Review Article

Gene Therapy Approaches for Treating HPV-Induced Damage in Human Tissues and Cells

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ABSTRACT

Human papillomavirus (HPV) is a leading cause of cervical and other anogenital and oropharyngeal cancers, with high- risk HPV types such as HPV-16 and HPV-18 playing a major role in oncogenesis. The viral oncoproteins E6 and E7 interfere with tumor suppressor proteins p53 and retinoblastoma (pRb), leading to uncontrolled cell proliferation, genetic instability, and resistance to apoptosis. While HPV vaccines have significantly reduced infection rates, they do not eliminate pre-existing infections or treat HPV- associated malignancies. This has driven interest in gene therapy as a potential treatment option for HPV- induced cellular damage. Gene therapy approaches, particularly CRISPR- Cas9, have demonstrated efficacy in selectively targeting and disrupting HPV oncogenes, leading to tumor regression. Other gene- editing techniques, such as zinc- finger nucleases (ZFNs) and transcription activator- like effector nucleases (TALENs), also show promise in HPV gene disruption. Efficient delivery systems, including viral and non- viral vectors such as nanoparticles, liposomes, and adeno-associated viruses (AAVs), are being optimized to enhance therapeutic outcomes. However, challenges remain, particularly concerning off- target effects, delivery efficiency, and potential immunogenic responses. This review explores the mechanisms of HPV-induced carcinogenesis, the role of gene therapy in targeting viral oncogenes, and advancements in gene-editing technologies. Future directions include integrating gene therapy with immunotherapy, developing personalized treatment strategies, and improving delivery mechanisms to enhance specificity and safety. While gene therapy holds significant promise for eradicating HPV- driven malignancies, further research is needed to refine these approaches for clinical application.

INTRODUCTION

Background on Human Papillomavirus (HPV)

The human papillomaviruses, or HPVs, are tiny, around 8000 base pair DNA double- strand viruses that have coexisted with the human species for dozens of millennia with very little alteration to their genetic makeup [1-8]. Though more than 200 HPV strains are now recognized, only a small subset is thought to cause cancer [6]. They are classified as high-risk HPV strains and probably cancer-related strains by the International Agency for Research on Cancer [6].

HPV types are commonly classified into "high- risk" and "low- risk" groups based on their propensity to induce oncogenesis. High-risk HPV strains encompass HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -73, and -82 [9]. Particularly benign anogenital lesions, such as laryngeal papillomas, low-grade squamous intraepithelial lesions (SILs) of the cervix, and genital warts (condylomata), are caused by low-risk HPV strains. These low-risk types are HPV-6, -11, -40, -42, -43, -44, -53, -54, -61, -72, and -81 [9].

With significant geographical variation, the prevalence of HPV infection is around 12% worldwide [3]. The human papillomavirus (HPV) is the most common virus that affects the reproductive system and is one of the leading causes of STDs worldwide [3,10]. HPV may infect mucosal membranes and skin in a variety of anatomical locations, including the oral cavity and anogenital area [3]. Regardless of other risk factors like sexual behavior, there is a very high correlation between the human papillomavirus (HPV) and cervical cancer [2,5,7,11-13]. Both physiologically and epidemiologically, the link between HPV and uterine cervix malignancies is well established [4,14,15]. Oesophageal, head and neck, vulvar, vaginal, penile, and anal cancers are among the non- cervical malignancies that HPV causes since it is an epitheliotropic virus. HPV infection is the primary cause of approximately 99.7% of invasive cervical cancer cases worldwide [16-17].

Globally, it is estimated that 530,000 new cases of invasive cervical cancer are diagnosed each year [3,16,19-23], and HPV DNA is detectable in approximately 99.7% of tumours, underscoring HPV as the near-universal causal factor in cervical carcinogenesis [3,9,10,16,24-27].

METHODS

Literature search strategy and selection criteria

A structured literature search was conducted by the authors across PubMed, Google Scholar, Scopus, and the electronic resources of King Abdulaziz University Library to identify relevant studies. The search encompassed literature published between 2005 and 2025, utilizing keywords such as "HPV gene therapy," "CRISPR-Cas9," "viral vectors," and "E6/E7 oncoproteins." Inclusion criteria prioritized peer-reviewed original research and clinical trials focusing on high-risk HPV types (HPV-16 and -18). Articles were excluded if they focused solely on prophylactic vaccination, lacked relevance to therapeutic gene- editing approaches, or were not available in English to maintain the focus on therapeutic interventions for established cellular damage.

DISCUSSION

The reasons behind the non-existence of HPV in the remaining 0.3% of cases of cervical cancer are as follows: poor detection techniques, the existence of undiscovered HPV strains, and HPV genome disruption during integration events [3,28]. Human papillomavirus (HPV)- associated carcinogenesis is a complex process that includes the buildup of genetic alterations in cells [16]. Replicative immortality, immunological inadequacies, genetic instability, prolonged angiogenesis, avoiding growth suppressors, dysregulation of cellular energetics, invasion, and metastasis are some of these alterations, which are summarized in Figure 1.

Six early genes (E1, E2, E4, E5, E6, and E7) and two late genes (L1 and L2) are encoded by the HPV genome [6,12].

Although the E1, E2, L1, and L2 proteins are necessary for all HPVs, different HPVs express different HPV proteins [6]. The oncoproteins E6 and E7, which are encoded by the viral genome, are the primary participants in this process. The E6 and E7 proteins are initially expressed at low levels because the viral E2 protein prevents their synthesis after infection [6,15,16].

