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APPLICATION OF CRISPR CAS SYSTEM IN THE TREATMENT OF GENETIC DISEASES AND COVID-19

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Abstract
Clustered Regularly Interspaced Palindromic Repeats (CRISPR–CAS) system, is an adaptive immunological system found in most prokaryotes.The field of genetic engineering has undergone a revolution as a result of this technology. Apart from its application in genetic engineering biotechnology, CRISPR/Cas's potential for treatment of disease has been explored for various diseases, such as sickle cell disease, neurologic disorders, cancer, etc., wherein CRISPR/Cas components such as ribonucleoprotein, sgRNA/mRNA, plasmid and Cas9/single guide RNA (sgRNA)were delivered. The CRISPR-Cas-based methods also helps in diagnosis of COVID19 infections. The CRISPR-Cas system is being studied for the development of antiviral drugs in addition to its capacity for diagnosis, however no CRISPR-based treatment has yet been authorised for use in humans. The purpose of this review is to describe prospective CRISPR-Cas-based techniques as an alternative therapy for combating genetic diseases and COVID 19 infection.

Introduction
By retaining viral DNA as memory in the genetic material of bacterial hosts, the CRISPR-Cas system is a prokaryotic adaptive defence mechanism that defends against phage invasion. A sort of repetitive sequence of nucleotide known as direct repeats surrounds the viral DNA in the system. CAS protein-coding sequences can be found close to the end of these direct repetitions. By using this approach to artificially control reprogrammed endonucleases, the target gene was guided. [1]. Using reprogrammable genome editing technology called CRISPR, internal DNA/RNA may be modified in

a sequence-specific manner; CAS proteins which are CRISPR-associated endonuclease has been employed in a variety of methods for gene editing, also known as gene modification. It has been successfully applied in the field of food industries, agriculture, bioenergy, in therapeutics and infectious agents. Gene-editing technology is anticipated to be able to regulate the emergence of diseases at the genetic level since many diseases are accompanied by changes in gene expression in vivo, especially those hereditary disorders brought on by substitution in a single gene [2]. Numerous cell types and creatures have employed CRISPR/Cas-based methods up to this point. CRISPR can be used for therapeutic genome modification in human cells or patients (in vivo) to address monogenic disorders. The CRISPR approaches employed to cure human monogenic disorders are the primary focus of this review.

BIOLOGY OF THE CRISPR-CAS SYSTEM

In the recent years, the field of gene editing, genomics, genome imaging and gene therapy has been revolutionized by the adaptable RNA-guided endonuclease (RGEN)-based CRISPR-Cas technology. This technology's broad variety of applications offers enormous potential for understanding and manipulating genetic or epigenetic components. CRISPR and CAS proteins are components of archaeal and bacterial natural adaptive defense systems against invading viruses [3]. A CRISPR array, which consists of hundreds of direct, usually palindromic repeats (35–45 bases) separated by distinctive spacer sequences (30–40 bases), makes up the CRISPR-Cas locus on the bacterial genome. One or more operons containing a cluster of CAS genes encoding the system's effector enzymes reside adjacent to the CRISPR array [4]. Three stages made up the immunological response provides by the CRISPR-Cas system: adaptation, pre-CRISPR RNA (crRNA) processing/expression, and interference. The expression of a complex of CAS proteins by the CRISPR-Cas loci initiates the adaptation stage. These CAS proteins then bind to the target DNA sequence and cause two double strand breaks based on the Protospacer Adjacent Motif (PAM), a distinctive short motif of 2-4 bases. The protospacer, a segment of the target DNA that has been released, is placed between two CRISPR array repeats and subsequently functions as a new spacer. [5]. The CRISPR array is transcribed during the expression processing step, producing a single, longer precrRNA that is then processed by a unique set of Cas proteins to form mature crRNA [6]. The next phase is interference, in which mature crRNA attached to the processing complex acts as a guide RNA to recognise identical sequences in the invading RNA of the virus, which is then cleaved and rendered inactive by one of the Cas proteins. [7]. CRISPR-Cas systems are divided into two categories, Class I and Class II. These are three further subtypes for each of the two classes, for Class I, Type I, III, and IV, and for Class II, Type II, V, and VI. [4]. In class I system, ribonucleoprotein (RNP) complex contains multiple subunits of proteins along with crRNA, while the class II system, RNP complex only contains one protein and crRNA to target invading viral RNAs. [8]. Pre-crRNA is processed by the Cas9 protein, which is found in the type II CRISPR-Cas system, together with tracrRNA and RNase III, whereas Pre-crRNA is processed by the Cas12 and Cas13 proteins in the type V and type VI systems, respectively[9, 10]. While the Cas13 protein cleaves the target ssRNA, the Cas12a and Cas12b proteins cleave the double-stranded target DNA upon recognition by mature crRNA. The target RNA's adjacent protospacer motif (PAM) is not necessary for Cas13 to function, whileunlike ssDNA, in the dsDNA target of Cas12 requires a PAM. The Cas13 protein is activated when complementary crRNA binds to the target RNA, and it subsequently degenerate the collateral ssRNA. [11]. The diagnosis of RNA virus infections has been demonstratedby this property of Cas13 [12]. Similarly, Cas12 proteins can be exploited for the identification of ssDNA viruses [14]. The Cas13 family has four subtypes (Cas13a, Cas13b, Cas13c, and Cas13d), each of which has two HEPN domains that impart RNase activity. [13]. Among all subtypes, Cas13d is significant because of its effective and reliable knockdown efficiency as well as easy of viral administration provided by the effector domain's short coding sequence.

