Research Progress of Microrna in The Treatment of Tumor Drugs

Yuxuan Hu [1, *], Ziling Shao [2] and Yuxiang Xu [3]

[1] School of Public Health, Southern Medical University, Guangzhou, China

[2] School of life science,Anhui Agriculture University,Hefei,China

[3] Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China

* Corresponding Author Email: huyuxuan@i.smu.edu.cn

Abstract. MicroRNA (miRNA) has great potential for treating various diseases and has advantages that other treatment methods do not have when treating many diseases. MiRNA therapy has been applied to treat various diseases including obesity and cancer, and has shown significant efficacy. Many scientists are committed to developing new miRNA drugs in order to achieve better efficacy of miRNA therapy and more comprehensive coverage of diseases. This article describes the mechanism of action of miRNA and introduces and summarizes several necessary procedures for the development and production of miRNA drugs, including designing, producing miRNA, and designing ways to transport miRNA to target cells and tissues. Firstly, a synthesized pre-mRNAs or adenovirus-based mMIRs vector is designed in vitro, then, deliver them into host through viral or non-viral mediated system, finally, the miRNA could complement to specific gene by base-pairing, regulating the function of protein via interfering the expression of target genes. We hope that this article can contribute to the future development of miRNA drugs.

Keywords: MiRNA threapy; Cancer; Synthesis of miRNA; Delivery of miRNA.

1. Introduction

MicroRNAs (miRNAs) are small noncoding RNAs that is a known regulator of basic biological processes in animals. It mainly regulates gene expression by interfering with transcription, translation, or epigenetic processes [1]. For the generation of miRNAs, it is currently believed that primary transcripts are synthesized by RNA polymerase II or RNA polymerase III to form primary miRNAs (pri-miRNAs) with typical hairpin structure. However, in order to become miRNAs with regulatory functions, the following steps are required: firstly, the double strands of pri-miRNAs are recognized by Di George critical region 8 (DGCR8) and bind to Drosha enzyme to form a microprocessing complex [2]. By the interaction between the basal UG motif and Drosha, as well as the alignment of DGCR8 and apical UGU motif, pri-miRNAs are correctly oriented for cleavage into smaller precursor miRNAs (pre-miRNAs) [3]; Then, pre-miRNA will be transported by Expotin 5 transporters and entering the cytoplasm from the nuclear pores [4]. In the cytoplasm, enzyme DICER and co-factors will process pre-miRNA to form a short double stranded miRNA molecule [5]; Finally, the Argonaute (AGO) protein can interact with enzyme DICER and binds to mature miRNA, causing the miRNA to be released and releasing one strand. The remaining strand (guide strand) will interact with AGO to form the RNA-induced silencing complex (RISC) [6].

MiRNA is related to the occurrence and development of many diseases. Some serious diseases can cause abnormal changes in miRNA levels, which often drive or accelerate the progression of these diseases. In a study targeting Sickle Cell Disease, scientists found that in K562 cells overexpressing miR-326, the content of KLF1 protein that can induce high-level expression of adult red blood cell genes is reduced and the expression level of HBG gene, which should have been gradually silenced in normal adult cells, increased. In addition, the expression of miR-326 is positively correlated with HbF levels in patients with β- thalassemia [7]. Therefore, the abnormally elevated level of miR-326 is closely related to Sickle Cell Disease. Previous studies on Alzheimer's disease (AD) have shown that the expression of miR-92a-3p, miR-181c-5p, miR-210-3p, miRNA-132 and miRNA-107 are

abnormal in the brains of AD patients. Further research suggests that overexpression of miRNA-22 may have neuroprotective effects on neurodevelopmental disorders and neurodegenerative diseases by inhibiting cell apoptosis. miR-132 promotes to dendritic growth of newly formed neurons in the hippocampus, and the absence of miR-132 and miR-212 can induce tubulin associated unit proteins aggregation and impair cognitive abilities [8]. Since 2008, MiRNA has been identified as a biomarker for cancer to detect diffuse large B-cell lymphoma in patients' serum [9,10]. MiR-15 and miR-16 are the two miRNAs that were first confirmed to be closely associated with cancer, and abnormal changes in their levels are a significant feature of B-cell leukemia [11]. Since then, abnormal miRNA levels have played a role as biomarkers in an increasing number of human disease detections. The abnormal level of circulating miRNA in human cancer has been published elsewhere. Figure 1 [12] briefly summarizes the regulation of circulating miRNA in plasma, serum and blood samples of six kinds of high incidence rate cancers.

