

Biomarkers for oesophageal squamous cell carcinoma and the role of HPV: Multi-omics approaches and current evidence (Review)

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Received October 21, 2025; Accepted April 1, 2026

DOI: 10.3892/ijo.2026.5887

Abstract. Oesophageal squamous cell carcinoma (ESCC) represents a major global health burden, particularly in regions with high incidence rates, significantly affecting patient quality of life and survival outcomes. Recent advances in multi-omics technologies have highlighted their potential in identifying prognostic markers for ESCC. Concurrently, the possible association between human papillomavirus (HPV) infection and ESCC development has been investigated, although epidemiological evidence remains heterogeneous and a definitive causal role has not been universally established. This narrative review examines the progress in multi-omics approaches for identifying prognostic markers of ESCC and provides a comprehensive analysis of the latest developments in HPV detection methods. Research from genomic, transcriptomic, proteomic, epigenomic, metabolomic, and immunomic studies was synthesized highlighting both promising biomarkers and the significant heterogeneity

in reported results, particularly regarding HPV prevalence rates across various geographical regions and detection methods. The research included not only offers novel insights into the pathogenesis of ESCC but also lays a theoretical foundation for early diagnosis and personalized treatment; however, most findings remain investigational and require prospective validation before clinical implementation. The clinical implications and future research directions are discussed with consideration of current limitations.

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Abbreviations: AI, artificial intelligence; CI, confidence interval; ctDNA, circulating tumour DNA; DFS, disease-free survival; EC, oesophageal cancer; ESCC, oesophageal squamous cell carcinoma; HPV, human papillomavirus; IHC, immunohistochemistry; ILK, integrin-related kinase; ISH, *in situ* hybridisation; L-2HG, L-2-hydroxyglutaric acid; MDH1, malate dehydrogenase 1; MSP, methylation-specific PCR; NGS, next-generation sequencing; NK, natural killer; OS, overall survival; PCR, polymerase chain reaction; ROS, reactive oxygen species; SPM, second primary malignant tumours

Key words: human papillomavirus, oesophageal squamous cell carcinoma, biomarkers, detection methods, multi-omics

1. Introduction

According to the World Health Organization, oesophageal cancer (EC) is the sixth most common cancer worldwide, with a poor prognosis and difficult early diagnosis. EC is primarily classified into two histological types: Squamous cell carcinoma and adenocarcinoma, with squamous cell carcinoma being the predominant form (1). The overall 5-year survival rate for ESCC is <20% (2). The development of oesophageal squamous cell carcinoma (ESCC) is driven by a complex interaction of genetic and environmental factors, including diet, smoking, alcohol consumption, and potentially viral infections (3,4). Recent advances in multi-omics technologies have opened new opportunities for identifying prognostic biomarkers for ESCC. Concurrently, the possible role of human papillomavirus (HPV) infection as a contributing factor in ESCC development has garnered increasing attention. Despite extensive research on the link between HPV and ESCC, the underlying mechanisms remain incompletely understood. Consequently, current research is focused on a comprehensive exploration of the role of HPV in ESCC and the development of reliable detection methods. However, findings from studies

investigating the relationship between HPV and ESCC show considerable variability.

HPV type 16 (HPV16) E6 has been shown to play a significant role in ESCC development (5), with HPV16 infection transforming basal esophageal cells and promoting cellular degeneration (6). Some studies, however, failed to detect high-risk HPV in ESCC samples (7), and in certain cases, HPV positivity was not significantly associated with clinicopathological features (8). These discrepancies may result from variations in study design, sample sources, testing methods, and geographical factors. Therefore, standardizing HPV testing protocols, particularly for ESCC, is essential. The aim of the present review is to outline multi-omics strategies for identifying prognostic markers of ESCC, explore the epidemiological and molecular mechanisms linking HPV infection to ESCC development, and evaluate the clinical applicability of current HPV detection methods. Ultimately, synthesizing these findings may provide novel insights into the diagnosis and treatment of ESCC, although some approaches discussed remain at an investigational stage.

2. Multiple factors promote ESCC formation

The onset of ESCC is closely linked to various factors, including lifestyle, environmental exposures, genetic susceptibility, dietary habits, and infections (Fig. 1).

Lifestyle and environmental exposure are associated with ESCC. Environmental factors and lifestyle significantly contribute to the development of ESCC. Research indicates that smoking and alcohol consumption are major risk factors (9), particularly in regions where these behaviours are prevalent. Smoking directly damages esophageal tissue and increases cancer risk by impairing immune function and promoting chronic inflammation. Alcohol, especially spirits, is strongly linked to ESCC development, and the combined effects of alcohol and tobacco use further increase the risk of EC (8). Drinking hot beverages at temperatures above 65°C notably increases the risk of EC (10). Moreover, deficiencies in vitamins C and E, as well as folic acid, have been associated with ESCC onset, as these deficiencies may impair antioxidant defences, thus increasing the risk of cancer (11). Regarding environmental exposures, individuals chronically exposed to certain chemicals, such as asbestos and industrial toxins, particularly in environments with high levels of smoke and toxic gases, are at significantly greater risk of developing ESCC (12).

Genetic susceptibility is associated with ESCC. Genetic susceptibility is also a key factor in ESCC development. Variants in the human leukocyte antigen gene region, for example, are recognized as risk factors for ESCC, potentially influencing the immune response of an individual to tumour cells (13). Tumour microenvironment alterations are closely linked to the progression of the disease. Cytokines secreted by tumour-infiltrating cells form a complex network that promotes tumourigenesis through mechanisms such as inflammation, immune editing, and immune escape (14). Chronic inflammation is particularly critical, as it can induce genetic mutations and foster tumourigenesis by altering the metabolic state and

microenvironment of cells (15). Future research should focus on understanding how genetic variants regulate the tumour microenvironment in ESCC and how these mechanisms can be harnessed to develop novel therapeutic strategies (16).

HPV infection is associated with ESCC. The association between HPV infection and ESCC has garnered considerable attention, although findings vary substantially across different studies (7,17). A comprehensive analysis of data from Asian countries revealed an overall HPV prevalence of 18.2% in ESCC, with a significant increase in cancer risk linked to HPV infection [odds ratio (OR)=3.81; 95% confidence interval (CI), 2.84-5.11; $P<0.001$] (18).

Geographical and methodological heterogeneity is evident in studying the association between HPV infection and ESCC formation. Several studies have highlighted substantial variation in HPV positivity rates among patients with ESCC. In some high-prevalence regions, the incidence of HPV infection is positively correlated with the incidence of ESCC, suggesting that HPV may be a significant contributing factor to the disease (19,20). For example, a meta-analysis of 26 studies involving 3,429 cases determined that the pooled prevalence of HPV16 infection was 38.1% (95% CI, 28.3-47.9%). The infection rate varied depending on the assay method (such as PCR primer selection) and the sample type (21,22). Conversely, negative results were consistently reported in studies from low-prevalence regions. A cohort study from a low-prevalence area found no cases of oesophageal squamous papilloma associated with squamous cell carcinoma (23), nor did it yield positive HPV RNA *in situ* hybridisation (ISH) results (24). Similarly, a Japanese study found no HPV DNA in any of the 31 EC samples (25), and a Korean study reported that all 129 oesophageal squamous cancer samples were HPV-negative (26). However, the HPV positivity rate was 9.6% (5/52 cases) in a Turkish study, with HPV type 39 being predominant (27). This heterogeneity underscores the need for standardized detection protocols and cautious interpretation of epidemiological data.

Different HPV types may play distinct roles in the pathogenesis of ESCC. HPV16 and HPV18, recognized as high-risk types, are linked to a variety of cancers, including ESCC. Higher frequencies of HPV16 infection have been observed in patients with ESCC and in those with Chagasic megaesophagus (CM)/ESCC (44.5 and 25.0%, respectively), and other high-risk types such as HPV31, HPV45, HPV51, HPV53, HPV56, HPV66, and HPV73 have also been detected (16,28). Additionally, co-infection with multiple HPV types has been documented, which may further increase the risk of cancer (29). Understanding the distribution of various HPV types and their association with cancer risk is essential for developing effective screening and prevention strategies.

Oncogenic mechanisms caused by HPV infection. HPV interacts with host cells in various ways, promoting its oncogenic effects. The primary oncogenic proteins, E6 and E7, disrupt normal cellular functions, particularly by interfering with cell cycle regulation. The E7 protein inhibits the function of retinoblastoma protein by binding to it, thereby inactivating the G₁/S phase checkpoint and facilitating progression into the S phase. This process promotes viral replication and proliferation (30).

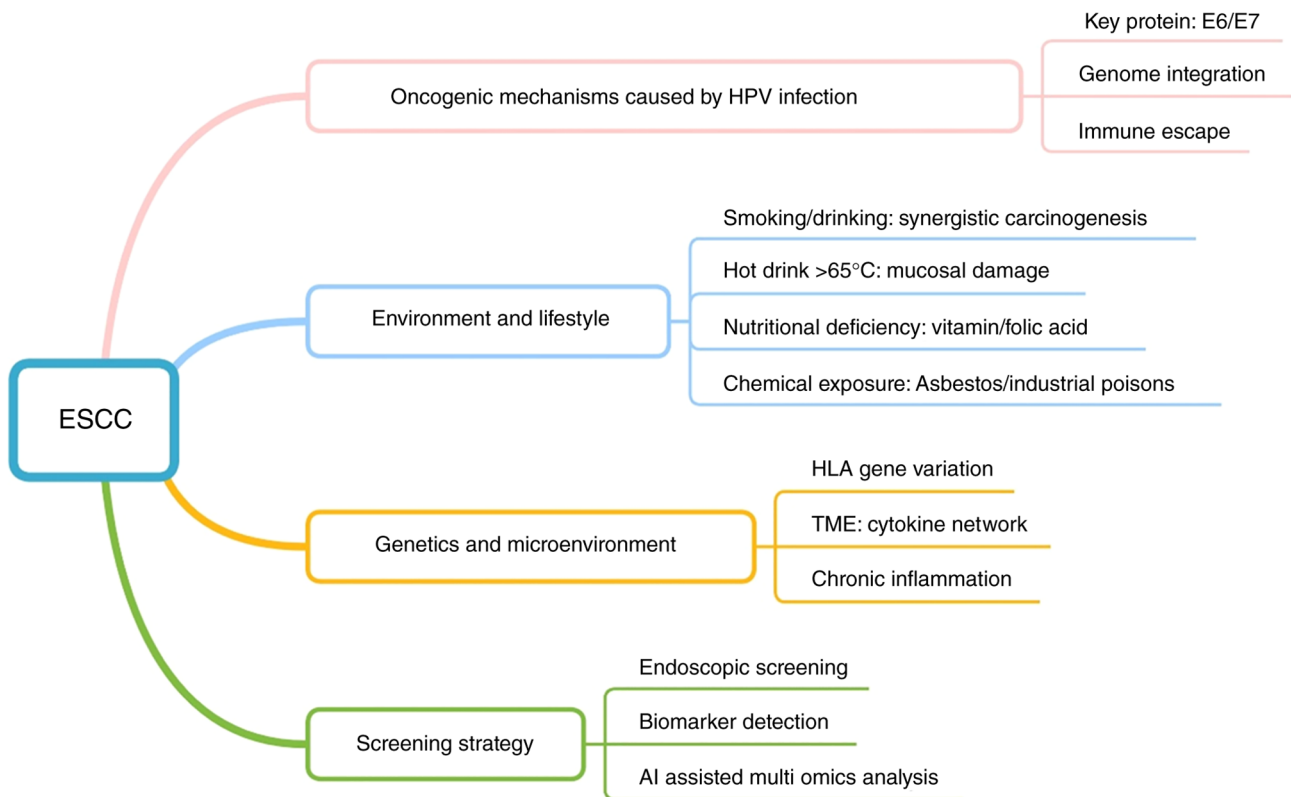


Figure 1. Factors associated with ESCC pathogenesis and screening strategy. ESCC formation is caused by multiple factors including environment and lifestyle, genetics and microenvironment. HPV infection is a pivotal environmental factor that induces ESCC tumorigenesis through genome integration, oncogenic protein expression and immune escape. ESCC, oesophageal squamous cell carcinoma; HPV, human papillomavirus; HLA, human leukocyte antigen; TME, tumour microenvironment.

