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The Evolving Biomarkers in Cervical Cancer: Integrating Multi-Omic and Emerging Approaches for Precision Cervical Cancer Diagnostics

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ABSTRACT: Cervical cancer remains a major global health burden despite being one of the most preventable malignancies. The transition from conventional morphology-based screening to human papillomavirus (HPV)-based testing has improved detection sensitivity; however, HPV positivity alone cannot reliably distinguish transient infection from biologically significant transformation. Cervical carcinogenesis is a multistep process involving persistent high-risk HPV infection, viral oncogene activity, host-cell cycle deregulation, epigenetic modification, genomic evolution, immune modulation and metabolic reprogramming. This review examines the evolving landscape of cervical cancer biomarkers and the transition from individual molecular markers toward integrated multi-omic and emerging approaches for improved detection, risk stratification, disease characterization and precision management.

HPV genotyping and viral persistence provide the initial framework for molecular risk assessment, while E6/E7 mRNA expression and p16/Ki-67 dual staining offer insights into transforming infection and host-cell deregulation. DNA methylation, non-coding RNAs, and genomic, transcriptomic, proteomic and metabolomic profiling further reveal the biological heterogeneity and functional consequences of cervical carcinogenesis. Immune biomarkers provide information on the tumour microenvironment and therapeutic responsiveness, whereas liquid-biopsy approaches, including circulating HPV DNA, circulating tumour DNA and extracellular vesicles, offer opportunities for minimally invasive disease monitoring.

Collectively, these biomarker classes interrogate complementary dimensions of cervical cancer biology. No single biomarker can adequately capture its biological complexity or fulfil every clinical requirement. The future of cervical cancer diagnostics and management therefore lies in integrated biomarker architectures combining viral, molecular, multi-omic and circulating tumour-derived information to improve diagnostic precision, risk stratification, longitudinal monitoring and personalized clinical management.

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INTRODUCTION

Cervical cancer remains one of the most preventable yet significant causes of cancer-related morbidity and mortality among women worldwide. Its development is characterized by a prolonged premalignant phase, during which progressive cellular and molecular abnormalities can be detected and treated before invasive disease develops. This biological characteristic has made cervical cancer particularly suitable for prevention through human papillomavirus (HPV) vaccination, screening and early treatment. Nevertheless, substantial disparities in vaccination coverage, screening infrastructure and access to diagnostic and therapeutic services continue to contribute to a disproportionately high disease burden in low- and middle-income countries [1,2].

Conventional cervical cancer screening has historically relied on Papanicolaou cytology, visual inspection with acetic acid (VIA), colposcopy and histopathology. Pap cytology has substantially contributed to reductions in cervical cancer incidence and mortality in organized screening programmes. However, its performance depends on adequate specimen collection, laboratory quality and expert interpretation. VIA offers a relatively inexpensive alternative in resource-limited settings but is strongly operator dependent. Histopathology remains essential for definitive diagnosis but requires invasive biopsy and is therefore unsuitable for population-level primary screening. These limitations have accelerated the transition towards HPV DNA testing, which provides greater sensitivity and reproducibility for the detection of clinically significant cervical precancer [2,3].

Cervical carcinogenesis is a multistep biological process in which persistent infection with oncogenic human papillomavirus (HPV) progressively interacts with host-cell regulatory pathways, producing genetic, epigenetic, transcriptional and phenotypic alterations. Although infection with a high-risk HPV genotype is the principal initiating event in the majority of cervical cancers, detection of viral DNA alone does not establish that an infected cell has undergone malignant transformation. This distinction is fundamental to contemporary cervical cancer diagnostics: the presence of HPV indicates exposure to, or persistence of, an oncogenic virus, whereas transformation-associated biomarkers provide evidence that the infection has begun to disrupt normal cellular regulation.

The attached review emphasizes the transition from conventional morphology-based screening towards molecular and metabolic approaches capable of identifying biologically significant transformation and improving risk stratification.

Molecular Biomarkers as Indicators of Cervical Carcinogenic Progression

1.1 HPV Genotyping and Viral Persistence

Persistent infection with high-risk human papillomavirus (hrHPV) represents the essential initiating event in the development of virtually all cervical cancers. However, HPV infection is highly prevalent, particularly among sexually active women, and the majority of infections are transient, asymptomatic, and eliminated by host immune mechanisms without progressing to clinically significant disease. Therefore, the detection of HPV alone does not necessarily indicate the presence of cervical precancer or an inevitable progression toward malignancy.

The critical biological distinction is between a transient infection and persistent infection with an oncogenic HPV genotype, particularly when viral persistence is accompanied by progressive molecular and cellular transformation [4–6].

The prolonged natural history of cervical carcinogenesis provides a valuable opportunity for early detection and prevention. Following persistent hrHPV infection, a subset of women may develop cervical intraepithelial neoplasia (CIN), which can subsequently progress to invasive carcinoma over several years or even decades. This multistep progression is influenced not only by viral persistence but also by HPV genotype, viral oncogene activity, host immune response, genetic susceptibility, epigenetic alterations, and accumulation of molecular abnormalities within infected epithelial cells [4,5,7]. Consequently, contemporary cervical cancer screening increasingly emphasizes biological risk stratification rather than simply identifying the presence or absence of HPV.

