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Exploring Novel Therapeutic Breakthroughs for Cancers: Potential Roles of miRNAs

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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs, 19–25 nucleotides in length, that regulate gene expression. miRNAs are involved in various cellular, biological, and pathological activities, including the development and progression of malignant tumors. Numerous studies have highlighted the significant roles of miRNA deregulation in various cancer types, involving critical pathways such as gene deletions and amplifications, abnormal epigenetic changes, and dysfunctional miRNA regulatory systems. miRNAs can function both as oncogenes and tumor suppressor genes in different contexts. Several cancer hallmarks are associated with deregulated miRNAs, including resistance to cell death, metastasis, sustained proliferative signaling, promotion of angiogenesis, and the suppression of growth factors. Recent research has demonstrated that miRNAs contribute to drug resistance in tumor cells by targeting genes linked to drug resistance or by influencing genes that regulate the cell cycle, apoptosis, and proliferation. A single miRNA often has tissue-specific regulatory functions and can affect multiple genes. Various miRNA types have been identified as potential biomarkers for tumor diagnosis and critical targets for novel therapeutic approaches. This review aims to explore the role of miRNAs in cancer progression, metastasis, and potential therapeutic interventions.

Keywords: MicroRNAs; Cancer; Treatment

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1. Introduction

Cancer is one of the deadliest diseases globally, posing a serious threat to human health. It remains one of the leading causes of death worldwide. According to WHO data, nearly 14.1 million new cancer cases were reported globally in 2012, resulting in 8.2 million deaths. Despite advancements in treatment methods, cancer

incidence continues to rise, with new cases projected to reach 23.6 million by 2030 [1]. Multiple factors contribute to cancer development and progression, including genetic and epigenetic alterations. This review summarizes the association between miRNAs and cancer proliferation, mechanisms of miRNA deregulation, and possible therapeutic strategies [2].

MicroRNAs (miRNAs) are small non-coding RNAs, consisting of 19–25 nucleotides, that regulate crucial biological functions and control gene expression [3]. miRNAs modulate the expression of target genes, either upregulating or downregulating them depending on the regulation mechanism involved. miRNAs are expressed at their genomic loci in either an intragenic manner (when located within an exon or intron of a protein-coding gene) or an intergenic manner (with their promoter controlling their expression). Like protein-coding genes, miRNA genes are transcriptionally regulated by factors such as transcription factors, DNA methylation, mutations, copy number variations, RNA stability, and cleavages [4]. The unique regulatory mechanisms of miRNAs often involve miRNA biogenesis, which results in global alterations in miRNA profiles linked to human malignancies.

Intracellular miRNA modulation has emerged as a novel feature of tumor microenvironment growth promotion. Recent studies have identified miRNAs as key mediators in communication between tumor cells and cancer-associated fibroblasts (CAFs), transported through extracellular vesicles. These extracellular miRNAs, originating from CAFs, promote development and survival in various tumor types, particularly colorectal, breast, and pancreatic cancers [5].

2. MicroRNAs and Cancers

Research has demonstrated that a single miRNA can target up to approximately 200 distinct elements, such as transcription factors, receptors, and vectors, each with a unique function. Around 30% of human mRNA expression related to cell division, development, and apoptosis may be regulated by miRNAs. Moreover, studies have shown that the expression of certain miRNA types varies significantly between normal and malignant tissues, indicating a critical role for these miRNAs in tumor initiation, growth, invasion, and metastasis. Based on their differential regulation of mRNA in cancer cells, miRNAs are classified as tumor suppressor miRNAs and oncogenic miRNAs, also known as oncomiRs [6].

The earliest known tumor-suppressive miRNAs that negatively regulate bcl-2 are *miR-15a* and *miR-16*. The anti-apoptotic gene *Bcl-2* is elevated in leukemia, lymphoma, and other cancers. Consequently, the downregulation or deletion of these two miRNAs increases the expression of bcl-2 and promotes the development of lymphoma and leukemia [7]. One of the earliest miRNAs discovered, *Let-7*, has been shown to repress the production of the oncogene *Ras*. *Ras* mutations are present in 15%–30% of human malignancies, and when induced by elevated bcl-2 protein levels, they result in cell transformation. Another study revealed that although let-7 expression was only slightly reduced in some tumor patients, it decreased significantly in lung cancer patients. The observed increase in let-7 in human lung cancer cells may inhibit malignant cell growth, suggesting that let-7 may function as a tumor suppressor gene in lung tissue. It is also notable that, on average, cancer cells produce less miRNA than normal cells [8]. This suggests that a subset of miRNAs inhibits tumor growth, and their depletion may increase the likelihood of tumor development.