Additionally, E6 contributes to immune evasion by promoting the degradation of p53 protein, inhibiting apoptosis, and enhancing cell survival, thereby creating a favorable environment for persistent viral infection (5,31). Therefore, it is important to distinguish between *TP53* gene mutations and p53 protein degradation in ESCC clinical samples, namely, mutations alter the gene sequence to express mutated p53 protein, while HPV E6-mediated degradation affects the level of p53 protein post-translationally. This distinction has implications for both biomarker interpretation and therapeutic targeting. These disruptions in cell cycle regulation foster HPV replication and establish conditions conducive to tumour formation.

Genomic integration of HPV plays a pivotal role in its oncogenic mechanism. This typically occurs in high-risk HPV-infected cells, where the viral genome integrates into the host genome. This integration leads to continuous expression of HPV E6 and E7 proteins, potentially inducing instability and accumulating mutations in the host genome, further promoting carcinogenesis (32). Genes located near the HPV integration site are significantly upregulated, contributing to oncogenic phenotypes. Specific genomic regions associated with HPV integration are linked to key biological processes such as cell-cycle regulation, signal transduction, and DNA repair (33). This discovery underscores the critical role of HPV integration in oesophageal carcinogenesis.

The immune escape mechanism is another key aspect of the oncogenic process of HPV. HPV alters signalling pathways in host cells to promote immune evasion. A key

strategy involves downregulating the expression of major histocompatibility complex class I on the cell surface, enabling infected cells to evade immune detection by CD8⁺ T cells (34). Furthermore, during cancer progression, the HPV oncoprotein E7 significantly reduces the expression of C-X-C motif chemokine ligand 14, a chemokine that recruits natural killer (NK) cells, thereby diminishing NK-cell and T-cell infiltration and promoting tumour progression (35). HPV may also influence the tumour immune microenvironment by altering macrophage polarization to a tumour-promoting phenotype (36). Moreover, HPV E7 can inhibit T-cell activity by downregulating Jumonji C histone demethylase 1B, leading to increased expression of the co-inhibitory molecule cytotoxic T lymphocyte-associated antigen 4, which further aids immune evasion and enhances tumourigenesis (37). This complex immune escape mechanism not only contributes to HPV-induced oncogenesis but also presents novel targets for cancer immunotherapy. Understanding the carcinogenic mechanisms is crucial for developing effective detection strategies, as different stages of viral integration and expression may require distinct diagnostic approaches.

3. Prognostic marker mining with multi-omics approaches

Mass screening and early detection are essential strategies for reducing the morbidity and mortality associated with ESCC. Given that ESCC is often asymptomatic in its early stages, with patients typically diagnosed at later stages, early detection

is critical (7). Current screening strategies for EC include endoscopic screening (38), cytological testing, and biomarker testing. With advancements in molecular biology and genomics, researchers are increasingly exploring multi-omics approaches and artificial intelligence (AI)-assisted screening, which can offer more accurate risk assessments and facilitate earlier diagnosis (39).

Genomics. Whole-genome sequencing has revealed driver gene mutation profiles in ESCC, with *TP53* mutations being particularly prevalent. These mutations disrupt cell cycle regulation and apoptotic pathways, thereby promoting tumourigenesis and progression. HPV18 E6 inhibits α -ketoglutarate-induced apoptosis in ESCC cells by promoting p53 protein degradation, which in turn downregulates malate dehydrogenase 1 (MDH1). MDH1 downregulates L-2-hydroxyglutaric acid (L-2HG) expression, thus preventing the increase of reactive oxygen species (ROS), as L-2HG is responsible for ROS accumulation (40). A meta-analysis demonstrated that patients with ESCC exhibiting high p53 protein expression had reduced overall survival (OS), regardless of tumour stage (41). Furthermore, the combined effects of HPV16 infection and *TP53* mutations can influence ESCC prognosis. Among *TP53* mutation-positive patients, those infected with HPV16 showed significantly improved OS compared with uninfected patients (median survival, 57 vs. 27 months), suggesting that HPV16 may serve as a prognostic marker for improved outcomes in this subset (42). Amplification of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α , cyclin D1, and SH3 and multiple ankyrin repeat domains protein 2 has been linked to tumour aggressiveness (43), while downregulation of chromosome 20 open reading frame 54 was revealed to contribute to the development, metastasis, and poor prognosis of ESCC (44).

Transcriptomics. Analysis of mRNA expression profiles has revealed distinct gene expression patterns associated with patient prognosis. For instance, E6/E7 mRNA induces alterations in cell cycle, proliferation, invasion, metastasis, and other key cancer cell behaviours by modulating downstream tumour-related signalling pathways (45). In HPV-infected patients with ESCC, mRNA expression profiles showed significant upregulation of genes involved in the cell cycle and DNA replication, including cyclin A2, DSN1 component of MIS12 kinetochore complex, and minichromosome maintenance 10 replication initiation factor (46). Additionally, microRNA (miR)-25 was shown to accelerate ESCC progression by directly inhibiting B-cell translocation gene 2 expression, with high miR-25 expression significantly associated with lymph node metastasis (47). Doublecortin-like kinase 1, short isoform promoted ESCC progression by activating the MAPK/ERK/MMP2 signalling axis and inducing epithelial-mesenchymal transition, presenting a potential prognostic biomarker and therapeutic target, although functional validation is required (48). Expression levels of C-terminal binding protein 2 (CtBP2) and cyclin H/cyclin-dependent kinase 7 were higher in ESCC tissues with lymph node metastasis compared with tissues without, with CtBP2 also shown to promote ESCC proliferation via negative transcriptional regulation of p16 (INK4A). Therefore, targeting the CtBP2 axis may reduce ESCC cell migration and represent a potential novel therapeutic strategy for ESCC (49).

Proteomics. Advancements in proteomics have enabled the identification of differentially expressed proteins in clinical samples, which may play a pivotal role in tumourigenesis and progression. For example, integrin-related kinase (ILK) has been found to be significantly overexpressed in ESCC compared with normal tissues, with its expression levels strongly associated with patient prognosis. This suggests that ILK could serve as an important prognostic marker for ESCC (50), while its therapeutic potential remains to be explored. Additionally, high expression levels of heat shock protein 27 and pyruvate kinase M2 in ESCC are associated with tumour aggressiveness and poor prognosis (51). A multi-omics approach integrating public databases, proteomics, and immunohistochemistry (IHC) has revealed that interferon γ -inducible protein 30 (IFI30) regulates the JNK and P21/P16 pathways, thereby promoting tumourigenesis in ESCC. Therefore, IFI30 may represent a potential novel therapeutic target for ESCC (52), although functional validation in preclinical models and assessment of druggability are required before clinical translation. The programmed death-ligand 1 (PD-L1) antibody (rabbit anti-human PD-L1 monoclonal, 1:25, clone SP142) has demonstrated efficacy when used alongside immune checkpoint inhibitors (53,54). Furthermore, significant progress has been made in constructing protein-protein interaction networks. Through bioinformatics tools, it was found that proteasome 26S subunit, non-ATPase 2 promotes tumour cell proliferation by inhibiting autophagy in ESCC cells, with its expression levels being closely linked to patient prognosis (55).

Epigenomics. DNA methylation alterations affect genes involved in cell cycle regulation, DNA damage repair, and cancer-related signalling pathways in ESCC (56). A study identified 120 genes whose DNA methylation levels were inversely correlated with their mRNA expression, including 16 key genes such as sine oculis homeobox homolog 4, cellular retinoic acid-binding protein 2, and EH domain-containing protein 3, which were validated in ESCC samples. The expression of these genes was significantly associated with OS and disease-free survival (DFS), suggesting their potential as prognostic markers in patients with ESCC (57). Additionally, the methylation status of the paired box 1 (PAX1) gene has been linked to ESCC development, and PAX1 methylation detection has shown high sensitivity and specificity for early ESCC screening (58), making it a potential screening marker. The integration of HPV genomic integration sites with host gene methylation (epigenetic changes) may help predict the risk of invasive cancer. Circulating tumour DNA (ctDNA) mutations in *TP53* and methylation of septin 9 and short stature homeobox 2 have been associated with reduced OS, DFS, and progression-free survival in individuals with HPV-negative cancer (59). Genome-wide methylation analyses have identified specific DNA methylation biomarkers that can distinguish tumour tissues from normal tissues in ESCC and its associated precancerous lesions (60). In patients with ESCC, hypermethylation is often associated with poorer survival prognosis, providing a novel basis for tumour risk assessment (61,62).

