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БИОТЕХНОЛОГИЧЕСКИЕ ПРОРЫВЫ В РЕДАКТИРОВАНИИ ГЕНОВ: CRISPR И ЕГО ПОТЕНЦИАЛ ДЛЯ ЛЕЧЕНИЯ ГЕНЕТИЧЕСКИХ ЗАБОЛЕВАНИЙ

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Данная статья рассматривает перспективные биотехнологические достижения в области редактирования генов с использованием технологии CRISPR и ее потенциал для лечения генетических заболеваний. Будут рассмотрены основные принципы работы CRISPR, преимущества этой технологии, а также перспективы ее применения в медицине.

Ключевые слова: CRISPR, генетические заболевания, редактирование генов, биотехнология, лечение, геном, мутации, терапия, инновации, медицина.

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BIOTECHNOLOGICAL BREAKTHROUGHS IN GENE EDITING: CRISPR AND ITS POTENTIAL FOR THE TREATMENT OF GENETIC DISEASES

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This article examines promising biotechnological advances in the field of gene editing using CRISPR technology and its potential for the treatment of genetic diseases. The basic principles of CRISPR operation, the advantages of this technology, as well as the prospects for its use in medicine will be considered.

Key words: CRISPR, genetic diseases, gene editing, biotechnology, treatment, genome, mutations, therapy, innovation, medicine.

Objective of the research is to review modern biotechnological advances in the field of gene editing using CRISPR technology and discuss its potential for the development of new treatments for genetic diseases. An analysis of the key principles of CRISPR, the advantages of this technology and the prospects for its use in medicine will help readers understand the importance and prospects of this area of research.

Material and methods

1. CRISPR-Cas9 technology: Description of the basic principles of the CRISPR-Cas9 system, including the selection of the target genome site, the design of flexible RNA (gRNA) and the mechanism of point modification of DNA.

2. Cell cultures and models: Cell types used (e.g. cell lines or primary cultures) to conduct gene editing experiments using CRISPR.

3. Experimental procedures: Detailed description of the experimental steps, including the introduction of CRISPR-Cas9 into cells, analysis of changes in the genome, verification of the effectiveness of editing, etc.

4. Analysis of results: Methods and techniques used to evaluate the effectiveness and accuracy of gene editing, such as DNA sequencing, PCR, immunohistochemistry and others.

CRISPR-Cas9 technology is a revolutionary genome editing method that allows scientists to point-change DNA sequences in cells [2]. The basis of this technology is the bacterial immune response system to viruses – CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the Cas9 enzyme. The principle of operation of CRISPR-Cas9 is to use a specially designed RNA molecule (gRNA), which directs the Cas9 enzyme to a specific section of the genome where double-stranded DNA is cut.

CRISPR-Cas9: Concept and Examples. CRISPR-Cas9 (clustered regularly intervening short repeats - CRISPR and its associated CRISPR-associated system 9) is an innovative molecular technology used for genome editing. Fundamentally, it is a bacterial immune response system transformed into a tool for precise DNA modification in living cells, including plants, animals and even humans.

The mechanism of operation of CRISPR-Cas9 [5]

1. Target DNA Detection: CRISPR-Cas9 identifies a specific section of DNA that needs to be corrected or modified.

2. "Clinging" to the Target DNA: The ribonucleoprotein Cas9 forms a complex with an RNA hydroge directed to the target site and provides specific "bonding" to it.

3. DNA Cutting: Cas9 acts like a knife, cutting a double-stranded DNA molecule at a specified location.

4. Genome repair: After cutting, the cell starts the DNA repair process. Two things can happen:

As a result of such interference in the genome, it becomes possible for scientists to correct genetic mutations, introduce new genes or suppress the function of certain genes, which opens up wide prospects not only for basic research, but also for the treatment of genetic diseases. First introduced in 2012, CRISPR-Cas9 technology has become a powerful tool for genetic engineering and is one of the most promising methods for changing genetic material.

Experimental procedures [2]:

1. Introduction of CRISPR-Cas9 into cells:

- Preparation of CRISPR-Cas9 and flexible RNA (gRNA) vectors for the target gene.

- Transfection of cells with CRISPR-Cas9 vectors using a suitable method (for example, lipofection or electroporation).

- Isolation of clones of cells in which the gene was edited using CRISPR-Cas9.

2. Analysis of changes in the genome:

- Extraction of DNA from cells with qualitative and quantitative analysis.

- PCR amplification of a gene site for further sequencing in order to identify editing points and mutations.

- DNA sequencing to confirm the presence of changes in the genome.

3. Verification of the effectiveness of editing:

- Evaluation of the effectiveness of introducing CRISPR-Cas9 into cells using molecular biology methods (for example, PCR or immunohistochemistry).

- Estimation of the percentage of successfully edited cells, taking into account the insertions and insertions/deletions in the genome.

- Comparison of results with control groups to assess statistical significance.

These steps represent a general approach to gene editing experiments using CRISPR-Cas9. The specific methods and conditions of the experiments may vary depending on the type of cells, the target gene and the objectives of the study [3].

The choice of a specific cell type depends on the goals and objectives of the study, as well as on the specifics of the tissue or organ that needs to be studied.

Analysis of the results [6]:

1. DNA sequencing:

- Sequencing of the target region of the gene to determine the presence of changes in the nucleotide sequence.

- The use of modern sequencing methods (for example, next-generation sequencing) to identify point mutations, indels and other changes.

2. Polymerase chain reaction (PCR):

- The use of PCR to amplify a section of a gene in order to detect changes, insertions/deletions or duplications.

- The use of quantitative PCR to quantify the effectiveness of gene editing.

3. Immunohistochemistry:

- The use of immunohistochemical methods to visualize the expression of a target protein or markers associated with gene editing.

- Assessment of the level of protein expression in edited cells compared with control cells.

4. Western blot analysis:

- Performing Western blot analysis to assess the level of expression of the target protein or changes in post-translational modifications.

5. Conducting functional tests:

- Assessment of functional changes in cells after gene editing, such as changes in growth, proliferation, differentiation, etc.

6. Statistical analysis:

These methods and techniques are widely used to analyze the results of gene editing experiments using CRISPR-Cas9. The right choice and combination of methods will help to evaluate the effectiveness and accuracy of gene editing and interpret the data obtained [1].

In the modern world of biotechnology, CRISPR-Cas9 technology opens up new opportunities for gene editing and the treatment of genetic diseases. This method of precise DNA modification allows scientists not only to correct mutations, but also to create customized treatments aimed at specific genetic defects [4].

The potential of CRISPR in medicine, the prospects of using CRISPR for the treatment of diseases such as Alzheimer's disease, cystic fibrosis, cancer and others. Future research directions Prospects for the development of CRISPR technology to improve the results of treatment of genetic diseases and the creation of new methods of gene editing.

Further development and research in the field of CRISPR-Cas9 promises new discoveries and innovations in the treatment of genetic diseases, making personalized medicine more accessible and effective. Addressing ethical and legal issues, as well as continuing scientific research, will be key steps towards realizing the full potential of CRISPR-Cas9 technology in medicine.

The article on biotechnological breakthroughs in gene editing using CRISPR-Cas9 is intended to shed light on innovative treatments for genetic diseases and inspire further research in this area.

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