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HPV-driven cancers: a looming threat and the potential of CRISPR/Cas9 for targeted therapy

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Abstract

Cervical and other anogenital malignancies are largely caused by E6 and E7 oncogenes of high-risk human papillomaviruses (HPVs), which inhibit important tumor suppressors like p53 and pRb when they are persistently activated. The main goal of traditional treatments is to physically or chemically kill cancer cells, but they frequently only offer temporary relief, have serious side effects, and have a high risk of recurrence. Exploring the efficacy and accuracy of CRISPR-Cas9 gene editing in both inducing death in HPV-infected cancer cells and restoring the activity of tumor suppressors is our main goal. In this study, we propose a novel precision oncology strategy that targets and inhibits the detrimental effects of the E6 and E7 oncogenes using the CRISPR-Cas9 gene editing system. In order to do this, we create unique guide RNAs that target the integrated HPV DNA and reactivate p53 and pRb. Reactivation is meant to halt aberrant cell development and restart the cell's natural dying pathways. This review discusses the potential of CRISPR/Cas9 in targeting HPV oncogenes, with a focus on studies that have demonstrated its promise in cancer treatment. Given the absence of a definitive treatment for papillomavirus infection and its subsequent association with various cancers, future clinical trials and experimental investigations appear essential to establish and evaluate the therapeutic potential of CRISPR-based approaches. This approach provides a less invasive alternative to conventional treatments and opens the door to personalized care that considers the genetic makeup of each patient's tumor.

Keywords HPV, CRISPR-Cas9, Gene editing, Oncogenes, Tumor suppressors

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Introduction

Numerous human malignancies, most notably cervical cancer, have been associated with high-risk human papillomavirus (HPV) infection [1]. The E6 and E7 oncoproteins, which are persistently expressed by these viruses and interfere with important tumor suppressors, including p53 and retinoblastoma protein (pRb), cause unchecked cell division and malignant transformation [2]. Most ano-genital HPV infection treatments available today are ablative and/or cytodestructive. Cryotherapy, scissor excision, laser therapy, and electrosurgery are examples of alternative treatment options for genital warts. In the short term, physically ablative therapies like cryotherapy are frequently quite effective, with clearances of 70–80%; nevertheless, even after several treatments, recurrence rates might reach 25–39% [3]. Although preventative vaccinations are effective, they do not completely eradicate pre-existing infections, and current therapies like chemotherapy, radiation, and surgery are typically invasive and non-specific; therefore, new, focused approaches are needed to fight HPV-driven cancers [4]. Several novel therapeutic strategies have emerged in recent years with the goal of directly targeting HPV-infected cells while limiting damage to healthy tissues. Immunotherapy, including therapeutic vaccinations and immune checkpoint inhibitors, has shown potential for boosting the immune response against HPV-driven malignancies [5]. Antisense oligonucleotides and small interfering RNAs (siRNAs) are examples of RNA-based treatments that provide gene-silencing methods to inhibit the expression of viral oncogenes [6]. Based on these findings, patients with HPV-driven malignancies may benefit from RNA-based approaches in terms of prognosis and response to treatment. However, it should be challenging to knock down ncRNAs utilizing RNA-based methods because many of them are found in the nucleus [6]. Moreover, epigenetic treatment is being investigated as a possible strategy to combat HPV oncogenesis since it modifies gene expression by altering DNA methylation and histone changes [7]. The CRISPR-Cas9 genome editing system has become a ground-breaking tool for precise gene targeting, providing a promising therapeutic approach for cancers linked to HPV [8]. CRISPR-Cas9 can disrupt the expression of the E6 and E7 genes by introducing targeted DNA double-strand breaks, which restore p53 and pRb function, initiate apoptosis, and stop tumor growth [9]. This study investigates how CRISPR-Cas9 technology, which inactivates HPV oncogenes, particularly E6 and E7, may be used as a targeted treatment strategy for HPV-associated malignancies. By examining the restoration of tumor suppressor functions (p53 and pRb), this research assesses the efficacy, difficulties, and potential uses of CRISPR-Cas9 technology in HPV treatment.

Silencing HPV oncogenes

Cervical cancer is among the four most common cancer diagnosed in women worldwide. The existence of HPV infection is the main reason for this neoplasm to progress and become apparent [10]. As of now, over 400 distinct types of HPV have been recognized [11]. HR-HPVs are linked to over 90% of cervical cancer cases, as well as being associated, albeit to a lesser degree, with other anogenital cancers and cancers of the head and neck [12]. Throughout the process of cancer development associated with HR-HPV, the viral DNA is often integrated into the chromosomes of host cells, and the proteins produced by the viral genes are essential contributors to the process of carcinogenesis. When a host is infected, the majority of HPV strains are eliminated within a few months; however, certain strains can persist over time. These persistent HPVs continuously express viral oncogenes that inhibit the function of tumor suppressor proteins p53 and Rb. This inhibition results in heightened genomic instability, the accumulation of somatic mutations, and in certain instances, the integration of HPV into the host's genome. Unlike stromal cells, HR-HPVs are located inside tumor cells, where they are the only cells that carry the viral oncogenes E6 and E7 [13]. The E1 and E2 proteins of HPV function as essential components that identify the replication origin, while the E4 and E5 proteins are thought to play roles in the viral life cycle. The E6 and E7 proteins serve as the primary oncoproteins associated with HPV. A significant mechanism by which the HPV E6 protein contributes to carcinogenesis involves the promotion of p53 degradation and the activation of telomerase, which subsequently leads to the down-regulation of genes that inhibit apoptosis [14]. The E6 protein, along with other significant oncogenic kinases like the epidermal growth factor receptor (EGFR) and tyrosine-protein kinase Met, may work together to enhance epigenetic regulation. Conversely, the HPV E7 protein plays a role in multiple signaling pathways, including the disruption of the retinoblastoma protein (pRb) and the cell cycle-dependent kinase inhibitor p21 [15, 16]. Alongside pRb, p21 is essential for controlling a number of epithelial processes and has unique roles as a tumor suppressor [17]. The E7 proteins associated with HR-HPV types, including HPV16 and HPV18, exhibit a significantly greater affinity for binding to the pRb. One of pRb's most important biochemical roles is its interaction with transcription factors of the E2F family, which mostly operate as repressors of replication enzyme-related gene expression. The tumor-suppressive characteristics of pRb are intimately associated with this interaction [18]. A recent investigation revealed that throughout the infectious phase, the HPV16 genome

exhibited a greater number of amino acid-altering variants, whereas the E7 protein remained genetically highly conserved [19].

The ongoing activity of viral oncoproteins E6 and E7 leads to increased instability in the genome, which contributes to the accumulation of mutations. This process ultimately disrupts the regulation of cell growth, paving the way for the development of cancer [20]. The persistent presence of E6 and E7, resulting from the integration of the viral genome into the host chromosome, is a defining characteristic of cervical cancer [21]. The exploration of E6 and E7 proteins as therapeutic targets has led to the investigation of small-molecule drugs, peptides, and therapeutic vaccines for the targeted treatment of cervical cancer [22]. Using targeted mutagenesis to permanently deactivate the viral E6 and/or E7 genes is an alternate method of treating HPV-related cancers. This technique is expected to result in the removal of HPV-transformed cells [21].

