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Review

Targeting HPV for the prevention, diagnosis, and treatment of cervical cancer

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Despite advances in screening and prevention, cervical cancer (CC) remains an unresolved public health issue and poses a significant global challenge, particularly for women in low-income regions. Human papillomavirus (HPV) infection, especially with the high-risk strains, is a primary driver of cervical carcinogenesis. Emerging evidence indicates that integrating HPV testing with existing approaches, such as cervical cytology and visual inspection, offers enhanced sensitivity and specificity in CC screening. HPV infection-associated biomarkers, including HPV E6/E7 oncogenes, p16ˆINK4a, DNA methylation signatures, and non-coding RNAs, offer valuable insights into disease progression and the development of personalized interventions. Preventive and therapeutic vaccination against HPV, along with tertiary prevention strategies such as the use of antiviral and immune-modulating drugs for HPV-related lesions, show great clinical potential. At the mechanistic level, single-cell RNA sequencing analysis and the development of organoid models for HPV infection provide new cellular and molecular insights into HPV-related CC pathogenesis. This review focuses on the crucial roles of HPV in the prevention, diagnosis, and treatment of CC, with particular emphasis on the latest advancements in screening and disease intervention.

Keywords: cervical cancer, human papillomavirus, screening, prevention, biomarkers

Introduction

Cervical cancer (CC) ranks fourth among the most prevalent cancers in women worldwide (Bray et al., 2024). According to data from the International Agency for Research on Cancer (IARC), the incidence of CC increased from 13.3 per 100000 people in 2020 to 14.1 per 100000 in 2022. During the same period, the mortality rate remained consistently high at ∼7 per 100000 (Sung et al., 2021; Bray et al., 2024). With the rising incidence rates and the persistently high mortality rates, the outlook for CC is concerning. More disturbingly, CC exhibits a disproportionate burden, with the majority of cases and deaths concentrated in low-income regions such as sub-Saharan Africa, Melanesia, South America, and Southeast Asia (Bray et al., 2018, 2024; Sung et al., 2021). Conversely, in high-income countries like the United States and several European nations, the incidence of CC has gradually decreased in the past three decades (Vaccarella et al., 2013; Bruni et al., 2022). Disparities in healthcare resource allocation, health awareness, screening accessibility, and treatment uptake are likely contributing factors to this imbalance (Qiu et al., 2021). Numerous studies have linked socioeconomic status, poor personal and sexual hygiene, early sexual activity, and multiple sexual partners to the development of CC (Chelimo et al., 2013; Cohen et al., 2019). Particularly noteworthy is the relationship between human papillomavirus (HPV) infection and cervical carcinogenesis. Persistent HPV infection has been recognized as a crucial factor in the development of CC (Arbyn et al., 2015; Wright et al., 2015), necessitating advancements in HPV testing technology

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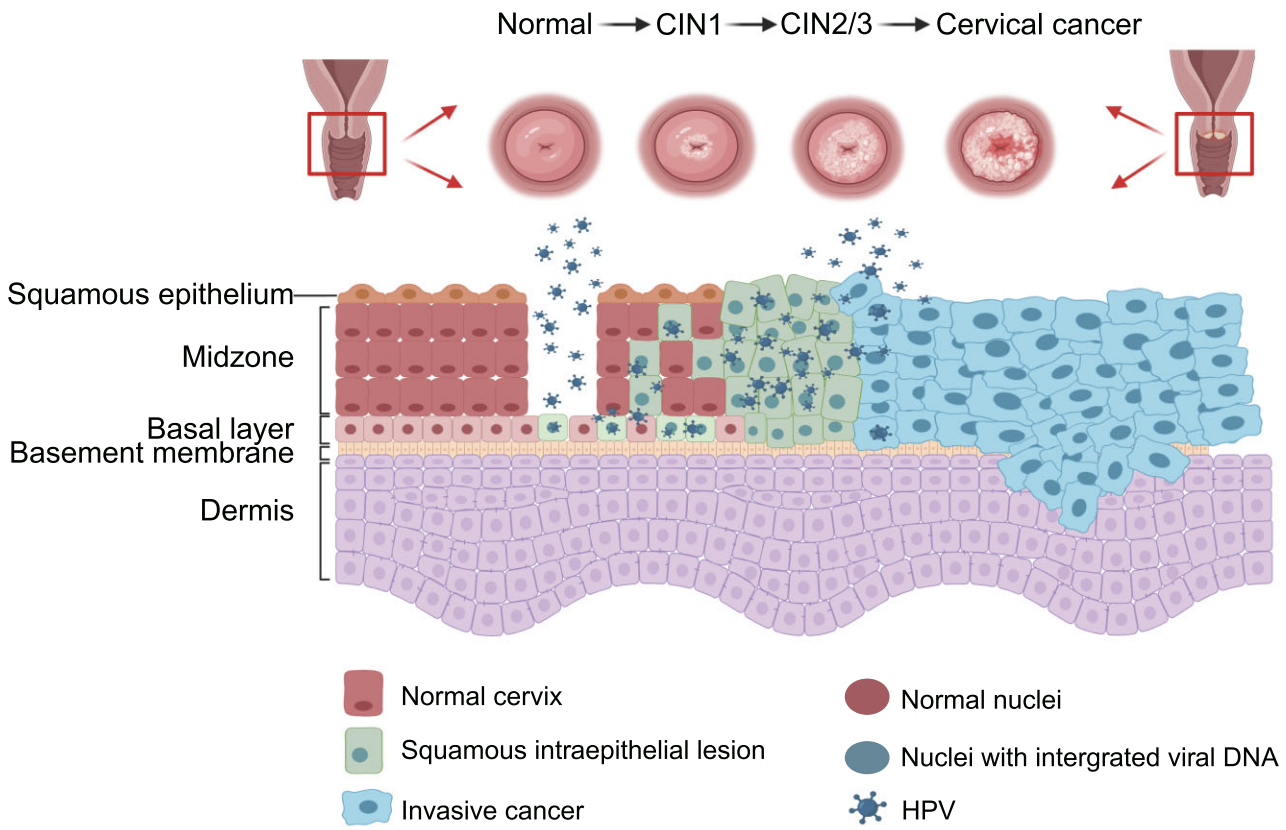

Figure 1 HPV life cycle and CC progression. The HPV life cycle begins with infection of basal epithelial cells, where the virus undergoes low-level replication. As the cells differentiate, the viral oncogenes are gradually expressed, leading to uncontrolled host cell proliferation. The virus then assembles and is released from differentiated epithelial cells. Persistent infection with HR-HPV types can cause CIN, progressing from mild (CIN1) to severe abnormalities (CIN3). Without timely reversal or treatment, this can ultimately develop into invasive CC. The image was created with BioRender.

and vaccines. A thorough understanding of the natural history of HPV infection and the progression of precancerous lesions is essential for developing effective screening, prevention, and treatment strategies for CC.

