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Crispr Cas – Revolutionizing Modern Therapies and Beyond

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Abstract

CRISPR-Cas technology has emerged as a transformative tool in modern molecular biology, revolutionizing both fundamental research and clinical applications. This RNA-guided gene-editing system enables precise and efficient genomic modifications, offering unprecedented potential for addressing genetic disorders, infectious diseases, and oncological conditions through innovative therapeutic interventions. The inherent specificity and programmability of CRISPR-Cas systems have facilitated breakthroughs in diverse fields, including precision medicine, regenerative therapies, and immuno-oncology. Beyond its therapeutic applications, CRISPR-Cas technology plays a critical role in agricultural biotechnology, environmental remediation, and synthetic biology, enabling the development of genetically modified organisms with improved traits, biosensors for pathogen detection, and novel bioengineered systems for sustainable bio-production. The ability to introduce targeted modifications at single-nucleotide resolution has expanded the frontiers of functional genomics, allowing researchers to systematically investigate gene function, regulatory networks, and epigenetic modifications with unprecedented accuracy. In contemporary medical therapies, CRISPR-Cas-based approaches are being explored for correcting monogenic disorders, engineering immune cells for adoptive cell therapies, and developing novel strategies for viral disease eradication. CRISPR-based diagnostic tools have also been harnessed for rapid, cost-effective detection of pathogenic infections and cancer biomarkers, paving the way for early disease intervention and personalized treatment regimens. Furthermore, theranostic applications integrating CRISPR-Cas technology with advanced imaging modalities are poised to enhance precision medicine by enabling simultaneous disease diagnosis and targeted therapeutic delivery. This article delves into the extensive impact of CRISPR-Cas systems on modern therapeutic paradigms, particularly in the fields of molecular diagnostics, targeted cancer therapies, and antiviral theranostics. Additionally, it examines the broader implications of this revolutionary technology for future biomedical research, ethical considerations, and translational medicine.

Keywords: CRISPR-Cas, immuno-oncology, molecular scissors, gRNA

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INTRODUCTION

Molecular scissors are the function of the CRISPR-Cas9 system. This opens the door for preclinical and clinical research to address a wide spectrum of treatment-resistant disorders by allowing scientists to change genetic material with unparalleled precision, efficiency, and flexibility. Numerous preclinical and clinical studies have been carried out using the CRISPR-Cas system to clarify genetic alterations monogenic human genetic illnesses, which impact individuals due to mutations in a single gene [1]. Additionally, some diseases with single or multiple gene mutation,

epigenetic regulation, and even precise point mutation may be rectified by the correction of genetic codes with CRISPR-Cas. Additionally, the CRISPR-Cas system has undergone significant development to fulfill its potential as a workable treatment strategy for a range of autoimmune conditions, malignancies, and infectious diseases [2, 3]. In 2018, the first clinical trial involving ex vivo editing of cells using the Cas9 system was approved for cancer treatment [4]. The Food and Drug Administration authorized the first in vivo clinical trial in 2019 [5]. A growing number of clinical trials indicate progress in various medical fields, including cancer, neurology, cardiovascular diseases, and infectious diseases, among others(https://clinicaltrials.gov).

Even though CRISPR-Cas technologies have therapeutic potential, several issues need to be resolved before they can be used in clinical settings. These include improving delivery methods, reducing off-target effects, managing immune responses, ensuring long-term safety, and addressing ethical concerns, such as germline editing. The CRISPR-Cas system has pioneered a transformative avenue in preclinical and clinical research, offering potential therapeutic strategies for a broad range of refractory diseases. Numerous monogenic human genetic disorders, which result from mutations in single genes, significantly impact human health. To address these conditions, several preclinical and clinical trials have leveraged CRISPR-Cas technology to target and correct the underlying genetic defects, thereby aiming to restore normal gene function and mitigate disease pathology [1, 3]. Notwithstanding its therapeutic potential, there are a few significant obstacles that must be overcome before CRISPR-Cas technologies may be used in clinical settings. These consist of enhancing delivery methods, reducing side effects, reducing immunological reactions, guaranteeing long-term safety and effectiveness, and attending to moral and legal issues.

Resolving these issues is essential for the successful translation of CRISPR-Cas technologies into effective clinical treatments for various diseases.

MECHANISM OF CRISPR

The components of Crispr Cas include a guide RNA (gRNA), the Cas9 protein, and a protospacer adjacent motif (PAM). The gRNA is a custom-designed RNA molecule that directs Cas9 to a specific DNA sequence through complementary base pairing. Cas9, a nuclease enzyme, then cuts the DNA at the target site, enabling edits through mechanisms like non-homologous end joining or homology-directed repair. For Cas9 to bind and function, a PAM sequence, such as NGG, must be present near the target DNA. Together, these components enable precise and efficient gene editing.