One study found that in both *in vivo* and *in vitro* experiments involving p53-mediated cell cycle stress or DNA damage, three miRNAs encoded by *miR-34* were identified in human cancers. Among these, *miR-34a* was found in a variety of tumor types. When these miRNAs are abnormally expressed, several cell cycle-regulating genes, including the anti-apoptotic protein Bcl-2, are downregulated. This can lead to cell cycle arrest or even cell death. Thus, *miR-34*

functions as a tumor suppressor gene and is essential for regulating cell division and apoptosis [9].

Oncogenic miRNAs have also been identified, characterized by their significant overexpression in tumor tissues and their role in inducing carcinogenesis. The first functional evidence for an oncomiR came with the identification of *miR-17-92*. The mature miRNAs and pri-miRNAs of *miR-17-92* were found to be significantly overexpressed in B-cell lymphoma and related cell lines. An *in vivo* analysis confirmed that the overexpression of *miR-17-92* promotes the development of myc-induced B-cell lymphoma and increases the frequency of lymphoma occurrence [10]. Additionally, it was discovered that the myc protein regulates the expression of *miR-17-92*. By directly binding to the *miR-17-92* locus, Myc stimulates its expression and suppresses the production of the transcription factor E2F1 protein, potentially inducing apoptosis. The co-expression of a single member of the *miR-17-92* family with c-Myc did not facilitate cancer growth, suggesting that the combined activity of all *miR-17* family members may be responsible for the cancer-promoting effects of the *miR-17* family. According to research, the *miR-17-92* gene cluster could represent a human oncogene [11].

Another oncomiR, *miR-21*, is significantly upregulated in several solid tumor types, including aggressive glioma, breast, prostate, and lung cancers, indicating its oncogenic role in cancer development. The transfection of the gastric cancer cell line HEK-293 with a *miR-21* inhibitor resulted in the suppression of cancer growth and the induction of cancer cell apoptosis [12].

3. The mechanisms of deregulated expression of miRNAs

Aberrant miRNA activity can lead to uncontrolled cell division or make tumor cells resistant to anti-tumor therapies. The mechanisms underlying the deregulation of miRNA expression in drug-resistant cells are crucial to understanding, as this knowledge can aid in developing strategies to counteract drug resistance. The primary causes of miRNA deregulation include amplification or deletion of miRNA genes, epigenetic regulation, degradation of transcription factors, and dysregulation of essential genes or proteins involved in miRNA biosynthesis and processing [13].

3.1. Gene amplification and deletion

Nearly 60% of the human miRNA gene pool is located in regions vulnerable to cancer-related events, such as translocation boundaries and areas of deletion or amplification. These regions make the genes more susceptible to amplification, relocation, or deletion. For example, chromosomal deletions in the 13q14 region are common. It has been shown that *miRNA-15a* and *miRNA-16-1* are located on 13q14 and are often reduced or deleted in more than 50% of chronic lymphocytic leukemia (CLL) cases [14]. In contrast, the *miRNA-17~92* family, found in the 13q31–q32 region, is frequently upregulated in various tumor types, which increases the number of mature miRNAs and promotes tumor growth. *MiRNA-21* has also been found to be overexpressed in many cancers, contributing to drug resistance in tumor cells. One study demonstrated that the amplification of the 17q23–25 chromosomal region, which results in reduced PTEN expression, was responsible for the overexpression of *miRNA-21* in ovarian cancer [15]. Another study showed that the amplification of 3q26.2 led to the overexpression of *miRNA-569* in some breast and ovarian cancers, which downregulated TP53INP1 and contributed to cell survival and proliferation [16].

3.2. Methylation and histone modifications of miRNA genes

Although histone modification and DNA methylation do not change the DNA sequence, they can alter gene activity. DNA methylation, which leads to gene silencing, is an alternative mechanism of gene suppression.