The E6 and E7 genes of high- risk (HR) oncogenic HPVs are regularly overexpressed in malignancies. Changes in a variety of signaling pathways brought on by the development of HPV E5, E6, and E7 oncoproteins can result in cervical cancer [16,27,29]. In order to facilitate viral amplification, key oncogenes E5, E6, and E7 promote host cell proliferation [21]. Protein kinase B (Akt), phosphoinositide 3- kinase (PI3K), and the Wnt and Notch pathways are all activated by E6. It may also attach to and deactivate the tumor suppressor protein 53 (p53) and PDZ domain–containing proteins such as hDlg, Scribble, and MAGI-1. E7 may activate the PI3K/ Akt pathway, release E2F to encourage the cell cycle, and bind to and inhibit the retinoblastoma protein (pRb) [8,16,21]. The stability and accumulation of p53 protein due to the E7-triggered cell cycle progression causes cell cycle arrest, senescence, and apoptosis [8].

The Role of Gene Therapy in Human Syndromes Treatment

Gene therapy is the scientific study of introducing targeted modifications into the human genome to enhance it or provide therapeutic benefits in disorders associated with genes [45-80]. Gene therapy makes it possible to treat any cell, tissue, or organ in the body by delivering therapeutic genetic material there [44]. Gene therapy was first used to treat monogenic diseases or cancer by delivering therapeutic transgenes using viral vectors. For thousands of patients with hereditary diseases and few alternatives for treatment, gene therapy's arrival at the clinic brought hope. Now, gene therapy is mostly used in research labs and is still in its experimental stages. The US, Europe, and Australia host most trials [44-47].

Somatic gene therapy, which includes introducing desired genes into somatic cells or patients, and germ-line gene therapy, which delivers genes into sperm or egg cells but is not frequently employed because of ethical problems, are the two primary forms of gene therapy [44,45,80]. In gene therapy, a disease-causing (aberrant) gene is replaced, repaired, or supplemented with a functional version to restore normal cellular function. Getting the gene delivered into the stem cell is one of the main issues with the procedure. A molecular carrier called a "vector" is used to deliver the gene. This vector must be highly specific, exhibit efficiency in releasing one or more genes of the sizes required for clinical applications, elude immune system recognition and be purified in large quantities and high concentrations to be produced and made widely available [44-47].

Direct editing, which includes gene insertion, ablation, and correction, has been possible due to the rapidly evolving science of gene editing, which has also significantly increased the ability to alter the genome. Among the gene editing tools that have emerged are meganucleases (MGs), zinc- finger nucleases (ZFNs), transcription activator- like effector nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/ Associated Proteins (CRISPR/ Cas9) [44-47,80]. These instruments induce double- strand breaks (DSBs) in the target DNA, which are then repaired primarily by error- prone non- homologous end joining (NHEJ), often disrupting the targeted gene [11,21,32].

There are two primary types of CRISPR-Cas systems that use the identification and cleavage method [26,32]. They are separated into a number of subtypes and six main groupings. Class 1 systems cleave using protein complexes, while Class 2 systems only cleave with one protein, potentially enabling genome editing [32]. Type II of Class 2 includes CRISPR- Cas9. It has multiple Cas genes, notably the cas9 gene, flanking a CRISPR array centrally located [33,34]. The CRISPR RNA transcribed from the CRISPR array (gRNA) directs the nuclease Cas9 to create a double-strand break (DSB) at the designated target site, as illustrated in figure 2. The discovery of the newest genome engineering technology, CRISPR/ *Cas9* , has greatly influenced gene editing [11,15,26,29,35-38].

Extensive research indicates that high-risk human papillomavirus (HPV) infection is a primary causative agent for nearly 99% of cervical malignancies. The two most prevalent high- risk subtypes are HPV16 and HPV18. The HPV genome comprises two primary regions: the early region (E) and the late region (L). The E region primarily regulates viral replication and life cycle, while the L region primarily encodes the capsid proteins responsible for viral structure [26,39].

The E6 and E7 genes within the HPV genome are recognized as the primary oncogenes [29,40–42]. The RIG-I signaling system (immune escape mechanism) and the p53 cancer suppressor pathway are blocked by the E6 oncogene, while the retinoblastoma protein (Rb) is suppressed

by the E7 oncogene, which also impacts p21 and other pathways [26]. Due to their distinct expression in cancer cells, these oncogenes are excellent targets for treatments based on CRISPR/*Cas9* [26,29]. This novel method makes it possible to introduce specific mutations into the viral genome that successfully prevent the virus from replicating, spreading, and causing cellular transformation [29]. Therefore, it is possible to specifically knock down E6 expression in cervical cancer cells using CRISPR-Cas9 [8,11,24].

Rationale for Using Gene Therapy to Target HPV

Recent years have seen the development of several novel strategies to combat cervical cancer. For gene therapy to be successful, safe gene vectors and gene delivery methods that allow for targeted cancer cells and minimize off-target side effects during treatment must be developed [43-45]. The delivery mechanisms for introducing foreign genes into target cells are called vectors. In experimental research, a vast array of vectors has been employed; nonetheless, they may be classified into two categories: viral and non-viral systems [43,47].

The most often used viruses as viral vectors include retrovirus, lentivirus, adenovirus (Ad), adeno-associated virus (AAV), and basic herpes viruses [43,48]. Non-viral vectors, in addition to physical methods like electroporation, gene guns, and ultrasound, can also be smart molecules like liposomes, polyplexes, nanoparticles, etc. that can enhance the therapeutic effect of currently advised treatment modalities like chemotherapy and radiation [43].