TARGET RECOGNITION

The gRNA binds to Cas9 and guides it to a specific DNA sequence with the complementary sequence in the genome.Cas9 searches for a PAM (Protospacer adjacent motif sequence) to ensure specificity and proper binding.

BINDING AND UNWINDING

The CRISPR-Cas9 system guided by a single-guide RNA (gRNA), precisely targets specific DNA sequences. This complex looks for a target DNA sequence in the genome that is next to a Protospacer Adjacent Motif (PAM), which is usually a brief conserved region.

Once the PAM site is recognized, the Cas9 protein binds to the DNA, inducing local unwinding of the double helix near the target sequence. This unwinding exposes the complementary strand of the DNA, allowing the gRNA to hybridize with its specific target sequence.

CLEVAGE

Cas9 introduces a double-strand break (DSB) at a precise location with three base pairs upstream of the PAM site.

CELLULAR REPAIR

After Cas9 creates a double-strand break (DSB) in the DNA, the cell initiates repair processes to maintain genomic stability. Two primary pathways are used to repair DSBs:

Non-Homologous End Joining (NHEJ) and **Homology-Directed Repair (HDR)**

Non-Homologous End Joining (NHEJ)

It is the dominant and faster repair mechanism in most cells, particularly during the G1 phase of the cell cycle when no homologous template is available. Without a homologous template, this process entails directly ligating the damaged DNA ends.

This is a quick repair process that might lead to insertion or deletion as a result can alter the gene sequence.

Homology-Directed Repair (HDR)

A precise repair mechanism using a provided DNA template to introduce desired changes into the genome. HDR is a highly precise but less frequently used repair pathway, active primarily during the S and G2 phases of the cell cycle when a homologous sequence is available. This pathway uses a homologous DNA sequence as a template to accurately repair the break.

APPLICATIONS

Crispr Case in Antiviral Therapies

CRISPR-Cas9 has demonstrated great potential in the quest for an effective HIV-1 treatment. The system works by using guide RNAs (gRNAs) to specifically target and cleave the viral genome, disrupting its ability to replicate. Targeting the long terminal repeat (LTR) sections of the HIV-1 genome using gRNAs is a particularly successful strategy in this area.

The LTRs are crucial sequences found at both ends of the HIV-1 genome, serving as regulatory regions essential for viral transcription, replication, and integration into the host genome. By targeting the LTR regions, CRISPR-Cas9 can potentially excise the integrated HIV-1 provirus from infected cells, effectively removing the viral genome and preventing reactivation of the virus from latent reservoirs [6]. Human T-cell in vitro research has shown that the CRISPR-Cas9 system is capable of effectively removing proviral DNA from the host genome.

This precise genomic editing results in a significant reduction in viral replication, highlighting the potential of CRISPR-Cas9 as a therapeutic strategy for targeting latent HIV-1 reservoirs and controlling viral persistence [7].

According to a study, Cas13-targeted HIV RNA genomes significantly lower viral RNA and prevent viral replication In CD + T-cells, Cas13 eradicated both latent and active HIV infection Targeting the CCR5 co-receptor, which serves as one of HIV-1's primary entrance routes into CD4+ T cells, is a similar strategy (Faivre N. et al., 2024).According to a study, viral infection in the MT4CCR5 cell line was successfully avoided by CRISPR-Cas9-mediated ablation of the CCR5 gene, a crucial co-receptor for HIV entrance, in conjunction with the HIV fusion inhibitor C46.

This dual strategy leverages CCR5 disruption to block HIV entry into host cells while using C46 to inhibit the fusion of the viral envelope with the host cell membrane, providing a robust barrier against infection [8]. This method attracted significant recognition after the "Berlin Patient" case, where a functional HIV cure was accomplished through a bone marrow transplant from a donor carrying the CCR5 Δ32 mutation, a genetic alteration that impairs CCR5 functionality [9]. The CRISPR-Cas9 system can be designed to create precise double-strand breaks (DSBs) in the Hepatitis B Virus (HBV) genome, effectively targeting and disrupting critical viral genes, like S, C, and X, which are essential for viral replication, thereby suppressing the virus's ability to reproduce [10].