High-risk HPV DNA testing has become the foundation of modern molecular cervical screening because of its high sensitivity for detecting women at risk of developing high-grade cervical lesions. Nevertheless, HPV-positive women constitute a biologically heterogeneous population. More than a dozen HPV genotypes are recognized as oncogenic, but their carcinogenic potential is not equivalent. HPV16 and HPV18 are responsible for a substantial proportion of cervical cancers

worldwide, with HPV16 showing a particularly strong association with cervical squamous cell carcinoma and HPV18 being relatively more frequently associated with cervical adenocarcinoma. Other oncogenic genotypes, including HPV31, HPV33, HPV35, HPV45, HPV52, and HPV58, also contribute significantly to high-grade cervical intraepithelial lesions and invasive cervical cancer [4,6,8].

This genotype-specific variation forms the basis for HPV genotyping as an initial level of molecular risk stratification. A binary HPV-positive result identifies women with oncogenic HPV infection, whereas partial or extended genotyping provides additional information regarding the relative risk associated with individual viral types. Women infected with HPV16 or HPV18 generally demonstrate a higher immediate and long-term risk of CIN3+ and invasive cervical cancer than women infected with several other high-risk genotypes [6,8,9]. Consequently, genotype information can assist in determining the intensity of follow-up, the requirement for triage testing, and the need for referral to colposcopy.

However, HPV genotype should be interpreted primarily as a risk biomarker rather than a direct marker of malignant transformation. Even infections involving highly carcinogenic types such as HPV16 or HPV18 may be transient and resolve spontaneously. Conversely, progression to high-grade lesions depends on the persistence of infection and the subsequent development of viral and host-cell abnormalities. Therefore, genotype alone cannot distinguish productive infection from transforming infection or reliably identify which individual HPV-positive woman will develop invasive disease [5,6].

Viral persistence provides an additional and biologically important dimension of risk assessment. Repeated detection of the same high-risk HPV genotype over time suggests failure of immune-mediated viral clearance and is strongly associated with an increased probability of cervical precancer. Persistent infection creates the conditions necessary for sustained expression of the viral E6 and E7 oncogenes, which interfere with critical tumour-suppressive pathways, including p53 and retinoblastoma protein (pRb), thereby promoting genomic instability and abnormal cell-cycle progression [5,10]. Nevertheless, persistence should not itself be equated with malignant transformation. Some persistent infections may remain non-progressive, while only a proportion acquire the molecular and cellular characteristics necessary for the development of CIN2+, CIN3+, or invasive carcinoma.

Thus, HPV genotype and persistence represent complementary but early components of a hierarchical molecular risk model. Genotyping identifies differences in inherent viral carcinogenic potential, while persistence indicates prolonged interaction between an oncogenic virus and the cervical epithelium. However, progression beyond persistent infection is influenced by additional biological events, including deregulated E6/E7 expression, disruption of host-cell-cycle control, epigenetic reprogramming, genomic instability, and alterations in the local immune microenvironment [5,10]. This biological complexity explains why HPV-based screening requires effective triage strategies to distinguish women with clinically significant transforming lesions from those with transient or low-risk infections.

HPV viral load has also been investigated as a potential indicator of disease severity and progression. Although an increased quantity of HPV DNA may reflect extensive viral infection, the relationship between viral burden and malignant potential is not straightforward. Productive infections may contain large quantities of episomal viral DNA without undergoing malignant transformation, whereas transformed lesions may exhibit relatively lower viral copy numbers but sustained or deregulated oncogene expression. In addition, measured viral load can be influenced by HPV genotype, sample cellularity, stage of infection, viral integration status, and methodological differences between analytical platforms [11,12]. Consequently, HPV viral load has not achieved sufficient consistency to serve as a universal independent marker of cervical cancer progression.

The principal limitation of HPV DNA testing is therefore that it detects the presence of oncogenic viral genetic material rather than the biological consequences of transformation. While this characteristic provides excellent sensitivity, it may reduce specificity because a positive result can represent either transient infection or clinically relevant precancer. Current screening strategies are consequently evolving toward sequential, risk-based models in which sensitive primary HPV testing is followed by transformation-specific triage biomarkers. These may include HPV E6/E7 mRNA detection, p16/Ki-67 dual staining, DNA methylation markers, and other emerging molecular signatures capable of identifying biologically significant disease [3,9,13].

Overall, HPV genotyping and assessment of viral persistence constitute the first molecular level of cervical cancer risk stratification. Their greatest clinical value lies not in diagnosing malignant transformation directly, but in identifying HPV-positive women who require further evaluation. Integration of genotype-specific risk, persistence, and downstream

transformation-associated biomarkers offers a more biologically informed approach to cervical screening, with the potential to maintain high sensitivity while improving specificity and reducing unnecessary colposcopy and overtreatment.