The study that employed the CRISPR/Cas system to target and cleave the E7 oncogene in HPV16-positive cervical cancer cell lines, demonstrated that the mutations induced by the CRISPR/Cas mechanism triggered apoptosis and inhibited cell growth specifically in HPV16-positive cells, with no observable effects on HPV16-negative cells. The disruption of the E7 gene and the subsequent elimination of the E7 oncoprotein led to the restoration of tumor suppressor pRb expression. These findings suggest that the HPV16-E7 gRNA-guided CRISPR/Cas approach holds promise as a potential therapeutic strategy for cervical cancer treatment [23]. Recent studies have further explored the application of CRISPR/Cas9 to target the promoter and open reading frame (ORF) of E6/E7 transcripts. This intervention resulted in reduced levels of E6 and E7 mRNA, increased P53 protein levels, decreased pRb levels, and enhanced apoptosis, alongside the inhibition of SiHa cell growth. Additionally, CRISPR/Cas9-transfected cells exhibited reduced growth in in vivo models, underscoring the therapeutic potential of this approach [23].

The CRISPR/Cas9 system has also been engineered to specifically target the E6/E7 mRNA of HPV16 and HPV18. Intertumoral administration of this system significantly reduced tumor growth and induced apoptosis in vivo, highlighting its potential as an adjuvant therapy for cervical cancer [8]. "In vitro experiments and mechanistic evidence strongly suggest that direct delivery of the CRISPR/Cas9 system to HPV-positive tumors (such as through intratumoral administration) could represent an effective strategy for suppressing tumor growth and inducing apoptosis. While the available data remain limited to cellular models and no in vivo intratumoral injection was performed, these findings provide a solid rationale for the future development of direct injection-based therapeutic approache [23]. Preclinical data have shown that intratumoral administration of CRISPR/Cas9 targeting the E7 oncogene effectively suppressed tumor growth and induced apoptosis in HPV-positive tumor models in mice [24]. Studies and clinical trials conducted so far have shown that intratumoral injection can effectively deliver CRISPR-Cas9 components to target cells within the tumor, improving the precision of the therapy and potentially minimizing off-target effects. However, the clinical translation of this method is still in early stages, and there are concerns about the potential risks of localized toxicity or immune reactions [25, 26]. Intratumoral administration, has shown promise in preclinical and clinical studies, particularly for localized cancers. It offers several advantages, such as high local concentrations of therapeutic agents, reduced systemic side effects, and the potential for more efficient targeting of the tumor [27]. However, in clinical settings, several challenges need to be addressed. One key issue is the tumor's heterogeneity, which can affect the distribution of the administered agent within the tumor. Tumors with poor vascularization or necrotic regions may be less responsive to intratumoral delivery, limiting its efficacy [28]. Additionally, the technical difficulty of delivering therapeutic agents precisely to deep or inaccessible tumors remains a challenge [29]. Despite these hurdles, intratumoral administration has been successfully used in some clinical trials for cancer immunotherapy and gene therapy. For example, intratumoral delivery of oncolytic viruses and immune checkpoint inhibitors has demonstrated encouraging results in melanoma and other skin cancers [30]. Although no large-scale clinical trials have yet validated the direct intratumoral delivery of CRISPR/Cas systems for cancer treatment, early-stage data remain highly promising [31]. In a related study, researchers developed several single-guide RNAs (sgRNAs) targeting the promoters and transcripts of the E6 and E7 oncogenes. This approach led to a marked increase in the expression levels of p53 and pRb, further supporting the therapeutic efficacy of CRISPR/Cas9 in cervical cancer [24]. In a notable in vivo study, systemic administration of E6 and E7 sgRNA via a CRISPR/Cas9 vector in mice demonstrated effective induction of tumor cell death through apoptosis. This study provided critical insights into the post-gene editing events within tumors, reinforcing the potential of CRISPR/Cas9 as a therapeutic tool [32]. Collectively, these findings indicate that genome editing methods targeting oncogenes hold significant clinical promise for cancer treatment. However, further investigation is necessary to fully establish their safety and effectiveness in clinical settings.

Crispr's role in cancer therapy

One of the most difficult problems facing contemporary medicine is cancer, which continues to be a major source of illness and mortality globally. A new era of hope for patients has been brought about by the quick advancements in precision oncology, which emphasize focused therapies that target certain cancer molecular pathways while causing the least amount of harm to healthy tissues. CRISPR-Cas9 technology, an extremely accurate DNA-editing instrument that has completely changed how we think about and treat cancer, is in the front of this change. The next phase of tailored cancer treatment might be driven by CRISPR when paired with other innovative technologies like AI-driven systems and PROTACs (PROteolysis Targeting Chimeras) [33]. Cancer research has placed a strong emphasis on gene therapy because to the crucial role that epigenetic and genetic modifications in oncogenes and tumor-suppressor genes play in the development of cancer. Unlike traditional treatments such as chemotherapy, gene therapy is associated with fewer side effects. Moreover, while conventional gene therapy methods often produce only transient results, advanced genetic modification tools like CRISPR-Cas9 offer the potential for durable and curative outcomes. In recent years, CRISPR-Cas9 has been effectively used in numerous research studies to alter gene expression in cells. This system enables site-specific mutagenesis and epigenetic modifications, providing precise control over gene regulation. Decades of research on genome-editing techniques have allowed for accurate modifications of target DNA sequences at the cellular level, deepening our understanding of the roles various genes play in tumor development. As a result, CRISPR-Cas9 has emerged as a state-of-the-art and highly efficient tool for treating a wide range of genetic disorders, particularly cancers [34]. In addition to its function in gene editing, CRISPR-Cas9 has demonstrated great promise for improving adoptive cell therapies, including treatments using chimeric antigen receptor (CAR) T cells. The effectiveness of these treatments can be increased by using CRISPR to edit inhibitory genes, which can boost immune responses [35]. CRISPR-Cas9 is used for much more than only altering immune cells. This technique is now being used to study the processes behind drug resistance, enable real-time gene expression manipulation, and change the tumor microenvironment [36].

The advent of CRISPR-Cas9 systems has revolutionized genome manipulation, significantly enhancing its efficiency and precision. In cancer gene therapy, CRISPR-Cas9's ability to induce somatic mutations within cells has enabled the identification of novel therapeutic targets. By delivering a combination of Cas9 and various sgRNAs, researchers can achieve multiple genetic alterations simultaneously in tumor cells, offering a powerful tool for studying and treating cancer [37]. The CRISPR system can be utilized to identify mutations that contribute to therapy resistance in cancer. An instance of this is the identification of NAMPT, which encodes nicotinamide phosphoribosyl transferase and serves as the target for the anticancer agent KPT-9274, made possible through CRISPR technology [38]. CRISPR technology is renowned for its exceptional capability to precisely target oncogenic mutations; however, it faces several challenges. One significant concern is the occurrence of off-target effects, which alter unexpected regions of the genome and lead to serious issues, especially when long-term therapies are involved. Additional challenges include immunological reactions to CRISPR components and chromosomal rearrangements. However, cutting-edge methods like base editing and prime editing are becoming more popular and offer more complex methods for precise DNA alterations. These advancements show promise in reducing off-target effects and increasing gene edit specificity, which will improve the safety and efficacy of CRISPR-based treatments in clinical settings [35]. Recent studies have shown that CRISPR-Cas9 has been trialed in various cancers. In cervical cancer, CRISPR was used to target HPV16/18 E6 and E7 oncogenes, restoring p53 and pRb functions [8]. In non-small cell lung cancer (NSCLC), CRISPR was applied to edit PD-1 in immune cells to enhance response to checkpoint blockade therapy [39]. Additionally, hematological malignancies such as leukemia have utilized CRISPR to target CD19 and CD22 genes, improving CAR-T cell therapy effectiveness [40].

