Journal of Pharmaceutical Research International

33(50A): 159-169, 2021; Article no.JPRI.76379
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Modern Solutions in the Treatment of HIV: From Antiretroviral Drug Therapy to Human Genome Editing

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i50A33392
Editor(s):
(1) Dr. Koteshwara Mudigonda, Propharmex Company, India.
Reviewers:
(1) Bernardino Geraldo Alves Souto, Federal University of São Carlos, Brazil.
(2) Annette d'Arqom, Universitas Airlangga, Indonesia.
(3) Fabio Mitsuo Lima, Centro Universitário São Camilo, Brazil.
Complete Peer review History: https://www.sdiarticle4.com/review-history/76379

Review Article

Received 01 September 2021
Accepted 05 November 2021
Published 16 November 2021

ABSTRACT

The human immunodeficiency virus (HIV) belongs to a group of anthroponotic viral diseases that cause HIV infection in the human body, apotheosely transforming into acquired immunodeficiency syndrome (AIDS). HIV infection, in people without adequate treatment, can lead to serious damage to the immune system (hereinafter referred to as IS), which leads to a sharp decrease in resistance to conditionally pathogenic microbes, as well as to the prevalence of oncological pathologies that may lead to death. Due to its simplicity, convenience, efficiency and cost-effectiveness, CRISPR/Cas has found application in a short period of time in a wide variety of fields of fundamental and applied medicine and biotechnology.

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Keywords: HIV; antiretroviral therapy; genetic engineering; genome editing.

1. INTRODUCTION

The term AIDS first appeared in 1982, a year after a rapidly progressive IP disorder was described in detail against the background of Kaposi's sarcoma or pneumocystis pneumonia in homosexual men. In the following year, a previously unknown virus was isolated from this cohort of patients, and this was done almost simultaneously by two researchers: French virologist Luc Antoine Montagnier and American scientist Robert Charles Gallo. Initially, the isolated pathogen was named a virus associated with lymphadenopathy, but three years later the International Committee of Experts on the Taxonomy of Viruses decided to name the agent Human Immunodeficiency Virus (HIV). In 2008, L. Montagnier, together with his colleague F. Barre-Sinussi, were honorably awarded the Nobel Prize in Medicine and Physiology for the discovery and description of a new pathogen [1].

After a while, the disease began to aggressively invade almost all continents, and now HIV and AIDS are present in all countries of the planet. The reasons for such a large-scale march are the extremely high sensitivity of people to HIV and the variety of ways of vírus transmission. Nevertheless, pharmacological antiretroviral therapy plays an extremely important role in improving the quality and duration of life and reducing the risk of complications in patients. 1980s of the last century, when certain principles of antiretroviral therapy did not yet exist, this diagnosis was immediately equivalent to a death sentence for the patient. And it was only with the advent of the latter that the mortality and morbidity rates decreased somewhat [2]. A novel method for CRISPR-induced sutureless mutation of biallelic CCR5delta32 in human iPS cells and HIV resistance of engineered monocytes / macrophages derived from iPS [3]. MedicineBiology12: 35 12 Sep. 2019 Difficulty 4.3

HIV patient receives CRISPR-edited blood cells for the first time NIAID / Flickr Chinese doctors tested CRISPR-edited cells on a patient with HIV infection and T-cell leukemia. After irradiation, the patient was injected with his own hematopoietic cells, in which the CCR5 gene was destroyed. The edited cells took root in the body and formed different types of blood cells. There were no serious side effects. True, they could not protect the patient from the virus - due to the low efficiency of editing [4].

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Despite the obvious successes in the fight against the virus, it continues to pose a threat. Therefore, the relevance in the study of HIV does not stop.

1.1 The Structure of the Human Immunodeficiency Virus

The human immunodeficiency virus is an RNA-containing virus. A characteristic feature of HIV is the presence of the reverse transcriptase enzyme (p66), RNase (p15), integrase (p32) and protease (p10). The virus needs enzymes to develop HIV inside the affected cell. In a private order, reverse transcriptase, or, as it is also called, DNA polymerase, participates in the synthesis of DNA on an RNA template; integrase is responsible for the catalysis of the incorporation of the formed viral DNA into the host cell chromosome; protease cleaves the resulting polyproteins into structural proteins [3, 4].

