The Impact of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in Healthcare: A Narrative Review

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ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are deoxyribonucleic acid segments that can be used in gene editing to provide a defence mechanism against viral DNA in prokaryotes. Compared to other genome editing techniques, the CRISPR/Cas9 system is simpler, more accessible, and highly efficient for precise DNA modifications. This review examines CRISPR's applications, particularly its potential in treating hereditary and chronic diseases. CRISPR technology offers advancement in treating diseases such as sickle cell disease, beta-thalassemia, haemophilia, cystic fibrosis, Duchenne muscular dystrophy, cardiovascular diseases, diabetes, and cancer. CRISPR enhances detection methods for infectious diseases like tuberculosis, human immunodeficiency virus and hepatitis B. By integrating CRISPR technology into healthcare, significant advancements can be made in the treatment and prevention of genetic diseases

Keywords: CRISPR, genome editing, hereditary diseases, cancer, chronic illness

INTRODUCTION

Over 3000 genes have been implicated in mutations that cause diseases.[1] Human diseases linked to these mutations include cystic fibrosis, cancer, Huntington's disease, and sickle-cell anaemia. Molecular approaches and specialized tools that ensure precise gene editing, are essential for comprehension and accurate treatment of disorders arising from genetic mutations.[2]

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has evolved as a revolutionary genetic defense system primarily occurring in certain prokaryotes. It offers several benefits in comparison to nuclease-based genome-editing approaches such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).[3,4] ZFNs are made up of zinc finger proteins (ZFPs) combined with a general Fok1 cleavage region.[5] Fok1 is a type of restriction enzyme that functions like a pair of molecular shears used to snip Deoxyribonucleic acid (DNA) at precise sites. In the context of gene editing, a set of two ZFNs are utilised, one positioned upstream and the other downstream of the target site, to create double-strand breaks (DSBs) in DNA.[6] One potential drawback is the constrained range of target site selection. TALENs were developed as an alternative to ZFNs in genome editing, facilitating more precise introduction of DSBs.[7] Like ZFNs, TALENs are composed of a combination of transcription activator-like effectors and Fok1 restriction enzyme. Transcription activator-like effectors are composed of amino acid sequences located near a DNA binding site. TALENs, in contrast to ZFNs, have a heightened precision in gene editing due to their capacity to target single nucleotides.[7] However, the larger size of TALENs compared to ZFNs poses a challenge in terms of packaging and delivery of genes.[1]

Compared to other gene editing techniques such as ZFNs and TALENs, the benefits that CRISPR offers include higher efficiency, precision, capability to target multiple genes, and cost-effectiveness.[8] Bacteria acquire immunity through a process known as horizontal gene transfer (HGT), allowing them to incorporate genetic material from viruses in their environment.[9] Initially, a bacterium may lack defence against a specific virus, but if it survives an initial infection, it integrates a fragment of the viral deoxyribonucleic acid (DNA) into its own genome, forming part of the CRISPR array. Over time, the bacterium accumulates a library of these viral DNA fragments. The phrase "Clustered Regularly Interspaced Short Palindromic Repeats"

(CRISPR) denotes the distinct portions of viral DNA that become integrated into the bacterium's genetic code. These sequences serve as a genetic memory, enabling the bacterium to recall and identify previous viral intruders.[10]

The process of CRISPR involves an intricate relationship between DNA, Ribonucleic acid (RNA), and proteins. In DNA, there are four bases: adenine (A), guanine (G), cytosine (C) and thymine (T), which pair up specifically: adenine with thymine and vice versa; cytosine with guanine and vice versa. In the realm of CRISPR, the bacterium synthesizes an RNA molecule from the CRISPR template and As replace Ts and Gs take the place of Cs. RNA utilises uracil (U) instead of thymine (T).[11]