CRISPR-CAS APPLICATIONS

The CRISPR-Cas system's gene-editing ability was initially recognized and used to create drugs for antiviral purposes. Its use as a gene-detection method was later recognized, and this revolutionized

the diagnostics field. Clinical settings can now quickly and accurately identify any pathogen because to point-of-care CRISPR diagnostics. The CRISPR-Cas system is currently applied to develop novel antiviral therapies, and it could be an advanced therapeutic choice for treating HIV infections. [15, 16].

Target genes for modification of the genome have been investigated using CRISPR/Cas systems so far. [17], splicing [18], cancers [20], transcription [19], immunological diseases [22], infectious diseases [23] and have been utilized to study and treat genetic disorders in the context of research [21]. Translational application of CRISPR/Cas in monogenic human inheritable diseases offers the potential to offer long-term therapy after a single treatment, which is one of the remarkable developments. In this part, we provide an overview of the most current uses of the CRISPR/Cas system for the development of disease models and the in vitro and in vivo therapy of genetic diseases. Beyond genome editing, detecting infectious and non-infectious diseases is one of the CRISPR-Cas system's newest uses. Since 2017, technologies for nucleic acid detection-based CRISPR-based diagnostics (CRISPR-DX) have been developed. By combining additional amplification and detection techniques, the design of CRISPR-DX platforms has been able to take into account most of the required characteristics to create ideal systems with excellent performance.

Application of CRISPR CAS System in genetic Diseases

Genetic eye disorder

A rare hereditary eye disorder known as Leber congenital amaurosis (LCA) causes significant vision loss at birth or in early childhood. LCA10 is a severe retinal degeneration brought on by CEP290 gene mutation. The CEP290 gene (~7.5 kb) is too big to be fit into a single AAV. To overcome this limitation, In order to correct the CEP290 splicing error in human cells and in humanized CEP290 mice, Editas Medicine created EDIT-101, a prospective genome editing therapy, for sub retinal administration.In this method, the abnormal splice donor produced by the IVS26 mutation is removed using SaCas9. When the AAV dose was not less than 1 1012 vg/ml, over 94% of the treated eyes in the human CEP290 IVS26 knock-in mice model reached the therapeutic target editing level (10%). [24].for the treatment of LCA10, Allergan and Editas Medicine have started a clinical development of EDIT-101. Gain-of-function GUCY2D mutations cause cone-rod dystrophy (CORD6), an autosomal dominant condition. GUCY2D's early coding sequence is selectively disrupted by CRISPR/Cas components given by AAV in the photoreceptors of mice and macaques through NHEJ. This study showed the possibility of CRISPR/Cas to treat hereditary retinal diseases by successfully performing somatic gene editing in monkeys using AAV-delivered CRISPR/Cas (up to 13% editing efficiency of GUCY2D mutated gene in macaque photoreceptor [25].