Metabolomics. The identification of characteristic metabolites in ESCC is a key component of metabolomics. Studies have shown that certain amino acid metabolites, such as

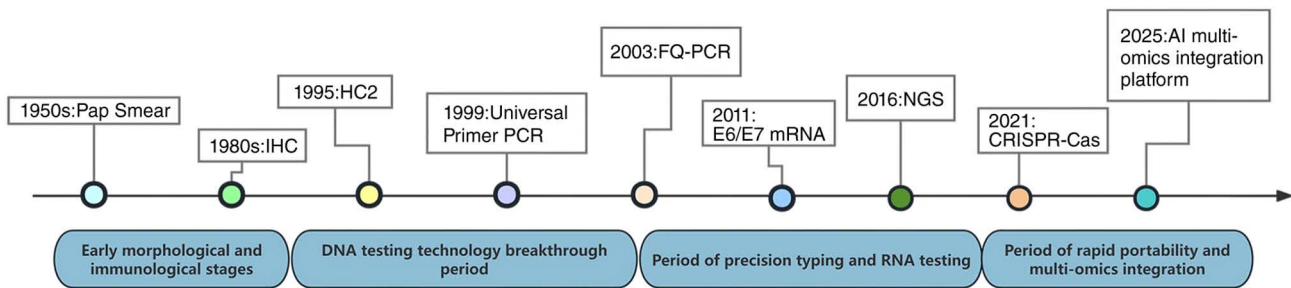


Figure 2. Timeline of the core development of HPV testing methods. The detection methods for HPV testing have gone through four important stages, including early morphological and immunological stage, DNA testing stage, precision typing and RNA testing stage, and rapid portability and multi-omics methods stage. HPV, human papillomavirus; IHC, immunohistochemistry; HC2, Hybrid Capture 2; PCR, polymerase chain reaction; FQ-PCR, fluorescence quantitative PCR; NGS, next-generation sequencing; CRISPR, clustered regularly interspaced short palindromic repeats; AI, artificial intelligence.

phenylalanine and tyrosine, are significantly increased, while others, such as L-tryptophan and 5-hydroxyindoleacetic acid, are decreased in the serum of patients with EC, suggesting a close relationship between these metabolites and tumorigenesis and progression (63–65). A cohort study indicated that nicotinamide and phospholipid metabolism were dysregulated in patients with HPV-positive tumours, while HPV-negative tumours exhibited increased purine and pyrimidine metabolism. This metabolism profile conferred an NAD^+ dependent survival advantage for the tumour cells, and targeting nicotinamide metabolism could specifically induce apoptosis in HPV⁺ cancer cells (66). Additionally, specific metabolites, including creatine and adenosine, play a significant role in the immunosuppressive microenvironment of EC, providing a theoretical basis for developing novel immunotherapeutic strategies (67). Alterations in various metabolites in body fluids or tissues are associated with cancer development and progression, and metabolomic analysis may uncover molecular biomarkers closely linked to ESCC development, capable of predicting cancer occurrence, progression, and prognosis.

Immunomics. A stronger immune response in the tumour microenvironment of HPV-positive patients, particularly the infiltration of CD8⁺ T cells, is associated with a favourable prognosis (68). Moreover, changes in immune-related protein concentrations are strongly associated with ESCC clinical outcomes. The p16 protein, commonly used as a surrogate marker for HPV infection, is closely linked to patient prognosis in EC. IHC expression of p16 shows a high positivity rate in patients with ESCC, especially in high-risk groups (19). HPV infection may upregulate host inflammatory factors, such as IL-6 and TNF- α , and detecting these markers through mass spectrometry or ELISA, in combination with HPV-positive results, may enhance the predictive accuracy of prognosis (69,70). Single-cell RNA sequencing has also revealed that specific immune cell subpopulations are significantly associated with tumour prognosis, providing a crucial foundation for the development of personalized immunotherapy regimens (71).

AI plus multi-omics. Gastrointestinal endoscopy exhibits limited sensitivity in detecting HPV-associated oropharyngeal cancer, but it can aid in distinguishing morphological features in ESCC. For instance, HPV-negative ESCC typically presents as flat erythematous lesions, while HPV-positive tumours, such

as those with *TP53* wild-type, may exhibit an exophytic growth pattern. Combining this with AI analysis may enhance early diagnostic accuracy (72). Radiomics effectively captures radiotypic features of HPV-positive tumours, such as intratumoural heterogeneity and infiltrative border patterns, demonstrating superior predictive performance for HPV-driven subtypes. This positions radiomics as a promising non-invasive biomarker for future HPV-stratified treatments (73). The integration of imaging feature identification, prognostic model development, and multi-omics data advances AI-driven early diagnosis and personalized therapy for ESCC. Currently, no authoritative guidelines recommend incorporating HPV testing into routine EC screening, and the multi-omics integration strategy remains in the preclinical research phase, lacking standardized screening protocols. Investigating the correlation between high-risk HPV subtypes and the geographical distribution of ESCC, developing integration models combining multi-omics data with imaging histology, and validating ctDNA methylation markers are promising research directions.

4. Methodological advances in HPV detection

In recent years, significant advancements in HPV detection methodologies have greatly enhanced the sensitivity and specificity of tests, providing more effective tools for clinical practice. The primary techniques used for HPV detection include polymerase chain reaction (PCR), IHC, genome sequencing, and liquid biopsy. The key developments in HPV testing methods over time are presented in Fig. 2.

Comparative analysis of traditional methods for HPV detection. Histocytological testing is commonly employed for diagnosis, involving the sectioning and staining of biopsy samples, followed by the examination of cellular morphology and tissue structure. This method enables the visualization of pathological features and is widely used in clinical settings. However, histocytological testing is limited in its ability to detect molecular features, particularly in early-stage lesions or poorly differentiated tumours, which can lead to misdiagnosis or missed diagnoses (74).

IHC plays a pivotal role in determining the degree of ESCC differentiation and its association with HPV infection. By using specific antibodies, IHC can detect the expression of key proteins in tumour cells, offering valuable insights into

Table I. Multi-dimensional comparisons of three methods for ISH.

Method	Signal type	Equipment requirement	Genotyping capability	Tissue localization	(Refs.)
CISH	Permanent color development signal (brown/red)	Optical microscope	Limited (single/double type)	Precise (localized to nucleus)	(105,106)
FISH	Transient fluorescent signal (needs to be stored away from light)	Fluorescence microscopes and image analysis systems	Multi-type (multiprobe required)	Precise (fluorescent signal may bleach)	(107,108)
Broad-spectrum probe ISH	Color developed or fluorescent signal, depending on probe labelling method	Selection of microscope according to probe type (normal or fluorescence)	Multi-type (broad spectrum coverage)	Precise (probe coverage affects localization specificity)	(109,110)

ISH, *in situ* hybridisation; CISH, chromogenic *in situ* hybridisation; FISH, fluorescence *in situ* hybridisation.

Table II. Multi-dimensional comparison of main traditional detection methods.

Methods	Sensitivity	Specificity	Genotyping capability	Tissue localization	(Refs.)
IHC	Moderate	Variable	None	Precisely locates the infected area	(111-113)
ISH	High (type-specific)	Moderate (dependent on probe design)	Limited (only partial subtypes)	Localizable viral DNA	(113-115)
PCR	Variable	High (type-specific)	Clear typing (multiple primers required)	Unorganized location information	(26,54,116)

IHC, immunohistochemistry; ISH, *in situ* hybridisation; PCR, polymerase chain reaction.

tumour biology and prognosis. For example, the overexpression of the p16 protein is frequently used as a marker for HPV infection, and its presence in ESCC is closely linked to HPV infection (75). Positive p16 expression is associated with a more favourable tumour prognosis, suggesting its potential value as a prognostic marker in clinical practice (76). However, IHC is highly dependent on the specificity and sensitivity of the antibodies used, which can result in false-positive or false-negative results. Additionally, the handling and fixation of the sample can influence the accuracy of IHC results. Therefore, it is important to interpret IHC findings in conjunction with clinicopathological features and other diagnostic tests to ensure greater diagnostic accuracy and reliability.

Molecular biology testing offers precise molecular insights, detecting gene mutations and viral DNA within cells and uncovering the molecular mechanisms behind tumours. ISH enables the direct observation of viral distribution and expression patterns in tissue sections, and the comparisons among three methods for ISH are depicted in Table I. Some studies have shown that HPV DNA-ISH is strongly associated with p16 IHC (77), providing significant pathological evidence for understanding the role of HPV in the development of

ESCC (78). PCR and next-generation sequencing (NGS) are currently the two principal molecular biology techniques used to detect HPV and its association with ESCC. PCR, using primers that target specific HPV genes, efficiently amplifies viral DNA for subsequent analysis. For example, PCR has been utilized to detect high-risk HPV types, such as HPV16 and HPV18, in EC samples, with certain studies reporting a high positivity rate (17/101, 16.8%) (79). By contrast, NGS offers a broader genomic analysis, detecting mutations and gene expression profiles simultaneously. A North American study identified rare *TP53* gene mutations associated with ESCC through NGS, opening novel avenues for targeted therapies (7).

The advantages and limitations of IHC, PCR, and ISH in detecting HPV-related cancers are summarized in Table II. Combining IHC with HPV RNA ISH offers advantages over using IHC or PCR alone, particularly in guiding therapeutic decisions and making prognostic assessments (80). This combination enhances diagnostic sensitivity and specificity, especially in cases of IHC-positive and PCR-negative HPV. Subsequent ISH testing can confirm or rule out active HPV infection, thus reducing the risk of misdiagnosis (81).

Table III. Comparison of HPV detection methods: Traditional vs. novel.

A, Traditional methods					
Method	Principle/ Technology	Advantages	Disadvantages	Multi-omics integration potential	(Refs.)
PCR (single gene)	Amplification of HPV DNA	High specificity; detects high-risk HPV types	Cannot distinguish episomal vs. integrated HPV; false negatives at low viral load	Moderate (genomic data; requires validation with other omics)	(117-119)
Hybrid Capture 2	RNA probes hybridize to HPV DNA for chemiluminescent detection	High-throughput; detects 13 high- risk HPV types	No genotyping; risk of cross- contamination	Low (single-omics data)	(102,117)
Immuno- cytochemistry	Detection of p16 (INK4a) overexpression via antibodies	Associated with HPV oncogenic activity; low cost	Limited specificity (p16 can be upregulated in non-HPV lesions)	Moderate (protein-level data; combinable with genomic assays)	(115,120)
B, Novel methods					
Next- generation sequencing	Whole-genome or targeted sequencing of HPV DNA/RNA	Detects HPV integration sites, mutations, and co-infections	High cost; complex bioinformatics analysis	High (integrates genomic, epigenomic, and host variation data)	(83,121)
Digital PCR	Absolute quantification of HPV DNA/RNA at single-molecule level	High sensitivity; precise viral load measurement	Expensive equipment; limited multiplexing capability	Moderate (quantitative data supports multi- omics validation)	(122-124)
CRISPR-Cas detection	CRISPR- Cas12a/9/3 enzymes target HPV DNA/RNA for signal amplification	Rapid (<1 h); portable; high specificity	Limited genotyping resolution; requires optimization	Moderate (requires protein-level validation)	(99,125, 126)
Methylation- specific PCR	Detection of methylated HPV DNA (L1/L2) or host genes (FAM19A4/ miR124-2)	Predicts progression to cancer; high specificity	Requires bisulfite conversion; variable thresholds across populations	High (epigenomic data enhances genomic/ transcriptomic models)	(89,127, 128)
Loop- mediated isothermal amplification	Isothermal amplification of HPV DNA with visual readout	Rapid; no specialized equipment needed	Limited genotyping; risk of primer dimerisation	Low (primarily genomic data)	(96,129)
Microfluidic biosensors	HPV DNA/RNA detection via electrochemical or optical signals on miniaturised chips	Portable; real- time results; low sample volume	Limited clinical validation; low multiplexing	Moderate (compatible with proteomic/ metabolomic integration)	(130-132)

Table III. Continued.