CRISPR-Cas9 technology offers a groundbreaking approach for the genetic identification and treatment of various cancers associated with HPV. This method boasts several advantages over conventional techniques, including ease of design, user-friendliness, and high editing efficiency, making it a promising avenue for medical applications. Nevertheless, comprehensive gene therapy strategies utilizing CRISPR-Cas9 remain largely experimental, with concerns regarding off-target effects and other safety risks. Overall, the innovative genetic treatment for HPV-related cancers is expected to advance significantly with the continued development of CRISPR-Cas9 technology [41]. In summary, CRISPR-Cas9 has profoundly transformed precision oncology, offering unprecedented opportunities for the genetic targeting of cancer. When integrated with complementary technologies such as PROTACs and AI-driven advancements, CRISPR-Cas9 is poised to unlock new possibilities in personalized cancer treatment [42]. (Table 1)

Table 1 CRISPR/Cas9 applications in HPV-Related cancer

Application	Findings/Outcomes	Ref
Targeting E7 Oncogene	Induces apoptosis in HPV16-positive cells; no effect on HPV16-negative cells	[23]
Effect on Tumor Suppressor Expression	Restoration of pRb expression upon disruption of E7	[9]
Reduction of E6/E7 mRNA Levels	Increased p53 levels, decreased pRb levels, promotion of apoptosis	[23]
In Vivo Tumor Growth Reduction	Significant tumor reduction when CRISPR/Cas9 is administered intratumorally	[8]
sgRNA Development	Increased expression levels of p53 and pRb through targeting E6/E7 promoters	[24]
Induction of Cell Death	Effective induction of apoptosis following systemic administration of sgRNA via CRISPR/Cas9 vector	[32]

Precision therapy for HPV related cancer

Cervical cancer is primarily linked to the ongoing infection caused by high-risk strains of HPV [43]. It has been established that persistent infection with high-risk types of HPV, particularly HPV types 16 and 18, is responsible for 90% of cervical cancer cases [22]. Extensive research has established that the viral oncogenes E6 and E7 play critical roles in the development and progression of cervical cancer. These genes exhibit oncogenic properties and are essential for maintaining the malignant phenotype of cancer cells [14, 44]. Accurate and timely detection of HPV infections is crucial for effective disease management and the prevention of HPV-related malignancies [45]. For the treatment of cervical precancerous lesions caused by oncogenic HPV, several surgical interventions are recommended. These include excisional procedures performed under local anesthesia, cryosurgery (freezing techniques), electrosurgical methods such as cone biopsy (conization) and loop electrosurgical excision procedure (LEEP), and laser therapy [46]. In addition to surgical options, the HPV vaccination initiative has been expanded to mitigate the increasing prevalence of malignancies linked to HPV, especially cervical cancer. Gardasil, Gardasil 9 (Merck Sharp & Dohme-MSD), and Cervarix (GlaxoSmithKline-GSK) are the three preventive vaccinations that have FDA approval. These vaccines are almost 100% effective at preventing cervical cancer owing to their composition of virus-like particles (VLPs) produced from the L1 protein via recombinant technology [47]. Current therapeutic approaches for cervical cancer include surgical interventions, adjuvant or neoadjuvant chemotherapy combined with radiation therapy, and total or radical hysterectomy [48]. Treatment strategies are tailored based on the disease stage, tumor growth patterns, and patient characteristics. Options may include radiation therapy (RT), chemotherapy, or a combination of both, either independently or alongside surgical procedures [49, 50]. For early-stage disease, modified radical hysterectomy (extra-fascial hysterectomy) is the preferred treatment. However, this approach is invasive and carries the risk of infertility [51]. In patients with comorbid conditions or functional challenges, radiotherapy may be a more suitable alternative to surgery [52]. Radiotherapy is a cornerstone of cervical cancer management across all stages. Techniques include whole pelvic radiation therapy, brachytherapy, and intracavitary radiation. Radiotherapy is particularly beneficial for patients with stage 1 A disease who are unsuitable for surgical resection and for those with advanced tumors. However, it is not without side effects [53]. While chemotherapy alone has a limited role in cervical cancer treatment, its combination with radiotherapy is the preferred strategy for managing the disease [54]. Although HPV is the primary cause of nearly all cervical cancer cases, the presence of the virus alone is insufficient to cause malignancy. Cancer development typically requires a prolonged period and additional tumor-promoting mechanisms, particularly the evasion of immune surveillance [55]. Consequently, innovative approaches such as immunotherapy hold significant promise for addressing HPV-related carcinogenesis [56]. The advent of CRISPR technology has enabled targeted modification of the E6 and E7 genes in HPV types 6, 11, 16, and 18. Studies have demonstrated the antiviral efficacy of CRISPR/Cas9 in various in vitro cell cultures and xenograft models of HPV-positive tumors. Disruption of the E6 and E7 genes reduced their respective viral protein levels, restoring the function of tumor suppressor proteins p53 and pR [31, 57]. Furthermore, the synergistic effects of combining CRISPR/Cas9 with chemotherapeutic or radiotherapeutic agents could lead to novel treatment strategies and improve clinical outcomes for cervical cancer patients [9].

CRISPR and immunotherapy

Decades of comprehensive research on genome-editing methodologies have enabled precise modifications of specific DNA sequences within cells, significantly advancing our understanding of the roles various genes play in tumorigenesis. Among these methodologies, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system has emerged as a powerful tool for diagnosing next-generation pathogens, facilitating gene editing, advancing drug discovery, and supporting therapeutic applications [58]. The CRISPR-Cas system is a critical component of the adaptive immune response in prokaryotes, providing defense against invasive plasmids and viruses. This system relies on Cas, an endonuclease associated with an RNA molecule derived from CRISPR sequences. When exposed

to foreign DNA from bacteriophages or plasmids, the CRISPR-Cas system is activated, enabling the identification and elimination of these invasive molecules. In bacteria and archaea, the Cas protein, guided by sgRNA derived from CRISPR sequences, recognizes and targets foreign DNA for destruction [59]. CRISPR-Cas9, in particular, has become the leading technology for genome editing due to its precision and versatility. It functions as an adaptive defense mechanism in microorganisms and has been widely adopted for genetic manipulation [60]. CRISPR-Cas systems are broadly classified into two categories: class 1 and class 2. Class 1 systems utilize multiprotein effector complexes, while class 2 systems rely on a single effector protein that binds to sgRNA to cleave target DNA sequences. The CRISPR-Cas9 system, a class 2 system, consists of the Cas9 endonuclease and sgRNA, which includes CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). The tracrRNA facilitates the interaction between Cas9 and sgRNA, ensuring accurate targeting of specific genomic sequences [34]. Recent studies have demonstrated the efficacy of CRISPR-Cas9 in modifying genes across various cell types. This system has been employed for site-specific mutagenesis, gene expression modulation, epigenetic marker alteration, and diagnostic applications targeting specific DNA and RNA sequences [61]. In recent years, CRISPR-Cas has emerged as a highly promising instrument for antiviral treatment. This system consists of two primary elements: the crRNA, which is engineered to attach to the complementary sequence of the target DNA, and the Cas proteins that cut the target DNA, resulting in a double-stranded break that is subsequently repaired by the cell's DNA repair mechanisms. The double-stranded breaks induced by Cas proteins interfere with genes essential for the viral life cycle and replication, thus effectively inhibiting viral replication within the host cell [62]. The CRISPR-Cas system combats viral infections by directly targeting viral DNA or RNA [63](Fig. 1). The presence of these Cas proteins is essential for the proper functioning of the adaptive immune response [64]. Among the various Cas proteins, Cas1 and Cas2 are universally present across all types of CRISPR-Cas systems and play a crucial role in the integration of spacers [65, 66]. CRISPR-Cas-based techniques exhibit significant versatility, cost efficiency and do not necessitate advanced equipment or specialized knowledge. Nonetheless, there are ethical dilemmas and scientific constraints that must be resolved before their application as a therapeutic option for humans. A primary challenge lies in the delivery mechanism for introducing CRISPR-Cas into target cells. The selected delivery method must facilitate the transfer of substantial quantities of CRISPR-Cas proteins to the intended cells. Furthermore, there are limitations regarding the size of Cas proteins that can be effectively delivered using lentiviral vectors, adenoviruses, and adeno-associated viruses [67].

