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Micro-RNAs: The new potential biomarkers in cancer diagnosis, prognosis and cancer therapy

B. Mansoori[1, 2], A. Mohammadi[1, 3], S. Shirjang[1] and B. Baradaran[1]✉

[1] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of genetic, East Azarbaijan Science and Research Branch, Islamic Azad University, Tabriz, Iran

Corresponding author: Behzad baradaran, Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran**.** E-mail: behzad_im@yahoo.com

Abstract

MicroRNAs (miRNAs) are a large class of small noncoding RNAs approximately 22 nucleotides in length. They are the main regulators of gene expression, regulating specific oncogenes, tumor suppressors, cancer stem cells and metastasis. MicroRNAs have become valuable to cancer research in recent years. They appear as a significant biomarker in tumorigenesis. Briefly, the capacities of miRNA to identify between tumor and normal tissue, to distinguish between various subgroups of tumors and to foretell results or responses to therapy have attracted scientist's attention to these small RNAs. MicroRNAs' remarkable stability in both the tissue and bloodstream of cancer patients has elevated the possibility that miRNAs may prove to be a novel diagnostic biomarker. This review focuses on the utility of miRNAs as key biomarkers in cancer diagnosis, cancer prognosis and cancer therapy.

Key words: miRNA, Biomarker, Cancer, Diagnosis, Prognosis, Therapy.

Introduction

It is assessed that 1.67 million new cancer cases and 0.59 million cancer-related deaths occurred in 2014 (1). Hence, cancer continues to be a worldwide epidemic with substantial socioeconomic impact (1). There are various components involved in fighting cancer, including the molecular mechanisms of cancer cell treatment, novel targeted therapeutic progression, micro marker discovery, primary detection and risk correction. It is clear that plenty of cancers are heterogenous in their molecular signatures and clinical appearances.

About 65% of the genome is transcribed into RNA, but only 2% is translated into functional proteins (2). Recent explorations demonstrated that large parts of DNA formerly considered to have no biological importance are transcribed into several types of non-protein coding RNAs. These RNAs of less than 300 nucleotides are collectively called small RNAs, and this group includes long non-coding RNAs (lncRNAs), small interfering RNAs (siRNA), small nuclear RNAs (snRNAs), PIWI-interacting RNAs (piRNAs), X-inactivation RNAs (xiRNAs), microRNAs (miRNAs), Promoter-associated RNAs (PARs), small nucleolar RNAs (snoRNAs) and so on (3).

MicroRNAs (miRNAs) were first identified in 1993 when Ambros and Ruvkun discovered *Lin-4* microRNAs from the nematode *C. elegans*. (4, 5). MicroRNAs are small noncoding RNAs (21-23 nucleotides) indispensable to canonic biological functions such as growth, invasion, angiogenesis, proliferation, differentiation, apoptosis and so on (4, 5). The majority (80%) of these small sequences are found inside introns of protein-encoding or non-encoding genes and are called "mirtrons" (6). miRNAs mainly bind imperfectly to the 3´UTR of target mRNAs (7) and regulate post-transcriptional gene expression negatively by inhibiting the translation and degradation of target mRNA. miRNAs are normally transcribed by RNA polymerase II into pri-miRNAs (hairpin structures) (8), then pri-miRNAs are processed by Drosha, producing a stem loop precursor called pre-miRNA. Later, precursor molecules are processed into the mature miRNA in two main steps. First, in the nucleus, the microprocessor complex (RNase III enzyme Drosha/DGCR8) in mammals cleaves the pri-miRNA into pre-miRNA(~70 nt), which is actively transported into cytoplasm by binding on Exportin-5 (Ran-GTP). Second, in the cytoplasm, the pre-miRNA is processed by another RNAse III enzyme Dicer that cleaves by binding with cofactors termed TRBP and PACT. These processes generate double-stranded small RNA (~20 note). Helicases unwind double-stranded small RNA; one of the strands, known as the "guide" strand is merged into a miRNA-induced silencing complex (miRISC), and the other, known as "passenger" strand, is released and degraded. However, studies have proved that, in some cases, the passenger strands can be loaded into miRISC and act as a mature miRNA (Fig. 1) (9). miRISC is formed by the Argonaute (Ago) families. Out of the four Ago proteins, only Ago2 can mediate endonucleolytic cleavage of the target mRNA; this occurs when there is complete complementarity between a miRNA and a target site of mRNA (10). Generally, nucleotides 2-8 of the miRNA, known as the "seed" region, are the most important region for targeting the 3' UTRs site of target mRNA (11). Additionally, oligonucleotide miRNA microarrays, deep sequencing (next generation sequencing), bead-flow cytometry, quantitative real-time polymerase chain reaction and high-throughput array-based Klenow enzyme assay proved these small molecules are

