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MicroRNAs and small interfering RNAs as tools for the directed regulation of cellular processes for cancer therapy

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ABSTRACT

MicroRNAs and small interfering RNAs (siRNAs) belong to an extensive class of small non-coding RNAs and play an important role in gene expression regulation in cells. It is shown that changes in the amount or activity of these molecules may lead to the development of various diseases, including cancer. This made it possible to consider them as promising diagnostic and prognostic markers, as well as tools for the directed regulation of protein synthesis in the cell and targets for therapy. This review summarizes the basic knowledge about the biogenesis, distribution and the mechanisms of action of microRNA and siRNA, as well as currently used ways of target genes expression management with their help. Possible methods of these molecules delivery into the cell *in vitro* and *in vivo* are considered.

Key words: small non-coding RNA, gene expression regulation, target therapy, cancer.

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МикроРНК и малые интерферирующие РНК как инструменты направленной регуляции клеточных процессов для терапии онкологических заболеваний

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РЕЗЮМЕ

МикроРНК и малые интерферирующие РНК (миРНК) относятся к обширному классу малых некодирующих РНК и играют важную роль в регуляции экспрессии генов в клетках. Показано, что изменения в количестве или эффективности воздействия этих молекул могут сопровождать развитие различных заболеваний, включая онкологические. Это позволило рассматривать их как перспективные диагностические и прогностические маркеры, а также инструменты для направленной регуляции синтеза белков в клетке и мишени для терапии. В данном обзоре суммированы основные знания о биогенезе, распространении и механизмах воздействия микроРНК и миРНК, а также способы направленного влияния на экспрессию генов с их помощью, используемые в настоящее время. Рассмотрены возможные варианты доставки молекул в клетку *in vitro* и *in vivo*.

Ключевые слова: малые некодирующие РНК, регуляция экспрессии генов, направленная терапия, онкологические заболевания.

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INTRODUCTION

MicroRNAs and small interfering RNAs (siRNAs) belong to the large and heterogeneous class of small non-coding RNAs, an important function of which is the regulation of gene expression in the cell. The history of the study of non-coding RNA began in 1993 with the publication by Victor Ambros and colleagues of data on the discovery of short RNA molecules that affect the translation of the lin-14 protein of the nematode Caenorhabditis elegans [1]. Since then, studies of small non-coding RNAs have been very intensive. But it was microRNAs and siRNAs that attracted the most attention and were studied more thoroughly. The accumulated knowledge allows the use of these molecules to affect living cells and the directed regulation of cellular processes. They have been used both in research projects and in the development of drugs in practical medicine. This is especially true in the treatment of malignant neoplasms, where the transformation of a cell into a tumor is accompanied by a significant shift in gene expression.

MicroRNA AND SiRNA

MicroRNA is a class of non-coding protein RNA molecules with a length of 18–24 nucleotides. They are important participants in the process of gene expression, regulating its intensity. Today, several thousand different microRNAs are known, each of which is able to control the synthesis of one to several hundred proteins. As a result, more than 60% of human genes are expressed with the participation of microRNAs.

A change in the level or activity of microRNAs can cause disturbances in the synthesis of certain proteins, which can lead to the development of a disease. It has been shown that a number of diseases are accompanied by deviations in the work of various microRNAs. A detailed study of the relationship between the work of individual microRNAs and the pathophysiology of diseases suggests the possibility of their use as molecular markers for diagnosing and predicting the course of the disease, as well as targets for targeted therapy.

SiRNAs are largely similar to microRNAs. These are molecules of 21–23 nucleotides in size that have a similar maturation path with microRNAs and a similar principle of action, but at the same time possess a number of features that separate them into a separate class [2]. In general terms, differences between microRNAs and siRNAs are presented in Table 1, and are described in more detail below.