CRISPR's exceptional capability to precisely target oncogenic mutations is unmatched; however, it faces several challenges. Off-target effects, which involve the editing of unintended genomic regions, continue to be a major issue, especially in the context of long-term treatments. Furthermore, chromosomal rearrangements and immune responses to CRISPR components present additional obstacles. Nevertheless, innovative approaches like base editing and prime editing provide more sophisticated strategies for accurate DNA modifications [35]. Gene modification facilitated by Cas9 necessitates the stable delivery of CRISPR components. This can be achieved through the transfection of plasmid DNA that encodes either the Cas9 protein along with sgRNAs or the Cas9–sgRNA ribonucleoprotein complexes [68]. One major obstacle to the practical use of CRISPR-mediated genome editing is the possibility of unwanted and destructive mutations. A primary factor contributing to off-target effects is the insufficient specificity of gRNAs. Therefore, it is essential to account for genetic variations among individuals when designing gRNAs [69]. Due to ethical considerations and safety concerns, the direct application of CRISPR-Cas9 technology in the human body is currently deemed inappropriate. However, in vitro modifications of T cells, such as CAR-T and TIL therapies, can be performed, followed by reinfusion into patients. While preclinical studies have shown promising results and the effectiveness of CRISPR technology in cancer treatment, several challenges must be resolved before its use in human therapies (Table 2). These challenges include off-target effects, immune responses triggered by Cas9 proteins, and the selection of appropriate target cells, among others [70]. Despite the significant advancements in the treatment of hematological malignancies, a considerable number of patients have encountered challenges with CAR-T adoptive cell therapy. This is partly attributable to the immunosuppressive tumor microenvironment and T-cell exhaustion [71]. Due to the recognized function of co-inhibitory molecules, including PD-1, CTLA-4, LAG-3, and TIM-3, in the dysfunction of T cells, the CRISPR/Cas9 system has been utilized to target and disrupt these inhibitory genes to improve CAR-T cell efficacy. The depletion of PD-1 through CRISPR/Cas9 has been demonstrated to enhance the capacity of CAR-T cells to eliminate tumor cells in vitro and to eradicate PD-L1 + tumor xenografts in vivo [72]. The ablation of Diacylglycerol Kinase (DGK) in CAR-T cells, alongside the presence of co-inhibitory genes, leads to enhanced anti-tumor immunity [73] (Fig. 2). The deletion of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene has been shown to improve the functionality of CAR-T cells while also decreasing

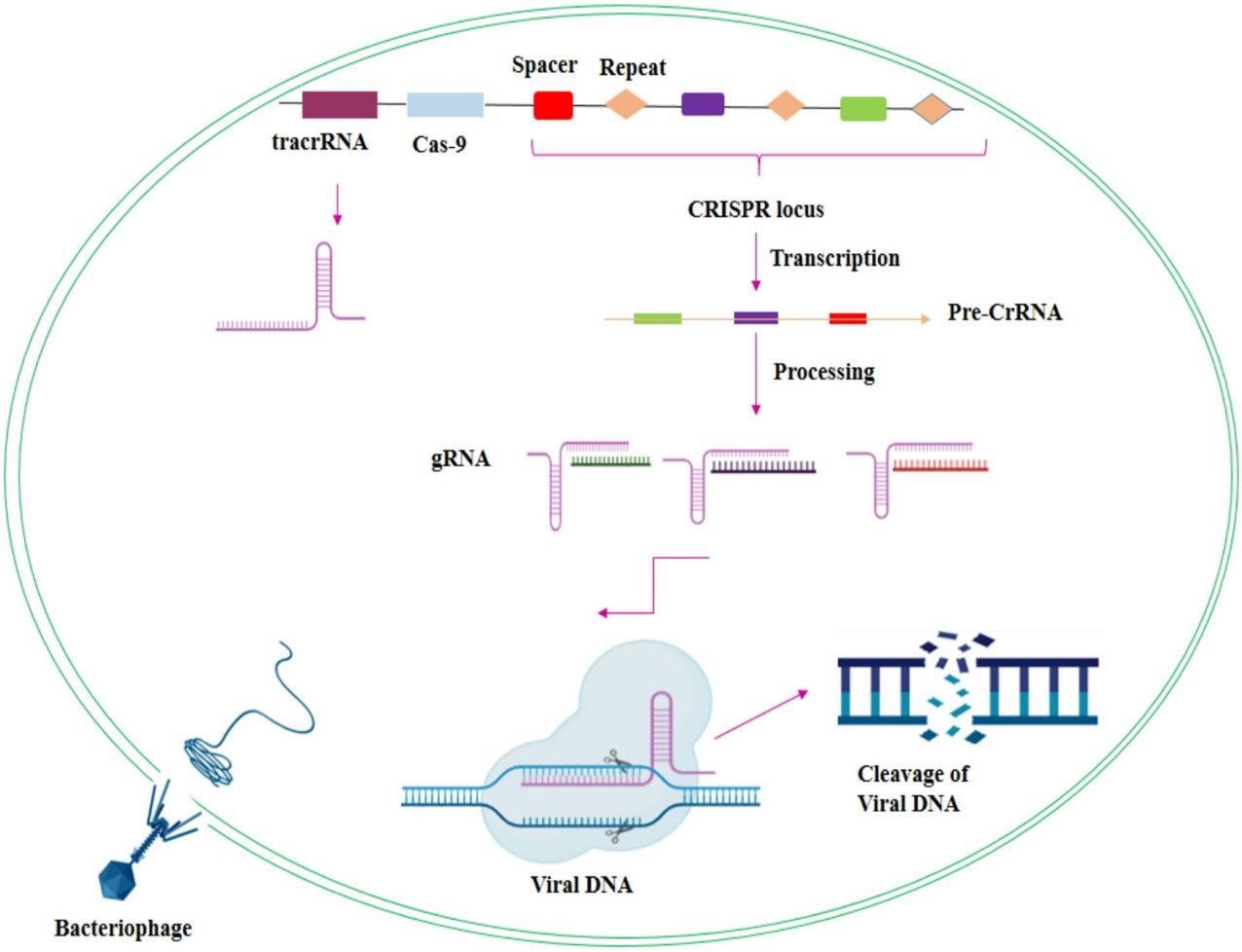

Fig. 1 Mechanism of CRISPR-Cas9 function in recognition and destruction of viral DNA. This picture shows the CRISPR-Cas9 immune mechanism in bacteria to fight against viral DNA (bacteriophage). First, the CRISPR locus including spacer and repeat sequences is located in the bacterial genome. After transcription and processing, a guide RNA (gRNA) is formed which binds to viral DNA together with Cas9 and cuts it. This process destroys the viral DNA and protects the bacteria against infection