Furthermore, CRISPR-Cas systems have been developed to facilitate the removal of covalently closed circular DNA (cccDNA), the stable viral reservoir within infected hepatocytes, which poses a significant obstacle to achieving a definitive cure [11, 12]. Recent research has demonstrated that CRISPR-based editing of HBV DNA lowers the viral load and decreases the production of viral antigens, such as hepatitis B surface antigen (HBsAg), which plays a key role in evading the immune response [13]. Although clinical trials have not yet begun, preclinical successes indicate that CRISPR-Cas9 could one day serve as a treatment for chronic HBV infection. Furthermore, CRISPR-Cas9 has been investigated as a possible treatment approach for human papillomavirus (HPV), particularly high-risk strains, like HPV16 and HPV18, that are closely associated with cervical cancer. The goal of this strategy is to interfere with the viral oncogenes E6 and E7, which cause infected cells to develop into malignant cells [14].

In vitro experiments with cervical cancer cell lines have shown that CRISPR-Cas9 can effectively target and disable these oncogenes, resulting in the apoptosis of infected cells and a decrease in tumor growth [15]. These results have been validated in vivo using mouse models, where CRISPR-Cas9 therapy has demonstrated effectiveness in shrinking the size of HPV-related tumors [16, 17]. The outbreak of SARS-CoV-2, the virus behind COVID-19, has led to growing interest in applying CRISPR technologies as antiviral solutions. Unlike other approaches, RNA-targeting CRISPR-Cas13 has been explored to cut the RNA genome of the SARS-CoV-2 virus. Targeting and degrading specific SARS-CoV-2 RNA using CRISPR-Cas13 has reduced viral replication in cell culture systems. This RNA-focused method holds significant potential for RNA viruses, like SARS-CoV-2, providing a swift and precise means of antiviral intervention [18, 19]. Although still mostly in preclinical phases, these studies collectively underscore CRISPR's immense promise in fighting viral infections by directly targeting viral genomes, key replication genes, or cellular receptors vital for viral entry, demonstrating encouraging outcomes against viruses that have long posed challenges to traditional treatment methods.

APPLICATION OF CRISPR CAS SYSTEM IN COVID 19

First discovered in late 2019, coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the worst pandemic in recent memory, affecting more than 215 nations and territories worldwide [20]. The CRISPR-Cas systems were created as rapid and targeted techniques for diagnosing SARS-CoV-2 [21–23]. In comparison to the conventional molecular diagnostic technique, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), CRISPR-based diagnostic platforms for SARS-CoV-2 detection offer advantages in terms of cost-effectiveness, specificity, sensitivity, and the absence of the need for complex instrumentation.

Consequently, this innovative approach holds the potential for rapid detection and streamlined application at the point-of-care. Furthermore, CRISPR-based detection may enhance test reliability by mitigating or eliminating issues associated with false negatives, false positives, or inconclusive results commonly observed in RT-qPCR assays [24]. Furthermore, CRISPR-Cas systems have been explored for therapeutic applications in COVID-19. One such tactic, known as PAC-MAN (prophylactic-antiviral CRISPR in human cells), targets and destroys the SARS-CoV-2 virus in human cells by utilizing Cas13d's RNA-guided RNA endonuclease activity.

This approach has been shown to effectively recognize and cleave SARS-CoV-2 RNA sequences using precisely designed guide RNAs in lung epithelial cells. Additionally, bioinformatics analyses have demonstrated that as few as six crRNAs can target 91% of 3051 sequenced coronavirus strains, suggesting that this strategy could provide a robust platform for combating future coronavirus-driven pandemics [25]. With the development of sophisticated, dependable, secure, and effective delivery mechanisms, the CRISPR-Cas13 system may provide an alternate treatment approach for COVID-19, whereas PAC-MAN is a proof-of-concept antiviral strategy.(Lotfi M. and others, 2020) [26].The creation of an efficient in vivo delivery system is necessary for the therapeutic use of CRISPR-Cas13 systems to treat COVID-19. Further validation of PAC-MAN in pertinent preclinical models, including

rhesus macaques, is necessary to evaluate its antiviral activity, specificity, and potential immunogenicity generated by Cas13d. As a possible therapeutic approach to fight COVID-19 and other viral dangers, PAC-MAN shows promise.

GENOME EDITING CRISPR BASED THERAPIES

Changes in an individual's genomic sequence, frequently brought on by mutations in certain genes, give rise to genetic illnesses. These mutations may lead to the creation of non-functional or dangerous proteins, producing cellular malfunction and a wide spectrum of disorders that vary in severity. Due to faulty genes, many genetic illnesses are transmitted and transferred from parents to children. These disorders are primarily caused by mutations, which interfere with regular biological functions and aid in the emergence of disease [27]. Such genetic variants can have serious negative effects on human health, including physical disabilities and chronic illnesses, which can often result in serious medical and financial difficulties for people as well as healthcare systems [28].