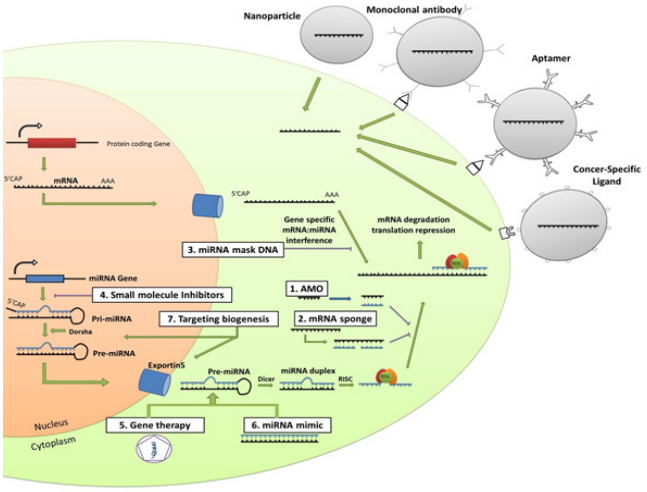

Figure 1. Schematic diagram of miRNA biogenesis and the therapeutic strategies with specific target delivery. Primarily, RNA polymerase II simplifies the transcription of the miRNA gene to pri-miRNA then pri-miRNA is processed by Drosha, producing a stem loop precursor called pre-miRNA in the nucleus. Then pre-miRNA is exported into the cytoplasm by exportin-5/ Ran-GTP. In the cytoplasm Dicer removes loop structures of pre-miRNAs and produces a duplex molecule including mature miRNA and an miRNA* fragment. Helicase unwinds the miRNA:miRNA* duplex; the miRNA* fragment is degraded by nuclease, whereas the mature miRNA molecule binds to RISC complex, the RISC–miRNA complex can then degrade or repress the translation of mRNA. 1. Anti-miRNA oligonucleotides (AMOs) paired with miRNA thus inhibit miRNA attachment to target mRNAs; 2 mRNA sponges include several binding sites for a particular miRNA which in turn prevent the attaching of this miRNA with its endogenous targets; 3 miRNA mask DNA is complementary to miRNA binding site, effecting gene interference of miRNA:mRNA interaction; 4 small molecule inhibitor inhibits the level of mature miRNA such as pri-miRNA; 5, 6 gene therapy using nanoparticles and virus delivery systems can induce the expression of specific tumor suppressive miRNA; 7 targeting miRNA biogenesis has been suggested. Enhancing the therapeutic miRNA stability encapsulated in nanoparticles is suggested. The delivery of therapeutic miRNA can be specified with nanoparticles conjugated to antibodies, aptamers or cancer-specific ligands.

important regulators in many diseases, including depression, heart disease, vascular diseases, cancer and so on. (7, 12). MicroRNAs act as oncogenes (oncomirs) or tumor suppressors in cancer pathways (13), and miRNA profiling experiments suggested that miRNAs are less abundant in tumors compared to normal tissue, which further suggests that miRNAs are preponderantly tumor suppressors rather than oncogenes (14).

Both in vivo and in vitro experiments demonstrated the importance of miRNA expression in the pathogenesis of cancer. Understanding MiRNA mechanisms in tumorigenesis, cancer maintenance and malignancies provides extraordinary information about cancer pathways, cancer diagnostics and cancer prognostics. Importantly, this information could also help in the progression of anticancer therapy.