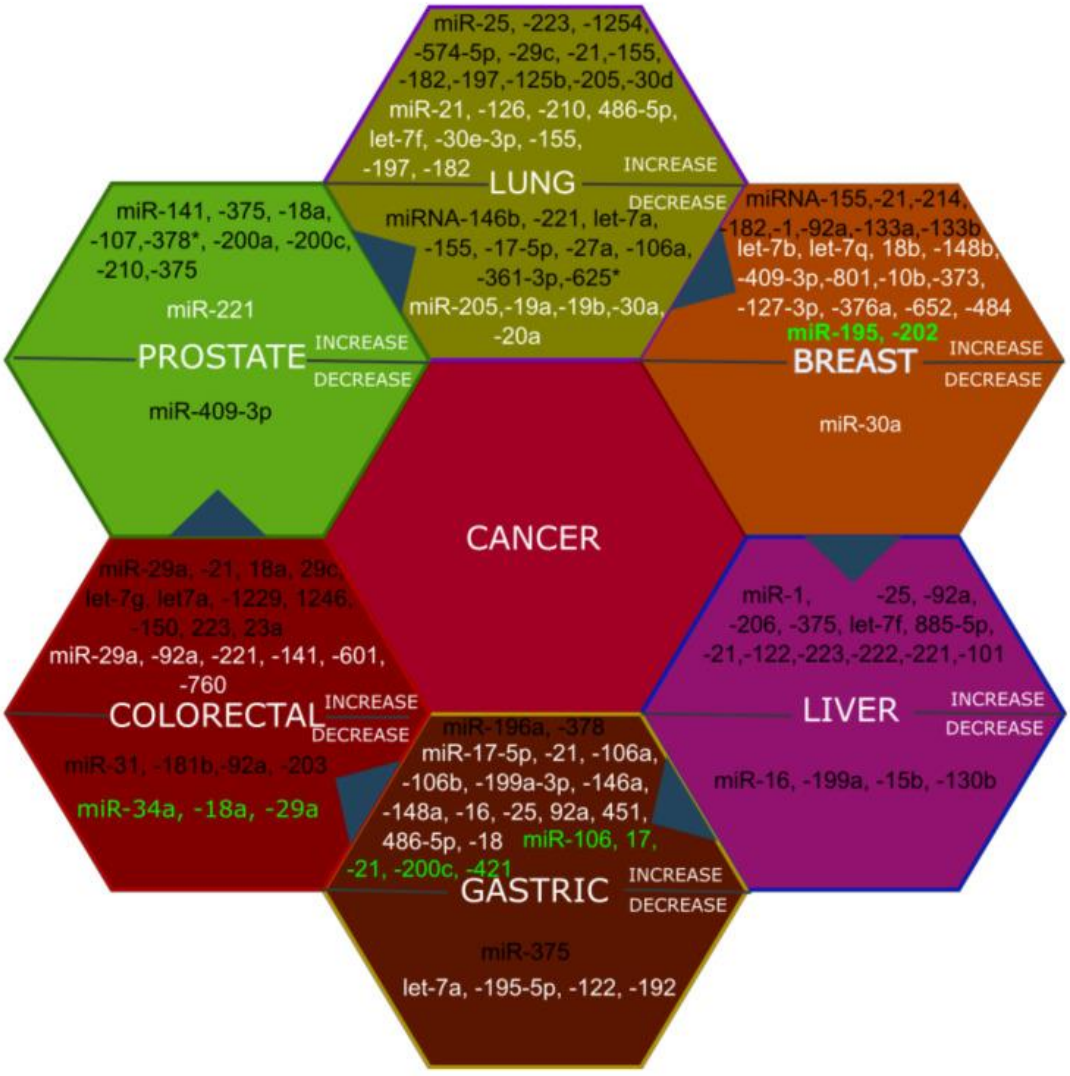

Figure 1. The regulation of circulating miRNA in plasma, serum and blood samples of six kinds of high incidence rate cancers [12].

For many human diseases, circulating miRNA has been considered a promising biomarker because they meet several criteria as preferred biomarkers, encompassing a high level of sensitivity, accessibility, and specificity [13]. MiRNA has been identified in numerous highly stable biological fluids. MiRNA can be easily extracted from blood or other fluid biopsy. MiRNA is also highly specific to tissue or cell types. The sensitivity of miRNA has been confirmed, and its level may vary depending on the progress of the disease or the treatment response. Given these benefits, miRNA

provides a non-invasive way for accurately diagnosing, predicting disease progression, guiding treatment and evaluating treatment responses. This essay aims to illustrate the application of miRNA in disease treatment, including its mechanism, production and delivery system.