The viral oncoproteins E6 and E7 are encoded by high-risk HPV, are essential for driving cellular transformation and oncogenesis. By subverting cellular processes such as apoptosis and growth suppression, these oncoproteins enable infected cells to acquire hallmarks of cancer, including uncontrolled proliferation, angiogenesis, invasion, metastasis, and telomerase activation. The indispensable role of E6 and E7 in maintaining HPV-associated malignancy has been demonstrated in numerous in vitro and in vivo studies. Depletion of E6 and E7 function in cancer cells results in cellular senescence or apoptosis, highlighting their critical role in sustaining malignant transformation [29,49]. E6 and E7 oncoproteins can induce a malignant phenotype by dysregulating key cellular processes. These proteins can contribute to the six hallmarks of cancer, including evasion of growth suppressors, resistance to cell death, sustained proliferative signaling, replicative immortality, angiogenesis, and invasion and metastasis [42,50]. Because E6 and E7 may eradicate all disease hallmarks and ensure the total eradication of cervical cancer cells, they are therefore the ideal candidates for therapeutic targeting.

Mechanisms of HPV-Induced Cellular Changes

Oncogenic Mechanism of HPV

The "pocket proteins"— Rb, p107, and p130 — of the cellular retinoblastoma family have been extensively studied as targets of the high-risk E7 proteins. [25]. The control and activity of the two viral oncogenes, E6 and E7, explain the molecular process of HPV carcinogenesis. When transfected into NIH 3T3 and Rat-1 cell lines, it has been shown that these two HPV 18 genes may transform. The E2 gene product controls the expression of the E6 and E7 genes. The integration frequently occurs at the E2 gene, which causes the E2 gene to be disrupted and the E6 and E7 genes to be derepressed. The tumor suppressor gene p53 is bound by the E6 gene product. The p53 protein is particularly ubiquitinated and destroyed when E6 attaches to it [5,42]. E7 targets the retinoblastoma gene product (pRb), another tumor suppressor protein. When E7 binds to pRb and alters its phosphorylation state, tumor suppressor proteins such as p53 — which normally enforces cell cycle control — become functionally inactive [51]. The transcription factor E2F, which is crucial for the G1 to S phase transition, is bound by pRb in a typical cell cycle. When E7 connects to pRb, it forms an inactive E7-pRb complex, but when E2F binds to pRb, it releases itself to bind DNA and promotes cell division and growth.

HPV's Impact on Cellular Pathways

Cells use signaling pathways to interact with one another and their surroundings as well as to determine their fate [22]. The amounts of gene transcription can be impacted by the activation of protein cascades caused by ligand binding to cell receptors. Cells use these intricate

mechanisms to convert outside inputs into biochemical signals that regulate several biological processes, including differentiation, proliferation, and death [22].

Several signaling pathways are known to be dysregulated in cancer. As a result, a lot of components that target these pathways have been suggested as potential treatment targets [22]. Key pathways that control cell proliferation and death, such as phosphatidylinositol 3-kinase/ protein kinase B (PI3K/ Akt), mitogen- activated protein kinase (MAPK)/ extracellular signal kinase (ERK), Notch, and Wnt/ catenin, have been found to consistently alter in several cancer types [22].

Numerous cancer types have been associated with mutations in various components of the Wnt/ β-catenin signaling pathway [22]. While mutations in genes such as CTNNB1 and AXIN1 are infrequent in HPV- associated neoplasms, alterations in β- catenin localization have been observed. In cervical cancer and oropharyngeal squamous cell carcinoma, increased cytoplasmic and nuclear β-catenin levels, coupled with decreased membrane-bound β-catenin, have been reported during cancer progression [22].

According to some research, the G protein- coupled receptor family member LGR5, which is gradually elevated in cervical neoplasia, activates the Wnt/ catenin pathway. This promotes the proliferation and carcinogenesis of cervical cancer cells [22].

There is strong evidence that hyperactivated Wnt pathways are responsible for HPV-associated cancers [22,53]. It is now known that HPV oncoproteins bind to and modify several cellular substrates involved in the regulation of the Wnt pathway, including hTERT, p53, p300/ CBP, Dvl, and PP2A. Additionally, details on possible viral regulatory mechanisms in this system are starting to emerge [22,54].

Perhaps due to HR-HPV oncoproteins, HPV-associated cancers have activated MAPK signaling pathways. Serine/ Threonine- Specific Protein Kinases (MAPKs) are activated by a variety of signaling pathways [25]. Angiogenesis, differentiation, proliferation, and cell survival are among the essential biological processes regulated by these pathways [39].

The MAPK families comprise p38/ stress- activated protein kinases (SAPKs), c- Jun N- terminal (JNKs), and extracellular signal- regulated kinases (ERKs), such as ERK1/2 and ERK5. Their individual MAPK phosphatases (MKPs) dephosphorylate Ser/ Thr residues deactivate their pathways, which are triggered by phosphorylation [39]. MAPK activation involves a common cascade of phosphorylations, in which a MAP kinase kinase (MAP3K) phosphorylates a MAP kinase (MAP2K), which in turn phosphorylates another MAP kinase (MAPK) [39].

Long-term effects of HPV infection on genomic stability and cell proliferation.

The term "genomic instability" describes a higher frequency of changes that are transferred to daughter cells during cell division [55]. Single nucleotide variants (SNVs) or large- scale structural variations (SVs) involving many chromosomes might be examples of these alterations. When some DNA repair mechanisms are dysregulated, the incidence of SV and SNV transmission can rise [55,56].

Genomic instability may be effectively studied using high-risk HPV-associated neoplasms as a model system [56]. This is further supported by the observation that chromosomally unstable cells may be seen in even pre- malignant lesions and that the production of two viral oncoproteins, HPV E6 and E7, promotes the development of cancer. This creates the possibility of using viral oncoproteins as instruments to comprehend the origins and effects of genomic instability [56].