B, Novel methods					
Method	Principle/ Technology	Advantages	Disadvantages	Multi-omics integration potential	(Refs.)
Liquid biopsy (ctDNA)	Detection of HPV DNA in ctDNA	Non-invasive; monitors treatment response	Low sensitivity in early-stage lesions	High (integrates genomic and transcriptomic profiling)	(59,133)
Single-cell sequencing	HPV integration and host transcrip- tome profiling at single-cell resolution	Reveals tumour heterogeneity and clonal evolution	Technically challenging; high cost	High (multi-omics data at cellular resolution)	(90,134)
AI-assisted cytology	Automated analysis of Pap smear images using deep learning	Reduces human error; improves screening speed	Requires large training datasets; high initial setup cost	Moderate (correlates with genomic/proteomic data)	(135-137)

HPV, human papillomavirus; PCR, polymerase chain reaction; CRISPR-Cas, clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins; ctDNA, circulating tumour DNA; AI, artificial intelligence.

Progress in novel technologies for HPV detection. Advances in high-throughput screening technologies have significantly facilitated the early detection of ESCC and the discovery of biomarkers. Recently, NGS technology has been widely adopted in cancer research, enabling rapid screening and characterization of HPV subtypes and their correlation with EC across large sample sizes and various specimen types (82,83). This technology can capture α -, β -, and γ -genus HPVs simultaneously, revealing multiple types and rare integrative events, providing a foundation for stratifying high-risk populations (84).

Droplet digital PCR can detect HPV DNA at concentrations as low as 0.1 copies/ μ l in plasma, making it ideal for early screening and postoperative surveillance, particularly in aggressive subsets of oesophageal adenocarcinomas, such as those with submucosal breakthroughs, where it exhibits a significantly higher detection rate (85). HPV E6/E7 mRNA assays, such as reverse transcription-quantitative PCR, reflect viral transcriptional activity and are more effective at distinguishing between latent and causative infections than DNA-based methods. These assays excel in differentiating latent infection from oncogenic status, with current research suggesting that methods capable of detecting viral transcription should be utilized to enhance the accuracy and sensitivity of testing (86,87). Digital PCR demonstrates 80% sensitivity (28/35) and 97% specificity (29/30) for HPV16 detection (88). Methylation-specific PCR (MSP) can measure the methylation levels of five tumour suppressor genes (RASSF1 α , p16 (INK4a), TIMP3, and PCQAP/MED15) in salivary DNA (89). Single-cell sequencing has revealed that HPV genes of different types exhibit distinct integration preferences across various samples and disease stages (90).

The HPV K-mer Index Tversky Estimator is a novel detection algorithm that analyses K-mer data and employs Tversky indexes for DNA and RNA sequences. It offers a fast, sensitive alternative for detecting HPV in macrogenomic and transcriptomic datasets. Recognized as one of the quickest and most accurate methods for identifying HPV genotypes from virtually any NGS data, it also stands out for its simplicity. Furthermore, this method is highly scalable and can be adapted for detecting other microorganisms beyond HPV (91).

Positron emission computed tomography (PET/CT) has been utilized to assess HPV infection status in patients with EC. The sensitivity and specificity of PET/CT for detecting second primary malignant tumours (SPM) were 0.73 (95% CI, 0.49-0.88) and 0.99 (95% CI, 0.98-1.00) (92), respectively. Further subsite analysis indicated that the sensitivity and specificity for oesophageal SPM detection were 0.47 (0.30-0.64) and 0.99 (0.98-1.00), respectively. By combining imaging techniques with molecular biology approaches, researchers have been able to more accurately assess tumour biology and its response to treatment.

The development of novel nanosensors and biosensors is also paving the way for more advanced tools in the early screening of EC (93). Portable HPV detection devices based on ELISA and PCR technologies, enhanced by emerging nanotechnology and biosensor innovations, have significantly improved sensitivity and accuracy (94). Clustered regularly interspaced short palindromic repeats (CRISPR)-based biosensors, widely adopted in both basic and applied research, represent a promising novel approach for nucleic acid detection. Technologies such as CRISPR-associated protein 9 (CRISPR-Cas9), CRISPR-Cas12, and CRISPR-Cas13 show considerable potential for HPV detection (95). A biosensing platform that combines loop-mediated isothermal amplification

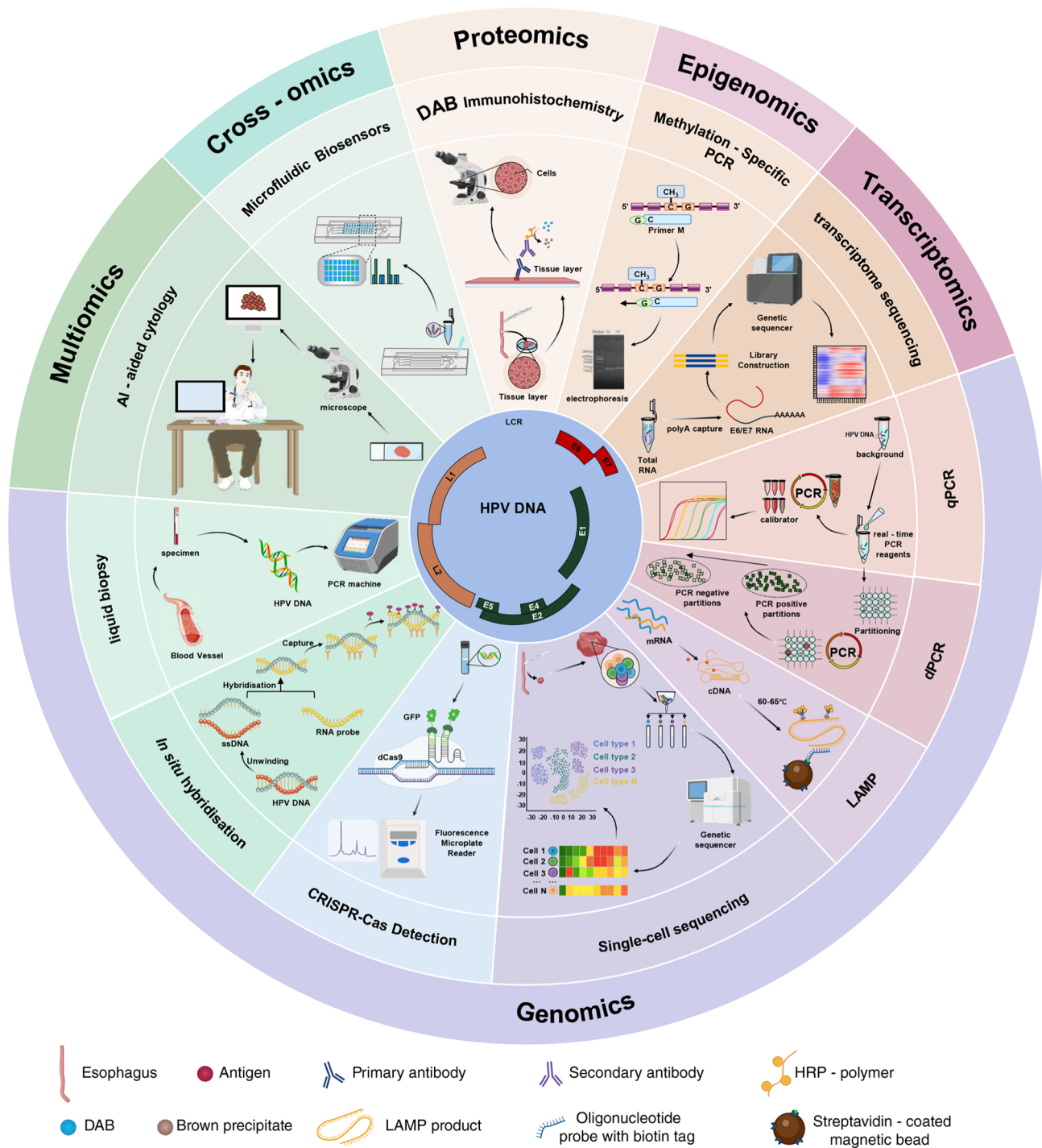


Figure 3. Schematic illustration of HPV detection principle. With the development of modern technology, there are multiple methods for HPV detection, mainly targeting the components or expression components of HPV. These methods include genomics, transcriptomics, epigenomics, proteomics, multi-omics, and cross-omics. HPV, human papillomavirus; DAB, 3,3'-diaminobenzidine; dPCR, digital polymerase chain reaction; qPCR, quantitative polymerase chain reaction; LAMP, loop-mediated isothermal amplification; CRISPR, clustered regularly interspaced short palindromic repeats; AI, artificial intelligence.

with electrochemical analysis can differentiate between free and integrated HPV16 types by targeting critical components, including E7 mRNA and E2 viral gene transcripts, which are absent after integration (96). Additionally, electrochemical sensing technology has introduced novel methods for detecting circulating tumour cells, a technique valued for its high sensitivity and rapid detection capabilities (97).

AI and big data hold significant promise in the analysis of HPV test results. AI technology can process and analyze the

large volumes of data generated by traditional testing methods, assisting in the automatic identification of potential lesion areas and enabling faster clinical decision-making (98). Big data analytics can also help researchers aggregate and analyze HPV infection data across multiple regions and populations, identifying trends in the prevalence of high-risk HPV types within specific groups (95). Furthermore, AI can be leveraged to develop novel HPV detection platforms that combine biosensors with CRISPR technology. These systems enable the

rapid detection of HPV and provide real-time results for field testing, greatly enhancing the convenience and accessibility of testing (99). As bioinformatics and AI technologies continue to evolve, the integration of these tools with big data analysis has the potential to enable personalized medicine approaches in HPV detection and management.

Comparison of methodological advances in HPV testing. A systematic comparison of traditional and novel methods for HPV detection is presented, examining their principles, advantages, disadvantages, potential for multi-omics integration, and clinical applicability (Table III). Traditional techniques, such as PCR, offer high specificity but struggle to differentiate between free and integrated HPV DNA, while p16 IHC is cost-effective yet controversial in terms of specificity (100). Emerging technologies such as NGS can integrate host variant data across multiple genomes, albeit at a high cost, while CRISPR-Cas and microfluidics enable rapid detection. Liquid biopsy provides a non-invasive monitoring method. However, traditional methods require careful handling to avoid issues such as aerosol contamination in PCR, cross-contamination in Hybrid Capture 2, and false negatives due to low viral loads. NGS necessitates specialized bioinformatics analysis and stringent quality control, while the CRISPR-Cas system requires optimization to enhance typing resolution, and liquid biopsies exhibit limited sensitivity in early-stage lesions.