MiRNA dysregulation in cancer

The first evidence of miRNA dysregulation in cancer came from Croce's group studies on chronic lymphocytic leukemia (CLL). These studies found the decorative region on chromosome 13q14 was mostly deleted in CLL, and they found two tumor suppressor microRNA genes, *miR*-15a and *miR*-16-1, were down-regulated in CLL (15). miRNAs may play a significant role as a novel class of oncogenes or tumor suppressor genes, and several miRNAs caused tumor formation and rapid regression, including oncogenic miRNAs, called "oncomirs," which increased in different cancers. Oncomirs generally promote tumor growth by negatively inhibiting tumor suppressor genes or genes that control cell differentiation or apoptosis (16) For example, *miRNA-21* was the first miRNA to be discovered as an oncomir because of overexpression of this miRNA in breast cancer, colorectal cancer, esophageal squamous cell carcinoma, human cholangiocarcinoma and pancreatic cancer (Table 2) (17). The molecular mechanism of *miRNA-21* has been explained through the recognition of specific downstream target genes, such as *PDCD4*, *SPRY1* and *PTEN* (18-20). The other oncogenic miRNAs are known as the *miR-17-92* cluster (termed OncomiR-1). The members of this cluster, including *miR-17-3p, miR-17-5p, miR-18a, miR-20a, miR-19a, miR-19b-1* and *miR-92a-1* are placed on chromosome 13, 7 and X, but in hematopoietic malignancies and B-cell lymphomas this cluster is overexpressed on chromosome 13 (21). Interestingly, the *miR-17-92* cluster targets many genes involved in apoptotic pathways, and by suppressing many target mRNAs are accountable for the anti-apoptotic effect (12, 22). Dr. Mendell (Johns Hopkins University, Baltimore, MD) considered *c-Myc*–regulated miRNAs in cellular transformation and tumorigenesis. He introduced data that *c-Myc* activates expression of a *miR-17* cluster on human chromosome 13. Chromatin immunoprecipitation demonstrated that *c-Myc* binds directly to this locus to activate transcription of current miRNAs. In addition, he represented that the *miR-17* cluster is widely overexpressed in human cancers and can increase tumorigenesis in animal models (23).

There are plenty of miRNAs known as tumor suppressors, such as *let-7* and *miR200c*, because their expression is reduced in malignant cells. Tumor suppressor miRNAs may act by negatively suppressing oncogenes or genes that suppress cell differentiation or apoptosis (Table 1) (14, 24, 25).

miRNA as a biomarker in cancer diagnosis

The National Cancer Institute (NCI) describes a biomarker as «a biological molecule found in blood, other body fluids, or tissues that are a sign of a normal or abnormal process or of a condition or disease" that "may be used to see how well the body responds to a treatment for a disease or condition" (52). Biomarkers can be of different molecular origins, including DNA, RNA or protein. Cancer biomarkers are potentially one of the most valuable tools for primary cancer detection, valid pretreatment staging, characterizing the response of cancer to chemotherapy treatment and monitoring disease development (53). As we said above, miRNAs are key regulators of gene expression and are associated with cancer progression, (29). In addition, Dr. Rosenfeld showed that miRNA expression profiles have been helpful in determining the origin of tissue for cancers of unknown primary origin (54). *MicroRNA* profiling

Table 1. micro-RNA altered expression in human cancer (26-46).

