# ANALYTICAL REVIEWS

# АНАЛИТИЧЕСКИЕ ОБЗОРЫ

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## GENETIC ENGINEERING APPROACHES TO THE DEVELOPMENT OF MODERN THERAPEUTICS

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The classic approach to production of protein-based therapeutics is their isolation from natural sources. This approach was associated with a number of difficulties, such as collecting the primary material from natural sources, isolating and purifying the protein, and its standardizing. With the development of recombinant DNA technology, it became possible to obtain large quantities of protein preparations lacking any contaminations. Human insulin produced using recombinant DNA technology is the first commercial therapeutic obtained by this way. Due to the rapid development of genetic engineering technologies, a large number of proteins have been obtained in *Escherichia coli* cells. In recent years, the approach for the development of drugs based on DNA molecules containing genes encoding therapeutic proteins has been developing more actively. Today, many scientists believe in the prospects of application of DNA vaccines. The ease of production, stability, the ability to mimic natural infections and elicit appropriate immune responses make this vaccine platform extremely attractive. Delivery and targeting of immunologically relevant cells are major tasks for maximizing the immunogenicity of DNA vaccines. Several different approaches that are currently being used to achieve this goal are discussed in this review. Pharmaceuticals based on nucleic acids have a number of undeniable advantages. The main options for prophylactic RNA vaccines, the methods used to deliver RNA to the cell, and methods for increasing the effectiveness of RNA vaccines are discussed. Usage of therapeutic drugs based on protein molecules and low molecular weight compounds is complicated by the fact that they cannot be targeted at a specific gene or its protein product, responsible for the occurrence of the disease. Action of nucleic acids can be directly directed to a particular DNA region in order to edit its nucleotide sequence. This method allows to correct a genetic defect, eliminating the cause of the disease. The principles of gene therapy and the successes achieved in this area are discussed. This review summarizes current achievements in the development of drugs based on recombinant proteins and nucleic acids.

**Keywords:** recombinant DNA technology; recombinant proteins; DNA; RNA; therapeutics; vaccines.

## ГЕННО-ИНЖЕНЕРНЫЕ ПОДХОДЫ К РАЗРАБОТКЕ СОВРЕМЕННЫХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ

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Классический способ получения белковых молекул, активных компонентов ряда лекарственных препаратов, заключался в их выделении из природных источников. Данный способ был сопряжен с рядом трудностей, таких как сбор источника препарата, выделение и очистка белка, его стандартизация. С появлением технологии рекомбинантной ДНК стало возможным получение больших количеств стандартизованных белковых препаратов, лишенных нежелательных примесей. Так, инсулин человека является первым коммерческим терапевтическим препаратом, созданным с использованием технологии рекомбинантной ДНК. Благодаря быстрому развитию технологий генной инженерии в последние годы большое количество белков было получено в клетках *Escherichia coli*, а также в других продуцентах. В настоящее время все активнее развивается направление разработки препаратов на основе молекул ДНК, содержащих гены, кодирующие белки, входящие в состав терапевтических препаратов. Многие ученые отмечают перспективность ДНК-вакцин. Простота производства, стабильность, способность имитировать естественные инфекции и вызывать соответствующие иммунные ответы делают эту платформу для производства вакцин чрезвычайно привлекательной. Обеспечение

адресной доставки и нацеливания на иммунологически релевантные клетки — основные задачи на пути достижения максимальной эффективности ДНК-вакцин. Новейшим подходом к разработке лекарственных препаратов, в том числе вакцинных, является технология рекомбинантной РНК. В данном обзоре обсуждены основные варианты профилактических РНК-вакцин, способы доставки РНК в клетку и способы увеличения эффективности РНК-вакцин. Лекарственные средства, созданные на базе нуклеиновых кислот, обладают рядом неоспоримых преимуществ. Применение терапевтических препаратов на основе белковых молекул и низкомолекулярных соединений осложнено тем, что они не могут быть нацелены на конкретный ген или его белковый продукт, ответственные за возникновение заболевания. Действие молекул нуклеиновых кислот может быть направлено на определенный участок ДНК с целью редактирования его нуклеотидной последовательности. Данный способ позволяет корректировать генетический дефект, устраняя причину заболевания, а не просто лечить его последствия. В обзоре представлены принципы генной терапии, суммированы современные достижения в области разработки препаратов на основе рекомбинантных белков и нуклеиновых кислот.

**Ключевые слова:** технология рекомбинантной ДНК; рекомбинантные белки; ДНК; РНК; лекарственные препараты; вакцины.

## Introduction

The rapid development of recombinant DNA technology and DNA and RNA sequencing methods have led to the emergence of tools for the study and treatment of diseases at the molecular level. Gene cloning enables the production of highly purified protein products in unlimited quantities. Human insulin [1], growth hormone [2], interferon [3], and factor VIII [4], obtained through genetic engineering methods in the prokaryotic expression systems were successfully applied in clinical practice for therapy in diabetes mellitus, growth hormone deficiency, hemophilia, and other diseases. Due to the production of large quantities of proteins such as somatostatin or interferon, which are usually present in a living organism in small quantities, their biological functions have been studied in greater detail. With the application of DNA technology, the molecular basis of several human diseases, such as sickle cell disease, thalassemia, and some types of oncological diseases, have been deciphered [5]. Presently, a variety of expression systems based on prokaryotic and eukaryotic cells are available. Among the prokaryotic expression systems, the greatest preference has been attributed to producers based on *Escherichia coli* cells due to their short life cycle, well-studied genetic characteristics, high productivity and, accordingly, the relatively low cost of drug production.

In the recent years, the development of drugs based on plasmid DNA molecules containing genes encoding therapeutic proteins has been increasingly developing. Competitive advantages of DNA preparations consist in the relative simplicity of their production and stability at the room temperature, which facilitates their storage and transportation. The ability to mimic natural infections and elicit an appropriate immune response makes this vaccine platform exceptionally attractive. Until date, none of the human DNA vaccines under development have been issues license yet, however the registration and licensing of several veterinary vaccines have demonstrated the prospectivity of such drugs. The main obstacle in achieving the maximum immunogenicity of DNA vaccines involves issues with their targeted delivery to immunologically relevant cells [6, 7].