2. Reasearch Progress on miRNA Drug Development and Application

2.1. The Mechanism of Action of miRNA as A Drug

2.1.1 MiRNAs in Tumor Cells

MiRNAs, which are endogenous non-coding RNA molecules, which are consisting of 20 to 22 nucleotides, act as primary regulators in post-transcriptional gene regulation. They play a significant role in initiation and advancement of different diseases [14]. In the context of cancer, miRNAs contribute to tumor development by transcriptionally activating oncogenes and dysregulating tumor suppressor genes. MiRNAs bind to the 3' untranslated region (UTR) of their target RNA, thereby modifying its expression. This process regulates diverse cellular activities including processes like cell growth, cell specialization, and programmed cell death [15]. It is significant to mention that miRNAs maintain a high degree of conservation across diverse species and exert influence over numerous functions through interactions with an array of factors, such as transcription factors, ribonucleoprotein (RNP) complexes, proteins, and elements involved in epigenetic modifications. [16].

Given the importance of microRNA in regulating gene expression and cell biology, It has been recognized as an indicator of cancer. In comparison to normal cells, tthe aberrantly expressed miRNA in cancer cells is related to various mechanisms, for example, chromosomal abnormalities, changes in transcription control, epigenetic changes, and miRNA biogenesis. Several studies that have utilized miRNA analysis and analyzing the sequencing data derived from tumor tissue samples demonstrate that miRNA can function as either a tumor inhibitor or carcinogenic miRNA, contingent upon whether or not they are downregulated or upregulated in different cancers [17].

As a tumor inhibitor, the reduction of miRNA in human malignant tumors will lead to an increase regarding the expression levels observed in the targeted mRNA, most of which are involved in regulation of cell growth, apoptosis, metabolism and invasion.

MicroRNA (miRNA) expression levels are subject to regulation by tumor suppressor genes.For instance, miRNA-34a exhibits dysregulation across a range of cancer types and is directly modulated by the tumor suppressor gene p53[18]. In models of non-small cell lung cancer, the upregulation of miRNA-34a has been shown to suppress the growth and progression of cancer cells. [19,20]. Additionally, miRNA-34a counteracts many carcinogenic processes by modulating genes involved in diverse cellular pathways [21]. Likewise, the miRNA-200 family is a widely studied group of miRNAs with numerous functions, such as increasing ZEB protein levels by downregulating E-cadherin, thereby inducing epithelial-mesenchymal transition (EMT) [22]. Studies also indicate that miRNA-200 indirectly impacts angiogenesis by downregulating CXCL1 and IL-8, which are key cytokines in the tumor microenvironment [23].

2.1.2 MiRNAs in Tumor Microenvironment

Cancer development is a multi-step process where normal cells are transformed into rapidly proliferating cells due to genetic alterations [24]. These fast-growing cells require a supportive environment to evolve into cancer, known as the tumor microenvironment (TME) [25]. As we gain more insights into the processes that contribute to the advancement of cancer, researchers are continually refining the selection of biomarkers used to identify different types of tumors.Recognition of diverse cell types within tumors has recently established TME as a significant cancer marker [26]. The characteristics of cancer highlight the crucial function of the microenvironment in promoting tumor development and spread, as well as affecting the efficacy of potential treatment strategies. [26]. Composed of various cell types—including tumor cells, cancer stem cells, stromal cells, and immune cells—and intricate interactions among them, TME forms a dense milieu [27]. Non-cellular elements

of the tumor's microenvironment encompass the extracellular matrix, comprised of collagen, fibrinolin, hyaluronic acid, and laminin. Interactions between tumor cells and non-malignant components within the tumor environment significantly influence tumor initiation and progression [28].

MiRNA expression patterns vary among tumor types, enabling differentiation from healthy tissue and other cancerous varieties. Upon tumor formation, tumor cells attract surrounding cells to participate in the development of the TME.

The emergence of a tumor prompts its cells to attract surrounding tissue cells. This supportive structure, consisting of cells, matrix, and blood vessels, forms the foundation for tumor growth and sustainability, commonly known as the Tumor Microenvironment (TME) [29].

In TME, miRNA (here called EV-miRNA) delivered from one cell to another through EV is now considered to be a factor related to cancer progression [30].

miRNA is a key messenger in the tumor microenvironment (TME), affecting various processes that lead to immunosuppression, tumor progression, metastasis and drug resistance. The strategy of regulating miRNA in TME is of great significance to overcome these challenges [17].