Pathological changes in the cervix caused by HPV infection

CC, primarily squamous cell carcinoma, typically originates in the cervical transformation zone, which is the junction of the squamous and columnar epithelia, anatomically located at the external os of the cervix (Bhatla et al., 2018). This region neighbours the vagina, harbouring diverse microbial communities and exposing it to infections by external viruses such as HPV. Microabrasions of the cervical epithelium lead to incomplete cervical mucosa and the exposure of the basement membrane. Infectious HPV DNA can enter the basal cells through these microabrasions. As the basal cells differentiate, the HPV DNA is expressed, leading to the transformation of HPV-infected basal cells into intermediate and superficial cells. Within the superficial layer, viral DNA is encapsulated in capsids to generate new viral particles, which are then released for subsequent rounds of infection (Figure 1).

Following HPV infection, especially with HR types, precursor lesions or cervical intraepithelial neoplasia (CIN) emerge (Cohen et al., 2019; Rahangdale et al., 2022). These lesions progressively evolve into CC under the influence of synergistic factors that enhance CC risk (Figure 1). The CIN can be categorized into CIN1, CIN2, and CIN3 based on the proportion of affected epithelial layers (Holowaty et al., 1999; Snijders et al., 2006). The majority of HPV-infected individuals clear the virus within 2 years, and the CIN1 or low-grade squamous intraepithelial lesion typically regresses spontaneously in >80% of cases within 1–2 years. However, in a fraction of patients experiencing persistent HPV infection, the precancerous lesions progress to CIN2 and CIN3, or high-grade squamous intraepithelial lesion (HSIL), which has a propensity to further transform into invasive CC. Understanding the biology and life cycle of HPV infection, as well as the progression of cervical precancerous lesions is pivotal for implementing effective screening, prevention, and treatment strategies to combat CC (Figure 1).

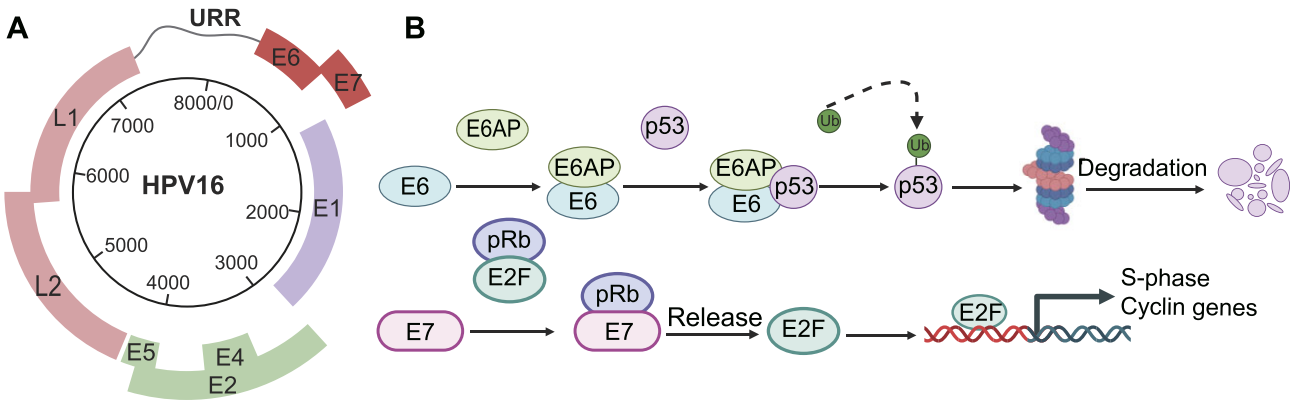

Figure 2 HPV genome and its function in CC pathogenesis. (**A**) Structure and arrangement of the HPV16 genome. (**B**) The E6 protein recruits E6AP to promote the ubiquitination of p53, leading to its degradation, thereby inhibiting apoptosis; the E7 protein binds to pRb, releasing E2F from the pRb–E2F complex, thereby causing uncontrolled cell proliferation. Ub, ubiquitin. The image was created with BioRender.

HPV infection and CC

Human papillomavirus

HPV is a non-enveloped virus with a circular double-stranded genome comprising three key regions: the early transcriptional region, late transcriptional region, and upstream regulatory region (Burd, 2003). The early transcriptional region encompasses E1, E2, E4, E5, E6, and E7, all of which participate in virus gene replication, transcription, and cellular transformation. Notably, the viral oncoproteins E6 and E7 are necessary for malignant conversion (Cardone et al., 2014; McBride, 2017). The late transcriptional region primarily consists of L1 and L2, which encode the major and minor capsid proteins, respectively. Capsid proteins possess the ability to self-assemble into virus-like particles (VLPs) that mimic the antigenic epitopes of natural viral particles. This mimicking elicits immune responses and generates protective antibodies. However, the VLPs without the viral genomes are non-infectious and non-pathogenic due to lack of DNA. The upstream regulatory region, located between E6 and L1, is non-transcriptional but controls virus transcription and replication (Doorbar et al., 2012; Piña-Sánchez, 2022). Understanding the molecular architecture of HPV is the cornerstone for developing effective diagnostic, preventive, and therapeutic strategies against HPV-related diseases (Figure 2).

Research estimates that ∼80% of sexually active women will contract HPV at some stage in their lives. However, >90% of individuals with HPV infection naturally clear the virus within 6–24 months due to their innate immune response. Nevertheless, in cases of persistent infection, HPV can lead to the development of CIN1 and/or CIN2/3, which may even advance to CC if left untreated. To date, >200 genotypes of HPV have been identified. However, not all genotypes are associated with CC. HPV types can be classified into LR (low-risk) or HR (high-risk) strains depending on their oncogenic potential. HR-HPV types are linked to precancerous lesions and CC, whereas LR-HPV types are associated with skin lesions (Doorbar et al., 2012). The epidemiological classification of HPV has identified at least 12 oncogenic HR-HPV types (HPV16/18/31/35/39/45/51/52/56/58/66/68), which account for >97% of CC cases (Muñoz et al., 2003). Notably, HPV16 and HPV18 are the most prevalent HR-HPV types, collectively responsible for ∼70% of CC and 80%–90% of other HPV-related tumours. In contrast, LR-HPV types (HPV6/11/40/42/43/44/54/61/72) may cause anogenital warts and oropharyngeal tumours. Understanding the association between specific HPV genotypes and CC is essential for implementing effective screening and developing preventive and therapeutic vaccines.

The mechanism of HPV-induced cervical carcinogenesis

HPV exhibits high host specificity, showing a strong affinity for epithelial cells in specific areas of the human body (de Villiers et al., 2004; Piña-Sánchez, 2022). HPV primarily infects mucosal epithelial cells of the genital tract, as well as cells lining the oral and pharyngeal mucosa, which can potentially lead to both benign and malignant tumours. HPV persistence is the key step for cervical carcinogenesis. First, HPV infects basal cells through damaged areas of the skin or mucosa, and persistent HPV infection will be developed once the DNA of HR-HPV types is integrated into the host cell genome. Viral DNA replication facilitated by viral oncoproteins E5, E6, and E7 results in a reduction in host immune defences, allowing the virus to evade immune surveillance (Balasubramaniam et al., 2019; Yadav et al., 2023). Next, during the differentiation and migration of infected basal cells towards the surface, HPV DNA moves upwards, leading to the keratinization of the infected cells. At this stage, the late genes L1 and L2 are activated and their protein products assemble into viral capsids that encapsulate newly synthesized viral DNA, resulting in the production of numerous virions within the differentiated cells. Following lysis of the superficial epithelial cells, virions are released onto the surface, primed for the next round of cellular infection. The intricate life cycle of HPV is crucial for the development of HPV-related diseases (Doorbar et al., 2012; de Sanjosé et al., 2018).