The use of therapeutic drugs based on protein molecules and low molecular weight compounds is complicated by the fact that they cannot be targeted to a specific gene or its protein product responsible for the development of a specific pathological process. For instance, the action of nucleic acid molecules can be targeted to a specific section of DNA in order to edit its nucleotide sequence. This method enables correction of a genetic defect by eliminating the cause of the disease and not just by treating its consequences.

Over the past several decades, candidate RNA drugs have been proposed with the aim of correction of pathology at the level of genes and RNA. Non-coding RNAs exhibit a therapeutic effect in various diseases by cleaving the messenger RNA (mRNA) due to the presence of antisense oligonucleotide sequences in their composition, the formation of duplexes with DNA, alternative splicing, RNA editing, chromatin modification, the modulation of transcription, translation, RNA masking, and gene silencing. Based on the mRNA, vaccine preparations are being developed aimed at protecting against pathogens, as well as combating tumor cells. Despite the fact

that, since 1990, nucleic acids have been used to modulate protein synthesis *in vivo* [8], the use of RNA in therapy has been limited by several factors. Single-stranded RNA is susceptible to degradation by nucleases, it can induce an immune response, it has a negative charge on the molecule, and it is extremely large to pass passively through the cell membranes; therefore, it is necessary to ensure its transport into the cell cytoplasm [9]. This problem is being addressed by the development of various delivery methods using carrier molecules.

## Recombinant DNA technologies for protein preparations

For the use of protein drugs, therapeutic proteins are usually required in large quantities. This production problem can be solved by the production of recombinant proteins, for which the expression systems that are based on prokaryotic and eukaryotic cells are used. These systems offer both advantages and disadvantages. The production of eukaryotic proteins in the eukaryotic producer cells is compelling because the resulting protein does not contain impurities that are typical for prokaryotic cells, which in turn simplifies its purification. Simultaneously, the technology for the production of recombinant proteins in eukaryotic cells, especially in cells of higher eukaryotes, is a long, laborious, and expensive process. In this regard, several researchers prefer the prokaryotic expression systems. *Escherichia coli* has one of the most popular expression systems due to its high cell growth rate, simplicity of genetic manipulations, and availability of a large number of recombinant proteins [10]. The ability to produce proteins in *E. coli* cells is based on the ability of the bacteria to reproduce the plasmid DNA present in their cells. Meanwhile, the expression vectors contain all the necessary elements for protein synthesis by using the enzymatic apparatus of the *E. coli* cells. In general, the scheme for obtaining a recombinant protein in *Escherichia coli* cells is presented in Figure 1.

The recombinant protein in *E. coli* cells is obtained in several stages, namely through chemical synthesis of the gene sequence encoding the recombinant protein, the creation of an expression plasmid by gene cloning in a plasmid vector, transformation of competent *E. coli* cells to create a strain-producer of recombinant protein, the selection of successfully transformed cell clones, cell cultivation for the production of recombinant proteins, and the subsequent purification of the protein.