Inherited Myopathy

Mutations in the gene that codes for dystrophin cause DMD, the most common type of degenerative muscular dystrophy, which is distinguished by weakness in the muscles, loss of mobility and early death. After local or systemic administration of CRISPR/Cas components by AAV, multiple groups have used NHEJ to get around a premature stop codon in exon 23 and restore the expression of dystrophin in adult and neonatal and mice. [26, 27]. Similar to this, after AAV-mediated systemic application of CRISPR gene editing components, CRISPR/Cas-induced NHEJ has been used to cure DMD in a DMD dog model. At 8 weeks after systemic delivery, 3 to 90% of dystrophin was recovered in skeletal muscle. The editing efficiency depended on the type of muscle, and the muscle histology was improved in treated dogs. [28]. Additionally, a trans-splicing AAV was administered locally by intramuscular injection to treat DMD in a mouse model using ABE. [29]. These research demonstrate how gene editing may be used to treat DMD in patients. One of the neuromuscular diseases, congenital muscular dystrophy type 1A (MDC1A), often exhibits at birth or early childhood. Myasthenia, amyotrophy, and hypotonia are its key characteristics. Loss-of-function mutations in LAMA2, which codes for laminin-2, are the cause of MDC1A. Ronald D. Cohn and his associates upregulated LAMA1, which encodes laminin-1 and is a structurally related protein to laminin-2, using CRISPRa to make up for the loss of laminin-2. In the MDC1A animal model, upregulating LAMA1

reduces muscular atrophy and paralysis and offers a unique, mutation-independent method for treating the disease. [30].

Inherited Hepatic disorder

Patients with loss-of-function FAH mutations and hereditary tyrosinemia type I (HTI) accumulate toxic compounds that damage the liver. CRISPR/Cas-mediated HDR has been utilised to repair FAHmut/mut in the HTI mouse model via hydrodynamic injection of plasmids expressing CRISPR/Cas components or by simultaneous administration of AAV bearing HDR template, sgRNA and of nanoparticles containing Cas9 mRNA [31] In order to treat HTI mice and FAH-knockout mice, VanLith et al. implanted edited hepatocytes containing corrected FAH into the recipients. [32]. In order to correct an FAH point mutation in an adult mouse model of HTI, Song et al. used ABE. [33]. For the treatment of HTI metabolic disorder and prevent the generation of toxic metabolites, multiple teams have knocked down hydroxyphenylpyruvate dioxygenase (HPD), an enzyme that is involved in the next phase of tyrosine catabolism and is an upstream enzyme of FAH [34]. Patients with alpha-1 antitrypsin deficiency (AATD) have progressive lung disease as a result of the loss of AAT antiprotease activity and liver disease as a result of a toxic gain-of-function mutant allele. Mutant AAT has been disrupted using CRISPR/Cas-mediated NHEJ to lessen the pathologic liver phenotype [35], while, AAT point mutations have been corrected with HDR [36].

Genetic Pulmonary disease

Inherited surfactant protein (SP) disorders and cystic fibrosis are the two kinds of congenital genetic lung diseases. [37].The perinatal serious respiratory failure death or chronic widespread lung disease with few treatment options are hallmarks of monogenic lung disorders brought on by mutations in the SP genes of the pulmonary epithelium. In order to inactivate the mutant SFTPCI73T gene by NHEJ, researchers cautiously timed intra-amniotic introduction of CRISPR/Cas9 components into a prenatal mouse model with the human SP gene SFTPCI73T mutation. In SFTPCI73T mutant mice, prenatal gene editing enhanced lung development, restored pulmonary pathophysiology, and increased survival to 22.8%. A mouse embryonic day 16 foetus was administered for intra-amniotic delivery into the amniotic cavity, where foetal breathing movements are suitable for foetal lung editing. In SFTPCI73T mice, embryonic day 19 foetuses with prenatal CRISPR delivery reached up to 32% SFTPC wild-type airway and alveolar epithelial cells, helped lung pathophysiology by immunohistology, increased lung development through decreasing the synthesis of mistrafficked SFTPC mutant proprotein, and boosted the rate of survival to 22.8%. [38]. Another fatal monogenic lung disorder caused by CFTR gene mutations is cystic fibrosis [39]. In induced pluripotent stem cells (iPSC) derived from cystic fibrosis patients, the homozygous F508 mutation (F508del) in exon 10 was accurately corrected using CRISPR. [40]and using piggyBac transposase as a selection marker, the average correction efficiency is increased up to 90%. CRISPR/Cas RNP electroporation increased 20% correction rate with F508del mutation in a patient-derived iPSC cell line [41].