Post-translational modifications, such as acetylation, methylation, and phosphorylation, occur on the amino-terminal histone tails and are closely associated with either gene activation or repression [17]. Acetylation of histones by histone acetyltransferases is often linked to gene activation, while hypoacetylation by histone deacetylases is linked to gene suppression. Histone methylation is associated with both gene activation and silencing. Epigenetic mechanisms, such as DNA methylation and histone modifications, contribute to the dysregulation of miRNA expression in cancers. These processes work together to silence tumor-suppressive miRNAs [18]. For instance, *miRNA-34a*, a known tumor-suppressor miRNA, is frequently downregulated in various tumor types, and its decreased expression is associated with increased tumor sensitivity to chemotherapy. A study found that 79.1% of primary prostate cancer samples had reduced *miRNA-34a* expression due to promoter methylation on the CpG island. Methylation of the *miRNA-34a* promoter was also observed in melanoma, bladder, lung, breast, kidney, pancreatic, and colon cancer cell lines [19].

3.3. Transcription elements mutation

Transcription factors are proteins that bind to cis-acting regions of a gene's promoter in eukaryotes, activating or suppressing gene transcription directly or indirectly, often in coordination with other proteins. In the context of miRNA regulation, p53 is the most well-known transcription factor. Following DNA damage, p53 expression increases and acts as a tumor suppressor, regulating hundreds of genes. In 2007, several studies simultaneously reported that p53 directly targets the *miRNA-34* family, including miRNA-34a, and significantly upregulates these genes. *MiRNA-34a*, a tumor suppressor miRNA, enhances cancer cell sensitivity to chemotherapy. p53 initiates transcription of the miRNA-34 family by directly recognizing their regulators. Two p53-related transcription factors, p63 and p73, have distinct roles, with p63 promoting cell division and survival, while p73 acts as a mediator of chemosensitivity. Research has shown that the expression of *miRNA-193a-5p* is regulated by p63 and p73 [19]. Pro-apoptotic isoforms of p73 *in vivo* stimulate *miRNA-193a-5p*, while p63 suppresses its expression in both normal and cancerous cells. Chemotherapy induces this miRNA in a p63/p73-dependent manner, which decreases chemosensitivity by inhibiting p73 through a miRNA-mediated feedback loop. Blocking *miRNA-193a* interrupts this feedback, reducing tumor cell survival and increasing their sensitivity to chemotherapy in both in vitro and in vivo experiments. Another study found that ΔNp63α, an isoform of p63 and a member of the p53 family, regulates epithelial-mesenchymal transition (EMT) and stimulates the transcription of *miRNA-205* in human bladder cancer cells. Knockdown of ΔNp63α reduced the expression of the *miRNA-205* host gene (*miRNA-205HG*), its primary and mature variants, and RNA Pol II interaction with the miRNA-205HG promoter [20].

Salmena *et al.* introduced the concept of competing endogenous RNAs (ceRNAs) in 2011. According to this theory, miRNA response elements (MREs) are present in pseudogenes, long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). These sequences act as "miRNA sponges," competing for limited pools of miRNAs, thereby reducing miRNA binding to their target genes [21]. The ceRNA hypothesis suggests that any RNA transcript containing MREs can sequester miRNAs from substrates with MREs, thus regulating the function of those targets [22]. Some studies indicate that ceRNAs may influence the regulation of chemoresistance-related miRNAs and their target transcripts. For instance, the tumor suppressor gene *PTEN* is frequently inactivated in cancer. Multiple miRNAs can bind to the 3'-UTR of *PTEN* mRNA, inhibiting its expression. *PTENP1*, a pseudogene of *PTEN*, shares significant homology with the same region in *PTEN* mRNA, particularly near the 3'-UTR. This similarity suggests that *PTENP1* can function as a ceRNA for *PTEN*. Both *PTENP1* and *PTEN* mRNA contain MREs capable of binding *miRNA-21* [23]. In clear-cell renal cell carcinoma (ccRCC), overexpression of *miRNA-21* promotes tumor growth and metastasis *in vivo*, as well

as cell proliferation, migration, and invasion *in vitro* [24]. Overexpression of *PTENP1* mimics the effects of *PTEN* activation by reducing cell proliferation, invasion, tumor formation, and metastasis in cells expressing *miRNA-21*. Additionally, the upregulation of *PTENP1* in ccRCC cells enhances their sensitivity to cisplatin (CDDP) and gemcitabine (GEM) treatments in both *in vitro* and *in vivo* settings. In patient samples, *PTENP1* and *PTEN* levels were inversely correlated with *miRNA-21* activity. Patients with ccRCC who did not express *PTENP1* had a lower chance of survival. These findings suggest that *PTENP1* acts as a ceRNA to inhibit cancer progression in ccRCC. Furthermore, ceRNAs are a relatively new concept, and many studies have shown a strong association between ceRNAs and cancer development and proliferation [25].