Cancer type	Over expression	Under expression
Solid		
Esophagus cancer	*miR-194, miR-192, miR-200c, miR-21*	*miR-203, miR-205*
Breast cancer	*miR-21, miR-22, miR-23, miR-29b-2,miR-96, miR-155, miR-191, miR-181,miR-182, miR-27a, miR-210, miR-195*	*miR-205, miR-143, miR-145, miR10b, miR- 125a/b, miR-155, miR17-5p, miR-27b, miR-9-3, miR-31, miR-34 family, let-7*
Thyroid cancer	*miR-146b, miR-221, miR-222, miR-181b, miR-155, miR- 197 , miR-224, miR-346*	*miR-30d, miR-125b, miR-26a,miR-30a-5p*
Hepatocellular cancer	*miR-18, miR-21, miR-33, miR-130b, miR-135a, miR- 221, miR-224, miR-301, miR-500*	*miR-199a/b, miR-195, miR-200a/b, miR-214, miR-223, miR-125a,miR-122a, miR-101, miR- 139, miR-150, miR-26a, miR-101*
Lung cancer	*miR-17-92 cluster, miR-21, miR-155, miR-191, miR-205, miR-210*	*let-7, miR-34 family, miR-143, miR-145, miR- 124a*
Colorectal cancer	*miR-18, miR-224, miR-10a, miR-17-92 cluster, miR-21, miR-24-1, miR29b-2, miR-31, miR-96, miR-135b, miR- 183*	*miR-143, miR-145, let-7, miR30 -3p, miR-124a, miR-129, miR133 b, miR328*
Ovarian cancer	*miR-200a/b/c, miR-141, miR-18a, miR-93, miR-429*	*miR-199a, miR-140, miR-145, miR-125a,b, let7*
Gastric and intestinal cancer	*miR-106b-25, miR-17-5p, miR-21, miR-106a*	*miR-15b, miR-16, let-7a*
Prostate cancer	*let-7d, miR-195, miR-203, miR-21,miR-181, miR-106, miR-363, miR-221*	*miR-128a, miR-101, miR-125a/b, miR-15a, miR-16-1, miR-143, miR-145, miR-23a/b, miR- 200, miR-330, miR-331*
Pancreas cancer	*miR-221, miR-376a, miR301, miR-21, miR-24-2, miR- 100, miR-103, miR107, miR-125b-1, miR-155, miR-181, miR-106, miR-363, miR-301, miR a, miR-212, miR- 34a376, miR-210*	*miR-375, let-7, miR-200, miR200b*
Bladder cancer	*miR-17, miR-23a,b, miR-26b, miR-103-1, miR-185, miR- 203 , miR-205, miR-221, miR-223*	*miR-29c, miR-26a, miR-30c, miR-30e-5p, miR-145, miR-30a-3p, miR-133a/b, miR-195, miR125b, miR-199a*
endometrial adenocarcinoma	*miR-205, miR-449, miR-429*	*miR-193a, miR-204, miR-99b*
Glioblastoma cancer	*miR-221, miR-222, miR-21*	*miR-181a, miR-181b, miR-181c, miR-125a, miR-125b*
Hematologic		
Acute myeloid leukemia	*miR-191, miR-199, miR155, miR-221, miR-222, miR- 125 a/b*	*miR-124a, miR-148a, miR-181a, miR-204, miR-223, miR-92a miR-181b*
Acute promyelocytic leukemia	*miR-15a, miR-15b, miR-16-1, let-7a-3, let-7c, let-7d, miR-223, miR-342, miR-107*	
Acute lymphoblastic leukemia	*miR-17-92 cluster, miR-125b-1, miR-128a, miR-128b, miR-204, miR218, miR-331, miR-181a, miR-181b, miR- 181 c, miR-142-3p, miR-142-5p, miR-150, miR-193a, miR-196b, miR30e-5p, miR-34b, miR-365, miR582, miR- 708*	*let-7b, miR-223, miR-100, miR-125b, miR-151- 5p, miR-99a, miR-92a*
Chronic myeloid leukemia	*miR-17-92 cluster, miR-17-5p, miR-17-3p, miR-18a, miR-19a miR-19b-1, miR-20a, miR-92a-1*	*miR-10a*
Chronic lymphocytic leukemia	*miR-21, miR-23b, miR-24-1, miR-146, miR-155, miR- 106b, miR-195, miR-221, miR-222*	*miR-15a, miR16-1, miR-29, miR143, miR-45, miR-30d, let-7a, miR-181a/b, miR-223, miR-92, miR-150*

could be applied to cancer classification, diagnosis and prognosis (Table 2) (55). miRNA profiles can not only distinguish between normal and cancerous tissue and distinguish the source of tissues, but they can also distinguish various subtypes of a specific cancer or even particular oncogenic abnormalities (56). For example, miRNA can specifically classify HER2/neu receptor, estrogen receptor and progesterone receptor status (28, 57) in breast cancer, and distinguish between basal and luminal breast cancer subtypes (58). Interestingly, *miR-155* overexpression and *let-7a* down-regulation can predict poor disease results (59), and some other studies have proved the importance of microRNAs as prognostic biomarkers (60, 61). Importantly, some teams in recent years have reported how *microRNA* profiling can foretell disease results or responses to therapy. Table 1 shows some miRNAs, their targets and their dysregulation in various cancers. For example, *miRNA-9-3* acting

