International Journal of Medicine and Medical Research

Journal homepage: https://ijmr.com.ua/
Volume 10, No. 2. 2024

UDC 577.2:616-085(048.6)
DOI: 10.61751/ijmmr/2.2024.81

Article's History:
Received: 29.06.2024; Revised: 26.09.2024; Accepted: 26.11.2024

Achievements in molecular biology: CRISPR/Cas9 in gene therapy (literature review)

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Abstract. CRISPR/Cas9 provides high precision and efficiency in altering genetic sequences, therefore significant in gene therapy. Mutations can be corrected, pathological genes can be removed, and the functional ability of proteins can be restored by CRISPR/Cas9. The review aimed to analyse the possibilities of using gene editing technology to treat cancer, genetic, and infectious diseases in the available studies published in 2013-2024. This publication analysed the use of CRISPR/Cas9 in experimental models for treating Duchenne muscular dystrophy, cystic fibrosis, sickle cell anaemia, acquired immunodeficiency syndrome and cancer. In Duchenne muscular dystrophy, genome editing helps increase the level of utrophin, which compensates for the dystrophin deficiency. In cystic fibrosis, CRISPR/Cas9 is used to correct defects in the CFTR gene, and in human immunodeficiency virus therapy, it is used to remove proviral deoxyribonucleic acid from infected cells. However, the technology also has certain limitations, such as the risk of off-target changes in the genome and the difficulty of delivering CRISPR/Cas9 into cells. Therefore, in 2024, CRISPR/Cas9 requires further improvement in clinical practice. CRISPR/Cas9 has great potential to change the approach to the treatment of incurable diseases in the future. The practical value of the study conducted by the authors is the presentation of a ready-made summary of the CRISPR/Cas9 system and a thorough analysis of the results of its use in the treatment of various diseases, which can be used to assess what prospects this technology has for future use

Keywords: genome editing technologies; adeno-associated viruses; Duchenne muscular dystrophy; cystic fibrosis; haemoglobinopathies; acquired immunodeficiency syndrome; cancer

Introduction

As of 2024, more than 9,000 diseases associated with changes in the human genome were identified, and effective treatments were found for less than 800 diseases [1]. Therefore, scientists are increasingly considering gene therapy as a priority treatment. This increased interest is supported by numerous studies and searches for technologies that could replace current, sometimes ineffective treatments. Over the past thirty years (1990-2020), many discoveries in the fields of molecular biology and genetics were made, for instance, "genetic scissors", polymerase chain reaction, and others. Well-known genome editing methods, such as mononucleases (MN) and zinc finger nucleases (ZFN), received considerable coverage. Despite the proven effectiveness of these methods, they often require careful and thorough planning when selecting genetic targets and may present various obstacles to accurate genome editing [2].

Suggest Citation:
Voroshchuk O, Yaremchuk O. Achievements in molecular biology: CRISPR/Cas9 in gene therapy (literature review). Int J Med Med Res. 2024;10(2):81–9. DOI: 10.61751/ijmmr/2.2024.81

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H. Yang *et al.* [3] highlighted the new immune system CRISPER/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats with Cas9-associated protein). The discovery of this technology is based on the study of the bacterial immune system, which uses CRISPR DNA sequences to fight viruses. Compared with conventional gene editing methods, this system, discovered in 2012, has greater potential due to its low cost, high accuracy, greater efficiency than MN/ZFN and multifunctionality in genome editing.

The mechanism of CRISPER/Cas9 functioning, also named "gene scissors", is based on the target gene being recognised by an artificially created RNA (sgRNA). This RNA then directs the Cas9 protein to break the DNA double strand, creating double-strand breaks (DSBs) in the corresponding region of the genome. Then, a natural process of repairing the damaged DNA occurs. In this process, targeted changes are made to the genome, including the removal of the mutated region or its replacement. H. Jiang *et al.* [4] reviewed how this unique technology demonstrated excellent and promising results in gene therapy and is used in the treatment of a wide variety of diseases, such as AIDS, cancer, Duchenne muscular dystrophy and many other genetic diseases. In addition, CRISPER/Cas9 is also used in agriculture to create genetically modified crops with increased resistance to parasites and weather conditions, in pharmaceuticals to develop therapeutic molecules based on the results of genome editing, and in other areas not related to medicine [5].