Inherited deafness

More than half of cases related to deafness occur due to the genetic mutation. More than 120 cases are identified related to deafness which are associated with genes, but very limited treatment is available. [42]. SaCas9-KKH/gRNA was found to specifically and safely recognise mutant Tmc1 but not the wildtype allele in vitro and in vivo by David P. Corey's team after screening 14 Cas9/sgRNA combinations. This discovery offers a method to effectively and specifically affect the dominant single nucleotide mutation rather than the wild-type alleles. [43].

Sickle Cell Disease

When glutamic acid is substituted for valine in the sixth codon of the -globin gene on chromosome 11, it results in a genetic monogenic disease known as sickle cell disease (SCD). SCD includes a set of disorders with varying clinical manifestations but a shared pathophysiologic outcome resulting

from a single monogenic mutation, whether acquired either in a homozygous condition or with another defective -globin gene. An abnormal haemoglobin is produced by the altered β-globin gene. The major transcription factor responsible for regulating the transition from foetal haemoglobin (HbF) to healthy adult haemoglobin (HbA) is BCL11A. A fetus's growth phase is when HbF is produced, and it differs slightly in structure from HbA. Instead of two -globin subunits, HbA is made up of two -globin subunits.Similarly,HbF is made up of two β-globin subunits as well, however it comprises two -globin subunits rather than β-globin. Foetalhaemoglobin levels drop and adult haemoglobin levels rise as the foetus develops. Although HbF has been utilised for foetal development, it performs identical functions and is a better substitute for sickled HbIn ex vivo cell culture systems and animal models, CRISPR/Cas9 may effectively be employed to repair the SCD mutation or stimulate HbF expression. Using hematopoietic stem and progenitor cells (HSPCs) and CRISPR-Cas9 gene editing, BCL11A expression in erythroid-lineage cells will reduced, β-globin synthesis will restored, and foetalhaemoglobin (HbF) production will reactivated [44]. However, there continual safety concerns, nevertheless, because to unexpected off-target effects and insufficient therapeutic efficacy. Ex vivo optimization studies should also be conducted, and larger animal models should be used to investigate the method's safety.

Huntington's disease

Psychiatric problems, motor issues, cognitive deficiency disorders trouble sleeping, and weight loss are all features of HD. HD is a hereditary neurodegenerative condition that causes motor impairment and neuron loss. [45]. Choroic movements, behavioural and cognitive issues, and dementia are just a few of HD's symptoms. HD is a hereditary condition that worsens over time. The HTT gene's CAG repeats expand in this condition, which leads to an extension of the polyglutamine pathway. Muscles, lungs, and the brain are where glutamate, the precursor of the neurotransmitter glutamine, is mostly synthesised. HTT protein mutations have also been linked to the development of HD, and this protein is necessary for neuronal development.

The CRISPR/Cas9 method effectively treats HD because it modifies the HTT gene's encoding without permanently altering the genome [47]. mHTT genes that target genetic variants linked to the CAG-expanded allele in patient-derived fibroblasts are likewise rendered inactive using the CRISPR/Cas9 system.