Genetic illnesses' financial effects on a pediatric accountable care organization are tremendous [29]. In terms of both financial expenses and human misery, the burden of hereditary diseases is immense. Because sickle cell disease and thalassemia are chronic conditions that require ongoing treatment, they significantly strain healthcare resources [28].

By enabling precise genome editing and providing novel therapeutic approaches for hereditary illnesses that were previously incurable and life-threatening, CRISPR-Cas technology has completely transformed genetic medicine. To restore normal gene activity, this method enables targeted double-strand breaks (DSBs) for precise gene knockouts, single-strand breaks, and particular nucleotide changes, insertions, or repairs. The goal of somatic gene editing with CRISPR is to alter non-reproductive cells while making sure that the genetic alterations are not transmissible. This strategy represents a big breakthrough in precision medicine and has great promise for treating genetic diseases and different types of cancer [29, 30]. Research employing hematopoietic progenitor cells (HPCs), animal models, and cell cultures has produced strong proof of the effectiveness of CRISPR-based treatments in repairing harmful mutations and successfully reestablishing normal gene activity [31]. By focusing on the α-globin locus in human hematopoietic stem/progenitor cells (HSPCs), a study showed that CRISPR-Cas9 has the potential to treat β-thalassemia. By deleting the HBA2 gene, the technique downregulated the expression of α-globin, while integrating a β-globin transgene increased the expression of β-globin. This technique maintained long-term hematopoietic repopulation in xenotransplanted animals and effectively repaired the diseased phenotype in cellular models.

Additionally, in HSPCs derived from β-thalassemia patients, the strategy effectively restored the α/β-globin balance. The use of a Cas9 nickase variant further improved the safety and precision of the editing process, reducing off-target effects [32].

Hemochromatosis, a common hereditary metabolic disorder among individuals of European descent, is frequently attributed to a C282Y mutation in the HFE gene. This mutation, characterized by a guanine-to-adenine substitution at nucleotide c.845, impairs proper folding of the HFE protein, preventing its localization to the cell membrane. The lack of membrane-bound HFE disrupts its interaction with transferrin receptors 1 and 2, resulting in dysregulated iron homeostasis and subsequent systemic iron overload [33]. In a study, optimized guide RNAs (gRNAs) were evaluated in cell culture, and an AAV8 split-vector system was employed to deliver the adenine base editor ABE7.10 along with a specific gRNA in 129-Hfetm1.1Nca mice. A single administration achieved correction of the target mutation in over 10% of hepatocytes, leading to significant improvements in hepatic iron metabolism. These findings highlight the potential of this approach as a gene correction therapy for addressing this prevalent hereditary disorder [34].

A variety of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene result in the recessive genetic condition known as cystic fibrosis (CF). This disorder causes poor

transport of chloride ions through epithelial cells, which causes thick mucus to build up in several organs. A subset of CF patients harbors mutations that are unresponsive to CFTR modulator therapies, leaving approximately 8% of affected individuals without viable treatment options [35]. A mutation-agnostic therapeutic strategy has been proposed utilizing CRISPR-Cas9 technology to insert cystic fibrosis transmembrane conductance regulator (CFTR) complementary DNA (cDNA), along with a transcription termination sequence, into exon 1 of the CFTR locus. Under the CFTR gene's inherent transcriptional control, this alteration makes it easier for functional CFTR mRNA to be expressed. This method is safe, according to preliminary research, with little indication of genomic reorganizations or a reduction in the ability of modified basal epithelial cells to regenerate. Notwithstanding these results, there are still unanswered questions regarding the strategy's potential as a long-term treatment option for cystic fibrosis. These include the possible influence of low-level chromosomal abnormalities (~1%) and the difficulties in achieving successful cell implantation and targeted in vivo delivery to basal cells of the airway epithelium [36].

Alzheimer's disease is a neurological condition that worsens over time and is characterized by personality changes, memory loss, and a slow deterioration in cognitive function.

These symptoms are primarily attributed to the pathological accumulation of amyloid-β plaques and tau neurofibrillary tangles within the brain, which disrupt cellular function and synaptic communication, leading to neuronal degeneration [37]. In vivo gene editing within adult brain neurons presents a promising therapeutic approach for treating neurological disorders, including Alzheimer's disease. A recent study developed CRISPR-Cas9-based nano-complexes to facilitate precise genome editing in the adult mouse brain. This innovative delivery system demonstrated high efficacy in targeting the central nervous system, while maintaining minimal off-target effects, thus ensuring both safety and specificity of gene editing. The study focused on the Bace1 gene, a key regulator of amyloid precursor protein processing, which plays a critical role in the pathogenesis of Alzheimer's disease. By targeting and disrupting the Bace1 gene, the CRISPR-Cas9 nano-complexes effectively reduced amyloid-β (Aβ) deposition, alleviating Aβ-related neuropathological features, such as plaque accumulation and neuroinflammation. Furthermore, the treatment ameliorated cognitive impairments observed in two distinct Alzheimer's disease mouse models These results highlight how CRISPR-Cas9 gene editing can be a revolutionary tool for diagnosing and treating neurodegenerative diseases, providing a new therapeutic approach for Alzheimer's disease and possibly other associated conditions [38].

