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CRISPR Gene Editing Technique: Advancements and Applications

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ABSTRACT

The fields of genomics, genetics, and molecular biology are at a crossroads. Thanks to the convergence of advanced techniques and collected knowledge, it is now feasible and very advantageous to edit individual base pairs or segments of DNA in cells and living organisms. When it comes to gene editing and regulation, CRISPR has proven to be a better option than zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN). CRISPR-Cas9 employs the same protein for multiple gene targets, unlike ZFN and TALEN, where the protein needs to be modified for each target. Modification of a brief guide RNA sequence is sufficient to trigger site-specific cleavage. Bioengineering applications have been greatly expanded by multiplexed CRISPR technologies, which involve the production of multiple gRNAs or Cas enzymes concurrently. The extent and efficiency of transcriptional control and genetic editing have been significantly increased by this method. Recent advances in machine learning models and empirical scoring methods have improved off-target prediction and sgRNA design.

Keywords: CRISPR-Cas9, Gene editing, Gene therapy, Cancer treatment

Introduction

Modern biotechnology has completely changed our understanding of the genetic foundation of human health. High throughput DNA microarrays and next-generation sequencing technology have made it possible to characterize the range of human genetic diversity through extensive, multinational joint initiatives. Spacers originating from bacteriophages and other extrachromosomal components make up a CRISPR locus. These spacers are separated by brief repetitive sequences that encode tiny non-messenger RNA. These spacers are adaptive; following a viral attack, bacteria incorporate a new spacer from the phage genome, and the removal or insertion of certain spacers modifies the bacteria's resistance to infection by their original viral strains [1]. The speed of plant breeding and agricultural research will change as a result of our capacity to identify and alter the genes by controlling plant characteristics fast. The two parts of the most widely used type II CRISPR system are the artificial single guide RNA (sgRNA), a fusion of Cas9 nuclease and a trans-activating CRISPR RNA (tracrRNA) and a CRISPR RNA (crRNA) [2]. When CRISPRs were discovered in the 1987 genome of *Escherichia coli*, they were described as exceptional sequence elements. These elements were made up of a set of 29 nucleotide repeats spaced 32 nucleotides apart, and they arose anytime bacteria came into contact with phage DNA [3]. The intricate dance between multiple Cas enzymes and CRISPR arrays underpins the function of CRISPR systems in bacterial adaptive immunity. The main argument of this review is to return to this "natural state" and highlights the benefits of expressing multiple Cas proteins or gRNAs simultaneously in vivo to edit or transcriptionally regulate multiple genetic loci in parallel [4]. The CRISPR/Cas genome editing technologies are currently being quickly altered to increase the scope of their possible applications. Other varieties of Cas nucleases and their orthologues were also shown to have the potential for genome editing after the Cas9 nuclease, the first Cas nuclease to be found, was employed for CRISPR genome editing. To improve CRISPR/Cas applications, scientists are also designing and altering the current Cas nucleases. There are now additional alternatives for choosing genome editing tools thanks to a range of CRISPR/Cas-derived genome editors, such as base editors and prime editors [5].

Recent Advancements in CRISPR

Not too long after SpCas9 was introduced into mammalian cells, other Cas9 proteins were investigated and created as tools for genome editing. For instance, the gene editing effectiveness of smaller Cas9 proteins from *Neisseria meningitidis* (Nme2Cas9) and *Staphylococcus aureus* (SaCas9) is similar to that of SpCas9. Comparing these smaller Cas9s to the larger SpCas9 (~4.3 kb), they are better suitable for in vivo delivery [6]. Over the past decade, RNA-programmed genome editors have been identified, developed, and employed for many applications. Causing targeted genetic disruption in a gene or gene regulatory region is the primary function of CRISPR-Cas9. This has made it possible for researchers to carry out multiplexed editing, genetic screening, and the rapid construction of knockout mice and other animal models. Thus, the introduction of tiny insertions or deletions (indels) via the highly error-prone non-homologous end-joining (NHEJ) or the high-fidelity homology-directed repair (HDR) is how the CRISPR/Cas9 gene editing system modifies the genome [7]. On the other hand, single nucleotide polymorphisms, or SNPs, are the primary cause of the most known genetic disorders. Thus, strategies to precisely alter a single base pair's sequence at a target spot without adding DSBs are required [8]. Over the past ten years, scientists have developed strategies to treat human diseases and alter crops by using CRISPR technology, an easily changeable tool for studying biological processes and genetic links.