If a section of the bacterium's CRISPR DNA reads ATTTGGCAC (a sequence previously encountered in a viral intruder), the bacterium will generate an RNA counterpart reading UAAACCGUG. The RNA molecule is often called the guide RNA, abbreviated as gRNA. This guide formed from the template in the bacterium's DNA, permeates through the cell of the bacterium. Along with the gRNA is the CRISPR-associated (Cas) protein, which is a microbial enzyme, a specialized protein. If, within the bacterium, there exists viral DNA containing the complementary sequence to the gRNA— in this case, ATTTGGCAC—the gRNA will bind to it with remarkable precision. The bases of the gRNA securely pair with their counterparts in the viral DNA.[11]

Next, the Cas protein functions akin to a set of molecular scissors, cleaving the viral DNA into fragments precisely at the location indicated by the gRNA. Unlike RNA, DNA consists of two interconnected strands of sequence, having the structure of a twisted ladder. The Cas protein cleaves the viral DNA across both strands. This cuts the virus, rendering it unable to replicate and thus safeguarding the microbe. Over time, this large family of Cas proteins was categorized, and the first to be identified was named Cas1.[11]

The acquired immunity is passed on to future generations of bacteria, providing them with protection against specific viruses they have encountered in the past.[12] This system serves as a natural immunity mechanism against invading viruses. This unique adaptive immune system has not only garnered immense interest from the scientific community but also paved the way for new opportunities in genetic engineering, disease treatment, and even the potential for altering the course of evolution itself.[11]

With the advent of CRISPR technology, healthcare has been significantly impacted, with more avenues being opened for advancements in precision medicine. By enabling precise manipulations of genetic material, CRISPR technology allows healthcare providers and researchers to explore precision medicine, laying the groundwork for the invention of targeted therapies and new approaches to disease treatment and prevention.[13]

CRISPR technology encompasses various systems, primarily CRISPR-Cas9, CRISPR-Cpf1 (Cas12a), and CRISPR-Cas13. Each system has unique characteristics that make them suitable for different applications:

CRISPR-Cas9: This the most widely used system for genome editing. It uses a gRNA to direct the Cas9 enzyme to a specific DNA sequence, where it creates a precise DSB. The break can then be repaired by the cell's natural repair mechanisms, allowing for gene knockout, insertion, or correction. CRISPR-Cas9 is employed in gene knockout studies, creating disease models, functional genomics, and developing potential therapies for genetic disorders such as sickle cell disease, cystic fibrosis, and certain types of cancer.[14]

CRISPR-Cpf1 (Cas12a): CRISPR-Cpf1, also known as Cas12a, differs from Cas9 in its cutting mechanism. It creates staggered cuts with sticky ends rather than blunt ends, which can facilitate more efficient and precise DNA fragment insertions. Cpf1 also requires a different protospacer adjacent motif (PAM) sequence, expanding the range of targetable genomic sites. This is particularly useful for gene insertion applications and targeting genomic regions that are inaccessible to Cas9. It is also used in creating animal models and exploring gene function.[15,16]

CRISPR-Cas13: Unlike Cas9 and Cpf1, CRISPR-Cas13 targets RNA instead of DNA. This makes it particularly useful for applications requiring RNA editing or interference, such as silencing viral RNA genomes, RNA interference (RNAi), studying RNA biology, transient gene knockdown, and developing RNA-based diagnostics like the Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK) platform for detecting viral RNA with high sensitivity and specificity.[17]

These systems provide researchers with versatile tools to address a wide range of genetic and therapeutic challenges.

METHODS

To investigate CRISPR's multidimensional role in healthcare, a comprehensive literature review was undertaken. The literature search was performed using PubMed as the primary database. Open-access peer-reviewed journal articles published in English between 01/01/2013 and 31/12/2023 were identified. Keywords used in the search were: “CRISPR”, “genome editing”, “hereditary diseases”, “cancer”, and “chronic illness”. Articles were chosen based on their direct relevance to CRISPR’s applications in treating hereditary and chronic diseases, diagnosing infectious diseases and targeted drug development.