Deficits in mice models of Alzheimer's disease are mitigated by in vivo neural gene editing using CRISPR–Cas9 amphiphilic nanocomplexes. The term "severe combined immunodeficiency" (SCID) describes a collection of uncommon, diverse hereditary diseases marked by significant deficits in the development and operation of lymphocytes, which are essential elements of the adaptive immune system.

These defects result in a marked deficiency of both T-cell and B-cell responses, leading to a severely compromised immune defense against infections. SCID patients are highly susceptible to life-threatening infections, and without early intervention, the condition is often fatal. The underlying genetic mutations that cause SCID can involve various genes, including those critical for lymphocyte development and signaling pathways essential for immune responses [39].

A recent study examined the possibility of using the CRISPR-Cas9 gene-editing technology to modify hematopoietic stem and progenitor cells (HSPCs) ex vivo to fix mutations that cause severe combined immunodeficiency (SCID).

To develop a robust model for studying gene correction strategies, the researchers first introduced SCID-associated mutations into healthy donor-derived CD34+ HSPCs. This model allowed for the examination of gene editing techniques aimed at restoring normal immune function. Using this system, the researchers successfully performed gene correction in HSPCs derived from a RAG2-SCID patient,

resulting in the generation of CD3+ T cells that exhibited a diverse repertoire of T cell receptors. These findings suggest that targeted CRISPR-Cas9-based gene editing in patient-derived HSPCs holds promise as a strategy for restoring functional immune responses and offers a potential therapeutic approach for SCID [40]. Recently, a growing number of genetic disorders have been addressed using CRISPR-Cas gene therapy, mainly through proof-of-principle studies.

CRISPR-CAS TECHNOLOGY IN CANCER THERAPY

CRISPR is increasingly recognized as a potent nucleic acid-based detection tool in cancer diagnostics due to its high precision and specificity. The Cas protein's endonuclease activity has been used to find mutations linked to cancer in patient samples [41, 42]. Fast and effective enrichment of small genomic areas and ultra-accurate sequencing with minimum DNA input are made possible by targeted genome fragmentation using CRISPR/Cas9 (CRISPR-DS). CRISPR-mediated ultrasensitive detection of target DNA (CUT-PCR), for instance, was developed by Iancu et al. [41] and allows for the exceptional sensitivity (<0.01%) and accuracy of detecting trace amounts of circulating tumor DNA (ctDNA), a potential cancer-specific biomarker, from patient blood samples.

This approach leverages CRISPR endonucleases, specifically Cas9 and Cas12, to selectively degrade the wild-type DNA sequence, thereby enriching the sample for mutant DNA. The mutant DNA is then amplified via polymerase chain reaction (PCR) and subsequently analyzed through sequencing. Importantly, this method has demonstrated its efficacy in detecting oncogenic mutations within ctDNA derived from the blood of colorectal cancer (CRC) patients, highlighting its potential for non-invasive cancer diagnostics [42].

In a different work, the CRISPR/Cas system was used to target the destruction of short tandem repeats (STRs) and then sequence the results to find microsatellites, a cancer biomarker [43]. Furthermore, another study employed a CRISPR/Cas9-based fragmentation strategy in combination with duplex sequencing (DS), which incorporates double-stranded molecular barcoding, referred to as CRISPRDS. This technique facilitates the efficient enrichment of targeted genomic regions, even from minimal quantities of input DNA. This method is currently being clinically evaluated for the identification of TP53 gene oncogenic mutations, particularly in the peritoneal fluid of women with ovarian malignancies. This technique has the potential to increase the sensitivity and precision of identifying genetic changes unique to tumors in liquid biopsy samples [42].

Furthermore, several sophisticated methods, such as DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) and Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK), use the CRISPR-associated proteins Cas12 or Cas13 in combination with isothermal amplification to identify nucleic acids at the single-molecule level, whether they are DNA or RNA [42]. These systems enable the detection of patient-specific mutations in tumor biopsies with exceptional specificity, including the ability to distinguish single-base mismatches, and exhibit remarkable sensitivity, with limits of detection as low as 2 attomolar. Such capabilities make these methods highly promising for precise, early detection of oncogenic mutations and other genetic alterations associated with cancer [44]. For instance, Chen et al. (2018) [45] created and applied the SHERLOCK platform to identify mutations linked to cancer in liquid biopsy samples taken from patients with lung cancer.

