

CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS – A NEW ERA IN GENOMIC TECHNOLOGY AND IT'S APPLICATIONS IN THERANOSTICS

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Received: February 07, 2023; Revised: August 03, 2023; Accepted: August 03, 2023

Abstract

Bacteria have “spacer” sequences of deoxyribonucleic acid that resemble genomic sequences of phage viruses between repetitive DNA sequences known as CRISPR. A CRISPR and spacer DNA template can be used to create transactivating tracrRNAs and targeting crRNAs that cleave DNA complementary to the crRNA, allowing the system to be engineered for cleaving any desired DNA sequence. In genetic engineering, it has revolutionized the field of medicine in treating various genetically-linked diseases that are difficult to treat such as Leber congenital amaurosis, retinitis pigmentosa, open angle glaucoma, haemophilia B, sickle cell disease or thalassemia, Duchenne muscular dystrophy, and malignancies such as malignant rhabdoid tumours. The CRISPR/Cas9 method has successfully accelerated and simplified editing of genes in CAR T cells with CD19-specific anti-tumor activity in malignancies such as lymphoma, malignant rhabdoid tumours and other embryonal tumours of the brain. The addition of small molecules as perturbations, functional genome screening with the CRISPR system could help to investigate interactions between genes and drugs, reveal changes in expression of genes following cancer drug therapy, and identify novel targets for precise treatment, while also shedding light on the genesis of disease, thereby making CRISPR-Cas technology, a recent tool, which is highly beneficial in theranostics.

Keywords: CRISPR Genomic editing; theranostics; therapeutics

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DOI: <https://doi.org/10.55766/sujst-2023-04-e0229>

Suranaree J. Sci. Technol. 30(4):030133(1-10)

Introduction

Ruud Janssen and Francisco Mojica proposed the acronym CRISPR in 2002 to describe microbial genomic loci made up of an interspaced repeat array (Hsu *et al.*, 2014). Cas9 is a nuclease enzyme that cuts DNA. Cas9 directs a targeting RNA to align to the target sequence in the DNA to be cut, then cuts this DNA as a way to destroy a virus. This ribonucleic acid-guided DNA nuclease system is dependent on a shorter proto spacer adjacent Motif (PAM). The sequence motif known as the PAM, which follows the sequence of the target genome, is essential for Cas9 to operate. Cas9 recognises the PAM sequence 5'-NGG, where N can be any nucleotide. In order to create space for the sgRNA to bind, Cas9 splits the DNA strands of the nearby sequence when it links to the PAM. If the sgRNA is complementary to that sequence, Cas9 cuts the DNA. Cas9 will unzip and match the DNA with the target RNA. If there is a complete match, Cas9 cuts the DNA, following which the cell tries to repair by itself but this is prone to error, leading to mutations that disables the gene. The Synthego CRISPR Design Tool is software that enables guide RNA design. CRISPR is used in any cell line, including stem cell cultures and fertilized eggs before embryo development. The opportunistic pathogen *Candida albicans*, baker's yeast (*Saccharomyces cerevisiae*), zebrafish (*Danio rerio*), fruit flies (*Drosophila melanogaster*), ants (*Harpegnathos saltator* and *Ooceraea biroi*), and mosquitoes have all been modified with CRISPR techniques (Hsu *et al.*, 2014).

Review

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. Repetitive DNA sequences, which were observed in bacteria with "spacer" DNA sequences in between the repeats that exactly match viral sequences (Hsu *et al.*, 2014). Its use in genome editing has made the CRISPR/CAS9-associated protein 9 (Cas9) system a well-known RNA-guided DNA targeting tool. It was initially obtained from the defence mechanism used by bacteria and archaea against phage invasion, but it has since been researched and used for its potential as a tool for genetic engineering and as a therapy. However, changes to the Cas9 protein have made it possible to cause single-stranded breaks (SSBs) and even epigenetic modifications of gene expression. The Cas9 protein normally generates double-stranded breaks (DSBs) in the target dsDNA sequence (Hsu *et al.*, 2014).

A brief "spacer DNA" sequence made from the genome of an earlier virus invasion follows each repetition (Hsu *et al.*, 2014). The CRISPR repeat-spacer units are then translated into two non-coding RNAs: a CRISPR RNA (crRNA) that contains nuclease guide sequences assembled from the spacers and a second non-coding RNA known as a trans-activating crRNA that is complementary to the repeat sequence (tracrRNA). This dual crRNA:tracrRNA may now recognise the viral DNA as foreign, base pair with the invader DNA, and engage Cas9 endonuclease to cause double strand DNA breaks (dsDNA) at the identification site, ultimately leading to the silencing of the viral gene (Hsu *et al.*, 2014).