Table 1

Differences in microRNAs and siRNAs		
Characteristic	MicroRNA	SiRNA
Molecule size	18–24 nucleotides	21–23 nucleotides
Structure	Single stranded	Double stranded
Beginning of biogenesis	From introns or individual sections of their own DNA (endogenous path)	From RNA of viruses or bacterial plasmids introduced into the cell, artificial vectors, etc. (exogenous pathway)
Immunogenicity	Own molecules, but artificially synthesized microRNAs can elicit an immune response	May elicit an immune response
Target Complementarity	Partial complementarity (the presence of a key «seed» region)	Complete complementarity
Targets	DNA, mRNA	mRNA
Specificity	One microRNA molecule regulates many DNA / RNA molecules, one DNA / RNA molecule can be a target for several microRNAs	Highly specific, one siRNA molecule binds one portion of mRNA, blocking the synthesis of one protein
Activity Result	Activation or repression of translation or transcription, mRNA degradation is possible	MRNA degradation, gene silencing
Activity sites	Cytoplasm, nucleus	Cytoplasm

BIOGENESIS OF microRNAs AND siRNAs

Understanding the biogenesis of microRNAs and siRNAs is very important for the possibility of influencing it. According to the canonical representation (Fig. 1), microRNA molecules are transcribed in the nucleus by RNA polymerase II from DNA regions that can be located both inside the genes encoding proteins (in introns) and in isolated regions of the genome under their own promoter. The resulting RNA transcript is called primary microRNA (pri-miRNA, pri-microRNA) and forms a secondary stem-loop structure with the presence of 7-methylguanosine molecules at the 5'-end, and at the 3'-end of poly (A)-"tail". After interacting with an enzyme complex consisting of RNase III (Drosha) and its companion DGCR8 (Pasha), pri-microRNA is converted to a microRNA precursor (pre-miRNA, pre-microRNA), consisting only of a stem-loop structure. Using exportin-5, pre-microRNA is transported from the nucleus to the cytoplasm, where the loop region is cleaved by another RNase III–Dicer, leaving a microRNA duplex consisting of two fully or partially complementary RNA strands 18–24 nucleotides in size. Subsequently, one of the duplex strands (host) forms a complex with proteins called the RNA-induced silencing complex (RISC), while the other ("passenger" strand), as a rule, is destroyed.

SiRNAs in the process of maturation pass a path similar to microRNAs. And although, unlike the latter, siRNA synthesis in mammalian cells does not begin from its own genome, but from vector molecules introduced into the cell from the outside (by bacteria, viruses or artificially), the same proteins (Dicer) participate in its biogenesis, and maturation is completed the formation of the RISC complex. In addition, siRNA can form in the cell as a result of cleavage of the RNA of the virus in a human cell. The mature siRNA molecule preserves the structure of the duplex.

TRANSPORT AND LOCALIZATION OF microRNAs IN THE BODY

After maturation, microRNA molecules can be transferred to different parts of the cell or beyond (Fig. 2). Some of them remain in the cytoplasm, where they interact with messenger RNA at various stages of translation, regulating protein synthesis. Another part of the molecules is transported into the intercellular space and circulates freely in the body in the form of ribonucleoprotein complexes or in exosomes [3, 4]. Exosomes are excreted by the cell and spread throughout the body, found in the extracellular space (extracellular matrix), blood plasma, synovial fluid, cerebrospinal fluid, saliva, urine and other liquid media and transferring microRNA from cell to cell. Exosomal microRNAs can act as markers for diagnosing or predicting the course of malignant neoplasms [5]. Another direction of movement of mature microRNAs is reverse transport to the nucleus. It has been shown that

most microRNAs are capable of such reverse transfer and are detected both in the nucleus and in the nucleoli [6]. In addition, gene expression is regulated in the nucleus with the participation of the RISC complex, for which the proteins involved in this process (AGO, TRPB, Dicer, TRNC6A) are freely transferred from the cytoplasm to the nucleus [7].