In 2020, the Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier, who heads the Division of Pathogen Sciences at the Max Planck Society in Berlin, and Jennifer Doudna from the University of California, Berkeley, for the discovery of one of the most powerful tools of gene technology – CRISPR/Cas9, or the so-called "genetic scissors". The technology of "genetic scissors" has brought life sciences to a new stage of development and is of great benefit to humanity. Even though genome editing technologies have certain drawbacks and limitations, and methods of their delivery to cells need to be improved, this discovery will allow for greater efficiency in gene therapy [6].

Despite all of the aforementioned advantages of this unique gene therapy approach, there are also many challenges and problems with its application, one of which is the difficulty of delivering the system to target cells, as discussed by W. Yang *et al.* [7]. As of 2024, the use of adeno-associated viruses (viral vectors) has significant limitations and the likelihood of transferring limited genetic material of the system, and the risks of inducing an immune response in the patient have also been noted. At the same time, the liposomal delivery system is inefficient and can damage the cell.

Nevertheless, there is an insufficiency, and sometimes the absence, of a comprehensive and structured presentation of the CRISPR/Cas9 technology. Most studies present either the structure of the system, its advantages, or its role in the treatment of one group of diseases. Therefore, this study was necessary to summarise most of this material in a less detailed form and present it in a structured and grouped way, from the mechanism of functioning and the structural design of the system to preclinical results in the treatment of various diseases. This is also necessary to provide a comprehensive assessment of the CRISPR/Cas9 gene tool and evaluate its prospects. Therefore, the purpose of this paper was to analyse the structure, advantages, disadvantages and medical applications of the latest promising CRISPR/Cas 9 technology according to studies published in 2013-2024.

The methodology of the study was as follows: the literature review was conducted by analysing numerous digital publications found on such library websites as PubMed, Scopus and Web of Science Core Collection. The analysis of literature sources covered the period from 2013 to November 2024; the search was carried out using MeSH (Medical Subject Headings) terms, synonyms and keyword searches. During the primary analysis, 55 literature sources were selected and reviewed. After further systematisation of the information using the methods of analysis, synthesis and generalisation, only 35 sources remained. Exclusion criteria: publications that did not meet the purpose of this study and the absence of significant results in the reviewed papers.

CRISPR/Cas9 Bacterial Immune System

CRISPR/Cas9 is a system that is a bacterial defence mechanism against phage infection [8, 9]. Based on the organisation of the effector protein, the CRISPR/Cas system is classified into two different classes. Class 1 CRISPR/Cas systems use multi-protein effector complexes, while class 2 CRISPR/Cas systems use single-protein effectors. These two systems use short DNA sequences (spacers) to guide the Cas proteins. The conserved sequence to facilitate targeting is called a protospacer flanking sequence (PFS) for RNA-targeted Cas proteins or a protospacer adjacent motif (PAM) for DNA-targeted Cas proteins [10]. The CRISPR/Cas9 system of *Streptococcus pyogenes* consists of Cas9 (CRISPR-associated protein 9) (Fig. 1), an endonuclease that causes double-stranded DNA breaks, which can be used to modify of the genome with a single guide RNA (sgRNA), which provides specificity [11].

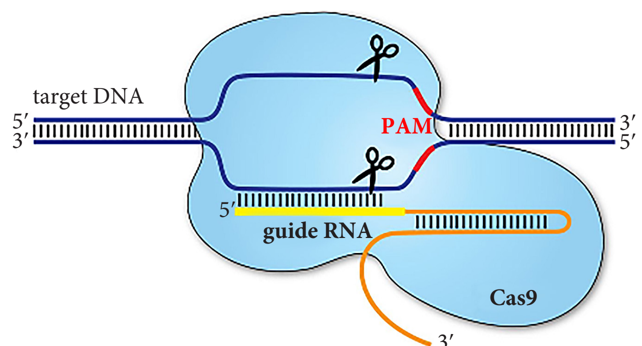

Figure 1. CRISPR/Cas9 technology
Source: M. Redman *et al.* [12]

