Potential in anti-cancer therapy using RNA - overview of therapies

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Abstract

The main targets for the production of mRNA-based tumor vaccines are tumor-associated antigens and tumor-specific neoepitopes exposed on the surface of tumor cells. Their mission is to stimulate the cellular response in the body of a sick patient. In producing of mRNA vaccines it is important to use an appropriate vector and method of delivery of the vaccine to the body. Recent years a lot of interest has been seen in using dendritic cells as a vector. Ugur Sahin and his associates were the first to create procedures for creating personalized mRNA mutanomic vaccines. Apart from mutanomes, miRNA – the type of interference RNAs regulating gene expression - played a role in the development of mRNA vaccines. It has been shown that miRNA dysregulation is observed in some cancers and plays a role in invasion, angiogenesis, apoptosis, avoidance of immune destruction or sensitivity to growth factors. Many of the functions of LncRNA affect gene expression by regulating chromatin function, affecting the stability and translation of cytoplasmic mRNA, and signaling pathways, may...
ultimately lead to cancer formation – and also can be used in anti-cancer therapy and as sensitive biomarkers. These discoveries led to the rapid development of RNA-based anti-cancer therapies and the emergence of the first therapeutics. In this review, we present the use of RNA in anti-cancer therapies and the review of therapies.

**Key words:** RNA; mRNA; LncRNA, miRNA; anti-cancer therapy; therapeutics

**Introduction**

Preferential tumor-associated antigens and tumor-specific neoepitopes (exposed on the surface of tumor cells; recognized by T lymphocytes on HLA molecules) are the main targets for the production of mRNA-based tumor vaccines. Most cancer vaccines are produced for cancer therapy, not prophylaxis. Their task is to stimulate the cellular response in the body of a sick patient. In optimizing the operation of such vaccines due to the different location and characteristics of tumors and the location of specific lymphocytes in the body, it is important to use an appropriate vector and method of delivery of the vaccine to the body [1,2,3].

Currently, the use of a vector in the form of dendritic cells ex vivo or in vivo is of great interest. Dendritic cells, as a component of the APC system, process antigens in both T and B lymphocytes, stimulating in this case the production of antibodies, hence it has become an important method of delivering mRNA vaccines in the transfection process [2].

**Overview of use of RNA in anti-cancer therapy**

Ugur Sahin (who also manages the production of the Sars-CoV-2 vaccine at BioNTech) and his associates were the first to create procedures for creating personalized mRNA mutanomic vaccines. Mutanoms encode tumor-specific neoantigens (m-peptides) that arise from mutations and appear first during tumor development. This method was successfully used for the first time in patients struggling with stage III and IV melanoma after the identification of mutations using intravenous vaccines. on dendritic cells) encoding TriMix as well as tumor antigens and ipilimumab, inducing a strong tumor-associated CD8 + T cell response, which resulted in highly beneficial treatment effects (the induced immune response is antigen-specific) - strong CD8+ T cell response in the vast majority of melanoma patients in stages III and IV [1,3,4].

The RNA-LPX vaccine by BioNTech, from the FixVac BNT111 cancer vaccine program, targets four non-mutated, tumor-associated antigens common in melanoma (NY-ESO-1, MAGE-A3, tyrosinase, TPTE). This therapy is currently in the first phase of clinical
trials, where the safety and tolerability of the preparations in patients with melanoma in stages III (b, c) and IV are investigated. The studies are carried out in seven cohorts, 89 people in total, three of which increase the dose (doses ranging from 7.2 μg to 400 μg) receive FixVac alone, and three more FixVac in combination with antibodies against the PD1 (programmed death receptor-1) [4].

RNA-based cancer therapies were also investigated by Reinhard et al., who patented the CAR-T chimeric antigen receptor showing high efficacy in patients with B-cell malignancies. This receptor reacts with the integral membrane protein CLDN6 (Claudin-6). The use of liposomal RNA vaccines encoding CLDN6 in mice studies significantly increased the stimulation of CAR-T cells with a sub-therapeutic dose and regression of large solid tumors in these animals. However, the challenge in the therapy of solid tumors with the use of this method is the reduction of tumor specificity and the instability of CAR-T cells [5].