1.2 HPV E6 and E7 Oncogene Expression: From Viral Detection to Biological Activity

A promising biomarker is HPV E6/E7 mRNA. Unlike HPV DNA testing, which confirms the presence of viral genetic material, E6/E7 mRNA testing provides evidence of active transcription of viral oncogenes. This makes it more closely associated with transforming infection [13,14]. The E6 oncoprotein promotes functional inactivation and degradation of the tumour suppressor p53, thereby impairing DNA repair, cell-cycle arrest and apoptosis. In parallel, E7 disrupts the retinoblastoma protein (pRb) pathway, releasing E2F transcription factors and promoting uncontrolled cellular proliferation. This disruption results in compensatory overexpression of p16INK4a, which has become an important biomarker of transforming HPV infection. When p16 overexpression occurs simultaneously with expression of the proliferation marker Ki-67 within the same cell, it indicates abnormal cell-cycle deregulation and provides a biologically meaningful signal of HPV-induced transformation [10,15].

Among viral biomarkers, E6 and E7 oncogene expression is particularly important because these genes are directly involved in HPV-mediated cellular transformation. HPV DNA testing demonstrates the presence of viral genetic material, whereas E6/E7 messenger RNA detection provides evidence that oncogenic viral genes are transcriptionally active. This distinction represents an important conceptual progression from identifying the causative agent to identifying its biological activity.

The E6 and E7 proteins interfere with two central tumour-suppressive pathways. High-risk HPV E6 promotes degradation and functional inactivation of p53, thereby impairing DNA-damage responses, cell-cycle arrest and apoptosis. E7 disrupts the retinoblastoma protein pathway, releasing E2F transcription factors and facilitating inappropriate entry into the cell cycle. Persistent activity of these viral oncogenes creates an environment conducive to genomic instability and accumulation of transforming cellular alterations. Thus, the distinction can be simplified as: HPV DNA positivity indicates viral presence whereas E6/E7 mRNA positivity is an evidence of transcriptionally active viral oncogenes

This difference may improve the specificity of molecular triage because E6/E7 mRNA is more closely associated with biologically active infection. Nevertheless, E6/E7 mRNA should not be regarded as an absolute marker of inevitable progression. Assay performance varies according to the HPV genotypes included, analytical platform, positivity threshold and clinical population. WHO has also specifically addressed HPV mRNA testing as an option within cervical screening programmes, reflecting the increasing clinical relevance of RNA-based viral detection [13,14].

Detection of E6 and E7 proteins represents an even more direct approach to identifying viral oncogenic activity. However, low concentrations of viral proteins, particularly in precursor lesions, create significant analytical challenges. E6/E7 protein assays therefore remain less widely established than HPV DNA testing and other transformation-associated approaches. Their greatest future value may lie in simplified multiplex or point-of-care diagnostic platforms capable of integrating viral and host-cell information.

One of the most important consequences of high-risk HPV E7 activity is disruption of the pRb–E2F regulatory pathway. Under normal physiological conditions, pRb restricts inappropriate progression through the cell cycle. When E7 functionally inactivates pRb, E2F transcriptional activity increases, promoting DNA synthesis and cellular proliferation. This abnormal signalling produces compensatory overexpression of the cyclin-dependent kinase inhibitor p16INK4a [10,15].

The biological sequence can therefore be represented as: HPV E7 activity → pRb inactivation

→ E2F release → abnormal cell-cycle progression → compensatory p16INK4a overexpression.

Accordingly, strong and diffuse p16 expression serves as an indirect biomarker of HPV-associated disruption of cell-cycle control. In cervical histopathology, p16 immunohistochemistry is particularly useful when morphological interpretation is equivocal.

However, p16 should not be interpreted as an isolated binary cancer marker because its diagnostic significance depends on staining pattern, tissue architecture and pathological context [15,16].

Ki-67 provides complementary information. It is a nuclear protein associated with actively proliferating cells and is widely

used as a marker of cellular proliferation. In normal cervical epithelium, proliferative activity is largely restricted to basal and parabasal layers. As cervical neoplasia progresses, proliferating cells extend into more superficial epithelial layers, reflecting disruption of normal maturation. However, Ki-67 is not specific to HPV-mediated neoplasia and may increase in other proliferative conditions. Its greatest diagnostic value therefore emerges when it is interpreted together with a marker of HPV-associated cell-cycle deregulation [15,16].

1.3. p16/Ki-67 dual staining as marker: Host-Cell Indicators of HPV-Induced Triage Transformation Strategy

Another clinically important approach is p16/Ki-67 dual staining. The simultaneous detection of p16 and Ki-67 within the same cervical epithelial cell represents one of the most biologically coherent molecular triage strategies currently available. Under normal cell-cycle conditions, strong p16-associated growth inhibition and active Ki-67-associated proliferation would generally not coexist within the same cell. Their simultaneous expression therefore indicates abnormal decoupling of cell-cycle regulatory mechanisms. Thus dual detection of p16, reflecting disruption of the pRb pathway, and Ki-67, indicating active proliferation, identifies cells undergoing abnormal HPV-induced cell-cycle deregulation. Large clinical studies have demonstrated the value of dual staining as a triage tool for HPV-positive women, with improved risk discrimination compared with cytology alone in selected clinical settings [17].

A dual-positive cell reflects: p16 positivity as an evidence of cell-cycle regulatory disruption and Ki-67 positivity as an evidence of active proliferation. Thus p16/Ki-67 co-expression indicates biologically abnormal transforming phenotype

The importance of this approach is that it identifies a molecular signature of transformation rather than relying exclusively on subjective morphological classification. The source document highlights the substantial prospective clinical development of dual staining and its role in HPV-positive triage populations.