WClustered Regularly Interspaced Palindromic Repeats (CRISPRs) are sequences in the bacterial genome

that, together with CRISPR-associated proteins (Cas), protect against viruses. One of these proteins is the Cas9 endonuclease, which cuts two DNA strands. Cas9 is guided to its target by a small-group RNA (sgRNA). To make a cut, a certain DNA sequence (from 2-5 nucleotides) must be located at the 3'-end of the sgRNA – known as RAM. DNA repair after the cut occurs in 2 ways: either by non-homologous end joining, which often leads to random DNA insertions/deletions, or by homologous repair. The latter uses a homologous DNA fragment as a template, allowing for precise editing. This pathway involves the delivery of a homologous DNA site with the desired modification (sequence change) using Cas9 protein and sgRNA, which ensures changes are made with an accuracy of one base pair [13].

CRISPRs protect against virus invasion in combination with a series of CRISPR-associated (Cas) proteins. Cas9, one of the associated proteins, is an endonuclease that cuts both strands of DNA. Cas9 is guided to its target by a stretch of RNA. This can be synthesised as a single strand called synthetic single-guided RNA (sgRNA); the stretch of RNA that binds to genomic DNA is 18-20 nucleotides. To cut, a specific DNA sequence of 2 to 5 nucleotides (the exact sequence depends on the bacterium that produces Cas9) must be located at the 3'-end of the guide RNA: this is called a protospacer adjacent motif (PAM). Repair after a DNA cut can occur in two ways: non-homologous end joining, which usually results in random DNA insertion/deletion, or homology-directed repair when a homologous DNA fragment is used as a repair matrix. It is the latter that allows for precise genome editing: a homologous DNA site with the required sequence change can be delivered using the Cas9 nuclease and sgRNA, theoretically allowing for changes as precise as a single base pair [13].

Bacteria develop this system by capturing DNA sequences and using it as a memory to identify themselves as an enemy and destroy it when they attack in the future. The great advantage of the CRISPR-Cas9 system is that it is not based on protein engineering (as in the case of ZFN) but on base pairing between sgRNA and DNA. CRISPR/Cas9 is easy to use as only a small new RNA fragment needs to be synthesised to target a new locus, which prevents the lengthy construction and cloning of complete protein domains, as in ZFN nuclease-based approaches. The synthesis of small fragments also allows for multi-complex applications, which allows for the targeting of multiple loci and the generation of chromosomal changes (deletions and translocations) [10, 11].

A bacterial immune mechanism for protection against bacteriophage infection and plasmid transfer in nature is the CRISPR/Cas9 system, which consists of the Cas9 endonuclease and a guide RNA (sgRNA) that provides specific recognition of the target DNA sequence. The Cas9 protein creates a double-stranded DNA break in the region complementary to the sgRNA in the presence of a protospacer adjacent motif (PAM). This technology allows not only for high-fidelity genome editing but also for modelling complex genomic changes, which makes it much easier to use than ZFN. The ease of use of CRISPR/Cas9, means that only sgRNA is needed to synthesise a precise target, therefore substantial for efficient genome editing and targeting multiple loci simultaneously.

Advantages of CRISPR/Cas9 in Genetic Engineering and Mechanisms of CRISPR/Cas9 Genome Editing

CRISPR/Cas9 is a revolution in the world of genetic research due to its versatility, accuracy and ease of use. Its success is based on the ability to precisely modify the genome by inducing double-strand breaks (DSBs) in DNA at predefined locations. This approach has opened new horizons for studying genetic functions, developing therapeutics, and creating organisms with specified characteristics [9, 14]. Unlike previous technologies, such as ZFN and TALEN, which required complex protein design for each new genetic locus, CRISPR/Cas9 uses a guide RNA (sgRNA). This RNA can be easily programmed to target any DNA sequence. As a result, the development of CRISPR tools requires less time and resources, which greatly simplifies the preparation for experimental studies [13, 14].