Table 2 Challenges and future directions in CRISPR technology

Challenge	Description/Implications	Ref
Off-Target Effects	Unintended genome alterations raise concerns for long-term treatments	[35]
Chromosomal Rearrangements	Potential complications from unintended modifications	[35]
Immune Responses	Possible adverse reactions to CRISPR components	[35]
Innovative Approaches	Base editing and prime editing as solutions to enhance specificity and reduce off-target effects	[56]

the likelihood of cytokine release syndrome (CRS) and inflammation [74]. Research has demonstrated that utilizing CRISPR/Cas9 technology to disrupt the endogenous TGF-β receptor II (TGFBR2) in CAR-T cells can reduce the exhaustion of these cells and enhance their efficacy in targeting solid tumors, both in vitro and in vivo [75]. Additionally, the use of CRISPR/Cas9 to eliminate CD7 and TRAC in CAR-T cells has enhanced the effectiveness of treatment for T-cell acute lymphoblastic leukemia (T-ALL) [76]. The immune system is crucial for immunosurveillance, as both adaptive and innate immune cells penetrate the tumor microenvironment (TME) and influence the regulation of tumor progression [77]. Tumor suppression is greatly aided by innate immune cells, which include natural killer cells, eosinophils, basophils, and other phagocytic cells such mast cells, neutrophils, monocytes, macrophages, and dendritic cells (DCs). Either by directly destroying tumor cells or by triggering adaptive immune responses, they do this [78]. B cells and T cells exemplify the lymphocytes constituting the adaptive immune system. While T cells are crucial for cell-mediated immune responses, B cells

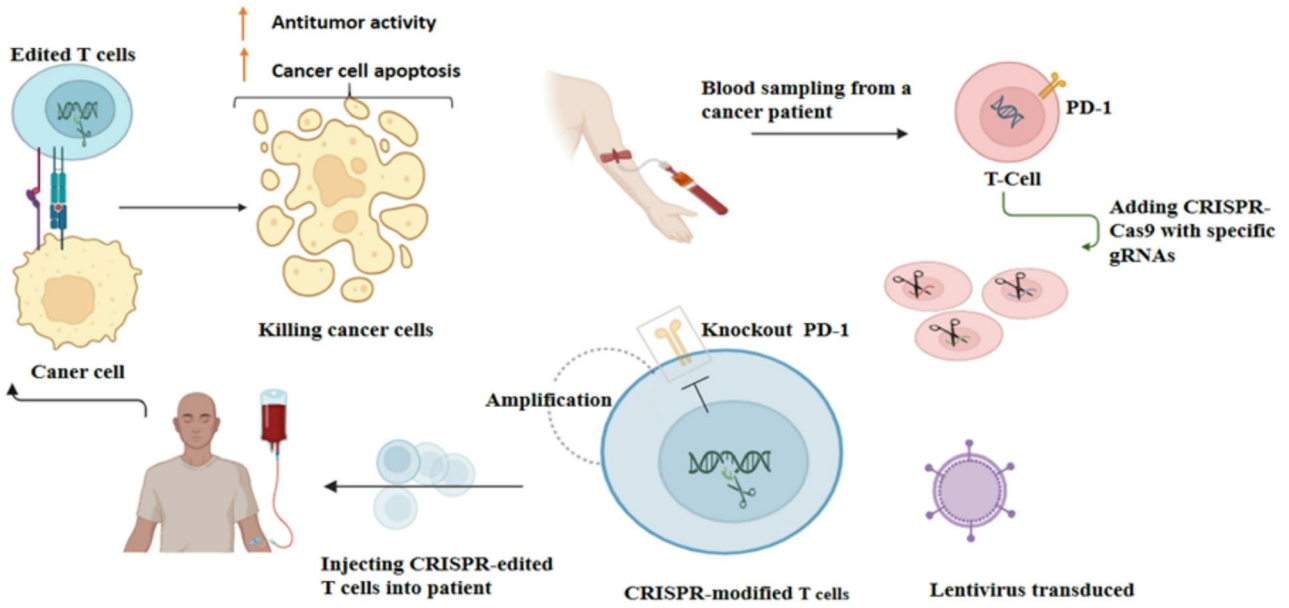

Fig. 2 Engineering T cells using CRISPR-Cas9 to increase anti-tumor immunity. The figure shows the process of engineering T cells using CRISPR-Cas9 to boost anti-tumor immunity. In this method, T cells are first extracted from a cancer patient. Then, using CRISPR-Cas9 technology, the PD-1 gene (an immune suppressor) and endogenous TCR in these cells is deactivated to increase their ability to recognize and kill cancer cells. After the modified cells have expanded, they are re-injected into the patient to generate a stronger anti-tumor immune response. This method is considered a novel and targeted approach in cancer immunotherapy that could help treat resistant cancers

are primarily responsible for humoral immune responses. Effective immune responses possess the capacity to eradicate malignant cells or modify their phenotypes and functions. Although the immune system can initially detect and monitor cancer cells effectively, these cells have developed various strategies to evade immune surveillance. These strategies include defects in antigen presentation mechanisms, the upregulation of inhibitory regulatory pathways, and the recruitment of immunosuppressive cell populations. Consequently, these adaptations hinder the effector functions of immune cells and diminish antitumor immune responses [79].

Immunotherapy is a form of cancer treatment that enhances the immune system's capacity to combat cancer. This approach functions by increasing the immune system's proficiency in recognizing and destroying cancerous cells [80]. Several immunotherapies have had impressive results, including oncolytic virus treatment (OVT), cancer vaccines, adoptive cell transfer (ACT), and immune checkpoint inhibitors (ICIs). However, when used in clinical settings, each of these therapies has unique drawbacks [81]. Monoclonal antibodies (mAbs), which function as immune checkpoint inhibitors (ICI) by blocking immunosuppressive signals on cancer or immune cells, are currently the most widely utilized immunotherapies in clinical practice. They have received numerous approvals from the US FDA for various solid tumors. However, it is important to note that immunotherapy does not yield positive results for every individual and may also lead to adverse side effects [82]. Cancer immunotherapies, which have been formulated through research into the mechanisms by which tumors evade the immune system, aim to manipulate immune responses to reactivate antitumor activity and counteract the escape pathways. Initial strategies in cancer immunotherapy focused on the use of cytokines to influence the functionality of immune cells [83]. Consequently, the immune system is more capable of recognizing and eliminating cancer cells. The Crispr-Cas9 technique has been employed to disable PD-1 and endogenous T-cell receptors in order to thwart the immune evasion strategies of tumor cells. In a particular study, the gene responsible for producing the PD-1 protein was effectively silenced using CRISPR-Cas9, resulting in an increased anti-tumor response from lymphocytes [84].

Recent advancements in adoptive cell therapy (ACT) demonstrate significant promise in the fight against tumors through the utilization of genetically engineered T cells [57]. Adoptive Cell Therapy (ACT) typically encompasses chimeric antigen receptor T-cell therapy, T-cell receptor (TCR) T-cell therapy, and tumor-infiltrating lymphocyte (TIL) therapy. Nevertheless, the effectiveness of ACT is often hindered by off-target toxicity and restricted efficacy [85]. This phenomenon is more pronounced in solid tumors compared to hematological malignancies. The recent advent of CRISPR/Cas9, a flexible tool for genetic modification, has the potential to overcome these challenges, thereby facilitating

the development of next-generation adoptive cellular therapies that can accurately target the desired cells and reduce adverse effects [86]. The potential uses of CRISPR-Cas9 technology go well beyond its role in gene editing within immune cells. This innovative tool is being employed to alter the tumor microenvironment, manage gene expression dynamically, and uncover mechanisms of drug resistance. In the realm of cancer immunotherapy, where immune checkpoint inhibitors (ICIs) have demonstrated significant success, CRISPR-based screening methods are revealing new targets that may enhance the effectiveness of these treatments [33].