Dysregulation of miRNAs - small, non-coding RNA molecules that regulate the expression of various target genes, play an important role in the progression of cancer. Research on the use of miRNAs began with the identification in 2002 by Calin and colleagues of two miRNAs (miR-15a and miR-16-1) in patients with chronic lymphocytic leukemia (CLL). These findings showed how miRNAs influence tumor suppression by inducing the process of apoptosis and inhibiting the cell cycle by regressing relevant genes - in this case related to cyclin [6]. These molecules play a key role in regulating genes involved in biological processes - such as the cell cycle, cell differentiation, proliferation, apoptosis, stress tolerance, energy metabolism and immune response, as well as pathological processes such as the formation of certain types of cancer by influencing molecular pathways. Involved in invasion, angiogenesis, apoptosis, avoidance of immune destruction or sensitivity to growth factors - research shows that all key neoplastic pathways are associated with miRNA alterations. miRNA is also involved in the process of malignant transformation of a tumor - it occurs through the participation of miRNA in the repression of tumor suppressor genes or by increasing the expression of oncogenes. The miRNA analysis also showed the expression of different patterns depending on the type of tumor, which initiated the discovery of biomarkers and also has the potential to discover therapeutic targets in cancer therapy. miRNA is divided into two groups - oncogenic miRNA (OncomiR) and tumor suppressor miRNA (TS-miRNA, TS-miR) - most tumors express some OncomiR (tumor suppression by inhibiting the translation of tumor-inhibiting mRNAs) and decreased expression of TS-miRNA (inhibition translation of oncoproteins) - therefore, in miRNA therapy, an antisense miRNA (anti-miR) or a miRNA used
to repress the oncomiR or restore TS-miR expression was created. Numerous studies show the involvement of miRNAs in drug resistance of cancer cells through the influence of miRNAs on genes involved in cell proliferation, their cycle and apoptosis [7].

RNA polymerase II (RNA polII) is involved in miRNA biosynthesis, which in the nucleus transforms into the primary miRNA (pri-miRNA) in the process of transcription, which is then processed by the Drosha / DGCR8 complex to release the intermediate miRNA precursor (pre-miRNA). This, in turn, binds to the Exp5 / Ran-GTP complex, which allows it to be delivered to the cytoplasm, where it is then processed by the Dicer / TRBP / PACT complex into double-stranded RNA. Double-stranded RNA is split into two single strands by the action of helicase - under physiological conditions, the RNA strand with less stability at the 5' end will be integrated with the RNA-induced silencing complex (RISC) and will become a mature miRNA, and the more stable strand at the 5' end will undergo degradation, while miRISC - the miRNA-induced silencing complex - will bind to the 3' untranslated regions (UTRs) of the target mRNA, thus inhibiting translation. However, this pathway may have some deviations - the processing of pri-miRNA into pre-miRNA may be independent of the Drosha / DGCR8 complex; a random strand may be integrated into the RISC complex or bind to RISC independent mRNA, and some miRISCs may bind to the 5'-UTR of the mRNA and increase its translation [8].

A key problem in miRNA delivery was the poor cellular uptake between the miRNA and the cell membrane (charge repulsion), hence the need to develop appropriate vectors. Viral vectors are most often used and synthetic systems (here the most common are lipid-based nanoparticles). The synthetic delivery system is easy to manufacture and less immunogenic than viral vector delivery, however synthetic transmission is non-specific, less efficient, and may be toxic. This problem was solved by coupling the synthetic vectors with PEG, which significantly increased the stability of these vectors as well as increased specificity. Additionally, they can be combined with peptides and antibodies that recognize specific antigens on the cell surface. The polyplexes formed by polyethyleneimine (PEI) together with miRNA proved to be very effective, which made it possible to obtain a positive charge of the molecule and efficiently deliver it through the negatively charged cell membrane. An innovative method of miRNA transmission turned out to be the enclosure of molecules in dendrimers (DEN). This method was highly effective in mice bearing MYC-induced tumors, where the complex mimicked let-7g, reducing the tumor size of the mice and increasing their survival at the same time. In the last decade, there has been increased interest in using
extracellular vesicles (EVs) as potential miRNA delivery vehicles represented by natural vesicles produced by all cells of varying size and biogenesis. These molecules could be used as tissue-specific natural transmitters in the treatment of cancer as well as brain tumors, as they are able to cross the blood-brain barrier [9].