Even after malignant transformation, significant expression of the HPV oncogenes is necessary for HPV-driven malignancies [55,57]. All carcinogenic HPV strains contain the oncogenes E6 and E7, which target and destroy the p53 and Rb family proteins, respectively. When these well-known tumor suppressors stop working, the genome becomes more open to the build- up of mutations, which can ultimately result in cancer [55,58]. Over the past 30 years, several investigations using cell culture have demonstrated that expression of E6 and E7 from HPV16 or HPV18 is sufficient to cause keratinocytes to become immortal [55]. The results show that

during carcinogenesis, both E6 and E7 expression are critical. E6 causes genomic instability and accelerates carcinogenesis, while E7 results in cell immortalization [27,55].

Gene Therapy Approaches to Target HPV

CRISPR-Cas9 for HPV Gene Editing

CRISPR/Cas, an RNA-guided endonuclease system, is a new programmable technology. And in a variety of species, such as prokaryotes, C. elegans, and zebrafish, it has become a potent tool for genome editing [59,61]. The Cas9 enzyme and site-specific single-guide RNA (sgRNA) at the 5'-N20NGG-3' site enable the system to virtually target any genomic region [59].

Double strand breaks (DSBs) are started by the Cas9 enzyme when it locates a specific genomic location that matches the sgRNA sequence. The mutagenic non- homologous end joining (NHEJ) repair pathway, which impairs the targeted gene, is the main method for fixing double-strand breaks (DSBs) [59,62].

Through the use of CRISPRa to activate genes, non-homologous end joining (NHEJ) to eliminate disease-causing genes, or homology-directed repair (HDR) to repair damaged genes, CRISPR/Cas editing provides a unique approach to treating many disorders [38,59,63].

Other Gene Editing Techniques

Programmable nucleases, such as zinc- finger nucleases (ZFNs), transcription activator- like endonucleases (TALENs), and CRISPR-associated Cas9 endonucleases, have been employed in the treatment of human cancer, infectious diseases, and genetic disorders [62,65-67]. Zinc finger nucleases (ZFNs) are chimeric nucleases consisting of a nonspecific FokI endonuclease domain fused to a sequence-specific DNA-binding domain [65]. This DNA-binding domain is composed of multiple zinc finger protein motifs, each typically recognizing a specific nucleotide triplet. The number and arrangement of these motifs determines both binding affinity and sequence specificity [65], as displayed in Figure 3.

The efficacy of a ZFN pair is determined by its binding affinity and sequence specificity. These factors also influence on-target modification and long-term stability. Notably, the number of zinc finger motifs directly impacts affinity and specificity. Pairings of 3+3 and 4+4 zinc fingers have demonstrated optimal activity compared to 5+5 and 6+6 configurations [68]. Several factors impact ZFNs' off-target behavior. For example, a negative relationship was shown by Cornu et al. (2008) between the toxicity linked to ZFN and the specificity of DNA binding. Furthermore, it has been demonstrated that off-target ZFN cleavage results from excess binding energy and that off-target activity may be reduced by varying spacer length [68]. Transcription Activator-Like Effector Nucleases (TALENs) are engineered restriction enzymes that combine the catalytic domain of FokI nucleases with the DNA-binding domain of Transcription Activator-Like Effectors (TALEs), as depicted in Figure 3 [68]. TALEs are naturally occurring virulence factors produced by Xanthomonas bacteria. These proteins bind to specific DNA sequences through a central DNA-binding domain, triggering the expression of host genes. The DNA-binding domain utilizes a unique one-to-one correspondence between individual amino acid repeats and specific DNA bases to recognize target sequences. TALENs are made up of TAL effectors bound to the FokI nuclease domain and identify DNA bases through conserved repeats that differ by two residues called the repeat variable residue (RVD), which gives specificity to individual bases. Along with their specificity, TALENs have varying levels of effectiveness, which vary depending on the kind of cell, particular target locations, length of impact, and delivery method. When compared to ZFNs and Meganucleases (MNs), TALENs' streamlined sequence recognition code offers advantages in terms of targetability and redesign [60,68].

Figure 3 (created with BioRender.com): Additional Gene-Editing Techniques TALEN, ZFN. The top illustration shows Zinc Finger Nucleases (ZFNs), which consist of a FokI nuclease domain fused to zinc-finger DNA-binding motifs positioned on opposite strands of the target DNA, where each zinc finger recognizes a specific nucleotide triplet. DNA cleavage occurs upon dimerization of the FokI nuclease domains. The bottom illustration shows Transcription Activator- Like Effector Nucleases (TALENs), which combine the FokI nuclease with TALE DNA- binding domains arranged along the target sequence. TALENs achieve high specificity through a one-to-

one correspondence between individual amino acid repeats (RVDs) and specific DNA bases. This figure illustrates the structural organization and DNA recognition mechanisms of both programmable nuclease systems.

Comparison of CRISPR-Cas9 with other gene-editing tools in terms of efficiency and specificity.

One of the main issues with clinical applications is the programmable nucleases' associated off-target activity and on-target efficiency. Multiple experimental methods have been established to objectively detect off- target activity in ZFNs, TALENs, and CRISPR- Cas9, despite the inconsistent outcomes [65,68].

For example, CRISPR- Cas9 has reportedly been shown to exhibit more editing effectiveness than TALENs, yet CRISPRs appear to be more prone to off-target actions than TALENs [65,69]. However, in HEK293FT cells and human induced pluripotent stem cells (iPSCs), additional research revealed that CRISPR- Cas9 and TALENs could achieve similar editing efficiency and high specificity [65]. For a more thorough knowledge of the three generations of artificial nucleases, direct comparison data between ZFN, TALEN, and CRISPR are currently absent [65].

