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Precision therapeutic targets for HPV-positive cancers: an overview and new insights

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Abstract
The increasing incidence and mortality rates of HPV-positive cancers, particularly HPV-positive head and neck cancer, in recent years have emphasized the pressing need for more efficacious treatment options. Recent studies have elucidated the molecular distinctions between HPV-positive and HPV-negative cancers, which are crucial for developing precise and effective therapeutic strategies. This review updates the most recent findings on the molecular variances between HPV-positive and HPV-negative cancers, evaluates current treatments for HPV-positive cancers, and summarizes emerging frontiers in HPV-targeted therapies aimed at developing more effective and precise interventions against these cancers.

Keywords HPV-positive cancers, Precision therapy, Head and neck cancer, Cervical cancer, HPV vaccine

Introduction

Human papillomavirus (HPV) is easily transmitted through damaged skin or mucous membranes and affects millions globally. It is a leading cause of several malignancies, including cervical, oropharyngeal, anal, vulvar, vaginal, and penile cancers [1]. According to the World Health Organization, HPV caused an estimated 620,000 cancer cases in women and 70,000 cancer cases in men in 2019 [2, 3]. Among them, cervical and head and neck squamous cell carcinomas (HNSCC) are the most common situations in HPV-positive cancers, and the involvement of HPV in HNSCCs keeps rising while decreasing in cervical cancer [4]. To combat HPV-positive cancers, the development of prophylactic HPV vaccines has been a significant milestone. Despite their effectiveness, these vaccines have several limitations. While the vaccine is highly effective when administered before HPV exposure, it offers limited therapeutic benefit for individuals with pre-existing infections. Moreover, the accessibility of vaccines is restricted by age and gender in some areas. Approximately one-third of men over the age of 15 worldwide are infected with at least one type of HPV, and 1/5 are infected with cancer-causing HPV types [5]. This indicates that HPV infection is more widespread in males [5]. Given that vaccines alone cannot fully address the challenge of HPV-positive cancers, novel strategies to make up for the limitation are in urgent need.

Compared with HPV-negative cancer cells, HPV-positive cells are believed to have a lower mutational burden since HPV is similar to the first "hit" in the "two-hit theory" [6]. Infection with the HPV virus lowers the

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threshold for carcinogenesis and does not necessitate the accumulation of numerous aberrations for carcinogenesis to occur. The unique characteristics of HPV-positive cancers suggest that precision treatment approaches could effectively target specific mechanisms involved in HPV-induced carcinogenesis. Precision treatment with fewer side effects and better efficacy may significantly improve the life quality of patients. This review aims to summarize current research on precision treatment and further explore potential treatment targets.

The molecular differences between HPV-positive and HPV-negative cancers

Understanding the molecular disparities between HPV-positive and HPV-negative cancers is crucial for advancing our knowledge of carcinogenesis and developing more effective treatment strategies. An examination of the HPV genome's structure and function serves as a foundational step. It can be divided into three regions: the early region (ER), which encodes early proteins (E1, E2, E4, E5, E6, E7); the late region (LR), responsible for the viral capsid proteins L1 and L2 and the long control region (LCR), a noncoding region containing regulatory elements for viral transcription and replication [7]. Importantly, the carcinogenic potential of HPV lies in the ER, particularly E6 and E7, which play pivotal roles in cellular transformation and carcinogenesis. Unlike the loss of p16 function in HPV-positive cancers, the E6-mediated degradation of p53 and E7-mediated silencing of pRb are distinctive features of HPV-positive cancers. E6 targets the tumor suppressor protein p53 for degradation, disrupting cell cycle control and apoptosis, while E7 binds to the retinoblastoma protein (Rb), overriding cell cycle arrest, and fostering uncontrolled cell growth [8]. The E2 protein negatively regulates the expression of E6 and E7 and is responsible for HPV's integration. Additionally, HPV employs E5 to evade the immune system for its ability to retain human leukocyte antigen (HLA) in the endoplasmic reticulum [9, 10]. Besides the ER, the LCR also plays a vital role. The LCR contains multiple transcription factor (TF) binding sites, including Sp1, AP1, NF1, TEF1, OCT1, YY1, BRN-3a, NF-IL6, KRF-1, NF-kB, FOXA1, GATA3, etc [7]., which modulate the expression of viral genes. The hijack of TFs by HPV plays a role in continuous cell proliferation [11]. The intricate interplay between HPV and human cells underscores the complexity of HPV-induced carcinogenesis. Besides, recent studies have further revealed specific chromosomal alterations involved in HPV integration [12]. HPV-positive HNSCCs are reported to be enriched in 3q24-27 chromosomal amplifications, a region coding for oncogenes PIK3CA, TP63, SOX2, CCNL1, and PARP1. The integration also perturbs cellular signaling pathways, such as Phosphoinositide-3-kinase (PI3K), dysregulating growth, proliferation, and survival [13]. By comparison, HPV-negative events are more complex and less distinguishable [14]. In HPV-positive cervical cancers, a significantly higher EMT mRNA score and a higher frequency of the APOBEC mutagenesis signature are reported [12]. Other HPV-positive cancers also show speciality. HPV-positive penile cancers are said to be associated with somatic mutations in ARPP21, CMYA5, RPGRIP, and CSPG4 [15]. The genetic analysis of HPV-positive and HPV-negative bladder cancers shows that the mutation frequency in cell cycle regulatory genes is significantly reduced in HPV-positive urinary bladder cancer cells [16]. HPV-positive conjunctival squamous cell carcinomas and squamous cell carcinomas of the pelvic and perineal region all have PIK3CA activating mutations as the most common genomic event [17–20]. These studies demonstrate that HPV-positive cancer and HPV-negative cancer are two distinct types of cancer. The HPV infection status shares similar molecular backgrounds across a range of cancers, such as HNSCC, cervical cancer, penile cancer, bladder cancer, and conjunctival cancer. Consequently, HPV-positive cancers exhibit cross-organ properties, suggesting the potential for developing universal targeting strategies.

In conclusion, the molecular differences between HPV-positive and HPV-negative cancers underscore the complexity of carcinogenesis and emphasize the need for tailored treatment approaches. We can develop more precise therapeutic interventions by deepening our understanding of these disparities, ultimately improving patient outcomes and survival rates.