Reprogramming TAM as an anti-tumor phenotype has proved to be an effective strategy for cancer treatment.

2.2. Current Methods for Producing miRNA

At present, miRNA therapy is a popular research direction [31]. Meanwhile, due to the close association between miRNA and various diseases, it is also considered an important target for drug development. By delivering the designed miRNA into patients, the expression of disease-related target gene could be downregulated, thereby achieving therapeutic goals [32,33,34,35].

The effective manipulation of miRNA expression is helpful for studying the therapeutic applications of miRNA. Recent studies have shown that the use of antisense molecules such as anti-miRs, antagomiRs, miRNA sponge, etc. can downregulate or inhibit the function of miRNA [36, 37, 38, 39]. The achieving of upregulation of miRNAs could be used by chemically synthesized miRNA mimics or intronic miRNA expression vectors, which will express pri-miRNAs or pre-miRNAs [40,41,42]. Jiaming F et al. (June 20, 2019) developed a novel strategy of the expression of mature miRNAs(mMIRs). They use convergent U6 and H1 promoters to make the expression of miR-3p and miR-5p be increased in the opposite direction. The corresponding transcription signals (TTTTT or UUUUU) were separated and expressed in units U6 and H1. Nowadays, the mMIR expression system has been developed into adenovirus shuttle vectors and retroviral transfer vectors, making it easy to use for the production of recombinant adenovirus or retroviruses [43].

2.3. Ways to Deliver miRNA Drugs into The Body

As mentioned earlier, miRNA mainly acts in the cytoplasm of target cells to exert corresponding effects on intracellular translation and other processes. Therefore, to use miRNA for treating diseases, it is necessary to safely transport miRNA into the cytoplasm of target cells. MiRNA is a hydrophilic, negatively charged high molecular weight molecule with limited membrane diffusion potential and difficulty in being fully taken up by cells. The ubiquitous nucleases in the human environment can degrade miRNAs that are not under safe protection, and the human immune system can also hinder the transport of miRNAs [44]. The factors mentioned above impose quite strict requirements on the miRNA transport system. A qualified miRNA delivery system needs to be able to protect miRNA molecules from serum endonucleases, evade immune detection, not interact with unrelated proteins, not be affected or absorbed by non-target cells, and accurately locate and be absorbed by target cells. Here are several currently reliable modes of miRNA transport.

The First method is to transport the required oligonucleotides to specific cells and tissues through genetically modified viruses, and persistently increase gene expression levels to achieve the goal of transporting miRNA or anti miRNA. It is referred to as Virus-Based miRNA and Anti-miRNA Oligonucleotide Delivery Systems. In this process, it is necessary to eliminate the pathogenic

determinant cluster of the virus to ensure the reduction or non-toxicity of the vector, and to adapt the vector to transgenes. Various viral transport vectors have been developed over the past few decades to match specific transgenes, different therapeutic purposes, and target cell types. At present, the main delivery vehicles include retroviral, lentiviral, adenoviral and adeno associated virus, and bacteriophage-based virus like particle (VLP) vectors [45]. These different types of delivery vehicles have different characteristics, advantages, and limitations. As shown in Figure 2 [45].

Viral based delivery vectors	Advantages	Disadvantages
Retroviral vectors 	1. Stable transgene expression	1. High carcinogenic potential due to insertional mutagenesis 2. Cannot transduce non-dividing cells
Lentiviral vectors 	1. Stable transgene expression 2. Transduces dividing and non-dividing cells	1. Random genomic integration may cause insertional mutagenesis
Adeno-associated vectors 	1. Low immunogenic 2. High transduction efficiency over a wide variety of cells 3. Targeted tissue-specific gene expression based on serotype	1. Low packaging capacity 2. Production is tedious and expensive
Bacteriophage-based VLP vectors 	1. Low immunogenicity and cytotoxicity 2. Low carcinogenic potential	1. Low loading capacity 2. Further studies required

Figure 2. The advantages and disadvantages of various viral vectors [45].

The remaining miRNA delivery methods are referred to as Non-Viral-Based miRNA and Anti-miRNA Oligonucleotide Delivery Systems [45]. The second method of delivering miRNA is to utilize the negative charge of miRNA to form cationic complexes with lipid nanoparticles, which are then transported into the cell through endocytosis. It is also called Lipid-Based Delivery Systems. By loading miRNA into LNP or PEG-LNP nanoparticles, miRNA can be selectively delivered to target cells. Before being put into use, it is necessary to measure the particle size, zeta potential, miRNA complexity efficiency, and cytotoxicity of LNPs to ensure their specificity and safety [46]. This method is widely used in the treatment of obesity and cancer [46,47].