4. Role of miRNAs in cancer therapy

Restoring miRNA activity and inhibiting overexpressed miRNAs are two key strategies in developing miRNA-based cancer therapies, as imbalanced miRNA levels are linked to carcinogenesis. To restore endogenous miRNA function and reestablish the production of tumor-suppressor miRNAs, miRNA mimics and small molecules are typically employed [26]. On the other hand, small molecule inhibitors, which target specific oncomiRs elevated in cancer cells, are used to prevent overexpressed miRNAs. Since some miRNAs act as tumor suppressors, using miRNA mimics to restore reduced tumor-suppressor miRNA levels to healthy levels is a promising therapeutic approach [27].

Before the discovery of miRNAs, gene therapy techniques were used to restore gene activity in cancer cells. However, due to the limitations of viral carriers and DNA plasmids, these approaches had inconsistent efficacy. The recent advancements in miRNA research have provided alternative tools. Since miRNAs are much smaller than proteins, they can more easily enter cells through certain delivery methods.

Esquela-Kerscher *et al.* demonstrated that restoring *let-7* miRNA could significantly inhibit tumor growth in mouse models, marking the first validation of let-7's role as a tumor suppressor and its potential as a cancer treatment [28]. Since then, interest in using miRNA mimics to restore miRNA activity has surged. The *let-7* family has been shown to suppress well-known oncogenes such as *NIRF*, *myc*, *HMGA*, *STAT3*, and *Ras*. Furthermore, reduced *let-7* production has been associated with poor prognosis in lung cancer, making the restoration of its function a potential treatment strategy [29]. Various administration methods were tested for *let-7* in mouse lung cancer models. The results demonstrated that *let-7* mimics could derepress direct *let-7* targets *CDK6* and *Ras*, induce necrosis, and slow tumor progression.

Similarly, the *miR-34* family, transcriptionally regulated by the tumor suppressor p53, is often deleted or downregulated in several cancers. *MiR-34a* has been shown to prevent metastasis and slow tumor progression by targeting CD44. When lipid-complexed *miR-34a* mimics were administered in mouse lung cancer models, tumor sizes were significantly reduced within a well-tolerated dose range, similar to the effects observed with *let-7* mimics [30].

In myc-driven liver cancer cells, *miR-26a* levels were lower than in normal cells. Reintroducing *miR-26a* mimics suppressed cyclin D2 and E2, halting the cell cycle. When *miR-26a* was administered to tumors in vivo, a significant response was observed, suggesting that restoring miRNA function using mimics could be an effective cancer treatment strategy [31].

In terms of overexpressed oncomiRs in cancer cells, considerable research has focused on suppressing these oncomiRs to develop new miRNA-based therapies. Small molecule inhibitors and complementary oligonucleotides—such as anti-miRNA oligonucleotides (AMOs), locked nucleic acid (LNA)-AMOs, antagomirs, and miRNA sponges—are the main types of miRNA inhibitors [32]. AMOs work by binding to the

target miRNA's complementary sequence, blocking its effects. AMOs are short DNA oligonucleotide strands that specifically bind to endogenous miRNA or its precursors, preventing them from binding to their target mRNA and thereby inhibiting miRNA activity. Subsequent research led to improved AMOs, such as 2'-O-methyl AMOs, 2'-O-methoxyethyl AMOs, and LNA-AMOS. LNA-AMOS, which features a modified structure, offers better selectivity, stability, and efficacy than earlier versions [33].