This system demonstrated exceptional sensitivity, capable of identifying mutations present at frequencies as low as 0.1% of the total DNA in the sample. Such sensitivity underscores the potential of the SHERLOCK platform for non-invasive and early detection of cancer-related genetic alterations [45].

A Cas12-based one-pot isothermal test that combines rolling circle amplification (RCA) and Cas12a's endonuclease activity was recently developed to detect cancer-associated microRNAs (miRNAs) with great sensitivity.

This system demonstrated the capability to detect miRNAs in pancreatic cancer patients with remarkable sensitivity, achieving detection limits at femtomolar concentrations. Additionally, the assay exhibited single-nucleotide specificity, underscoring its potential for precise identification of miRNA biomarkers in cancer diagnostics [46, 47]. An assay for the sensitive detection of microRNAs based on one-pot isothermal Cas12. These developments highlight the CRISPR/Cas system's potential as a powerful platform for identifying biomarkers unique to cancer. With continuous progress and refinement in the field, the CRISPR/Cas system is poised to emerge as a highly sensitive and precise diagnostic tool, offering personalized approaches for the early detection and monitoring of cancer in individual patients.

CRISPR CAS IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING

The ability of stem cells, especially pluripotent stem cells (PSCs), to develop into a variety of cell lineages makes them extremely promising for use in regenerative medicine.

However, their therapeutic application is limited by several challenges, including genomic instability, suboptimal differentiation efficiency, and the risk of immunogenicity. These limitations pose obstacles to their clinical translation and underscore the need for advanced strategies to enhance the safety, efficacy, and reliability of stem cell-based therapies [47].

CRISPR-Cas technology provides a powerful tool for addressing these challenges by facilitating precise and targeted genetic modifications to enhance the therapeutic potential of stem cells. This approach enables the correction of pathogenic mutations, the enhancement of differentiation efficiency, and the reduction of immunogenicity through the modification of key genetic pathways. By leveraging its high specificity and versatility, CRISPR-Cas technology offers a means to improve the safety, functionality, and clinical applicability of stem cell-based therapies [48]. Correcting harmful mutations in induced pluripotent stem cells (iPSCs) to cure genetic illnesses is a well-known example of how CRISPR-Cas9 is used in regenerative medicine. In a groundbreaking study, iPSCs taken from patients with Duchenne muscular dystrophy (DMD) had the mutation that causes the disease accurately corrected using CRISPR-Cas9 technology. The potential of CRISPR-Cas9 to produce genetically modified, patient-specific iPSCs for therapeutic applications in regenerative medicine was demonstrated by this repair, which restored the expression of functional dystrophin protein (Li H. L. et al., 2014) [49]. Myogenic progenitor cells (MPCs) that could restore dystrophin expression were produced from the genetically modified iPSCs. These MPCs effectively incorporated into host muscle tissue and produced functional dystrophin protein in mouse muscle cells after being transplanted into a model of Duchenne muscular dystrophy (DMD).

This study exemplifies the therapeutic potential of CRISPR-Cas9 technology in regenerative medicine by demonstrating the successful correction of a monogenic mutation and the generation of functional muscle cells. These findings highlight the feasibility of using CRISPR-Cas9 as a gene-editing tool for developing targeted treatments for DMD and other monogenic disorders [50]. Through the targeting and modification of important transcription factors involved in cardiac differentiation, this method has been shown to produce cardiomyocytes from human embryonic stem cells (hESCs) [51].

A significant obstacle in stem cell transplantation is addressed by CRISPR-Cas technology, which makes it possible to create stem cell lines with decreased immunogenicity. In a noteworthy work, induced pluripotent stem cells (iPSCs) from mice and humans were genetically modified to overexpress the immunological checkpoint protein CD47 and disrupt MHC class I and II genes to reduce immune rejection.

These hypoimmunogenic iPSCs maintained their pluripotency and differentiation capacity, successfully differentiating into endothelial cells, smooth muscle cells, and cardiomyocytes capable of evading immune detection. Remarkably, these engineered cells demonstrated long-term survival in fully mismatched allogeneic recipients without the need for immunosuppressive therapy. These findings

underscore the potential of CRISPR-Cas-mediated genetic engineering to develop universal, immune-compatible cell therapies for regenerative medicine [52].