The early proteins E6 and E7 of HPV are closely associated with its carcinogenicity (Figure 2). E6 binds to the cellular ubiquitin

ligase E6-associated protein (E6AP), leading to a conformational change that results in the formation of a complex with E6, E6AP, and p53 (Mulvany et al., 2008; Gupta et al., 2010; Martinez-Zapien et al., 2016). This complex facilitates the degradation of the tumour suppressor protein p53, thereby inhibiting cell apoptosis (Martinez-Zapien et al., 2016; Sailer et al., 2018). Conversely, the E7 protein binds to pRb, leading to a robust interaction that dissociates E2F from the pRb–E2F complex, consequently activating its transcriptional regulatory function. This results in dysregulated cell cycle, uncontrolled proliferation, and indirect inhibition of cell apoptosis (Hwang et al., 2002; Liu et al., 2006). In summary, E6 and E7 proteins synergistically contribute to the replication and propagation of viral DNA, induce genetic mutations of infected cells, and ultimately immortalize and transform host cells. The concerted action of E6 and E7 is widely recognized as the pathogenic mechanism of HPV, and these two proteins have been extensively used as screening markers for CC in clinical practice.

Prevention and control of HPV in CC

The application of HPV vaccines in the prevention of CC

In recent years, HPV vaccines have emerged as a global research hotspot, with researchers worldwide dedicating efforts to develop efficient, attenuated, and cost-effective vaccines that cater to the diverse needs of different economic regions, thereby effectively preventing and treating HPV-induced CC. Vaccines are categorized into preventive and therapeutic based on their immunological functions (Oliver et al., 2017; Lei et al., 2020; Mix et al., 2021), and preventive HPV vaccines offer a broader range of applications compared to therapeutic vaccines.

Preventive vaccines are primarily administered to individuals to prevent HPV infection and its associated diseases (Pei et al., 2023). Currently, preventive vaccines mainly target the HPV capsid protein L1 antigen. Studies have shown that vaccines based on VLPs, constructed by expressing the virus capsid protein L1 in monkey kidney cells, induce specific immune responses in mice. Animal infection models with bovine papillomavirus and cottontail rabbit papillomavirus have confirmed the effectiveness of L1 VLP-based preventive vaccines, driving the development of HPV preventive vaccines based on L1-VLPs (Frazer, 2019). Six vaccines are currently on the market globally: HPV16/18 bivalent vaccines (GlaxoSmithKline Cervarix®, Xiamen Wantai Xincaning®, and Yuxi Zerun Wozehui®), HPV6/11/16/18 quadrivalent vaccines (Merck Gardasil® and Indian Serum Institute Cervavac®), and HPV6/11/16/18/31/33/45/52/58 nonavalent vaccines (Merck Gardasil®9), all utilizing HPV L1 VLPs as antigens. They effectively prevent infection by common oncogenic HPV types, estimated to prevent up to 90% of HPV-related cancers. In 2021, a new alum-adjuvanted bivalent vaccine protecting against HPV16 and HPV18 was licensed to further improve global supply and demand for HPV vaccines (Zou et al., 2020). Additionally, efforts are underway to develop next-generation vaccines based on the virus's minor structural protein L2, potentially preventing a broader range of HPV types (Schiller and Müller, 2015).

The application of HPV in screening of CC

The cost of CC vaccines poses a significant challenge for low and middle-income countries. To circumvent these barriers, effective screening methods are needed to prevent the progression of CC (Tsu et al., 2021). Cancer screening is the cornerstone of secondary prevention strategies for CC, providing powerful means for detecting and intervening at the precancerous stages of the disease. By implementing comprehensive screening plans and targeted interventions, we have the opportunity to significantly alleviate the global burden of CC and improve the health of women worldwide (Gavinski and DiNardo, 2023).

Cervical cytology (Pap smear), acetic acid (VIA)/Lugol's iodine (VILI), and ThinPrep cytology test (TCT) are the classical approaches for CC detection. In the 1940s, Greek physician Dr Papanicolaou pioneered the Pap smear technique, enabling early detection of cervical cell abnormalities through microscopic examination of cervical smears. This innovation reduces the incidence of CC by 80% among women aged 35–64 undergoing cytological screening every 3 years according to studies by the IARC. Since the 1990s, visual inspection with VIA or VILI has been widely used as the primary screening method for CC in developing countries. VIA/VILI involves applying acetic acid/iodine to the cervix for staining, allowing direct visual observation of cervical epithelial staining reactions to determine the presence of lesions, with the advantages of independence from equipment, ease of operation, and low cost. Despite both methods are simple and cost-effective, they may produce false-positive or false-negative results. Recognizing this limitation has spurred the emergence of a new generation of cytology methods, including TCT, a liquid-based cytology method representing a more advanced approach to examining cervical cytology. Similar to Pap smear, samples are collected and placed in a liquid carrier before being sent to the laboratory for analysis. TCT improves the traditional methods by preserving almost all of the sample collected, avoiding the artefacts caused by cell over-drying during conventional smearing, thus enhancing the accuracy and reliability of detection. Due to its significantly lower false-negative rate compared to Pap smear, TCT has been quickly adopted in developed regions worldwide.

The discovery of the link between CC and HPV infection marked a pivotal moment in CC screening since the 1970s. The development of HPV nucleic acid testing technology brought about a milestone shift in this field. Hybrid Capture II HPV-DNA testing (HC2) received FDA approval in 1999, becoming the first FDA-approved method for detecting HR-HPV. HC2 directly detects HR-HPV viruses at the DNA level using molecular biology techniques, addressing the limitations of cytological examination. By alerting potential cases during the infection stage before cellular abnormalities manifest, HC2 enables early intervention through avoidance of HR factors, regular check-ups, or timely intervention to prevent CC and maintain a healthy life. Therefore, the combination of cytological examination and HPV testing gradually becomes the guiding principle for early CC screening. While HC2 can detect 13 HR-HPV viruses, it is unable to distinguish individual virus types. With technological advancements,