History of CRISPR Genomic Editing Technology: *Escherichia coli*'s genome contains clustered regularly interspaced short palindromic repeats, which were first discovered in 1987 by Atsuo Nakata and his team at the Osaka University in Japan. These are the brief genomic sequences that can be found in prokaryotes' genomes. These sequences are known as palindromic repeats because they are identical when read from 5' to 3' on one strand of DNA and from 5' to 3' on the complementary strand of DNA. Gram-positive and Gram-negative bacteria as well as archaea, were the first to be described to have this, which sparked interest in CRISPR. The purpose and significance of CRISPR were initially discussed in prokaryotes in the middle of the 2000s. These prokaryotes are protected from invasion by viral DNA, bacteriophages, and plasmids by their adaptive immunity, which is developed following exposure to a pathogen or immunisation (Dukhovny *et al.*, 2019).

Spacers are the distinctive sequences that are sandwiched between these palindromic sequences; these are pieces of foreign DNA that do not belong to the bacteria and come from previously been infections by mobile genetic elements like bacteriophages, transposons, and plasmids. The spacers found in the CRISPR system were sequenced to reveal this, which led to the theories that this might be a defence mechanism used by bacteria to identify foreign DNA fragments. A little bit of foreign viral DNA is acquired by the bacteria during a viral infection, and it is integrated into the CRISPR locus to produce CRISPR arrays. These are made up of palindromic repeats with duplicate sequences from the bacterial genome flanked by variable sequences, or spacers, which are also derived from external genetic material. A bacteria

can recognise memories of a previous infection in this way.

Mechanism of CRISPR in Prokaryotes

Invasion : Once a virus attacks a bacterium, the infected viral genome is integrated in between the palindromic repeat genetic sequences. The CRISPR array undergoes transcription to form CRISPR-RNA (CrRNA). The longer strand named as pre-crRNA. Cas refers to CRISPR-associated nuclease protein is capable of cleaving DNA at specific nucleotide sequences. Cas9 is one of the nucleases found in *Streptococcus pyogenes*, one of the most characterised CRISPR-associated nuclease proteins. The tracr RNA is complementary to palindromic repeats and can anneal. For each spacer, a complex is formed consisting of pre-cr RNA, tracrRNA, and cas9 protein.

CrRNA Biogenesis: By nucleolytically cleaving a primary transcript (pre-crRNA), CRISPR-derived RNA (crRNA) libraries can be created containing complementary sequences to previously encountered foreign DNA sequences.

An enzyme called Ribonuclease III will cleave the strand in between these complexes into individual crRNA, which are called effector complexes. **Targeting:** When there is formation of effector complexes inside the bacterium, the bacterium is now ready to defend against the invader, whose genome produced that CrRNA.

Spacer acquisition: If this complex encounters a section of viral DNA that has a sequence which is complementary to the crRNA, the nuclease enzyme will coordinate and if it recognizes a short sequence unique to the viral genome called a Protospacer Adjacent Motif (PAM), then it snips both strands of DNA, just a few base pairs upstream from the PAM, thereby neutralizing the virus because the viral genome can no longer transcribe its genome properly to produce more virus particles, thereby infection is not possible (Figure 1).

When Jennifer Doudna, a genetic scientist from the University of California, and French microbiologist Emmanuelle Charpentier originally suggested that the bacterial CRISPR-Cas9 may be utilised as a programmable tool kit in other organisms, including humans, CRISPR technology was first applied in biotechnology. The molecules tracrRNA and crRNA are distinct from one another. These were fused together using a linker to create single guide sRNA, which marked the beginning of the first significant advancement for its application. This two-part mechanism can break DNA if the sRNA combines with the Cas9 protein. The complex will form, read the DNA until it identifies the proper sequence along with the PAM sequence, bind, and the DNA will be cleaved