The goal of tissue engineering is to create functioning tissue constructions that can replace diseased or damaged tissues. CRISPR-Cas technology has emerged as a powerful tool for enhancing tissue regeneration by facilitating precise modifications of key genetic pathways that regulate cellular processes, including proliferation, differentiation, and extracellular matrix (ECM) production. Increasing the ability of endogenous cells to regenerate is a key use of CRISPR-Cas in tissue regeneration. In animal models of muscle injury, groundbreaking work showed that activation of the Wnt/β-catenin signaling pathway via CRISPR-Cas9 significantly enhanced muscle regeneration. This was accomplished by focusing on regulatory components in the pathway to raise muscle stem cells' β-catenin levels, which improved the cells' capacity for self-renewal and differentiation. Muscle progenitor cells, which are essential for the regeneration and repair of muscle fibers, proliferated due to this pathway's activation. These results highlight how CRISPR-Cas technologies can alter important signaling pathways, providing a viable approach to developing tissue engineering and regenerative medicine [53]. Because CRISPR-Cas technology allows cells to be precisely engineered to produce therapeutic factors in response to tissue injury, it holds great potential for the advancement of regenerative medicine.

By utilizing this gene-editing tool, researchers can precisely control the expression of specific genes within targeted cells, thereby enabling the dynamic synthesis of critical factors, such as growth factors, cytokines, or extracellular matrix components, exclusively at sites of injury. This tailored approach could optimize the regeneration process, enhancing tissue repair and functional recovery while minimizing off-target effects. The ability to modulate gene expression with high specificity opens new avenues for developing targeted therapies that promote tissue regeneration and healing [54]. A revolutionary step forward in the creation of innovative treatment approaches for a variety of illnesses and tissue damage is the use of CRISPR-Cas technology in tissue engineering and regenerative medicine. By enabling precise genome editing of stem cells, CRISPR-Cas facilitates the targeted modulation of gene expression, thereby enhancing the regenerative potential of tissues. This technology allows for the correction of genetic defects, the promotion of cellular reprogramming, and the optimization of tissue regeneration processes. Moreover, CRISPR-Cas not only addresses longstanding challenges in cellular engineering and therapeutic intervention but also paves the way for the development of innovative, personalized approaches to tissue repair and regeneration, offering new possibilities for precision medicine.

CRISPR CAS IN MEDICAL DIAGNOSTICS

The creation of CRISPR-based detection tools, like SHERLOCK, is one of the advances in CRISPR-Cas-based diagnostics [55, 56]. Technologies based on CRISPR-Cas13 and Cas9 can both target certain DNA and RNA sequences. Cas13 changes conformation when it binds to its target RNA, triggering its ribonuclease activity and causing non-specific cleavage of neighboring RNA molecules.

In certain cases, this ribonuclease activity can extend to DNA if the system is engineered for such specificity. When integrated with reporter probes conjugated to quenchers, this catalytic property results in the generation of a detectable signal, which facilitates the sensitive detection of nucleic acids, even at low abundance. This mechanism allows for highly sensitive, real-time nucleic acid detection, enhancing the ability to detect specific genetic material in various biological and diagnostic applications [56]. DETECTR, on the other hand, targets DNA using Cas12. Like Cas13, Cas12 activates its collateral cleavage activity by binding to its target DNA and causing cleavage in reporter probes that are close to the target to provide a signal that can be detected [44].

Both CRISPR-Cas12 and Cas13 platforms leverage their distinctive collateral cleavage activities to amplify detection signals, enabling the precise and rapid identification of trace amounts of nucleic acids. These catalytic activities, which result in non-specific cleavage of adjacent nucleic acids upon target

binding, significantly enhance signal output, allowing for highly sensitive detection. These features make these systems invaluable for a wide range of diagnostic applications, including viral pathogen detection and the analysis of genetic mutations. The ability to detect minute quantities of nucleic acids with high specificity and speed positions CRISPR-based platforms as powerful tools in molecular diagnostics and precision medicine [57] has been successfully employed to detect RNA viruses, including Zika and Dengue, as well as bacterial pathogens, such as *Mycobacterium tuberculosis*. This platform integrates CRISPR-Cas13a, a ribonuclease with collateral cleavage activity, with isothermal amplification techniques, notably recombinase polymerase amplification (RPA). The combination of these technologies enables the rapid and sensitive detection of target nucleic acids without the need for complex thermal cycling equipment, thus simplifying diagnostic workflows. SHERLOCK is a useful tool for point-of-care testing in environments with limited resources because of its ability to do quick, high-sensitivity diagnostics and its low infrastructure requirements [58].

Similarly, DETECTR has demonstrated great specificity and sensitivity in identifying DNA viruses, including the Human Papillomavirus, utilizing Cas12a (Yin L. et al., 2021). To manage infectious disease epidemics and facilitate prompt medical intervention, CRISPR-based diagnostic platforms have become essential point-of-care testing tools. Compared to traditional PCR-based techniques, these systems have several benefits, especially in environments with limited resources. CRISPR-based diagnostics' mobility, simplicity of usage, and quick turnaround time greatly increase their usefulness in a variety of clinical and field settings.