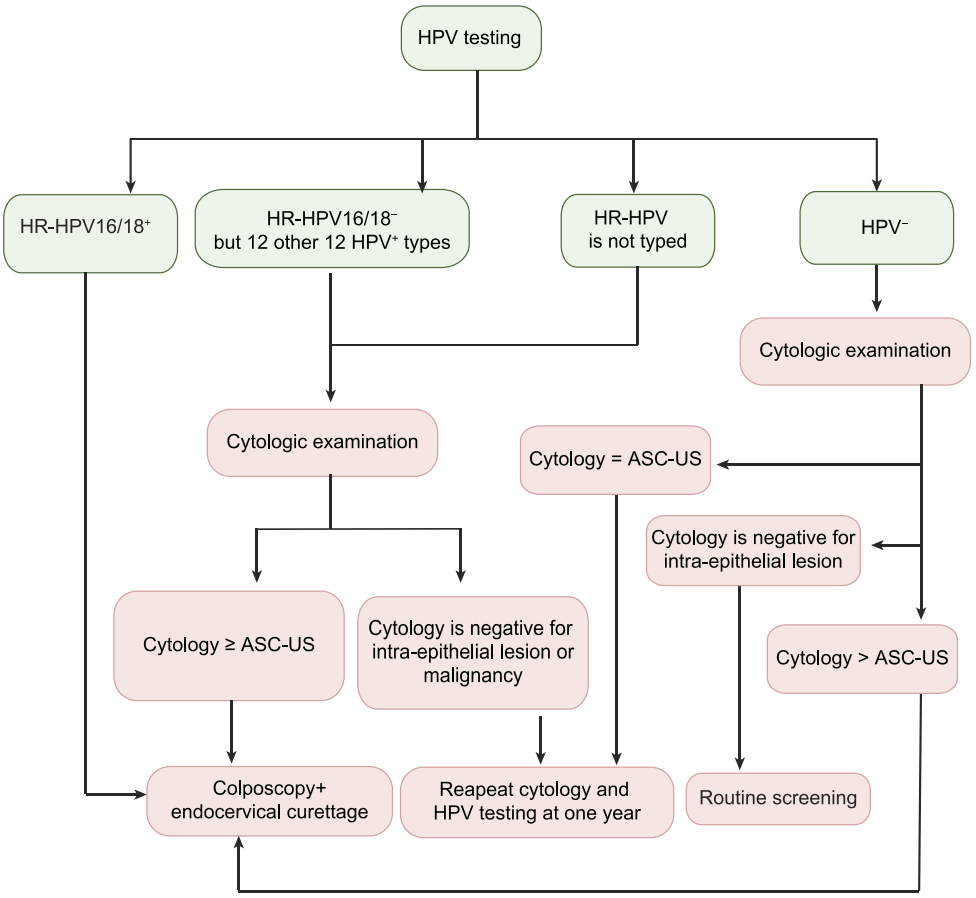

Figure 3 Cancer screening for HPV-infected patients. HPV^{-} individuals can undergo regular screening, while HPV^{+} patients are categorized based on the types of HPV infection. The HPV16/18^{+} patients undergo colposcopy directly, while those positive for other 12 types of HR-HPV require further evaluation based on cytology results.

researchers have identified HPV types 16 and 18 as posing HR factors for CC induction (Campos et al., 2015). HPV DNA typing technology has matured, evolving towards HPV nucleic acid testing as the primary screening method (Jeronimo et al., 2017; Curry et al., 2018; Fontham et al., 2020; Kyrgiou et al., 2020). A large-scale study in China comparing HPV testing and combined screening in the general population demonstrated that primary screening for HPV16/18 types yields similar high-grade lesion detection rates as combined screening but with higher sensitivity and negative predictive value, offering the best cost-effectiveness in the first screening round (Wu et al., 2017). Subsequent histological testing for HPV16^{+} or HPV18^{+} individuals ensures high detection sensitivity and accurately triages the HR populations, therefore effectively preventing the misuse of medical resources (Bray et al., 2018; Dong et al., 2021).

However, most HPV infections are harmless and additional tests are required to identify women with progressing infections or precancer. To bolster CC prevention, HPV testing alone or with cytology was introduced as an alternative to cytology screening. Women who test negative for HPV will resume routine screening annually. For the women who were infected with HPV, the integration of HPV genotyping has emerged as a vital measure for risk assessment. Specifically, triaging HPV16/18^{+} individuals without cytology testing is recognized as more indicative of precancerous conditions than pooled HPV^{+} cases with atypical squamous cells of undetermined significance (ASC-US) (Saslow et al., 2012; Melnikow et al., 2018). Currently, US screening guidelines have recommended incorporating co-testing of HPV16/18 triage for those who test positive for HPV but negative for intraepithelial lesion or malignancy, facilitating prompt referral for colposcopy upon identification (Wentzensen et al., 2016). For those cases infected with other HR-HPV types, it is recommended to complement the results with a cytology examination (Mix et al., 2021). If the cytology results are normal, the cytology and HPV testing should be repeated in 1 year. Conversely, if the cytology results show abnormalities such as ASC-US or a higher grade, immediate colposcopy is required (Cheng et al., 2018). When both HPV and cytology results are negative, only routine screening is necessary (Figure 3).

Tertiary prevention for CC targeting HPV

Surgery, radiation, and chemotherapy, either individually or in combination, are the primary therapeutic approaches for

both HPV^+ and HPV^- CC patients. The choice of treatment is tailored to the stage and extent of CC progression. Traditional methods are generally effective in managing the disease and can even result in the conversion of HPV^+ status to HPV^-. For patients with active HPV infections, therapeutic vaccines and antiviral drugs are recommended as adjuvant treatments (Supplementary Figure S1).

To optimize the management of cervical lesions caused by persistent HPV infection, treatment plans should be tailored to the severity of the disease. Cervical precancers resulting from HPV infection encompass CIN1, CIN2, CIN3, and adenocarcinoma *in situ* (AIS). For patients with CIN1, regular follow-up and observation are typically recommended. However, without appropriate intervention, CIN2, CIN3, and AIS carry a significant risk of progression to invasive adenocarcinoma. Cervical excision therapy, which includes loop electrosurgical excision procedure (LEEP) and cold knife conization (CKC), is recommended by clinical guidelines as an effective approach for managing precancerous lesions, offering both diagnostic and therapeutic benefits. Although there are no significant differences in recurrence rate, positive margins, or residual lesions between LEEP and CKC in the treatment of CIN, LEEP has a lower incidence of complications such as bleeding, infection, cervical stenosis, and adverse pregnancy outcomes (Jiang et al., 2016). Therefore, LEEP has become the mainstay of excisional surgery. Total hysterectomy is not a preferred treatment for precursor lesions and is performed only in certain special circumstances.

Early-stage CC is classified according to the International Federation of Gynecology and Obstetrics (FIGO) staging system into IA1, IA2, IB1, and IIA1 (tumour <4 cm). Surgery is the primary treatment option for patients with early-stage CC except for patients with surgical contraindications. Microinvasive cancer (stage IA) without lymphovascular space invasion (LVSI) is typically managed with conization to ensure complete removal of the lesion. For patients with stage IA1 plus LVSI or stage IA2, treatment options include conization, radical hysterectomy alone, or combined with pelvic lymphadenectomy. Additionally, radical hysterectomy remains the standard treatment for patients with visually identifiable small-volume tumours (stages IB1 and IIA1). Postoperative adjuvant therapy may not be necessary for early-stage CC patients. However, for those at HR early-stage patients with positive pelvic lymph nodes, parametrial invasion, or positive surgical margins, postoperative concurrent chemoradiotherapy is recommended.