Current treatment for HPV-positive cancers

The latest National Comprehensive Cancer Network (NCCN) guidelines for both cervical (2024.V4) and head and neck cancers (HNC) (2025.V1) advocate universal HPV testing for precise treatment guidance [21, 22]. HPV infection is nearly universal in cervical cancers and significant in oral squamous cell carcinoma within HNC. It is noteworthy that HPV-positive HNC patients exhibit significantly different epidemiological, clinicopathological, and prognostic characteristics compared to HPV-negative patients [22–24]. Furthermore, the American Joint Committee on Cancer has discussed HPV-associated oropharyngeal cancer as a separate category since the 8th Vision. Additionally, HNC NCCN guideline has highlighted the significance of HPV infection in assessing prognosis, recognizing HPV-associated HNSCC as a unique subtype since 2016. The pathogenicity of HPV should be acknowledged and leveraged to prevent and reduce the incidence and mortality rates of related cancers. However, specific therapy for HPV-positive cancers is not achievable clinically. The mainstay of treatment for HPV-positive cancers, akin to HPV-negative cancers,

remains surgery, radiotherapy (RT), and chemotherapy. Given their heightened sensitivity to these modalities and improved immune responses, immunotherapy is increasingly recognized as a first- or second-line option [21, 25, 26]. Herein, we concisely overview current treatments and highlight recent advancements and improvements in therapeutic strategies.

Surgery

The primary treatment of early-stage cervical cancers and HNC is either surgery or RT. The surgical approaches for HPV-positive cervical cancer patients differ between those who have and have not given birth. Radical hysterectomy may be employed for non-fertility sparing, whereas cone biopsy with or without pelvic lymphadenectomy might be considered for fertility sparing, with the ultimate choice of procedure determined by the patient's specific clinical presentation and oncological requirements [21]. For HPV-positive HNC patients, the traditional surgical approach is mandibulotomy. However, mandibulotomy disrupts the integrity of the lower lip and mandible, resulting in significant trauma, slow recovery, and a relatively high incidence of postoperative functional disorders such as swallowing and voicing issues [27]. To improve prognosis, new approaches such as transoral robotic surgery (TROS) and transoral laser microsurgery were proposed and have been included by the NCCN [28–30].

Radiotherapy

RT is an important treatment strategy for both cervical cancers and HNC. In patients with an intact cervix and without prior surgery, the primary tumor and regional lymphatics at risk are treated with definitive external beam radiotherapy to 40–50 Gy. Brachytherapy boosts the cervical tumor with an additional 30–40 Gy, totaling 80 Gy for small tumors or ≥ 85 Gy for larger ones. In HNC, the RT dose for the primary tumor and involved lymph nodes is 66–70 Gy; for the sites of suspected subclinical spread, it is 44–50 Gy. However, the threshold dose for the toxic and side effects of radiotherapy is 50–60 Gy and after exceeding 55 Gy, progressive dysphagia can occur with every additional 10 Gy [29]. To reduce side effects, some researchers have proposed a decreased intensity in RT since HPV-positive cancers are more sensitive to radiation [31, 32]. NCCN pointed out that de-escalation to 50 Gy may be considered in patients with HPV-positive oropharynx cancer who have ≤ 4 positive lymph nodes, T1–T2 resected to negative or close margins (< 3 mm), and/or N1–N2 disease with ≤ 1 mm extranodal extension. Many clinical trials have also verified the long-term benefit of de-intensity RT for HPV-positive patients. Typical trials like MC1273 and NCT06563479 indicated that reducing the dose of RT holds great potential for improving life quality for patients with HPV-positive cancers [33, 34]. However, it is crucial to carefully select suitable patients and closely monitor toxicity to ensure effective treatment outcomes.

Chemotherapy

Concurrent chemoradiotherapy is currently one of the standard treatment regimens for advanced carcinoma. Cisplatin remains as the preferred radiosensitizing agent in the primary treatment for patients with locally advanced HPV-positive cervical cancer and HNC when used concomitantly with RT and carboplatin as a preferred radiosensitizing agent for patients who are cisplatin intolerant. The NCCN Panel has noted for all chemoradiation agents that the cost and toxicity profiles of these radiosensitizing agents should be considered and is especially critical when these regimens are being used for extended field radiation therapy where toxicities may be more severe [21, 22]. Thus, alternative drugs for cisplatin have been proposed to reduce toxicity. A clinical research, ARTSCANIII, adapted cetuximab to replace cisplatin [35]. However, the 3-year failure rate was higher in the cetuximab group compared to the cisplatin group (23% vs. 9%, $P = 0.0036$), suggesting that cetuximab has inferior control compared to cisplatin. The outcomes of various innovative chemotherapy regimens vary. At this stage, more attempts are still needed to explore the indications of different or new regimens with better promotion prospects.

Immunotherapy

Immunotherapy nowadays brings hope to many patients [36]. Immune checkpoint drugs like CTLA-4 or PD-L1 antibodies have been proven effective in precision treatment [37, 38].

For PD-L1 + cervical cancers, pembrolizumab/pembrolizumab + cisplatin/paclitaxel ± bevacizumab has been the first-line therapy according to NCCN. For HPV-positive HNC, some clinical trials, such as CheckMate-141, has also applied nivolumab (nivo, anti-PD-1) [39]. Another phase 2 trial, NCT03172624, applied nivolumab plus ipilimumab (ipi, anti-CTLA-4) in advanced salivary gland cancer [40]. Both studies indicated positive objective response rates. However, the optimal timing and indications for immunotherapy in clinical practice are still unclear. This is due to various factors including patients' immune infiltration status, tumor-specific antigens, immune escape mechanisms, and tumor cell heterogeneity, which can all impact clinical outcomes. Additionally, the efficacy and resistance of immunotherapy often necessitate its combination with traditional treatments like surgery and RT. Consequently, further clinical trials are essential to establish definitive guidelines for immunotherapy and ascertain its clinical value.

The distinction made in the NCCN guidelines between treatment protocols for HPV-positive and HPV-negative cancers adequately reflects the significance attached to HPV-positive cancers. Despite better prognoses, HPV-positive HNC remains lethal, and the long-term impacts of surgery and chemoradiotherapy on young patients' quality of life are significant. Nevertheless, HPV infection can serve as a prioritized target due to its high degree of specificity. Therefore, there is a potential and need to develop precise therapy specifically tailored for HPV-positive cancers. Leveraging current knowledge of HPV-dependent pathways and interactions, novel precision therapeutic approaches may be designed to improve patients' prognoses.