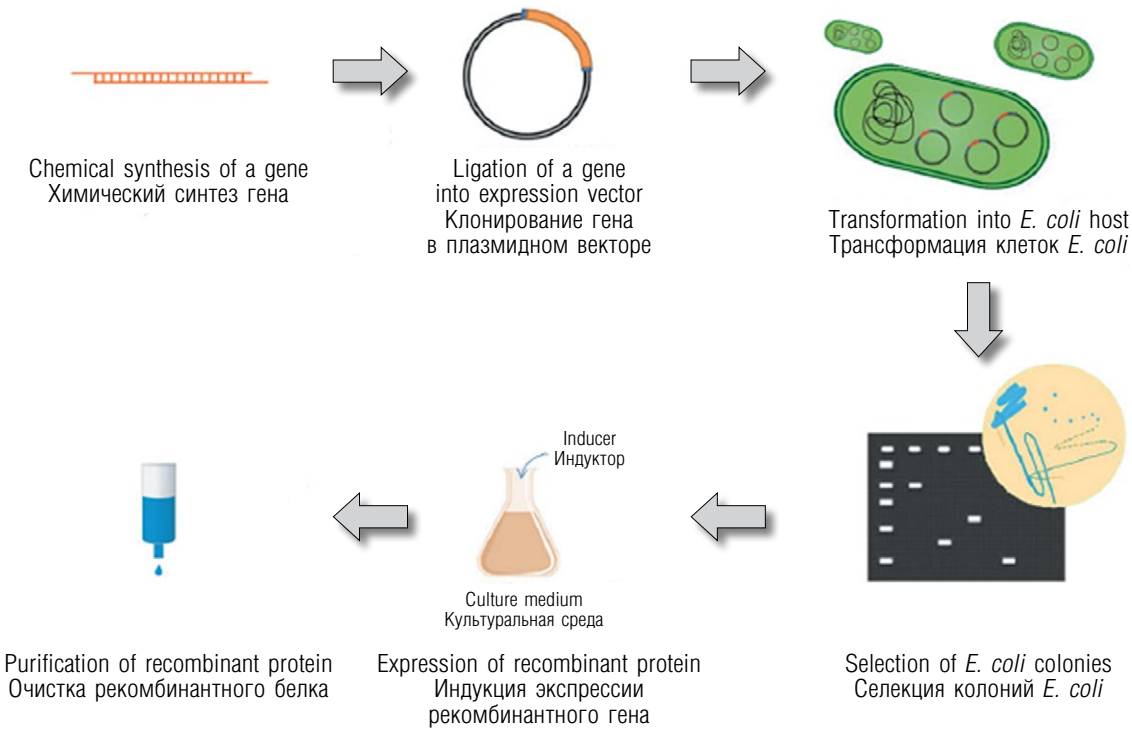


**Fig. 1.** Scheme for producing recombinant protein [11]

**Рис. 1.** Схема получения рекомбинантного белка [11]

Undoubtedly, DNA technology has potential for creating pharmaceuticals. Human insulin is be a classic example of successful transition from a drug obtained through extraction from animal tissues to a recombinant one. In 1922, Friederich Bunting and Charles Best were the first to use an insulin preparation obtained from an isolated animal pancreas successfully to treat insulin-dependent patients [12]. Until 1972, the only insulin available was a drug obtained through purification from the pancreas of pigs and cows. Due to the recombinant DNA technology, in 1982, it became possible to obtain a preparation of recombinant human insulin. Recombinant human insulin offers two distinct advantages over an insulin preparation obtained through the extraction from the pancreas, for instance, it provides an almost unlimited supply of recombinant protein, as there is no need to collect its source; in addition, recombinant human insulin is completely identical to the natural human insulin. Recombinant insulin is less immunogenic than the corresponding animal protein. It has been revealed that, long-term therapy with insulin isolated from the pig pancreas can lead to allergic reactions. Currently, a large number of insulin variants are available that differ in terms of the time of action in the body, and drugs with improved efficacy have also been developed [13].

In the recent years, the field of obtaining recombinant enzymes has been developing increasingly actively [14]. For example, a recombinant L-asparaginase (EC 3.5.1.1) has been obtained and used in the therapy of acute lymphoblastic leukemia. The recombinant enzyme is characterized by its high affinity for L-asparagine and minimal activity toward glutamine as a substrate, with the half-life in *in vitro* experiments of approximately 40 h. Meanwhile, the enzyme demonstrated deglycosylation activity, which could create an additional barrier for proliferating cancer cells [15].

In addition, the use of recombinant DNA technology enables the study of the molecular mechanisms in the development of various diseases. The mutation of the γ-sarcoglycan coding gene (SGCG), an integral membrane protein responsible for maintaining the integrity of the sarcolemma of muscle cells, leads to limb-lumbar muscular dystrophy, which is a congenital disease for which there is currently no therapy available [16]. A full-length SGCG protein has been obtained in *E. coli* cells, which has been used to study the pathogenesis of muscular dystrophy of the extremities and the lumbar spine as well as to develop etiotropic therapy [17].

Due to the recombinant DNA technology, the physiological functions of various natural compounds have been studied, which has resulted in newer possibilities for their clinical application. Thus, for example, antimicrobial peptides, particularly, human defensins, and cathelicidin, were initially characterized in *in vitro* experiments as bactericidal agents, but later they showed immunomodulatory properties. Human defensins modulate the immune response to foreign nucleic acids, particularly, human β-defensin 3 stimulated the synthesis of interferon alpha in response to the penetration of viral double-stranded DNA. The study of recombinant human β-defensin 3 in *in vitro* experiments using cell lines (such as mouse dendritic cells and mononuclear cells of human peripheral blood) revealed that the immunomodulatory effect was implemented due to the activation of toll-like receptors-9 (TLR-9). Due to this reason, human β-defensin 3 can be positioned as a promising adjuvant in vaccine compositions [18].

In addition to the production of recombinant analogs of natural proteins, the recombinant DNA technology enables obtaining chimeric proteins with preset properties [19, 20]. The development of protein preparations with an increased half-life as well as therapeutic and prophylactic proteins with increased efficiency has been performed [21].

About 30% of all protein drugs are produced using an expression system based on *E. coli* cells. There are, however, a number of limitations and obstacles with using this type of a producer, including the need to purify the recombinant protein from impurities of other molecules of the producer strain (such as proteins, DNA, and lipopolysaccharides). Being highly immunogenic in humans, these drug impurities can cause several undesirable side-effects. In this regard, the amount of impurity molecules must be strictly controlled and standardized. Another difficulty that researchers face when developing a technology for the production of a recombinant protein in *E. coli* cells consists in obtaining it in a native soluble form. At a high level and the rate of synthesis, a foreign protein accumulates in prokaryotic cells in the form of agglomerates

of a partially folded protein, namely the inclusion bodies that must subsequently be refolded in order to obtain a therapeutically active component. Nevertheless, the refolding protocols for each protein are individual and should be carefully designed.

## Preventive DNA vaccines

Another promising approach to the development of immunobiological drugs is their production based on DNA molecules. The scheme for the production of such preparations based on plasmid DNA in *E. coli* cells is similar to that for the production of a recombinant protein. The production process involves the stages of synthesis of a gene encoding a therapeutic protein, cloning a gene in a plasmid vector, transformation of competent *E. coli* cells, selection of transformants containing a plasmid, subsequent production of cell biomass of a strain producing plasmid DNA, and the purification of plasmid DNA. One of the main differences between the production of recombinant plasmid DNA and protein is the structure of the vectors used that encode antigens under the control of effective eukaryotic promoters, which ensures protein synthesis in the cells of the drug recipient organism. In this case, *E. coli* cells were used for the replication of plasmid DNA.

Several DNA vaccines have already been approved for use in veterinary medicine, including a vaccine against infectious necrosis of hematopoietic tissues in salmon, for the treatment of canine melanoma, and for the prevention of equine fever caused by West Nile virus [22]. For several reasons, until date, none of the developed DNA vaccines have been licensed for use in humans. One of these reasons is the relatively low immunogenicity of these vaccines compared to those of the classically used vaccines. To address this obstacle, a large number of different strategies are being developed to enhance the immunogenicity of DNA vaccines, including the modification of vaccine vectors, codon optimization, the use of traditional and molecular adjuvants, the use of antigen co-expression with a molecular adjuvant, the administration of DNA vaccines via electroporation, and prime-boost strategies.

To increase the effectiveness of an immunobiological preparation based on plasmid DNA, it must be delivered into cells to induce antigen expression *in vivo*. In case of DNA vaccines, this approach is required for the presentation of the protein antigen by the major histocompatibility complex (MHC) molecules and the subsequent recognition by immunocompetent cells. Antigens synthesized from genes encoded on plasmid DNA activate both class I and class II MHCs, thereby stimulating both $CD4^+$ and $CD8^+$ T cells [23].