AI-enhanced cytology improves screening efficiency but depends heavily on big data training. Emerging technologies significantly surpass traditional methods in terms of sensitivity and the integration of multi-omics data, particularly genomic, epigenomic, and single-cell data. However, clinical validation and cost constraints remain major challenges. To address these, a combined multi-omics approach, such as integrating DNA methylation testing with traditional HPV DNA testing and cytology, could comprehensively guide clinical decision-making, optimizing treatment plans and enhancing clinical efficacy by mitigating the limitations of individual technologies. Although not extensively discussed in the literature, HPV infection may induce aberrant methylation of host genes (such as p16 and adenomatous polyposis coli) via E6/E7 proteins. Combining this with MSP or pyrophosphate sequencing could lead to the development of an 'HPV infection + methylation profile' screening model, enhancing specificity and potentially reducing false positives in non-cancerous HPV carriers (22). To facilitate early diagnosis, enhancing the specificity of cancer screening through the combination of HPV DNA typing (genomic) with p16/Ki67 double staining (proteomic) is essential (101-103). Moreover, co-infection of HPV with Merkel cell polyomavirus significantly increases the risk of ESCC (OR=2.45), suggesting that multiplex PCR or macrogenomic sequencing could be used to assess infection-related cancer risk more comprehensively (104). A summary of the principles behind various HPV detection methods is provided in Fig. 3.

5. Conclusions

Based on their translational relevance, the biomarkers involved in this review can be categorized into three distinct groups including diagnostic biomarkers, prognostic biomarkers, and

therapeutic biomarkers according to their intended clinical application. The first group comprises diagnostic biomarkers, which aid in the detection or confirmation of ESCC, such as PAX1 methylation for early screening. The second group contains prognostic biomarkers, which provide information on patient outcomes, including survival or recurrence risk, for example *TP53* mutation status, p16 immunohistochemical expression, and DNA methylation signatures associated with overall survival. The third group consists of potential therapeutic targets, which are molecules or pathways that may be amenable to pharmacological intervention, such as ILK, IFI30, CtBP2, and components of the MAPK/ERK pathway. It is important to emphasize that diagnostic and prognostic biomarkers have demonstrated clinical utility, while the majority of therapeutic targets remain at a preclinical stage and require further functional validation and drug development efforts before clinical translation can be considered. The integrated application of multi-omics technologies provides a novel perspective for biomarker discovery in ESCC, enhancing our understanding of its complex biological features, particularly in studies related to HPV infection. By combining multi-level data from genomics, transcriptomics, proteomics, and other domains, this approach offers deeper insights into the mechanisms underlying ESCC. However, it is important to acknowledge that the majority of findings discussed in this review derive from retrospective or exploratory studies, these results require prospective validation in well-designed clinical cohorts. The heterogeneity in HPV detection rates across different studies underscores the need for standardized testing protocols before clinical implementation. Future research should focus on the following aspects: Firstly, developing standardized HPV testing methods with clear analytical and clinical validity; secondly, establishing the clinical utility of multi-omics biomarkers using prospective studies; thirdly, exploring the function of potential therapeutic targets in appropriate model systems; finally, investigating the epidemiological characteristics of HPV across different populations and geographical regions, and sources of heterogeneity. Although challenges remain, continued research and technological advances may eventually yield more precise and effective strategies for ESCC management. Currently, the role of HPV in ESCC and the application of multi-omics technologies have uncovered complex mechanisms of tumorigenesis, and they also highlighted avenues for further investigation that may ultimately benefit patients with ESCC.

Acknowledgements

Not applicable.

Funding

The present review was supported by the National Natural Science Foundation of China (grant no. 82273044), the Science Fund for Distinguished Young Scholars of Fujian Province (grant no. 2021D034), the Science Fund from the Health Commission of Fujian (grant no. 2023GGB04), and the Nantong University Clinical Medicine Special Scientific Research Fund Project (grant no. 2024LZ014).

Availability of data and materials

Not applicable.

Authors' contributions

LZ designed the scope and structure of the review, performed the structured literature searches, critically synthesised and interpreted the findings, and wrote major sections of the manuscript. KL and XZ jointly contributed to the design of the review framework, participated in the critical synthesis and interpretation of findings, and revised key sections of the manuscript. HZ, JZ, JX, XA, XQ, YY and LZ contributed to the structured literature searches, and helped draft and revise specific sections. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Pennathur A, Gibson MK, Jobe BA and Luketich JD: Oesophageal carcinoma. *Lancet* 381: 400-412, 2013.
- Ilson DH and van Hillebergersberg R: Management of patients with adenocarcinoma or squamous cancer of the esophagus. *Gastroenterology* 154: 437-451, 2018.
- Talukdar FR, Ghosh SK, Laskar RS and Mondal R: Epigenetic, genetic and environmental interactions in esophageal squamous cell carcinoma from northeast India. *PLoS One* 8: e60996, 2013.
- Conway E, Wu H and Tian L: Overview of risk factors for esophageal squamous cell carcinoma in China. *Cancers (Basel)* 15: 5604-5627, 2023.
- Hu J, Ji Y, Miao T, Zheng S, Cui X, Hu J, Yang L and Li F: HPV 16 E6 promotes growth and metastasis of esophageal squamous cell carcinoma cells in vitro. *Mol Biol Rep* 50: 1181-1190, 2023.
- Zhao H, Wei Y, Zhang J, Zhang K, Tian L, Liu Y, Zhang S, Zhou Y, Wang Z, Shi S, *et al*: HPV16 infection promotes the malignant transformation of the esophagus and progression of esophageal squamous cell carcinoma. *J Med Virol* 95: e29132, 2023.
- Bauer AH, Alkhateeb KJ, Agoston AT, Odze RD, Joshi MG, Huffman BM, Enzinger P, Perez K, Deshpande V, Cleary JM, *et al*: Transcriptionally active human papillomavirus infection in a minority of esophageal squamous cell carcinomas in North America. *Am J Surg Pathol* 48: 883-889, 2024.
- Inoue M, Shimizu Y, Ishikawa M, Abiko S, Shimoda Y, Tanaka I, Kinowaki S, Ono M, Yamamoto K, Ono S and Sakamoto N: Relationships of early esophageal cancer with human papillomavirus and alcohol metabolism. *World J Gastroenterol* 26: 6047-6056, 2020.
- Castro C, Peleteiro B and Lunet N: Modifiable factors and esophageal cancer: A systematic review of published meta-analyses. *J Gastroenterol* 53: 37-51, 2018.
- Luo H and Ge H: Hot tea consumption and esophageal cancer risk: A Meta-analysis of observational studies. *Front Nutr* 9: 831567, 2022.
- Cai Y, Lin J, Wei W, Chen P and Yao K: Burden of esophageal cancer and its attributable risk factors in 204 countries and territories from 1990 to 2019. *Front Public Health* 10: 952087, 2022.
- Abnet CC, Arnold M and Wei WQ: Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology* 154: 360-373, 2018.
- Hu JM, Li L, Chen YZ, Liu C, Cui X, Yin L, Yang L, Zou H, Pang L, Zhao J, *et al*: HLA-DRB1 and HLA-DQB1 methylation changes promote the occurrence and progression of Kazakh ESCC. *Epigenetics* 9: 1366-1373, 2014.
- DeNardo DG, Andreu P and Coussens LM: Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumor immunity. *Cancer Metastasis Rev* 29: 309-316, 2010.
- Papamentzelopoulou M and Pitririga VC: Unlocking the interactions between the Whole-body microbiome and HPV infection: A literature review. *Pathogens* 14: 293, 2025.
- Ndemela LM, Ottoman OM, Chitemo HD, Minja CA, Rambau PF and Kidenya BR: Epidemiological distribution of high-risk human papillomavirus genotypes and associated factors among patients with esophageal carcinoma at Bugando medical center in Mwanza, Tanzania. *BMC Cancer* 24: 932-941, 2024.
- Guo F, Liu Y, Wang X, He Z, Weiss NS, Madeleine MM, Liu F, Tian X, Song Y, Pan Y, *et al*: Human papillomavirus infection and esophageal squamous cell carcinoma: A case-control study. *Cancer Epidemiol Biomarkers Prev* 21: 780-785, 2012.
- Petrelli F, De Santi G, Rampulla V, Ghidini A, Mercurio P, Mariani M, Manara M, Rausa E, Lonati V, Viti M, *et al*: Human papillomavirus (HPV) types 16 and 18 infection and esophageal squamous cell carcinoma: A systematic review and meta-analysis. *J Cancer Res Clin Oncol* 147: 3011-3023, 2021.
- Woellner LFA, Medeiros JS, Ribas CAPM, Nassif PAN, Ribas-Filho JM, Sobral ACL, Ariede BL, Costa DAPDD and Malafaia O: Is there correlation between human papillomavirus (Hpv) and esophageal epidermoid carcinoma? *Arq Bras Cir Dig* 34: e1528, 2021.
- He D, Zhang DK, Lam KY, Ma L, Ngan HY, Liu SS and Tsao SW: Prevalence of HPV infection in esophageal squamous cell carcinoma in Chinese patients and its relationship to the p53 gene mutation. *Int J Cancer* 72: 959-964, 1997.
- Zhang SK, Guo LW, Chen Q, Zhang M, Liu SZ, Quan PL, Lu JB and Sun XB: Prevalence of human papillomavirus 16 in esophageal cancer among the Chinese population: A systematic review and meta-analysis. *Asian Pac J Cancer Prev* 15: 10143-10149, 2014.
- Li SY, Li Y, Shen LP, Wu XZ, Zhao XY, Zhou L, Liu HT and Zeng Y: Meta analysis on etiological relationship between human papillomavirus and esophageal carcinoma. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 23: 85-87, 2009 (In Chinese).
- Antunes LC, Prolla JC, de Barros Lopes A, da Rocha MP and Fagundes RB: No evidence of HPV DNA in esophageal squamous cell carcinoma in a population of Southern Brazil. *World J Gastroenterol* 19: 6598-6603, 2013.
- Li Y, Lin F, Ling Q, Xiao Y, Xue X, Zhou W and Wang HL: Detection of human papillomavirus in squamous papilloma of the esophagus. *Int J Surg Pathol* 32: 748-757, 2024.
- Akutsu N, Shirasawa H, Nakano K, Tanzawa H, Asano T, Kobayashi S, Isono K and Simizu B: Rare association of human papillomavirus DNA with esophageal cancer in Japan. *J Infect Dis* 171: 425-428, 1995.
- Koh JS, Lee SS, Baek HJ and Kim YI: No association of High-risk human papillomavirus with esophageal squamous cell carcinomas among Koreans, as determined by polymerase chain reaction. *Dis Esophagus* 21: 114-117, 2008.
- Turkay DO, Vural C, Sayan M and Gurbuz Y: Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an institutional experience from Turkey and review of literature. *Pathol Res Pract* 212: 77-82, 2016.
- Munari FF, Sichero L, Carloni AC, Lacerda CF, Nunes EM, de Oliveira ATT, Scapulatempo-Neto C, da Silva SRM, Crema E, Adad SJ, *et al*: Frequency of human papillomavirus detection in chagasic megaesophagus associated or not with esophageal squamous cell carcinoma. *Pathobiology* 89: 29-37, 2021.
- Zheng Y, Fan YG, Zeng Y, Liu SY and Gao LM: Different genotype distribution of human papillomavirus between cervical and esophageal cancers: A study in both High-Incidence Areas, Xinjiang, China. *Biomed Res Int* 2020: 7926754, 2020.
- Anna Szymonowicz K and Chen J: Biological and clinical aspects of HPV-related cancers. *Cancer Biol Med* 17: 864-878, 2020.
- Astori G, Merluzzi S, Arzese A, Brosolo P, de Pretis G, Maieron R, Pipan C and Botta GA: Detection of human papillomavirus DNA and p53 gene mutations in esophageal cancer samples and adjacent normal mucosa. *Digestion* 64: 9-14, 2001.

