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CRISPR-CAS9: A Groundbreaking Approach For Human Disease Rehabilitation

Aditi Patil , Dr.Shivani Kakkar Khanna, Dr. Pooja Gupta

Mohan Suberbia, L2 302, phase 2, Ambernath west, India, 421501

ABSTRACT: The field of medicine and research can greatly benefit from the application of genetic engineering. The Cas-9 protein and clustered regularly interspaced short palindromic repeats (CRISPR) are linked to a groundbreaking method in genetic engineering that allows for precise gene editing. CRISPR/Cas9 is an RNA domain-containing endonuclease-based genome engineering tool that has showed potential in cancer rehabilitation owing to its high precision, accuracy, time-saving, and cost-effective tactics. It has been utilized to enhance the clinical condition of Huntington's disease (HD), a rare neurological ailment caused by a CAG trinucleotide increase in the Huntingtin gene. It is distinguished by the considerable penetration of a single mutation, and it has been used to repair CF-causing mutations, making them effective therapeutic agents for combating HIV and other infectious diseases. In recent years, RNA-editing technologies based on clustered regularly interspaced short palindromic repeats (CRISPR)-associated nucleases 9 (CAS9) have come to light as potential treatments for this insidious illness. In this article the problem statement is find out how crispr cas 9 technology is used for effective treatment of various human diseases. Diseases to be covered in this article are cancer, huntington’s disease, cystic fibrosis, human immunodeficiency virus (hiv) and alzheimers disease.

KEYWORDS: Crispr-cas9, Cancer, Neurological disease, Genome, DNA editing, Cystic fibrosis, Targeted Drug Delivery, Genome Engineering, human disease.

INTRODUCTION: Recent advances in genome alteration, such as the CRISPR-CAS9 gene editing tool and technology, have completely changed the scientific sector. Genome engineering is an important aspect if biomedical research and medicine are taken into consideration. These are short palindromic repeats that are clustered regularly interspaced and linked to the cas9 protein. With the aid of this revolutionary technology, scientists will be able to alter the architecture and structures of DNA to prevent the spread of some diseases and improve the management of some genetic illnesses. It is extensively employed in the treatment of several human illnesses, targeted medication delivery, research on plants and animals, and therapies. It can be used

to treat a wide range of illnesses in people, including cancer, sickle cell anemia, thalssemia, and Huntington's disease. With CRISPR CAS9 technology, DNA segments may have parts added, removed, or edited. It has demonstrated how to use genetic editing to correct particular mutations that are unique to a patient, therefore improving the untreatable ailments' state. Synthetic RNA molecules have been incorporated into eukaryotic genomes to make it possible for selective editing and the targeting to particular genomic regions. Further effective investigation and patient application are needed to fully realize its promise in the medical area. The technique identifies and bonds the sgRNA/cas9 ribonucleoprotein complex to the target DNA employing the single guided RNA (sgRNA) sequence and CAS9 endonuclease. The target DNA is rectified by the insertion, deletion, addition, and inversion operations, which are carried out at the site by the CAS9. In humans, repair processes happen to be carried out by both the individualized DNA sequences and our inherent natural repair mechanisms. CRISPR was discovered in Escherichia coli's IAP gene. The gene is composed of short Palindromic DNA repeats which are ubiquitous in many microorganisms. CRISPR plays a specialized role in the resistance of the bacteria against the foreign DNA. Bacterial strains may adapt to phage resistance through the introduction of new spacer sequences. These sequences resemble to the invasive nucleotides from the CRISPR phage gene, triggering gene disruption. Short 2-5 bp sequences in the area have been identified as protospacer adjacent motifs (PAMs). CRISPR and Cas nucleases in Bacteria. Cas9, a pathogen-specific enzyme, has been extensively assessed in invertebrates and mammals due to its specificity and flexibility in DNA sequence. Makarova et al. (2006) demonstrated that the Cas9 protein cleaves DNA at defined locations, highlighting parallels between CRISPR/Cas9 technology and RNA interference. Some Cas proteins, such as Cas13, also cut RNA. Although CRISPR CAS9 technology ensures target specificity and is based on the ribonucleotide complex for efficient therapy, it is a more economical and effective strategy for treating patients than other genome editing approaches. Other benefits include targeted medication distribution, base editions, generating numerous modifications at once, DNA editing in the embryo, and treating chronic conditions. This method is also employed for creation of genetically inhibited animal models for research purposes and it also has been successful in entering clinical trials against certain lethal conditions like cancer. Clinical trials that are both secure as well as successful are likely to arise from these recent advances.