The plasmid vector transforms myocytes, keratinocytes, and dendritic cells. Dendritic cells can independently implement antigen presentation, while myocytes and keratinocytes implement this process through cross paths (Fig. 2).

The gene expression of a DNA vaccine is usually controlled by type II polymerase promoters. The endogenous promoters of mammalian polymerase II are not as strong as viral promoters, such as cytomegalovirus (CMV) or SV40 (which are used particularly in vectors pcDNA3.1, pVAX1, pVIVO2, pCI, pCMV, and pSV2). The cytomegalovirus promoter is highly active in most cell types, making it a widely used one for the development of DNA vaccines. Past studies using DNA vaccines containing the HIV-1 *env* have shown that stronger promoters induced more active protein synthesis and the formation of more powerful immune responses [25]. Although viral promoters effectively stimulate the synthesis of the vaccine antigen, this does

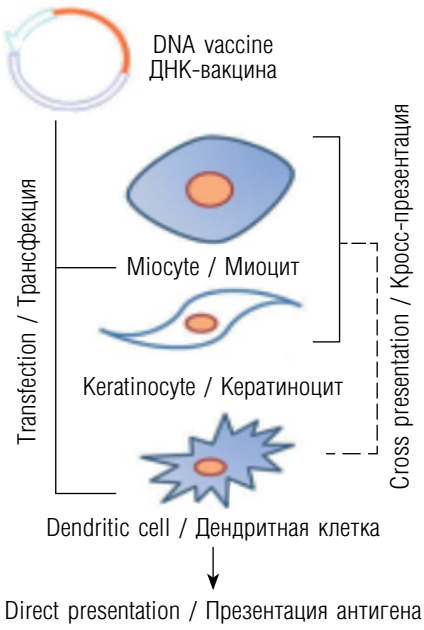


**Fig. 2.** Schematic representation of a DNA vaccine action [24]

**Рис. 2.** Схематическое изображение действия ДНК-вакцины [24]

not always correlate with the vaccine efficiency. The reason for this is that pro-inflammatory cytokines such as tumor necrosis factor α or interferon γ can inhibit viral promoters. It has been revealed that promoters of the class II MHC are insensitive to these factors [26]. Thus, despite the fact that the CMV promoter, which provides a high level of gene expression, remains the main one in the development of most DNA vaccines, and alternative promoters can increase the vaccination efficiency. Notably, the optimization of a nucleotide sequence by replacing rare codes does not always increase the efficiency of vaccination with DNA plasmids. For example, during the development of a DNA vaccine for the prevention of malaria, the native antigen nucleotide sequence was found to elicit a more powerful T-cell response against *Plasmodium yoelii* than the codon-optimized sequence for expression in mammalian cells [27]. Thus, when developing DNA vaccines, it becomes necessary to compare the efficiency of the optimized and native antigen sequences.

Another approach to increasing the efficiency of vaccination using plasmid DNA is the improvement of the methods of its delivery into the cell cytoplasm. For these reasons, liposomes are being developed, which are spherical vesicles consisting of a lipid bilayer comprising of phospholipids and cholesterol. Liposomes capture or bind plasmid DNA and facilitate its penetration into the target cell [28]. Interestingly, when creating pharmacological preparations for intramuscular administration using liposomes based on plasmid DNA, their local irritating effect is expelled. However, despite that, they remain promising candidates for drug development for use on mucous membranes. A recent study on laboratory C57BL/6 mice revealed that oral vaccination with a vector based on pcDNA3.1 plasmid DNA encoding the M1 protein gene of influenza A virus, encapsulated in cationic liposomes, could induce both humoral and cellular immune responses, which imparted protection against respiratory infections [29]. Liposomes are also effective in intranasal vaccination of laboratory Balb/c mice with a candidate vaccine against Eastern equine encephalitis virus based on plasmid DNA pcDNA3.1 containing the C, E3, E2, 6k, and E1 virus genes [30].

DNA vaccines offer several advantages over the conventional vaccines, including the construct aspects of DNA vaccines, for whose creation, as a rule, only one-step cloning into a plasmid vector is required. This approach reduces their cost and production time. In addition, the *in vivo* expression of a gene driven by a eukaryotic promoter and endogenous post-translational modification lead to the formation of native protein structures, which ensures correct immune presentation. From the safety perspective, cloning and synthesizing nucleic acids encoding an antigen, instead of isolating it from natural sources, enable avoidance of the use of pathogenic microorganisms in the production of vaccines. With the use of recombinant DNA technology, almost any type of molecular manipulation with plasmid DNA can be performed, including the introduction of mutations into a gene *in vitro*, which enables to quickly change the design of the antigens. This aspect is extremely important in the development of prophylactic drugs against pathogens such as influenza virus, which is characterized by high variability due to possible antigenic drift. Plasmid DNA has a good safety profile without causing irritation at the injection site, which is the most common side-effect of drugs used in intramuscular administration. Plasmid DNA is stable at the room temperature, which eliminates the need to organize a cold chain during its transportation as the need for any special storage conditions [31].

## Prophylactic RNA vaccines

Several years ago, mRNA was recognized as a new class of therapeutic compounds with its own international nonproprietary nomenclature [32]. RNA-based vaccines are being developed that can induce an immune response against pathogens and tumor cells [33]. This is quite a recent field. New methodological approaches are being developed in relation to the aspects of RNA molecules, namely, their instability and rapid degradation in the mammalian body, the need for an efficient method of delivery into cells for the translation process, as well as with the solution of the problems caused by the emergence of a powerful pro-inflammatory response upon RNA administration. In the past decade, the last of the listed problems was largely overcome by including modified nucleosides in the RNA vaccine sequence, which optimized the RNA sequence, and allowed thorough purifica-

tion from the by-products (especially double-stranded RNA [dsRNA]), which in turn enabled reduction of the toxicity of therapeutic mRNA and increment in its degree of translation in the body [34].