32. Jeon S and Lambert PF: Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: Implications for cervical carcinogenesis. *Proc Natl Acad Sci USA* 92: 1654-1658, 1995.
33. Mima M, Okabe A, Hoshii T, Nakagawa T, Kurokawa T, Kondo S, Mizokami H, Fukuyo M, Fujiki R, Rahmutulla B, *et al*: Tumorigenic activation around HPV integrated sites in head and neck squamous cell carcinoma. *Int J Cancer* 152: 1847-1862, 2023.
34. Piersma SJ: Immunosuppressive tumor microenvironment in cervical cancer patients. *Cancer Microenvironment* 4: 361-375, 2011.
35. Westrich JA, Vermeer DW, Silva A, Bonney S, Berger JN, Cicchini L, Greer RO, Song JI, Raben D, Slansky JE, *et al*: CXCL14 suppresses human papillomavirus-associated head and neck cancer through antigen-specific CD8+ T-cell responses by upregulating MHC-I expression. *Oncogene* 38: 7166-7180, 2019.
36. Yuan X, Liu K, Li Y, Zhang AZ, Wang XL, Jiang CH, Liang WH, Zhang HJ, Pang LJ, Li M, *et al*: HPV16 infection promotes an M2 macrophage phenotype to promote the invasion and metastasis of esophageal squamous cell carcinoma. *Clin Transl Oncol* 23: 2382-2393, 2021.
37. Zhou Q, Chen L, Song Y, Ma L, Xiao P, Chen L, Zhen H, Han R, Chen X, Sun S, *et al*: Induction of co-inhibitory molecule CTLA-4 by human papillomavirus E7 protein through downregulation of histone methyltransferase JHDM1B expression. *Virology* 538: 111-118, 2019.
38. Liu W, Yuan X, Guo L, Pan F, Wu C, Sun Z, Tian F, Yuan C, Zhang W, Bai S, *et al*: Artificial Intelligence for Detecting and Delineating Margins of Early ESCC Under WLI Endoscopy. *Clin Transl Gastroenterol* 13: e00433, 2022.
39. Meng QQ, Gao Y, Lin H, Wang TJ, Zhang YR, Feng J, Li ZS, Xin L and Wang LW: Application of an artificial intelligence system for endoscopic diagnosis of superficial esophageal squamous cell carcinoma. *World J Gastroenterol* 28: 5483-5493, 2022.
40. Tang D, Zheng Y, Wang G, Sheng C, Liu Z, Wang B, Zong Q, Zhang Y, Hou X, Yao M and Zhou Z: HPV18 E6 inhibits alpha-ketoglutarate-induced pyroptosis of esophageal squamous cell carcinoma cells via the P53/MDH1/ROS/GSDMC pathway. *FEBS Open Bio* 13: 1522-1535, 2023.
41. Zhao Z, Wang P, Gao Y and He J: The high expression instead of mutation of p53 is predictive of overall survival in patients with esophageal squamous-cell carcinoma: A meta-analysis. *Cancer Med* 6: 54-66, 2017.
42. Qu F, Ji C, Wang Y, Zhu R, Hu W, Liu S, Zhao X, Li J, Miao G, Zhang M, *et al*: Survival benefits of human papillomavirus 16 infection in patients with esophageal squamous cell carcinoma undergoing chemoradiotherapy: A retrospective cohort study. *J Med Virol* 96: e29592, 2024.
43. Erkizan HV, Sukhadia S, Natarajan TG, Marino G, Notario V, Lichy JH and Wadleigh RG: Exome sequencing identifies novel somatic variants in African American esophageal squamous cell carcinoma. *Sci Rep* 11: 14814, 2021.
44. Li M, Kong J, Wang L, Yan H, Liang W, Wang N and Zhao J: Defective expression of C20orf54 in esophageal dysplasia: A possible biomarker of esophageal carcinoma for early detection. *World J Surg Oncol* 20: 155, 2022.
45. Peng Q, Wang L, Zuo L, Gao S, Jiang X, Han Y, Lin J, Peng M, Wu N, Tang Y, *et al*: HPV E6/E7: Insights into their regulatory role and mechanism in signaling pathways in HPV-associated tumor. *Cancer Gene Ther* 31: 9-17, 2024.
46. Shafiq MO, Cakir MO, Bilge U, Pasha Y and Ashrafi GH: Transcriptomic analysis of HPV-positive oesophageal tissue reveals upregulation of genes linked to cell cycle and DNA replication. *Int J Mol Sci* 26: 56, 2024.
47. Guo B and Tian Z: Mir-25 Promotes metastasis of esophageal cancer by targeting BTG2. *Appl Biochem Biotechnol* 195: 5365-5378, 2023.
48. Ge Y, Fan X, Huang X, Weygant N, Xiao Z, Yan R, Liu H, Liu J, An G and Yao J: DCLK1-Short splice variant promotes esophageal squamous cell carcinoma progression via the MAPK/ERK/MMP2 pathway. *Mol Cancer Res* 19: 1980-1991, 2021.
49. Zhang J, Zhu J, Yang L, Guan C, Ni R, Wang Y, Ji L and Tian Y: Interaction with CCNH/CDK7 facilitates CtBP2 promoting esophageal squamous cell carcinoma (ESCC) metastasis via upregulating epithelial-mesenchymal transition (EMT) progression. *Tumour Biol* 36: 6701-6714, 2015.
50. Ma XL, Yao H, Wang X, Wei Y, Cao LY, Zhang Q and Zhang L: ILK predicts the efficacy of chemoradiotherapy and the prognosis of patients with esophageal squamous cell carcinoma. *Oncol Lett* 18: 4114-4125, 2019.
51. Zhang X, Liu T, Zheng S, Liu Q, Shen T, Han X, Zhang Q, Yang L and Lu X: SUMOylation of HSP27 regulates PKM2 to promote esophageal squamous cell carcinoma progression. *Oncol Rep* 44: 1355-1364, 2020.
52. Xie W, Wei S, Feng C, Fu Y, Zhang Z, Dai S, Zhang C, Zhao L and Shan B: IFI30 knockdown inhibits ESCC progression by promoting apoptosis and senescence via activation of JNK and P21/P16 pathways. *Thorac Cancer* 16: e70063, 2025.
53. Hongo T, Yamamoto H, Jiromaru R, Yasumatsu R, Kuga R, Nozaki Y, Hashimoto K, Matsuo M, Wakasaki T, Tamae A, *et al*: PD-L1 expression, tumor-infiltrating lymphocytes, mismatch repair deficiency, EGFR alteration and HPV infection in sino-nasal squamous cell carcinoma. *Mod Pathol* 34: 1966-1978, 2021.
54. Lee HK, Kwon MJ, Ra YJ, Lee HS, Kim HS, Nam ES, Cho SJ, Park HR, Min SK, Seo J, *et al*: Significance of druggable targets (PD-L1, KRAS, BRAF, PIK3CA, MSI, and HPV) on curatively resected esophageal squamous cell carcinoma. *Diagn Pathol* 15: 126, 2020.
55. Liu Y, Wu M, Xu S, Niu X, Liu W, Miao C, Lin A, Xu Y and Yu L: PSMD2 contributes to the progression of esophageal squamous cell carcinoma by repressing autophagy. *Cell Biosci* 13: 67, 2023.
56. Lin L, Cheng X and Yin D: Aberrant DNA methylation in esophageal squamous cell carcinoma: Biological and clinical implications. *Front Oncol* 10: 549850, 2020.
57. Chen Y, Liao LD, Wu ZY, Yang Q, Guo JC, He JZ, Wang SH, Xu XE, Wu JY, Pan F, *et al*: Identification of key genes by integrating DNA methylation and next-generation transcriptome sequencing for esophageal squamous cell carcinoma. *Aging (Albany NY)* 12: 1332-1365, 2020.
58. Fang C, Wang SY, Liou YL, Chen MH, Ouyang W and Duan KM: The promising role of PAX1 (aliases: HUP48, OFC2) gene methylation in cancer screening. *Mol Genet Genomic Med* 7: e506, 2019.
59. Kaorey N, Dickinson K, Agnihotram VR, Zeitouni A, Sadeghi N and Burnier JV: The role of ctDNA from liquid biopsy in predicting survival outcomes in HPV-negative head and neck cancer: A meta-analysis. *Oral Oncol* 161: 107148, 2025.
60. Liu J, Dai L, Wang Q, Li C, Liu Z, Gong T, Xu H, Jia Z, Sun W, Wang X, *et al*: Multimodal analysis of cfDNA methylomes for early detecting esophageal squamous cell carcinoma and precancerous lesions. *Nat Commun* 15: 3700-3715, 2024.
61. Tian Z, Li Z, Zhu Y, Meng L, Liu F, Sang M and Wang G: Hypermethylation-mediated inactivation of miR-124 predicts poor prognosis and promotes tumor growth at least partially through targeting EZH2/H3K27me3 in ESCC. *Clin Exp Metastasis* 36: 381-391, 2019.
62. Song G, Xu J, He L, Sun X, Xiong R, Luo Y, Hu X, Zhang R, Yue Q, Liu K and Feng G: Systematic profiling identifies PDLIM2 as a novel prognostic predictor for oesophageal squamous cell carcinoma (ESCC). *J Cell Mol Med* 23: 5751-5761, 2019.
63. Li X, Zhao L, Wei M, Lv J, Sun Y, Shen X, Zhao D, Xue F, Zhang T and Wang J: Serum metabolomics analysis for the progression of esophageal squamous cell carcinoma. *J Cancer* 12: 3190-3197, 2021.
64. Cheng J, Liu Q, Jin H, Zeng D, Liao Y, Zhao Y, Gao X and Zheng G: Integrating transcriptome and metabolome variability to reveal pathogenesis of esophageal squamous cell carcinoma. *Biochim Biophys Acta Mol Basis Dis* 1867: 165966, 2021.
65. Zhang S, Lu X, Hu C, Li Y, Yang H, Yan H, Fan J, Xu G, Abnet CC and Qiao Y: Serum metabolomics for biomarker screening of esophageal squamous cell carcinoma and esophageal squamous dysplasia using gas Chromatography-mass spectrometry. *ACS Omega* 5: 26402-26412, 2020.
66. Jaiswal S, Mishra V, Majumder S, Wangikar PP and Sengupta S: Metabolomic profiling reveals grade-specific niacinamide accumulation and its therapeutic potential via SIRT1-CD38-EMT axis modulation in cervical cancer progression. *Biochim Biophys Acta Mol Cell Res* 1872: 119994, 2025.
67. Gao Y, He S, Meng X, Zheng K, Cui H, Cheng Y, Shen X, Zhai Y, Zou B, Wang F, *et al*: Multi-omics analysis reveals immunosuppression in oesophageal squamous cell carcinoma induced by creatine accumulation and HK3 deficiency. *Genome Med* 17: 44-68, 2025.
68. Zhang D, Tang WJ, Tang D, Zhou J, Chou L, Tao L and Lu LM: The ratio of CD4/CD8 T-cells in human papillomavirus-positive laryngeal squamous cell carcinoma accounts for improved outcome. *Acta Otolaryngol* 136: 826-833, 2016.
69. Li X, Escoffier H, Sauter T and Tavassoli M: Targeting Fibroblast-derived interleukin 6: A strategy to overcome Epithelial-mesenchymal transition and radioresistance in head and neck cancer. *Cancers (Basel)* 17: 267, 2025.