Individuals with certain genetic disorders are treated using the CRISPR/Cas9 method through the development and analysis of patient-derived pluripotent stem cells (iPSCs) obtained from such individuals. Because of their capacity for self-renewal and pluripotency, iPSCs are similar to embryonic stem cells. They are derived from human iPSC fibroblasts using virus-mediated induction. According to reports, HTT has developed HD-iPSC lines with various CAG repeat lengths and control iPSC lines. [48]. A combination of sgRNA and a Cas9 D10A nickase can be used to repair certain iPSC clones, according to studies, after antibiotic selection. The wild-type Cas9 protein catalyses DSBs that are targeted by sgRNA. On the other hand, sgRNA targets DNA that has been single-stranded broken by Cas9 D10A nickase. Cas9 D10A is thought to have improved target sequence selectivity.

Parkinson's disease

A prevalent progressive neurodegenerative state called Parkinson's disease (PD) is characterised by the decline of dopaminergic (DAergic) neurons in the substantia nigra (SN) [49]. Degenerative Parkinsonism, a clinical motor condition brought on by Parkinson's disease (PD), is characterised by muscular stiffness, postural instability, bradykinesia, and resting tremor. Leucine repeat kinase 2 (LRRK2) and -synuclein (SNCA) both exhibit autosomal-dominant Parkinson's disease (PD), whereas mutations in parkin, PTEN-induced putative kinase 1 (PINK1), DaisukeJunko-1 (DJ-1) [50], RAB39B, P13, ghrelin, Prokineticin 2, and Protein Kinase C (PKCδ) exhibit autosomal recessive mutation.

Parkinson's disease research with CRISPR is taking on a new perspective that may have seismic effects on how the condition and other neurological disorders are treated.

Different genetic abnormalities relaed to PD with their CRISPR CAS approach are:

1. One of the frequent risk loci linked to sporadic PD is SNCA. It is important to note that none of the sporadic single-nucleotide polymorphisms (SNPs) linked to PD modify the a-synuclein protein coding sequence [51]. Because it has been suggested these variations may increase gene expression levels, [50] therefore, the accumulation of aberrant protein is a typical pathogenic aspect of Parkinson's disease (PD). Utilising the CRISPR/Cas9 technology, distinct SNCA variants that include SNP may be found and modified.
2. Mitophagy and mitochondrial quality control depend on PINK1 and PARKIN. The PINK1/PARKIN-mediated mitophagy regulators have been found using RNAi screening. Researchers have employed CRISPR/Cas9 gene-editing technology as a screening method that looks to outperform RNAi for phenotypic screening to clarify the set mitophagy threshold of the cells. [52].
3. P13 is suggested as a therapeutic target for Parkinson's disease (PD) since it is involved in the control of mitochondrial activity. CRISPR/Cas9 knockout of this gene in mouse studies.

4. PK2 has a significant function in mitochondrial biogenesis. According to reports, postmortem brains from PD patients had increased PK2 signalling. This gene and its receptor have been blocked using the CRISPR/Cas9 technology. These in vitro investigations' findings demonstrated that a PK2 deletion enhanced the sensitivity of neurons to cell death brought on by neurotoxic agents. [52].
5. PKCδ regulates the apoptosis that endosulfan causes. CRISPR/Cas9 was used to knock down PKCδ in N27 dopaminergic cells. This stable knock-down cell line demonstrated the decrease in endosulfan-induced caspase-3 levels.

Alzheimer's disease

The two main etiological aspects of Alzheimer's disease (AD) are the gradual loss of cognitive function and irreversible loss of neurons. Cognitive function and memory formation steadily decline as a result of an irreversible neuron loss in AD, a chronic neurodegenerative disease. The development and deposition of amyloid-beta 42 (Ab42), mutations in presenilins 1 and 2 (PSEN1), phosphorylated Tau, and excessive glial cell activation are its defining features. Additionally, impaired neurotrophin signalling and decreased synapse function are crucial aspects of AD. Memory loss, apathy, sadness, and impatience are some of the primary symptoms [54]. A PSEN2N141I mutation patient's basal forebrain cholinergic induced pluripotent stem cells (iPSC)-derived neurons were edited using the CRISPR/Cas9 technology to remove autosomal dominant mutations that might have decreased the formation of amyloid-beta 42. [55]. Another important risk factor for late-onset AD is the APOE4 allele. As a possible editing tool, CRISPR/Cas9 may potentially be used to change APOE4 to APOE2 or E3, one of the common alleles that does not seem to increase the risk of AD. As a result, changing one of these amino acids using CRISPR/Cas9 can successfully offset the risk associated with the APOE4 allele.