Direct targeting of HPV for precision treatment

Direct targeted therapy for HPV-related cancers aims to precisely inhibit HPV-associated factors including the ER, the LR, the LCR and proteins encoded by them. Among them, E6 and E7 have been considered attractive therapeutic targets due to their leading role in tumorigenesis. Targeting E6 and E7 offers a particular treatment option, minimizing side effects by sparing uninfected healthy tissues that lack these targets. Numerous approaches have been proposed to interfere with the expression of viral oncogenes, including RNA interference (RNAi), gene editing, protein-targeting with small molecules, and immunotherapies (Fig. 1).

Small nucleic acid drugs

Small nucleic acid drugs stand at the strategic forefront of biopharmaceutical innovation. As early as the 1990s,

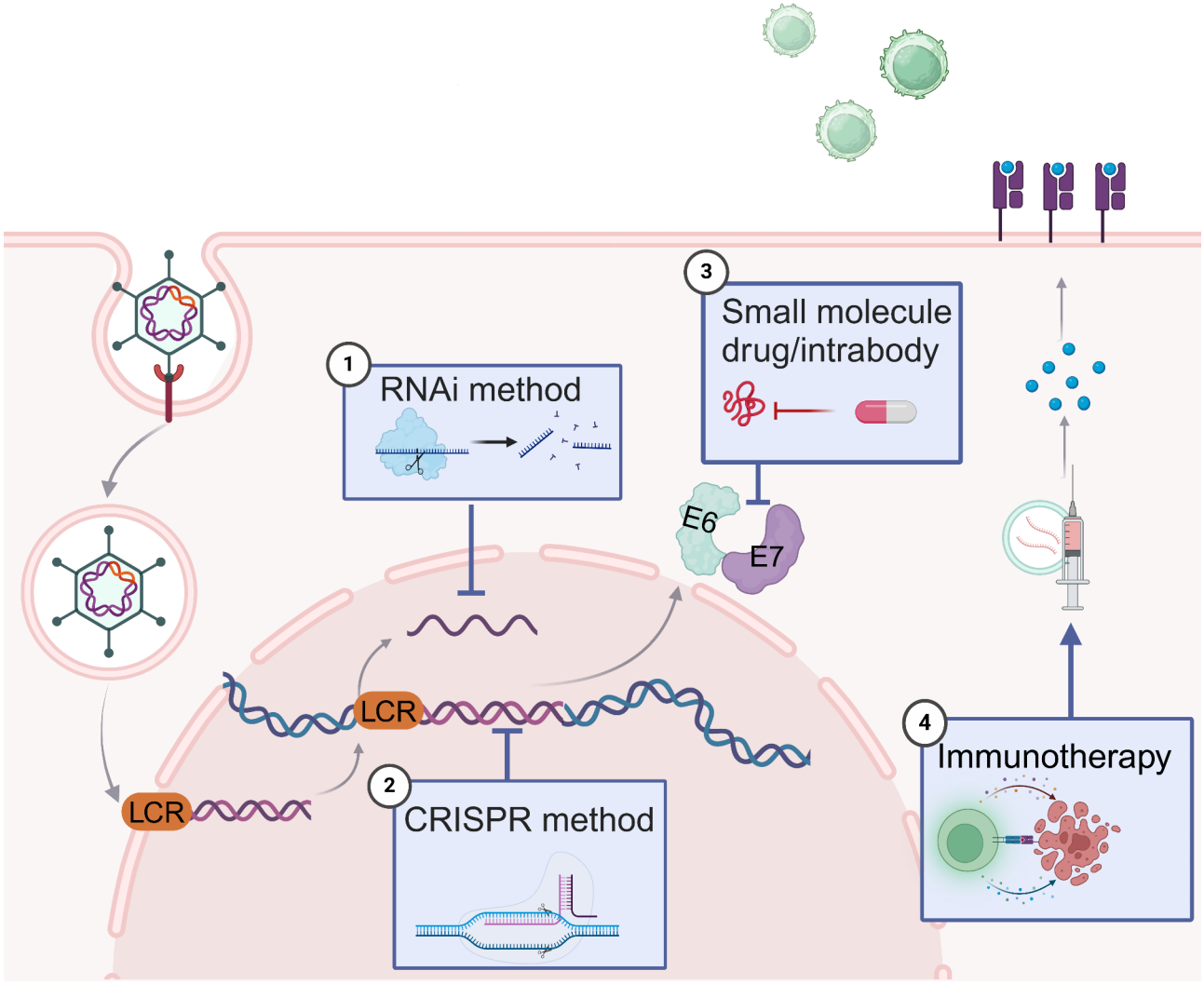

Fig. 1 Direct targeting of HPV. Illustration of RNAi, gene editing, protein-targeting with small molecules/antibodies and immunotherapy. The RNAi leads to homology-dependent degradation of the target mRNA. The CRISPR system recognizes and cleaves the HPV genome. Small molecule drugs/antibodies directly bind to oncoproteins and inhibit their function. Immunotherapy promotes the immune response of the host. Created in BioRender.com

the mechanism of small nucleic acid drugs was elucidated [41], opening the door for small nucleic acid drugs. In HPV-positive cancers, many studies have adopted small nucleic acid drugs to silence E6 and E7 [42–46]. Serval attempts also target functional regions in LCR [47–50]. Nevertheless, cancer treatment *via* systemic administration of siRNA or shRNA remains a major challenge because the negatively charged siRNA prevents passive diffusion into the cytoplasm across the cell membranes and is also rapidly eliminated through enzymatic degradation. Over the past decade, extensive research has led to the emergence and optimization of delivery systems to address these challenges. Since serval adeno-associated virus (AAV) based drugs have gained FDA approval [51, 52], AAV-shE6E7 has been designed to knockout E6 and E7 oncogene in HPV + cancers in vitro [46]. However, some researchers think lipid nanoparticles (LNP) are safer than AVV for lower hepatotoxicity and immunogenicity [53]. The current focus on LNP is enhancing tissue specificity uptake and biodistribution. For instance, biological conjugation of anti-EGFR mAbs for LNP facilitates cargo delivery for HPV-positive cancer cells and mediates anti-tumor activity [43]. Meanwhile, other carriers like polymers have also been put into use. Notably, the cRGD ligands conjugated on the micellar surface targeted αvβ3 and αvβ5 integrin receptors dramatically facilitated the uptake of siRNA payloads by cultured HeLa cell, an HPV + cervical cancer cell line [42]. As research continues to progress, the field of siRNA delivery holds significant potential for addressing previously challenging medical conditions and driving innovations in precision medicine. These advances all together paved the way for the clinical use of small nucleic acid.

