully bridging the gap between basic research and clinical application.

DISCUSSION

The core value of CC biomarkers in early screening and diagnosis is to enhance detection sensitivity and specificity, and to provide precise risk stratification for abnormal results. HPV DNA testing and genotyping represent the most sensitive screening methods; individuals positive for HPV16 or HPV18 can be directly referred for colposcopy. However, their specificity is relatively low, making it difficult to distinguish transient from persistent infections, which may lead to unnecessary clinical interventions. HPV *E6/E7* mRNA testing directly reflects viral oncogene transcriptional activity, enabling the differentiation of transient from persistent infections with greater specificity than HPV DNA testing. This assay demonstrates substantial diagnostic value, particularly for high-grade lesions, and is recommended by the WHO as a supplementary screening tool. By detecting the co-expression of a cell cycle inhibitor and a proliferation marker, p16/Ki-67 dual staining exhibits high diagnostic accuracy for cervical intraepithelial neoplasia (CIN); however, its cost and technical barriers limit widespread adoption at the primary care level. Gene methylation assays (e.g., *PAX1*, *SOX1*, and *JAM3*) exhibit methylation levels that correlate positively with lesion severity. These assays are suitable for self-collected samples, offer distinct advantages in resource-limited settings, and have been identified by the WHO as potential screening methods.

In the triage of abnormal screening results, these biomarkers primarily facilitate the effective risk stratification of individuals with HPV positivity or cytological abnormalities. Specifically, p16/Ki-67 dual staining, HPV *E6/E7* mRNA detection, and gene methylation analysis can all be employed to further stratify HR-HPV-positive cases, prioritizing the identification of clinically substantial lesions and reducing unnecessary colposcopy referrals. Currently in

China, initial screening combines HPV DNA testing with liquid-based cytology. Individuals with dual positivity or HPV16/18 positivity are directly referred for colposcopy, whereas those infected with other HR-HPV genotypes undergo precise molecular biomarker stratification to achieve "equal risk, equal management." Although the combined use of biomarkers substantially enhances screening efficiency, their health economic feasibility and accessibility at the primary care level require further attention, and future strategies should be optimized through large-scale studies (48).

CONCLUSION

This review systematically summarizes key advances in HPV-related biomarkers for CC, providing theoretical support for precision prevention and treatment — specifically, the earlier and more accurate identification of high-risk populations, the avoidance of overtreatment, and the guidance of individualized therapy. At the molecular level, virus-derived markers (e.g., HPV E6/E7) and epigenetic markers (e.g., host gene methylation) provide highly specific early diagnostic signals regarding viral oncogenic activity and host cell malignant transformation, respectively. At the cellular level, protein markers such as p16 and Ki-67 directly reveal HPV-induced cell cycle dysregulation and abnormal proliferation, serving as crucial foundations for pathological diagnosis and risk stratification. At the systemic immune level, the dynamic monitoring of immune cell subsets (e.g., CD8+ T cells, Tregs, and M1 macrophages) within the tumor microenvironment, coupled with the analysis of key cytokine, chemokine, and immune checkpoint networks, not only deepens our understanding of immune evasion mechanisms but also guides efficacy predictions for immunotherapies and the development of novel targets. In summary, a multidimensional biomarker system encompassing viral, host epigenetic, cellular protein, and immune microenvironment factors is propelling CC clinical management from traditional morphological assessment toward a new era of precision medicine based on molecular subtyping, ultimately providing powerful tools and strategies to reduce CC incidence and mortality.

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