Nevertheless, p16/Ki-67 dual staining is not intended to replace histopathological confirmation of disease. Instead, it improves risk stratification among HPV-positive women by identifying individuals more likely to harbour clinically significant high-grade lesions. Its clinical relevance demonstrates a broader principle in cervical biomarker research: the most useful diagnostic biomarkers are often those that reflect a specific biological mechanism of transformation rather than simply identifying the presence of the causative virus [17].

1.4 DNA methylation markers- as an Epigenetic Biomarker of Cervical Progression

Epigenetic dysregulation represents an important component of cervical carcinogenesis and has emerged as a promising source of molecular biomarkers for the detection and risk stratification of HPV-associated cervical disease. Among epigenetic mechanisms, DNA methylation is particularly relevant because alterations in methylation patterns can modify gene expression without changing the underlying DNA sequence. Methylation commonly occurs at cytosine residues within CpG-rich regions of gene regulatory sequences, and abnormal hypermethylation may contribute to transcriptional silencing of genes involved in cell-cycle regulation, cellular adhesion, differentiation, apoptosis, and tumour suppression. During the progression from persistent high-risk human papillomavirus (hrHPV) infection to high-grade cervical intraepithelial neoplasia and invasive carcinoma, both host-cell and viral genomes may undergo progressive epigenetic alterations [18–20].

The principal clinical value of methylation biomarkers lies in their potential to distinguish transient HPV infection from biologically advanced transforming disease. Although hrHPV testing is highly sensitive, many HPV-positive women will not develop clinically significant cervical lesions. Consequently, there is a need for objective triage markers capable of identifying women with a greater likelihood of harbouring high-grade precancer or invasive cancer. Progressive methylation of specific host-cell genes appears to reflect the accumulation of molecular alterations associated with long-term HPV-mediated transformation, making methylation analysis a biologically relevant complement to HPV-based screening [19,20].

Several host genes have been investigated as candidate methylation biomarkers, including FAM19A4, miR124-2, CADM1, MAL, EPB41L3, ASCL1, and LHX8. Among these, the

combined FAM19A4/miR124-2 methylation assay has received considerable clinical attention. Studies have demonstrated that increased methylation of these targets is associated with advanced cervical disease and that the assay can identify a high proportion of cervical carcinomas and advanced precancerous lesions among HPV-positive women [21–23]. Longitudinal studies have further suggested that HPV-positive women with negative FAM19A4/miR124-2 methylation results have a

relatively low short- to medium-term risk of cervical cancer, supporting the potential utility of this biomarker combination in molecular triage strategies [22,23].

The relationship between methylation and cervical carcinogenesis is consistent with the multistep nature of disease progression. Early or transient HPV infections may not exhibit substantial host-cell epigenetic alterations, whereas persistent infection associated with progressive transformation can lead to increasing disruption of normal gene regulation. Consequently, methylation biomarkers may provide information that is complementary to viral detection. HPV testing identifies the presence of an oncogenic infection, whereas methylation analysis may indicate whether the infected epithelium has acquired molecular characteristics associated with advanced transformation. This distinction is particularly valuable because it shifts cervical screening from simple detection of viral exposure toward a more biologically informed assessment of disease risk [19,20].

An additional advantage of methylation-based testing is its analytical objectivity. Unlike cytology, which requires morphological interpretation and may be influenced by observer variability, molecular methylation assays can generate quantitative or threshold-based results using standardized analytical platforms. This may improve reproducibility and facilitate large-scale screening. Furthermore, methylation analysis can potentially be performed using self-collected cervicovaginal samples, creating opportunities to expand screening participation among women who face geographical, cultural, economic, or healthcare-related barriers to clinician-based sampling. Studies evaluating FAM19A4/miR124-2 have demonstrated the feasibility of detecting clinically relevant methylation changes in different self-sampling formats [22,24].

Despite these advantages, several challenges remain before methylation biomarkers can be universally integrated into cervical cancer screening algorithms. Different studies have evaluated distinct gene panels, laboratory methodologies, normalization procedures, and positivity thresholds, making direct comparison between studies difficult. Diagnostic performance may also vary according to the population studied, HPV genotype distribution, age, disease prevalence, and clinical endpoint used for evaluation. Therefore, favourable results obtained in individual cohorts require independent external and prospective validation before widespread implementation [20,23].

Overall, DNA methylation biomarkers represent a significant advancement in the transition toward precision-based cervical cancer screening. Rather than replacing HPV testing, their greatest value may lie in a complementary, sequential diagnostic approach, in which highly sensitive HPV detection is followed by methylation-based or other transformation-specific triage tests. Integration with cytology, HPV genotyping, p16/Ki-67 dual staining, or additional molecular biomarkers may further improve diagnostic accuracy. Thus, methylation profiling offers a biologically meaningful and analytically objective strategy for identifying HPV-positive women at increased risk of advanced cervical precancer or invasive cancer, while potentially reducing unnecessary colposcopy and overtreatment [21–24].