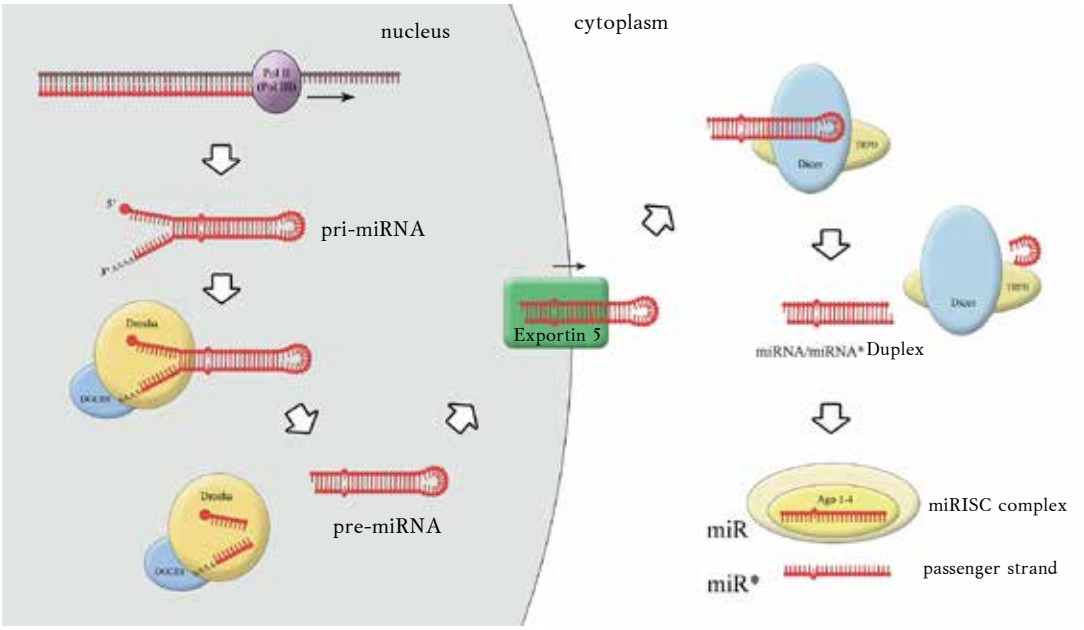

Fig. 1. The canonical scheme of microRNA biogenesis

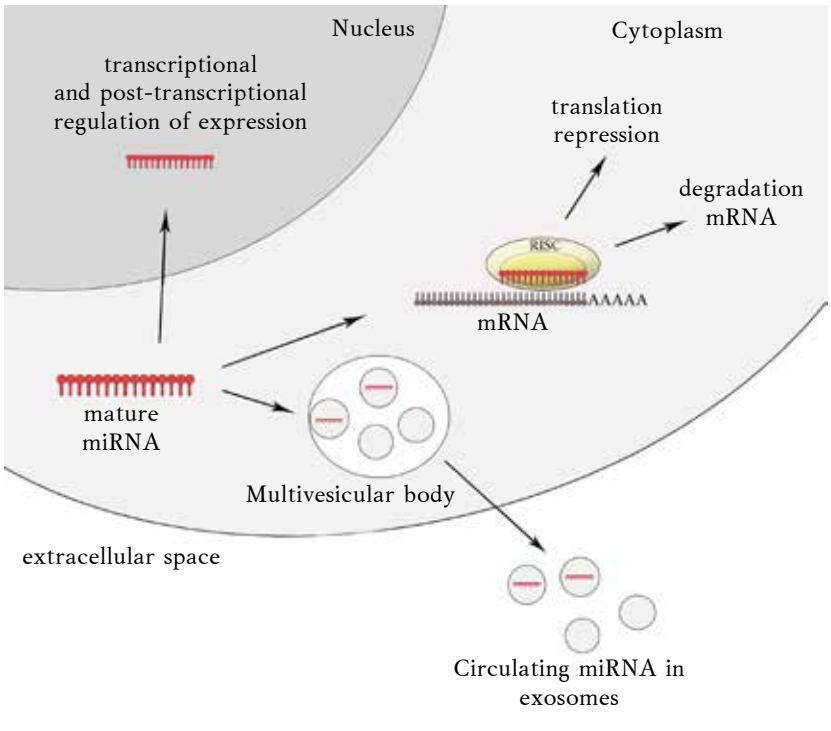

Fig. 2. Transport of mature microRNA in the body

When ingested or formed in a cell, siRNAs are usually stored in the cytoplasm, regulating via interaction with mRNA. In 2004, H. Kawasaki and K. Taira showed that siRNAs are able to induce cell DNA methylation by interacting with CpG islands in the promoter part of the gene. However, free penetration and the constant presence of siRNAs in the cell nucleus were not proven [8]. The mechanism of siRNA delivery to genomic DNA is still not fully understood.

WAYS OF REGULATING GENE EXPRESSION INVOLVING microRNA

The most studied method for regulating protein synthesis is the interaction of microRNA with messenger RNA in the cell cytoplasm. MicroRNAs bind to mRNA in specific complementary regions (target sites). Most often, these sites are found in the 3'-untranslated region of the mRNA (3'-UTR), but, in general, are found in the coding sequence (CDS), and even in the 5'-UTR [9]. As a rule, the binding site on the mRNA molecule is highly conserved so that random mutations or polymorphisms do not interfere with protein synthesis. Moreover, one mRNA can be a target for many microRNAs, and their joint participation determines the degree of suppression of protein synthesis.