Cystic fibrosis

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene lead to cystic fibrosis (CF), an autosomal recessive monogenic disease. [56] causing damage to the digestive and lungs systems. The CFTR protein, an ion channel that transports electrically charged atoms and molecules back and forth across cells, distributes chloride ions normally from inside to outside of cells in healthy individuals, enabling cilia movement. Chloride ions are stuck inside cells as a result of the mutations, preventing water from passing through the cell. Regular mucosal levels raise and become stickier as a result of water deficiency. The cilia move more slowly when the mucus is thicker. When the cilia stop moving normally, mucus starts to clog the airways and bacteria start to grow. [57]. Mucus develops in the lungs, pancreas, and intestines of CF patients, causing malnutrition, stunted development, recurrent respiratory infections, breathing problems, and finally irreversible lung damage.

A Cas9 nickase linked to an engineered reverse transcriptase (RT) and complexed with a prime editing guide RNA (pegRNA) make up the CRISPR-based technique known as prime editing. [58]. The pegRNA varies from the typical gRNA in that it contains a sequence that the RT module reverse transcribes to create a donor DNA template. According to the pegRNA design, the result of prime editing is either a high degree of precision insertion, deletion, or substitution. Therefore, the range of disease-causing mutations that may be resolved with the use of this new technique may be considerably expanded, including CF. By introducing three nucleotides into the altered F508 locus, primary editing may be used to treat the most prevalent cause of CF, as was suggested in the original article. [58]. To further understand CF pathophysiology, CRISPR-Cas9-mediated CFTR gene disruption was also used to create the sheep models (CFTR+/− and CFTR−/−) [59]. The enhanced research and development of new CF treatments is intended to benefit from the use of the mutant CFTR sheep model.

Cancer

In cancer, one of the most common diseases and causes of mortality in the world, a variety of signalling pathways relating to angiogenesis, proliferating cells, dissemination, and resistance to apoptotic evasion are dysregulated. [60] Combining CRISPR/Cas9 technology with immunotherapy, such as Chimeric Antigen Receptor (CAR) T cell therapy, might be an effective strategy for treating cancer [61]

Finding genotype-specific vulnerabilities is one of the main goals of CRISPR/Cas9 screening in oncology. These genes may be specifically deleted, which offers a method to identify prospective treatment targets by reducing the viability of cancer cells. [62]. Finding genes that work in synergy with drugs or result in drug resistance is another use for CRISPR screening, which can provide insight into how cancer responds to drug therapy. Inhibitors of the Receptor tyrosine kinase RTK/Ras/MAPK pathway are frequently utilised for the treatment of lung cancer and other malignancies, although the majority of patients still fail to cope well with treatment. CRISPR/Cas9 gene deletion analysis in lung cancer cells revealed that KEAP1 loss affects cell metabolism, allowing cells to proliferate without MAPK signalling in the presence of various specific RTK/Ras/MAPK pathway inhibitors. [63]. Thus, Loss-of-function testing can assist determine the effectiveness of associated drugs in clinical studies and help patients choose their treatments.

Thalassemia

A genetic hematological disease called thalassemia results from abnormalities in the α globin and β globin genes. Each year, 0.3 to 0.4 million infants worldwide are born with hemoglobinopathies. Streptococcus pyogenes-isolated CRISPR/Cas9 may be modified and utilised to knock off genes in human cells. Genome editing carried out by CRISPR/Cas9 can also restore the normal function of β-globin with little adverse consequences. [64]. Reactivating γ-globin, which can take the place of damaged -globin, is another method of treating thalassemia. EIF2AK1, also known as heme-regulated inhibitor (HRI), participates in protein translation and inhibits HbF expression. An enzyme called HRI, which is regulated by red blood cells, prevents HbF from being translated. In addition, HbF is inhibited by BCL11A. Low BCL11A expression prevents HRI formation, which thereby increases HbF production [65]. By reducing the synthesis of γ-globin and reducing the binding of BCL11A to its binding site, CRISPR/Cas9 is utilised to reactivate the genes involved with γ-globin. This reduces the clinical severity of β-thalassemia and increases the production of γ-globin. [66].