Gene editing

Gene editing therapy like the CRSIPR-based and TALEN-based techniques is currently in the spotlight for accurate and effective gene manipulation ability. With feasible delivery systems, serval attempts of CRISPR-based therapy have been conducted in HPV-positive cancers and showed that using designated TALEN and CRISPR/Cas9 as genome editing tools could produce disruption of HPV16 and HPV18 E6/E7 DNA, significantly decreasing the expression of E6/E7, inducing cell apoptosis and inhibiting cell lines growth [54, 55]. However, in a clinical trial, NCT03057912, gene editing for HPV + patients showed unknown results, indicating latent defects for gene-editing methods. Different from RNAi, the CRISPR system directly and irreversibly modifies genes, off-target effects caused by unwanted cutting of Cas proteins can lead to more serious and irreversible outcomes. Thus, ensuring precision remains a primary consideration for clinical translation. To minimize off-target effects, the device of single guide RNA (sgRNA) is of paramount importance, as the final treatment outcome can vary significantly based on the choice of sgRNAs. However, the availability of sgRNAs is currently limited, and identifying the optimal sgRNAs requires substantial experimentation. Nevertheless, the CRISPR method still holds great potential, exemplified by the recent approval of a CRISPR-based drug in the United Kingdom. With its capabilities for base editing and gene insertion, CRISPR applications are expected to expand further [56].

Small molecule drugs or antibody drugs

Besides inhibiting oncogene expression, directly targeting key proteins in the carcinogenic process of HPV using intracellular antibodies or small molecules is a viable option. Intrabodies and small molecule drugs can be delivered into cells through vectors and directed against oncoproteins like E6 and E7 [57–59]. To devise effective intrabodies or small molecule drugs, deciphering the functions of each domain in the oncoprotein is necessary [60]. With advancements in protein structure prediction and docking analysis [61], our understanding of the structural and functional domains of HPV oncoproteins has reached a new frontier, enabling precise interference. An uncompleted clinical trial, NCT04278326, uses mRNA to produce nanobodies targeting E6 and E7 in organoids built from precancerous cervicovaginal lesions or cervical cancer. But overall, clinical translation has not been realized in this field. The challenges arise from the relatively small molecular size of HPV oncoproteins, complicating the development of this therapeutic strategy. Furthermore, the functional domains of HPV oncoproteins bear structural similarities to those of normal functional proteins, thus interfering with the domains of oncoproteins may disrupt normal physiological activities. For instance, E6's zinc finger domain and PSD95-Discs large-ZO1 (PDZ) domain are commonly seen in human enzymes so zinc-ejection and PDZ-binder can lead to inevitable off-target effects [62]. Considering these challenges, an alternative approach is to target human proteins that interact with HPV oncoproteins. It is known that oncoproteins heavily rely on normal host proteins to carry out their functions, co-opting these normal proteins to aid in HPV survival and propagation. Directly targeting these normal proteins may not be a wise choice, as they play essential roles in normal cellular functions. Therefore, targeting the complex formed by oncoproteins and normal proteins is more feasible. For example, the E6-E6AP-p53 complex forms characteristic structural domains, and small molecule drugs have the potential to impede their interaction and prevent p53 degradation [60]. Research also tried to prevent pRb degradation [63], but it has been reported that pRb-targeted therapies performed poorer due to less contribution to cell death. Therefore, p53 may be a better therapeutic target [64].

Nonetheless, the use of intrabodies and small molecule drugs represents innovative approaches in cancer treatment, offering distinct alternatives for precision therapy.

Immunotherapeutic approach

Immunotherapy has been in the spotlight in cancer treatment, boasting a diverse array of specific modalities. Immune checkpoint inhibitors and chimeric antigen receptor T-cell immunotherapy (CAR-T) are prominent strategies in clinical practice. Immune checkpoint inhibitors, such as PD-1 and PD-L1 inhibitors, work by disrupting immune checkpoints to rejuvenate T-cell activity, thereby boosting anti-tumor immune responses. Nevertheless, the complex immune evasion mechanisms of tumor cells may render immune checkpoint inhibitors ineffective. CAR-T therapy [65] utilizes genetic engineering to modify patients' T-cells, making them specifically recognize and eradicate tumor cells. While promising, CAR-T therapy is associated with high costs and limited success rates in solid tumors. In order to make up for the shortcomings of the above treatments and expand the coverage of immunotherapy, therapeutic vaccines stand out due to their specificity, flexibility, and low cost [66–68].

Therapeutic vaccines are designed based on the antigen presentation process of tumor cells. In tumor cells, mutated proteins within tumor cells can be processed into short peptides and bind to HLA-I molecules, forming complexes that are expressed on the cell surface. CD8 + T cells are activated by these complexes and kill tumor cells expressing the corresponding antigen. Therapeutic vaccines are delivered into the body in the form of nucleic acids or peptides, which results in the increased presence of tumor proteins or peptides within the body, thereby promoting the recognition and expansion of T cells (Fig. 2). In HPV-positive cancers, therapeutic tumor vaccines typically contain specific antigens of HPV. Vaccines encoding optimized E6 [69], E7 [70], and E2 [71] have been proposed. Considering that E6 and E7 themselves are oncogenes, the potential carcinogenicity of using them as mRNA vaccines remains to be discussed. Since the E2 proteins perform as a repressor of E6 and E7 oncogene [72], it seems to be a better choice. In 2014 and 2021, two nucleic-acid vaccine candidates named MAV E2 [71, 73, 74] and VGX-3100 [75, 76] completed phase III clinical trials, encoding modified E2, E6 + E7 respectively. Pitifully, their clinical efficacy both proved modest in treating HPV-positive cancers. Further improvement is still needed to enhance tissue specificity and explore more targets [77].