1.5 Viral DNA Methylation and HPV Integration

In addition to host-cell methylation, epigenetic changes within the HPV genome itself have been investigated. Methylation of specific regions within the viral genome, including portions of L1, L2 and regulatory regions, may change during persistent infection and progression toward high-grade disease. Viral methylation is biologically attractive because it examines alterations within the causative agent while simultaneously reflecting changes in the host–virus interaction. However, interpretation is complicated by genotype-specific differences, disease stage and the physical state of viral DNA [18,28].

HPV integration into the host genome represents another molecular event associated with cervical transformation. During productive infection, HPV genomes may persist as episomes. In many cervical cancers, viral DNA becomes integrated into host chromosomes. Integration can disrupt viral regulatory regions, particularly those involving E2, resulting in deregulated E6 and E7 expression. Integration may also contribute to genomic instability and influence neighbouring host genes [10,29].

Nevertheless, integration is not an obligatory event in every cervical cancer. Some tumours retain episomal viral DNA, and patterns of integration vary between HPV genotypes and tumours. Therefore, HPV integration should be regarded as an informative molecular characteristic rather than a universal stand-alone diagnostic marker. Its greatest value may lie in molecular characterization of tumour heterogeneity and incorporation into future integrated risk models [29].

2. METABOLIC BIOMARKERS

An emerging and particularly important dimension is the study of metabolic biomarkers. Malignant transformation is accompanied by extensive metabolic reprogramming to support continuous proliferation, survival and invasion. Cervical cancer cells may exhibit increased glucose utilization, enhanced glycolysis, lactate production, altered mitochondrial function, amino-acid and glutamine metabolism, lipid remodelling and changes in oxidative and redox homeostasis. These biochemical alterations generate measurable metabolite patterns that may distinguish normal cervical tissue from precancerous lesions and invasive carcinoma [25–27].

3. NON-CODING RNAS AS MOLECULAR BIOMARKERS

MicroRNAs are small non-coding RNA molecules that regulate gene expression post-transcriptionally. Because a single microRNA may influence multiple target transcripts, these molecules can occupy important regulatory positions within cancer-associated signalling networks. Cervical carcinogenesis is accompanied by substantial changes in microRNA expression, with different molecules implicated in proliferation, apoptosis, invasion, metastasis and therapeutic resistance [30,31].

Frequently investigated candidates include miR-21, miR-34a, miR-155, miR-196a, miR-203, miR-214, miR-218, members of the miR-29 family and miR-10a. Findings suggest that some microRNAs may exhibit oncogenic behaviour whereas others possess tumour-suppressive functions. For example, miR-21 has frequently been associated with cervical carcinogenesis, whereas reduced expression of tumour-suppressive microRNAs such as miR-34a or miR-218 has been described in specific disease contexts [30–32].

An important advantage is the relative stability of microRNAs in biological fluids. Their detection in plasma, serum, urine or cervicovaginal secretions raises the possibility of minimally invasive testing. However, substantial variation between studies remains a major obstacle. Differences in sample type, RNA extraction, normalization, analytical platform and patient population can produce inconsistent results. Consequently, individual microRNAs should presently be regarded primarily as emerging biomarkers rather than established clinical tests [30,31].

Long non-coding RNAs and circular RNAs extend this regulatory landscape. lncRNAs may influence chromatin organization, transcription, RNA stability and protein interactions, while circRNAs possess covalently closed structures that may confer increased stability. Both have been implicated in cervical cancer proliferation, invasion, epithelial–mesenchymal transition and therapeutic resistance. However, the clinical evidence remains less mature than that supporting HPV testing, p16/Ki-67 dual staining or selected methylation assays. Small cohorts, heterogeneous analytical methods and limited external validation currently restrict routine clinical translation [31,33].

4. IMMUNE BIOMARKERS AND THE TUMOUR MICROENVIRONMENT

Persistent HPV infection and cervical cancer progression occur within a complex immunological environment. Effective clearance of HPV depends on coordinated innate and adaptive immune responses, whereas persistent infection is associated with immune evasion. Established tumours can further develop mechanisms that suppress antitumour immunity [5,34].

Potential immune biomarkers include PD-L1 expression, tumour-infiltrating lymphocytes, immune-checkpoint molecules, cytokine profiles, HLA-associated alterations and immune gene-expression signatures. These markers are generally more relevant to prognosis and therapeutic stratification than to primary population screening [34,37]. The increasing importance of immunotherapy in advanced cervical cancer has intensified interest in identifying biomarkers capable of predicting therapeutic response. However, reliance on a single immune marker may oversimplify a highly complex tumour microenvironment. PD-L1 expression alone does not fully represent immune-cell composition, tumour antigenicity or the functional state of antitumour immunity. Future predictive models may therefore require integration of immune markers with genomic, transcriptomic and other tumour-specific information [37].

5. LIQUID BIOPSY AND CIRCULATING TUMOUR-DERIVED BIOMARKERS

Liquid biopsy represents a major conceptual development in molecular oncology. Instead of relying exclusively on tissue or cervical-cell specimens, liquid-biopsy approaches attempt to detect tumour-derived material released into accessible biological fluids. Potential analytes include circulating tumour DNA, circulating cell-free HPV DNA, microRNAs, extracellular vesicles,

circulating tumour cells and tumour-derived proteins or metabolites [38,39].