CRISPR/Cas9 provides a high level of editing accuracy. Efficiency is achieved using the Cas9 protein, which cuts DNA, and guides RNA, which provides specific targeting. Once a break is created, the cell triggers repair mechanisms such as nonhomologous end joining (NHEJ) or homologous directional repair (HDR). NHEJ usually causes small insertions or deletions that can shift the reading frame and cause gene knockout. HDR, in turn, can be used to insert specific sequences or make point mutations using donor DNA as a matrix [11, 13].

CRISPR/Cas9 can edit multiple genes simultaneously in a single experiment, which significantly accelerates the study of complex genetic networks. This creates opportunities for research into polygenic diseases caused by the interaction of many genes. In addition, CRISPR/Cas9 adaptations, such as CRISPRa (activation) and CRISPRi (inhibition), allow for changes in gene expression without editing their sequences. This is particularly useful for functional studies when it is important to assess the impact of changes in expression levels on cellular processes [15-16]. Compared to ZFN and TALEN, the development of CRISPR tools is much faster and cheaper. As of 2024, there are numerous ready-made sgRNAs for different genomes, which simplifies the preparation for experiments. Laboratories around the world can easily integrate CRISPR into their research, which contributes to its spread [9, 15].

CRISPR/Cas9 technology has enormous potential in medicine. It is being considered for the treatment of genetic diseases, cancer, infectious diseases, etc. The uniqueness of this technology is determined by its ability to create stable genetic modifications that do not disappear over time, contrary to the temporary changes typical of RNAi [10, 14]. CRISPR variants, such as base editing, can alter individual nucleotides without creating DNA breaks. Prime editing is another innovative approach that offers the ability to edit

DNA with high precision. These modernisations expand the range of capabilities of the technology [9].

CRISPR/Cas9 is not simply a genetic editing technique, but a system that is changing approaches to genome research and disease treatment. Its advantages in accuracy, versatility, speed and accessibility render this technology indispensable in modern and future biology and medicine [13]. The CRISPR/Cas9 genome editing mechanism is divided into three stages: the first is recognition, the second is cleavage, and the third is repair. The constructed sgRNA directs Cas9 and recognises the target sequence in the gene of interest using a complementary base pair of the 5'crRNA. The Cas9 protein is inactive in the absence of sgRNA. The Cas9 nuclease produces double-stranded breaks (DSBs) at 3 bases upstream of the PAM. The PAM sequence is a short (2-5 base pairs) conserved DNA sequence downstream of the cut site [16].

The Cas9 protein is a nuclease that is most used in genome editing technology, recognising the PAM sequence at 5'-NGG-3' (N can be any nucleotide base). When Cas9 finds a target site with a matching PAM, it triggers local DNA melting to form an RNA-DNA complex, but the mechanism of how the Cas9 enzyme melts the target DNA sequence is not yet clearly understood. The Cas9 protein is then activated to cleave the DNA. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand of the target DNA to produce predominantly blunt-ended DSBs [17].

CRISPR/Cas9 is one of the most revolutionary technologies in the field of genetic engineering that combines versatility, precision and ease of use. Thanks to guide RNA (sgRNA) and the Cas9 endonuclease, this system can accurately target specific DNA sites, causing double strand breaks that stimulate the cell's natural repair mechanisms. This creates great prospects for creating gene knockouts, and point mutations, editing single nucleotides, and studying complex genetic networks. The advantages of CRISPR/Cas9, such as low cost, high efficiency and accessibility, render it an indispensable technology in molecular biology and medicine.

The Use of CRISPR/Cas-9 in the Treatment of Genetic Diseases

One of the areas of application of CRISPR/Cas9 is its potential use for the treatment of diseases caused by single gene mutations, certain infectious diseases, various types of cancer, etc. This approach was tested only in preclinical models, although expectations are that it can be applied in clinical practice. J. Wang *et al.* [18] investigated the impact of CRISPR/Cas9 on Burkitt's lymphoma. This is a cancer of the lymphatic system, in particular B lymphocytes, that develops in the germinal centre of the lymph node, caused by mutations in the cMyc gene. J. Wang and team used a CRISPR/Cas9 system against Epstein-Barr virus (EBV) and found a reduction in the effect of the virus on tumour proliferation. In addition, F.A. Khan *et al.* [19] determined that when editing the tumour suppressor gene *Trp53* in *Arf-/-Eμ -Myc* lymphomas, overexpression of the Myc gene is responsible for several types of lymphoma cancer. *Mll3* is another substantial tumour suppressor gene disruption by CRISPR *ex vivo* in acute myeloid leukaemia [20]. The use of the TALENs genome editing tool in a girl with leukaemia by C. Chen *et al.* [21] created grounds for more effective use of CRISPR in the clinic.