Currently, several miRNA-based drugs in cancer therapy are being tested in clinical and preclinical trials. One of them is MiR-10b with promising results in research into the treatment of glioblastoma multiforme. MiR-10b is involved in the regulation of cell migration, invasion and metastasis and has proven its role in numerous studies in the case of metastatic tumors - reducing metastatic tumors in mice with cancer. Similarly, in metastatic cancers, MiR-221 caused a reduction in the size and number of tumors in this case in the liver of the transgenic mouse. A study by Tyler E. Miller et al. showed that inhibition of miRNA-221 and miRNA-222 of the human breast adenocarcinoma cell line (MCF-7) with reduced p27 and ERα expression may increase tamoxifen sensitivity by increasing the tissue inhibitor of metalloproteinases-3 (TIMP3), which would improve the treatment of drug-resistant breast adenocarcinoma. Nucleolin, which is part of the Drosha / DGCR8 microprocessor complex, may influence the regulation of the expression of several miRNAs (miR-21, miR-221/222, miR-103). Expression of these miRNAs is responsible for the malignancy and drug resistance of breast cancer, additionally Falkenberg et al. reported that expression of miRNA-221 and miRNA-222 is associated with the degree of tumor aggressiveness and its distant metastasis, and therefore may be an important marker in the prognosis of breast cancer. Nucleolin and its effect on specific miRNAs can be used in the treatment of drug-resistant breast tumors by increasing their sensitivity to fulvestrant - nucleolin-directed treatment would reduce cell growth and increase apoptosis of neoplastic cells [10,11,12].

It has been shown that miR-221/222 expression also correlates with tumor size, liver cirrhosis, tumor stage and influences the prognosis of hepatocellular carcinoma, hence the use of miRNA silencing technology could be used in HCC treatment and patient prognosis. Similarly, overexpression of miR-221/222 causes pancreatic cancer progression (tumor tissues have increased expression of both miRNAs) - in this case by expressing MMP-2 and MMP-9 by direct targeting the tissue inhibitor MMP-2, which facilitates the invasion of pancreatic cancer. This discovery could allow the synergistic use of sunitinib, used as a chemotherapeutic in cancer therapy, together with anti-miRNA oligonucleotides directed against miR-21, miR-221/222 and miR-10, which would allow for high therapeutic effects of pancreatic ductal adenocarcinoma, and high expression of miR-221 and miR-222 in neoplastic cells would allow
the use of both miRNAs as biomarkers and predictors. Inhibition of miRNA-221 expression could also be achieved through the use of metformin - according to Tanaka et al. metformin suppresses the G1 phase of the cell cycle and apoptosis by upregulating p27, the death receptor 5 (DR5), and Bim in pancreatic cancer cells. Overexpression of miR-221 and miR-222 also affects the progression of prostate cancer - overexpression of both miRNAs causes a decrease in the level of the p27 cell cycle inhibitor and ARHI responsible for the cell cycle and apoptosis. Mercatelli et al. thanks to the carrying out in vivo tests, they confirmed the effectiveness of miR-221/222 inhibition in inhibiting tumor growth by upregulating p27. Overexpression of miRNA-221 and miRNA-222 and the effectiveness of inhibition in anti-cancer therapy have also been noticed in gastric cancer, colorectal cancer, multiple myeloma, glioma and MML [10].

Loss of miR-16 has been observed to be associated with a variety of cancers, including NSCLC, prostate cancer, and pleural mesothelioma. In this case, Reid et al. studied miR-16 and showed significant inhibition of pleural mesothelioma tumor growth in diseased mice by targeting Bcl-2 and CCND1 against the normal pleura. Researchers used minicells targeting EGFR-specific antibodies for delivery in mice models of pleural mesothelioma. MesomiR-1, a miR-16 mimic, delivered by targeted bacterial minicells from ENgeneIC, has been tested in a Phase 1 clinical trial (which is completed) in mesothelioma patients [9,13]. miR-15a / miR-16 influence the regulation of genes, among which we can distinguish BCL2, MCL1, CCND1 and WNT3A - both miRNAs are down-regulated in CLL, pituitary adenoma and in prostate cancer. The rate of loss of miR-15a / miR-16 heterozygosity was highest in metastatic prostate cancers, which shows a correlation between deletions and the formation of distant metastases. The research by WeiJin et al. showed that the sequence on the miR15a / 16 genes can bind to the 3'-UTR of several components in the TGF-β signaling pathways, which in turn are responsible for tumor progression and metastasis [14].