RESULTS

Treatment of Sickle-cell Disease

Casgevy®, a CRISPR-based gene therapy, was approved in December 2023 by the European Medicines Agency (EMA) as a therapy for sickle cell disease.[18] In an ongoing clinical trial involving 29 individuals, including six adolescents, who were dealing with severe sickle cell disease, 28 patients experienced significant improvement in health, evidenced by the absence of vaso-occlusive crises (VOC) for a continuous period of at least 12 months.[19]

Treatment of Beta-thalassemia

The application of CRISPR technology in the treatment of beta-thalassemia is primarily based on the increased production of foetal haemoglobin to make up for the absence of functional adult haemoglobin in patients.[20] In the CLIMB-Thal-111 (Phase 2/3) trial, every patient treated with Casgevy® showed clinical improvement. Over time, the average percentage of modified BCL11A alleles, a key regulator of the repression foetal haemoglobin in adults, remained consistent. [21]

Treatment of Haemophilia

In an investigation carried out by Luo et al., the creation of a Haemophilia A mouse model was successfully established through Factor VIII (F8) gene editing. CRISPR/SaCas9 technology was subsequently employed to perform in vivo gene editing, where donor DNA was integrated at the desired location, resulting in the restoration of F8 expression. This genetic correction resulted in substantial improvements in blood coagulation capabilities, evident from reduced activated partial thromboplastin time values and increased survival in a tail-clip bleeding challenge.[22]

Haemophilia B arises from mutations in the Factor IX (FIX) gene.[23] To correct this mutation, Lyu et al. concentrated on creating induced pluripotent stem cells (iPSCs) tailored to the patient from peripheral blood mononuclear cells (PBMNCs) through the application of CRISPR-Cas9 for precise genome modification. This resulted in the successful insertion of the full-length F9 complementary DNA into the adeno-associated virus (AAV) integration site 1 locus of the iPSCs, without detection of unintended mutations. Subsequently, the iPSCs were transformed into hepatocytes capable of secreting human FIX.[24]

Treatment of Cystic Fibrosis

In an investigation done by Walker et al., CRISPR-Cas9 was used to target a deep intronic splicing mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, responsible for cystic fibrosis. The method involved delivering ribonucleoprotein complexes to cystic fibrosis basal epithelial cells using non-viral receptor-targeted nanocomplexes. This targeted approach successfully restored canonical CFTR messenger RNA splicing, resulting in the expression of functional CFTR protein. Electrophysiological assessments confirmed the recovery of CFTR function in cultures of airway epithelial air-liquid interface. The study reported no off-target editing.[25]

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Treatment of Duchenne Muscular Dystrophy

Researchers used CRISPR genome editing technology to correct Duchenne Muscular Dystrophy (DMD) in mice for more than a year. The study involved a systemic treatment that not only showed functional dystrophin restoration and increased muscle strength but also maintained its effectiveness despite immune responses and alternative gene editing outcomes.[26]

In a different study done by Kita et al., researchers aimed to restore dystrophin protein by inducing multi-exon skipping in the dystrophin gene, whose mutation is responsible for DMD. To enhance the population of genome-edited cells, they created a two-colour single-stranded annealing (SSA) based reporter system for Cas3. This approach successfully induced large deletions, up to 340 kilobases in size. This method was tested on patient-derived iPSCs.[27]

Treatment of Cardiovascular Diseases

The study by Ishizu et al. focused on targeted genome replacement in non-dividing cardiomyocytes. The principle was based on direct correction of genetic defects in heart cells that typically do not divide. CRISPR-Cas9 was used for homology directed repair (HDR), a method in which the Cas9 enzyme breaks the DNA at a specific location and then uses the cell's own repair mechanisms to add a new genetic sequence. With this, HDR was confirmed to be effective for genome editing in non-dividing cardiomyocytes and this holds significant promise as a therapeutic approach for addressing cardiomyopathy caused by pathological mutations. [28]