To explore more effective targets, it is needed to precisely identify antigenic epitopes, and such approach is the identification of tumor-specific antigens (TSAs) [78]. TSAs are antigens expressed only on tumor cells and at deficient levels on healthy tissues. In the context of HPV + cancer, the infection of HPV serves as the foundation for the generation of TSA. Antigenic peptides derived from HPV proteins are inherently absent in normal somatic cells, thus theoretically reducing the likelihood of eliciting autoimmune reactions when targeted for therapeutic purposes. Once TSAs are identified, the therapeutic vaccine can follow to amplifying TSA signals and summon T cell reaction[85]. An ongoing clinical trial, NCT05061940, also tried to assess the patient's TSA and/or HPV-16 E6/E7 expression profiles. Based on the results, it will be determined if a patient is eligible for multi-antigen cytokine-enhanced T cell therapy. However, the potential interference of E5 in antigen peptide presentation may counteract the amplification of TSA peptides. E5 is reported to retain HLA in the endoplasmic reticulum [9, 10]. The mechanism can result in little viral antigen expression in tumor cells and inactivation of immune cells, limiting the clinic use of immunotherapy in HPV-positive patients [79] (Fig. 3). However, according to the 'missing self' hypothesis, E5-induced downregulation of HLA I should be offset by the NK cells' elimination [80], which is not seen in HPV-positive cancer. It indicates that immune evasion in HPV-positive cancers involves complicated and unknown mechanisms, which makes blocking E5 a doubtable option. Nevertheless, inhibiting E5 represents a possible approach that can be employed independently or in combination with other strategies, and has already been used in preclinical studies [81].

Immunotherapy is one of the crucial directions in cancer treatment, and the integration of therapeutic vaccines with TSA is poised to inject fresh vitality into this field of immunotherapy. Currently, there is a growing interest in the identification of TSA serves as a cornerstone for mRNA therapies. However, progress in TSA identification is hampered by various challenges, primarily cost constraints. If more effective methods for identifying neoantigens can be found, immunotherapy for HPV-positive cancer could take a significant step forward.

Indirect targeting strategy in HPV-positive cancers

Given the challenges of directly targeting key HPV molecules, we can shift our attention to interfering with crucial stages of the HPV lifecycle to indirectly inhibit its ability to induce malignant transformation. Indirect targeting does not directly interact with HPV-specific molecules but instead influences or modulates biological processes or signaling networks associated with carcinogenesis. In HPV-positive cancer, this approach may involve targeting the process by which HPV hijacks the host's transcriptional regulatory system or transcription factors and signaling pathways to regulate cellular signaling or gene expression, aiming to block gene expression

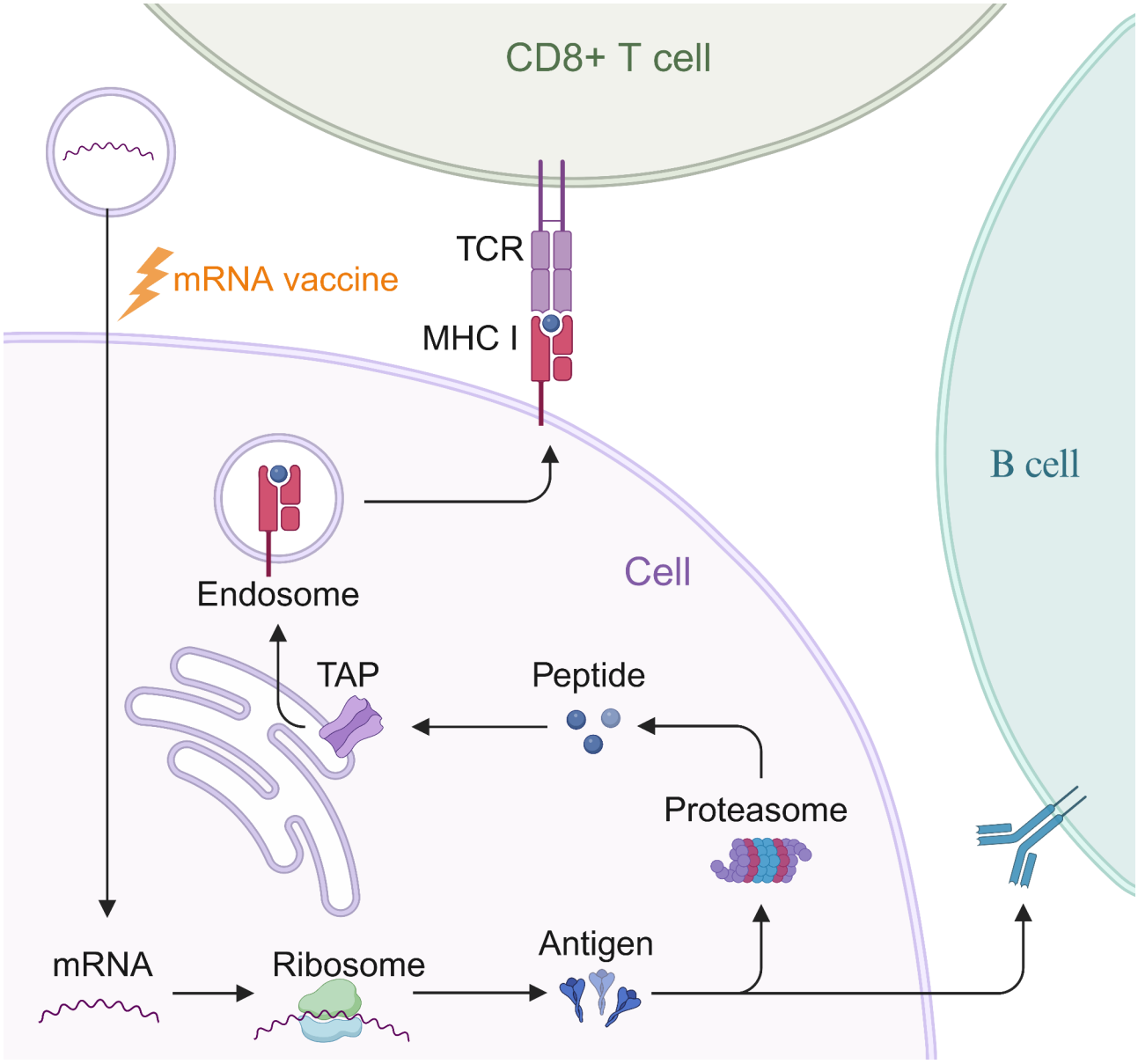

Fig. 2 Mechanism of therapeutic mRNA vaccine against tumors. The mRNA is translated into the specific antigen proteins by ribosomes within the cell. These proteins are then processed and presented on the cell surface, along with MHC molecules to activate T cells of the adaptive immune system. Additionally, the vaccine can also stimulate the production of antibodies by B cells, further bolstering the immune response against tumors. Created in BioRender.com

at the regulatory level, thereby inhibiting tumor growth or metastasis.