Gene editing meets cancer therapy

Genome editing reached an important turning point in 2012 with the discovery of CRISPR technology [87]. CRISPR-based gene editing technology can completely change the way cancer is treated by enabling accurate and effective genome alteration to target certain genetic abnormalities that fuel tumor development and metastasis [88]. Numerous preclinical investigations and clinical trials have shown encouraging findings in the expanding corpus of research studying the application of CRISPR-based gene editing in cancer treatment in recent years [89]. The two primary components of the CRISPR tool are a DNA-cutting enzyme, usually known as Cas9, and a guide RNA [90]. Cas9 enzyme functions as a set of "molecular scissors" that can split the two DNA strands at a particular spot in the genome, allowing for the addition or removal of DNA fragments. an RNA fragment known as gRNA is made up of a little, about a 20-base-long segment of a pre-designed RNA sequence embedded in a larger RNA scaffold [91]. The pre-made sequence "guides" Cas9 to the correct region of the genome while the scaffold portion attaches to DNA and this guarantees that the Cas9 enzyme performs its cut at the appropriate location inside the genome [92]. Because its RNA bases are complementary to those of the target DNA sequence in the genome, the guide RNA is made to locate and attach to a specific sequence in the DNA; theoretically, this means that the guide RNA will only bind to the target sequence and not to any other parts of the genome [93]. The Cas9 cuts through both strands of DNA after following the guide RNA to the same spot in the sequence and at this point, the cell repairs the damaged DNA [94]. Researchers can alter one or more genes in the genome of a target cell by using the DNA repair mechanism [95]. The first step in the process of CRISPR-based oncogene inactivation techniques is identifying certain oncogenes that are essential for the development of cancer [96]. Oncogenes, which are altered forms of normal genes known as proto-oncogenes, can produce too much protein and cause cells to grow uncontrollably, which is a sign of the onset of cancer [97]. Following the identification of the target oncogene, scientists create a gRNA that selectively identifies and attaches to the amplified or mutant oncogene region [96]. One strategy is inactivating the genes responsible for tumor growth. For instance, one possible tactic to treat cervical cancer is to deactivate the E6 and E7 oncogenes, which are encoded by the HPV. These genes are important for tumor growth in cancers linked to HPV, and their silencing can dramatically reduce tumor cell proliferation and cause cell death [98]. To increase effectiveness and enhance treatment results, CRISPR-based strategies can be effectively combined with existing cancer medicines [99]. For example, precisely altering drug-resistant genes using CRISPR and chemotherapy makes cancer cells more sensitive to chemotherapeutic drugs [100]. Furthermore, in conjunction with CAR-T cell therapy, CRISPR may be utilized to modify patient-derived immune cells, including T cells, to express CARs that improve their capacity to target tumors [101]. Additionally, CRISPR enhances the efficacy of immunotherapies such as immune checkpoint inhibitors by mutating immune checkpoint genes in cancer cells [102]. Another instance is the combination of CRISPR with targeted medicines, which can overcome resistance and enhance the benefits of targeted medications by simultaneously targeting numerous important pathways using gene editing [103]. Researchers can improve the penetration of therapies and increase the effectiveness of different cancer treatments by using CRISPR to change tumor cells or the tumor microenvironment [88]. These illustrations show how CRISPR may work in concert with existing cancer treatments and prepare the ground for more individualized and efficient cancer treatment strategies [104].

Targeting HPV oncogenes with CRISPR

Viral DNA is commonly incorporated into host cell chromosomes during HPV-driven cancer formation, and the proteins that are produced by viral genes are essential for carcinogenesis [13]. several studies have documented several advancements in the field of cervical cancer in recent decades, with the most significant and early advancements being made in the mechanism related to HPV. Another research investigated cervical cancer prevention and therapy, using it as a point of entry for other cancers caused by HPV [105]. The CRISPR-Cas9 system is the most advanced genome editing technique and is an adaptive defensive network found in microorganisms [41]. It has the benefit of being programmable using short RNAs, which makes it simpler to utilize than other genome editing methods like ZFNs and TALENs [106]. Researchers have already shown that CRISPR-Cas9 targets DNA and has been shown to be capable of editing human chromosome DNA [107, 108]. Based on these findings, a phase I clinical study using CRISPR-Cas9 was

Table 3 Clinical trials on CRISPR and immunotherapy in HPV-Related cancers

Study Name	Cancer Type	Study Phase	Target Gene	CRISPR Strategy	Type of Immunotherapy	Mechanism	Key Finding	Ref
NCT03745287	Cervical Cancer	Preclinical (Animal Studies)	HPV16 E6/E7	CRISPR disables HPV16 E6/E7 oncogenes	Immune Checkpoint Therapy	Restores tumor-suppressor function, triggers cancer cell death	Reduced tumor growth in lab models	[23]
NCT04170057	Head and Neck Cancer	Early Clinical (Phase I/II)	PD-1	CRISPR removes PD-1 from immune cells	PD-1 Blockade Therapy	Boosts the immune system's ability to fight cancer	Stronger immune response seen in early results	[9]
NCT03616860	Cervical Cancer	Preclinical (Animal Studies)	HPV E6/E7, BCMA	CRISPR-engineered CAR-T cells	CAR-T Cell Therapy	Enhances immune cells' ability to recognize and attack tumors	Early success, further trials ongoing	[22]
NCT03166812	HPV-Positive Oropharyngeal Cancer	Phase I Clinical	HPV E7, PD-1	CRISPR modifies immune cells to better detect HPV	Checkpoint Blockade + CRISPR	Restores immune response by removing inhibitory signals	Partial remission in early patients	[115]
NCT03399448	HPV-Positive Anal Cancer	Phase I/II Clinical	PD-1	CRISPR edits PD-1 in immune cells	PD-1 Blockade Therapy	Helps immune system stay active against cancer	Tumor shrinkage observed	[32]
NCT03057912	Cervical Precancerous Lesions	Phase I Clinical	HPV E6/E7	CRISPR targets HPV oncogenes directly	Not Applicable	Aims to stop early-stage cancer progression	Assessing safety and effectiveness	[117]
NCT03545815	Mesothelin-Positive Solid Tumors	Phase I Clinical	PD-1	CRISPR removes PD-1 from CAR-T cells	CAR-T Cell Therapy	Increases CAR-T cell persistence and attack on cancer	Evaluating safety and long-term effects	[118]