Table 2. miRNA and their targets in cancer(15, 29, 47-51).

micro RNA	miRNA dysregulation in cancer	miRNA targets	Molecular mechanism
miR-9-3	Down regulated in breast cancer	*P53*	acted in programed cell death (P53) pathway
miR-16 , miR-15a	Down regulated in CLL	*cyclin D1* *cyclin D2* *cyclin E1*	induced cell cycle arrest in G0-G1
miR-17-92	Up regulated in B cell lymphoma and medulloblastoma	*P21, N-Myc*	facilitating the transition of the cell cycle from G1 to S from sonic hedgehog pathway and promote proliferation
miR-25	Up regulated in gastric cancer	*P57*	Inhibit cip/kip families, kinase dependent cyclin inhibitors from 3' UTR
miR-101	Down regulated in liver carcinoma	*Mcl-1*	Forwarded apoptosis and inhibited tumorigenesis
miR-122	Down regulated in liver carcinoma	*cyclin G1, P53*	increased p53 protein levels and, activity through its negative regulation of cyclin G1
miR-128	Down regulated in glioma cancer	*E2F3a*	Inhibited cell growth
miR-143	Down regulated in colorectal cancer	*KRAS*	Inhibited kinase phosphorylation
miR-145	Down regulated in lung adenocarcinoma and colon cancer	*EGFR, IGF-1R*	Inhibited cancer cell growth in mutated *VEGFR* cancers
miR-512-5*P*	Down regulated in gastric cancer	*Mcl-1*	Induced programed cell death in cancerous cells

in programmed cell death depends on P53 pathway, there was down-regulated in breast cancers with presence of lymph node metastasis, down regulation of *the miRNA-9-3 cause* of tumor progression, (28) or *miR-17-92* facilitating the transition of the cell cycle from G1 to S from the sonic hedgehog pathway and promote proliferation, these miRNAs targeted *p21* and *N-myc* caused up-regulation in B cell lymphoma and medulloblastoma. Dr. Lawrie showed the serum levels of *miR-21* were related to relapse-free durability in patients with diffuse large B-cell lymphoma; therefore, *miR-21* may have capacity as a diagnostic biomarker for this disease (62). Dr. Mitchell proved that the serum levels of *miR-141* could differentiate between patients with prostate cancer and healthy subjects, and also found the attendance of circulating tumor-derived miRNAs in the blood by using a mouse prostate cancer xenograft model (63). Dr.

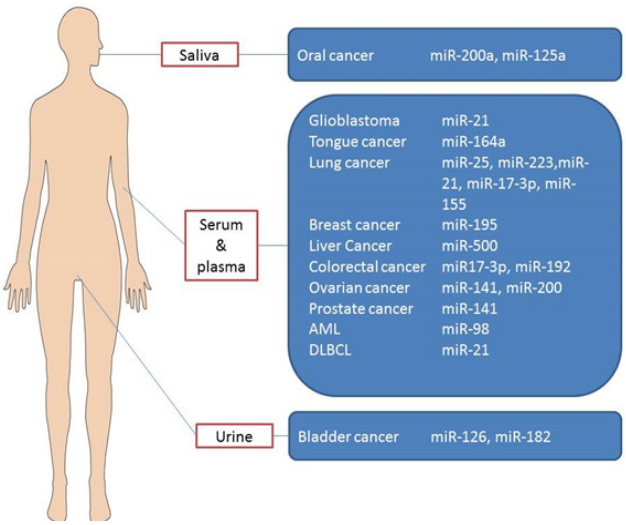

Figure 2. miRNAs as a cancer diagnostic biomarkers in human body fluids. AML, acute myeloid leukemia; DLBCL, diffuse large B-cell lymphoma.