The structure of RNA vaccines includes an open reading frame that encodes an antigen and its flanking sequences, namely 5′- and 3′-untranslated regions on both the sides of the open reading frame, 5′-end 7-methylguanosine cap and 3′-polyadenylic tail (polyA). These elements are required to maximize the translation rate and/or persistence of the vector in the transformed cells as a result of interactions with regulatory proteins, other RNAs, and metabolites [34]. RNA vaccines can occur in both the form of a non-replicating vector and in the form of a self-replicating replicon [35]. The construct of self-replicating vectors contains additional viral elements that are responsible for replication [36]. RNA replicons are obtained by replacing the structural genes of single-stranded RNA viruses (for example, alphaviruses, flaviviruses, and picornaviruses) with the target antigen gene while preserving the non-structural genes that are responsible for the synthesis of proteins that ensure replication. The main advantage of this approach over the use of non-replicating mRNAs involves the fact that, due to the self-replication of the vector *in vivo*, a high level of expression of the target antigen is registered. The introduction of such vectors triggers an immune response that is closest to that of a natural infection, as a result, the vaccine vector begins to replicate in the host cell to form dsRNA intermediates, which are the ligand of TLR-3. Signaling through TLR-3 leads to the production of interleukin-12, which is a cytokine that induces the synthesis of interferons which activate the response of T-helpers 1 and then that of cytotoxic T cells [37]. However, until date, the technology of obtaining such molecules with a high yield has not been developed considering that they are significantly larger than non-replicating vectors. In addition, RNA vaccines based on self-replicating replicon are also susceptible to enzymatic cleavage when administered into the body, and the hydrophilic nature and the high molecular weight cause the problem of an efficient delivery system into the cell cytoplasm [38].

The delivery of RNA into the cell cytoplasm is a necessary condition for the target antigen synthesis. This problem is solved by developing carrier molecules that protect mRNA from rapid degradation and deliver it to the cytoplasm without manifesting any significant toxic effect. There exist viral and non-viral RNA delivery systems. Despite the known efficiency of viral systems in delivering nucleic acids into cells, their use are limited by the possibility of an immune response to the viral vector [39]. Non-viral delivery methods imply the association of mRNA with carrier molecules, namely lipids, polymers, and peptides. All of them have demonstrated good perspectives in preclinical and some clinical studies [40]. In the development of polymer carriers of RNA molecules, polyethylene imine was initially used successfully, but, presently, only preclinical data of its use are available. More recently, lipid-containing polymers called charge-altering releasable transporters were developed, and they were capable of delivering mRNA directly to T-lymphocytes, making them extremely promising for the development of anticancer drugs [41]. Presently, lipid nanoparticles are the most commonly used method of mRNA delivery. In experiments on laboratory mice, it has been revealed that mRNA encapsulated in lipid nanoparticles is transcribed efficiently *in vivo* through various routes of administration into the body [42].

While nucleic acid-based vaccines generally demonstrate significant advantages over the conventional vaccines in terms of safety, the induction of both B- and T-cell responses, mRNA vaccines offer advantages over DNA-based vaccines [43]. The methodological problem associated with DNA vaccines is to ensure efficient delivery to the cell nucleus, where the antigen transcription takes place. In addition, DNA vaccines bear the potential risk of integration into the host genome, which can lead to insertion mutagenesis. There are no such risks in mRNA-based vaccines, since their reproduction occurs in the cell cytoplasm [44]. The relatively short half-life results in transient and more controlled expression of the encoded antigen. In addition, mRNA can be obtained in a cell-free environment through *in vitro* transcription, which eliminates the need for using bacterial cells, resulting in the elimination of the problems associated with the presence of impurities of the producer strain molecules. This approach provides simple cleaning and reduces the cost

of the drug production [37]. All this facts support that RNA is a promising platform for drug development.

## Nucleic acid-based therapeutic drugs

The human genome sequencing and the study of the role of certain genes in the pathogenesis of diseases have stimulated the development of therapeutic drugs based on nucleic acids. One of the significant advantages of drugs based on DNA and RNA over the classical low molecular weight drugs are their more selective recognition of molecular targets that impart their action with a strict specificity. These drugs, used both in the acute course of diseases and in their prevention and therapy at an early stage, facilitate the prevention of progression of pathological processes as well as the development of complications. Gene therapy involves the correction of a defective gene via administration and expression of its correct copy that manifests itself, as in the synthesis of an essential protein product. Similarly, nucleic acid-based drugs for genetic ablation interfere with the expression of certain genes that provides specificity in disease control management. In addition, the negative side-effects of gene therapy can be minimized when compared with the classical pharmaceutical drugs that are usually less specific and, as a result, have a less pronounced systemic effect on the body.

Over the past few decades, the generally accepted concept of the role of RNA in supporting the process of protein synthesis has changed, and it is now believed that RNA can also be used for therapeutic purposes. Due to the implementation of 2 international projects, Functional Annotation of the Mammalian Genome and the Encyclopedia of DNA Elements (ENCODE), <2% of the mammalian genome has been cited responsible for protein synthesis and almost 98% has been transcribed into protein non-coding RNA (ncRNA), which plays a regulatory role in the implementation of molecular and cellular functions by controlling the gene expression [45]. ncRNA is classified into long non-coding RNA (lncRNA), short non-coding RNA (sncRNA), and translational/structural RNA, which perform different functions. It has been demonstrated that ncRNA affects the processes of transcription, translation, and post-translational modifications. Non-coding RNAs regulate the gene expression under both the normal and pathological conditions to modulate the development and progression of the disease [46]. The characterization of the ncRNA coding sequences and deciphering the mechanisms of their action can also help in the diagnostics of the disease, the study of its development, and the elaboration of specific therapies. Due to the uniqueness of the mechanisms of action of ncRNAs, they represent promising targets for the development of a new class of therapeutic drugs. Non-coding RNAs can exhibit a therapeutic effect in various diseases by cleaving mRNA due to the presence of antisense oligonucleotides in their sequence, the formation of duplexes with DNA, alternative splicing, RNA editing, chromatin modification, transcription modulation, translation, RNA masking, and gene silencing.