MiRNA sponges, like antagomirs, block miRNA from binding to its targets. Sponges are longer nucleic acids containing multiple miRNA-binding motifs and are typically DNA plasmids or transcribed RNA rather than short oligonucleotide strands. The study of pharmacological sites is crucial to developing new drugs [34]. Since miRNA plays a central role in gene regulation, it has emerged as a valuable tool for studying therapeutic targets. Traditional drugs are small chemical compounds that target individual cancer proteins, which has limitations in clinical settings. In contrast, miRNAs function at the core of multi-target regulatory networks and inherently regulate many receptor genes. Furthermore, miRNA production is tightly regulated by signaling pathways involving key enzymes, making the entire signaling cascade a potential therapeutic target.

Moreover, miRNA-based drugs can target molecules that conventional chemical or antibody-based drugs cannot reach, offering new avenues for treating diseases, particularly cancer, where traditional treatments have shown limited efficacy. As a result, miRNAs have gained increasing interest as therapeutic targets in drug development [35].

Certain small molecules, such as hypomethylating agents, can restore downregulated miRNAs. For example, the drugs decitabine and 5-azacytidine, used to treat myelodysplastic syndromes, increase the expression of several miRNAs. Additionally, enoxacin has been shown to stimulate miRNA production, leading to a general increase in miRNA levels in cell culture. In mouse xenograft models, enoxacin inhibited tumor growth by upregulating 24 mature miRNAs. These examples highlight the potential of small molecules to restore miRNA function in cancer treatment [36].

Another targeted strategy for miRNA restoration is the use of miRNA mimics—synthetic double-stranded RNA molecules that imitate endogenous miRNAs to regulate their activity. The primary challenge in applying miRNA mimics is developing an effective delivery system, such as nanoparticles, lipid emulsions, atelocollagen formulations, or adeno-associated viruses, due to the volatile nature of miRNA mimics in biological systems. In colon xenograft mouse models, the targeted delivery of *miR-34a* and *let-7* mimics via lipid formulations dramatically suppressed cancer proliferation. Similarly, *miR-26a* delivery using an adeno-associated virus vector inhibited cancer cell growth and reduced tumor size. Notably, a method for miRNA replenishment has been established in clinical trials for liver cancer using liposome-formulated *miR-34* mimic (MRX34) [37].

MiRNA sponges, which can absorb complementary miRNA molecules, are another effective miRNA regulator. By binding miRNAs, sponges prevent them from attaching to their target receptors, thus inhibiting miRNA function. Long non-coding RNA and circular RNA are known to function as natural miRNA sponges, binding miRNAs and interacting with miRNA target particles to negatively regulate their activity [38]. Shu *et al.* developed a sponge-based system that expresses circular miRNA inhibitors targeting *miR-223* and *miR-21*. This system was more effective at inhibiting cancer cell proliferation than its linear counterparts. Additionally, small molecule inhibitors of miRNA, as chemical entities, can be developed using conventional drug discovery methods. These inhibitors come in various forms, each with a distinct mechanism of action [39].

5. Altered microRNA expression in cancer cells

In the past decade, it has been discovered that cancerous cells and tissues exhibit aberrant miRNA expression

patterns. Certain miRNAs regulate key genes necessary for maintaining cellular homeostasis. When these genes are altered, abnormal biological processes, such as unchecked cell division, angiogenesis, metabolic dysregulation, and apoptosis, can occur, leading to cancer development [40]. Altered miRNA expression has been associated with numerous cancer hallmarks, including growth signaling (*let-7* family, *miR-21*), insensitivity to antigrowth signals, evasion of apoptosis, angiogenesis, invasion and metastasis (*miR-10b*, *miR-31*, *miR-200* family, *miR-21*), immune evasion (*miR-15b*), tumor-promoting inflammation (*miR-23b*, *miR-155*, *let-7d*), and genomic instability [41]. Cancer alters miRNA expression patterns that are specific to individual cells and cellular conditions, which differ from those seen in healthy tissues. Moreover, miRNAs can drive cancer progression by either upregulating oncogenes or suppressing tumor suppressor genes. This underscores the significance of miRNAs in cancer development and their potential as biomarkers for further subclassification of cancer types.