Yamamoto showed that *miR-500* is an oncofetal miRNA in liver cancer; in mouse liver development, *miR-500* expression is high in the fetal liver and down-regulated in the evolutionary process and then up-regulated in the liver cirrhosis procedure (64). To utilize circulating miRNAs as a diagnostic biomarker, it is necessary to obtain a better understanding of the mechanisms by which miRNAs are released into the bloodstream. Several studies have shown that the serum miRNAs are resistant to RNase digestion, and suggest that lipid or lipoprotein complexes, exosomes, microvesicles, prostasomes and apoptotic bodies protected plasma RNA from degradation (65-74). Thus, serum miRNAs are good biomarkers for detecting cancer (75, 76). Many kinds of circulating miRNAs have been introduced in different types of cancers, as certain cancers cannot be diagnosed by noticing serum biomarkers. In such cases, circulating miRNAs in serum, urine and saliva are good candidates for further use (Fig 2) (77).

miRNA as a biomarker in cancer prognosis

MicroRNA as a biomarker of treatment response

Measuring the potential dependence of miRNA expressions with clinical results in gastric cancer patients shows *miR-451* down-regulation is associated with a worse prognosis. Overexpression of *miR-451* in gastric cancer cells regulates the oncogene macrophage migration inhibitory factor (MIF) generation, decreases cell proliferation and enhances sensitivity to radiotherapy. These approaches suggest the role of *miR-451* as a prognosis biomarker for gastric cancer (78). Dr. Geoffrey showed low levels of *hsa-miR-205* and *hsa-let-7d* expression in head and neck squamous cell carcinoma are associated with poor head and neck cancer survival, and he showed that miRNA expression levels can be applied as prognostic markers of head and neck cancer (79).

CLL (chronic lymphocytic leukemia) with 17p deletion and *TP53* (tumor protein 53) mutation is resistant to chemotherapy. Low expression of *miR-34a* in CLL is thought to be associated with *p53* inactivation. Also, it is suggested that *miR-34a* has a role in chemotherapy resistance and thus may serve as a biomarker for poor prognosis in CLL (80). Dr. Coulouarn has shown that the loss of *miR-122* expression in hepatocellular carcinoma (HCC) tumor cells separates, with particular gene expression profiles joining to HCC development. This miRNA is specifically suppressed in a subset of early HCCs that are determined by poor prognosis. *miR-122* is indicated to be a potential diagnostic and prognostic marker for HCC progression (81).

MicroRNA as a biomarker for predicting progression and metastasis

Dr. Volinia showed the unique role of *miR-210* in invasion and prognosis in breast cancer, exhibiting up-regulation of *miR-210* in DCIS (Ductal carcinoma in situ) and down-regulation of this miRNA in IDC (invasive ductal carcinoma) (82). In another study, *miR-21* and *miR-155* expression was evaluated in tumor tissue and in adjacent normal tissue of 156 CRC (colorectal cancer) patients. High *miR-21* expression was mainly associated with liver metastasis, venous invasion and tumor stage, and high *miR-155* expression was mainly associated with lymph node metastases (83). A study team has determined that *miR-129* has prognostic potential for foretelling disease progression in bladder cancer. A direct link between *miR-129* and the two putative targets *SOX4* and *GALNT1* was confirmed using luciferase assays (84). A new study has observed that the expression of *miR-196a* is higher in Barrett's esophagus, dysplastic lesions and esophageal adenocarcinoma compared with normal squamous mucosa, and in high-grade dysplasia compared with Barrett's esophagus and low-grade dysplasia, this proved *miR-196a* particularly targets *SPRR2C* (small proline-rich protein 2C), *KRT5* (keratin 5) and *S100A9* (S100 calcium-binding protein A9) (85). In one study, it was shown that *miR-10b* is highly expressed in metastatic breast cancer cells and positively regulates cell invasion. Also, they showed that the overexpression of *miR-10b* in otherwise non-metastatic breast tumors can lead to invasion and metastasis. *miR-10b* expression is induced by the transcription factor Twist, which binds directly to the promoter of *mir-10b* (*MIRN10B)*. The *miR-10b* induced by Twist works to inhibit translation of the mRNA encoding homeobox D10, effecting enhanced expression of a well-defined pro-metastatic gene, *RHOC* (86). Another study has determined *miR-21* post-transcriptionally down-regulates tumor suppressor *Pdcd4* and stimulates migration, invasion and metastasis in colorectal cancer (87).