Another serious genetic disease is Duchenne muscular dystrophy (DMD). According to the research of Canadian scientists D. Wojtal *et al.* [22], CRISPR/Cas9 exhibits a therapeutic effect in Duchenne muscular dystrophy (DMD) caused by mutations in the dystrophin gene, thanks to its genome editing mechanism by increasing the expression of utrophin (UTRN). UTRN is a protein that is functionally similar to dystrophin and partially compensates for its loss. In these studies, CRISPR/Cas9 was used to activate the A and B promoters of the UTRN gene to increase the synthesis of utrophin. The increase in the level of urotrophin (1.7-6.9 times compared to baseline values) would allow compensation for the functions of dystrophin, in particular: restoring the amount of β-dystroglycan, a protein that is critical for the stability of muscle cell sarcolemma. This approach allows not to correct the dystrophin gene directly, but to compensate for its absence, reducing the progression of DMD [21, 22].

For the treatment of DMD, CRISPR/Cas9 can also be used to remove mutations in the dystrophin gene that cause a reading frame shift. Removing the defective exons can be used to restore the reading frame, resulting in the synthesis of a functional, albeit reduced, dystrophin protein. This approach resembles the phenotype of Becker muscular dystrophy, which is a less severe form of the disease. M. Tabebordbar *et al.* [23] used the delivery of CRISPR/Cas9 endonucleases using adeno-associated virus (AAV) to restore dystrophin expression in mice with experimental DMD by deleting the exon containing the original mutation. As a result, a shorter but functional protein is synthesised. Mice treated with CRISPR/Cas9 for DMD partially recovered functional muscle dysfunction. This study demonstrated that the dystrophin gene was edited in muscle stem cells that replenish mature muscle tissue. This is necessary to ensure that any therapeutic effects of CRISPR/Cas9 do not disappear over time.

Multiple studies by C.E. Nelson *et al.* [24] provide substantial evidence supporting the effectiveness of *in vivo* genome editing to correct destructive mutations in DMD in the corresponding dystrophic mouse model. Programmable CRISPR complexes can be delivered locally and systemically to terminally differentiated skeletal muscle fibres and cardiomyocytes, as well as to muscle satellite cells in newborn and adult mice, where they mediate targeted gene modification, restore dystrophin protein expression, and partially recover functional deficiencies in dystrophic muscles.

G. Schwank *et al.* [25] were the first to use CRISPR/Cas9 to study the treatment of cystic fibrosis (CF). Cystic fibrosis is caused by mutations in the CFTR gene, particularly the most common mutation F508 del, which leads to the formation of a non-functional CFTR protein. This

disrupts the transport of chlorine ions across cell membranes, causing the accumulation of thick mucus in the respiratory tract, digestive tract and other organs. In the study, adult intestinal stem cells were first taken from two patients with cystic fibrosis, and then intestinal organoids were grown from these cells to mimic the structure and function of real tissue. The CRISPR/Cas9 system was then used to correct F508 del. To fix the mutation, the researchers provided the cell with a donor DNA template that contained the correct sequence of the CFTR gene. The cell uses this template to repair the DNA break through the HDR mechanism, which replaces the F508 del mutation with a normal sequence. Adeno-associated viruses (AAVs) were used to deliver CRISPR/Cas9 components into cells. They effectively delivered Cas9 and sgRNA to organoid cells. As a result, they successfully corrected the most common mutation causing cystic fibrosis in intestinal organoids. The authors demonstrated that once the mutation was corrected, the function of the CF transmembrane conductor receptor (CFTR) was restored.