Locally advanced CC (LACC) refers to large lesions of CC confined to the cervix or involving only the pelvic region. It is characterized by large tumour volume, difficulty in local control, challenging surgery, and a high risk of postoperative recurrence and metastasis. According to the National Comprehensive Cancer Network guidelines, unlike early-stage CC patients, the preferred treatment for LACC is concurrent chemoradiotherapy. In regions where radiation therapy is not available, radical surgery may serve as an alternative treatment strategy for patients with FIGO stage IIA2-IB3. Radical surgery can effectively remove the primary cervical lesions and the surrounding involved or potentially involved tissues, thereby reducing the rates of distant metastasis and local recurrence to achieve better prognostic outcomes (Koensgen et al., 2017). Additionally, both FIGO guidelines and the European Society of Medical Oncology guidelines acknowledge the role of neoadjuvant chemotherapy in LACC treatment, with a local control rate of $>80\%$ with concurrent chemoradiotherapy (Mileshkin et al., 2023). Meanwhile, this treatment paradigm reduces the probability of recurrence or metastasis after surgery, providing young patients with the opportunity to preserve fertility (Robova et al., 2015).

In addition to traditional treatments for HPV infection, novel advanced therapies have emerged in recent years. For instance, monoclonal antibodies targeting immune checkpoints have exhibited favourable outcomes for patients with CC (Liu et al., 2017; De Felice et al., 2018). Importantly, the combination of antiviral drugs and immunotherapy is being investigated for HPV-related lesions (Burd, 2003). Cidofovir, effective against DNA viruses, has shown promise in inhibiting HPV-infected cell growth and improving prognosis in patients with severe CIN (Andrei et al., 1998; Snoeck et al., 2000). Similarly, another antiviral drug podophyllin combined with vidarabine also reduced HPV activity and cell growth, offering potential therapeutic benefits (Okamoto et al., 1999). Furthermore, the development of therapeutic vaccines for individuals already infected with HPV has also drawn intensive attention. Therapeutic vaccine strategies encompass DNA, RNA, peptides, proteins, and viral vectors, primarily targeting HPV E6/E7 oncogenes (Prudden et al., 2022). Following vaccination, antigens are presented to the immune system, eliciting immune responses against established persistent HPV infections to treat the associated lesions (Garbuglia et al., 2020). However, due to the complex mechanism of action and unresolved issues, effective therapeutic HPV vaccines are currently unavailable in clinics. These advancements underscore the evolving landscape of CC treatment, offering new avenues for improving the outcomes and quality of life of HPV-related patients.

The role of HPV in novel diagnostic and treatment approaches for CC

Diagnostic biomarkers for HPV-infected patients with CC

Early screening and diagnosis of CC allow for timely prevention and treatment. Primary screening methods, such as cervical cytology, HR-HPV testing, and colposcopy, have been instrumental in early detection. However, these methods show notable limitations that affect their clinical efficacy. Cervical cytology is influenced by sampling techniques, the classification of cervical transformation zone, and the subjective judgement of the examiner, leading to variability in sensitivity and potential for missed diagnoses (Oyouni, 2023; Perkins et al., 2023). HR-HPV DNA testing can detect the presence of the virus but is unable to distinguish between transient and persistent infections or identify precancerous lesions and cancer, resulting in a high false-positive rate and imposing a significant psychological burden on patients (Koliopoulos et al., 2017). Colposcopy is an invasive procedure, with results influenced by the classification of the

Table 1 Diagnostic biomarkers for HPV-infected patients with CC.

Marker	Function	Advantages	Disadvantages	Detective methods
E6/E7 mRNA/oncoproteins	Leading to uncontrolled cell cycle progression and continuous cell proliferation	Directly reflecting the status of HPV infection, simple to perform, and high sensitivity	Requiring high-quality samples, high cost	Real-time PCR (RT-PCR)
p16ˆINK4a	Promoting tumour cell proliferation and cellular abnormalities	Correlating with HPV infection, objective	Requiring tissue biopsy, low specificity	IHC
DNA methylation	Hypermethylation of promoters and other regions of tumour suppressor genes leads to transcriptional silencing, impacting tumour signalling pathways	Correlating with HR-HPV infection, high sensitivity and specificity, non-invasive, high sample stability	High cost, technical complexity, and variability in detection sensitivity	Quantitative methylation-specific PCR, pyrosequencing
ncRNAs	Downregulation of tumour suppressor genes or upregulation of oncogenes	Non-invasive or minimally invasive testing, early detection, and dynamic monitoring	Not determined	PCR, RT-PCR, RNA-seq
HERVs	Inducing gene transcription and chromatin remodelling	High specificity, sensitivity, and non-invasive detection	Not determined	PCR, RT-PCR, RNA-seq

cervical transformation zone and subjectivity of the examiner, requiring lengthy examination times and substantial resources, making it less suitable for large-scale screening (Benites-Zapata et al., 2023). Therefore, the identification of reliable genetic, molecular, and immunohistochemical biomarkers for the early diagnosis of cervical precancerous lesions and tumours is essential in current oncological research. The critical role of HPV in the development of CC shapes the advancement of new diagnostic and therapeutic approaches for the disease. Here, we discuss several types of diagnostic biomarkers explored in CC patients with HPV infection (Table 1).

The presence of HPV E6/E7 mRNA indicates persistent HPV infection, leading to uncontrolled cell cycle progression and continuous cell proliferation, thereby increasing the risk of CIN and CC. Therefore, HPV E6/E7 mRNA detection offers a more efficient CC screening method, decreasing referrals for women with cytological abnormalities to colposcopy (Monsonego et al., 2011; Rossi et al., 2021; Tiiti et al., 2022). Overall, the detection of HPV E6 and E7 mRNA transcripts or proteins represents a more precise method for diagnosing CC, assessing disease progression, and developing personalized treatment strategies.

The cell cycle regulator p16ˆINK4a is a hallmark of HPV-related cervical tumours, offering a potential avenue for CC detection (Kalof and Cooper, 2006; Mimica et al., 2010). Elevated expression of p16ˆINK4a, triggered by the HPV E7 oncoprotein, leads to abnormal cell proliferation and thus functions as a surrogate marker for HPV-driven transformation (Mulvany et al., 2008). For early CC screening, p16ˆIINK4a can accurately identify both malignant and atypical cells in cervical cell smears, making it more effective than HPV testing (Horn et al., 2008; Leon et al., 2021). Importantly, immunohistochemical staining of p16ˆINK4a is commonly used in histopathological analysis to distinguish between benign and malignant cervical lesions and assess the degree of atypical proliferation. Therefore, p16ˆINK4a detection not only avoids the subjectivity of morphological diagnosis but also offers straightforward detection methods, easy operation, and low cost, positioning it as an ideal auxiliary detection marker for CC.