miRNA-targeted cancer therapy

The expansion of new therapies has contributed significantly to enhanced 5-year survival and a decrease in overall mortality rates (88, 89). With developments in profiling, cancer therapies can now be customized for each individual. More than 2000 gene-therapy-based clinical trials are in development for different diseases, but only one involves miRNA therapy (90). Therapy can be targeted toward miRNAs in two pathways, including miRNA reduction and miRNA replacement. miRNA reduction therapy involves inactivating miRNAs that are up-regulated or overexpressed in tumor cells, including microRNA sponges, anti-miRNA oligonucleotides, miRNA masking and small molecule inhibitors. In the anti-miRNA oligonucleotides strategy, the binding of miRNAs to their binding targets are simply and nicely controlled by the rules of the Watson–Crick base pairing model. Hence, clear inhibitory molecules of miRNA are anti-miRNA oligonucleotides (AMOs), which block the interactions between miRNA and its target mRNAs by the constitution. AMOs are chemically modified to improve its stability. Locked nucleic acid (LNA) is an example of an AMO (91, 92). A microRNA sponge is distinguished as a synthetic mRNA including several binding sites for an endogenous miRNA, hence preventing the interplay between miRNA and its endogenous targets. In in vitro assays, these "sponges" repressed miRNA targets as powerfully as chemically modified AMOs (such as LNA) (93). In respect to this, the efficacy of the stable expression of sponges in applications in vivo need to be appraised. AMOs may evoke off-target side effects and undesirable toxicity because AMOs are sequence-specific but not gene-specific. Xiao et al. designed another strategy called miRNA masking (miR-mask) which refers to a sequence with complete complementary to the binding site of an endogenous miRNA in the target gene with higher affinity, hence blocking the availability of endogenous miRNA to its binding site without the potential off-target side effects of mRNA degradation by AMOs (94). Small molecule inhibitors against specific miRNAs have also been assayed. Azobenzene was identified as a specific and effective inhibitor of biogenesis of *mir-21* from a screening. These specific inhibitors of the miRNA pathways prepare not only unique tools for the examination of miRNA functions, but also promising reagents to raise patient response to available stand-alone cancer drugs or chemotherapies (Fig 1) (95).

miRNA replacement therapy strategies focus on the representation of the miRNAs which are down-regulated or deleted in the tumor cells, including restoring suppressor miRNAs and enhancing miRNA biogenesis processing (96).

Restoring Suppressor miRNAs

It has been suggested that restoration of tumor suppressive miRNAs has antitumor effects so they introduced the method of restoring suppressor miRNAs, and studies on various tumor suppressor miRNAs proved this hypothesis. Various *in vitro* studies showed overexpressing *Let-7* in lung cancer cell lines inhibited cell growth (97-100). *Lin28* has been known to block *Let-7* processing and finally cause *pre-let-7* degradation (101, 102), Therefore inhibiting *Lin28* will restitute *let-7* expression and inhibit tumorigenesis. Another example of restoring suppressor miRNAs is that *miRNA-15* and *miRNA-16* are often deleted in CLL patients, which targeted *BCL2* (45, 46). Studies have shown that transfecting *miR-15/16* expressing construct effected in reduction of BCL2 protein levels and enhanced apoptosis in cancer cell lines (46, 103).

Table3. Summary of studies using Nano particles for miRNA delivery in cancer therapy (108-112).