The principal advantages of liquid biopsy include minimal invasiveness and the possibility of repeated longitudinal sampling. These characteristics make it particularly attractive for monitoring treatment response, identifying minimal residual disease and detecting molecular evidence of recurrence. However, its utility differs according to disease stage. Early precursor lesions may release extremely small quantities of tumour-derived material, limiting sensitivity for population screening. Consequently, current evidence is stronger for monitoring established cancer than for replacing cervical screening in asymptomatic populations [38–40].

6. CIRCULATING HPV DNA AND MOLECULAR MONITORING

HPV-associated cervical cancer possesses a distinctive advantage for liquid-biopsy development: tumour cells contain viral DNA sequences that can potentially serve as highly specific molecular targets. Cell-free HPV DNA can be detected using sensitive technologies such as digital PCR and next-generation sequencing [40,41].

Studies have demonstrated that circulating HPV DNA may correlate with disease burden and change dynamically during treatment. Persistent or re-emergent detection following definitive therapy may therefore provide evidence of residual or recurrent disease. The following discussion highlights the potential for molecular recurrence detection to precede conventional clinical recognition [40,41].

Prospective research has supported this concept, while more recent studies continue to investigate the kinetics of cell-free HPV DNA during chemoradiotherapy and its potential relationship with treatment response and disease outcome [40–42]. Although highly promising, circulating HPV DNA requires further standardization regarding sampling time points, assay sensitivity, genotype coverage and interpretation thresholds. At present, its most compelling potential applications lie in treatment monitoring, molecular residual-disease assessment and recurrence surveillance rather than primary screening [40,41].

7. CELL-FREE DNA, EXTRACELLULAR VESICLES AND CIRCULATING TUMOUR CELLS

Circulating cell-free DNA contains fragments released into the bloodstream from normal and pathological tissues. The tumour-derived fraction, termed circulating tumour DNA, may contain somatic mutations, copy-number alterations or methylation changes. In cervical cancer, ctDNA approaches may complement circulating HPV DNA by providing information about host genomic alterations and tumour evolution [38,39].

However, tumour-derived DNA may be present at very low concentrations, particularly in early-stage disease. High analytical sensitivity is therefore essential, and interpretation may be complicated by non-tumour somatic alterations arising from processes such as clonal haematopoiesis. Thus, ctDNA remains more promising for monitoring and tumour characterization than for population-level screening [38].

Extracellular vesicles, including exosomes, provide another potential source of biomarkers. Tumour-derived vesicles may contain proteins, lipids, microRNAs, lncRNAs and other molecular components that reflect tumour biology. Their membrane structure can protect molecular cargo from degradation, potentially improving analytical stability.

Nevertheless, exosomal biomarker development is limited by challenges involving isolation, purification, normalization and identification of tumour-specific vesicles. Distinguishing tumour-derived extracellular vesicles from the large background population of physiologically produced vesicles remains technically difficult. These biomarkers should therefore currently be considered investigational [43].

Similarly, circulating tumour cells provide the opportunity to study intact malignant cells within the bloodstream. Their low abundance, particularly in localized disease, creates substantial technical challenges. Their greatest potential may therefore be in advanced disease, metastasis research and therapeutic characterization rather than early screening [39].

8. THE FUTURISTIC AND -OMICS BIOMARKERS

8.1 Genomic Biomarkers and the Somatic Landscape of Cervical Cancer

Next-generation sequencing has fundamentally expanded understanding of cervical cancer biology. Large-scale molecular characterization has demonstrated that cervical cancer is not a uniform disease but consists of biologically heterogeneous

tumours with distinct genomic and molecular features. The TCGA integrated analysis of primary cervical cancers identified recurrent alterations involving pathways related to PI3K signalling, TGF-β signalling, chromatin regulation and immune biology. More than 70% of analysed tumours contained alterations affecting PI3K–MAPK and/or TGF-β pathways [34].

Frequently altered genes include PIK3CA, EP300, FBXW7, PTEN, ARID1A, ERBB3, CASP8, HLA-A and TGFBR2. Among these, PIK3CA is particularly important because alterations in this gene can activate the PI3K/AKT signalling network, a pathway involved in proliferation, metabolism and survival. Genomic abnormalities affecting chromatin-remodelling genes also illustrate the close interaction between genetic and epigenetic mechanisms during tumour evolution [34].

Genomic biomarkers are particularly valuable when they move beyond disease identification toward tumour subclassification and therapeutic stratification. Molecular alterations may be categorized according to their clinical role as: Diagnostic biomarkers that support disease identification or classification, Prognostic biomarkers,those providing information regarding expected disease behaviour and Predictive biomarkers,indicating the likelihood of response to a specific treatment.

This distinction is essential because the presence of a molecular alteration does not automatically imply clinical actionability. Therapeutic relevance depends on functional significance, tumour context, drug availability and clinical evidence. However, exisiting lierature appropriately cautions against assuming that every recurrent genomic alteration represents a useful therapeutic target.