exactly where it is needed. Each of the two domains of Cas9 will cut one DNA strand. The target DNA is then subjected to the normal DNA mechanism following the DNA incision. The cleaved dsDNA can be repaired in one of two ways: by non-homologous end joining (NHEJ) or by homology-directed repairs (HDR). A DNA with a comparable sequence can serve as a template for the NHEJ pathway because it directly ligates damaged double-stranded DNA without the need for a homologous template. The NHEJ process can also add or remove certain sequences at joining ends, resulting in what are known as Indels. Indels are DNA strands that have nucleotide sequences either inserted or deleted. Non-uniformly sized DNA strands are produced by the NHEJ. The HDR pathway, an alternative mechanism, is frequently present in bacterial and archaeal cells. The eukaryotic domain has a higher prevalence of the NHEJ mechanism. Although more complicated than NHEJ, the HDR pathway also makes use of a similar DNA template. The neighbouring regions near the site of cleavage are homologous to the homologous DNA template, allowing it to incorporate additional DNA pieces. The template directs the repair procedure and reduces the likelihood of mistakes. In contrast to NHEJ, the HDR pathway maintains consistency in the size of the resultant dsDNA since there is no addition or deletion of nucleotide sequences (Figure 2).

Microhomology-mediated end joining (MMEJ) is a distinct end-joining method applied in CRISPR/Cas9 gene editing. MMEJ is active when microhomology (5-25 bp) exists both upstream and downstream of DSBs. This enables the annealing of two microhomology sequences because the intermediate region has been removed. Recently,

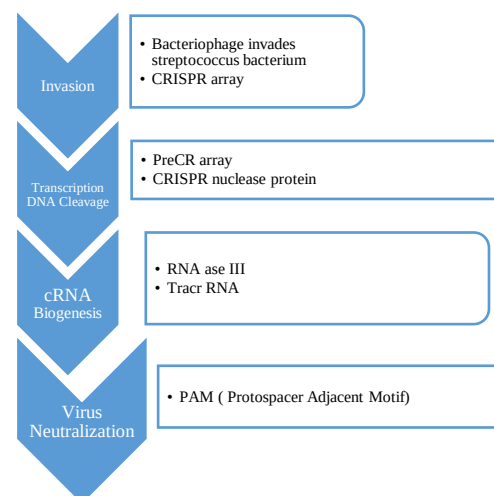


Figure 1. CRISPR-Cas9 Genomic editing

a new MMEJ-based method dubbed precise integration into target chromosomal (PITCh) was developed to achieve the targeted knockin of transgenes. The microhomology sequences of the PITCh donor vector and the genome are exposed by Cas9, causing MMEJ to promote transgene integration at the DSBs. MMEJ is active when HDR is inactive, which is during the M and early S phase. The fact that MMEJ is two to three times more effective than HDR is a key point.

CRISPR is the name of a modified bacterial defence mechanism. Bacteriophages (viruses that infect bacteria) attack bacteria by injecting their DNA through the bacterial cell membrane. This bacteriophage DNA can rewire the invading bacterial cell once it has entered it and cause it to start producing more bacteriophages. One defense method is CRISPR. A bacterial cell snips off a little chunk of the invading bacteriophage's DNA and stores it in its own genome each time it comes into touch with a new bacteriophage. If the bacterial cell ever comes into touch with that specific bacteriophage type again, it will be able to "pull out" that stored piece of DNA and utilise it to recognise and destroy the invader (Dukhovny *et al.*, 2019).

The bacteria use the DNA they store as a defence mechanism against bacteriophages in the following ways:

A region of the bacterial genome called the CRISPR locus is changed by DNA from the invading virus. The DNA (A, T, G, and C) in the CRISPR locus corresponding to the subsequent bacteriophage invasion is transcribed into a short segment of RNA (A, U, G, and C) known as a CRISPR RNA (or crRNA). CrRNAs bind to the effector complex, a huge protein, directing it towards the bacteriophage DNA, which has a complementary sequence to the crRNA sequence. The effector complex and its crRNA bind to the bacteriophage DNA that is invading using Watson-Crick base pairing. The invading bacteriophage DNA is cut, and the CRISPR locus can store it for additional encroachments. Some CRISPR systems process the crRNA by requiring a second RNA called a tracrRNA. The tracrRNA and the pre-processed crRNAs complement one another. Upon RNase III, a ribonuclease, cleaving, a crRNA/tracrRNA hybrid forms and instructs the effector complex to obliterate the bacteriophage DNA that is invading. The various CRISPR-Cas-9 Delivery systems are enumerated in Table 1.

