CRISPR-Cas System for Genetic Disease Therapy

Subjects: Cell & Tissue Engineering

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CRISPRs (clusters of regularly inter-spaced palindromic repeats) is a term that refers to regular and repetitive nucleotide sequences that are universally present in bacterial DNA. It was first discovered in 1987 by Professor Nakada's team at Osaka University in Japan. Since then, several researchers have discovered that this repeating sequence is the microorganism's defense system for fighting virus invasion. That is, when a microorganism is infected with a bacteria-specific virus, some of the surviving individuals cut part of the virus's DNA and insert it into their genome one after another. Bacteria defend themselves against phages and plasmids by using the CRISPR-CRISPR-associated protein (Cas) system, which is a genetically encoded RNA-mediated adaptive immunity system. The CRISPR-Cas system is extremely diverse, and it is broadly classified into two classes (I and II), which are further classified into six types (I-VI) and several subtypes. Cascade is the effector complex for CRISPR interference in class I/type I systems. It is composed of CRISPR RNA (crRNA), a large and small Cas subunit, as well as a variable number of Cas5, Cas6, and Cas7 that recognize the target and recruit Cas3, cleaving the target DNA. On the other hand, the RNP surveillance complex in class II systems is made up of a single Cas protein, Cas9 or Cpf1, and this effector complex participates in both target recognition and cleavage.

Keywords: CRISPR-Cas9 ; Cas13 ; Immunotherapy ; gene editing

1. Introduction

RNAs are transcribed from the CRISPRs (clusters of regularly inter-spaced palindromic repeats) locus and processed to produce crRNA and sequence-invariant trans-activating crRNA (tracrRNA), with a nucleotide sequence corresponding to the spacer [1]. These RNAs combine with a protein called Cas9 (CRISPR-associated protein 9) to form a sequence-specific endonuclease [2]. Using this principle, the possibility of gene-editing technology using RNA has been reported, unlike the previously used ZFN and TALEN gene-editing technologies. For gene editing using CRISPR, single-guide RNA (sgRNA) can be made by linking the major regions of crRNA and tracrRNA [3]. With the Cas9 system derived from S. pyogenes, it was demonstrated that the Cas9-sgRNA complex can cleave target DNA with a nucleotide sequence complementary to that of sgRNA in vitro [4].

Genetic disease occurs when a specific mutation of a parent's gene is inherited and does not function normally [5]. When a genetic disease developed symptoms, it was possible to avoid the substances that cause it or to provide symptomatic treatment to alleviate the symptoms, but the underlying treatment method has yet to be widely adopted [6]. To overcome this, and for a more fundamental treatment, researchers use gene-editing technology, i.e., the CRISPR-Cas9 system, to treat the defective gene [7][8][9]. The CRISPR-Cas9 system is used in treatment to correct genes such as blood and somatic cells taken from the patient's body in a laboratory and then injected back into the patient's body [10]. When this treatment is repeated several times, the percentage of normal cells in the body increases, which can completely cure the disease or relieve symptoms [11]. In addition, a method of injecting a genetically modified material tailored to the patient's condition into the human body is used. When the CRISPR-Cas9 system enters the body, it comes into contact with somatic cells, which precisely locate the target DNA and begin gene editing [12]. So now, the CRISPR–Cas system is now widely used to treat human genetic diseases, such as cystic fibrosis (CF), Duchenne muscular dystrophy (DMD), Huntington's disease (HD), hemophilia, and hematopoietic diseases.

2. Cystic Fibrosis

Cystic fibrosis (CF) is a disease caused by a defect in the CTFR proteins, which affects several organs and causes a loss of control over electrolyte and osmole because mucus is not created normally [13]. It was thought to be an excellent candidate for a genetic engineering-based treatment approach. Since it was discovered that utilizing CRISPR-cas9 in iPSCs extracted from CF patients, it is feasible to efficiently fix the homozygous deletion of F508 in the CFTR gene, which is the major mutation of disease [14]. Since then, methods for accurately and safely differentiating ex vivo edited cells by

injecting them back into the body are being studied [15][16]. Furthermore, research is being performed to circumvent the limits of existing approaches for correcting genes by targeting epithelial or basal cells directly. Novel approaches are also being developed continuously, such as the double nickase approach and numerous permutations of size and symmetry in repair template homology arms [17].

3. Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most common hereditary disease among progressive muscular dystrophy and is caused by a dystrophin gene mutation [18]. Dystrophin is part of a group of proteins (a protein complex) that work together to strengthen and protect muscle fibers from injury [19]. As a result, in DMD patients, gradual degeneration occurs primarily in skeletal muscle, with connective tissue or fat replacing the muscle, resulting in pseudohypertrophy and decreased muscle strength. Exon 50 of dystrophin's rod domain is one of the most common deletions in DMD patients, putting exon 51 out of frame with preceding exons [20]. Previous research has shown that exon 51 can be skipped or reframed, and dystrophin expression can be restored by injecting two adeno-associated viruses of serotype 9 (AAV9) which encode the CRISPR-Cas9 gene and sgRNAs into a canine model [21]. Another study proposed two strategies for correcting this mutation by CRISPR-Cas9-mediated skipping of surrounding exons, causing splicing of exon 43 to exon 45, and introducing a premature termination codon in exon 44 of the dystrophin gene [22].

4. Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disorder that is caused by a CAG trinucleotide repeat expansion in exon 1 of the huntingtin (HTT) gene, which results in the production of abnormal proteins that gradually damage brain cells [23]. Using SaCas9-induced indels, researchers used the CRISPR-Cas9 system to disrupt the expression of the mutant HTT gene in a mouse model of HD, resulting in a nearly 50% reduction in neuronal inclusions, as well as a significant improvement in life span and some motor impairments [24].

5. Hemophilia

Hemophilia is a congenital hemorrhagic disease caused by mutations in the blood coagulation genes factor VIII (FVIII) or factor IX (FIX) [25]. Hemophilia is a promising target for gene therapy because it is caused by a single genetic defect. As a result, many researchers are attempting experiments with the CRISPR-Cas9 system to correct the defective coagulation factor gene [26], and many clinical trials for hemophilia A and hemophilia B are currently underway [27]. Nonetheless, many limitations remain, such as the immune response to the AAV used to deliver the CRISPR-Cas9 system or tracking the long-term effect after treatment, and various methods for circumventing these issues are currently being proposed [28].

6. Hemoglobinopathy

Hemoglobinopathies, which include β -thalassemia and sickle cell disease (SCD), are genetic diseases caused by disorder in the proteins that form the structure of hemoglobin, which transports oxygen [29]. The study was carried out in patients with these diseases under the assumption that if fetal hemoglobin was reactivated, clinical symptoms in patients with reduced oxygen transport capacity due to abnormal hemoglobin would be mild [30]. Researchers developed the CRISPR-Cas9 genome-editing strategy in KU-812, KG-1, and K562 cell lines by deleting a 200 bp genomic region within the human erythroid-specific BCL11A (B-cell lymphoma/leukemia 11A) enhancer. The deletion of 200 bp of the BCL11A erythroid enhancer, which includes the GATAA motif, results in a significant increase in γ -hemoglobin expression in K562 cells [31].

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