Nucleic acid-based treatment medications include plasmid DNAs encoding proteins for gene therapy, oligonucleotides, ribozymes, DNAzymes, aptamers, and small-interfering RNAs (siRNAs). Although most nucleic acid-based drugs are tested in the early stages of clinical trials, they are promising candidates for the development of therapeutic drugs against a wide range of diseases, including cancer, neurological (such as Parkinson's disease and Alzheimer's disease), and cardiovascular diseases. Gene therapy is aimed to achieve long-term and efficient expression of genes administered at the therapeutic level. However, there are various other options. Therefore, a normal copy of a mutated gene can be administered, the changes can be made in the nucleotide sequence or in the expression of genes responsible for the onset of the disease that can be suppressed.

The purpose of gene administration involves restoration of the normal cellular function by administration a functioning copy of the gene without affecting the mutant one that remains in the cell. This approach can be illustrated by treatment of diseases such as spinal muscular atrophy [47] and primary immunodeficiency. For example, in Huntington disease, the cellular function was lost as a result of the defective protein accumulation. In this case, the genes were suppressed in order to restore the normal cell functions by reducing the expression of the mutated genes through the RNA interference. Another gene therapy approach is gene editing. Although the use of nuclease is not necessary for

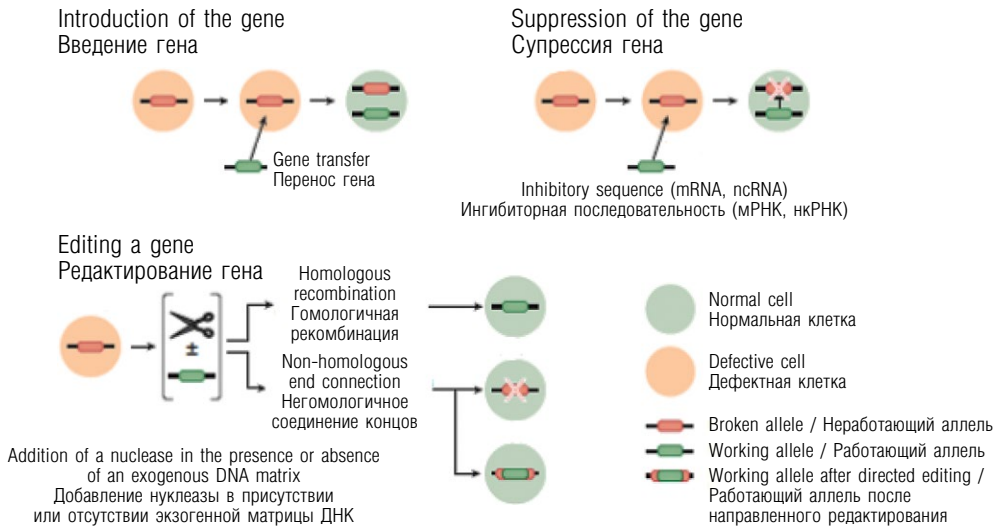


**Fig. 3.** Schematic representation of the principles of gene therapy [48]

**Рис. 3.** Схематическое изображение принципов генной терапии [по 48]

genome editing, the efficiency of gene-specific editing in the mammalian cells is usually enhanced by inducing DNA double-strand breaks at the target site. The choice of one of the DNA repair mechanisms will determine the result of genome editing. After breaking, DNA can bind to a non-homologous end, so that there is a possibility of gene knockdown. In the presence of an exogenous matrix of a functional gene, DNA repair can lead to the correction of the mutant gene via homologous recombination. The third potential result is the insertion of a DNA matrix through a non-homologous end connection, which can lead to the addition of a nucleotide sequence to the gene [48] (Fig. 3).

Until date, several gene therapy drugs have been approved for use in Europe and the USA. These include the drug Glybera, which was licensed in 2012 by the European Medicines Agency (EMA), for the treatment of familial lipoprotein lipase deficiency, based on an adenoassociated virus [49]. In 2015, the Food and Drug Administration (FDA) approved the drug IMLYGIC, which is a genetically modified herpes simplex virus type 1, for the topical treatment of inoperable lesions in patients with melanoma. In 2016, the γ-retrovirus-based drug Strimvelis for the treatment of severe combined immunodeficiency caused by adenosine deaminase deficiency (ADA-SCID) was approved by the EMA [50]. In 2017, the FDA approved 3 gene therapy drugs.

KYMRIAH and YESCARTA target $CD19^+$ T-lymphocytes for the treatment of lymphoma. KYMRIAH is also indicated for the treatment of acute lymphoblastic leukemia [51, 52]. LUXTURNA is a drug based on a recombinant adenoassociated virus for the treatment of hereditary retinal degeneration caused by the RPE-65 (IRD-RPE-65) mutation [53]. In 2018, the FDA approved a drug based on siRNAs encapsulated in lipid nanoparticles. The drug is used for the treatment of polyneuropathies associated with hereditary autosomal dominant disease and transthyretin amyloidosis, which causes damage to the peripheral nervous system and internal organs [54].

## Conclusion

The advancements in the genetic engineering techniques and the creation of recombinant DNA molecules has stimulated the development of life sciences. Owing to the powerful tools of recombinant DNA technology that have been actively developed over the past decades, DNA manipulation has become a common practice. Gene cloning has become relatively simple, which has led to a breakthrough in understanding the pathogenesis of several diseases, the methods of their diagnoses, as well as the associated prevention and therapy.

Contemporary methods of genetic engineering enables obtaining natural proteins in quantities not limited by the presence of their natural sources and without the use of pathogenic microorganisms. In fact, drugs with desired properties can be obtained using chimeric molecules.

The great interest in the creation of drugs based on DNA and RNA, as noted in the recent years, has opened up the prospect of developing not only innovative drugs for the treatment of various types of pathology but also developed methods for eliminating the causes of diseases induced by gene mutations.

All methodological approaches described so far offer certain advantages and are characterized by some limitations in their application. The choice of a platform for the development of a particular drug thus depends, to a large extent, on the specific task facing the researcher.