The virus has the sha pe of a sphere with a diameter of 100-120 nm (Fig. 1).

If you look at an image of a particle with a transmission electron microscope, you will see a circle with a clear, dark color around the circle that contains the nucleocapsid. The nucleocapsid includes 2 identical single-stranded plus-RNA molecules (associated with the nucleocapsid protein p7: it has two amino acid residues containing large amounts of cysteine and

histidine and zinc atoms) and the aforementioned enzymes [5]. Next, the external and internal structure of the virus particle will be considered in detail.

In the program for modeling the structure of molecules, viruses PyMOL v2.5 Release Notes, first a model of the HIV virus, then Fig. 2 shows two variants of the structure of the capsid of the human immunodeficiency virus HIV determined experimentally using a combination of various methods: X-ray diffraction analysis, electron microscopy, cryo-electron tomography and computer modeling [6]. The PyMOL v2.5 Release Notes program allowed us to compare different methods of studying viros, compare the structure of the virus. So that in the future it would be possible to compare virus models in genome sequencing.

In the program, the CA protein (these are proteins that are involved in the signaling pathways of calcium cells by binding to Ca2 +) molecules are collected in groups of six and form hexamers - symmetrical hexagonal structures. Then the hexamers fit together like a hexagonal tile. Also, CA protein molecules can be assembled in five pieces into pentamers - symmetrical pentagons. These pentagons (highlighted in purple in the figure) are joined with hexagons (blue), creating fragments with a smaller radius of curvature and allowing the capsid to "round off" and close. Therefore, the structures of the HIV capsid are often compared with the structure of fullerenes- carbon nanoparticles in the form of closed polyhedra, which also consist of five- and hexagonal faces. The capsid variant (Fig. 2) shown on the right consists of 186 hexamers and 12 pentamers. The variant pictured on the left includes 216 hexamers and 12 pentamers - this is the largest biomolecular structure ever determined experimentally and uploaded to the Protein Data Bank database: it has a molecular weight of 34,853 kDa (35 million units of molecular weight) and consists of 1356 (216*6 + 12*5 = 1356) identical protein chains.

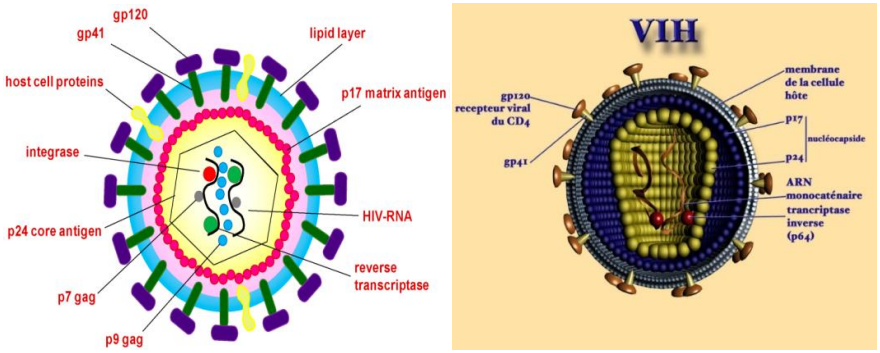

Fig. 1. The figure shows the main proteins of the membrane and matrix of the viral particle, as well as schematically marked enzymes and an element of the genetic apparatus