Target transcriptional factors for precision treatment

As HPV heavily depends on the proteins and factors within the host cell to support its reproduction, many TFs have been hijacked to bind to the LCR, regulating the expression of downstream oncogenes. Research has investigated the possibility of inhibition of some TFs and explored the therapeutic potential. Various TFs have been proposed as potential targets. Activator protein 1 (AP-1) [82], Nuclear factor κB (NF-κB) [83], YY1 [84, 85], and the signal transducer and activator of the transcription (STAT) family [86, 87] etc. are all shown to be hijacked during HPV infection and can be interrupted through certain drugs (Fig. 4). A few experiments were carried to the next stage, in vivo experiments, but the outcomes were not satisfying [88–92]. In addition, most of these studies have not revealed the detailed mechanisms through which these drugs modulate TFs. In all, achieving precision therapy through direct targeting of TFs is currently unattainable. The current challenge

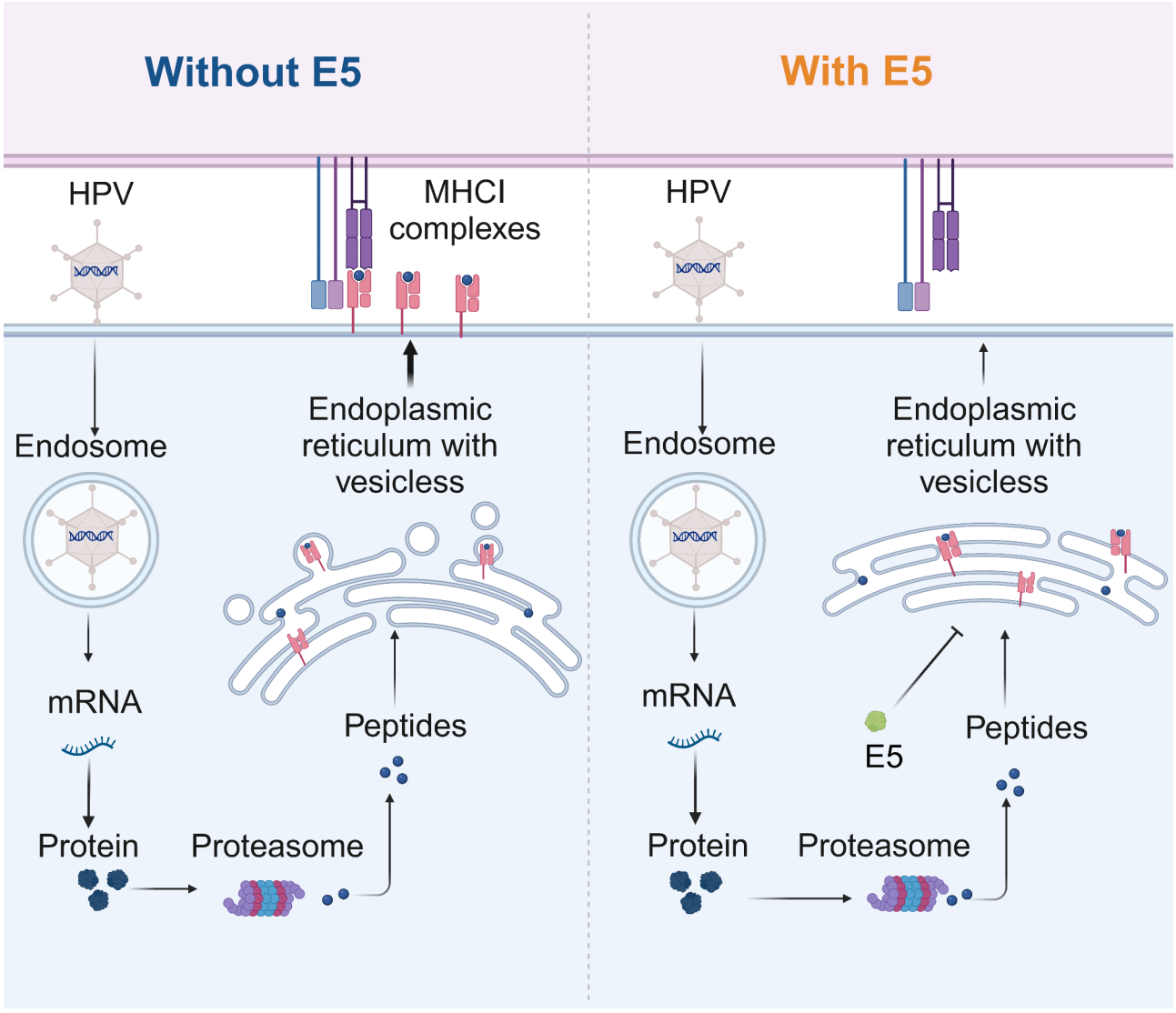

Fig. 3 Immune evasion leads by HPV E5 protein. Without E5, the antigens are proteolytically degraded to antigenic peptides, which bind to MHC in the endoplasmic reticulum to become the MHC complex and are delivered to the cell membrane. However, in the presence of E5, MHC is retained in the endoplasmic reticulum. Created in BioRender.com

lies in the unclear understanding of the mechanisms by which TFs, through their interaction with the LCR, hijack host cellular activities, leading to the malignant proliferation of tumors. This implies that we do not yet know the significance of these TFs in the process of cancer development, thus it is not possible to identify ideal and effective targets. To achieve precision targeting through TFs, a comprehensive screening of TFs interacting with HPV LCR needs to be conducted, elucidating their roles and mechanisms to select suitable targets. Subsequent precision targeting can be achieved through methods such as small-molecule drugs or nanobodies. However, even so, there are inevitable challenges in targeting TFs. TFs often play indispensable roles in normal cells, regulating vital life processes, and interfering with crucial TFs may result in significant side effects. Therefore, targeting the binding sites of TFs on the LCR, disrupting HPV's hijacking of TFs, may serve as a better choice.