Applications for genetic diseases

The discovery of the genes responsible for monogenic human diseases has advanced significantly, yet treating these illnesses remains extremely difficult. They are thought to be responsible for over 10,000 known human illnesses. 5000–8000 monogenic diseases genetic illnesses resulting from changes in a single gene are included in this group. Over 75,000 harmful genetic variations have been found in the ClinVar database [3]. Since gene therapy may alter the faulty repair the hematopoietic system and insert a gene into autologous hematopoietic stem cells (HSCs)., it is a promising treatment option for inherited blood disorders. Two hereditary blood illnesses are sickle cell disease and β-thalassemia. β-globin production is decreased or absent in β-thalassemia due to a variety of mutations in the β-globin gene, including minor insertions, single point mutations, and deletions 47. A Glu->Val mutation in the β-globin subunit of haemoglobin 48, 49 causes sickle cell disease by resulting in aberrant hemoglobin S. A common method to improve both β-globin diseases is to get the paralogous γ-globin genes to express again. using CRISPR/Cas to cleave the erythroid enhancer's GATA1 binding region was utilized by the Bauer group. This method raises the expression of γ-globin by suppressing the Expression of the γ-globin repressor BCL11A on erythroid cells. It is clinically feasible to achieve long-lasting fetal hemoglobin induction with this method [9].

Applications for virus infections

The antiviral defense mechanisms found in CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are a class of bacteria and archaea systems that have lately been used as molecular tools in medicinal research. CRISPR-associated (Cas) proteins work in tandem with certain endonucleases to facilitate the cleavage and engineering of nucleic acids, primarily dsDNA. A short guide (sg)RNA can be used to route the Cas9 endonuclease to a specific location inside a dsDNA molecule, where it then causes a dsDNA break. The cellular DNA repair mechanisms then heal this injury, mostly through the non-homologous end joining (NHEJ) system [10]. Human dendritic cells, macrophages, and CD4+ T-cells are all infected by the ssRNA retrovirus HIV. A significant decrease in CD4+ T-cells during active infection might result in a reduction in cell-mediated defense and an increased chance of opportunistic infections. A possible method for inactivating the integrated provirus or stopping naïve cells from becoming infected in the first place is CRISPR-Cas9. CRISPR-Cas9-based therapeutic strategies find the HIV long terminal repeats (LTRs), which are located at both the 5′ and 3′ ends of the pro-viral DNA and function as the binding site(s) for transcription factors, to be appealing targets. employing such a tactic. caused a latently infected cell

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line to either have the integrated viral sequence completely removed or to have a segment between two target sites within the same LTR removed [11].

Application for treatment of Cancer

A number of genes linked to cancer stem cells, oncogenes, tumor suppressors, chemo-resistant genes, metabolism-related genes, and chemo-resistant genes, are dysregulated expressed and mutated during the development and spread of cancer. Restricting tumor development and progression by targeted mutation correction and the restoration of dysregulated gene expression is the ultimate aim of cancer therapy. In the fundamental study on cancer, the CRISPR/Cas9 gene editing method has been widely applied, and some promising results have been obtained [7]. Several tumor suppressor genes have been effectively rendered inactive with CRISPR/Cas9 in order to generate cancer disease models. For instance, medulloblastoma and glioblastoma formed in a mouse brain when P53, Nf1, Pten, and Ptch1 were deleted by CRISPR. As was previously mentioned, cancer cells gradually amass a variety of mutations and genetic abnormalities. With the use of CRISPR/Cas9, we can generate cancer models with various mutations in order to pinpoint the early triggers of mutagenesis and genomic instability [12]. While medication resistance or tumor recurrence often result in a poor prognosis, conventional cancer treatment methods (such as radiation, chemotherapy, and surgery) can delay recurrence and increase the length of time that cancer patients survive. Furthermore, the lack of specificity in radiation and chemotherapy might have fatal consequences as well as dangerous side effects. Therefore, there is a continued need for novel cancer treatments, and the CRISPR/Cas9 system has the potential to bring about dramatic improvements in cancer care [8].

Conclusion:

The genetic foundation of human health has been completely transformed by modern biotechnology, which has made it possible to extensively characterize the genetic variety of humans using cutting-edge tools like next-generation sequencing and high-throughput DNA microarrays. Spacers from bacteriophages are inserted into bacterial genomes to increase resistance to viral infections, and CRISPR systems which have their roots in bacterial adaptive immunity have developed into potent genetic editing instruments. Regarding human health, CRISPR/Cas9 and its offshoots have demonstrated potential in treating genetic illnesses, particularly those that are monogenic. Certain genetic defects may be precisely and specifically corrected by techniques like base editing and prime editing, which may lead to therapeutic therapies for illnesses like β-thalassemia and sickle cell disease. Moreover, CRISPR has been modified for use in antiviral treatments, most notably to target the HIV genome and obstruct viral latency and replication. Furthermore, by repairing errors and restoring normal gene activity, CRISPR has made it possible to create realistic disease models in cancer research and may one day lead to the development of innovative cancer medicines. However, the breadth and effectiveness of CRISPR-based therapies continue to grow because of continuous advancements in precision and delivery techniques. In conclusion, the quick development of CRISPR technology has led to new directions in genetic study and treatment, with potential big effects on agriculture, the treatment of diseases, and our general understanding of genetics. The potential uses of CRISPR are expected to increase as scientists continue to improve these instruments, providing creative answers to some of the most important problems facing science and health.

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