Diseases to be covered in this article are as follows:

Cancer: Cancer has been one of the most alarming public health concern approxiamately casuing more than 8 billion deaths annually. Recent investigation demonstrates that CRISPR/Cas9 significantly inhibits tumor cell growth in an an array of tumor types. CRISPR/Cas9 is an RNA domain-containing endonuclease-based genome engineering technology which has shown potential in cancer rehabilitation due to its high precision, accuracy, time-reducing, and economically viable strategies. Targets for this approach include tumor-suppressive genes, oncogenes, and pharmaceuticals used in chemotherapy. CRISPR/Cas9 could represent a beneficial therapeutic target for controlling the progression of cancer. Over the past couple of decades, there has been a notable advancement with regard to cancer diagnosis and research. The use of customized treatment options for cancer have been rendered conceivable by the molecular profiling of cancer patients.

But the diagnostic techniques used today are pricey and need specialized tools. Only a tiny percentage of cancer driver proteins undergo therapy with targeted cancer therapies, such as monoclonal antibodies and small-molecule medications, which could result in off-target side effects. Tools that are versatile, successful, and precise are required for cancer diagnosis and focused therapy. Particularly the RNA-targeting CRISPR-Cas13 system, the selection of CRISPR-based genome and transcriptome modification tools has grown quickly. Cas13-based diagnostic techniques eliminates the need for complicated equipment and permit rapid detection and surveillance of cancer indicators from liquid biopsy samples.

Resistance to medications and relapse are common results of the many molecular markers that describe cancer.

Using molecular markers, precise medicine aims to pair patients with the right medications.

Accurate medicine necessitates an understanding of drug resistance and cancer.

The CRISPR-cas9 system is a powerful instrument for rapid screening and gene editing.

Finding intriguing therapeutic targets, identifying biomarkers, and comprehending drug resistance procedures are a few examples of breakthroughs in CRISPR-cas9-based screening.

The fight against cancer has made significant developments in gene engineering, with CRISPR/Cas9 technology displaying tremendous potential. CRISPR/Cas9 technology may avoid development of tumors and restore function through elimination of oncogenes and reestablishing tumor suppressor genes. This approach has been actively investigated for a variety of cancers, including lung, breast, head, colorectal, and hepatocellular carcinoma. Numerous target genes have been implicated, including EGFR, p53, FAK, Nestin, BRCA, HER2/Neu, TERT, ALK, KRAS, BRAF, NOTCH1, and PTEN. Experiments have demonstrated implementing CRISPR/Cas9 technology to specifically target oncogenic KRAS mutant alleles could possibly significantly decrease tumor development. CRISPR/Cas9 technology is also operational for looking into disease etiology as well as assessing the involvement of novel oncogenes or tumor suppressor genes in the clinical process. As an illustration, small cell lung cancer (SCLC) is a high-grade neuroendocrine tumour that accounts for 15% to 20% of all lung cancers. Owing to studies, p107 and p130 contain returning mutations in around 6% of human SCLC tumors, which could have been validated by CRISPR/Cas9 screening. CRISPR/Cas9 systems not only specifically target oncogenes in order to avoid tumor growth, but they also do large-scale cancer gene screening in order to boost the efficiency of anticancer medication development. Researchers used CRISPR/Cas9 screening on over 300 human cancer cell lines from 30 distinct kinds of cancer, integrating cellular adaptive effects, genetic biomarkers, and drug development targets to locate novel targets for specific tissues and genotypes. A clinical experiment in China employed CRISPR/Cas9 gene editing to treat non-small-cell lung cancer patients. The approach targeted the PD-1 gene in T cells in sufferers' peripheral blood, which enhanced the therapy's efficiency. Three patients with advanced refractory cancer took part in a phase 1 in-human experiment employing CRISPR-Cas9 technology. Patients sustained engineered T cells for up to 9 months that follows reintroduction. Another trial offered CAR-T-cell treatment for relapsed hematological tumors, that focuses on CD19 tumor cells. In a 2019 in vivo clinical study, AGN-151587, a CRISPR-Cas9 gene therapy medication, was supplied directly to patients with Leber's congenital

amaurosis 10 (LCA10). The study resulted in 19 registered CRISPR-Cas9 gene editing interventional clinical trials.