Target HPV cancer-dependent/preferred signaling pathway for precision treatment

As the understanding of the nature of carcinogenesis evolves, it becomes increasingly clear that the dysregulation of cellular signaling pathways leads to uncontrolled cell proliferation. The focus of drug development is shifting from traditional cytotoxic drugs to drugs that inhibit aberrant signaling pathways in tumor cells. Currently, drugs targeting abnormal signaling pathways are continuously being developed and approved. In instances where substantial aberrations in specific pathways in

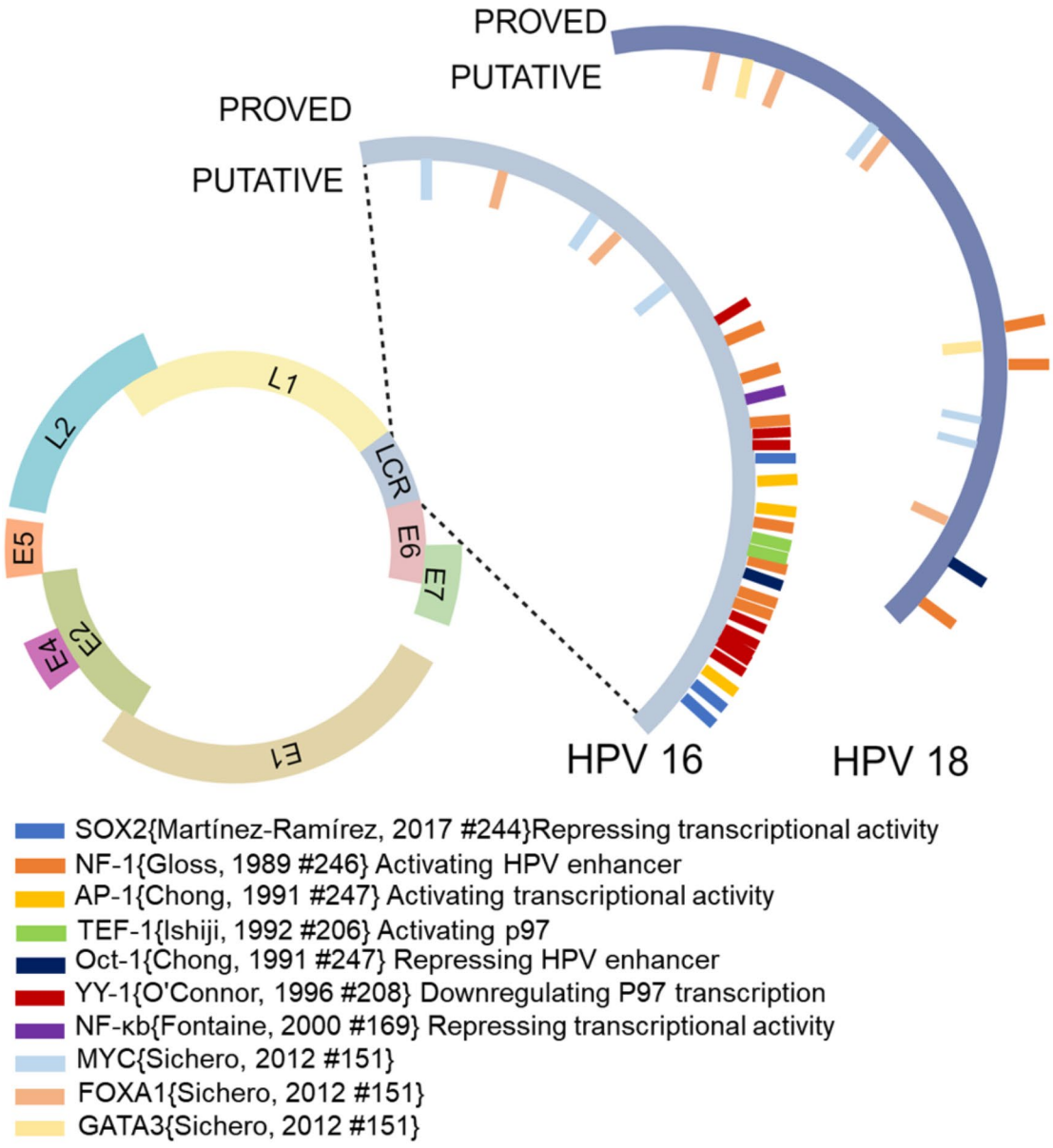

Fig. 4 TFs interacted with the LCR. Multiple TFs are proven or putative to interact with LCR of HPV16 and HPV18. YY-1, NF-1, NF-κb, SOX2, TEF-1, AP-1 and Oct-1 are proven to interact with LCR of HPV 16, while MYC and FOXA1 have putative interaction. For HPV 18, role of NF-1 and Oct-1 are proved and those of FOXA1, GATA3, MYC are putative

HPV-positive cancers, the existing signaling-targeted drugs can be directly employed for precision treatment. Analysis using The Cancer Genome Atlas database reveals that signaling pathways such as PI3K and EGFR are often aberrantly activated in HPV-positive tumors [93]. Therefore, there is potential to directly apply mature signaling pathway drugs to HPV-positive tumors for targeted treatment.

PI3K inhibitors

PI3KCA is reported to have mutations in 56% of tumors in HPV-positive HNSCC [93], comparing to HPV-negative which is around 18%. PI3K is a large family composed of lipid and serine/threonine kinases, including several phosphoinositide kinases and DNA-dependent protein kinases. The PI3K-AKT-mTOR signaling pathway has become a promising anti-cancer treatment target, and its blockade has already been applied in breast cancer treatment [94]. Focusing on the PI3K/Akt/mTOR pathway, several experiments for relevant agents are undergoing, such as the pan-PI3K pathway inhibitor Buparlisib/BKM120 and the dual inhibitor targeting the catalytic sites of PI3K and mTOR, Omipalisib/GSK2126458 [95]. Hideyuki Takahashi and colleagues

[96] demonstrated AKT3's importance in the prognosis of HNSCC and it could be studied as a therapeutic target. Given the successful application of PI3K inhibitors in breast cancer and the prevalent mutations in the PI3K pathway observed in HPV + cancers, there is considerable potential for PI3K inhibitors to serve as a therapeutic option for HPV + cancers. The mechanisms by which HPV induces mutations in the PI3K signaling pathway are still not fully understood. Further exploration of these mechanisms holds promise to unlock additional possibilities for the therapeutic application of PI3K signaling pathway in HPV-related diseases.