Delivery Systems for Gene Therapy

Nanoparticle-Based Delivery

CRISPR- Cas9- mediated gene editing is more versatile, efficient, and accurate than previous gene- editing discoveries like zinc- finger nucleases and transcription activator- like effector nucleases [66,69]. Unfortunately, CRISPR-Cas9 cannot be used directly as a treatment due to its weak pharmacokinetic and pharmacodynamic properties. One further challenging obstacle to effective gene editing is the safe and effective delivery of CRISPR- Cas9 components to target cells and tissues [69]. Strong nucleic acid carriers are therefore required in order to deliver CRISPR- Cas9 into target cells for therapeutic applications [69]. Plasmids containing CRISPR-Cas9, sgRNA, or a Cas9 endonuclease protein complex with sgRNA are transferred via nanocarriers in this technique. It is challenging for CRISPR/ Cas9, irrespective of the delivery payload, to enter cells [69]. It's interesting to note that liposomes show promise as CRISPR/ Cas9 delivery systems.

PEGylation, or the surface coating of nanoparticles with poly (ethylene glycol) (PEG), is a widely used technique to improve the structural stability of the particles and prolong the duration of their blood circulation [7]. To achieve the necessary circulation time for CRISPR/ Cas9 delivery, liposomal carriers can be PEGylated. However, it would also decrease endosomal escape and cellular absorption at the same time, which lowers the overall efficacy of gene silencing [7].

Research recently reported on a CRISPR/Cas9 delivery method based on PEGylated liposomes. These scientists looked at the possibility of using PEGylated liposomes to transfer and maintain naked (unprotected) CRISPR/ Cas9 plasmids in serum [69]. They found that for up to six hours, PEGylated liposomes may shield plasmids from hydrolysis in serum. Moreover, within the four hours after the injection into the circulation, no DNA was found. By encasing CRISPR/Cas9 with the sgRNA specific to HPV E6 and E7 oncogenes within the freeze-dried matrix technology for cancer therapy, tumor development was effectively reduced and treatment was administered in vivo [38,69].

Advantages and Limitations of Nanoparticle Delivery Systems.

Developing tailored drug delivery systems for a variety of disorders, including cancer cells, is a potential area of study in nanomedicine [34]. Due to cancer cells' high rates of proliferation and resistance to conventional treatment approaches, researchers are concentrating on lipid-based drug delivery, which employs nanoparticles to encapsulate and transport medications to certain cells or tissues [34,66,69]. The therapy of cancer involves the use of many kinds of nanoparticles. Chemotherapy medications can be directly delivered to cancer cells using nanoparticles, increasing their efficacy and lowering their negative effects. By causing hyperthermia, they can also heat up and kill cancer cells. Furthermore, by improving medical imaging methods, nanoparticles can improve tumor surveillance and localization [34,44].

Furthermore, drugs can use nanoparticles to actively evade drug-resistant mechanisms in cancer cells, allowing the drugs to reach their targeted locations and provide therapeutic effects. Drugs can be released from nanoparticles in a regulated way, maintaining drug levels and reducing adverse effects [34]. Nanoparticle-based drug delivery systems provide several advantages in the treatment of cancer, such as improved drug targeting and penetration into tumor cells, which increase therapeutic effectiveness [66]. Nevertheless, these methods do have certain disadvantages, including potential toxicity problems and risks related to immune system recognition and clearance. For safe and efficient clinical applications, a great deal of research is required to optimize these systems and solve their shortcomings [34,44,66].

Viral Vectors for Gene Therapy

The CRISPR/ Cas9 system can be delivered to cells via various methods, including protein, mRNA, or DNA. Plasmids and adeno-associated viruses (AAVs) are commonly used viral vectors for introducing DNA encoding gRNA and Cas9. AAVs have a strong track record of safety and efficacy in clinical trials, making them a promising gene delivery vehicle. CRISPR/Cas9 delivered by AAV has been successfully employed to target numerous genetic diseases [23]. In the context of HPV18-related cancers, the AAV-E6-CRISPR/Cas9 system has been utilized to disrupt the E6 oncogene in HeLa cells. This disruption resulted in increased cell death and decreased cell growth, along with the restoration of p53 expression [23,26].

Potential challenges related to immunogenicity and off-target effects when using viral vectors.

The AAV vector's primary disadvantage is immunity to the AAV capsid, which can be partially attributed to the virus's non-enveloped nature and protein shell. The host immune response can therefore easily produce neutralizing antibodies [30]. These anti-capsid antibodies prevent AAV particles from entering target cells by neutralizing them even at low titers [30]. This implies that unless a new serotype is utilized, AAV vector-mediated distribution is essentially a one-time treatment that cannot be repeated [30].

Immunogenicity to AAV can be resolved by enrolling people who test negative for neutralizing antibodies; however, this approach is not ideal as it excludes a sizable section of the population [30,71]. One of the biggest concerns with genome editing therapy is the potential for off-target mutagenesis. The degree of danger is contingent upon the expression level and duration of Cas9/gRNA [30]. Therefore, transitory expression rather than long-term expression (like AAV) is preferable from a delivery standpoint to lower the possibility of off-target mutagenesis [30].

Efficacy and Outcomes of Gene Therapy for HPV

In Vivo Studies and Tumor Clearance

In vivo studies have demonstrated that AAV2-mediated delivery of granulocyte-macrophage colony-stimulating factor (GM-CSF) and B7-1 significantly reduced tumor growth and improved survival; however, transduction and transgene expression were largely confined to the needle track, limiting overall tumor transduction.