70. Jenkins BH, Tracy I, Rodrigues MFSD, Smith MJL, Martinez BR, Edmond M, Mahadevan S, Rao A, Zong H, Liu K, *et al*: Single cell and spatial analysis of immune-hot and immune-cold tumours identifies fibroblast subtypes associated with distinct immunological niches and positive immunotherapy response. *Mol Cancer* 24: 3, 2025.
71. Gao G, Deng A, Liang S, Liu S, Fu X, Zhao X and Yu Z: Integration of bulk RNA sequencing and Single-cell RNA sequencing to reveal uveal melanoma tumor heterogeneity and cells related to survival. *Front Immunol* 13: 898925, 2022.
72. Tayama S, Miyamoto H, Waki K, Honda M, Matsuno K, Yamasaki A, Gushima R, Nagaoka K, Naoe H, Imuta M, *et al*: Impact of HPV status on oropharyngeal cancer detection via gastrointestinal endoscopy: A retrospective study. *Int J Clin Oncol* 30: 696-704, 2025.
73. Huang C, Cintra M, Brennan K, Zhou M, Colevas AD, Fischbein N, Zhu S and Gevaert O: Development and validation of radiomic signatures of head and neck squamous cell carcinoma molecular features and subtypes. *EBioMedicine* 45: 70-80, 2019.
74. Vallejo C, Gheit Y, Qi J, Nagi TK, Suarez ZK, Haider MA and Zahra T: Management of esophageal squamous cell carcinoma with esophageal stent placement in an elderly patient with dysphagia. *Cureus* 15: e50483, 2023.
75. Ishida H, Kasajima A, Fujishima F, Akaishi R, Ueki S, Yamazaki Y, Onodera Y, Gao X, Okamoto H, Taniyama Y, *et al*: p16 in highly malignant esophageal carcinomas: The correlation with clinicopathological factors and human papillomavirus infection. *Virchows Arch* 478: 219-229, 2021.
76. Zhang CJ, Zhang JX, Wang ZZ, Li P, Shang JW and Guo YJ: Prognostic evaluation of human papillomavirus and p16 in esophageal squamous cell carcinoma. *Chin Med J (Engl)* 133: 751-752, 2020.
77. Chi J, Preeshagul IR, Sheikh-Fayyaz S, Teckie S, Kohn N, Ziemba Y, Laser A, Frank D, Ghaly M, Kamdar D, *et al*: Evaluating of HPV-DNA ISH as an adjunct to p16 testing in oropharyngeal cancer. *Future Sci OA* 6: FSO606, 2020.
78. Chang F, Syrjänen S and Syrjänen K: Demonstration of human papillomavirus (HPV) type 30 in esophageal squamous-cell carcinomas by in situ hybridization. *Int J Cancer* 55: 171-173, 1993.
79. Chang F, Syrjänen S, Shen Q, Cintonino M, Santopietro R, Tosi P and Syrjänen K: Evaluation of HPV, CMV, HSV and EBV in esophageal squamous cell carcinomas from a high-incidence area of China. *Anticancer Res* 20: 3935-3940, 2000.
80. Pakkanen P, Silvoniemi A, Aro K, Bäck L, Irjala H, Aaltonen LM, Hagström J, Haglund C, Laine J, Minn H and Huvila J: Simultaneous p53 and p16 immunostaining for molecular subclassification of head and neck squamous cell carcinomas. *Head Neck Pathol* 18: 73-82, 2024.
81. Nissi L, Huusko T, Routila J, Vaittinen S, Leivo I, Irjala H and Ventelä S: Added value of HPV-DNA in situ hybridization as an adjunct to p16 Immunohistochemistry in oropharyngeal squamous cell carcinoma. *Acta Otolaryngol* 145: 340-347, 2025.
82. Lippert J, Bonlokke S, Utke A, Knudsen BR, Sorensen BS, Steiniche T and Stougaard M: Targeted next generation sequencing panel for HPV genotyping in cervical cancer. *Exp Mol Pathol* 118: 104568, 2021.
83. Ren J, Ma N, Seckar T, Bassa S, Zetola N, Grover S, Wei Z and Robertson E: Characterization of the genomic landscape in HPV-positive cervical and head and neck squamous cell carcinomas by whole genome next generation sequencing. *Cancer Genomics Proteomics* 22: 188-207, 2025.
84. Agalliu I, Chen Z, Wang T, Hayes RB, Freedman ND, Gapstur SM and Burk RD: Oral Alpha, Beta, and Gamma HPV Types and Risk of Incident Esophageal Cancer. *Cancer Epidemiol Biomarkers Prev* 27: 1168-1175, 2018.
85. Parameshwaran K, Sharma P, Rajendra S, Stelzer-Braid S, Xuan W and Rawlinson WD: Circulating human papillomavirus DNA detection in Barrett's dysplasia and esophageal adenocarcinoma. *Dis Esophagus* 32: doz064, 2019.
86. Rajendra S, Pavey D, McKay O, Merrett N and Gautam SD: Human papillomavirus infection in esophageal squamous cell carcinoma and esophageal adenocarcinoma: A concise review. *Ann N Y Acad Sci* 1482: 36-48, 2020.
87. Benvari S, Aslanimehr M, Samiee-Rad F, Naserpour-Farivar T and Sadeghi H: Comparative efficacy of HPV 16/18 DNA and E6/E7 mRNA testing in detecting high-grade cervical lesions (CIN2+) in women with cervical biopsies. *Diagn Microbiol Infect Dis* 111: 116668, 2025.
88. Rosing F, Meier M, Schroeder L, Laban S, Hoffmann T, Kaufmann A, Siefer O, Wuerdemann N, Klußmann JP, Rieckmann T, *et al*: Quantification of human papillomavirus cell-free DNA from low-volume blood plasma samples by digital PCR. *Microbiol Spectr* 12: e0002424, 2024.
89. Lim Y, Wan Y, Vagenas D, Ovchinnikov DA, Perry CF, Davis MJ and Punyadeera C: Salivary DNA methylation panel to diagnose HPV-positive and HPV-negative head and neck cancers. *BMC Cancer* 16: 749, 2016.
90. Zeng X, Peng F, Wang Z, Teng Q, Sha Y, Leung RK, Christopher LAIKC, Li G, Huang X and Lin S: New insights into tumor microenvironment and HPV integrations in cervical cancer pathogenesis revealed by single-cell transcriptome data. *Hum Mol Genet* 34: 920-933, 2025.
91. Nowicki M, Mroczek M, Mukhedkar D, Bala P, Nikolai Pimenoff V and Arroyo Muhr LS: HPV-KITE: Sequence analysis software for rapid HPV genotype detection. *Brief Bioinform* 26: bbaf155, 2025.
92. Ershadifar S, Larsson J, Young K, Abouyared M, Bewley A and Birkeland AC: Efficacy of 18FDG-PET/CT in detecting synchronous malignancies in patients with head and neck cancer: A systematic review and Meta-analysis. *Otolaryngol Head Neck Surg* 171: 1639-1649, 2024.
93. Li P, Liang D, Yang E, Zeb M, Huang H, Sun H, Zhang W, Peng C, Zhao Y and Ma W: Bio-nanopore technology for biomolecules detection. *Adv Biotechnol (Singap)* 2: 45, 2024.
94. Ghaleh HEG, Shahriary A, Izadi M and Farzanehpour M: Advances in early diagnosis of cervical cancer based on biosensors. *Biotechnol Bioeng* 119: 2305-2312, 2022.
95. He C, Li Y, Liu J, Li Z, Li X, Choi JW, Li H, Liu S and Li CZ: Application of CRISPR-Cas system in human papillomavirus detection using biosensor devices and Point-of-Care Technologies. *BME Front* 6: 0114, 2025.
96. Izadi N, Strmiskova J, Anton M, Hausnerova J and Bartosik M: LAMP-based electrochemical platform for monitoring HPV genome integration at the mRNA level associated with higher risk of cervical cancer progression. *J Med Virol* 96: e70008, 2024.
97. Xu T, Zhou J, Li X, Ke W, Liu J, Gao H and Dai H: Electrochemical sensing technology for liquid biopsy of circulating tumor cells-a review. *Bioelectrochemistry* 140: 107823-107835, 2021.
98. Goldstein A, Gersh M, Skovronsky G and Moss C: The future of cervical cancer screening. *Int J Womens Health* 16: 1715-1731, 2024.
99. Yin L, Zhao Z, Wang C, Zhou C, Wu X, Gao B, Wang L, Man S, Cheng X, Wu Q, *et al*: Development and evaluation of a CRISPR/Cas12a-based diagnostic test for rapid detection and genotyping of HR-HPV in clinical specimens. *Microbiol Spectr* 13: e0225324, 2025.
100. Wang WJ, Wu MJ, Ren JL, Xie P, Chang J, Hu GM and Wu HF: p16INK4a is not a reliable screening marker of HPV infection in esophageal squamous cell carcinoma: Evidence from a meta-analysis. *Int J Biol Markers* 31: e431-e439, 2016.
101. Pineda Contreras S, Quiroz Lagos A, Herrera Soto J, Reyes Vergara C, de la Barra Vivallos T, Elgorriaga Islas E and Montenegro Heredia S: Impact of HPV detection and p16-Ki67 expression on prognosis in anal cancer patients. *Rev Esp Patol* 58: 100806, 2025.
102. De Marco L, Bisanzi S, Ronco G, Mancuso P, Carozzi F, Allia E, Rizzolo R, Gustinucci D, Frayle H, Viti J, *et al*: Extended HPV genotyping by the BD Onclarity assay: Concordance with screening HPV-DNA assays, triage biomarkers, and histopathology in women from the NTCC2 study. *Microbiol Spectr* 13: e0089724, 2025.
103. Zappacosta R, Colasante A, Viola P, D'Antuono T, Lattanzio G, Capanna S, Gatta DM and Rosini S: Chromogenic in situ hybridization and p16/Ki67 dual staining on formalin-fixed paraffin-embedded cervical specimens: Correlation with HPV-DNA test, E6/E7 mRNA test, and potential clinical applications. *Biomed Res Int* 2013: 453606-453616, 2013.
104. Yahyapour Y, Sadeghi F, Alizadeh A, Rajabnia R and Siadati S: Detection of merkel cell polyomavirus and human papillomavirus in esophageal squamous cell carcinomas and Non-Cancerous esophageal samples in northern Iran. *Pathol Oncol Res* 22: 667-672, 2016.
105. Cabibi D, Giannone AG, Quattrocchi A, Lo Coco R, Formisano E, Porcasi R, Benfante V, Comelli A and Capra G: High-risk HPV CISH detection in cervical biopsies with weak and/or focal p16 Immunohistochemical positivity. *Int J Mol Sci* 25: 5354, 2024.