Unlike plants, in animals, complete complementarity of microRNAs to the target gene is practically not found, but it has been proved that in this case, for the effective binding of the RISC complex, it is sufficient to complement the target molecule with only the second to eighth nucleotide region at the 5'-end of the microRNA molecule, called the key ("seed") region. Although there are microRNAs that regulate in a different way: it has been proven that miR-24 is involved in the regulation of cellular processes by acting on target genes that do not contain a site complementary to its "seed" region [10]. In any case, the result of microRNA attachment is the effect of the protein part of the RISC complex on the target mRNA.

The main protein of the RISC complex in humans is an enzyme of the Argonaute AGO2 family, which is a structural analogue of RNase H, and therefore has the ability to cleave mRNA molecules directly at sites determined by microRNAs. Due to its action, degradation of the mRNA molecule is possible after meeting with microRNAs. However, it was shown that this occurs only in 29% of cases, whereas in approximately half of the cases (48%), interaction with microRNAs results in repression of translation without matrix destruction, and in 23% there is a simultaneous course of these two processes [11]. It is assumed that the method of regulation may depend on the site of landing of microRNAs. The binding of microRNAs to mRNAs in the 3'-non-coding region (3'-UTR) is more likely to lead to the degradation of target RNA due to deadenylation of the poly (A) "tail" and, as a result, destabilization and rapid degradation of the molecule [12–14] . Landing on the coding part of the gene is more likely to suppress the synthesis of the polypeptide on the ribosome [15]. And when binding to a 5'-non-coding region (5'-UTR), messenger RNA can result in mRNA degradation due to preliminary decapping of the 5' end [16], as well as activation of translation [17]. Switching the microRNA-protein complex from the inhibition function to translation activation may depend both on the action of specific factors (for example, eIF4E) and on the state of the cell or phase of its cell cycle [18]. For example, miR-206 inhibits KLF4 protein synthesis in proliferating epithelial cells, but it activates in immortalized epithelial cells of the MCF10A line [19]. Like repression, activation can range from mild stimulatory effects to a significant increase in polypeptide synthesis.

Nuclear microRNAs are capable of influencing gene expression at post-transcriptional or transcriptional levels. Thus, they are able to control the synthesis or cause degradation of other microRNAs or long non-coding RNAs [20]. The ability of microRNAs to bind to single or even double stranded DNA leads to inhibition of [21] or activation of [22] gene transcription. It is noteworthy that in the nucleus, microRNA also acts in combination with the Argonaute protein, as well as other components of the RISC complex.

SiRNA REGULATION

The binding of RNA to the target mRNA molecule is its main difference from microRNA. The siRNA molecule does not have a "seed" region, but has complete complementarity to the mRNA molecule. For this reason, its action very specifically extends to the synthesis of only one protein. The result of its binding is the cleavage of the target with proteins of the RISC complex in the region between the 10th and 11th nucleotides of siRNA and complete cessation of translation [23].

DISORDERS OF microRNA IN MALIGNANT NEOPLASMS

Disorders associated with the development of malignant neoplasms can occur at the stages of biogenesis or work of microRNAs. These can be mutations of genes encoding microRNAs themselves or proteins important for their synthesis. Despite the general conservatism, polymorphisms in mRNA are also possible at the sites of binding to microRNAs. Also, the development of a malignant neoplasm can be associated with a change in the levels of certain microRNAs in the cell.

Table 2 presents examples of various abnormalities associated with biogenesis and the action of microRNA in the cell.