finished in 2020 in patients with advanced non-small cell lung cancer, marking the start of the technique for oncology clinical therapy [39]. CRISPR-Cas9 has been employed extensively in recent years to target the HPV genes that cause the majority of cervical cancer tumors [41]. When CRISPR-Cas9 was used in 2014 to target the HPV virus's E6 and E7 genes, tumor cells died [8]. An increasing amount of research has shown that the E6 and E7 genes are essential for facilitating the induction of cell cycle arrest and apoptosis [109]. As a result, several researchers have extensively examined the E6 and E7 genes as the primary target locus [98, 110, 111]. The initial recognition of HPV16-E7 as a target of the CRISPR-Cas9 system for gene therapy of HPV virus-positive cervical cancer came in 2014 [23]. A different study that same year showed that the CRISPR-Cas9 system, which targets the E6 and E7 loci, caused an enormous increase in p53 and p21 and this resulted in a significant reduction in the proliferation of cervical cancer cells in vitro [24]. In addition to various other impacts, it was shown in later research that knocking down the E6 and E7 genes might decrease the proliferation of cervical cancer cells. Targeted silencing of the HPV16 E6/E7 gene may also be a high-quality sensitizer of CDDP (Cisplatin) chemotherapy in cervical cancer, according to a 2016 research, which offered fresh concepts for further gene therapy approaches [112]. The identical research team discovered once more in 2019 that inhibiting the HPV16 E6/E7 gene and the PD-1 pathway could function in concert to increase the anticancer impact [113]. Using the CRISPR-Cas9 technique, some researchers are focusing on examining additional HPV oncogenic processes in addition to the therapy that targets the E6/E7 gene. When the SIRT1 gene was silenced using CRISPR-Cas9 in 2017, it was evident how crucial the cytosolic enzyme SIRT1 is for controlling HPV16 replication [114]. The previous study used CRISPR-Cas9 technology to continue examining the regulatory function of the SIRT1-WRN axis in 2020, noting the viral replication cycle's reliance on WRN [115]. Additionally, some researchers have begun to investigate the host proteins that are associated with the E6/E7 gene transcription products. By binding to the host cell's tumor suppressor protein, PTPN14, the E7 oncoprotein from both HPV16 and HPV18 viruses effectively inhibits the expression of genes responsible for cell differentiation, according to a 2020 study. Additional experiments using CRISPR-Cas9 to mutate the PTPN14 gene in cervical cancer cells revealed a significant reduction in the oncogenic potential of the cancer cells, underscoring the crucial role of PTPN14 in suppressing tumor development caused by high-risk HPV strains [116]. Numerous experiments are being conducted on gene therapy targeting the CRISPR-Cas9 system. (Table 3)

Oncogene suppression simplified

Numerous genetic mutations are diverse and accumulate in cancer, including oncogenes (RAS), activation of tumor suppressor (TP53), alterations in genes such as multidrug resistance genes (MDR1/Pgp and ABC), and changes in epigenetic factors (DNMT1) [119]. The medical field has introduced a variety of therapies in recent decades to improve the prognosis of cancer patients. These therapies include immunotherapy, hormone therapy, chemotherapy, targeted drug therapy, stem cell transplantation,

immunotherapy, and surgery. These treatments greatly reduce or eradicate the malignant cells, extending the maximum lifetime by around five years [120, 121]. Cancer persists regardless of these treatments, eventually leading to molecular heterogeneity that is ascribed to a variety of molecular processes, including transcriptome, genomic, phenotypic, and epigenetic alterations [122]. However, these treatments did not significantly increase patient lifespan because of their numerous drawbacks, such as toxicity, high expense, and side effects (which impair healthy cells and result in partial or whole loss of organ functions) [119]. Immunotherapy has opened a new chapter in the treatment of solid tumors, although it still has limitations and has to be developed to overcome side effects. Furthermore, molecular-level gene analysis and modification can further accelerate the creation of sophisticated technologies [123]. A genome editing engineering technique that targets any gene in the afflicted region and produces knock-in and knock-out alternations has been presented recently to cure cancer and surpass the limitations of immunotherapy [124]. A novel RNA domain-containing endonuclease-based genome engineering tool called CRISPR-Cas9 has been created for the treatment of cancer. It has significantly changed gene expression and gene therapy because it is easy to use and has high accuracy and efficiency [125]. In contrast to non-solid tumors like leukemia, solid tumors such as breast, lung, liver, prostate, and colorectal cancer are the most prevalent and have made less progress in their treatment with gene therapies. This quickly changed with the advent of CRISPR/Cas-9 [119, 126]. According to previously released research, CRISPR/Cas-9 may efficiently target cancer cells and stop tumor development by triggering apoptosis and preventing cell division and metastasis [127, 128]. The endonuclease enzyme Cas-9 and single guide RNA (sgRNA) are essential components of the CRISPR system, which has motivated the application of CRISPR technology in the treatment of cancer cells. The Cas-9 protein induces double-stranded DNA breaks (DSBs) at targeted genomic loci. This initiates the DNA repair process, often resulting in the insertion or deletion of short sequences via the non-homologous end joining (NHEJ) repair mechanism [125]. Nevertheless, Cas-9 may also engage in alternative repair mechanisms, such as template DNA-based homology-directed repair (HDR). Approximately a decade ago, the CRISPR/Cas-9 method was initially utilized in human and animal cells. It emerged as a highly innovative and adaptable approach for combating cancer, demonstrating potential in treatment experiments [121, 129]. The development of a novel multifunctional treatment medicine or instrument is necessary due to the multi-step transformation progression of cancer. However, since CRISPR/Cas9 can edit several genes at once, it is regarded as a direct and parallel target and is acknowledged as an effective test center tool against genetic alterations linked to cancer in both in vitro and in vivo research [130].

Crispr against HPV cancers

Scientists discovered that the HPVs were able to integrate their genes into the human genome, which appeared to be a crucial step in the development of cancer. Because of its integration, the HPV oncogene expresses itself persistently, making its eradication challenging. Patients with chronic HPV infection or HPV gene integration currently lack a viable therapy [41]. Cervical cancer, anal cancer, oral cancer, oropharyngeal cancer, and other malignancies are all considered HPV-driven cancers [41]. Various investigations have documented numerous advancements in the field of cervical cancer in recent decades, with the earliest and most profound progress being made on the mechanism relevant to HPV [131]. A study examined cervical cancer prevention and treatment and used it as the point of entry for other cancers caused by HPV [105]. CRISPR/cas9 has emerged as a potential new technique for preventing the expression of certain genes and the treatment of the majority of malignancies caused by HPV from a particular angle. Another study has previously demonstrated that CRISPR-Cas9 targets DNA and can modify human chromosomal DNA [107, 108]. Researchers simply need to create the gRNA complementary to the target DNA sequence for the CRISPR/Cas9 system. Given its speed and ease of design, the CRISPR/Cas9 system may be a perfect substitute for ZFN and TALEN in causing targeted gene editing [132]. According to certain earlier research, HPV-related cervical cancer may benefit from treatment using the CRISPR/Cas9 system, which targets the HPV oncogene [24]. A 2021 study found that CRISPR/Cas9 targeting HPV16 E7 may successfully reverse HPV-related cervical carcinogenesis in vitro and in K14-HPV16 transgenic mice, which has demonstrated significant promise in the clinical therapy of cervical precancerous lesions [133]. CRISPR/cas9 liposome delivery may efficiently eliminate HPV, which then causes autophagy and immunological activation linked to cell death by releasing molecular patterns linked to harm [134]. gRNA-liposome-mediated HPV gene deletion can significantly inhibit the development of human cervical cancer cells both in vitro and in vivo [135]. These HPV-targeting gRNA-liposomes can produce damage-related molecular pattern (DAMP), trigger autophagy, increase the production of more DAMP and autophagosomes, and restore the sensitivity of tumor cells to death [134]. Additionally, a cervical carcinoma model was used to assess the anticancer effect of CRISPR in conjunction with anti-PD-1 immunotherapy, demonstrating the amazing therapeutic effect and immunological memory of this combination approach. For several malignant tumors,

CRISPR nanodrugs' tumor inhibitory repair may increase the sensitivity of immune checkpoint blockade (ICB) and offer a potent combination therapy [134]. Nevertheless, nearly comprehensive gene treatment plans related to CRISPR-Cas9 remain in the experimental stage, with current off-target effects and other safety risks. All things considered, the advancement of CRISPR-Cas9 technology can be expected to bring about a novel genetic cure for the majority of cancers caused by HPV [136].