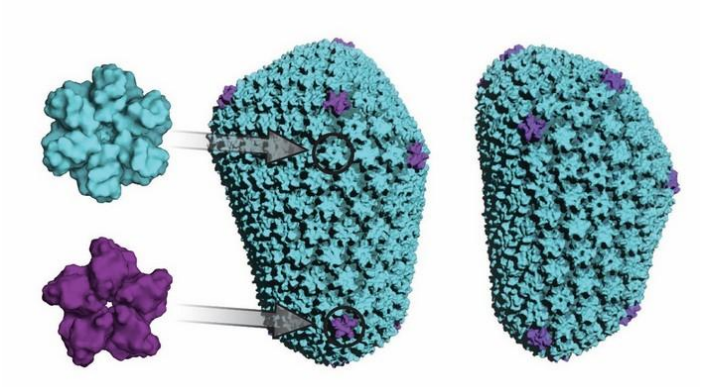

Fig. 2. Two variants of the human immunodeficiency virus capsid structure

From the outside, the virus has a super-capsid two-layer lipid envelope, permeated with 72 mushroom-like spikes of glycoprotein complexes. Each such complex is a synchronization of three gp120 molecules (needed to fix the virion to CD4 receptors and the coreceptor of infected cells) and three transmembrane gp 41 molecules penetrating the lipid envelope. The latter, it is important to note, promotes the fusion of the supercapsid with the cell membrane of cells. These two glycoproteins are, among other things, extremely important for the diagnosis and therapy of the disease.

We have studied what is also the cause of CD4-lymphocyte death, the micrograph (Fig. 3) shows the fusion of the membranes of infected helpers with the formation of functionally inactive syncytium (CXCR4-X4).

A layer of matrix protein p17 with a thickness of about 6 nm is embedded under the supercapsid shell. Thematrix protein surround sthenucleocapsid [7].

The capsid is a truncated protein cone, which contains protein p24 with a molecular weight of more than 24 kDa. A protein-nucleic complex (genome, enzymes and protein p7) is enclosed inside the capsid. This structure of HIV-1 (protein p24) is associated with 200 copies of cyclophilin A, borrowed by the virus from an infected cell. Cyclophilin A is a protein (peptidolprolyzomerase) that is involved in the fixation of protein p17 and the conduction of signals in T-lymphocytes. Inside the capsid there is also another Vhr protein involved in the processes of reproduction and assembly of virions. The Vhr protein interferes with the action of antiviral systems – a separate functional feature that plays a huge role in "survival" inside the body [8,9].

The virus genome can exist in two variants – in the form of genomic RNA and in the form of DNA synthesized on genomic RNA and embedded in some chromosome of the host cell. The second form is called provirus. At both ends, on the left and right, the RNA genome has long terminal repeats (LTR – long terminal repeat) that control the processes of creating daughter virions. These repeats are "turned on" by both the proteins of the virus and the proteins of the infected cell itself. The nucleocapsid protein p7 serves to link the capsid to the genome – this is the most important functional and structural link. In the capsid, among other things there is also a seed RNA molecule *(tRNAlys)* [9, 10].

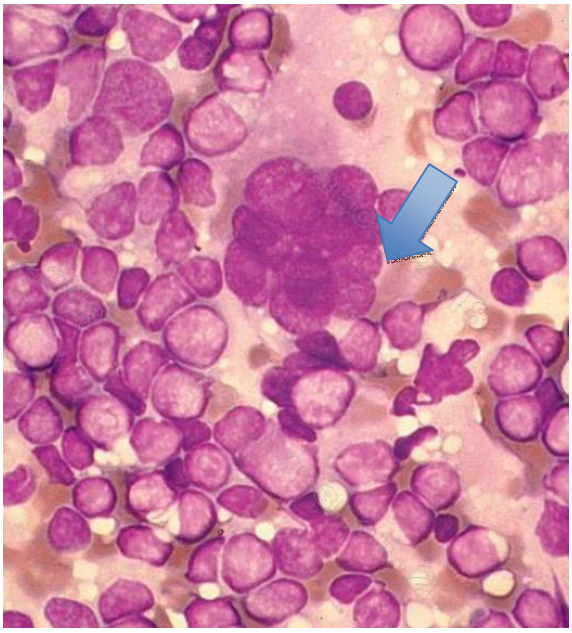

Fig. 3. Death of CD4 lymphocytes under microscopy, micrograph shows the fusion of membranes of infected helpers with the formation of functionally inactive syncytium (CXCR4-X4)

The genome is diploid, consists of 9 genes, 3 of which are structural *(gag, pol, env)*, 6 – regulatory *(tat, rev, nef, vif, vpr, vpu)*. Each of the genes is responsible for encoding unique structural fragments and processes. For example, the gag gene determines the synthesis of nucleoid and matrix proteins, the pol gene encodes reverse transcriptase and other nucleoid proteins, and the env gene monitors the formation of shell proteins [6,11].