Advantages of CRISPR Technology

CRISPR technology helps in the precise identification of the pathogen. It helps in identifying many mutated genes involved in complex genetic diseases in humans. It aids in studying the

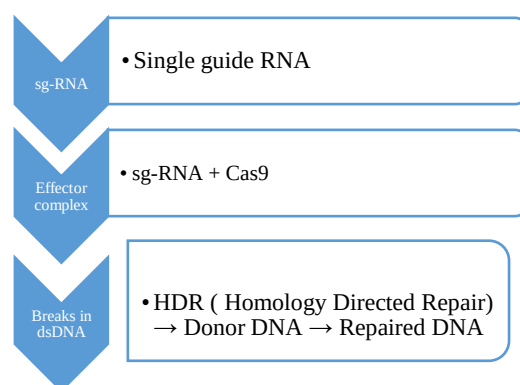


Figure 2. NHEH and NDR pathways in CRISPR Cas9 genomic editing

Table 1. CRISPR-Cas9 delivery systems in Mammalians

CRISPR-Cas9 delivery systems	Types	Advantages	Disadvantages
Viral mediated delivery	Adeno associated virus	Non-integrative, low-immunogenicity in humans, Replication defective	Too large to accommodate, limited cloning capacity (<4.7 kb) capacity
	Lenti virus	Efficient delivery, Large cloning capacity	Random integration, insertional mutagenesis
Non-viral mediated delivery	Electroporation Lonza 4D-Nucleofector system using nucleocuvette strip	Efficient delivery, easy to operate	Difficult to work <i>in vivo</i>
	Lipid/gold based nanoparticles	Low stress to cells	Requires extensive optimization

phylogenetic conversion of pathogens like bacteria, viruses, and xanthomonads. It helps in regression of latency period in HIV-infected cells. It provides a screening tool for cancer. It has a valuable therapeutic application CART-Cell therapy in the treatment of cancer immunotherapy, in which the T-cells are modified for specialization in recognizing and killing cancer cells. haematological malignancies and solid tumours. This includes breast or lung cancer, relapsed tumours with no further curative treatment options, such as melanoma, multiple myeloma, round cell or myxoid liposarcoma, and synovial sarcoma. It is used to study and identify the phylogenetic variations of the pathogenic microorganisms. It helps to treat complex syndrome like Wolfram syndrome characterized by optic atrophy and Type II Diabetes mellitus. It helps to identify harmful genomic sequences and remove such deleterious genes in

human embryos. It is also used to identify disease-causing genes in human embryos.

Disadvantages of CRISPR Technology

Sequencing is restricted only to target sites. The possibility of unexpected outcomes at the target site, such as chromosomal rearrangements, massive deletions, and the insertion of undesirable sequences can occur after CRISPR genomic editing. Off-target mutagenesis can arise in the genome at undesired locations. CRISPR genomic editing cannot be done effectively in the main viral vector used for human therapeutics, adeno-associated virus (AAV). Immunological cross reactivity can occur against Cas-9 expressing cells, during CRISPR genomic editing.

Applications of CRISPR Genome Editing

Differentiate various strains of Zika virus: SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking), a new CRISPR technology that targets RNA, is used to differentiate between American and African strains of the Zika virus by identifying the genomic sequences detect molecules of one genetic material among mixed samples, and can distinguish between genetic sequences that differ by only one nucleotide (Dukhovny *et al.*, 2019).

Sickle cell anemia and β -Thalassemia: CRISPR technology is used to treat genetically linked sickle cell anemia and β -thalassemia. For patients with sickle-cell anaemia Vertex Pharmaceuticals and CRISPR Therapeutics conducted the first clinical trial in the US employing CRISPR to catalyse disruption of genes for therapeutic benefit. They later conducted trials for β -thalassemia. This treatment, known as CTX001, raises the amount of fetal haemoglobin (HbF), which has high affinity for one or two of the four haemoglobin binding spaces on red blood cells and consequently help major hemoglobin disorders including SCD and β -thalassemia (Frangoul *et al.*, 2021).

Type 1 and 2 Diabetes Mellitus: CRISPR is used in a liposomal drug delivery system to treat Type 2 diabetes mellitus. PEC-QT (VCTX-210 partnered with CRISPR), a new therapy that places genetically modified stem cells implanted in the patient's body to treat all non-insulin dependent Type I Diabetes mellitus and insulin dependent Type II Diabetes mellitus. Gene-corrected WS SC beta1 cell gene expression by CARISPR-Cas genomic editing exhibit dynamic insulin secretion driven by glucose and express cell markers resulting in efficient glucose utilization, thus aiding in treatment of Type I Diabetes mellitus (Cho *et al.*, 2019).

Wolfram Syndrome: CRISPR technology treats Wolfram syndrome, characterized by diabetes mellitus and optic atrophy. CRISPR genomic editing helps in replacing the defective WFS1 gene with wild-type WFS1 alleles, that could be relocated into the retinal ganglion cells of patients with Wolfram syndrome (Maxwell *et al.*, 2020).