The first miRNA-based cancer therapy in the first phase of clinical trials conducted in humans with advanced solid tumors was the 2016 MRX34 therapy by MiRNA Therapeutics (now Synlogic) mimicking miR-34a encapsulated in a liposomal nanoparticle, inhibiting tumor growth by downregulating the p53 gene. The therapy has shown results as a one-component therapy, including confirmed partial responses in patients with renal cell carcinoma, acral melanoma and hepatocellular carcinoma. However, clinical trials of MRX34 were discontinued due to the reporting of numerous serious adverse reactions, including disease progression in some cases and even deaths [9,15].
Miragen is currently conducting a Phase 2 trial on the Cobomarsen (MRG-106) oligonucleotide for cutaneous T-cell lymphoma (CTCL) patients to inhibit miR-155. miR-155 is involved in the regulation of the B and PI3K signaling pathway in CTCL and leads to the uncontrolled expansion of clonal cells and tumor progression. The study demonstrated good patient tolerance and significantly improved the quality of life of people with CTCL. The best results were obtained with the injection of 300 mg of Cobomarsen and the average waiting time for results was 276 days - compared to methotrexate, which responds after 20 months, interferon-alpha - after 24 months and bexarotene - 12 months [16,17].

In early 2019, Regulus announced clinical trials of the miRNA drug (RGLS5579) targeting miR-10b in patients with glioblastoma multiforme, but it is currently in preclinical studies [18].

Research carried out by scientists over the past decade has shown that long non-coding RNAs (LncRNAs) play an important role in gene regulation, which, unlike the aforementioned siRNAs, miRNAs and other short RNAs, are not translated into proteins. Many of the functions of LncRNA affect gene expression by regulating chromatin function (and its remodeling), affecting the stability and translation of cytoplasmic mRNA (including target mRNA degradation and inhibition of translation), and signaling pathways, which may ultimately lead to cancer formation [19]. Among the head and neck cancers (HNC), one of the most common cancers is cancer of the larynx (about 1/3 of head and neck cancers, the majority of which is squamous cell carcinoma of the larynx – LSCC), in the treatment of which a satisfactory strategy has not yet been achieved despite advances in general treatment [20]. For this reason, it is important to identify specific molecular signatures that, as biomarkers, could be a predictive factor in treatment and allow the determination of therapeutic targets in anti-cancer therapy. LncRNA, which is also considered as factor responsible for cancer progression, is considered an innovative molecular biomarker - dysregulation of long non-coding RNA may be associated with tumor growth, angiogenesis and metastasis. Exosomal LncRNA can be detected in body fluids (such as urine, blood, saliva) where it is very stable, so that LncRNA can be used in the future as a non-invasive biomarker to be detected using molecular biology techniques (as PCR or sequencing) as an alternative to invasive biopsies. According to reports, LncRNA interacting with NF-κB (NKILA) serves as a tumor suppressor in some cancers, including laryngeal cancer, binding to the NF-κB: IκB complex and causing inhibition of NF-κB activation - in the case of laryngeal cancer, low levels of NKILA were observed correlating with a correspondingly shorter survival period. NKILA, by modifying its activity, can also be used to enhance the
cytotoxicity of X-rays and counteract the radio-resistance of laryngeal cancer [21]. It has been shown that cytoplasmic LncRNAs can affect miRNA stability by stimulating or attenuating activity, acting like a miRNA sponge - for example, in liver cancer (HULC), downregulation of miRNA activity, including miR-372, has been observed with elevated LncRNA levels [22,23]. Some of the LncRNAs have already been recognized as sensitive biomarkers in various types of cancer, and recent studies have found that individual LncRNAs such as H19, MALAT1 and HOTAIR are overexpressed in various tumor cell lines - HOTAIR in particular is overexpressed in GIST tumors, and MALAT1 is a metastatic marker and an unfavorable prognosis for early-stage non-small cell lung cancer. Inhibition of target LncRNAs in cells may reduce tumor progression and may become the future of cancer therapy and development of new therapeutic strategies, as well as the use of LncRNAs as biomarkers [24,25].

**Conclusion**

RNA is a unique macromolecule with huge potential in medicine, which has not yet been used and fully discovered. Thanks to the numerous studies of scientists on ribonucleic acid, newer and newer methods of treating cancers are available. The above review demonstrated the great potential of the RNA as well as the successes in using the first RNA-based therapeutics in anti-cancer therapies, of which there will certainly be even more in the future.

**References**


