Review article

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MicroRNAs: New non-invasive diagnostic biomarkers and therapeutic method for cancer treatment

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ABSTRACT

Background: Cancer is a global health problem and the main cause of mortality. Most cancer-associated cases of mortality are the consequences of lack of effective treatment and biomarkers for early diagnosis. New hopes for the improvement of the early diagnosis and treatment of cancer synchronize with the emergence of microRNAs (miRNAs). MicroRNAs are small, noncoding, single-stranded RNAs, the length of which is approximately 18–25 nucleotides and which bind to 3' untranslated region (3'UTR) of the target messenger RNAs (mRNAs), leading to mRNA degradation or translational inhibition; thereby regulating gene expression post-transcriptionally.

Aim: Using microRNAs as promising and potential biomarkers for diagnosis and therapeutic targets.

Methods: The microRNA expression changes in peripheral blood and can be assayed using non-invasive, low-cost, precise, and rapid tools.

Results: It is noteworthy that miRNAs participate in multiple cancer-related biological processes, including proliferation, apoptosis, angiogenesis, drug resistance, invasion, and metastasis. Interestingly, the identified cancer-associated miRNAs, including over-expressed oncogenic miRNAs (oncomiRs) or underexpressed tumor-suppressive miRNAs, are diverse and specific for different tissues and cancer types.

Conclusion: The genetic testing of microRNAs opens up the exciting possibility of early diagnosis and treatment before the onset of metastasis.

Keywords: microRNAs, gene silencing, circulating biomarkers, cancer diagnosis, anticancer therapy, miRNAs detection.

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INTRODUCTION

Cancer was not very common in the past centuries. The increase in its incidence during the 21st century may be a consequence of changing lifestyles and habits as well as increased life expectancy (1). In recent years, it has become a worldwide health problem and the main cause of death, with an increase in its frequency in more developed countries (2). The survival of cancer patients depends on early detection of cancer cells, which can lead to their total removal through surgery. However, detection is impossible at an early stage in most cases of cancer (1). Therefore, mortality is a result of a lack of effective treatment and biomarkers for early diagnosis. Thus, it is necessary to improve the diagnosis and treatment of cancer. New hopes have been given by the emerging microRNAs (miRNAs) as the new biomarkers for early diagnosis and therapeutic targets (3)(4)(5). MicroRNAs are small, noncoding, single-stranded RNAs that are 18–25 nucleotides in length and regulate gene expressions post-transcriptionally by binding to the 3' untranslated region (3'UTR) of target messenger RNAs (mRNAs), leading to mRNA degradation or translational suppression (6).

Molecular mechanisms of gene silencing by microRNA

MicroRNAs regulate 30–90% of mRNAs, and more than 5300 human genes are targeted by miRNAs. One miRNA can target several mRNAs, and there are multiple sites of single mRNA for binding with different miRNAs(7).

Briefly, as shown in Figure 1, in the nucleus RNA, polymerase II transcribes miRNA into primary miRNA. Then, this primary-miRNA is processed by the microprocessor complex Drosha and Pasha, also known as DGCR8, which produces precursor miRNA, that is subsequently transported into the cytoplasm via the Ran-GTP-dependent nuclear transmembrane protein (exportin5). In the cytoplasm, double-stranded miRNA results from the cleavage of the precursor miRNA by the Dicer enzyme. Afterward, this double-stranded miRNA undergoes the loss of one of its strands in order to produce mature single-stranded miRNA (8).

The post-transcriptional gene silencing occurs when mature single-stranded miRNA is associated with the Argonaute family of proteins (AGOs) to perfectly target the complementary sequence of mRNA and the seed region of miRNA (2–7 nucleotides at 5' end) binds with the 3' untranslated region (3'UTR) of mRNA, forming miRNA-induced silencing complex (miRISC). This complex induces the silencing of gene expression through mRNA degradation or suppression of its translation (Figure 1). Notably, in the cases of partially complementary sequences, the Argonaute proteins need to interact with additional proteins of the GW182 family to promote the gene silencing (9).

As shown in Figure 1, miRISC-mediated translational repression occurs at the initiation or post-initiation (such as elongation and termination) steps. Block in translation initiation requires the existence of both the 5-cap structure and poly (A) tail in mRNA. They are important for stimulating eIF4G recruitment, which is a central step in early initiation. Thus, the molecular target of miRISC to inhibit translation initiation is the cap-binding protein eukaryotic initiation factor (eIF) 4E and impairs its function. EIF4E acts with the poly(A)-binding protein (PABP) to recruit eIF4G to the mRNA. Additionally, PABP also has a role in the 60S ribosomal subunit joining for the initiation of the translation (10). Moreover, miRISC can repress translation initiation by interacting with the ribosome anti-association factor (eIF6) to block the association between the 60S and the 40S ribosomal subunits (11).

Repression of translation mediated by miRNA complexes can also take place post-initiation during elongation by dropping off or

releasing the translating ribosomes at multiple sites within the open reading frame (ORF) of mRNA that leads to decrease in polyribosomes loading and, subsequently, in the synthesis of full-length polypeptides. Furthermore, the decrease in transfer RNA (tRNA) activities induced by miRNA complexes causes premature termination. This repression at the termination step can be also caused by recruitment of termination factors (e.g., eRF3) (12).