Since the introduction of gene therapy as a cancer treatment tool, immunotherapy-related strategies to boost immune responses against cancer cells have been employed. These include genetic vaccination against antigens unique to cancer cells, introducing immune stimulatory genes such as cytokines or costimulatory genes into cancer cells, and enhancing antigen presentation through the manipulation of antigen-presenting cells (APCs). Adeno-associated virus (AAV) vectors are widely used in cancer gene therapy research because they support efficient transgene delivery, can mediate long-term expression, and generally exhibit a favorable immunogenicity profile in vivo. In vivo, AAV very slightly stimulates the host immune system; long-term transgenic expression may be accomplished, and many therapeutic transgenes relevant to cancer fall within the packaging capacity of recombinant AAV (rAAV).

Immunogenic Cell Death (ICD)

One kind of apoptosis known as immunogenic cell death (ICD) is identified by the release of certain molecules into the environment that are members of the danger-associated molecular

patterns (DAMPs) family [72]. DAMPs are intracellular compounds that are often undetectable to live cells but exhibit immunostimulatory properties when they are exposed to or released by dying cells. Some anticancer medications can accelerate this process [73]. The induction of ICD is believed to be stressor-dependent as exposure to certain DAMPs requires the production of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress [73]. Stress activates a complicated signaling cascade in the ER, a eukaryotic organelle. ER stress is now a common "enabler" of ICD, described as disrupted ER homeostasis.

Research indicates that by accurately knocking down the cyclin-dependent kinase 5 (CDK5) gene, encapsulated CRISPR/Cas9 in nanoparticles may dramatically lower the expression of programmed death ligand-1 (PD-L1) on cancer cells [73]. Paclitaxel (PTX), which has a significant ability to transform "cold" tumors into "hot" tumors, can significantly lower regulatory T lymphocyte counts, improve antitumor immunity, cause immunogenic cell death, and repolarize tumor-associated macrophages when it is encapsulated in well-known nanoparticles [73].

Challenges in Achieving ICD Through CRISPR-Mediated Gene Therapy

Smart nanotechnology strategies can synergize chemotherapy with CRISPR technology to target immune-related genes. Chemotherapeutic agents such as doxorubicin (DOX), paclitaxel (PTX), and cisplatin are commonly used to induce tumor cell death; however, their ability to trigger bona fide immunogenic cell death (ICD) is often limited by tumor-intrinsic resistance mechanisms and immunosuppressive signaling in the tumor microenvironment.

In contrast to small-molecule chemotherapeutic agents, CRISPR/Cas9 components—such as plasmid DNA or Cas9-sgRNA ribonucleoprotein complexes—are large macromolecules.

This substantial size hinders the efficient co-delivery of multiple CRISPR components within a single nanocarrier [34].

Challenges and Limitations of Using Gene Therapy for HPV

Off-Target Effects

Despite CRISPR/Cas systems' great potential in translational medicine, off-target consequences remain a major challenge [74]. Cas9's cleavages on untargeted genomic sites may have undesirable consequences, referred to as "off-target effects." The off-target locations are often sgRNA-dependent since Cas9 has been demonstrated to tolerate up to three mismatches between sgRNA and genomic DNA [74]. Since off-target changes may have negative effects including cytotoxicity, genotoxicity, or likely chromosomal rearrangements, they have attracted a lot of interest in the field of genome editing for human therapeutic applications. One important factor in this process is determining the frequency of off-target edits throughout the entire genome.

Delivery and Efficiency Issues

Barriers to effective delivery of gene therapy tools to HPV-infected tissues. Since viral vectors may attach to fusogenic cell receptors, they often have greater transduction efficiencies. However, clinical translation of viral-vector-based CRISPR/Cas9 delivery still faces several serious challenges, including pre-existing immunity to AAV capsid proteins, restrictions on repeat dosing, and the risk of off-target genome editing arising from prolonged Cas9 expression. Conversely, non-viral vectors are often safer and less immunogenic, although they are less successful at transfection than viral vectors [43]. Live vector-based vaccinations utilize viral or bacterial vectors to deliver antigens. These vectors efficiently distribute antigens within host cells, resulting in strong immune responses. E6 and E7 antigens are delivered to dendritic cells (DCs), which present the antigens to T cells via MHC class I and II pathways. While these vaccines offer several advantages, there are potential risks, including adverse effects in immunocompromised individuals and limitations in achieving long-lasting immunity with repeated vaccinations [1].

Long-Term Implications and Safety Concerns

When used for genome editing, the RNA-guided bacterial antiviral defense system CRISPR-Cas (clustered regularly interspaced short palindromic repeats– CRISPR- associated protein) is emerging as a formidable new tool for creating effective treatments for hereditary illnesses [75-76]. The long-term effectiveness of CRISPR-Cas-Based in vivo gene treatments in treating a variety of ailments, from hematologic disorders to metabolic and muscular problems, has been shown by preclinical research conducted in mice, bigger animals, and non- human primate models. AAV and other conventional gene therapy vectors are used in many of these methods.

There are several established methods to lessen the possibility of a negative immune reaction after CRISPR-Cas9 gene therapy, including using Cas9 orthologs from nonpathogenic bacteria, promoting immunological tolerance or immune suppression, or focusing on immune-privileged tissues like the eye [76].