The study by Ma et al. achieved results in correcting a genetic mutation associated with hypertrophic cardiomyopathy (HCM). Utilizing the novel ABEmax-NG system, an advanced adenine base editor, the target locus in a mouse model was edited. The application of ABEmax-NG in embryos from R404Q/+ HCM mice brought about a substantial correction rate (62.5% to 70.8%) of the Myh6 c.1211C>T mutation. As a result, postnatal mice and their progeny no longer exhibited HCM symptoms and mutant RNA levels were reduced.[29]

Treatment of Diabetes

A research team at Washington University School of Medicine investigated the application of CRISPR-Cas9 gene editing in conjunction with iPSC technology to treat diabetes. This approach involved transforming skin cells from patients into iPSC, using CRISPR-Cas9 to rectify a single nucleotide alteration in the Wolfram syndrome type 1 gene (WSF1) gene. The corrected iPSCs were then differentiated into beta cells that synthesize insulin. The beta cells were then implanted into mice with severe diabetes, resulting in consistent regulation of insulin and blood sugar levels for a duration of up to six months.[30]

CRISPR Therapeutics and ViaCyte collaborated to develop a treatment named VCTX210, which is based on gene editing and derived from stem cells. This therapy is aimed at treating Type 1 Diabetes and insulin-dependent Type 2 Diabetes. As of early 2022, the Phase I clinical trials primarily focused on assessing the safety of VCTX210.[31]

Treatment of Cancer

CRISPR-Cas9 screenings on a genomic scale across 324 cancer cell lines from 30 distinct types of human cancers was conducted. A structure that combined the impact of gene deletions on cellular health with genomic biomarkers and the feasibility of targeting these genes for drug development was established. This approach allowed for the systematic prioritization of novel targets in specific tissue types and genetic makeups. One notable outcome was the recognition of Werner syndrome ATP-dependent helicase as a potential target for treatment in various cancers exhibiting microsatellite instability.[32]

Another approach involved the use of CRISPR-Cas9 mechanism to control gene activity through CRISPR activation and CRISPR interference. This resulted to temporary changes in transcription and epigenetic regulation.[33]

A recent application of CRISPR technology is its use in treating glioblastoma, a highly aggressive and difficult-to-treat brain cancer. Researchers developed a cancer shredding technique that targets the non-coding genome and temozolomide signature using CRISPR, facilitating the destruction of glioma cells through CRISPR technology.[34]

Detection of Infectious Conditions

Researchers developed a CRISPR-based test called Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK) which utilizes the Cas13 enzyme. This system identifies SARS-CoV-2 in RNA extracts from respiratory swabs.[35] In a study, SHERLOCK was used to test a total of 534 clinical samples and, demonstrated high specificity and sensitivity. Specifically, it showed a sensitivity of 96% using fluorescence detection and 88% using lateral-flow detection. The sensitivity threshold of the technique was around 42 units per reaction.[36]

CRISPR technology's ability to detect mutations in the *Mycobacterium tuberculosis* genome, responsible for conferring resistance to specific antibiotics, has also been explored. The CRISPR region in *Mycobacterium tuberculosis* has shown a conserved structure, characterized by a solitary type IIIa CRISPR-Cas system in almost all *Mycobacterium tuberculosis* complex strains. Recent research suggests that the CRISPR-Cas mechanism in *Mycobacterium tuberculosis* is at least partially active, which could be utilized in gene silencing strategies.[37]

CRISPR technology has been further adapted in the detection of Hepatitis B virus (HBV). A detection system based on CRISPR-Cas12, termed Cas12a-DETECTR, has been created for highly sensitive and precise point-of-care identification of HBV. This technique uses multiple cross displacement amplification for swift initial amplification, succeeded by detection based on Cas12b to analyse the targets. The ultimate detection outcome is observable through real-time fluorescence and a lateral flow biosensor, showing a detection threshold of 10 copies per test.[38]