The third method is through Polymer Delivery Systems. There are currently multiple feasible methods. The first method is to use polymers with positively charged amino groups such as polyethyleneimine (PEIs) to bind with anionic miRNAs to form complexes, in order to protect miRNAs and ensure that they can be taken up and function by cells [45]. The second type is peptide nanoparticles, also known as cell penetrating peptides (CPP). CPP is often rich in arginine or lysine, therefore it carries a positive charge or exhibits amphiphilicity. CPP can form electrostatic interactions with miRNA, stabilizing and delivering them to cells [44]. The third type is polysaccharide-based delivery systems. Due to its unique physical, chemical, and biological properties including high biocompatibility, low cost, and good mucosal adhesion, chitosan is a highly prominent candidate in polysaccharide-based delivery systems [48].

The fourth method is Inorganic Compound Based Delivery Systems. In this method, the inorganic compounds involved in miRNA delivery mainly include nanoparticles of gold, iron oxide, and silica. The related therapy of embedding miRNA into inorganic compound nanoparticles has shown its potential in treating cancer and restoring heart function damaged by diabetes [45].

The last method is Extracellular Vesicle Based Delivery Systems. This method mainly includes two strategies. The first strategy is to induce cell lines that overexpress the target miRNA, in order to produce a large number of extracellular vesicles containing the target miRNA that bind to the target cells to achieve therapeutic effects. The second strategy is to first isolate exosomes and then enrich them with miRNA [45].

3. Conclusions

MiRNAs are pivotal in facilitating communication between cells within the tumor microenvironment and are recognized for their ability to influence the immune context of tumors.This article elucidates the mechanism of action of miRNA and provides a succinct overview of the essential steps involved in the development and production of miRNA-based therapeutics,including the design, production of miRNA and the method of transporting miRNA to target cells and tissues. In cancer research, miRNA can be manifested as a tumor inhibitor or carcinogenic miRNA, and its function depends on downgrade or upregulation in different cancers. The emergence of cancer is characterized by its intricate progression.Normal cells are transformed into fast-growing cells through a series of genetic changes, which eventually need a suitable environment to develop into cancer. MiRNA is closely related to a variety of diseases, so it is regarded as an important target for drug development. By upregulating or downregulating miRNA, a corresponding drug delivery system can be designed to transport specific miRNA to the patient, so as to regulate the expression of disease-related genes. This regulation can help inhibit disease-related target genes or reduce certain overexpressed miRNAs, so as to achieve therapeutic effects.Effective miRNA expression regulation provides the basis for the study of its therapeutic application. miRNA mainly acts on the cytoplasm of target cells, affecting intracellular translation and other processes. Therefore, the safe delivery of miRNA to the cytoplasm of target cells is the key to miRNA treatment of diseases. Because miRNA is a hydrophilic and negatively charged high molecular weight molecule, its membrane diffusion capacity is limited, and it is vulnerable to the degradation of nuclease in the body and the immune system, so strict requirements are put forward for the delivery system of miRNA. An effective miRNA delivery system needs to protect miRNA from degradation, avoid immune testing, and ensure that miRNA can be accurately absorbed by target cells. Numerous miRNAs have demonstrated potential in overcoming immunosuppression and chemotherapy resistance across a spectrum of tumor prototypes. Efficient transfer these miRNAs to the tumor microenvironment (TME) is paramount, necessitating a sophisticated delivery system. Recent developments in delivery mechanisms, especially the demonstrated effectiveness of lipid nanoparticles as seen in the COVID-19 vaccine, indicate that targeting miRNA delivery to tumors could represent a significant breakthrough in the field of clinical oncology.Nonetheless, target specificity of miRNAs is a crucial consideration due to their potential multi-target nature and the consequential off-target effects within the TME. Furthermore, a comprehensive evaluation of the kinetics of these miRNAs, akin to other anti-cancer adjunct therapies, is imperative.Nevertheless, RNA therapy in the clinical environment will surge, and miRNA may certainly be an important part of these treatments[17].

To propel the progression of miRNA-centered pharmaceuticals, a comprehensive exploration of the therapeutic capabilities of miRNAs is essential. This investigation should focus on their potential as a standalone and adjunctive approach in cancer treatment.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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