Tumor suppressor miRNAs (TS-miRNAs) and oncomiRs are the two main categories of tumor-related miRNAs. Lower expression of TS-miRNAs (e.g., *miR-133a*, *miR-145*, *miR-143*) allows for the translation of oncoproteins, while elevated expression of certain oncomiRs promotes cancer by inhibiting the translation of tumor-suppressing mRNAs (e.g., *miR-17-92*, *miR-125b*, *miR-125*) [42]. The dynamic nature of miRNA expression highlights their potential in cancer treatment strategies. As a result, miRNA mimics and antisense miRNA (anti-miRs) therapies have been developed and are commonly used to either replace TS-miRs or inhibit oncomiRs, respectively [43].

miRNAs are present in both the body's extrinsic and intrinsic environments. Recently, circulating miRNAs have emerged as a promising new class of biomarkers. For example, significantly elevated levels of *miR-31* have been found in the tissue and serum of individuals with oral cancer. *MiR-31* targets the enzyme SIRT3 (NAD-dependent deacetylase), which regulates metabolism and energy production [43]. Similarly, patients with gastric and germ cell cancers exhibit higher levels of *miR-371-3* in their tissue and serum, which directly targets the tumor suppressor gene *TOB1*, promoting cancer development and metastasis [44]. Furthermore, changes in miRNA expression have been associated with chemotherapy resistance. Overexpression of *miR-155* and *miR-221*/*miR-222* has been linked to poor outcomes in lung cancer and unfavorable responses to anti-estrogen therapies. The role of miRNAs in diagnostics and treatment is highly variable across different types of cancer.

6. MiRNA editing as cancer therapy

Some miRNAs hold potential as therapies because they are key regulators of carcinogenesis in certain types of cancer. For instance, in xenograft mice with stomach and breast tumors, augmenting the decreased levels of *miR-29b*, a driver of cell proliferation, growth, and angiogenesis, led to a reduction in tumor size. Another promising miRNA is miR-34a, which is typically underexpressed in various cancer types, particularly breast cancer. Some of miR-34a's key targets include Fos-related antigen (Fra-1), which regulates apoptosis, development, and transformation; and NAD^+-dependent histone deacetylase Sirtuin-1 (SIRT1), which removes the tumor suppressor p53 [45]. While miRNA regulates numerous biological processes, there are challenges in delivering miRNAs effectively. Crude miRNAs become unstable when exposed to different blood ribonucleases unless removed by phagocytosis through the reticuloendothelial system (RES). Additionally, the negative charge of crude miRNAs prevents them from crossing the vascular endothelium or cell membrane. To overcome these issues, various delivery methods for miRNAs are urgently needed [46].

Reducing oncomiRs, which are often overexpressed in human malignancies, restores tumor-suppressor activity, making it a potential therapeutic approach. Commonly used miRNA inhibitors either block miRNA production or inhibit miRNA-mRNA interaction. Examples include single-stranded antisense anti-miR

oligonucleotides (AMOs), locked nucleic acid (LNA) anti-miRs, antagomiRs, miRNA sponges, and small molecule inhibitors of miRNAs (SMIRs). For instance, antagomirs against *miR-16*, *miR-122*, *miR-192*, and *miR-194* were intravenously injected to significantly reduce their natural expression without inducing an immune response. Another example is the oncogenic *miR-21* antagonist, which inhibited EMT and angiogenesis in breast cancer by suppressing AKT and subsequently activating the Mitogen-Activated Protein Kinase (MAPK) pathway. MiRNA sponges, which are RNA compounds with repeated miRNA-binding sequences, can sequester specific miRNAs. This effectively suppresses the expression of *miR-23b in vitro* and *in vivo*, reducing angiogenesis, invasion, and migration of gliomas [47].

Another therapeutic approach is to introduce tumor-suppressive miRNAs into cancer cells. Tumor-promoting mRNAs can be targeted using pre-miR, plasmid-encoded miRNA genes, or synthetic double-stranded miRNA mimics, which compensate for lost tumor-suppressor miRNAs. The *miR-34* family, which is mutated in approximately 50% of human cancers, plays a key role in inhibiting tumor formation [48]. When *miR-34* mimics were introduced into cancer cells, they demonstrated a growth-inhibiting effect, confirming the potential of miRNA mimics as cancer therapies. However, challenges such as clearance by the RES and ribonucleases, as well as the negative charge of miRNAs, hinder their ability to cross the vascular endothelium and cell membrane. Even when they enter a cell, miRNAs are degraded by endolysosomes. Preserving normal tissue is essential for the successful delivery of cancer-specific medications. Tumor microenvironments, including compromised blood perfusion, act as barriers, limiting systemic delivery of miRNAs. Macrophages, neutrophils, and monocytes in the tumor microenvironment can nonspecifically absorb miRNAs carried within delivery mechanisms. Researchers have explored various strategies for delivering therapeutic miRNA mimics or blockers to address these issues [49].