Identification of emerging human pathogens: CRISPR genomic technology is useful in identifying *Stenotrophomonas maltophilia*, an emerging noscomial opportunistic human pathogen that has the ability to colonize indwelling catheters and airways. The distribution of CRISPR locus across the *Stenotrophomonas* genus, including *S. terrae*, and *S. acidaminiphila* along with genomic sequences helps to identify the specific species of *S. maltophilia* (Looney *et al.*, 2019).

Leber's hereditary optic neuropathy: An inherited eye disorder, which is rare that results in optic atrophy and blindness in newborns caused by biallelic functional loss due to a mutation in the *CEP290* gene. Therapy using a CRISPR technology called EDIT-101 targets the *CEP290* locus with an IVS 26 mutation that enables subsequent translation of functional protein rather than splicing (Simonelli *et al.*, 2010).

Parkinsonism: CRISPR technology is used in the treatment of Parkinsonism, a neurological disorder characterized by tremors of muscle, pill-rolling tremors, decreased mobility, and mask-like facial expression. CRISPR-Cas 9 technology is used to knockdown PKC delta expression in dopaminergic neuronal cells. CRISPR-Cas9 editing of mutated Leucine-rich repeat kinase2 (LRRK 2) reduced neurite complexity in dopaminergic neurons (Mehmood *et al.*, 2021).

Cancer Therapy: Chimeric Antigen Receptor T-cell Mediated Immunotherapy (CART) targeted immunotherapies for solid malignancies, including lymphoma, leukemia, prostate cancer, glioma, sarcoma, melanoma and breast cancer.

In addition to being safe, NY-ESO-1(CTA New York Esophageal Squamous Cell Carcinoma-1) is also known as cancer-testis antigen 1B (CTAG1B) TCR-engineered T cells trafficked to marrow showed prolonged persistence correlated with clinical activity against antigen-positive myeloma.

Gene editing using CRISPR-Cas9 permits the creation of CAR-T cells with enhanced anti-cancer properties. (Baghini *et al.*, 2022).

HIV therapy: CRISPR-associated nuclease-9 (Cas9) is used in the treatment of HIV. CRISPR-Cas9 conferred resistance by genetic mutation of CCR532 against the CCR5 (C-X-C Chemokine receptor 5) gene. CRISPR -Cas9 target RNA

is (RNA interference), that block viral entry, and also HIV dsDNA, formed by viral transcription through reverse transcriptase and integration of proviral DNA (Xiao *et al.*, 2019).

Developing selective antimicrobials for bacterial pathogens: To create selective and titratable antimicrobials for the elimination of dangerous bacteria like *Salmonella enterica* and *E. coli*, CRISPR technology is being applied. Conventional antibiotics can be replaced by CRISPR antimicrobials, and combination therapies may make use of them more successfully. For instance, *E. coli*-infected *G. mellonella* larvae were treated with ceftriaxone and CRISPR/Cas9 targeting blaTEM-1, which confers beta-lactam resistance. As a result, 70% of the dual-treated larvae survived compared to 30% of the larvae treated with antibiotics alone. Instead of treating infections, CRISPR antimicrobials may be employed to eradicate antibiotic-resistant microorganisms. Antibiotic resistance genes could be targeted using CRISPR technology in order to prevent the spread of resistant bacteria after antibiotic use. After receiving antibiotic therapy, the amount of erythromycin-resistant *E. faecalis* in the stomach was dramatically decreased by CRISPR/Cas9 targeting of the ermB gene in *E. faecalis* (Yadav *et al.*, 2021).

Treatment of Hepatitis B infections: CRISPR technology is used to treat Hepatitis-B viral infection by targeting and depletion of ccc DNAs (closed circular covalently bound DNAs) of Hepatitis -B virus and also by DNA editing using CRISPR-Cas. CRISPR-Cas9 based editing of Hepatitis B Virus Genome by designing a guide RNA targeting the core, polymerase, surface and HBx region (Kennedy *et al.*, 2015).

Filariasis infections: CRISPR is able to parasitize nematode *Caenorhabditis elegans*, hence used in the treatment of Lymphatic filariasis. CRISPR-Cas9 genome editing helps to mediate the deletion of LmjF_LEM3171 PFR2 locus (Sollelis *et al.*, 2015).