HUNTINGTONS DISEASE: Huntington's disease (HD) is a rare neurological disorder caused by a CAG trinucleotide elevate in the Huntingtin gene. CRISPR, a simple and effective genome editing application is gaining traction in biomedical research for HD therapy. HD's monogenic nature makes it a good model for examining pathogenic pathways as well as developing options for therapy. Huntington's disease (HD) is an ailment that causes nerve cells in the brain to gradually degrade and die, affecting regions responsible for voluntary movement. People with HD develop involuntary movements and odd bodily postures, as well as behavioral, emotional, and cognitive issues. Early warning signs of HD might range from moderate clumsiness to cognitive or mental problems and abnormalities in behavior. Cognitive changes might include challenges with attention or judgment, problem solving or decision making, including driving, prioritizing, and organizing. These cognitive abnormalities elevate as the disease advances, eventually leading to dementia, in which the person is unable to function in everyday life. Behavior changes may include variations in mood, impatience, depression, or aggression. These symptoms may fade as the disease develops, but in rare cases, they might persist, including furious outbursts, suicidal ideation, profound despair, and psychosis. People with HD might withdraw from social activities.

CRISPR technology is an innovative technique that has been employed to rectify the clinical state of Huntington's disease (HD), a mutagenic disorder characterized by significant penetration of a single mutation. However, there are various obstacles to overcome, including difficulties in cell targeting, the use of viral vectors in clinical trials, and ethical concerns in practice. The proximate root of the disease is the CAG-expanded HTT gene, and mHTT has been found to be damaging to neurons. Kolli et al. (2017) successfully muted the HTT gene by blocking the synthesis of the damaging mutant huntingtin protein by an organized approach. They revealed distinctive polymorphic locations that differentiate between common haplotypes in HD and normal populations. CRISPR/CAS9 technology was proposed to disable the mutant allele by targeting polymorphisms in DNA that result in PAM sequences. A technique for precise predicted haplotype inactivation was developed, based on two gRNAs to target locations with changes that affect the MAP and cause a pre-projected deletion on the mutant chromosome. Yang et al. (2020) evaluated the role of RAN gene translation in the etiology of Huntington's disease employing in in vivo investigations using CRISPR/Cas9. They altered the genome of two HD140QI knockin mouse models (KI) for Huntington's disease (HD) and made use of a guide RNA (gRNA) to generate mutations in exon 1 HTT (E1) that triggered the expression of RAN but not the full huntingtin protein. Animals E1#1 and E1#2 had a similar start codon, ATG, but mutations inhibited huntingtin from synthesizing proteins. Additional studies has demonstrated that CRISPR technology is helpful in the therapeutic management of Huntington's disease without causing unnecessary genetic changes or the emergence of off-targets. Therefore, CRISPR/CAS9 technology has shown potential in treating Huntington's disease by deleting repetitive CAG trinucleotides without triggering offtarget effects. Clinical investigations and long-term monitoring are of the utmost importance for using CRISPR/Cas9 therapy to treat Huntington's disease.