7. Approaches for miRNA therapeutic delivery

Intrathecal injection of miRNA mimics or blockers is a more efficient delivery method than systemic approaches due to better absorption and lower toxicity. However, local administration is restricted to easily accessible and localized primary solid tumors, such as those in cervical, breast, and melanoma cancers. One advantage of local administration is minimal nonspecific uptake by healthy organs, which reduces unintended toxicity and immunogenicity [50]. miRNAs should be delivered to targeted cells, protected from early circulatory degradation, and allowed to enter cells easily without triggering an immune response. Systemic miRNA delivery systems and oligonucleotide modifications have been explored as enhanced therapeutic delivery methods for miRNA inhibitors and mimics. Chemically modified miRNA oligonucleotides increase stability and resist degradation by circulating nucleases. Both viral and non-viral vectors are frequently used for miRNA delivery. Viral vectors are effective but can elicit adverse immune responses, making non-viral vectors preferable for clinical research. Many labs are also investigating the use of nanoparticles as an alternative delivery mechanism for miRNAs [51].

Viral vectors deliver pre-miR or mature miRNA encoded in a plasmid into tumor cells, produce mature miRNA, use viral promoters to stimulate expression, and subsequently repress or degrade target mRNAs. Lentivirus, adenovirus, and adeno-associated virus (AAV) have all been effective in delivering miRNA mimics or antagonists to tumor cells, resulting in miRNA production and activation [52]. Kasar *et al.* demonstrated that *miR-15a/16* production, which was absent in chronic lymphocytic leukemia (CLL), was restored when these miRNAs were delivered via lentiviral vectors to a CLL model in New Zealand Black mice. In another study, intravenous delivery of lentiviruses encoding *miR-494* antagonists reduced the function of myeloid-derived suppressor cells (MDSCs), which promote angiogenesis and tumor formation [53,54]. Genetic modification

allows for the conjugation of targeting moieties to viral capsid proteins, improving the vectors' affinity for cancer-specific receptors and enabling targeted delivery into tumors [55]. However, it is important to note that lentiviruses integrate their viral DNA into the host genome, which may cause mutations and activate oncogenic pathways. In contrast, AAVs offer a safer alternative for therapeutic administration due to their episomal genome structure. Although viral vectors are an efficient way to deliver functional miRNA antagonists or mimics to tumor tissues, immunogenic responses remain a significant concern in practical applications. As a result, several non-viral delivery methods have been developed as safer alternatives for miRNA delivery [56].

Current research suggests that nanoparticles are a leading non-viral carrier for delivering exogenous nucleic acids, including DNA, mRNA, siRNA, and miRNA. However, concerns about safety and efficacy limit their practical use as therapeutic vectors, despite widespread application [57]. Nanoparticle-mediated miRNA delivery must overcome various barriers, including pharmacokinetics, endosomal escape, rapid renal clearance, and degradation. Notably, nanoparticles accumulate in tumors through the enhanced permeability and retention (EPR) effect, and particle size significantly impacts optimized targeting. Lipid-based vectors, the most common type of nanoparticles for in vivo gene therapy, consist of spherical structures made of phospholipid bilayers enclosing nucleic acids or drugs within an aqueous core. Liposome-based nanoparticles are favored for their low immunogenicity, flexibility, biocompatibility, and multiple delivery options. Advances in liposome design and modeling have enabled customization of their pharmacokinetics and absorption for specific clinical contexts [58].