Therapy for Human Immunodeficiency Virus

CRISPR-Cas9 can remove the HIV-1 provirus embedded in human myeloid cells. Successful excision of the HIV-1 provirus led to the suppression of viral replication.[39] Following the success in human myeloid cells, researchers expanded their studies to include CD4+ T-cells cultured in a living organism from patients with infections. The research utilised a modified form of the Cas9 enzyme, known as saCas9, along with multiple gRNAs aimed at the viral DNA in the 5′-LTR and Gag gene regions. The study was conducted on transgenic mice and rats genetically engineered to include the HIV-1 genome. Injecting these animals intravenously with a recombinant adeno-associated virus 9 (rAAV9) vector, which carried saCas9 and gRNAs, led to the cutting of the integrated HIV-1 DNA and the removal of a significant fragment of this DNA. This excision was observed in various organs and tissues of the animals, including spleen, liver, heart, lung, kidney, and the lymphocytes circulating in the bloodstream.[40,41]

In another study, the combination of CRISPR-Cas9 with long-acting slow-effective release antiviral therapy (LASER ART) was tested in humanized mouse models infected with HIV-1. LASER ART, comprising nanoparticles with hydrophilic and lipophilic antiretroviral prodrugs, aids in decreasing the regularity of antiviral treatment. The integration of CRISPR-Cas9 and LASER ART resulted in the absence of viral rebound in some treated mice.[42] In a non-human primate study, AAVs were employed as carriers for the delivery of CRISPR-Cas9. A one-time intravenous administration of AAV9-CRISPR-Cas9 succeeded in removing segments of the integrated proviral DNA of Simian Immunodeficiency Virus (SIV).[43]

Another study was done on the use of the CRISPR-Cas13a system for rapid and remarkably accurate diagnosis of HIV-1 infection. This research led to the creation of a method for detecting HIV-1 RNA by integrating the CRISPR-Cas13a lateral flow strip with reverse transcriptase recombinase-aided amplification (RT-RAA) technology. The research involved designing reverse transcriptase recombinase-aided amplification primers and developing an easy-readout and sensitivity enhanced detection method for HIV-1 RNA. The ability of the CRISPR-based lateral-flow strip test to sensitively detect HIV-1 RNA was evaluated using clinical samples. A detection threshold as minimal as 1 copy/μL was achieved and clinical samples with a viral load down to 112 copies/mL could be detected.[44]

Use of CRISPR in the Production of Recombinant Proteins

In a study by Srila and colleagues, CRISPR/Cpf1 was employed to target and knock out glutamine synthetase (GS) genes within Chinese hamster ovary (CHO) cells. This was achieved by deleting both a gene with high expression, GS5, and another with low expression, GS1. The outcome was enhanced selection efficiency for stable cell lines capable of producing therapeutic antibodies.[45]

In another study, researchers designed gRNAs specific to the Caspase-3 gene. The CHO cells then underwent transfection with the elements of CRISPR-Cas9. Editing the caspase-3 gene in CHO cells resulted in a significant increase in the survival rate of the cells and a substantial decrease in caspase-3 protein expression levels. The manipulated cells showed a higher threshold for cell death compared to the cells in the control group when stimulated by oleuropein, indicating improved resistance to cell death. The manipulated cell lines demonstrated a higher proliferation rate compared to the control cells when exposed to a substance that induced cell death. The edited CHO cells produced significantly more recombinant erythropoietin, especially when exposed to oleuropein, in contrast to the control cells.[46]

Table 1: Use of Crispr in Chronic Conditions

CONDITION	OUTCOME
Sickle-cell disease	Absence of vaso-occlusive crises[19]
Beta-thallasemia	Increased production of foetal haemoglobin[21]
Haemophilia	Restoration of factor VIII and IX expression[22–24]
Cystic fibrosis	Expression of cystic fibrosis transmembrane conductance regulator protein[25]
Duchenne muscular dystrophy	Functional dystrophin restoration[26,27]
Cardiovascular diseases	Elimination of hypertrophic cardiomyopathy symptoms[28,29]
Diabetes	Insulin synthesis and regulation of blood sugar level[30]
Cancer	Destruction of glioma cells[34]