Table 2

Various forms of microRNA disorders with malignant neoplasms

Type of disorder	Diseases	Source
Polymorphisms and mutations in the genes of proteins associated with biogenesis and the action of microRNAs	Dicer1 syndrome: the presence of mutations in the *Dicer1* gene leads to a change in the structure and functions of the protein and, as a result, to a disruption of the synthesis of various microRNAs. The result is malignant neoplasms: pleuropulmonary blastoma, Sertoli-Leydig cell tumor, neuroblastoma, rhabdomyosarcoma, etc.	[24–26]
Change or loss of the gene encoding microRNA during chromosomal rearrangements	Deletion of a 13q14 chromosome fragment in chronic B-cell lymphoblastic leukemia leads to the loss of microRNA miR-15 and miR-16 coding regions, which are negative regulators of BCL2 protein synthesis. The result is a decrease in the ability of cells to apoptosis	[27, 28]
Mutations and polymorphisms in a gene encoding microRNA	Single nucleotide substitution G> C (rs2910164) in the gene encoding miR-146a leads to a change in the production of this microRNA and is associated with an increased risk of developing renal cell carcinoma, glioma, early manifestation of familial breast or ovarian cancer	[29–31]
Mutations and polymorphisms in mRNA at sites of interaction with microRNAs	*HIF1A* gene polymorphism in the region near the binding of the "seed" region of miR-199a leads to increased protein synthesis, which is associated with poor prognosis for the ductal adenocarcinoma of the pancreas. Single nucleotide substitution in the 3' non-coding region of the *SET8* gene disrupts the miR-502 binding site, which increases the risk of early development of breast cancer	[32, 33]
Reduced microRNA	MiR-143 and miR-145 have a reduced level in the cells of cancer of the gastrointestinal tract and exhibit oncosuppressive properties upon endogenous administration	[34]
Increased amount of microRNAs	MicroRNA miR-21 has a relatively high level in six types of solid tumors (breast, lung, prostate, stomach, pancreas and rectum cancer), as well as glioblastoma	[35, 36]

To correct molecular processes in the cell using microRNA, it is possible to regulate both the number of regulatory molecules and the quality of regulation by controlling the ability of the RISC complex to bind specifically to the target site.

WAYS OF TARGETED REGULATION OF GENE EXPRESSION

Imitators (mimics) of microRNA. To artificially increase the level of a given microRNA in a cell, synthetic copies of this molecule are introduced into it – imitators, or mimics (replacement therapy). These can be mature molecules,direct copies of microRNAs that have the same properties of binding molecules as their prototype, or its predecessors, and even genes encoding it. The introduction of mature microRNA is a more convenient and quick method of exposure. Due to its small size, the molecule easily penetrates through the cell membrane as part of RNA-lipid complexes (lipid transfection, or lipofection). Using mature mimics, for example, the possibility of suppressing the proliferation of gastric and colorectal cancer cells using miR-375 *in vitro* has been demonstrated [37, 38]. However, in the case of using microRNA mimicry to enhance the effect of repression of protein synthesis, it is important to remember that for active operation of microRNA it must form the RISC ribonucleoprotein complex. It was shown that the introduction of individual microRNA molecules into the cell, as a rule, meets a limited protein pool, and to form the ribonucleoprotein complex, they are forced to compete with the endogenous microRNA of the cell for the protein

components of the RISC complex and can deplete their reserves. As a result, this can lead not only to an insignificant effect of suppressing translation of target mRNA, but also to an increase in the translation of other proteins due to impaired regulatory functions of the cell's own microRNAs [39]. At the same time, even small amounts of exogenous microRNA are introduced into the cell together with plasmids expressing AGO2, leading to a significant effect of its activity [40].

The introduction of microRNA precursors allows at least partially solving the problem with the formation of the RISC complex: the synthesis and maturation of a molecule in a cell is more likely to lead to its natural encounter with AGO2. It was even shown that the introduction of a microRNA duplex consisting of the leading and passenger strands is more likely to lead to the formation of an active microRNA molecule than the introduction of a mature single-stranded molecule [41]. The possibilities of introducing pre-miRNA or pri-miRNA into the cell are also described.

In general, the use of imitators opens up wide possibilities for substitution therapy for the fight against malignant neoplasms. Thus, increased miR-4779 microRNA activity due to the use of mimics led to the suppression of tumor growth, blocking of the cell cycle and stimulation of cancer cell apoptosis in colorectal cancer [42]. In another study, targeted management of the miR-29b simulator allowed the suppression of the development of acute myeloid leukemia [43].

MicroRNA inhibitors. The antisense inhibitor is an RNA oligonucleotide complementary to the target microRNA. Upon their binding, a sufficiently strong duplex is formed, which prevents the microRNA from landing on mRNA and thereby removes the ban on translation. One of the natural regulators of microRNA activity in the body is competitive endogenous RNA (ceRNA), including long non-coding RNA molecules (long ncRNA, lncRNA) [44], ring RNA [45], pseudogenes. Due to the presence of microRNA landing sites in their nucleotide sequence, these molecules are capable of acting as a so-called molecular sponge, which assumes an attack of microRNAs and thereby removes the block from real messenger RNA [46]. With the help of such a "sponge", almost complete removal of active miR-221/222 from the cell was achieved, which led to increased apoptosis of squamous cell carcinoma of the oral cavity [47].