Unlike traditional PCR, which often requires specialized equipment and infrastructure, CRISPR diagnostics can be deployed with minimal technological resources, facilitating widespread access to accurate and timely testing. This makes CRISPR-based approaches particularly valuable for fast response in public health emergencies and for providing essential diagnostics in low-resource environments [59, 60].

Furthermore, the continuous refinement of CRISPR-based systems to recognize an expanded range of genomic and transcriptomic targets, coupled with their integration into complementary diagnostic platforms, such as lateral flow assays and microfluidic systems, promises to enhance their versatility and utility. The advent of CRISPR-Cas technology is revolutionizing medical diagnostics by providing more sensitive, specific, and rapid methods for detecting a wide array of genetic markers and pathogens. In addition to increasing diagnostic precision, this integration makes point-of-care testing easier, allowing for quicker and more effective disease monitoring and diagnosis.

The ongoing advancements in CRISPR-based diagnostics are poised to significantly impact clinical decision-making and disease management, heralding a new era in precision medicine.

CONCLUSION

CRISPR-Cas9 has ushered in a new era of potential within the biosciences, offering groundbreaking possibilities across various fields, from medicine to agriculture. Its future holds both promise and uncertainty, with applications that address some of humanity's most significant challenges. However, as this novel frontier is navigated, it is crucial that the research is proceeded with caution, adhering to ethical principles and maintaining a collective responsibility for societal welfare. Researchers are expanding the potential of this technology as regulatory environments continue to change.

Potential applications include the treatment of previously incurable genetic disorders and significant advancements in the understanding of complex diseases, such as Alzheimer's and HIV. CRISPR-Cas9 encourages a reimagining of the limits of human ingenuity and invites a collaborative effort in shaping the future of science and medicine.

The ideal gene therapy should be cost-effective, simple, highly specific, rapid, portable, user-friendly, safe, and exhibit robust efficacy. The CRISPR-Cas systems have emerged as the most prominent and

widely studied tools for precise genome editing, earning recognition as one of the most powerful platforms for therapeutic gene modification across a broad spectrum of diseases. However, despite their promise, significant challenges remain in translating these technologies into widespread clinical applications for patients suffering from currently untreatable conditions. These obstacles are largely due to issues, such as delivery efficiency, tissue specificity, off-target effects, and immunogenic responses. The ongoing evolution of CRISPR-Cas systems continues to enrich the molecular toolkit available for gene editing, driving the exploration of novel strategies to improve therapeutic outcomes. As a cutting-edge genome-editing approach, CRISPR-based technologies have already demonstrated promising results in modulating disease pathology, offering potential avenues for ameliorating or even curing various genetic disorders. Nevertheless, to achieve clinical applicability, further research is required to optimize delivery mechanisms, ensure tissue-specific expression, enhance gene correction precision, and minimize off-target mutations and immune responses.

Moreover, CRISPR gene-editing technologies must meet the rigorous standards of clinical care, comparable to existing approved therapeutic modalities. In conclusion, given the numerous advantages of CRISPR-Cas systems over previous gene-editing methods, coupled with the concerted efforts of researchers worldwide, it is anticipated that these technologies will eventually realize their full potential to mitigate or cure a wide array of human diseases, thereby revolutionizing the field of medicine. CRISPR technology has greatly expanded the field of genetic research in just ten years since it was first used as a gene-editing tool in mammalian cells, allowing for accurate and effective genome manipulation.

This transformative technology has not only facilitated the targeted correction of genetic disorders but has also introduced new opportunities in epigenome editing, offering potential therapeutic strategies for non-genetic diseases.

Moreover, recent innovations in base editing and prime editing have substantially enhanced our ability to model and characterize disease-associated mutations, providing invaluable insights into the molecular underpinnings of various pathologies. These advanced editing techniques are poised to play a pivotal role in identifying novel therapeutic targets and elucidating mechanisms of drug resistance. Such advancements are particularly promising in the context of oncology, where CRISPR-mediated gene-editing strategies will likely contribute to the development of precision medicine approaches tailored to individual cancer profiles. As the field progresses, continued advancements in CRISPR-based technologies will accelerate drug discovery, optimize treatment regimens, and enhance diagnostic capabilities, ultimately leading to more effective and personalized therapeutic interventions. The potential of CRISPR technology in advancing both our fundamental understanding of disease mechanisms and its clinical applications underscores its immense promise for the future of medicine.

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