The human immunodeficiency virus is characterized by significant variability, largely related to the variability of gp120. This allows the virus to survive in the environment of an infected organism that is aggressively tuned against it. According to some data, the variability is 100 thousand - 1 million times higher than that of the influenza virus. As a result of periodic DNA polymerase failures, serologically different virus clones can be detected in the patient [12].

The main HIV antigens are group-specific and type-specific antigens (core and nuclear p24), as well as type-specific gp41 and gp120 [13].

HIV resistance in the external environment has been discussed in the course of numerous experiments. The disappearance of viral activity was observed when cell cultures were dried at a temperature of 24 to 28 degrees Celsius only after 3-7 days. In a liquid medium at a given temperature, HIV remains active for 15 days, and at a temperature close to normal in humans - for 11-12 days. When heated to 51 degrees Celsius, the virus dies in 8-10 minutes. There is an opinion that HIV can remain viable in dried blood for a long time. Viral particles are extremely sensitive to chemicals (20% ethyl ether, acetone and 0.2% sodium hypochlorite solution). They show resistance to ultraviolet radiation [14,15].

1.2 HIV Life Cycle

The life cycle of the human immunodeficiency virus consists of 6 consecutive stages:

1. Adsorption and penetration into an infected cell by endocytosis;
2. Release of viral RNA;
3. Synthesis of DNA-provirus, embedding into the genome of the host cell;
4. Synthesis of RNA of daughter virions;
5. Synthesis of proteins of daughter virions;
6. Assembly and release of newly formed daughter virions from the cell by budding [16,17].

The full life cycle takes place within 1-2 days. Penetrating into the human body, viral particles act according to the following scenario. GP120 glycoprotein on the surface of the sphere binds to the CD4 receptor of T-lymphocytes, dendritic and microglial cells and macrophages using a special device in the form of a pocket. After a short period of time, the virus additionally binds to another auxiliary transmembrane coreceptor, which acts as the CCR5 receptor on macrophages (M-tropic viruses) or CXCR4 on T cells.

Then there is a clear process in its functionality: the core of the virus enters the cell by merging the supercapsid with the plasma membrane of the cell [16].

The virion nucleus follows towards the nucleus, dividing along the way. There is a process of deproteinization. After the maximum convergence of the "bare" core with the nucleus of the infected cell, thanks to reverse transcriptase, complementary double-stranded DNA is synthesized on the viral RNA matrix. This process takes place completely in the cytoplasm of the lymphocyte, and it requires the presence of viral RNA, tRNA primer, reverse transcriptase and nucleosides.

As a result, a provirus (i.e. viral DNA) is formed, which is sent directly to the cell nucleus and, thanks to the integrase enzyme, is embedded in the nuclear DNA. In the future, the proviral element is considered as a matrix for the synthesis and production of genomic RNA of daughter virions and mRNA that provides synthesis of proteins of daughter virions. mRNAs are transported back to the cytoplasmic matrix of the cell, where the production of structural and regulatory proteins is carried out on ribosomes. After that, the RNAi molecules of viral proteins are directed to the assembly sites of daughter virions [16, 18].

Ready-made virions exit the cell subunit by budding, while the core is "dressed" due to the modified plasma membrane of the cell, on the inner side of which glycoprotein spikes have been established. After the release of all daughter virions, the cell immediately dies.

During the period of persistent infection, viral particles can only be detected using a special PCR method. The activation of the virus occurs due to some kind of trigger - the productive phase immediately begins, during which the

active reproduction of HIV particles inside the cell occurs [6,16,19].

2. MODERN APPROACHES TO NON-DRUG THERAPY OF HIV-1

Before proceeding to scientific data, it is necessary to identify some important historical points. There is a phenomenon of HIV resistance, officially established in 1995. Several people were identified who, despite the "common needle" and sexual relations with infected people, did not get sick, although their partners subsequently died. Along with the CD4 receptor, a co-receptor - a receptor for chemokines – CCR5 is needed to penetrate into the HIV T cell. In 1% of the world's population, due to mutations, the CCR5 receptor is abnormal. This feature gives resistance to HIV infection, blocking the fixation of viral particles to the target cell in the body. Approximately 10% of people belonging to the Caucasian race have this inherited mutation (in a heterozygous state). In different regions of the planet, the frequency of occurrence of this phenomenon is less. As a result: the virus cannot get into the cell [20, 21].