Exogenous inhibitors also show effective inhibition of the activity of target microRNAs. But at the same time, it becomes necessary to deliver the molecule into the cell through the membrane and maintain its stability in the cytoplasm, since an exogenous RNA molecule that does not have protection can be rapidly destroyed by RNases. These problems are partially solved by one of the latest generations of microRNA inhibitors, created on the basis of "closed" ("locked") nucleic acids, or LNA-inhibitors (locked nucleic acid). They are oligomers 12–14 nucleotides long, having a methylene bridge between the 2'-O and 4'-C ribose rings in the nucleotide part. As a result, such an LNA molecule is more resistant to the action of endonucleases, forms a stronger duplex with the RNA or target DNA, and also more easily penetrates the cell membrane (due to its small size) [48], and does not show significant toxicity to the body in experiments on mice [49]. All this makes LNA inhibitors promising for the development of drugs based on the principle of inhibition of microRNA activity. It was with their help that it was possible to degrade miR-21 and thereby achieve increased apoptosis and suppressed proliferation of hepatocellular carcinoma cells [50].

SYNTHETIC REGULATORS

Sometimes, when researching or developing a method for treating a disease, it becomes necessary to affect certain regions of messenger RNA that do not have a natural site for regulatory molecule landing. For example, if an oncogenic gene mutation occurs that leads to a change in the structure of the protein, it would be convenient to block the synthesis of the mutant form of the protein, while maintaining the normal protein. This requires regulation with recognition of mutations in messenger RNA. In this case, artificial microRNA molecules come to the rescue. In 2017, M. Acunzo and colleagues vividly demonstrated the feasibility and benefits of creating artificial microRNAs for targeted inhibition of translation of the *KRAS* gene containing the G12S point mutation to increase the sensitivity of lung cancer cells (cell line A549) to treatment with gefitinib. It was demonstrated that even a single nucleotide substitution changes the regulatory effect [51]. However, since microRNAs can affect several mRNAs at the same time, this can lead to undesirable side effects and make it difficult to use for one purpose. A more specific option is the use of siRNA molecules. But the complete complementarity of siRNA makes it less sensitive to polymorphisms and mutations. In addition, siRNA activity *in vivo* may be limited by the occurrence of an immune response. For example, when studying the effect of siRNAs on the growth of cells carrying the V617F mutation of the *JAK2* gene,

a significant decrease in the effect of inhibition by cytokines was shown [52].

Another type of synthetic microRNA inhibitors is peptide nucleic acids (PNA). These are chemical compounds that are linear polymer molecules similar to DNA, but with N- (2-aminoethyl) glycine instead of sugar. The advantage of PNAs is their resistance to degradation by nucleases and proteases, as well as the independence of hybridization with DNA or RNA from the concentration of salts in the medium [53]. Another important property of PNA is sensitivity to non-complementary bases. Even one mismatching nucleotide is capable of changing the melting temperature of the PNA-DNA duplex to 15 ° C, which makes them promising molecules for the selective inhibition of targets containing single nucleotide mutations [54]. In addition, it was shown that PNAs can be modified in such a way that their penetration into the cell is ensured without the participation of an additional transfection reagent [55]. To date, this type of molecule has been successfully used to inhibit microRNAs *in vivo* [56].

Proper design and optimization of the conditions for the use of artificial molecules can achieve the desired effect of reducing or disabling protein synthesis in the cell, reduce nonspecific effects, and avoid toxicity or immunogenicity of the introduced substance. This makes these molecules promising therapeutic agents in medicine in general and oncology in particular.

DELIVERY OF SMALL NON-CODING RNA TO CELLS

The delivery of microRNAs and siRNAs to cells *in vitro* and *in vivo* is still a challenge and is a field of active development. First, free RNA molecules without any protection or modification are easily destroyed by nucleases, and in the animal body they are also excreted by the kidneys and liver [57] or are retained in non-target organs. Secondly, other tissues and structures (blood vessel walls, connective tissues, tumor microenvironment), which have different permeability, as a rule, arise on the way to the tumor cell in the body, which can significantly complicate the delivery. Thirdly, foreign RNA molecules can initiate an immune response and cause unwanted side effects [58]. Fourthly, even in an *in vitro* culture, where the achievement of the goal is possible by introducing molecules into the nutrient medium, the membrane barrier is required to penetrate the cell. Finally, a molecule entering the cell may be denied access to the target as a result of inclusion in the endosome [57, 59] or non-specific interaction with non-complementary or partially complementary RNA molecules.