M.C. Canver *et al.* [26] conducted a study that demonstrated the possibility of using the CRISPR/Cas9 system to treat such serious diseases as sickle cell disease (SCD) and β-thalassaemia. Haemoglobinopathies are caused by mutations in the β-globin (HBB) gene, which causes disorders of haemoglobin (HbA) synthesis. Traditional treatment approaches include replacement therapy or bone marrow transplantation, but they are limited and ineffective. M.C. Canver *et al.* [26] also explored an alternative approach – reactivation of fetal haemoglobin (HbF) synthesis. This type of haemoglobin is expressed in the embryonic and perinatal periods and compensates for the functions of adult haemoglobin. With the transition from HbF to HbA in the postnatal period, HbF activity is inhibited by the protein BCL11A. The identification of the role of this protein as a key suppressor of fetal haemoglobin has become the basis for the development of therapeutic strategies [12]. M.C. Canver and the team used the CRISPR/Cas9 system to target the BCL11A enhancer, which is a regulatory element in the genome that controls the expression of this protein. In mice with a model of sickle cell anaemia, scientists used CRISPR/Cas9 to edit the BCL11A enhancer, substantially increasing HbF levels. The high level of fetal haemoglobin significantly reduced the symptoms of the disease, improved haematopoiesis and restored red blood cell function. In human cells, the researchers used primary erythroblasts, the cells involved in the formation of red blood cells (the so-called precursors). Editing the BCL11A enhancer using CRISPR/Cas9 led to the activation of HbF, which functionally replaced HbA. This proved that the technique can be effective for human cells as well. The results of their research are extremely promising. Reactivating HbF is a more realistic approach than introducing a full-length β-globin gene because of the difficulty of delivering such large genes into cells.

The use of the unique CRISPR/Cas9 system has yielded positive results and great potential in the future for the treatment of various genetic diseases and more. This technology can be used for precise changes in the genome, which creates new prospects for the treatment of diseases such as Duchenne muscular dystrophy, cystic fibrosis, sickle cell anaemia and various types of cancer. CRISPR/Cas9 can also be used to correct mutations, activate genes and change protein expression.

CRISPR/Cas9 in the Treatment of AIDS

Another potential clinical application of CRISPR/Cas9 is the treatment of complex infectious diseases such as HIV. Acquired immunodeficiency syndrome (AIDS) is one of the most serious infectious diseases in the world [27-28]. As of 2024, 41 years have passed since the discovery of human immunodeficiency virus type 1 (HIV), but there is still no vaccine against HIV infection. Combination antiretroviral therapy (cART) has been used for the past ten years to keep HIV replication under control. Although cART can inhibit the replication of the virus, the virus can persist in some cells. As a result, patients must take antiretroviral drugs for life [29].

Long-term use of antiretroviral drugs causes side effects. Thus, the search for alternative strategies to combat HIV is highly relevant. A prominent field is gene therapy, which is needed to deliver antiviral gene reagents that would prevent the virus from replicating in cells that may be infected with HIV. Technologies for modifying gene expression through RNA interference (RNAi) or genome editing based on clustered regularly interspaced short palindromic repeats (CRISPR) offer new opportunities for HIV inhibition.

Firstly, it is necessary to mention the fact that complex antiretroviral therapy does not remove the viral DNA integrated into the cell genome (although it actively suppresses HIV replication). This viral DNA creates latent reservoirs that remain a source of new infection after ART is stopped. E. Herrera-Carrillo *et al.* [27] examined the CRISPER/Cas9 technology in HIV therapy. The mechanism of action of CRISPR-Cas9 against HIV in their studies involves the use of a guide RNA (sgRNA) that provides precise targeting of the Cas9 protein to the proviral DNA integrated into the cell genome. Cas9 creates double-stranded breaks in the viral DNA, causing damage. Then, breaks in important regions of the viral genome, such as LTR (long terminal repeats) regulatory regions or genes encoding viral proteins (gag, pol, env), block the activation of the proviral DNA or cause its inactivation. This approach effectively inhibits virus replication and prevents its spread to new cells.