EGFR inhibitors

Receptor tyrosine kinase variations also exist in HPV-positive tumors, with EGFR [97], DDR2, VEGFR, and ERBB2 being proved in HPV-positive tumors, with protein tyrosine kinases regulating a series of physiological and biochemical processes such as cell growth, differentiation, and apoptosis, closely related to tumor initiation and progression [93]. Different tyrosine kinases belonging to other families are being screened as targets for anti-cancer drug development, including the EGFR, vascular endothelial growth factor receptor, and more. Aiming at the EFGR pathway, Cetuximab, a compound competing with ligands in the extracellular domain and blocking EGFR activation, has been the most accepted targeting treatment for HPV-associated cancer so far. Other medicines like nilotinib, dasatinib, erlotinib, and gefitinib also seem to downregulate EGFR and VEGFR-2 expression in vitro [98]. In addition, intracellular ATP-competitive small molecular inhibitors have also been proven effective [99]. Nevertheless, EGFR exhibits insufficient specificity for HPV-positive patients. On one hand, the occurrence and progression of OPC-HPV + may be closely related to E6 and E7. On the other hand, patients have a relatively high detection rate of mutations in the PI3K pathway. Therefore, targeted therapy can be approached from these two pathways.

Combined inhibitors

Combined inhibition by drugs such as multi-kinase inhibitors may be more suitable for experimentation in HPV-positive HNSCC. Given the complexity of tumor initiation and development, where most tumors do not rely on a single signaling pathway to sustain their growth and survival, there are cross-interactions and compensatory effects between different signaling pathways. Combined inhibition drugs can achieve synergistic treatment and overcome resistance by inhibiting multiple signaling pathways or multiple molecules in a single pathway. This concept has gained convincing clinical evidence, with two multi-target small molecule compounds, sunitinib [100] and sorafenib [101], recently approved by the FDA for monotherapy in renal cancer. However, increased toxicity associated with blocking multiple pathways also pose a problem.

In summary, drug development based on pathways still facing severe challenges. The pathways of signals are intricate, with close connections between various signaling pathways. Currently, the understanding of signal pathways is not clear, posing difficulties in selecting targets. Additionally, most signal pathways are involved in essential life activities of normal cells, and disrupting these pathways may lead to unpredictable side effects. Therefore, the selection of targets becomes crucial, yet effective targets are actually quite limited. The signal pathways abnormally activated in HPV-positive tumor cells are not well understood. To achieve direct clinical translation, it is still most important to elucidate the specific mechanisms by which signaling pathways regulate tumor proliferation in HPV-positive cases. However, given the complexity of signal pathways themselves, precise treatment based on these pathways is extremely challenging.

Summary and future directions

The escalating prevalence of HPV-related cancers has emerged as a global concern. Alongside the toxic side effects and functional impairment of corresponding organs resulting from the non-specificity of current treatment modalities, the imperative for efficacious treatment alternatives is evident. Distinct molecular mechanisms underlie cancer development in HPV-infected and non-infected tumors, offering specific avenues for breakthrough treatments. Nevertheless, current approach still harbors its own limitations and is still far away from clinical use. In the context of precision-targeted therapy for HPV-positive tumors, we propose several promising directions.

Firstly, the continuous development of delivery systems and chemical modifications have made nucleic acid drugs a hopeful candidate. In addition, the substantial differences between the viral DNA and the human genome provide the foundation for low off-target effects in gene-silencing therapy. In terms of target selection, besides classical targets such as the E6 and E7 genes, the virus's E2, E5 genes, and LCR could also serve as potential candidate targets. To explore more potential target, identifying TSAs may open the door for effective immunotherapy or vaccines. In the future, extensive experiments are still required to explore effective antisense nucleotide sequences and modification methods for therapeutic purposes.

Secondly, directly interfering with key proteins of the HPV virus is also a rational approach. While synthesizing corresponding antibodies is one of the most direct methods, the complexity in development and the risk of

triggering abnormal immune responses limit its application. With the advent of accurate protein structure prediction, we can now more precisely synthesize small molecule drugs targeting specific domains of oncoproteins to hinder their functions. However, due to the relatively small molecular size of HPV oncoproteins, direct targeting poses challenges. Therefore, interfering with oncoproteins forming functional complexes (such as E6-E6AP) is a feasible approach. As protein structure analysis and prediction become a reality, future research should further unveil the proteins interacting with oncoproteins and their significance in the development of cancer, facilitating the selection of appropriate targets.

Thirdly, amplifying aberrant antigens for cancer treatment is a cutting-edge strategy in immunotherapy. Aberrant antigens expressed in tumor cells but not in normal cells or at lower levels, can serve as potential target, triggering immune attacks on cancer cells. Several approaches aim to amplify aberrant antigens for cancer treatment. Personalized cancer vaccines, carrying tumor-specific antigens can stimulate the patient's immune system. CAR-T cell therapy involves extracting T cells from a patient, genetically modifying or transfecting them to express chimeric antigen receptors specific to tumor surface aberrant antigens. After activation and expansion, these CAR-T cells are reintroduced into the patient to combat cancer cells. These strategies are particular suitable for HPV-positive cancers in theory since viral infection stimulates exogenous antigen-processing. However, it is challenge to identify proper tumor antigens due to immune evasion of tumor cells, considerable cost and low yield of antigen-separation method.

Lastly, the indirect targeting approach, specifically targeting HPV-related TFs and signaling pathways, also holds some feasibility. However, due to the complexity of the relevant molecular mechanisms, research on TF and signaling pathways lacks clear theoretical foundations. In HPV-positive tumors, identified targets in the proposed abnormal pathways are currently scarce. Consequently, indirect targeting is currently significantly constrained.

In conclusion, recent advances in the directions we have mentioned have paved the way for innovative treatments in HPV-positive cancers. Building upon refining delivery systems for gene-silencing therapies, developing inhibitors that disrupt HPV oncoproteins, amplifying aberrant antigens for immune attack, and indirectly targeting HPV-associated transcription factors and signaling pathway, it is hopeful that precision therapy for HPV-positive tumors becomes a plausible prospect in the future.

Author contributions

Yixi Huang, Jiayi Wang, Wenbin Yang conceptualized and wrote the main manuscript text and prepared Figs. 1, 2, 3 and 4. Feifei Hou and Xiaodong Feng conceptualized, reviewed end edited the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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