HPV-related DNA methylation signatures also play a pivotal role in identifying subtypes of CC and stratifying the prognosis of patients. Among epigenetic modifications, host DNA methylation has been heavily studied in CC (Xie et al., 2018; Li et al., 2020). Abnormal DNA methylation patterns, such as hypermethylation of tumour suppressor genes' promoters and hypomethylation of oncogenes' promoters, lead to dysregulated transcription of these genes and disrupt tumour-related signalling pathways. This phenomenon is a common feature of HPV-associated CC. Abnormal DNA methylation usually becomes detectable in the precancerous stage, reaching its peak after HR-HPV-induced tumours occur (Verlaat et al., 2018). DNA hypermethylation increases with the severity of CC (Kremer et al., 2018; Zummeren et al., 2018). DNA methylation markers, such as methylated CpG islands in specific genes (e.g. p16ˆINK4a, CADM1, ANKRD18CP, C13orf18, EPB41L3, JAM3, SOX1, ZSCAN1, and PRDM14), have been studied as diagnostic biomarkers for CC (Varghese et al., 2018; Verlaat et al., 2018; Kyrgiou et al., 2020). In HPV^{+} women, DNA methylation markers demonstrate superior sensitivity and specificity in diagnosing HSIL compared to abnormal cytology and HPV16/18 genotyping (Cook et al., 2019). This provides a more accurate triage management model for the timely identification of HR populations requiring clinical intervention.

Non-coding RNAs (ncRNAs) may also facilitate the diagnosis of CC. Dysregulated expression profiles of ncRNAs have been observed in HPV-infected CC patients (The Cancer Genome Atlas Research Network, 2017; Yeo-Teh et al., 2018). For instance, infection of HR-HPV enhances the expression of lnc-FANCI-2 through the viral oncoprotein E7 and YY1 (Liu et al., 2021). Upregulation of LINC00290, LINC02500, and LENG9 transcripts has been found in CC, associated with increased proliferation,

migration, and invasion of CC cells (Zhou et al., 2022). Moreover, long non-coding RNAs (lncRNAs) such as HOTAIR, MALAT1, and HOXA11-AS, can act as oncogenes and contribute to cancer initiation and progression. Additionally, specific microRNAs (miRNAs) such as miR-21, miR-155, and miR-375 are dysregulated in CC, leading to downregulation of tumour suppressors or upregulation of oncogenic proteins (Pardini et al., 2018). Some ncRNAs are resistant to degradation even under extreme conditions, making them suitable as diagnostic biomarkers.

More recently, an intricate relationship between HPV-related CC and the human endogenous retroviruses (HERVs) in the genome has been gradually uncovered. HERVs are repetitive sequences containing long terminal repeats as well as internal coding elements such as gag, pol, and env (Johnson, 2019). HERVs are located primarily in heterochromatic regions and suppressed by epigenetic mechanisms (Lander et al., 2001). However, under pathological conditions, HERVs can be abnormally activated, producing various RNA species and proteins including miRNAs, lncRNA, gag, and env (Jönsson et al., 2020). These molecules contribute to tumour formation and progression by participating in gene transcription regulation, maintaining chromatin structure, and impacting host immune responses (Yi et al., 2006; Panda et al., 2018; Rezaei et al., 2021). In CC cells, HPV infection alters HERVs expression, affecting various subfamilies such as HERVK, HERVH, HERVE, HERVI, and HERVL (Curty et al., 2020, 2021; Stricker et al., 2023). HERVs also manifest altered expressions among CC patients, demonstrating various associations with HPV types, tumour stages, and patient survival outcomes. HERVs like MER41A, HERVH-int, and HERVK9 exhibit marked upregulation in CC tissues (Alldredge et al., 2023). Notably, HERVK shows dynamic expression patterns during the progression of CC (Ko et al., 2021; Tavakolian et al., 2021; Soleimani-Jelodar et al., 2024). Compared to normal tissues, the expression of HERVK env was reduced in CIN1, CIN2, CIN3, and CC tissues. In contrast, levels of HERVK gag and rec were elevated in CIN1 and CIN2 tissues but significantly decreased in CIN3 and CC tissues. Therefore, HERVs may serve as a new class of prognostic biomarkers for CC, underscoring the importance of further investigations into the relationship between HERVs' activation and HPV infection.

New treatment opportunities for CC patients with HPV infection

Immunotherapy has emerged as a transformative approach, significantly improving outcomes and survival rates for cancer patients (Cohen et al., 2020; Figure 4). CC, as a virus-driven malignancy, is recognized as highly immunogenic, with abundant tumour cell mutations, neoantigen formation, and immune cell infiltration, making it particularly responsive to immunotherapy (Wang et al., 2019). In 2014, the FDA-approved pembrolizumab, a PD-1 inhibitor, for the treatment of advanced CC, marking the onset of the targeted therapy era in CC treatment. The KEYNOTE-158 study validated the therapeutic efficacy of pembrolizumab in advanced CC. The KEYNOTE-826 established the use of immune checkpoint inhibitors in combination with pembrolizumab, with or without chemotherapy, as the first-line treatment for recurrent or metastatic CC (Chung et al., 2019). However, recent immunotherapy studies focusing on monotherapy for advanced-stage patients have not shown significant advantages over traditional treatments. This highlights the urgent need to explore combination therapies to enhance the effectiveness of immunotherapy (Colombo et al., 2021). Promisingly, the results of the Phase I NRG-GY017 randomized trial (NCT03738228) indicated that the concurrent use of atezolizumab or the initiation of atezolizumab in combination with chemoradiotherapy demonstrated good safety and tolerability, suggesting the potential efficacy of induction immunotherapy (Mayadev et al., 2020). Additionally, another study showed that patients receiving concurrent and adjuvant durvalumab with chemoradiotherapy had better survival outcomes than those receiving chemoradiotherapy alone in cases of LACC (Mayadev et al., 2020). Further studies are needed to validate the effectiveness of combining immunotherapy with chemotherapy, radiation, and other treatment regimens in CC.

Currently, there are three types of prophylactic HPV vaccines targeting the L1 capsid protein available on the market. However, the L1 capsid protein is absent in infected basal epithelial cells, making these vaccines ineffective at eliminating existing infections. Thus, there is an urgent need to develop therapeutic HPV vaccines for individuals already infected with HPV. Researchers have developed several types of therapeutic vaccines, including live vector, nucleic acid, peptide, protein-based, and cell-based vaccines, all of which have shown promising results in clinical trials (Cheng et al., 2018). For example, VGX-3100, consisting of two DNA plasmids encoding optimized synthetic consensus E6 and E7 genes of HPV16 and HPV18, has completed Phase I and Phase II clinical trials. The Phase III clinical trial (CTR20201547) of VGX-3100 for the treatment of CIN patients also showed good safety and higher response rate in the treatment group than in the placebo group. Specifically, in terms of viral clearance, the viral clearance rate in the treatment group was 37.3% (50/134), compared to 8.7% (6/69) in the placebo group. Therefore, VGX-3100 is expected to fundamentally transform the existing treatment regimens for HPV-related cervical precancerous lesions, preventing the development of CC. In addition, a live attenuated bacterial therapeutic HPV vaccine has been selected for Phase II trials and is currently undergoing clinical evaluation for various cancers including CC (NCT01266460), due to its favourable safety profile and effectiveness (Maciag et al., 2009). Furthermore, a Phase III clinical trial (AIM2CERV) is investigating the efficacy of ADXS11-001 as an adjuvant immunotherapy for HR, LACC patients following chemotherapy or radiotherapy (NCT02853604). Preclinical studies have also made corresponding progress in the development of CC vaccines or adjuvants. As early as 2019, Ugur Sahin and colleagues developed the E7 RNA-LPX HPV therapeutic vaccine, which was able to completely eradicate tumour tissues and establish a protective T-cell memory response in $HPV16^{+}$ mice (Grunwitz et al., 2019). Many efforts have also been dedicated to improving the antitumour effects of existing mRNA vaccines. Eric Huang developed an mRNA encoding the gene adjuvant STINGV155M to enhance