The results of *in vitro* experiments [29, 30] demonstrate a significant reduction in the amount of proviral DNA in infected cells, which indicates the effectiveness of CRISPR-Cas9 in fighting both active and latent forms of the virus. Even in the case of incomplete deletion of proviral DNA, editing causes mutations that make the virus replication defective. The use of multiple sgRNAs to simultaneously target multiple regions of the HIV genome reduces the risk of mutations that allow the virus to avoid CRISPR. Despite the encouraging results, several challenges need to

be overcome for the successful use of CRISPR in HIV treatment. These include the high mutability of the virus, which makes targeting difficult, the possibility of off-target effects that can damage the host cell genome, and the difficulty of delivering CRISPR-Cas9 to all infected cells in the body. Delivery is one of the key technical challenges, and methods based on viral vectors (e.g., adeno-associated viruses, AAV) or nanoparticles are being developed for this purpose.

E. Herrera-Carrillo *et al.* [27] argue that CRISPR/Cas9 has significant potential to become a revolutionary approach to HIV treatment, as it allows eliminating pro-viral DNA and blocking viral replication. At the same time, further research is needed to implement this technology in practice to optimise the safety and efficacy of the method, as well as to develop effective approaches to delivering CRISPR into the body.

CRISPR/Cas9 technology has proved to be effective in the treatment of AIDS and opens new prospects for treating this incurable disease. Thanks to its ability to precisely edit the genome, this system can not only block virus replication but also effectively destroy the pro-viral DNA that is integrated inside cells. Despite the encouraging results of *in vitro* studies, there are certain obstacles to the clinical application of CRISPR/Cas9 in the treatment of HIV and AIDS, such as the high mutability of the virus, problems with the delivery of the CRISPR/Cas9 system to the affected target cells, and others.

CRISPR/Cas9 as a Method of Fighting Cancer

Cancer is the primary cause of death due to genetic mutations in the world. Oncogenes, tumour suppressor genes and DNA repair genes are substantial in the mechanisms of carcinogenesis. By using CRISPER gene editing, research biologists found a way to treat cancer with immunotherapy. Gene editing is possible not only in cell cultures and mammals but also in humans. In addition, CRISPR/Cas9 has been used to create an oncolytic virus that is used to treat cancer due to its ability to specifically infect and lysate cancer cells, ideally preserving normal cells for cancer treatment [31-32].

According to research conducted by L. Wang *et al.* [31], CRISPR/Cas9 technologies achieved success in cancer immunotherapy by providing a precision genetic editing technology. This new method can be used to make precise changes to the genome and correct or remove mutations that cause cancer. The use of CRISPR/Cas9 can improve the ability of the immune system to detect and destroy cancer cells, which is progress in cancer treatment. The precision of CRISPR/Cas9 in genetic modification is beneficial for the treatment of T cells, which is an important aspect of cancer therapy. CRISPR/Cas9 improves the specificity and efficacy of chimeric antigen receptor (CAR) and T-cell receptor (TCR) agents that target tumour agents, thus enhancing the immune response against cancer. This approach inhibits the immune checkpoint inhibitors (PD-1/PD-L1) that cancers use to prevent them from being recognised by the immune system, thus allowing the immune system to effectively fight cancer [33].

In other studies, conducted by A. Saber *et al.* [32] in oncology, CRISPER/Cas9 demonstrated the ability to address tumour heterogeneity and drug resistance. Tumour heterogeneity, the diversity of tumour cells within cells in a single patient, hinders treatment by creating a pool of genetic variants that can lead to therapeutic resistance and ineffectiveness of treatment. Congenital and acquired drug resistance often leads to relapse and death [34]. CRISPR/Cas9 was used to explore therapeutic targets to create drugs that do not develop resistance. CRISPR/Cas9 is a method for improving existing cancer treatments and has all the prospects for successful application in the future in clinical oncology as well. T-cell therapy with chimeric antigen receptor (CAR), gene screening, and tumour modelling were improved, but the effects of genome editing remain poorly understood and may increase the risk of additional genetic mutations [35].