CYSTIC FIBROSIS: Cystic fibrosis (CF) is an inherited condition which impacts the body's mucus and sweat glands, leading to thick, sticky mucus that can cause blockages, impairment, and infections. Previously lethal in childhood, survival has gotten better as an outcome of medical discoveries and breakthroughs in birth screening, pharmaceuticals, nutrition, and lung transplants. Roughly 100,000 individuals globally, have cystic fibrosis. Some people have mild symptoms, while others have severe symptoms or life-threatening problems. Common repercussions include lung difficulties, such as recurrent exacerbations, which are usually triggered by significant lung infections. Medical professionals recommend therapies to enhance lung function to prevent or cope with problems, which improves quality of life and allows individuals to live longer. Recent advances in CF pharmacology provide promise for people with particular genotypes, yet many remain 'undruggable'. CRISPR-Cas9 technology, which emerged in the mid-2010s, has broadened the potential for precise gene editing in mammalian cells. It has been utilized to fix CF-causing mutations, and CRISPR-Cas9 technologies may be repurposed for innovative CF therapies. Cystic fibrosis, is a genetic condition caused by mutation in the CFTR gene, which impacts the lungs and digestive system. The CRISPR-Cas9 technique has been proposed as a potential method for rectifying these alterations. CRISPR-Cas9 has been verified in studies to be effective in correcting CFTR mutations in iPSCs, sheep models, and cultured CF patient stem cells. This could represent an important resource for researching intriguing CF therapies. In primary adult stem cells, the CFTR gene is altered via homologous recombination. Thus, CRISPR technology may one day be utilized for curing cystic fibrosis. The current understanding of the CRISPR and Cas9 nuclease system has opened the possibility of targeting genes for personalized medicine. This advancement permits precise repair of the CFTR gene as part of a precision medicine strategy. Organoids offer a step toward employing these models for customized therapy, as they have been utilized exclusively for this purpose. Precision medicine, on the other hand, focuses on the first discovery of genotypic, environmental, and phenotypic components driving patients' disease and response to medications, which is done using massive databases and big data.

HUMAN IMMUNODEFICIENCY VIRUS (HIV): HIV-1 infection is a substantial worldwide health hazard, and combination antiretroviral treatment (cART) is ineffective at eradicating proviral DNA from the human genome. In recent years, gene-editing technologies based on clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease 9 (Cas9) have come to light as viable treatment for HIV-1 infection. New gene-targeting techniques based on or equivalent to CRISPR/Cas9, such as base editor, prime editing, SHERLOCK, DETECTR, PAC-MAN, and pfAGO, have been developed and improved for pathogen identification and disease repair. Recent investigations on HIV-1/AIDS gene therapy have revealed more gene-editing targets based on research into the underlying basis of HIV-1 infection. These technologies offer prospective approaches and uses for HIV/AIDS therapy in the future. Recently created clustered regularly short palindromic repeats (CRISPR) system permit selective manipulation of gene expression, making them viable therapeutic agents for fighting HIV infection and other infectious disorders. CRISPR has emerged as the most potent genome editing technology for editing the HIV-1 genome in infected cells. Ebina et al. demonstrated the first successful CRISPR targeting of the HIV-1 genome in infected HEK293T and HeLa cells, exhibiting CRISPR/Cas9's potential to effectively restrict viral production. Liao et al. targeted

numerous locations, including the LTR sections, and found that regardless of the amount of viral DNA that had been incorporated, protein expression declined. Kaminski et al. put the Cas9 gene under the command of a Tat-activating promoter, which provoked viral DNA cleavage, demonstrating that the promoter was transactivated for Cas9 production. Zhu et al. utilized Jurkat cell lines latently infected with HIV-1 to develop gRNAs targeting 10 conserved spots and tumor necrosis factor alpha (TNFα) to stimulate viral gene expression. The results exhibited a tenfold drop in GFP reporter expression and a ~20-fold reduction in p24 expression. Lebbink et al. observed that two sgRNAs targeting distinct target sites hampered viral proliferation and escape. In latently infected Jurkat cells, this multifunctional strategy was applied to target viral matrix protein along with three key enzymes: reverse transcriptase, integrase, and protease. The dual-gRNA combinations were more successful at halting virus replication. Herskovitz et al. generated a library of gRNAs that are capable of harming five distinct HIV-1 exons: tat1-2, rev1-2, and gp41. Transfection, electroporation, lentivirus, and lipid nanoparticles (LNP) were amongst the delivery methods employed. The results revealed infection shrinks in all patients, with 82% and 94% reductions following transfection and lentivirus treatments, respectively. In addition to targeting proviral DNA that has been incorporated into the host genome, a few investigations have attempted to target pre-integrated HIV-1 DNA in the cytoplasm. Liao et al. executed in vitro tests that targeted the HIV-1 cDNA produced via reverse transcriptase in an attempt to block its integration and subsequent infection. The RNA-editing Cas13 system was subsequently evaluated against HIV-1 infected cells, and it efficiently repressed HIV-1 infection in primary CD4+ T cells while minimizing reactivated HIV-1 in latently infected cells. In addition to targeting proviral DNA that has been incorporated into the host genome, a few investigations have attempted to target pre-integrated HIV-1 DNA in the cytoplasm. Liao et al. executed in vitro tests that targeted the HIV-1 cDNA produced via reverse transcriptase in an attempt to block its integration and subsequent infection. The RNA-editing Cas13 system was subsequently evaluated against HIV-1 infected cells, and it efficiently repressed HIV-1 infection in primary CD4+ T cells while minimizing reactivated HIV-1 in latently infected cells.