**Conflict of interest.** The authors declare no conflict of interest.

## References

1. Araki E, Araki H, Senokuchi T, Motoshima H. New perspectives on the insulin therapy. *J Diabetes Investig*. 2020;11(4):795-797. https://doi.org/10.1111/jdi.13263.
2. Richmond E, Rogol AD. Treatment of growth hormone deficiency in children, adolescents and at the transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):749-755. https://doi.org/10.1016/j.beem.2016.11.005.
3. Razaghi A, Owens L, Heimann K. Review of the recombinant human interferon gamma as an immunotherapeutic: Impacts of production platforms and glycosylation. *J Biotechnol*. 2016;240:48-60. https://doi.org/10.1016/j.jbiotec.2016.10.022.
4. Raso S, Hermans C. Recombinant factor VIII: past, present and future of treatment of hemophilia A. *Drugs Today (Barc)*. 2018;54(4):269-281. https://doi.org/10.1358/dot.2018.54.4.2800622.
5. Ladisch MR, Kohlmann KL. Recombinant human insulin. *Biotechnol Prog*. 1992;8(6):469-478. https://doi.org/10.1021/bp00018a001.
6. Goryaev AA, Savkina MV, Obukhov YI, et al. DNA and RNA vaccines: Current status, quality requirements and specific aspects of preclinical studies. *BIOpreparations. Prevention, Diagnosis, Treatment*. 2019;19(2):72-80. https://doi.org/10.30895/2221-996X-2019-19-2-72-80.
7. Porter KR, Raviprakash K. DNA vaccine delivery and improved immunogenicity. *Curr Issues Mol Biol*. 2017;22:129-138. https://doi.org/10.21775/cimb.022.129.
8. Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle *in vivo*. *Science*. 199023;247(4949 Pt 1):1465-1468. https://doi.org/10.1126/science.1690918.
9. Kaczmarek JC, Kowalski PS, Anderson DG. Advances in the delivery of RNA therapeutics: From concept to clinical reality. *Genome Med*. 2017;9(1):60. https://doi.org/10.1186/s13073-017-0450-0.
10. Baeshen MN, Al-Hejin AM, Bora RS, et al. Production of biopharmaceuticals in *E. coli*: current scenario and future perspectives. *J Microbiol Biotechnol*. 2015;25(7):953-962. https://doi.org/10.4014/jmb.1412.12079.
11. Huang W, Rollett A, Kaplan DL. Silk-elastin-like protein biomaterials for the controlled delivery of therapeutics. *Expert Opin Drug Deliv*. 2015;12(5):779-791. https://doi.org/10.1517/17425247.2015.989830.
12. Barfoed HC. Insulin production technology. *Chem Eng Prog*. 1987;83:49-54.
13. Insulin. B LiverTox: clinical and research information on drug-induced liver injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
14. Arbige MV, Shetty JK, Chotani GK. Industrial enzymology: The next chapter. *Trends Biotechnol*. 2019;37(12):1355-1366. https://doi.org/10.1016/j.tibtech.2019.09.010.
15. Sindhu R, Manonmani HK. Expression and characterization of recombinant L-asparaginase from pseudomonas fluorescens. *Protein Expr Purif*. 2018;143:83-91. https://doi.org/10.1016/j.pep.2017.09.009.
16. Шнайдер Н.А., Николаева Т.Я., Бороева Е.Н., и др. Конечностно-поясная мышечная дистрофия с аутосомно-доминантным типом наследования: пельвиофеморальная форма Лейдена – Мебиуса // Нервно-мышечные болезни. – 2013. – № 1. – С. 46–61. [Shnayder NA, Nikolayeva TY, Boroeva EN, et al. Autosomal dominant limb-girdle muscular dystrophy: Leyden–Möbius pelvifemoral form. *Neuromuscular Diseases*. 2013;(1):46-61. (In Russ.)]. https://doi.org/10.17650/2222-8721-2013-0-1-46-61.
17. Jamaladdine M, Harris MS, Liyanage L, et al. Expression, purification, and structural analysis of the full-length human integral membrane protein γ-Sarcoglycan. *Protein Expression and Purification*. 2019;167:105525. https://doi.org/10.1016/j.pep.2019.105525.
18. Ferris LK, Mburu YK, Mathers AR, et al. Human beta-defensin 3 induces maturation of human langerhans cell-like dendritic cells: An antimicrobial peptide that functions as an endogenous adjuvant. *J Invest Dermatol*. 2013;133(2):460-468. https://doi.org/10.1038/jid.2012.319.
19. Prejit N, Pratheesh PT, Nimisha S, et al. Expression and purification of an immunogenic SUMO-OmpC fusion protein of salmonella typhimurium in *Escherichia coli*. *Biologicals*. 2019;62:22-26. https://doi.org/10.1016/j.biologicals.2019.10.010.
20. Singh SK, Thrane S, Chourasia BK, et al. Pfs230 and Pfs48/45 fusion proteins elicit strong transmission-blocking antibody responses against plasmodium falciparum. *Front Immunol*. 2019;10:1256. https://doi.org/10.3389/fimmu.2019.01256.