In 2007, an American patient named Timothy Ray Brown underwent for the first time an operation to transplant hematopoietic bone marrow stem cells (HSCT), or otherwise, the so-called hematopoietic stem cell transplantation procedure for the treatment of leukemia. HIV diagnosis/AIDS was diagnosed to him in 1995, while studying in the German capital. According to the official version, this is the reason why Ray was named "The Berlin Patient" [22-24]. The operation was carried out by a group of doctors led by German hematologist GeroHutter, after which the case was widely sanctified in the media of the whole planet [20,22].

The selection of the donor was very tough: out of 60 suitable candidates, a homozygous person with two genetic copies of a rare variant of the cellular receptor was selected for the CCR5 coding gene.

The operation was carried out successfully, but leukemia returned a year later. The procedure of allogeneic hematopoietic stem cell transplantation was repeated, and a few years later, doctors found no signs of HIV infection in Ray Brown's blood and other biological fluids. The scientists also noted a decrease in specific antibodies, which indicates that the virus was indeed excluded from Brown's body. Why did the applicant not repeat himself for many years after success?

Many experts who have studied this case have argued that Brown's HIV remission is unusual. A patient other than HIV/AIDS, suffered from potentially fatal leukoencephalopathy. This means that transplantation should not be performed on other HIV patients, even if a suitable donor can be found. Experts at the time expressed doubts that Ray Brown's cure was due to the unusual nature of the stem cells he received [22-24].

However, the skepticism of the world community has somewhat diminished after an international group of scientists from the UK, Singapore, Spain and the Netherlands, under the auspices of an Indian-born physician Ravindra Gupta, published an article in the journal Nature on March 5, 2019, which talks about the second successful cure of a patient with HIV "HIV-1 remission after CCR53232/Δ32 hematopoietic stem cell transplantation." They did this after a successful operation carried out as part of a study funded by the amfAR Foundation. The study involved 40 people infected with HIV, and only one of them gave a positive result. He was called the "London patient" [23,25].

In Gupta's article, it was reported that the second patient with HIV-1 underwent allogeneic stem cell transplantation (HSCT) using cells from a CCR5Δ32/Δ32 donor, and after 16 months, antiretroviral therapy was interrupted. HIV-1 remission persisted for 18 months, after which HIV-1 RNA molecules were detected in plasma, but in extremely small amounts (together with undetectable HIV-1 DNA in peripheral CD4 T lymphocytes).

Quantitative analyses of the viral growth of peripheral CD4 T lymphocytes did not show a reactivated virus using a total of 24 million "resting" CD4 T cells. CCR5-tropical, but not CXCR4-tropical viruses were identified in HIV-1 DNA from CD4 T cells of the patient before transplantation. CD4 T cells isolated from peripheral blood after transplantation did not express CCR5 and were sensitive only to CXCR4, an ex-vivo tropical virus.

HIV-1 Gag-specific CD4 and CD8 T-cell responses were leveled after surgery, while cytomegalovirus-specific responses were detected. Similarly, HIV-1-specific antibodies dropped to levels comparable to those of the "Berlin patient" Timothy Ray Brown.

In the journal Science, Ravindra Gupta himself avoids using the term "healing", preferring "long-term remission" [23,25,26].

The effectiveness of transplantation is influenced by many factors and triggers. The most important task is to find a genetically ideal donor. He must have both mutant copies of the gene, and there are very few such people. Secondly, it is impossible to be sure with accuracy that the operation will be successful, because everything is taken into account: the physical condition of the donor and the patient's organisms (meaning concomitant diseases and predispositions in the anamnesis), the immune "aggressiveness" of the transplant and even minor hereditary differences between them, which may be key. These issues are extremely complex, and scientists have not yet figured out all the nuances, and given the fact that even the selection and appointment of antiretroviral therapy causes difficulties, the rebus remains unsolved until now.