Future Directions in Gene Therapy for HPV Treatment

Emerging Gene-Editing Technologies

In 2019, David R. Liu's team published a study demonstrating that Prime editing (PE) enables all 12 possible base- to- base substitutions, as well as precise small insertions and deletions, without requiring donor DNA templates or double-strand breaks (DSBs). This greatly broadens the scope of therapeutic genome editing and, in principle, could correct approximately 89% of known pathogenic human variants. [31,77]. Furthermore, despite its relatively recent inception, PE has been extensively utilized to correct pathologic mutations from genetic disorders both in vitro and in vivo. This has significant promise to advance the science of gene therapy from bench to bedside [31].

Prime editors (PEs) and prime editing guide RNA (pegRNA) make up the PE system. Prime editors are proteins that combine a modified reverse transcriptase (RT) domain with a Streptococcus pyogenes Cas9 (SpCas9) H840A nickase mutant. PegRNA acts as the donor template for the desired alteration, whereas the correct genomic location on the non-edited DNA strand is located by the sgRNA which targets the nicking enzymes from the PE system [31,78].

The tool makes use of the specificity that is obtained from the traditional CRISPR Cas9, but it also includes a reverse transcriptase enzyme, an edit template, and guide RNA that must be inserted into the DNA target region [77,78]. Prime editing has attracted a lot of interest as a possible cancer therapy strategy throughout time. Radiation- and chemotherapy- based approaches have significant shortcomings that Prime Editing can overcome. A site- specific mutagenesis method with excellent genome editing accuracy and efficiency is called prime editing [77].

Personalized Medicine Approaches

Targeting patient-specific mutations and oncogene profiles is a fascinating and developing field of study in personalized gene treatments for HPV (human papillomavirus)-related malignancies [43]. Cervical, anal, oropharyngeal, and other genital malignancies are among the many cancers that have HPV as a major contributing factor. Certain oncogenes expressed by the virus, most notably E6 and E7, interfere with tumor suppressor proteins including p53 and Rb (retinoblastoma protein), which accelerates the development of cancer [79].

Recently, new antiviral RNA interference (RNAi) treatments using small interfering RNAs (siRNAs) have been created and evaluated in clinical studies. In mammalian cells, siRNAs can selectively silence endogenous genes, and in illnesses caused by viruses, they can selectively silence viral genes [79]. Remarkably, it has been shown that RNA interference (RNAi) targeting E7 or E6/ E7 increases TP53 and/ or pRB accumulation, which in turn causes HPV16- positive cervical cancer cell lines and HPV18-positive human cervical cancer cells to undergo apoptosis and/or senescence [79].

Integration with Immunotherapy

A very promising method for enhancing the immune system's innate anti-tumor responses is the

genetic alteration of immune cells. Immune cells may now target and eradicate tumor cells with increased anti- tumor specificity while protecting healthy tissue thanks to this strategy [29]. Chimeric antigen receptors (CARs), which are frequently produced by tumor cells, are integrated into immune cells to enable more accurate antigen identification and binding [29,80]. In the context of HR- HPV- induced cervical cancers, T cell receptor (TCR)- based therapies find viral proteins to be attractive targets since they are noticeably lacking from healthy cells. Targeting the oncoproteins HPV E6 and E7 is especially interesting because of their high expression in cervical cancer and excellent evolutionary conservation.

Preclinical studies produced encouraging findings, as modified E7 TCR- T cells successfully promoted the regression of tumors positive for HPV in mice models [29].

Conclusion

With the methods described in this paper, gene therapy has made amazing strides in treating genetic diseases that were previously incurable, including cervical cancer, even if there are still many challenges to be solved. While the potential of personalized medicine, innovative delivery strategies, and next-generation editing technologies promise to transform healthcare, research gaps in safety, long-term efficacy, and delivery must be filled. The trajectory of this science has the potential to change medicine in the years to come by treating complicated, age-related, and multifactorial diseases. This framework may be a useful place to start when talking about the gaps in the field's knowledge and potential future developments in gene.

AUTHOR CONTRIBUTIONS

Abdullah Bandar Baraka Almutiri: Conceptualization, literature search, drafting of the original manuscript, data organization, and preparation of all figures and tables.

Saad Obaid Alfraidi: Contributed to manuscript review and assisted in addressing reviewers' comments and editorial revisions during the submission process.

Abdallah Ali Hausawi: Contributed to early-stage manuscript review.

Mohammed Alsaeed: Provided expert input from an infectious diseases perspective and reviewed relevant virological aspects of the manuscript.

Fadhel Zaben Alotaibi: Reviewed the manuscript from a histopathology perspective and provided expert consultation on tissue pathology aspects.

Sari S. Sabban: Contributed to manuscript review and academic guidance.

Mohamed Morsi Mohamed Ahmed: Provided overall supervision, scientific guidance, and critical review of the manuscript from a molecular genetics perspective.

All authors reviewed and approved the final version of the manuscript.

Figures

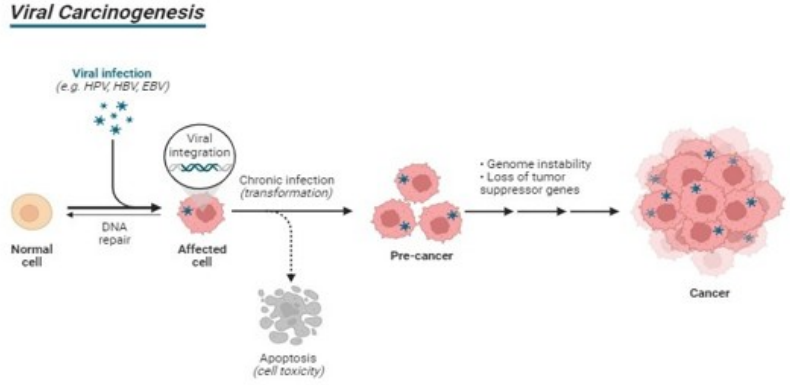

Figure 1: Schematic overview of HPV-driven carcinogenesis, including immune evasion, genomic instability, sustained angiogenesis, evasion of growth suppressors, replicative immortality, maintenance of cell survival, and dysregulated cellular energetics.