ALZHEIMER’S DISEASE: Alzheimer's disease (AD) is a widespread condition characterised by cognitive impairment and enduring neuronal death. It is a neurodegenerative condition that arises from accumulation of tau and amyloid-β (Aβ), leading to neurofibrillary tangles within the cell and extracellular amyloid plaques. An accurate diagnosis of Alzheimer's disease is determined after a postmortem examination of the brain, although new ways for an easy, non-invasive diagnosis are being explored. A small percentage of AD cases can be attributed to predominant, autosomal mutations in one of three genes, amyloid precursor protein (APP), presenilin-1 and 2. Primary symptoms include memory loss, apathy, sadness, and impatience. The present therapies are symptomatic and supportive, with side effects including disorientation, dizziness, depression, constipation, and diarrhea. CRISPR/Cas9 gene editing, a very simple and affordable method, has sparked interest for its potential benefits in Alzheimer's disease treatment. This method has showed promise in other neurodegenerative illnesses, such as Huntington's and Parkinson's. However, its potential for managing Alzheimer's disease has not been properly examined. In recent years, CRISPR-Cas9 technology has been employed to build novel AD models that illustrate a more precise disease phenotype, articulate pathogenesis procedures, pursuit pathogenic genes, and develop a therapeutic for this insidious disease.

CRISPR-Cas9 was employed to generate protective deletions within the 3′-UTR of APP, which greatly decreased Aβ pathology in APP-KI mice. Bart De Strooper and his team altered the native mouse and rat APP genes into the human version by introducing three distinct point mutations: G676R, F681Y, and R684H. Tau knockout mice serve as essential for studying the role of this protein in Alzheimer's disease. Tan and colleagues hired CRISPR-Cas9 in order to generate a small deletion in the transcriptional start codon in exon 1 of the Mapt gene, that produces tau, resulting in a novel animal model that is resistant to excitotoxicity and lacks deficits in memory. CRISPR-Cas9 was additionally used to assess how known AD risk factors impact the onset of the disease. Alzheimer's disease has been associated to the gene Plcγ2, which is expressed in microglia. Multiple variants of the Plcγ2 gene are associated with high or low risk of acquiring Alzheimer's disease. Christian Haass and colleagues created a Plcγ2-P522R knock-in mouse model using CRISPR-Cas9 and reported that this variation may lessen the risk of Alzheimer's disease through enhanced microglial function. CRISPR can improve AD-like disease in animal models by targeting non-Aβ-producing genes. Overall, CRISPR-Cas9's potential applications in the field of Alzheimer's disease have been efficiently probed in a number of animal models implementing a variety of methodologies.

RESULTS: The results suggested that the infection declines among all patients, with 82% and 94% reductions following transfection and lentivirus therapies, respectively.

FUTURE WORK: Additional research indicates that CRISPR technology can aid in the therapeutic treatment of Huntington's disease sans producing undesirable genetic modifications or the emergence of off-targets. CRISPR/CAS9 technology has shown potential in treating Huntington's illness through the elimination of repeated CAG trinucleotides without leading to off-target consequences. Clinical studies and long-term monitoring are of the utmost importance for employing CRISPR/Cas9 treatment to treat Huntington's disease.

CONCLUSION: In a nutshell, the CRISPR-CAS9 system has proven to be one of the most efficient research tools in medical research. It has successfully alleviated a variety of life-threatening ailments and discovered effective solutions for incurable diseases. The crispr cas9 system has offered fresh hope for neurological illnesses such as Huntington's and Alzheimer's. Many different kinds of cancers, sickle cell anaemia, and viral infections such as HIV, which are lethal and extremely harmful, have discovered an impeccable means of being healed owing to advances in CRISPR. To summarize, every breakthrough in CRISPR CAS9 Technology has shown to be beneficial in the field of medicine and offered us an assurance to ease and treat several grave ailments as indicated.

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