And, finally, another significant factor: the "Berlin patient" Brown received intensive treatment before transplantation in the form of a long course of chemotherapy and full body irradiation due to leukemia. Gupta's patient, who also suffers from Hodgkin's lymphoma, only went through a course of chemotherapy and underwent surgery once.

One should not lose sight of the fact that Timothy Ray Brown was heterozygous for the CCR5 gene, and the cells of the "London patient" Gupta were homozygous and did not contain mutant alleles [22-24].

3. HUMAN GENOME EDITING: ZINC FINGERS AND CRISPR/AS IN HIV THERAPY

In November 2018, genetically modified children were born in China. Scientist He Jiankuya, the project manager, said that during genome editing, he made children immune to HIV by intentionally introducing a mutation into the CCR5 gene, as in donors in the case of "Berlin" and "London" patients. This event caused a truly atomic explosion of disputes and shock from the public, but this does not negate the fact that humanity has crossed the threshold of a new era [27-29].

The driving force behind the development of medicine today is the computer revolution and quantum theory. They made it possible to see the most detailed models of molecular structures. Man has learned to build DNA atom by atom, and gene sequencing, which used to be time-consuming and infinitely expensive, has become an automated procedure that is now available to an increasing number of people.

The discovery of restriction enzymes and DNA ligases at the end of the twentieth century was the trigger for the emergence of genetic engineering, since with their help it turned out to be possible to split the strands of the molecule of life into given loci, connect them and form new constructions. Thus, different combinations of bacterial and viral genomes are now being obtained [30].

In 1999, scientists isolated a gene that was then called the "smart mouse gene". This gene makes the memory and intelligence of its owners much more effective: animals pass mazes faster, remember information better. Some of the first successful experiments with changing the genome of small mammals were obtained by scientists at Princeton University. Joseph Qian created a line of genetically modified mice that have an additional gene called NR2B, which triggers the synthesis of the neurotransmitters N-methyl-D-aspartate (hereinafter NMDA) in the anterior part of the mouse brain. Such experiments at the time confirmed the Hebb rule: learning occurs when certain neural highways in the brain are amplified. They can be strengthened by controlling the work of synapses. These data allowed scientists to advance further in their experiments on experimental animals and create new lines of genomic reconstructions. In particular, what He Jiankuya did with CCR5 [31].

Between these two events, the discovery of the "smart mouse genome" and the birth of genetically modified children in China, several key moments occurred. A few years ago, scientists created, based on the mechanisms of "bacterial immunity", a method of genetic engineering of CRISP/AC, which provides an accurate effect on the specified locations of the DNA molecule. This method allowed manipulation of the genome at a completely new level, allowing to make point mutations, correct, embed, eliminate fragments of genes or even the genes themselves [26].

To manipulate the genome, scientists usually use nucleases- enzymes that determine certain nucleotide sequences and cleave them. For the

reverse reunification of the sites, enzymatic units of DNA ligases are used, which are included in the composition of natural enzyme complexes that repair "broken sites" in the DNA structure [27].

The "crosslinking" of the molecule is carried out by enzyme complexes of the recombination system, thanks to which homologous sites in the genomic DNA are exchanged during the formation of germ cells.

And although the discovery of such tools inspired scientists around the world, such a set of methods turned out to be very scarce. It was practically impossible to use it in manipulations of higher complex organisms, let alone humans. The difficulties lay in the fact that restriction enzymes can recognize short DNA sequences of bacteria and viruses, and such specificity of enzymatic units for working with plants and animals a priori was not enough. Therefore, after some time, so-called chimeric nucleases were created - complex proteins consisting of two units responsible for splitting DNA on the one hand and selective binding to certain nucleotide sequences on the other. Such proteins can be created in the cells themselves, which is a great convenience: vectors (autonomous genetic carriers) marked with a nuclear localization signal (proteins that ensure the penetration of the construction complex into the nucleus) and coding nucleases are introduced into the cells. The first among the "chimeric nucleases" were "zinc fingers"[27].