21. Kim TY, Park JH, Shim HE, et al. Prolonged half-life of small-sized therapeutic protein using serum albumin-specific protein binder. *J Control Release*. 2019;315:31-39. https://doi.org/10.1016/j.jconrel.2019.09.017.
22. Kutzler MA, Weiner DB. DNA vaccines: Ready for prime time? *Nat Rev Genet*. 2008;9(10):776-788. https://doi.org/10.1038/nrg2432.
23. Liu MA. DNA vaccines: An historical perspective and view to the future. *Immunol Rev*. 2011;239(1):62-84. https://doi.org/10.1111/j.1600-065X.2010.00980.x.
24. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev Vaccines*. 2016;15(3):313-329. https://doi.org/10.1586/14760584.2016.1124762.
25. Wang S, Farfan-Arribas DJ, Shen S. Relative contributions of codon usage, promoter efficiency and leader sequence to the antigen expression and immunogenicity of HIV-1 Env DNA vaccine. *Vaccine*. 2006;24(21):4531-4540. https://doi.org/10.1016/j.vaccine.2005.08.023.
26. Vanniasinkam T, Reddy ST, Erel HC. DNA immunization using a non-viral promoter. *Virology*. 2006;344(2):412-420. https://doi.org/10.1016/j.virol.2005.08.040.
27. Dobano C, Sedegah M, Rogers WO, et al. Plasmodium: Mammalian codon optimization of malaria plasmid DNA vaccines enhances antibody responses but not T cell responses nor protective immunity. *Exp Parasitol*. 2009;122(2):112-123. https://doi.org/10.1016/j.exppara.2009.02.010.
28. Karkada M, Weir GM, Quinton T, et al. A liposome-based platform, VacciMax, and its modified water-free platform DepoVax enhance efficacy of *in vivo* nucleic acid delivery. *Vaccine*. 2010;28(38):6176-6182. https://doi.org/10.1016/j.vaccine.2010.07.025.
29. Liu J, Wu J, Wang B. Oral vaccination with a liposome-encapsulated influenza DNA vaccine protects mice against respiratory challenge infection. *J Med Virol*. 2014;86(5):886-894. https://doi.org/10.1002/jmv.23768.
30. Ma J, Wang H, Zheng X. CpG/Poly (I:C) mixed adjuvant priming enhances the immunogenicity of a DNA vaccine against eastern equine encephalitis virus in mice. *Int Immunopharmacol*. 2014;19(1):74-80. https://doi.org/10.1016/j.intimp.2014.01.002.
31. Gary EN, Weiner DB. DNA vaccines: Prime time is now. *Curr Opin Immunol*. 2020;65:21-27. https://doi.org/10.1016/j.coi.2020.01.006.
32. International Nonproprietary Names for Pharmaceutical Substances (INN). *WHO Drug Information*. 2015;29(2):113.
33. Qadir MI, Bukhat S, Rasul S, et al. RNA therapeutics: Identification of novel targets leading to drug discovery. *J Cell Biochem*. 2020;121(2):898-929. https://doi.org/10.1002/jcb.29364.
34. Pardi N, Hogan MJ, Weissman D. Recent advances in mRNA vaccine technology. *Curr Opin Immunol*. 2020;65:14-20. https://doi.org/10.1016/j.coi.2020.01.008.
35. Brito LA, Kommareddy S, Maione D. Self-amplifying mRNA vaccines. *Adv Genet*. 2015;89:179-233. https://doi.org/10.1016/bs.adgen.2014.10.005.
36. Ulmer JB, Mason PW, Geall A, et al. RNA-based vaccines. *Vaccine*. 2012;30(30):4414-4418. https://doi.org/10.1016/j.vaccine.2012.04.060.
37. Iavarone C, O'hagan DT, Yu D, et al. Mechanism of action of MRNA-based vaccines. *Expert Rev Vaccines*. 2017;16(9):871-881. https://doi.org/10.1080/14760584.2017.1355245.
38. Anderluzzi G, Lou G, Gallorini S, et al. Investigating the impact of delivery system design on the efficacy of self-amplifying RNA vaccines. *Vaccines (Basel)*. 2020;8(2):E212. https://doi.org/10.3390/vaccines8020212.
39. Wadhwa A, Aljabbari A, Lokras A, et al. Opportunities and challenges in the delivery of mRNA-based vaccines. *Pharmaceutics*. 2020;12(2):E102. https://doi.org/10.3390/pharmaceutics12020102.
40. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. *Mol Ther*. 2019;27(4):710-728. https://doi.org/10.1016/j.ymthe.2019.02.012.
41. McKinlay CJ, Benner NL, Haabeth OA, et al. Enhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of charge-altering releasable transporters. *Proc Natl Acad Sci U S A*. 2018;115(26):E5859-E5866. https://doi.org/10.1073/pnas.1805358115.
42. Pardi N, Tuyishime S, Muramatsu H, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J Control Release*. 2015;217:345-351. https://doi.org/10.1016/j.jconrel.2015.08.007.
43. Pardi N, Hogan MJ, Porter FW, Weissman, D. mRNA vaccines – a new era in vaccinology. *Nat Rev Drug Discov*. 2018;17(4):261-279. https://doi.org/10.1038/nrd.2017.243.
44. Jahanafrooz Z, Baradaran B, Mosafer J, et al. Comparison of DNA and mRNA vaccines against cancer. *Drug Discov Today*. 2020;25(3):552-560. https://doi.org/10.1016/j.drudis.2019.12.003.
45. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489(7414):57-74. https://doi.org/10.1038/nature11247.
46. Ulitsky I, Bartel DP. lincRNAs: Genomics, evolution, and mechanisms. *Cell*. 2013;154(1):26-46. https://doi.org/10.1016/j.cell.2013.06.020.
47. Arnold ES, Fischbeck KH. Spinal muscular atrophy. *Handb Clin Neurol*. 2018;148:591-601. https://doi.org/10.1016/B978-0-444-64076-5.00038-7.
48. Anguela XM, High KA. Entering the modern era of gene therapy. *Annu Rev Med*. 2019;70:273-288. https://doi.org/10.1146/annurev-med-012017-043332.
49. Gaudet D, Méthot J, Déry S, et al. Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for

lipoprotein lipase deficiency: An open-label trial. *Gene Ther*. 2013;20(4):361-369. https://doi.org/10.1038/gt.2012.43.
50. Schimmer J, Breazzano S. Investor outlook: Rising from the ashes; GSK's European approval of Strimvelis for ADA-SCID. *Hum Gene Ther Clin Dev*. 2016;27(2):57-61. https://doi.org/10.1089/humc.2016.29010.ind.
51. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018; 378(5):439-448. https://doi.org/10.1056/NEJMoa1709866.
52. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531-2544. https://doi.org/10.1056/NEJMoa1707447.
53. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, Phase 3 trial. *Lancet*. 2017;390(10097):849-860. https://doi.org/10.1016/S0140-6736(17)31868-8.
54. Akinc A, Maier MA, Manoharan M, et al. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nat Nanotechnol*. 2019;14(12):1084-1087. https://doi.org/10.1038/s41565-019-0591-y.

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