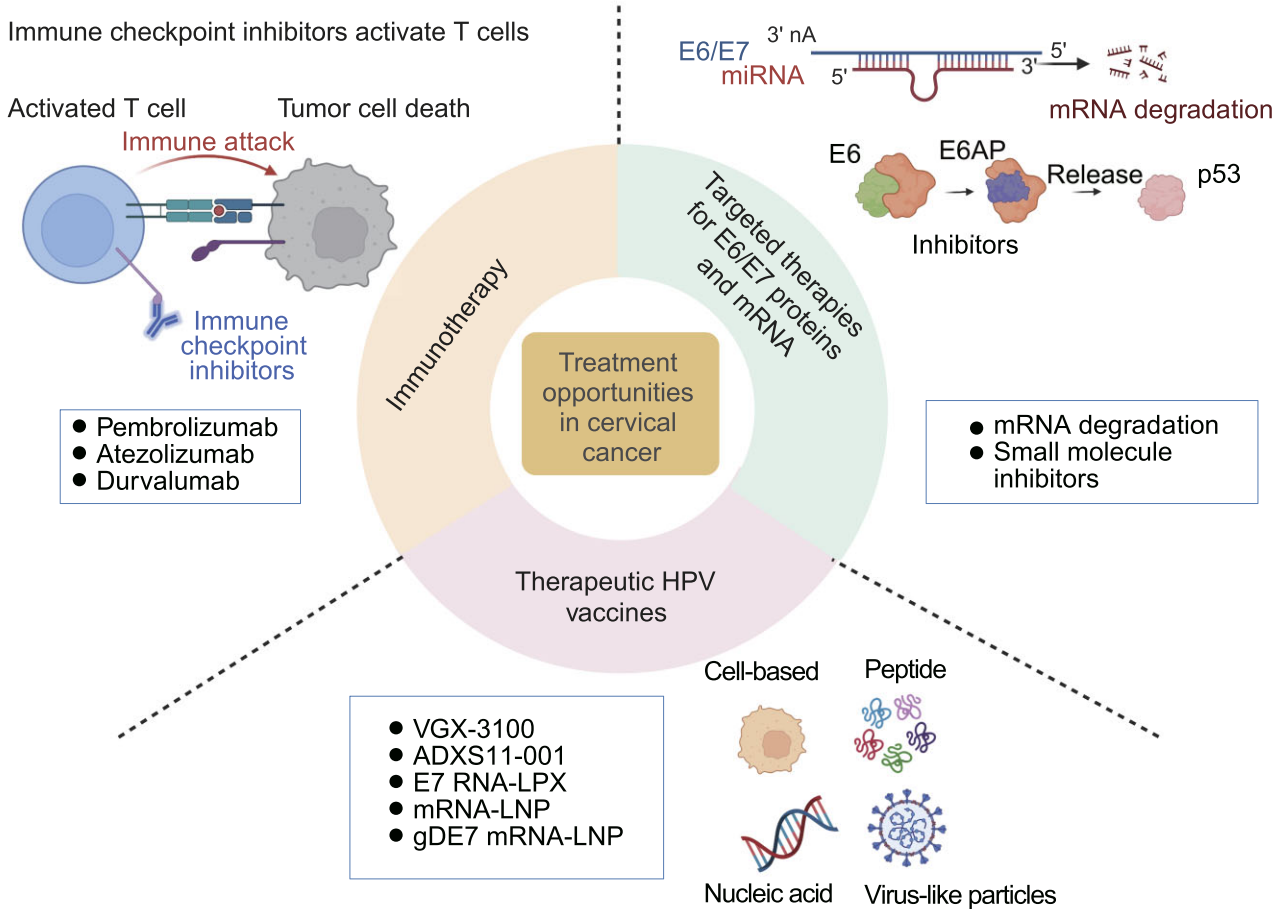

Figure 4 Treatment opportunities for CC patients with HPV infection. Immunotherapy, therapies targeting E6 and E7 oncogenes and mRNA, and HPV vaccines represent promising new treatment approaches for CC. The image was created with BioRender.

the anti-tumour effects of an mRNA-LNP vaccine encoding the E6/E7 oncoproteins. The administration of this adjuvant inhibited the growth of HPV TC-1 tumour cells and prolonged the survival of vaccinated mice (Tse et al., 2021). An mRNA therapy encoding anti-CD40 (NCT03418480) for the treatment of HPV16^{+} head and neck squamous cell carcinoma is currently in Phase 1/2 clinical trials. An mRNA-LNP vaccine encoding the HPV16 E7 oncoprotein and the HSV1 herpes virus glycoprotein gDE7 fusion protein, known as the gDE7 antigen, was developed (Ramos da Silva et al., 2023). This vaccine comprises three types of mRNA: modified/unmodified conventional mRNA and self-amplifying mRNA. Excitingly, a single immunization with gDE7 mRNA-LNP vaccines effectively eradicates tumours and shows more robust anti-tumour activity compared to gDE7 plasmid DNA and gDE7 recombinant protein-based vaccines (Ramos da Silva et al., 2023). Taken together, therapeutic vaccines targeting HPV could serve as a potential strategy to improve clinical outcomes. However, efforts to identify ideal targets remain to be defined.

Accumulating evidence demonstrates that HR-HPV E6/E7 oncogenes are associated with HR-HPV integration and the progression of cervical SIL (Yeo-Teh et al., 2018). HPV E6 and E7 oncoproteins promote the tumorigenesis of CC by inducing cell proliferation, inhibiting apoptosis, disrupting DNA repair mechanisms, and stimulating angiogenesis (Hoppe-Seyler et al., 2018; Balasubramaniam et al., 2019; Pal and Kundu, 2019). The impact of HPV E6/E7 oncoproteins on the therapeutic response of CC patients has also been defined. Thus, it is increasingly clear that targeting HPV E6 and E7 oncoproteins may represent an important adjunct to current therapeutic modalities. On one hand, vaccination targeting HPV E6/E7 oncogenes has demonstrated promising potential in combating persistent HPV infection. On the other hand, therapeutics targeting E6/E7 proteins also show the potential to treat CC. Inhibiting E6/E7 protein expression or altering their structure and activity can effectively prevent the progression of CC (Bhattacharjee et al., 2022; Zheng et al., 2022). Recently, numerous small molecular inhibitors have been developed to target the binding interface between E6 and E6AP, leading to the reactivation of p53 and apoptosis of HPV^{+} cells (Malecka et al., 2014; Rietz et al., 2016; Yuan et al., 2016). Additionally, efforts to develop the monoclonal antibodies targeting HPV E6/E7 oncoproteins in CC to enable better therapeutic efficacy in patients are still underway.