Table 2: Use of Crispr in Infectious Diseases

CONDITION	OUTCOME
SARS-CoV-2	Identification of SARS-CoV-2 with high sensitivity and specificity[35,36]
Hepatitis B	Detection of hepatitis B[38]
Human immunodeficiency virus (HIV)	Detection of HIV-1 infection and suppression of viral replication[39–41,44]

DISCUSSION

CRISPR has opened doors to precision medicine, offering unprecedented precision and versatility in gene editing. Its application extends from combating genetic disorders to advancing cancer therapies and exploring new frontiers in disease prevention and cure.

The approval of Casgevy® for the management of sickle cell disease and beta-thalassemia, represents a significant milestone in medical science, particularly in the field of genetic disorders. Casgevy®, approved for sickle cell disease and beta-thalassemia, utilizes CRISPR-Cas9 to modify patients' hematopoietic stem cells, inducing foetal haemoglobin production to alleviate symptoms. Stem cells are edited outside the body, then implanted after chemotherapy. Increased foetal haemoglobin combats sickling of red blood cells. For beta-thalassemia, Casgevy® disrupts BCL11A to boost foetal haemoglobin. Low platelet and white blood cell count, mouth sores, nausea, musculoskeletal pain, abdominal pain, vomiting, febrile neutropenia, headaches, and itching were among the common adverse events seen in the trials. These effects are mainly associated with the procedures necessary for the altered blood cells to engraft and take the place of the original stem cells.[18] Casgevy®'s approval is particularly significant for patients aged 12 years and older who suffer from either severe sickle cell disease or beta-thalassemia.[18] The significance of Casgevy® in healthcare lies in its ability to offer a one-time, potentially curative treatment for these genetic disorders, reducing the need for continuous

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management with drugs such as hydroxyurea and regular blood transfusions. This advancement shifts the focus from symptomatic treatment to genetic correction, which could drastically improve patient outcomes and quality of life.

CRISPR-Cas9 holds promise for treating haemophilia by targeting F8 or FIX genes.[24] Its ability to correct mutations in the F8 and FIX genes offers a long-term solution for restoring normal coagulation functions. Preclinical models showing restored coagulation function suggest that CRISPR could reduce dependency on factor replacement therapies, which are both costly and require frequent administration.[47] This represents a paradigm shift in managing haemophilia. Transitioning from regular prophylactic treatments to potentially curative gene therapies will necessitate new healthcare frameworks for patient education, monitoring gene therapy-related adverse effects, and ensuring the long-term efficacy and safety of these innovative treatments.

In the management of cystic fibrosis, CRISPR technology's precision in targeting and correcting CFTR gene mutations without off-target effects opens new avenues for treating cystic fibrosis.[25] The ability to correct the underlying genetic defect in cystic fibrosis represents a shift from managing symptoms with antibiotics, mucolytics, and other supportive therapies to addressing the root cause of the disease.

CRISPR-Cas9's application in DMD, particularly in correcting dystrophin gene mutations, showcases the potential for reversing or halting disease progression.[26] The CRISPR-Cas3 gene editing system also demonstrated its ability to reestablish dystrophin activity in stem cells taken from individuals with DMD.[27] The restoration of functional dystrophin protein in muscle cells indicates significant therapeutic benefits. This could revolutionize the treatment of DMD, moving from symptomatic management to addressing the root genetic cause.

One of the key applications of CRISPR in cardiology is in the treatment of HCM, a common genetic heart disease. The success in correcting Myh6 c.1211C>T mutation underscores CRISPR's potential in treating hereditary heart diseases, paving the way for transformative therapies.[29] Correcting the genetic root of HCM offers a potentially curative treatment, surpassing symptom management.