Treatment of neuropathic pain in spinal cord injuries : By triggering the suppression of glial cell maturation factor (GMF) and lowering the signalling of the p38 mitogen-activated protein kinase pathway, CRISPR/Cas9 is utilised to treat spinal cord lesions brought on by trauma. This helps reduce neuropathic pain. On the other side, C3 knockdown in astrocytes may enhance axonal regeneration, which may aid in the restoration of motor function. Additionally, PTEN (phosphatase and tensin homolog) knockdown in neurons may encourage an increase in neurite length (Paschon *et al.*, 2020).

Treatment of cervical cancers caused by HPV: Cervical cancer cells can be stalled by treatment that inactivates the human papillomavirus E6 or E7 gene using a bacterial CRISPR-Cas RNA-guided endonuclease. By inducing senescence in cancer cells, HPV16-E7 gRNA-guided CRISPR/Cas represents a viable therapeutic approach for the treatment of HPV-induced cervical cancer (Kennedy *et al.*, 2014).

Cockayne syndrome: CRISPR technology is utilised to cure Cockayne syndrome, an uncommon autosomal recessive hereditary condition that manifests as rapid aging progressive neurological deterioration, cachexic dwarfism and heightened sensitivity to sunlight. CS-MSCs corrected by Integration-free induced pluripotent stem cells (iPSCs), Cas9-2A-GFP, gRNA-mCherry, and single stranded oligodeoxynucleotide (ssODN) helps to knock out gene ERCC6 that causes Cockayne syndrome. Gene corrected GC-MSC (Mutant Stem Cells) exhibit restored DNA repair ability and resistance to UV-induced apoptosis. The autologous GC-MSC free of pathogenic mutation helps to produce novel therapeutic strategies for treatment of cockayne syndrome (Wang *et al.*, 2020).

Atherogenic Dyslipidaemia: CRISPR-Cas9 is used in the treatment of atherosclerosis caused by disruption of genes, which are atherogenic, such as the PCSK9 gene, which produces a protein that causes atherogenic dyslipidaemia. It helps to suppress the genes that increase the risk of atherosclerosis (Chadwick *et al.*, 2018).

Hypertrophic cardiomyopathy: CRISPR is used in the regeneration of damaged myocytes in hypertrophic cardiomyopathy by the safety of Adeno Associated Virus 1-mediated gene transfer and improvement of the exercise capacity and symptoms in patients with advanced cardiac hypertrophy (Behrens-Gawlik *et al.*, 2014).

Hemophilia: CRISPR genome editing is also beneficial in treating haemophilia B, an X-linked inherited bleeding disorder by gene repair, and also promotes differentiation of induced pluripotent stem cells to hepatocyte-like cells (HLCs). CRISPR-Cas9 genomic sequence modification was used in the treatment of haemophilia B, an inherited disease caused by mutation of the factor IX gene. The Y371D mutation was successfully modified by CRISPR-Cas9 genomic editing (Morishige *et al.*, 2020).

Cystic fibrosis: Cystic fibrosis is a genetic disorder caused by defects in the CFTR (cystic fibrosis transmembrane conductance regulator) protein. In the airway surface liquid (ASL), which is composed of mucus above the periciliary liquid layer (PCL), CFTR forms chloride and bicarbonate

ion channels on the apical surface of epithelial cells. In order to maintain the thin, watery consistency of the PCL, ions create osmotic pressure that draws water into the ASL. Epithelial cells retain chloride ions when the CFTR protein is not functional. As a consequence, sodium hyperabsorption is associated with loss of CFTR-mediated inhibition of the epithelial sodium channel, ENaC, leading to excessive water loss from the ASL. CRISPR is beneficial in treating cystic fibrosis (CF), which affects the lung, pancreas, gut, liver, and reproductive organs. CFTR variants, mutations in the CF gene alter CFTR expression, protein levels, or function. CRISPR is helpful in treating cystic fibrosis. A few of the compromised epithelial cell functions include abnormalities in the transfer of chloride and bicarbonate via this channel, elevated sweat chloride concentrations, changes in pancreatic hormone regulation, intestinal blockage, and more. CRISPR editing of the human SP gene *SFTPC173T* reduced pulmonary pathogenesis and improved development of lungs. Markers which are selective, such as piggyBac transposase with CRISPR, accurately fixes CFTR bearing homozygous F508 deletions (F508del) on 10th exon in iPSCs isolated from individuals with this disease (Graham and Hart, 2021).

Malaria: CRISPR genome editing is used for genomic splicing in mosquitoes that cause malaria and is beneficial in preventing its spread by facilitating gene editing in the *Plasmodium berghei* malarial species. The creation of a CRISPR/Cas9 system targeted the ACT2 locus employing a linear donor template and ribozymes for sgRNA production allows for gene editing in *P.berghei* (Lee MCS *et al.*, 2019).