Broader relevance of CRISPR-Cas9 therapy in HPV-associated cancers

Although cervical cancer is the most well-known malignancy associated with high-risk human papillomavirus (HPV) infection, other cancers are also significantly linked to HPV [137]. Notably, HPV-positive head and neck squamous cell carcinoma (HNSCC), particularly oropharyngeal squamous cell carcinoma, has seen a marked increase in incidence [138]. As with cervical cancer, HPV genome integration in host cells is linked to carcinogenesis in HNSCC. This integration frequently results in genetic changes, such as activation of oncogenes or disruption of tumor suppressor genes, which may decrease responsiveness to therapy [139]. Preclinical studies have explored the application of CRISPR-Cas9 technologies in HNSCC models, targeting the E6 and E7 oncogenes to induce apoptosis and reduce tumor growth. In the context of HNSCC, CRISPR/Cas9 systems have shown considerable promise by enabling precise modification of oncogenes and tumor suppressor genes frequently altered in these malignancies, including TP53, NOTCH1, and PIK3CA [88, 140]. The use of CRISPR-mediated approaches aims not only to inhibit tumor growth but also to counteract therapeutic resistance, a major hurdle in the management of HNSCC. By targeting resistance-associated genes, CRISPR has the potential to enhance the efficacy of conventional treatments such as chemotherapy and radiotherapy. Furthermore, gene editing technologies are being utilized to reprogram immune cells, particularly T lymphocytes, to improve tumor recognition and cytotoxicity [141]. However, the clinical translation of CRISPR therapies faces several obstacles, including the development of efficient and targeted delivery systems, the prevention of off-target genetic modifications, and the mitigation of immune reactions triggered by CRISPR components [142]. Nevertheless, advancements in delivery methods and editing precision continue to drive the field forward, suggesting that CRISPR-based therapies could become integral to future personalized treatment strategies for HNSCC [143]. Several preclinical models of HPV-associated head and neck squamous cell carcinoma (HNSCC) have been established to facilitate the study of viral oncogenesis and therapeutic interventions. These models include both cell lines and animal systems characterized by either integrated or episomal HPV genomes [144]. For instance, established cell lines such as UM-SCC-47 and UPCI-SCC-090, which harbor integrated HPV16 genomes, continue to serve as essential in vitro platforms. In contrast, 93-VU-147T maintains episomal HPV16 DNA, offering a distinct model to study non-integrated viral forms [145, 146]. More recently, patient-derived xenograft (PDX) models, including the JHU029 and JHU022 lines, have been developed to closely mimic the heterogeneity and genomic landscape of HPV-positive tumors in vivo [147]. These models provide critical systems to evaluate the efficacy of genome-editing technologies like CRISPR-Cas9 and facilitate the translation of targeted therapies into clinical practice [148]. Moreover, HPV-related anogenital cancers, including anal, vulvar, vaginal, and penile cancers, also present potential targets for CRISPR-Cas9-based interventions [149]. These cancers share common oncogenic pathways mediated by persistent expression of HPV E6 and E7, suggesting that therapeutic strategies developed for cervical cancer may be translatable to these malignancies as well. Expanding the therapeutic scope to include these cancers could significantly broaden the clinical impact of CRISPR-Cas9 technology in HPV-associated malignancies [150].

Future perspectives and conclusion

A long-standing objective of researchers over the last 20 years has been to create dependable and affordable methods for making precisely targeted changes to living cells' genomes. A wide range of areas can benefit greatly from the use of genome editing to solve challenges. It is possible to eliminate the gene causing a specific genetic condition in humans by gene therapy [151]. In recent years, the scientific world has embraced CRISPR-based technologies because they allow for the effective targeting and modification of DNA in live cells from dozens of species, including humans and other eukaryotes [152]. Precise editing can be applied in customized gene therapy to rectify inherited monoclonal disorders or sequence-specific targeting of pathogens to cure infectious diseases, as well as for many other uses. However, the implementation of CRISPR concurrently offers various practical and technological hurdles principally linked with delivery tactics, the management of repair pathways, off-target and on-target consequences, and significant ethical concerns [153]. The CRISPR/Cas genome-editing system is a promising therapeutic approach that may complement or perhaps replace the present treatments of radiation, chemotherapy, and surgery. The massive volume of research on CRISPR/Cas suggests that this novel approach to cancer treatment is the way of the future [46]. Prior to being used in clinical practice, these techniques will still need to be optimized for efficacy, safety, and specificity.

the future of precise gene therapy for cervical cancer is bright as long as we continue to understand the nature and function of programmable Cas nuclease. In the near future, CRISPR technology could play a significant role in enabling humans recover from genetic diseases [154]. The largest obstacle to the broad clinical application of CRISPR/Cas9 in human medicine is still the development of safe and efficient in vivo delivery. Viral vectors are used in the majority of current clinical investigations; nonetheless, issues including immunogenicity, cytotoxicity, and carcinogenicity still need to be resolved [155]. The potential of lipids and carrier polymers is boosted by the rapid development of nanotechnology. Nanocarriers can reduce off-target effects and effectively package and protect various forms of CRISPR/Cas9 components. They can also improve blood circulation, cell uptake, and precise targeting by altering their surface. However, in order to achieve CRISPR/Cas editing at the tumor site and carry out targeted therapy, a sustained-release system of nanocarriers still has to go beyond a number of physical challenges [124]. Even if current nanocarriers are unable to satisfy the demands of extensive clinical applications, the development of biomaterials will contribute to the future expansion of genome editing's medicinal uses [156]. Despite its early stages, CRISPR screening holds significant potential for identifying key cancer genes and potential treatment targets. While future research will concentrate on other immune regulatory cells to identify new regulatory pathways in the tumor microenvironment (TME), earlier studies employed CRISPR screening to uncover the role of immune cells and methods of interaction with cancer cells [62]. Universal CAR-T cells can be employed extensively in clinical practice due to the ability of CRISPR gene editing of human primary T cells to create allogeneic T cells with stronger anticancer activity and fewer side effects, in addition to certain advances in immuno-oncology [157]. Additionally, the CRISPR/Cas9 system has a lot of potential for targeting viruses that cause cancer. Targeting HPV18-E6 or HPV16-E6 with the CRISPR/Cas9 system led to decreased proliferation capacity and increased apoptosis in cervical cancer cell lines that were positive for HPV, while HPV-negative cells remained unaffected, offering a novel approach to gene therapy in cancer [158].

Furthermore, recent advances in CRISPR technology are paving the way for more refined and safer applications. Base editing and prime editing systems, which enable precise nucleotide modifications without generating double-stranded breaks, offer promising alternatives to traditional CRISPR-Cas9 and may reduce the risk of off-target effects [159, 160]. Improving delivery methods also remains a crucial next step; the development of viral vectors, lipid nanoparticles, and extracellular vesicle-based delivery systems could enhance the specificity and efficiency of gene editing in clinical settings [161, 162]. Additionally, combining CRISPR-mediated gene editing with immune checkpoint blockade therapies or engineered T-cell therapies (such as CAR-T cells) could synergistically improve treatment outcomes in HPV-associated malignancies [40, 163]. Future clinical trials focusing on safety, long-term efficacy, and personalized CRISPR strategies tailored to individual tumor genomic profiles are essential to fully realize the transformative potential of this technology [164]. In conclusion, The CRISPR system has significantly influenced cancer research and will remain vital in the future.

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