Extracellular vesicles (EVs), such as exosomes or lipid-bilayer membrane-enclosed nanoparticles, are naturally produced by cells and facilitate intercellular communication by transferring nucleic acids (DNA, RNA) and proteins. EVs show potential for therapeutic and diagnostic applications, and much research is being conducted on their use in clinical settings. EVs can be broadly classified into apoptotic bodies, microvesicles, and exosomes. Microvesicles (50–1,000 nm) are released from the plasma membrane, while exosomes (30–150 nm) originate from the endosomal system. For convenience, "EV" is used as a general term for both exosomes and microvesicles of approximately 100 nm in size [59]. Although these subpopulations have different biosynthesis pathways, there is currently no reliable way to distinguish them. Compared to other delivery methods, EV-mediated miRNA transport offers several advantages as a biocompatible molecular carrier. EV surface receptors or proteins selectively deliver miRNAs to specific cell types. Unlike synthetic nanoparticles, EVs avoid endosomal entrapment within the cell, allowing miRNAs to act more quickly and effectively by suppressing gene targets [60]. EVs also protect miRNAs from RNase degradation and harsh conditions, enabling encapsulated miRNAs to travel through bodily fluids. EV-mediated miRNA delivery has been successfully demonstrated in the treatment of colon cancer (*miR-143*), gliomas (*miR-146b*), and lung cancer (*miR-145*). To improve cell selectivity, peptides can be conjugated to EV membranes. Ohno et al. developed EVs conjugated with the GE11 peptide, which targets epidermal growth factor receptor (EGFR) and was used to deliver *let-7a* to breast cancer cells expressing EGFR. These studies highlight EVs' potential to revolutionize therapeutic delivery strategies [61]. However, large-scale production and efficient encapsulation techniques are still under development.

8. Challenges and future perspectives of miRNA-based therapeutic approaches

While advancements have been made in miRNA-based therapies, several obstacles remain that hinder the transition of miRNA from research to clinical practice. The first challenge is delivery efficacy. Current chemically synthesized miRNA delivery methods exhibit limited cellular uptake capabilities. For therapeutic

benefits, miRNA must effectively cross cell membranes and navigate the complex vascular systems of various tissues [62,63]. The second challenge involves miRNA selectivity and off-target effects, which complicate the delivery of miRNAs to target cells. Unlike siRNA, the "multi-targeting" nature of miRNA can be both advantageous and problematic. While it may assist in treating diseases by influencing multiple factors involved in disease progression, it also leads to off-target effects. Despite sequence-specific targeting, miRNAs can inadvertently bind to unintended mRNAs with only partial complementarity, complicating precision targeting [64]. The third issue relates to miRNA-induced toxicity. Research has shown that some miRNAs can regulate the expression of enzymes involved in drug metabolism, such as bile acid synthase CYP7A1 and cytochrome P450s (CYPs). Dysregulation of CYP expression by certain miRNAs can disrupt drug metabolism, cause drug accumulation, and potentially lead to toxicity [65]. The fourth challenge is overcoming the rapid clearance of miRNA by the circulatory system.

Despite these limitations, miRNA holds great promise in cancer therapy. Certain miRNAs can directly influence cancer by regulating cell division, proliferation, and apoptosis, while others may have an indirect impact by targeting tumor suppressor genes and oncogenes. The study of miRNA's role in tumor development and progression has garnered significant attention. Advances in molecular biology have improved techniques for detecting miRNAs, leading to the identification of an increasing number of cancer-associated miRNAs and a clearer understanding of the relationship between miRNA and target mRNA. Changes in miRNA expression patterns are key factors in tumor formation and carcinogenesis.

Since identifying miRNAs in cancer patients can aid in cancer prediction, treatment, and diagnosis, miRNAs may emerge as novel tumor biomarkers. As miRNAs regulate target genes that contribute to the biological characteristics of cancers, research-based therapeutics involving miRNAs hold promising applications in the coming decades. The use of miRNA in medicine depends on low-cost, high-efficiency, and sensitive detection techniques. However, limitations in current detection methods hinder the widespread clinical application of miRNAs. Once these challenges are addressed, miRNA detection and therapy are expected to enter clinical practice successfully and become novel targets for cancer treatment. The recently discovered natural delivery system involving exosomes/EVs, when combined with targeting ligands, offers great potential as a biocompatible delivery structure specific to cancer cell types. The expression of miRNAs is influenced by numerous factors, and dysregulated miRNA expression often leads to resistance to anticancer drugs. The entire regulatory network of miRNAs, which includes their interactions with mRNA, protein, and other non-coding RNAs, expands the possibilities for their use in future research and clinical applications.

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Disclosure statement

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