8.2 Transcriptomic and Proteomic Biomarkers

Transcriptomic profiling provides an additional layer of information between genomic alteration and functional phenotype. Gene-expression signatures can identify activated signalling pathways, immune states, proliferative programmes and molecular subgroups that may not be apparent from DNA sequencing alone. HPV-mediated transformation influences transcriptional networks involved in cell-cycle regulation, DNA repair, immune responses, metabolism and epithelial differentiation [34,35].

Multigene signatures may therefore provide greater biological resolution than single-gene biomarkers. However, RNA instability, sample quality requirements, platform variation and computational complexity currently limit widespread implementation in population screening. Transcriptomic approaches are consequently more established in research and tumour characterization than in routine cervical screening [35].

Proteomic biomarkers provide a closer representation of functional cellular activity because proteins and their post-translational modifications execute many of the biological consequences of genomic and transcriptional alterations. The clinical success of p16 and Ki-67 demonstrates how protein-level biomarkers can be translated into diagnostic practice. High-throughput proteomics expands this concept by allowing simultaneous analysis of numerous proteins, signalling pathways and post-translational modifications.

An important implication is that DNA alterations alone cannot completely predict tumour phenotype. Protein abundance, phosphorylation and other post-translational events may significantly influence proliferation, immune interactions and therapeutic response. Therefore, proteomic analysis represents an important bridge between molecular architecture and functional cancer biology [35,36].

Metabolomics is particularly essential for all the -omic based markers because it provides a functional representation of cellular activity. Whereas genomics identifies genetic potential and transcriptomics reflects gene expression, metabolomics captures downstream biochemical consequences of molecular and environmental interactions. Consequently, metabolic signatures may complement molecular biomarkers by revealing the functional phenotype associated with HPV-driven transformation. However, unlike HPV DNA testing or established molecular triage assays, metabolic biomarkers have not yet been standardized into universally accepted clinical screening platforms and require extensive multicentre validation [25–27].

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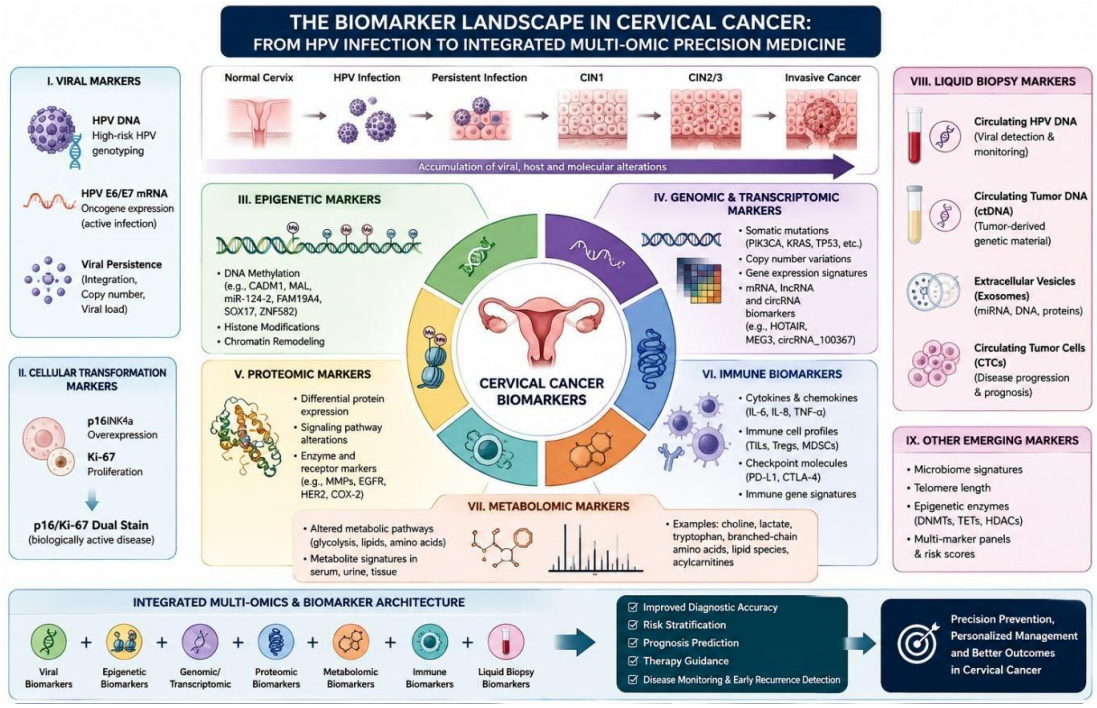

Molecular Biomarkers as a Bridge to Other Biomarkers

The molecular events driving cervical carcinogenesis inevitably generate functional biochemical consequences. Persistent E6/E7 activity promotes uncontrolled proliferation, while genomic and epigenetic abnormalities modify signalling networks, nutrient utilization and cellular survival. Alterations involving pathways such as PI3K/AKT can influence growth, protein synthesis and metabolism. Hypoxia, mitochondrial dysfunction and oxidative stress further contribute to metabolic adaptation [10,25,34].