Identification of foodborne pathogens: CRISPR genome editing is used for the detection of foodborne pathogens. The quantitative and qualitative analysis of infectious pathogens has enormous potential for the CRISPR-Cas12a-based biosensor analytical technology. A CRISPR-Cas 13a system, has been successfully used for detection of drug resistant *E. coli*, *S. aureus* and *Shigella* (Shin *et al.*, 2021).

Transthyretin Amyloidosis: CASPR editing was used in production of the therapeutic drug NTLA 2001, an *in vivo* gene editing therapeutic agent in transthyretin amyloidosis, characterized by progressive accumulation of misfolded transthyretin protein. CRISPR/Cas9-based therapy (NTLA-2001) was used to knockout the transthyretin gene, which significantly reduced the production of transthyretin from hepatocytes with a single course therapy (Gillmore *et al.*, 2021).

SARS-COV2 DETECTR ASSAY: This assay is based on CRISPR-CAS12 technology and detects

nucleocapsid (N) and envelope (E) genes of SARS-CoV-2. The assay uses a principle of RNA extraction and simultaneous amplification isothermally by Reverse Transcription - Loop-mediated isothermal amplification (RT-LAMP) method. The targeted gene of SARS-CoV-2 are visualised by a fluorescent reporter molecule. The assay also shows a laminar flow strip in which less than a single band indicates negative infection and above one or two bands, indicates SARS-CoV-2 infection. This assay has a rapid turn over time of 40 minutes with 95% accuracy (Uddin *et al.*, 2020).

PACT (Prophylactic Antiviral CRISPR in human cells): This uses the VI-D CRISPR-Cas13d version from *Ruminococcus flavefaciens*, which is tiny enough to be packaged more easily in viral vehicles and has strong catalysis and high specificity in human cells. In order to tackle coronavirus variations, this method was devised to simultaneously target numerous areas for RNA degradation (Uddin *et al.*, 2020).

Fabry's disease: A genetic deficiency of the lysosomal enzyme alpha-galactosidase A results in Fabry's disease, a glycosphingolipid storage disorder that affects the vascular, cardiac, renal, and nervous systems and is characterised by neuropathic pain. The glycolipid substrate globotriaosylceramide (Gb3) accumulates in different cell types throughout the body as a result. Using the CRISPR-Cas9 gene editing technique, it is now possible to target the GLA gene to create genetically modified engineered cells, which provides therapeutic potential for Fabry's disease (Kaneski *et al.*, 2022).

Rheumatoid arthritis: The genes *IL20RA*, *IL22RA2*, *IFNGR1*, *MYC*, *FOXO1*, *OLIG3*, and *TNFAIP3* can be disrupted by the SNP rs6927172 using CRISPR-Cas9. The micro RNA micro-RNA155 (miR-155), an important proinflammatory factor in the pathogenesis of rheumatoid arthritis can be knocked out in the RAW 264.7 cell line of macrophage, resulting in the diminution of proinflammatory cytokines (Lee *et al.*, 2022).

Crohn's disease: The gene knockout of SGK2 gene by CRISPRgRNA resulted in decreasing the progression of Crohn's disease. The loss of functional mutation was associated with rs7234029 SNP. rs6651252 enhances the regulation and expression of c-MYC, that are elevated in patients with Crohn's disease (Lee *et al.*, 2022).

Psoriasis: CRISPR-Cas9 genome editing is used to knockout the desmoglein 1 gene that aids in the differentiation of epidermis, regulation of inflammatory processes and ERAP1, the key genes involved in the etiopathogenesis of psoriasis. The epistasis between HLA-C*06:02 and ERAP1 variations impacts psoriasis, which eventually involves ADAMTS-like protein 5 (ADAMTSL5)

and immunological sensitisation of melanocytes, according to CRISPR-Cas9 editing (Lee *et al.*, 2022).

Systemic Lupus erythematosus: CRISPR-Cas9 editing knockdown of CXorf21 resulted in reduced expression of interleukin-6 and tumour necrosis factor-alpha and resulted in regression of systemic lupus erythematosus (Lee *et al.*, 2022).

Multiple sclerosis: CRISPR-Cas9 genomic editing helps to knock out TNFRSF1A gene that causes Multiple Sclerosis. The IL7R exon 6 rs6897932 SNP has an impact on gene expression. Multiple sclerosis risk is exacerbated by an epistatic interplay between rs2523506 in DDX39B and rs6897932 in IL7R, which controls IL7R exon 6 splicing.

