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
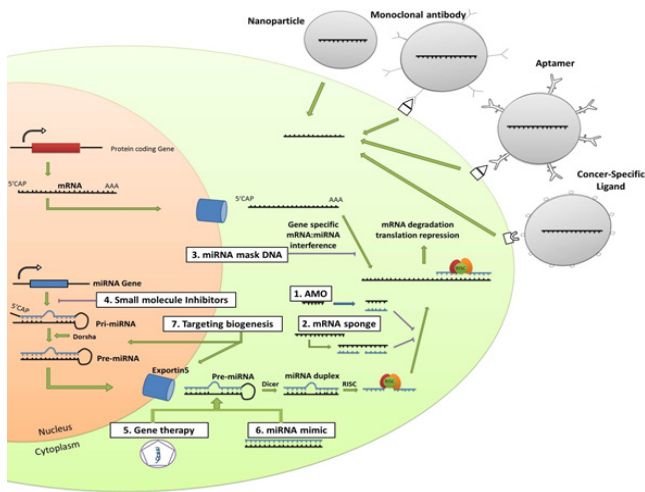
Figure 1. Schematic diagram of miRNA biogenesis and the therapeutic strategies with specific target delivery. Primarily, RNA polymerase II simplifies the transcription of the miRNA gene to pri-miRNA then pri-miRNA is processed by Drosha, producing a stem loop precursor called pre-miRNA in the nucleus. 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, *miR-NA-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*
Acute promyelocytic leukemia	*miR-15a, miR-15b, miR-16-1, let-7a-3, let-7c, let-7d, miR-223, miR-342, miR-107*	*miR-181b*
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-512-5P	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).
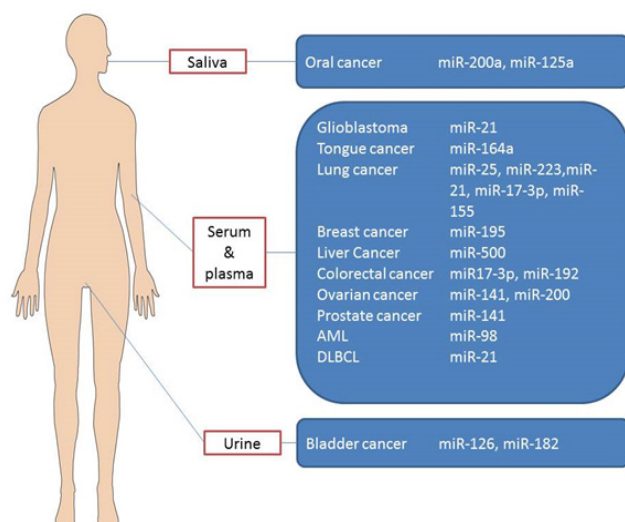
CLL (chronic lymphocytic leukemia) with 17p deletion and *TP53* (tumor protein 53) mutation is resistant to chemotherapy. Low expression of *miR-34a* in CLL is thought to be associated with *p53* inactivation. Also, it is suggested that *miR-34a* has a role in chemotherapy resistance and thus may serve 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.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA: a cancer journal for clinicians*. 2014, **64**(1):9-29. doi: 10.3322/caac.21208

2. Carninci P, Kasukawa T, Katayama S, Gough J, Frith M, Maeda N, et al. The transcriptional landscape of the mammalian genome. *Science*. 2005, **309**(5740):1559-63.

3. Mansoori B, Shotorbani SS, Baradaran B. RNA Interference and its Role in Cancer Therapy. *Advanced pharmaceutical bulletin*. 2014, **4**(4):313.

4. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nature reviews Molecular cell biology*. 2014, **15**(8):509-24. doi:10.1038/nrm3838

5. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene< i> lin-14</i> by< i> lin-4</i> mediates temporal pattern formation in C. elegans. *Cell*. 1993, **75**(5):855-62. doi: 10.1016/0092-8674(93)90530-4

6. Grund SE, Polycarpou-Schwarz M, Luo C, Eichmüller SB, Diederichs S. Rare Drosha splice variants are deficient in microRNA processing but do not affect general microRNA expression in cancer cells. *Neoplasia*. 2012, **14**(3):238-IN26. doi:10.1093/nar/gkt1181

7. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Developmental biology*. 2007, **302**(1):1-12. doi: 10.1016/j.ydbio.2006.08.028

8. Baer C, Claus R, Plass C. Genome-wide epigenetic regulation of miRNAs in cancer. *Cancer research*. 2013, **73**(2):473-7. doi: 10.1158/0008-5472

9. Chiang HR, Schoenfeld LW, Ruby JG, Auyeung VC, Spies N, Baek D, et al. Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes & development*. 2010, **24**(10):992-1009. doi: 10.1101/gad.1884710

10. Wang B, Li S, Qi HH, Chowdhury D, Shi Y, Novina CD. Distinct passenger strand and mRNA cleavage activities of human Argonaute proteins. *Nature structural & molecular biology*. 2009, **16**(12):1259-66. doi: 10.1038/nsmb.1712

11. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009, **136**(2):215-33. doi: 10.1016/j.cell.2009.01.002

12. Li C, Feng Y, Coukos G, Zhang L. Therapeutic microRNA strategies in human cancer. *The AAPS journal*. 2009, **11**(4):747-57. doi: 10.1208/s12248-009-9145-9

13. Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. *Annual review of medicine*. 2009, **60**:167-79. doi: 10.1146/annurev.med.59.053006.104707

14. Murray MJ, Raby KL, Saini HK, Bailey S, Wool SV, Tunnacliffe JM, et al. Solid tumors of childhood display specific serum microRNA profiles. *Cancer Epidemiology Biomarkers & Prevention*. 2015, **24**(2):350-60. doi: 10.1158/1055-9965

15. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences of the United States of America*. 2004, **101**(9):2999-3004. doi: 10.1073/pnas.0307323101

16. Marwah A. Potential Role of miRNA in Oncogenesis. *International Journal of Cell Biology and Cellular Processes*. 2015, **1**(1):13-27.

17. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. *Annual review of pathology*. 2014, **9**:287. doi: 10.1146/annurev-pathol-012513-104715

18. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *Journal of Biological Chemistry*. 2008, **283**(2):1026-33. doi: 10.1074/jbc.M707224200

19. Meng F, Henson R, Wehbe–Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*. 2007, **133**(2):647-58. doi: 10.1053/j.gastro.2007.05.022

20. Thorsen SB, Obad S, Jensen NF, Stenvang J, Kauppinen S. The therapeutic potential of microRNAs in cancer. *The Cancer Journal*. 2012, **18**(3):275-84. doi: 10.1097/PPO.0b013e318258b5d6

21. Ota A, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, et al. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer research*. 2004, **64**(9):3087-95. doi: 10.1158/0008-5472.CAN-03-3773

22. Olive V, Li Q, He L. mir-17-92: a polycistronic oncomir with pleiotropic functions. *Immunological reviews*. 2013, **253**(1):158-66. doi: 10.1111/imr.12054

23. Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. *The lancet oncology*. 2012, **13**(6):e249-e58. doi:10.1016/S1470-2045(12)70073-6

24. Bandyopadhyay S, Mitra R, Maulik U, Zhang MQ. Development of the human cancer microRNA network. *Silence*. 2010, **1**(1):6. doi: 10.1186/1758-907X-1-6

25. Dou J, He X, Cao W, Zhao F, Wang X, Liu Y, et al. Overexpression of microRNA-200c in CD44+ CD133+ CSCS inhibits the cellular migratory and invasion as well as tumorigenicity in mice. *Cell Mol Biol*. 2013, **59**:OL1861-OL8.

26. Croce CM, Calin GA, Volinia S. Methods for Diagnosing Breast Cancer Using miR-21, miR-125-1, miR-125b-2 and miR-145. *Google Patents*; 2012.