COVID-19

The third major human pandemic of its family to affect humans in the past 20 years was brought on by the SARS-CoV-2 virus, a member of the Betacoronavirus genus and the cause of Coronavirus Disease 2019 (COVID-19). Due to its non-segmented enveloped positive-sense single-stranded RNA, large genomic size, and highly zoonotic and transmission from human to human rates in comparison to other members of its family [67]. Due to the unusual self-replication phenomena of this newly emerged virus, the genomic sequence is very susceptible to mutation and recombination [68]. The

SARS-CoV-2 genome contains fourteen open reading frames (ORF), which together code for four structural proteins—membrane (M), envelope (E), spike (S) and nucleocapsid (N)—as well as 9 accessory proteins and sixteen nonstructural proteins that make up the replicase complex. Cathepsin L and transmembrane protease serine 2 (TMPRSS2), two host cell proteases, activate the two functional segments (S1 and S2) of spike protein. [69]. The receptor-binding domain (RBD) of the SARS-CoV-2 spike protein attaches to the host cell receptors, Angiotensin-Converting Enzyme 2 (ACE2), whereas the spike protein of other human coronaviruses can attach to a variety of cellular entry receptors, and determines the tropism and pathogenicity of the virus. Compared to SARS-CoV and MERS-CoV, an RBD mutation increases the virus's affinity for human ACE2 and, as a result, its transmissibility occur. [67].

Understanding the biology and pathology of the virus will aid in the development of diagnostic procedures, therapeutic plans, and vaccines. In the development of anti-viral molecular therapy platforms, intervention tactics, such as targeting the virus cell entry route, which includes RBD, as well as the virus replication complex, which includes RdRp, are crucial targets. [70].

Traditional approaches to treating viral diseases often focus on avoiding viral infections by targeting macromolecules along the entry route up to proliferation. Drug repurposing, which employs already-existing medications to expedite treatment in the event of the advent of viruses, is the initial stage in anti-viral therapy. The second strategy may involve creating drugs with a target-based approach that focuses on the genomes and proteomics of the targeted virus. [71]. Based on these two strategies, drug candidates for the treatment of SARS-CoV-2 virus infection typically concentrate on innate immunity support or suppression of the excessive inflammatory response, disruption of viral enzymatic function (especially RNA-dependent RNA polymerase), inhibiting viral proteins (nucleocapsid, envelope, membrane, and accessory proteins), prevention of virus endocytosis and prevention of SARS-CoV-2 replication. [72]. On the other hand, outdated drugs and vaccinations typically have little effect on viruses that have undergone new mutations and spread pandemics. These restrictions on the COVID-19's treatment and prevention may also apply to other viruses in the same family, such SARS and MERS. Novel therapies that focus on the virus' genetic sequences are therefore urgently needed in these circumstances.

Antiviral treatment uses biotechnological methods like siRNA or the CRISPR-Cas system that disrupt gene function. Identifying the virus' DNA or RNA sequence in order to cut it and render it useless is one of the most well-known uses of the CRISPR-Cas system. [73]. The CRISPR system's Cas9 and Cas13 endonuclease enzyme subunits can recognise the RNA and DNA of viruses in mammalian cells that have been infected. Studies have revealed that Cas9 can produce off-targets in the DNA sequence of virus-infected host cells and has a decreased cleavage efficacy for ssRNA. [74]. The crRNA targets ssRNA coding regions of the virus without affecting with the human transcriptome, causing deterioration of sequence, stopping gene expression, and ultimately inhibiting viral activity. The CRISPR-Cas13 system is not dependent on the detection of PAM [75].