Forensics as DNA analysis tools: Mixed, and deteriorated samples can all be examined using the CRISPR-Cas9 approach. This method of changing DNA can result in the creation of ghost DNA profiles. A ghost DNA profile may be discovered using alternative genetic markers and methylation patterns (Dash and Arora, 2022).

Alzheimer Disease: Thioredoxin-interacting protein (Txnip) expression is reduced as a consequence of CRISPR-Cas9 genome editing in HT22 cells, which is an effective way to reduce amyloid-induced protein cysteine oxidative alteration. According to the research, Txnip is a promising therapeutic target for the management of Alzheimer's disease. By removing the background of endogenous -secretase, it is possible to knock out the Presenilin 1 (PSEN1) genes in N2a cells. It has been discovered that exogenous addition of recombinant protein obtained from PSEN1 mutations can reduce the generation of A-42 and A-40 (Lu *et al.*, 2021).

Huntington's Disease: A uncommon genetic illness called Huntington's disease causes the brain's neurons to perish, which causes mood swings, aggressive behaviour, memory problems, and difficulty concentrating. By multiplexing various sgRNAs into the lentiviral vectors, CRISPR/Cas9 can simultaneously target many genes. In order to remove exon 1 of the mutant HTT allele, which causes Huntington's disease, one may therefore combine two sgRNAs. Targeting a region of the HTT gene (before the CAG expansion) in Exon 1 to cause a frame shift and obstruct transcription and translation results in non-allele-specific editing. (b) Transcription-disrupting methods based on allele-specific approaches and the existence of SNPs in the promoter region that are heterozygous in people with Huntington's disease. (c) The deletion of HTT exon 1 might be induced by combining two sgRNAs (inside the promoters and the first intron). To cause an exon 1 deletion unique to an allele, one

of the two sgRNAs should target a sequence that encodes an SNP (Vachey and Déglon, 2018).

Cure Blindness: CRISPR-Cas9 genomic editing technology is used to cure ocular diseases. For instance, subretinal Adeno Associated Virus-facilitated transport of SaCas9 combined with a couple of genomic RNAs to mark a profound intronic mutation in the CEP290 gene was used in treatment of Leber congenital amaurosis (Ahmad, 2022).

Inhibition of biofilm:

CRISPR-Cas technology to alter *S. Mutans'* behaviour in a way that prevents them from producing a matrix and essentially prevents the development of biofilms (Gong *et al.*, 2022).

Precision T cell replacement for Personalized cell therapy: Neo T-cells engineered by CRISPR-Cas9 genomic editing are used for precise replacement of T-cells in personalized cell therapy (Foy *et al.*, 2023).

Treatment of malignant rhamdoid tumours and other embryonal tumours of the brain: Targeting PLK4 with small-molecule inhibitors using CRISPR-Cas9 genomic editing proves a novel treatment strategy for the treatment of aggressive therapy resistant malignant rhomboid tumours and other paediatric embryonal tumours of the brain (Sredni *et al.*, 2017).

Conclusions

CRISPR-Cas methodology has gained tremendous importance in genomic technology to counteract refractory diseases that do not respond to treatment by interfering in the genomic sequence of the pathogen. CRISPR genomic editing. CRISPR genome editing has provided a valuable tool for scientists for its wider utility in the treatment of pathological diseases like HIV, Parkinsonism, cystic fibrosis, Hepatitis-B, malaria, and Zika virus infections. CRISPR-Cas also helps in the treatment of premature ageing caused by Cockayne syndrome. CRISPR-Cas genomic technology has also proved to be efficacious in the treatment of pain caused by injury to spinal cord or Fabry's disease. The integration of CRISPR in biosensors can help in the quick identification and provide a valuable tool in theranostics. Further research on CRISPR genomic editing is essential for its use in integration in biosensors, that help in the identification of rare strains of pathogens.

The use of CRISPR-Cas must imply legal and bioethical principles that protect humans and safeguard the integrity of the patient, and their

genetic information to avoid illegal and inappropriate uses.

Future vision in CRISPR-Cas system is aimed to cure rare or catastrophic diseases, such as cancer, diabetes, or congenital anemia, fight against diseases such as HIV, malaria, dengue, Zika virus or the recent pandemic threat SARS-CoV-2, interruption of bacterial resistance to antibiotics, and decreasing the virulence of microorganisms that cause infections.

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