In diabetes treatment, CRISPR-Cas9 was used to correct genetic mutations in patient-derived iPSCs, which were then differentiated into insulin-producing beta cells.[30] This method offers a novel way to create functional autologous cell replacements, potentially providing a long-term solution for insulin regulation. VCTX210, the first gene-edited diabetes treatment in clinical trials, utilizes CRISPR-Cas9 to engineer allogeneic stem cells for beta cell replacement. Designed to evade immune response, it aims to eliminate the need for immunosuppression in type 1 diabetes patients.[48] Healthcare providers would be responsible for managing the storage and preparation of CRISPR components, educating patients on the new therapy, and monitoring for any adverse effects. This approach could shift diabetes management from lifelong insulin therapy to a one-time genetic intervention.

In cancer therapy, CRISPR has been employed in distinguishing between mutations that drive cancer development and those that are benign. This has led to the generation of complex cancer models, including organoid cultures and animal models, essential for comprehending the role of genes in cancer development and progression.[49] This represents a comprehensive resource for cancer dependencies and creates a framework to prioritize oncology targets, nominating specific new targets and informing early phases of drug advancement with a varied and potent collection of targets in oncology.[32]

The introduction of multiplexed activation of endogenous genes as an immunotherapy (MAEGI), utilizes CRISPR activation to boost tumor antigen visibility and induce robust anti-tumor immune responses. Proven effective in preventive and curative contexts across various cancers, MAEGI alters the tumor microenvironment, enhancing T cell infiltration and immunological signatures against tumors.[33] This represents a significant advancement in cancer immunotherapy, providing a versatile approach to eliciting potent immune responses against cancer.

Further advancing the field, a novel concept in cancer therapy termed cancer shredding, applied specifically to glioblastoma, a lethal brain cancer, has been introduced. The approach used leveraged on CRISPR-mediated

targeting of repetitive sequences in tumour genomes, leading to rapid elimination of cancer cells. It focused on exploiting temozolomide-induced mutational signatures in hypermutated glioblastomas, enabling cancer-specific cell ablation.[34] This method offers a potential new avenue for treating gliomas with CRISPR has also been increasingly explored for its potential in diagnosing and managing a wide variety of infectious illnesses. SHERLOCK, a CRISPR-based system, has been employed to identify SARS-CoV-2. The system functions by detecting SARS-CoV-2 virus nucleic acid sequences.[50] SHERLOCK can also be used to address the limitations of conventional reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) techniques. Utilizing SHERLOCK allows for precise and sensitive identification of SARS-CoV-2 RNA in a format suitable for high-volume testing.[36]

CRISPR-Cas technology has also been recognized for its prospect in tuberculosis diagnostics, particularly in identifying antibiotic resistance genes. CRISPR-Cas systems particularly address the challenges of detecting paucibacillary and extrapulmonary tuberculosis cases. They have been has been used to explore *Mycobacterium tuberculosis* biology and how it interacts with the immune system of the host, underlining the technology's significance in revolutionizing tuberculosis research and control.[37]

A rapid and precise HBV detection system was developed using CRISPR-Cas12, overcoming nucleic acid extraction hurdles. The system offers results via lateral flow strips and fluorescence, with a 1 copy/µL detection limit and processing in 13-20 minutes. Demonstrating high sensitivity, specificity, and comparability to quantitative polymerase chain reaction, this method holds significant promise for point-of-care HBV detection, especially in medically disadvantaged areas.[38]

CRISPR-Cas9 has shown significant promise in targeting and modifying HIV-1 DNA integrated into the human genome, offering a potential strategy for HIV treatment and opening new avenues for managing this persistent virus. In a study, the precise removal of proviral DNA from infected cells and tissues in simian immunodeficiency virus-infected macaques using AAV9-CRISPR-Cas9 was demonstrated. This approach successfully reduced proviral DNA levels, indicating significant progress towards eliminating HIV reservoirs. These findings suggest that CRISPR-based gene editing could be instrumental in achieving a functional cure for HIV, significantly advancing current management strategies and setting the stage for potential phase 1/2 clinical trials.[38,51]