Target miRNA	Local delivery	Function	Cancer Model	Results
miR-34a	LPH-PEG-GC4	Mimics	Lung cancer	Reduction of tumor growth, induction of apoptosis, inhibition of survivin expression and downregulation of MAPK pathway
miR-34a	Neutral lipid	Mimics	NSCLC	Inhibition of tumor growth
miR-155	PLGA-penetratin	Antagonists	Lymphoma	Induction of apoptosis and reduction of tumor growth
miR-34a	Silica nanoparticles	Mimics	Neuroblastoma	Induction of apoptosis, reduction in vascular density of tumors and inhibition of tumor growth
miR-26a	Adeno-associated viruses (AAVs)	Mimics	Hepatocellular carcinoma	Inhibition of tumor cell proliferation and induction of apoptosis
miR-143	Cationic liposomes	Mimics	Colorectal carcinoma	Inhibition of tumor growth
miR-21	Seed-targeting tiny LNAs	Antagonist	Breast cancer	Repression of the miR-21 function in tumor
miR-33a	Polyethyleneimine (PEI)	Mimics	Colon carcinoma xenograft	Induction of apoptosis, inhibition of tumor growth and downregulation of the oncogenic kinase Pim-1
miR-375	Cholesterol-conjugated 2′-O-methyl-modified	Mimics	Hepatoma xenograft	Inhibition of tumor growth
miR-122	LNP-DP1	Mimics	Hepatocellular carcinoma	Inhibition of angiogenesis and tumor growth

Enhancing miRNA Biogenesis Processing

Reduced miRNA biogenesis has been associated with tumor development. For instance, decreased Dicer1 expression in a subset of lung cancers has been discovered to correlate with poor prognosis (104), and other studies have shown that high Dicer and Drosha expression were associated with enhanced median survival in several cancers (105-107).

The delivery of miRNA is still a challenge and has limited the application of nucleic acid drugs. Because of the small size and low molecular weight of miRNAs, it is possible to be formulated into an effective delivery system for prescription as attractive options for clinical cancer therapy progression achieve effective gene knockdown in cancerous cells, the strategies for efficient invivio delivery and escape from blood clearance, intracellular trapingare (such as endosome) and enzyme degradation are in progress. Some of the strategies employed in *in vivo* miRNA delivery studies for cancer therapy are summarized in Table3 (108-.113).

Conclusions and future perspectives

The Nobel Prize of 2006 in Physiology or Medicine was given to Andrew Fire and Craig Mello for their discovery of RNA interference (RNAi). miRNAs suppress their target mRNAs by complementary attaching and induction of the RNAi pathway. The finding of hundreds of miRNAs has enhanced the field of biomedical RNAi to the current level of substantial recognition. Abundant studies in patients have disclosed that oncomir profiling can identify cancers and foretell patient results with high accuracy. Multiple studies proposed target analysis combining genomics, miRomics and proteomics might help determine the aspect of targets that are regulated by miRNAs. Due to the rapid development over the past several years, it is probable that miRNAs have a promising future in the area of cancer diagnostics, prognosis and treatment. Several miRNAs are qualified by epigenetic alterations in cancer cells, including histone modification and DNA methylation. However, each miRNA can qualify hundreds of target genes, which leaves a major challenge in identifying the exact miRNA targets for cancer investigation. The advantage of miRNAs for the diagnosis, prognosis and treatment of human cancer will depend on carefully designed experimental studies. In addition, it will require the selection of the best methods for sample amassment, miRNA isolation, miRNA evaluation and data analysis. To simplify this, we require a better understanding of particular miRNA specifications, including how targeting of several mRNAs by individual miRNA affects data declaration in marker studies and the effect of miRNA isoforms on diagnostic yield. Fewer successes are reported in the progression of miRNA therapeutic strategies. Rather, the basic subjects are the discovery of the microRNAs which play a serious role in the biology of a particular tumor type by changing a whole network of target proteins; the confirmation of the targets and exact anticipation of the putative undesirable off-target effects; and the progression of effective methods of a specific drug delivery. With all the efforts and progressions in developing miRNA-mediated therapy three main obstacles still remain: first maintaining target specificity and off-target gene silencing, second, achieving high therapeutic efficiency and third, finding an efficient delivery system. Factors that

can restrict miRNA therapeutic performance are the abundance of target gene modulation and the number of cells that can be targeted.

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