27. Babashah S, Soleimani M. The oncogenic and tumour suppressive roles of microRNAs in cancer and apoptosis. *European journal of cancer*. 2011, **47**(8):1127-37. doi: 10.1016/j.ejca.2011.02.008

28. Ferracin M, Lupini L. MicroRNA expression profiling and its clinical impact in breast cancer. *MicroRNAs: Key Regulators of Oncogenesis: Springer*; 2014. p. 355-67. doi:10.1007/978-3-319-03725-7_14

29. Cho W. MicroRNAs in cancer—from research to therapy. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2010, **1805**(2):209-17. doi: 10.1016/j.bbcan.2009.11.003

30. Lowery AJ, Miller N, McNeill RE, Kerin MJ. MicroRNAs as prognostic indicators and therapeutic targets: potential effect on breast cancer management. *Clinical Cancer Research*. 2008, **14**(2):360-5. doi: 10.1158/1078-0432.CCR-07-0992

31. Guo R, Sherman-Baust C, Abdelmohsen K. miRNA-Based Ovarian Cancer Diagnosis and Therapy. *MicroRNA Targeted Cancer Therapy: Springer*; 2014. p. 115-27. doi: 10.1007/978-3-319-05134-5_6

32. Barbarotto E, Schmittgen TD, Calin GA. MicroRNAs and cancer: profile, profile, profile. *International journal of cancer*. 2008, **122**(5):969-77. doi: 10.1158/0008-5472.CAN-07-1936

33. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer research*. 2005, **5**(21):9628-32. doi: 10.1158/0008-5472.CAN-05-2352

34. Xia L, Zhang D, Du R, Pan Y, Zhao L, Sun S, et al. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *International journal of cancer*. 2008, **123**(2):372-9.

35. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, De Martino I, et al. E2F1-regulated microRNAs impair TGFβ-

dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer cell*. 2008, **13**(3):272-86.

36. Gartel AL, Kandel ES, editors. miRNAs: Little known mediators of oncogenesis. *Seminars in cancer biology*; 2008: Elsevier. doi: 10.1016/j.semcancer.2008.01.008

37. Chen Z, Wang D, Gu C, Liu X, Pei W, Li J, et al. Down-regulation of Let-7 microRNA Increased K-ras Expression in Lung Damage Induced by Radon. *Environmental Toxicology and Pharmacology*. 2015. doi:10.1016/j.etap.2015.08.009

38. Gottardo F, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P, et al., editors. Micro-RNA profiling in kidney and bladder cancers. *Urologic Oncology: Seminars and Original Investigations*; 2007: Elsevier. doi: 10.1016/j.urolonc.2007.01.019

39. Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, et al. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. *Proceedings of the National Academy of Sciences*. 2008, **105**(10):3945-50. doi: 10.1073/pnas.0800135105

40. Zhao H, Wang D, Du W, Gu D, Yang R. MicroRNA and leukemia: tiny molecule, great function. *Critical reviews in oncology/hematology*. 2010, **74**(3):149-55. doi: 10.1016/j.critrevonc.2009.05.001

41. Zanette D, Rivadavia F, Molfetta G, Barbuzano F, Proto-Siqueira R, Falcão R, et al. miRNA expression profiles in chronic lymphocytic and acute lymphocytic leukemia. *Brazilian Journal of Medical and Biological Research*. 2007, **40**(11):1435-40. doi: 10.1590/S0100-879X2007001100003

42. Wu W, Lin Z, Zhuang Z, Liang X. Expression profile of mammalian microRNAs in endometrioid adenocarcinoma. *European Journal of Cancer Prevention*. 2009, **18**(1):50-5. doi: 10.1097/CEJ.0b013e328305a07a

43. Jiang J, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, et al. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clinical Cancer Research*. 2008, **14**(2):419-27. doi: 10.1158/1078-0432.CCR-07-0523

44. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *New England Journal of Medicine*. 2005, **353**(17):1793-801. doi: 10.1056/NEJMoa050995

45. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences*. 2002, **99**(24):15524-9.

46. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proceedings of the National Academy of Sciences of the United States of America*. 2005, **102**(39):13944-9. doi: 10.1073/pnas.0506654102

47. Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer research*. 2009, **69**(14):5761-7. doi: 10.1158/0008-5472.CAN-08-4797

48. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene*. 2009, **28**(10):1385-92. doi: 10.1038/onc.2008.474

49. Su H, Yang J-R, Xu T, Huang J, Xu L, Yuan Y, et al. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer research*. 2009, **69**(3):1135-42. doi: 10.1158/0008-5472.CAN-08-2886