Therefore, molecular transformation is ultimately accompanied by reprogramming of metabolic functions like glucose utilization, mitochondrial energetics, lipid metabolism, antioxidant defence and cellular redox balance. Thus this understanding correctly positions metabolic biomarkers as a downstream functional complement to molecular biomarkers. Molecular analysis addresses the question: Which oncogenic mechanisms are altered? Metabolic analysis addresses the complementary question: What biochemical phenotype has resulted from those alterations?

This relationship provides a strong conceptual basis for an integrated molecular–metabolic biomarker strategy. Genomic and epigenetic alterations may establish the potential for malignant behaviour, whereas the metabolome provides a more immediate representation of the functional biochemical state of the transformed cell. Metabolic changes may therefore complement molecular biomarkers in disease detection, risk stratification, prognosis and treatment monitoring [25–27].

Molecular Biomarker Testing, Self-Sampling & Clinical Translation

An important advantage of molecular testing is its potential compatibility with self-collected cervicovaginal specimens. Traditional cytology depends heavily on obtaining appropriately preserved cells from the cervical transformation zone, making clinician-collected samples particularly important. Molecular assays, in contrast, may detect nucleic acids without requiring preservation of detailed cellular morphology.

HPV DNA testing is therefore particularly suitable for self-sampling, and selected DNA methylation assays may also be adaptable to this approach. The potential public-health implications are substantial. Self-sampling could reduce barriers related to geographical access, shortage of trained healthcare personnel, discomfort with pelvic examination and other obstacles to participation in cervical screening [2,3,24].

The value of a biomarker should consequently not be assessed only according to analytical sensitivity and specificity. Scalability, affordability, accessibility and compatibility with decentralized sampling may be equally important when

considering implementation in real-world screening programmes.

Despite substantial progress, many promising molecular biomarkers have not entered routine clinical practice. One major obstacle is biological heterogeneity. Cervical cancers vary according to HPV genotype, histological subtype, stage, host biology and immune microenvironment. Therefore, a biomarker that performs well in one clinical population may not demonstrate identical performance elsewhere.

Pre-analytical variability also represents a major concern. Sampling technique, specimen type, transport conditions, storage duration, extraction method and cellular composition may influence molecular measurements independently of disease biology. Analytical variation further complicates translation. PCR, quantitative PCR, digital PCR, sequencing, immunocytochemistry and methylation platforms differ in detection limits, normalization procedures and positivity thresholds. A clinically useful biomarker requires robust analytical validity and reproducibility [44].

Population characteristics must also be considered. Age distribution, HPV prevalence, vaccination coverage, geographical genotype patterns and background disease prevalence can influence diagnostic performance. Independent external validation is therefore essential before widespread implementation.

Sensitivity alone is also insufficient for determining biomarker value. In triage settings, very high sensitivity accompanied by poor specificity may lead to excessive colposcopy and biopsy. Positive and negative predictive values additionally depend on disease prevalence. Therefore, biomarker performance must always be interpreted within its intended clinical application and population [44].

CONCLUSION:

Why No Single Molecular Biomarker Is Sufficient?

A major conclusion emerging from cervical biomarker research is that searching for a single universally superior biomarker may be biologically unrealistic. Cervical carcinogenesis is a sequential and multidimensional process, and each biomarker interrogates only a particular component of that process. For example, HPV DNA identifies oncogenic viral infection with high sensitivity whereas HPV genotype and persistence refine long-term carcinogenic risk Similarly E6/E7 mRNA indicates transcriptionally active viral oncogenes whereas p16/Ki-67 dual staining identifies abnormal host-cell cycle deregulation and proliferation.

Thus, evidently these biomarkers therefore answer different clinical questions. Hence an effective multimarker strategy for screening and precancer detection might combine HPV genotype, viral oncogene activity, host-cell transformation markers and DNA methylation can be a better strategy than a one biomarker–one diagnosis. In contrast, biomarker panels for established invasive cancer may incorporate somatic genomic alterations, immune features, circulating HPV DNA and functional proteomic or metabolic information. Thus, biomarker architecture should be tailored to the clinical context.

From Molecular Detection to Precision Cervical Cancer Management

The development of cervical diagnostics can be viewed as a progression through increasing biological resolution i.e From detection of morphological abnormalities, HPV Genotype testing to Intergated multi-omic molecular metabolic signatures where Genomics, transcriptomics, proteomics and liquid biopsy characterize tumour heterogeneity and therapeutic vulnerabilities can be established.

Molecular biomarker research has fundamentally transformed the understanding of cervical carcinogenesis. Cervical cancer is no longer viewed exclusively as a disease identified through morphological abnormalities; instead, it is increasingly understood as a biologically ordered continuum extending from persistent oncogenic HPV infection through viral oncogene activity, host-cell-cycle deregulation, epigenetic modification, genomic evolution and tumour progression.

The central conclusion is that no single molecular biomarker can adequately represent every stage of cervical carcinogenesis. HPV DNA identifies infection; E6/E7 expression reflects viral oncogenic activity; p16/Ki-67 indicates host-cell transformation; DNA methylation reflects epigenetic progression; genomic and proteomic analyses characterize tumour biology; and circulating tumour-derived analytes provide opportunities for longitudinal monitoring.

The future of cervical cancer precision diagnostics therefore lies in integrated biomarker architectures rather than isolated

molecular measurements.

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