Zinc fingers – are protein domains or as they are also sometimes called structural motifs of proteins that are stabilized at both ends (or from one) by zinc ions associated with amino acid residues [28]. As a rule, there are about 20 such residues. In shape, such structures resemble a finger, which is why they were given this name.

Since antiretroviral therapy implies lifelong continuous treatment, the "zinc fingers" have been adopted in this matter. It must be said that the domains of such are in the composition of human transcription factors -proteins capable of regulating the synthesis of RNA molecules with matrix DNA. In the synthesis of artificial nucleases, a zinc-finger chain can be made so that it recognizes certain loci of the DNA molecule. If such a chain is long enough, it can recognize relatively extended sequences of genetic material consisting of a number of trinucleotides, which means that there is a real possibility of pinpoint exposure to specified areas in large complex genomes [32].

Unfortunately, this was not enough. The method of "zinc fingers" was found to have shortcomings, because of which they could not be fully implemented in clinical practice: 1). Lack of clear recognition of trinucleotide repeats, which leads to a noticeable number of DNA cleavages in "non-target" sites; 2). The method turned out to be very labor-intensive and financially difficult, since each DNA sequence required the synthesis of its optimized protein structure zinc-finger nuclease.

Complicated complexes based on chimeric nucleases called TALENs (Transcription Activator-like Effector Nucleases) have also become a more promising solution for selective exposure to DNA [32-, 34].

The role of DNA-recognizing structures in them is played by protein domains, each of which "sees" only one nucleotide. The natural prototype of such domains were the TAL-effectors of some bacteria. These bacterial proteins, getting into the nuclears of plant cells, mimic transcription factors and bind to DNA sites, thus activating genes necessary for the survival of parasitic bacteria. Since the mechanism of calculating DNA in this case means the formula "one nucleotide = one protein domain", then obtaining a design that specifically recognizes the nucleotide sequence needed by the researcher is a relatively easy task. And although TALENs turned out to be much better compared to the "zinc fingers", it did not help much in the treatment of HIV. An even more complete solution was required, and in 2012-2013. An epoch—making event took place in the field of genetic engineering: CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats - short palindromic repeats regularly arranged in groups), which opened the way for work at the genome level of higher organisms. This tool is quite simple and convenient to use and provides a focused impact on the target sites in the DNA itself. In addition, CRISPR/Cas are available in a separate laboratory on the planet.

Unlike chimeric nucleases, in CRISPR/Cas, the structures that calculate DNA are not proteins, but relatively short RNA chains [9]. When CRISPR/Cas was created, the idea was based on the mechanisms of protection of living bacteria from their natural enemies, bacteriophages. CRISPR loci do gigantic

amounts of work. Loci consist of standard repetitions of non-coding DNA sequences, and spacers separate these repeats.

Using the example of the same bacteria: when a virus already known to it is re-penetrated into a bacterial cell, a long primary RNA encoded in the CRISPR locus is rapidly assembled [35]. As a result: the "maturation" of such primary DNA gives rise to a line of rRNAs, each of which consists of a specific site corresponding to a spacer and universal sites corresponding to palindromic repeats of bacterial DNA. Bacterial DNA is responsible for attracting Cas proteins, and spacer plays the role of a "dispatcher": it interacts with a complementary section of viral DNA, after which Cas proteins cut it, eliminating the pathogen. Due to its high specificity and the ability to instantly adjust the CRISPR/Cas algorithm provides bacteria with protective properties. To date, several such algorithms have been studied. The most widely described and understood is type II CRISPR/Cas, found in Streptococcus pyogenes [36].

4. CONCLUSION

As a result, CRISPR/Cas has gained a lot of popularity. Due to its simplicity, convenience, efficiency and cost-effectiveness, CRISPR/Cas has found application in a short period of time in a wide variety of fields of fundamental and applied medicine and biotechnology. And although CRISPR is not a panacea for all problems and diseases, it is considered a reason for high hopes for the effective elimination of HIV and AIDS due to the point insertion of a mutation into the CCR5 gene with further bone marrow transplantation to HIV patients without the presence of such severe procedures as chemotherapy and radiation in therapy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://ffhdj.com/index.php/BioactiveCompounds/article/view/802

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/76379