Another crucial aspect of CRISPR's application in HIV treatment involves RNA-based gene editing. Studies targeting the HIV-1 long terminal repeat U3 region with Cas9/gRNA have effectively halted virus expression and replication across various cell types without causing genotoxicity. This approach not only successfully removed proviral DNA fragments but also prevented new infections, highlighting a promising pathway for targeted AIDS treatment and HIV-1 prevention.[39]

Combining CRISPR with long-acting slow-effective release antiviral therapy (LASER ART) showed that this integrated approach could suppress HIV-1 replication and remove integrated HIV-1 DNA, potentially achieving a functional cure. The absence of viral rebound in treated mice reinforces the strategy's promise for HIV-1 treatment.[42]

The development of CRISPR-based lateral-flow strips offers a user-friendly tool for precise HIV-1 nucleic acid detection, aiding early diagnosis and monitoring in clinical settings. This technology enables rapid, accurate detection of HIV-1 RNA, significantly improving the early diagnosis and treatment monitoring of HIV.[44]

CRISPR's ability to target and modify HIV-1 DNA provides a potential pathway to eliminate the virus from infected individuals, addressing one of the most challenging aspects of HIV management—the persistence of viral reservoirs. By removing proviral DNA and preventing new infections, CRISPR-based therapies could transform the landscape of HIV treatment, moving from lifelong antiretroviral therapy to potential cures.

Healthcare providers can assist in counselling patients on the results of CRISPR-based diagnostics and integrating these tools into routine healthcare settings. The ability to quickly and accurately diagnose infectious diseases can significantly improve patient outcomes and reduce the spread of infections.

CRISPR technology enhances recombinant protein synthesis, notably in CHO cells crucial for biopharmaceutical manufacturing. CRISPR-Cas9 has been used to adjust antibody fucosylation, vital for therapeutic effectivenes.[33] Effective GS gene knockout in CHO cells using CRISPR/Cpf1 has been demonstrated, boosting selection efficiency for stable antibody-producing cell lines.[45] This advancement streamlines biopharmaceutical production, reducing time and costs in drug development. It also enhances therapeutic protein quality and yield, potentially increasing accessibility, and affordability, particularly in lower to middle-income countries.[52]

CRISPR-Cas9's efficacy in enhancing biopharmaceutical production in CHO cells by modifying apoptosis-related genes has been demonstrated. This technology enables targeted gene editing, fostering the development of advanced CHO cell factories.[46] Such advancements promise improved protein production and quality, driving more effective and affordable biopharmaceuticals. Additionally, CRISPR-Cas9 elucidates molecular mechanisms underlying protein production, aiding in the rational design of optimized CHO cell factories for specific biopharmaceutical requirements.[52] Healthcare providers can be involved in ensuring the quality control of these biopharmaceuticals, providing patient education on new biologics, and monitoring for adverse reactions. This advancement could lead to more accessible and affordable biopharmaceuticals, improving patient access to essential medications.

Limitations of the Study

A major constraint of this research was the scarcity of literature specifically addressing the direct employment of CRISPR technology in healthcare. This gap in academic and practical resources limits the depth of analysis and understanding that can be achieved.

RECOMMENDATIONS

For future studies, it is essential to focus on expanding the scope and depth of research concerning the use of CRISPR technology in healthcare. Collaborative research projects between academic institutions, biotechnology companies, and medical organizations could explore the practical applications of CRISPR in healthcare, including case studies and pilot programs. Such collaborations could also examine the ethical, legal, and social implications of integrating CRISPR into pharmacy.

CONCLUSION

The study underscores the revolutionary possibilities of CRISPR technology in healthcare. CRISPR's applications, ranging from genetic disorder treatments to advancements in cancer therapy and other health conditions, highlight its versatility and precision.

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