The CRISPR/Cas9 system created new opportunities in cancer therapy. By editing the genome, CRISPR/Cas9 can be used to modify immune cells to fight tumours more effectively, as well as to create oncolytic viruses that selectively destroy cancer cells. This technology can also overcome the problem of tumour heterogeneity and the development of drug resistance. Despite the significant results and great potential, CRISPR/Cas9 still requires further research to be studied in detail and approved for mainstream cancer treatment.

Conclusions

CRISPR/Cas9 technology has become a breakthrough in the field of genetic engineering, creating new opportunities for gene therapy by eliminating mutated genomic regions. The emergence of this innovative technology, based on the natural mechanism of bacterial defence against viruses, has become a vivid example of how basic research can change practical medicine. The CRISPR/Cas9 mechanism of action allows for genome editing with high precision, simplicity and efficiency, rendering this technology a unique treatment for genetics, cancer and many other diseases. According to the results of the analysed studies, in Duchenne muscular dystrophy, the use of CRISPR/Cas9 has increased the level of utrophin, which partially compensates for the loss of dystrophin, helping stabilise muscle tissue.

In cystic fibrosis, the F508 del mutation in the CFTR gene was successfully corrected in intestinal organoid cells of patients. The restored function of the CFTR protein improved ion transport in tissues. In haemoglobinopathies such as sickle cell anaemia and β-thalassaemia, CRISPR/Cas9 allowed reactivating foetal haemoglobin (HbF) by editing the BCL11A protein enhancer, which compensates for the β-globin deficiency. In HIV, CRISPR/Cas9 has effectively removed proviral DNA from the cell genome, reducing viral replication and inducing mutations that render HIV inactive.

At the same time, there are successful cases of this technology being used in cancer, where CRISPER/Cas9 is used to edit immune cells (T-lymphocytes) to increase their ability to detect and destroy tumour cells. In addition, CRISPR is capable of creating oncolytic viruses and modifying immune checkpoint inhibitors, which enhances

the effectiveness of immunotherapy. However, despite all the advantages, CRISPR/Cas9 requires further research, in particular, to study various possible side effects and reduce the risk of their impact on the body, improve the technology of CRISPR/Cas9 delivery into the cell, etc.

Acknowledgements

None.

Conflict of Interest

None.

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Досягнення молекулярної біології: CRISPR/Cas9 у генній терапії (огляд літератури)

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Анотація. CRISPR/Cas9 забезпечує високу точність і ефективність у зміні генетичних послідовностей, що робить її важливим інструментом у генній терапії. Завдяки CRISPR/Cas9 можна коригувати мутації, видаляти патологічні гени та відновлювати функціональну здатність протеїнів. Метою цього огляду було проаналізувати можливості використання технології редагування генів для лікування онкологічних, генетичних, інфекційних захворювань у наявних дослідженнях, які опубліковані в період 2013-2024 років. У даній публікації проаналізовано застосування CRISPR/Cas9 на експериментальних моделях у терапії м'язової дистрофії Дюшена, муковісцидозу, серповидно-клітинної анемії, синдрому набутого імунодифіциту та онкологічних захворювань. При м'язовій дистрофії Дюшена редагування геному сприяє підвищенню рівня утрофіну, що компенсує дефіцит дистрофіну. При муковісцидозі CRISPR/Cas9 використовується для корекції дефектів у гені CFTR, а при терапії вірусу імунодифіциту людини – для видалення провірусної дезоксирибонуклеїнової кислоти в інфікованих клітин. Однак технологія має і певні обмеження – ризик позацільових змін у геномі, складність доставки CRISPR/Cas9 у клітини. Отже, на 2024 рік залишається необхідність подальшого вдосконалення методів застосування CRISPR/Cas9 в клінічній практиці. CRISPR/Cas9 має великий потенціал в майбутньому змінити підхід до терапії невиліковних захворювань. Практичною цінністю проведеного авторами дослідження є подання готової короткої інформації про систему CRISPR/Cas9 та грунтовний аналіз результатів її застосування при терапії різних захворювань, що дозволяє оцінити, які перспективи має ця технологія у застосуванні в майбутньому

Ключові слова: технології редагування геному; аденоасоційовані віруси; м'язова дистрофія Дюшена; муковісцидоз; гемоглобінопатії; синдром набутого імунодифіциту людини; рак