106. Outh-Gauer S, Augustin J, Mandavit M, Grard O, Denize T, Nervo M, Lépine C, Rassy M, Tartour E and Badoual C: Chromogenic in situ hybridization as a tool for HPV-related head and neck cancer diagnosis. *J Vis Exp*: Jun 14, 2019 doi: 10.3791/59422.
107. Xiong J, Cheng J, Shen H, Ren C, Wang L, Gao C, Zhu T, Li X, Ding W, Zhu D and Wang H: Detection of HPV and human chromosome sites by Dual-color fluorescence in situ hybridization reveals recurrent HPV integration sites and heterogeneity in cervical cancer. *Front Oncol* 11: 734758, 2021.
108. Houldsworth J: FHACT: The FISH-based HPV-associated cancer test that detects nonrandom gain at four genomic loci as biomarkers of disease progression. *Expert Rev Mol Diagn* 14: 921-934, 2014.
109. Zhang W, Kapadia M, Sugarman M, Free H, Upchurch C, Gniewek R, White K, Miller M, Vladich F, Ferenczy A, *et al*: Adjunctive HPV in-situ hybridization (ISH) assay as an aid in the diagnosis of cervical intraepithelial neoplasia in cervical tissue specimens: An analytical and functional characterization. *Int J Gynecol Pathol* 31: 588-595, 2012.
110. Zito Marino F, Ronchi A, Stilo M, Cozzolino I, La Mantia E, Colacurci N, Colella G and Franco R: Multiplex HPV RNA in situ hybridization/p16 immunohistochemistry: A novel approach to detect papillomavirus in HPV-related cancers. A novel multiplex ISH/IHC assay to detect HPV. *Infect Agent Cancer* 15: 46, 2020.
111. Kim HK, Lee YP, Kim H, Yang Y, Kwon J, Lee KH, Son SM and Han HS: Differential diagnosis of synchronous double primary squamous cell carcinomas of the esophagus and occult primary oropharynx through HPV testing: A case report. *Medicine (Baltimore)* 104: e42243, 2025.
112. Pastrez PRA, Mariano VS, da Costa AM, Silva EM, Scapulatempo-Neto C, Guimarães DP, Fava G, Neto SAZ, Nunes EM, Sicheiro L, *et al*: The relation of HPV infection and expression of p53 and p16 proteins in esophageal squamous cells carcinoma. *J Cancer* 8: 1062, 2017.
113. Moreas H, Tsiambas E, Lazaris AC, Nonni A, Karameris A, Metaxas GE, Armatas HE and Patsouris E: Impact of HPV detection in colorectal adenocarcinoma: HPV protein and chromogenic in situ hybridization analysis based on tissue microarrays. *J BUON* 19: 91-96, 2014.
114. Tripodi S, Chang F, Syrjänen S, Shen Q, Cintonino M, Alia L, Santopietro R, Tosi P and Syrjänen K: Quantitative image analysis of oesophageal squamous cell carcinoma from the high-incidence area of China, with special reference to tumour progression and papillomavirus (HPV) involvement. *Anticancer Res* 20: 3855-3862, 2000.
115. Malik SM, Nevin DT, Cohen S, Hunt JL and Palazzo JP: Assessment of immunohistochemistry for p16INK4 and high-risk HPV DNA by in situ hybridization in esophageal squamous cell carcinoma. *Int J Surg Pathol* 19: 31-34, 2011.
116. Smits HL, Tjong AHSP, Ter Schegget J, Nooter K and Kok T: Absence of human papillomavirus DNA from esophageal carcinoma as determined by multiple broad spectrum polymerase chain reactions. *J Med Virol* 46: 213-215, 1995.
117. Bae JM, Min KT, Shin JY, Shin SK, Kim SN, Lee HP, Kim SO and Hong SP: Comparison of digene hybrid capture 2, GeneMatrix PapilloScreen, and a PCR sequencing assay in detecting high-risk and probable high-risk oncogenic HPV genotypes in specimens from Korean women. *Arch Virol* 159: 1909-1916, 2014.
118. Broccolo F: A multiplex real-time PCR-platform integrated into automated extraction method for the rapid detection and measurement of oncogenic HPV type-specific viral DNA load from cervical samples. *Methods Mol Biol* 1160: 87-97, 2014.
119. Mattox AK, D'Souza G, Khan Z, Allen H, Henson S, Seiwert TY, Koch W, Pardoll DM and Fakhry C: Comparison of next generation sequencing, droplet digital PCR, and quantitative real-time PCR for the earlier detection and quantification of HPV in HPV-positive oropharyngeal cancer. *Oral Oncol* 128: 105805, 2022.
120. Kumar R, Ghosh SK, Verma AK, Talukdar A, Deka MK, Wagh M, Bahar HM, Tapkire R, Chakraborty KP and Kannan RR: p16 Expression as a surrogate marker for HPV infection in esophageal squamous cell carcinoma can predict response to Neo-adjuvant chemotherapy. *Asian Pac J Cancer Prev* 16: 7161-7165, 2015.
121. Sasivimolrattana T, Liewchalermwong S, Chantrattita W, Sensorn I, Chaiwongkot A and Bhattarakosol P: Virome capture sequencing for comprehensive HPV genotyping in cervical samples. *Sci Prog* 108: 368504251334515, 2025.
122. Gupta A, Dagar G, Das SK, Chauhan R, Shankar A, Sharma DN, Suri V, Khan MA, Macha MA, Ahmed I, *et al*: Prognostic value of circulating HPV cell-free DNA in cervical cancer using liquid biopsy. *Sci Rep* 15: 11480, 2025.
123. Almeren AO, Waenerlund M, Landstrom F, von Beckerath M, Qvick A, Carlsson J and Helenius G: Circulating tumour DNA as a complementary tool for treatment evaluation in HPV-Associated head and neck squamous cell carcinoma: An observational cohort study. *Clin Otolaryngol* 50: 831-839, 2025.
124. Bryan ME, Aye L, Das D, Hirayama S, Al-Inaya Y, Mendel J, Naegele S, Efthymiou V, Alzumaili B, Faquin WC, *et al*: Direct comparison of alternative Blood-based approaches for early detection and diagnosis of HPV-Associated head and neck cancers. *Clin Cancer Res* 31: 3483-3493, 2025.
125. Nguyen HV, Hwang S, Lee SW, Jin E and Lee MH: Detection of HPV 16 and 18 L1 genes by a nucleic acid amplification-free electrochemical biosensor powered by CRISPR/Cas9. *Bioelectrochemistry* 162: 108861, 2025.
126. Hu T, Ji Q, Ke X, Zhou H, Zhang S, Ma S, Yu C, Ju W, Lu M, Lin Y, *et al*: Repurposing type I-A CRISPR-Cas3 for a robust diagnosis of human papillomavirus (HPV). *Commun Biol* 7: 858-869, 2024.
127. Verhoef L, Bleeker MCG, Polman N, Steenbergen RDM, Ebisch RMF, Melchers WJG, Bekkers RLM, Molijn AC, Quint WG, van Kemenade F, *et al*: Evaluation of DNA methylation biomarkers ASCL1 and LHX8 on HPV-positive self-collected samples from primary HPV-based screening. *Br J Cancer* 129: 104-111, 2023.
128. Vink FJ, Meijer C, Hesselink AT, Floore AN, Lissenberg-Witte BI, Bonde JH, Pedersen H, Cuschieri K, Bhatia R, Poljak M, *et al*: FAM19A4/miR124-2 methylation testing and human papillomavirus (HPV) 16/18 genotyping in HPV-positive Women under the age of 30 years. *Clin Infect Dis* 76: e827-e834, 2023.
129. Wang J, Jing G, Huang W, Xin L, Du J, Cai X, Xu Y, Lu X and Chen W: Rapid in situ hydrogel LAMP for On-Site Large-Scale parallel Single-cell HPV detection. *Anal Chem* 94: 18083-18091, 2022.
130. Ma C, Zou M, Xu N, Liu Y and Wang Y: Portable, and ultrasensitive HR-HPV tests based on nucleic acid biosensors. *Front Cell Infect Microbiol* 14: 1357090, 2024.
131. Shah SS, Senapati S, Klacsann F, Miller DL, Johnson JJ, Chang HC and Stack MS: Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. *Cancers (Basel)* 8: 85, 2016.
132. Yu L, Peng Y, Sheng M, Wang Q, Huang J and Yang X: Sensitive and Amplification-Free electrochemiluminescence biosensor for HPV-16 detection based on CRISPR/Cas12a and DNA tetrahedron nanostructures. *ACS Sens* 8: 2852-2858, 2023.
133. Parisi FM, Lentini M, Chiesa-Estomba CM, Mayo-Yanez M, Leichen JR, White M, Giurdanella G, Cocuzza S, Bianco MR, Fakhry N and Maniaci A: Liquid biopsy in HPV-Associated head and neck cancer: A comprehensive review. *Cancers (Basel)* 17: 977, 2025.
134. Zhang Y, Zhang Y, Pan C, Wang W and Yu Y: HPV-driven heterogeneity in cervical cancer: Study on the role of epithelial cells and myofibroblasts in the tumor progression based on single-cell RNA sequencing analysis. *PeerJ* 12: e18158, 2024.
135. Song B, Leroy A, Yang K, Dam T, Wang X, Maurya H, Pathak T, Lee J, Stock S, Li XT, *et al*: Deep learning informed multimodal fusion of radiology and pathology to predict outcomes in HPV-associated oropharyngeal squamous cell carcinoma. *EBioMedicine* 114: 105663, 2025.
136. Hieronimimon HM, Trzcinska A, Wen FT, Howard FM, Dolezal JM, Dyer E, Kochanny S, Schulte JJ, Wang C, Chen H, *et al*: Analysis of AI foundation model features decodes the histopathologic landscape of HPV-positive head and neck squamous cell carcinomas. *Oral Oncol* 163: 107207, 2025.
137. Ardila CM and Yadalam PK: AI-driven histopathologic insights in HPV-positive head and neck squamous cell carcinomas. *Oral Oncol* 163: 107261, 2025.