50. Zhang Y, Chao T, Li R, Liu W, Chen Y, Yan X, et al. MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a. *Journal of Molecular Medicine*. 2009, **87**(1):43-51. doi: 10.1007/s00109-008-0403-6

51. Saito Y, Suzuki H, Tsugawa H, Nakagawa I, Matsuzaki J, Kanai Y, et al. Chromatin remodeling at Alu repeats by epigenetic treatment activates silenced microRNA-512-5p with downregulation of Mcl-1 in human gastric cancer cells. *Oncogene*. 2009, **28**(30):2738-44. doi: 10.1038/onc.2009.140

52. NCI. Biomarker: NCI; 2009. Available from: http://www.cancer.gov/dictionary/?searchTxt=biomarker.

53. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends in molecular medicine*. 2014, **20**(8):460-9. doi:10.1016/j.molmed.2014.06.005

54. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, et al. MicroRNAs accurately identify cancer tissue origin. *Nature biotechnology*. 2008, **26**(4):462-9. doi: 10.1038/nbt1392

55. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. *Nature Reviews Cancer*. 2015, **15**(6):321-33. doi:10.1038/nrc3932

56. Iorio MV, Croce CM. microRNA involvement in human cancer. *Carcinogenesis*. 2012, **33**(6):1126-33. doi: 10.1093/carcin/bgs140

57. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, et al. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Molecular cancer*. 2006, **5**(1):24.

58. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin S-F, Dunning MJ, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. *Genome Biol*. 2007, **8**(10):R214. doi: 10.1186/gb-2007-8-10-r214

59. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer cell*. 2006, **9**(3):189-98. doi: 10.1016/j.ccr.2006.01.025

60. Li X, Zhang Y, Zhang Y, Ding J, Wu K, Fan D. Survival prediction of gastric cancer by a seven-microRNA signature. *Gut*. 2010, **59**(5):579-85. doi: 10.1136/gut.2008.175497

61. Caramuta S, Egyhazi S, Rodolfo M, Witten D, Hansson J, Larsson C, et al. MicroRNA expression profiles associated with mutational status and survival in malignant melanoma. *Journal of Investigative Dermatology*. 2010, **130**(8):2062-70. doi: 10.1038/jid.2010.63

62. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *British journal of haematology*. 2008, **141**(5):672-5.

63. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences*. 2008, **105**(30):10513-8. doi: 10.1073/pnas.0804549105

64. Yamamoto Y, Kosaka N, Tanaka M, Koizumi F, Kanai Y, Mizutani T, et al. MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. *Biomarkers*. 2009, **14**(7):529-38. doi: 10.3109/13547500903150771

65. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. *Trends in cell biology*. 2009, **19**(2):43-51. doi: 10.1016/j.tcb.2008.11.003

66. Ghosh AK, Secreto CR, Knox TR, Ding W, Mukhopadhyay D, Kay NE. Circulating microvesicles in B-cell chronic lymphocytic leukemia can stimulate marrow stromal cells: implications for disease progression. *Blood*. 2010, **115**(9):1755-64. doi: 10.1182/blood-2009-09-242719

67. Lima LG, Chammas R, Monteiro RQ, Moreira MEC, Barcinski MA. Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer letters*. 2009, **283**(2):168-75. doi: 10.1016/j.can-

let.2009.03.041

68. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature cell biology*. 2008, **10**(12):1470-6. doi: 10.1038/ncb1800

69. Saleem SN, Abdel-Mageed AB. Tumor-derived exosomes in oncogenic reprogramming and cancer progression. *Cellular and Molecular Life Sciences*. 2015, **72**(1):1-10. doi: 10.1007/s00018-014-1710-4

70. Burden H, Holmes C, Persad R, Whittington K. Prostasomes—their effects on human male reproduction and fertility. *Human reproduction update*. 2006, **12**(3):283-92. doi: 10.1093/humupd/dmi052

71. Babiker AA, Nilsson B, Ronquist G, Carlsson L, Ekdahl KN. Transfer of functional prostasomal CD59 of metastatic prostatic cancer cell origin protects cells against complement attack. *The Prostate*. 2005, **62**(2):105-14. doi: 10.1002/pros.20102