In human lung epithelial cells, prophylactic anti-viral CRISPR (PAC-MAN) may successfully recognise and destroy virus sequences and their mutations. The CRISPR-Cas13d class 2, type VI-D, system used by the PAC-MAN system was obtained from Ruminococcus flavefaciens XPD3002. By concurrently detecting protected areas (such ORF1ab, N genes and RdRp) and viral ssRNA in their replication and transcription phases utilising a combination of 6 crRNAs, PAC-MAN can identify more than 90% of all coronaviruses. [76]. High flexibility in recognising viral sequences, directness against the virus and rapid detection are the benefits of CRISPR-based anti-viral systems. [76]. The adeno-associated virus-packaging system (AAV delivery approach) has a strong tropism towards respiratory tissues, making it possible to transfer the Cas13d expression vector there and express it under the stimulation of expression promoters in certain tissues, most notably airway cells. [75].

Delivery of CRISPR-CAS System

The most prominent gene editing technique, the CRISPR/Ca system, enables multipurpose and very precise genome alteration, enabling the treatment of a wide range of serious disorders, including

cancers, genetic disorders, and infectious diseases. In order to perform its role in the treatment of diseases, the CRISPR/Cas system often requires delivery techniques such as in vitro, in vivo, and ex vivo. Effective distribution to target cells is a crucial need for the CRISPR/Cas9 system to work. Different delivery methods are:

- **Physical Methods:** Hydrodynamic injection, electroporation, microinjection and other techniques including deformation of membrane, lance array nanoinjection (LAN) and sonoporation are examples of physical delivery methods depending on brief membrane disruption. [77].
- **Delivery through Viral vector:** Viral vectors, which are among the most widely used delivery techniques, primarily comprise adenoviruses (AdVs), adeno-associated viruses (AAVs) and lentiviruses. [78]. In order to make viral-like particles containing Cas9 and sgRNA, HEK 293T cells must be packed according to the virus delivery strategy. Target cells are then infected and either brought into the body or used for in vitro research.
- **Delivery through Non-Viral vector:** Delivery by non-viral vectors is a new area of study. The concept behind utilizing a non-viral vector is to use the physicochemical characteristics of artificial or naturally occurring vectors to facilitate the transfer of CRISPR components. The majority of non-viral vectors reported in the literature at this time are nanoparticles, and nanoparticles of lipid polymer nanoparticles, DNA nanoparticles, and inorganic nanoparticles are among the nanomaterials often employed to carry CRISPR components. These vectors may be utilized as virus particles in vitro or in vivo and are devoid of any viral components, which helps to increase safety and decrease immunogenicity.

Limitations and Challenges

Therapeutics using CRISPR today still have its limitations. First, the likelihood of off-target mutagenesis is one of the most important concerns in genome editing therapy. Numerous initiatives have been undertaken to increase specificity, such as the creation of a sgRNA designer that takes into account numerous factors. [79]. Second, on-target mutagenesis commonly occurred in double-strand breaks by single-guided RNA/Cas9, leading to massive deletions spanning several kilobases and complicated rearrangements of genome at the specified site, which might have pathogenic impacts. [80]. Third, several physiological hurdles make the effective, secure, and targetable administration of the CRISPR/Cas9 system in vivo is a serious therapeutic issue [81]. Fourth, the immunogenicity of the human body to the bacterial Cas9 protein is another obstacle in using CRISPR/Cas9. [82]. Fifth, CRISPR-Cas9-based therapeutic treatments raise the probability of restoration events or rearrangement of genome following sgRNA-induced double-stranded breaks [83]. The deletion, inversion, and incomplete or inaccurate insertion of donor DNA sequence at the site of integration are unexpected effects of DNA repair systems that may result in unexpected mutations [84].

Conclusion

Since its discovery, CRISPR/Cas technology has revolutionized several disciplines, including biology, healthcare, and even agriculture. As this technology advances, it will become increasingly simple to precisely change the genes of both prokaryotes and eukaryotes, and it will also become able to treat diseases that currently go untreated. Using this technology to alter human embryos for the treatment of disease raises ethical concerns in addition to the previously discussed ones. There is no doubt that CRISPR technology has a promising future, but it has to be advanced by solving the aforementioned issues as soon as possible.

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