In recent years, single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for deciphering the diverse

cell populations and intercellular communications within the tumour microenvironment, providing unprecedented molecular insights into various cancers including CC. To investigate epithelial heterogeneity and identify epithelial-specific gene signatures in HPV^+ CC, Li and Hua (2022) utilized scRNA-seq analysis on tissue samples from a patient with CC and revealed novel cell layers exhibiting dysregulation of cell differentiation, extracellular matrix structure, and cell cycle dynamics. Notably, these undefined epithelial layers maintained a high level of E6 and E7 expression. Besides, by exploring the heterogeneity between HPV^+ and cells in each epithelial cluster, they found that HPV^+ cells exhibited a preference for epithelial–mesenchymal transition, extracellular matrix interactions, and cellular differentiation signals within a specific epithelial layer (Yue et al., 2023). In a separate scRNA-seq investigation, samples from nine patients, including two HPV^- normal, two HPV^+ normal, two HPV^+ HSIL, and three HPV^+ cancer samples, revealed significant heterogeneity of immune microenvironment across four key stages of epithelial malignant transformation in the development of CC (Guo, 2023). During the progression of the HPV-infected cervix towards precancer, the microenvironment promotes the progression of CC by influencing HPV infection and clearance. In the early HPV infection stage, plasmacytoid dendritic cell (pDC) activation might negatively regulate viral replication, whereas as the HPV infection persists, pDCs derived from CIN exhibit immune tolerance and may even facilitate immune escape. The most key aspect is that the loss of $CD4^+$ Th17/Treg homeostasis might lead to the failure of HPV clearance, ultimately contributing to carcinogenesis (Guo, 2023). Tregs and NK cells are the most critical cell types that contribute to immune evasion in CC. Li et al. (2022) identified four $CD4^+$ T cell subclusters in normal cervical tissues, CIN, as well as early and advanced cervical squamous cell carcinoma (CESC) tissues: naïve $CD4^+$ T cells ($CCR7^+$), Th17 cells ($IL17A^+$), TNFRSF9 high Tregs, and TNFRSF9 low Tregs (Cheng et al., 2018). Another study conducted by Liu et al. (2023) observed an increased abundance of Th17 cells and TNFRSF9 high Tregs in CESC tissues, which correlated with elevated immune inhibition scores and poorer survival outcomes. Liu et al. (2023) also identified two subsets of NK cells in CESC based on transcriptomic profiling: $CD16^+$ ($FCGR3A^+$) and $CD56^+$ NK cells ($NCAM1^+$). The abundance of $CD16^+$ NK cells, which exhibited high levels of cytotoxic genes (FGFBP2, NKG7, and GZMH), were significantly lower in CESC samples than in normal cervical samples. Moreover, a higher infiltration of $CD16^+$ and lower infiltration of $CD56^+$ NK cells were associated with better survival outcomes in CESC patients. In contrast, two additional NK cell subsets, $FGFBP2^+$ and $XCL2^+$ NK cells, were identified in cervical adenosquamous carcinoma. Moreover, a higher infiltration of $FGFBP2^+$ NK cells, characterized by elevated levels of cytotoxic genes (GNLY, PRF1, GZMA, and GZMB) and low levels of inhibitory genes (PDCD1, CTLA4, and TIGIT), was associated with better survival outcomes in CC patients (Liu et al., 2023). Furthermore, a significant immune ecosystem heterogeneity was identified at different stages of CC development (Cheng et al., 2018). For instance, HSIL samples were mainly infiltrated by effector NK cells, tissue-resident $CD8^+$ T cells, DC1, pDC, Treg cells, and M1-like macrophages, forming a low-activity but activated microenvironment. In contrast, tumour tissues were highly enriched with M2-like macrophages, resident NK cells, and exhausted $CD8^+$ T cells, displaying a marked immunosuppressive state (Li and Hua, 2022). However, in cases of CC with metastatic lymph nodes, there was an enrichment of naïve $CD4^+$ T cells, central memory $CD4^+$ T cells, effector memory $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, and circulating NK cells (Li and Hua, 2022). This study indicates dynamic changes in the abundance and molecular profiles of cell infiltrates involved in CC initiation and progression (Li and Hua, 2022). These comprehensive scRNA-seq analyses elucidate the cellular landscape and dynamics throughout the progression of CC, shedding light on how HPV infection impacts gene expression during epithelial malignant transformation. We believe that the targets identified by the scRNA-seq analyses, either the HPV-infected epithelial cells or the microenvironment, warrant further functional studies to develop new treatment opportunities for HPV-related CC patients.

Conclusion and perspectives

CC continues to impose a significant global health burden, calling for the identification of new diagnostic and therapeutic targets to combat this malignancy. The abundant repetitive elements in the human genome such as HERVs have drawn increasing attention in recent years as a novel player involved in many human diseases (Yu et al., 2022; Copley and Shorter, 2023; Dhillon et al., 2023). HERVs are differentially expressed in HPV^+ and HPV^- populations, impacting the transcription of nearby genes associated with CC development and prognosis (Li et al., 2017; Curty et al., 2020, 2021; Ko et al., 2021; Tavakolian et al., 2021; Alldredge et al., 2023; Soleimani-Jelodar et al., 2024). Although a significant association between HPV infection and HERV activation has been established, its underlying mechanism remains incompletely understood (Curty et al., 2021). HPV infection may activate HERVs via the E6 and E6AP-mediated degradation of p53. Additionally, HPV infection can induce restructuring of the host genome and changes in DNA methylation levels, which promote the transcription of HERVs (Curty et al., 2020, 2021). Furthermore, HPV infection may suppress the antiviral immune response in host cells, creating conditions favourable for HERVs' activation. Further interrogations of the relationship between HPV infection and HERVs' activation are expected to not only elucidate the pathogenic mechanisms of CC but also provide new insights and targets for its prevention, diagnosis, and treatment.

The future advancements in new methodologies as well as novel disease models are also crucial for the development of better diagnostic and treatment strategies for CC. The cutting-edge scRNA-seq technologies provide a comprehensive insight into HPV infection and CC, shedding light on both genetic factors and microenvironment aspects. Meanwhile, recent studies have successfully utilized patient-derived cervical organoids to

simulate the dynamics of individual and co-infections of HPV and Chlamydia (Koster et al., 2022). The cervical ectocervical stem cells can be genetically manipulated to express the HPV16 E6/E7 oncogenes, and the cervical organoids derived from these engineered stem cells develop features similar to precancerous lesions while maintaining self-renewal capabilities and organizing into mature stratified epithelia (Koster et al., 2022). These new organoid models for CC, together with the explorations of the dynamic alterations of CC tissues and the microenvironment by scRNA-seq, will enable researchers to fully uncover the cellular and molecular processes of CC initiation and progression, offering new insights into the intricate interactions between pathogen infection and tumour development, therefore facilitating the advancement of more effective prevention and treatment modalities.

Lastly, in addition to the development of new intervention strategies, crucial efforts should be made to increase public awareness, reduce costs, expand the accessibility of screening services, and implement appropriate immunization programs to enhance vaccine coverage. The holistic actions of the whole human society worldwide will eventually achieve the ultimate goal of eliminating CC.

Supplementary material

Supplementary material is available at *Journal of Molecular Cell Biology* online.

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