72. Holmgren L, Szeles A, Rajnavölgyi E, Folkman J, Klein G, Ernberg I, et al. Horizontal transfer of DNA by the uptake of apoptotic bodies. *Blood*. 1999, **93**(11):3956-63. doi: 10.1111/j.1423-0410.2002.tb05323.x

73. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Science signaling*. 2009, **2**(100):ra81. doi: 10.1126/scisignal.2000610

74. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature cell biology*. 2007, **9**(6):654-9. doi: 10.1038/ncb1596

75. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell research*. 2008, **18**(10):997-1006. doi: 10.1038/cr.2008.282

76. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell research*. 2008, **18**(10):997-1006. doi: 10.1038/cr.2008.282

77. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer science*. 2010, **101**(10):2087-92. doi: 10.1111/j.1349-7006.2010.01650.x

78. Bandres E, Bitarte N, Arias F, Agorreta J, Fortes P, Agirre X, et al. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clinical Cancer Research*. 2009, **15**(7):2281-90. doi: 10.1158/1078-0432.CCR-08-1818

79. Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M, et al. Low-Level Expression of MicroRNAs< i> let-7d</i> and< i> miR-205</i> Are Prognostic Markers of Head and Neck Squamous Cell Carcinoma. *The American journal of pathology*. 2009, **174**(3):736-45. doi: 10.2353/ajpath.2009.080731

80. Zenz T, Mohr J, Eldering E, Kater AP, Bühler A, Kienle D, et al. miR-34a as part of the resistance network in chronic lymphocytic leukemia. *Blood*. 2009, **113**(16):3801-8. doi: 10.1182/blood-2008-08-172254

81. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene*. 2009, **28**(40):3526-36. doi: 10.1038/onc.2009.211

82. Volinia S, Galasso M, Sana ME, Wise TF, Palatini J, Huebner K, et al. Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. *Proceedings of the National Academy of Sciences*. 2012, **109**(8):3024-9. doi: 10.1073/pnas.1200010109

83. Shibuya H, Iinuma H, Shimada R, Horiuchi A, Watanabe T. Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. *Oncology*. 2011, **79**(3-4):313-20. doi: 10.1159/000323283

84. Dyrskjøt L, Ostenfeld MS, Bramsen JB, Silahtaroglu AN, Lamy P, Ramanathan R, et al. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. *Cancer research*. 2009, **69**(11):4851-60.

85. Maru DM, Singh RR, Hannah C, Albarracin CT, Li YX, Abraham R, et al. MicroRNA-196a is a potential marker of progression during Barrett's metaplasia-dysplasia-invasive adenocarcinoma sequence in esophagus. *The American journal of pathology*. 2009, **174**(5):1940-8. doi: 10.2353/ajpath.2009.080718

86. Ahmad A, Sethi S, Chen W, Ali-Fehmi R, Mittal S, Sarkar FH. Up-regulation of microRNA-10b is associated with the development of breast cancer brain metastasis. *American journal of translational research*. 2014, **6**(4):384.

87. Asangani I, Rasheed S, Nikolova D, Leupold J, Colburn N, Post S, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008, **27**(15):2128-36. doi: 10.1038/sj.onc.1210856

88. Zhao H, Shen J, Medico L, Wang D, Ambrosone CB, Liu S. A pilot study of circulating miRNAs as potential biomarkers of early stage breast cancer. **PLoS One**. 2010, **5**(10):e13735. doi: 10.1371/journal.pone.0013735

89. Berrino F, De Angelis R, Sant M, Rosso S, Lasota MB, Coebergh JW, et al. Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995–99: results of the EUROCARE-4 study. *The lancet oncology*. 2007, **8**(9):773-83.

90. Yue J. miRNA and vascular cell movement. *Advanced drug delivery reviews*. 2011, **63**(8):616-22. doi: 10.1016/j.addr.2011.01.001

91. Weiler J, Hunziker J, Hall J. Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? *Gene therapy*. 2006, **13**(6):496-502. doi: 10.1038/sj.gt.3302654

92. Elmén J, Lindow M, Schütz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. *Nature*. 2008, **452**(7189):896-9.

93. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nature methods*. 2007, **4**(9):721-6. doi: 10.1038/nmeth1079

94. Xiao J, Yang B, Lin H, Lu Y, Luo X, Wang Z. Retracted: Novel approaches for gene-specific interference via manipulating actions of microRNAs: Examination on the pacemaker channel genes HCN2 and HCN4. *Journal of cellular physiology*. 2007, **212**(2):285-92. doi: 10.1002/jcp.21062

95. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A. Small-Molecule Inhibitors of MicroRNA *miR-21* Function. *Angewandte Chemie International Edition*. 2008, **47**(39):7482-4.

96. Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. *The lancet oncology*. 2012, **13**(6):e249-e58.

97. Kumar MS, Erkeland SJ, Pester RE, Chen CY, Ebert MS, Sharp PA, et al. Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proceedings of the National Academy of Sciences*. 2008, **105**(10):3903-8.

98. Lee S-T, Chu K, Oh H-J, Im W-S, Lim J-Y, Kim S-K, et al. Let-7 microRNA inhibits the proliferation of human glioblastoma cells. *Journal of neuro-oncology*. 2011, **102**(1):19-24. doi: 10.1007/s11060-010-0286-6

99. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, et al. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *CELL CYCLE-LANDES BIOSCI-*

ENCE-. 2008, **7**(6):759.

100. Heegaard NH, Schetter AJ, Welsh JA, Yoneda M, Bowman ED, Harris CC. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. *International journal of cancer*. 2012, **130**(6):1378-86. doi: 10.1002/ijc.26153

101. Yang G, Zhang W, Yu C, Ren J, An Z. MicroRNA let-7: Regulation, single nucleotide polymorphism, and therapy in lung cancer. *Journal of Cancer Research and Therapeutics*. 2015, **11**(5):1. doi: 10.4103/0973-1482.163830

102. Osaki M, Okada F, Ochiya T. miRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. 2015. doi: 10.4155/tde.14.122

103. Bonci D, Coppola V, Patrizii M, Addario A, Cannistraci A, Francescangeli F, et al. A microRNA code for prostate cancer metastasis. *Oncogene*. 2015. doi: 10.1038/onc.2015.176

104. Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K, et al. Reduced expression of Dicer associated with poor prognosis in lung cancer patients. *Cancer science*. 2005, **96**(2):111-5. doi: 10.1111/j.1349-7006.2005.00015.x

105. Flavin RJ, Smyth PC, Finn SP, Laios A, O'Toole SA, Barrett C, et al. Altered eIF6 and Dicer expression is associated with clinico-pathological features in ovarian serous carcinoma patients. *Modern Pathology*. 2008, **21**(6):676-84. doi: 10.1038/modpathol.2008.33

106. Foulkes WD, Priest JR, Duchaine TF. DICER1: mutations, microRNAs and mechanisms. *Nature Reviews Cancer*. 2014. doi: 10.1038/nrc3802

107. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature genetics*. 2007, **39**(5):673-7. doi: 10.1038/ng2003

108. Yamada Y, Tabata M, Yasuzaki Y, Nomura M, Shibata A, Ibayashi Y, et al. A nanocarrier system for the delivery of nucleic acids targeted to a pancreatic beta cell line. *Biomaterials*. 2014, **35**(24):6430-8. doi: 10.1016/j.biomaterials.2014.04.017

109. Thakral S, Ghoshal K. miR-122 is a unique molecule with great potential in diagnosis, prognosis of liver disease, and therapy both as miRNA mimic and antimir. *Current gene therapy*. 2015, **15**(2):142. doi: 10.2174/1566523214666141224095610

110. Guinea-Viniegra J, Jiménez M, Schonthaler HB, Navarro R, Delgado Y, Concha-Garzón MJ, et al. Targeting miR-21 to treat psoriasis. *Science translational medicine*. 2014, **6**(225):225re1-re1. doi: 10.1126/scitranslmed.3008089

111. Hagiwara K, Ochiya T, Kosaka N. A paradigm shift for extracellular vesicles as small RNA carriers: from cellular waste elimination to therapeutic applications. *Drug delivery and translational research*. 2014, **4**(1):31-7. doi: 10.1007/s13346-013-0180-9

112. Simonson B, Das S. MicroRNA Therapeutics: the Next Magic Bullet? *Mini reviews in medicinal chemistry*. 2015, **15**(6):467-74. doi: 10.2174/1389557515666150324123208