Various methods are currently being developed for delivering RNA molecules to tumor cells: chemical, physical, biological. Chemical methods include the use of polymer complexes (polyethyleneimine), lipid nanoparticles (liposomes), dendrimers, inorganic compounds (iron oxide, gold, silicate nanoparticles) [60, 61], etc. It is believed that chemical delivery methods initially have low transfection efficiency in comparison with biological. This is partly due to the short lifespan of molecules *in vivo*, their ability to bind to serum proteins in the blood. However, modifications of chemical compounds make it possible to overcome these difficulties by creating stable constructs that provide more specific delivery of microRNAs to cells. Examples are nanostructured lipid carriers that have a positive charge on the surface of the bilipid layer, or molecules recognized by cell receptors that have been successfully used to deliver microRNAs to cell cultures and *in vivo* in mouse models [62, 63]. Chemical delivery methods can also include modifications of the RNAs themselves, which increase the stability of molecules, reduce their toxicity and (or) facilitate penetration into the cell (LNA, PNA). Chemical methods are effectively used in *in vivo* studies.

Physical delivery methods are suitable for *in vitro* cultures and include magnetofection, biobalistics, electroporation, sonoporation, laser irradiation, etc. The most common method is electroporation, which allows an electrical impulse to break a gap in the membrane, thereby ensuring direct penetration of nucleic acids into the cell cytoplasm, or placing the desired molecule in the exosome for subsequent delivery to the cells. The use of exosomes increases the stability of molecules and facilitates the *in vivo* delivery method [64], while direct delivery to cells in *vitro* by electroporation is highly regarded for its simplicity and efficiency [65].

Chemical and physical delivery methods provide, as a rule, the transient nature of gene expression, having a relatively short life span of the introduced molecules. For a longer effect, biological methods are used, namely delivery as part of viral vectors (transduction). The DNA of adenoviruses, retroviruses, lentiviruses can be used as a vector.

Adenoviral vectors are double-stranded DNA molecules, are characterized by relative ease of use and are successfully used for fast and short-term introduction of molecules, since they are not able to introduce the desired gene into the genomic DNA of a eukaryotic cell and ensure constant synthesis of a given RNA. However, another important advantage

is their ability to introduce foreign DNA fragments up to 38 kb into the cell. In contrast, retroviral RNA vectors contain no more than 8 kb of a foreign nucleotide sequence. However, it is introduced into the genome of the host cell at the stage of mitotic division. Lentiviral vectors are similar to retroviral ones, but differ in their ability to introduce a foreign sequence into the genome of both dividing and non-dividing cells located in the postmitotic period or at the stage of terminal differentiation. Retroviral and lentiviral vectors are used for stable transfection of dividing cells *in vitro* and *in vivo*, demonstrating the high efficiency of introducing both mimics and microRNA inhibitors [66]. The disadvantage of viral vectors is the high immunogenicity and potential toxicity of molecules, as well as the instability of the viral genome and the likelihood of the virus reversing to the "wild" type with the loss of the insert of interest [67]. Recent developments are aimed at modifying designs to reduce or eliminate the negative effects of these vectors [68], which opens up wide possibilities for their use in science and clinical practice for the treatment of diseases.

CONCLUSION

The use of regulatory microRNA and siRNA molecules to control gene expression in a cell seems to be a powerful technology for studying normal cellular processes and pathology, as well as for treating diseases, in particular, malignant neoplasms. Patents have already been registered and a number of microRNA or siRNA-based drugs are being registered for the treatment of diseases, including chronic lymphocytic leukemia (regulation of the *BCL-2* gene), liver cancer (regulation of *VEGF* and *KSP* expression), and other solid tumors, including later stages of progression [69]. Therapies due to the directed regulation of gene expression using microRNAs and siRNAs predict a great future, calling these drugs new-generation drugs. However, much of the mechanisms of such regulation remains incompletely studied, and the world of regulatory molecules still requires a deep and multifaceted study.

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