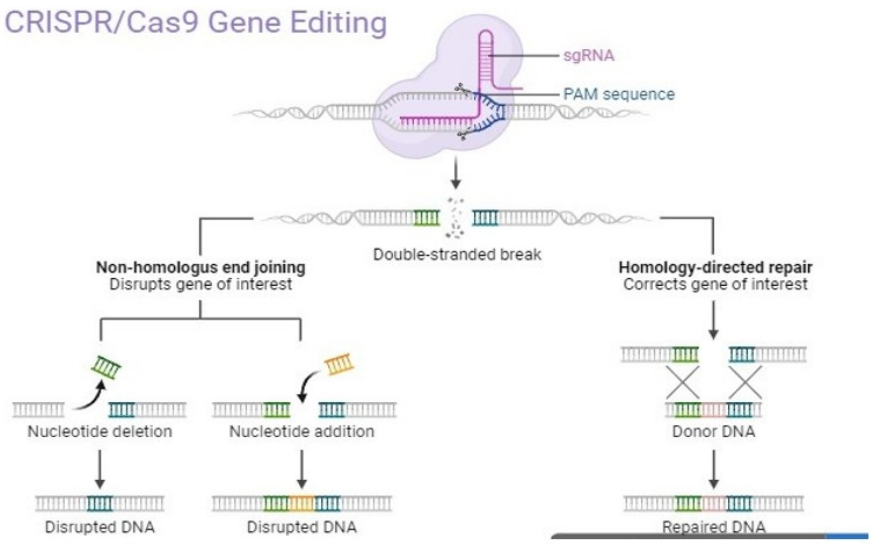

Figure 2: CRISPR-Cas9 genome editing workflow: Cas9/sgRNA introduces a double-strand break (DSB) at the target site, enabling gene disruption or repair via NHEJ or HDR pathways.

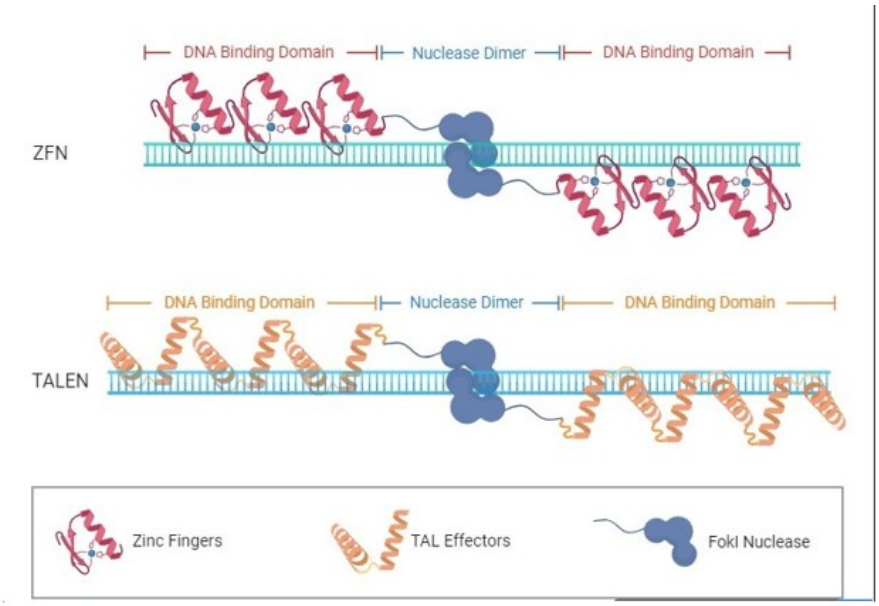

Figure 3: Additional Gene-Editing Techniques TALEN, ZFN.

Tables

S/n	Study type	Results	Limitations	References
1	In vitro	Results demonstrated that HPV16-E7 single-guide RNA (sgRNA) guided CRISPR/Cas system could break HPV16-E7 DNA at specific locations, resulting in growth inhibition and death in HPV-positive SiHa and Caski cells but not in HPV-negative C33A and HEK293 cells respectively. This was the first instance of this happening.	A primary concern regarding the use of the CRISPR/Cas system as an antiviral agent is its potential for off-target effects. The relatively short recognition sequence, 5′-N20NGG-3′, increases the likelihood of unintended cleavage events, particularly when mismatches occur at the 5′ end.	[59]
2	In vitro	The results demonstrated that producing a bacterial Cas9 RNA-guided endonuclease in combination with single guide RNAs (sgRNAs) specific for either E6 or E7 could cleave the HPV genome. This causes the E6 or E7 gene to acquire inactivating deletion and insertion mutations. Consequently, p53 or Rb are triggered, resulting in cell cycle arrest and, eventually, cell death.	It will be necessary to create adeno-associated virus (AAV)-based vectors that carry both a Cas9 and a sgRNA expression cassette in order to eliminate HPV-caused cancers in vivo.	[15]
3	In vivo	This study showed that the intravenous delivery of Cas9/16E7sgRNA plasmids packed in stealth liposomes significantly increased the survival rate of syngeneic mice by successfully clearing existing 16E7-driven tumours. These outcomes are like effectiveness trials conducted on immunocompromised mice.	Low liver toxicity was seen after systemic CRISR-lipoplex dosing.	[64]

Table 1: Studies demonstrating the efficacy of CRISPR-Cas9 in preclinical models (e.g., in vitro studies and mouse models).

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