Additional mechanisms of silencing result in mRNA destabilization and degradation via the loss of the poly(A) tail, which is caused by a complex of CCR4 and CAF1 deadenylases and NOT protein. (13) (14) This

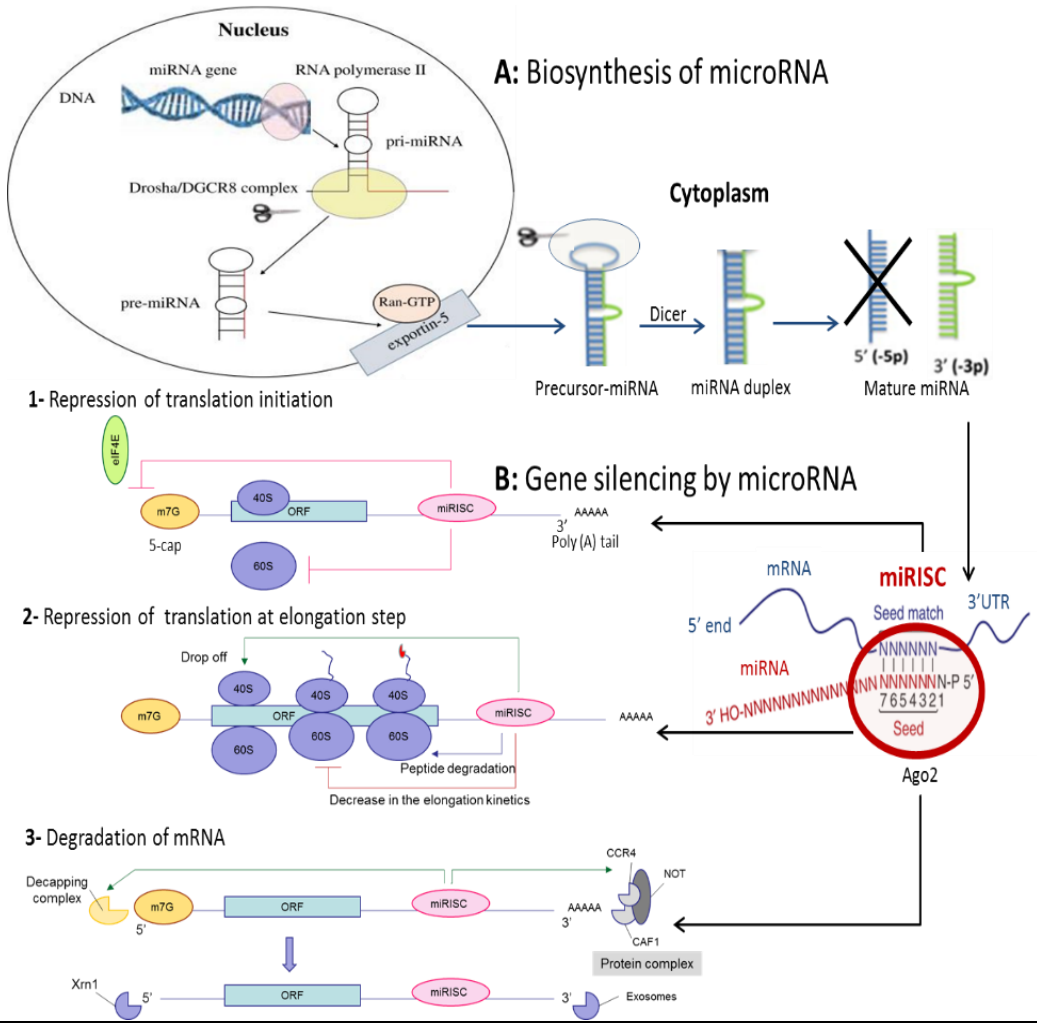

Figure 1: MicroRNAs biogenesis and regulation. (A) MicroRNAs biosynthesize at the beginning within the nucleus in order to produce primary miRNA. Then, they are transported into cytoplasm as precursor miRNAs where they undergo further processing, resulting in temporary miRNA duplex, which converts into mature miRNA by losing one of its double strands. **(B)** Gene silencing takes place in the cytoplasm when a mature miRNA associates with Argonaute proteins and, subsequently, targets complementary sequence of mRNA at its 3'UTR to form miRISC. This complex inhibits gene expression via three mechanisms: repression of translation initiation, suppression of translation post-initiation, or mRNA decay with modifications (14) (9).

losing leads to cap removal and then mRNA decay by exonuclease such as Xrn1 at its 5'end or by the exosomes at its 3'end (Figure 1) (15).

The role of oncogenic and tumor-suppressive miRNAs in cancer-related biological processes

Dysregulation of miRNAs triggers cancer development by disrupting hundreds of the target genes implicated in critical biological processes. For example, miR-101 can modulate diverse target genes involved in various cancer-associated biological processes, including proliferation, apoptosis, angiogenesis, drug resistance, invasion, and metastasis (5). In human esophageal cancer, miR-10b induces migration and invasion by directly targeting the tumor-suppressor gene KLF4 (16). Another example is miR-126, which negatively regulates the invasion in breast cancer by targeting ADAM9 (17). Additionally, expression profiling of serum miRNAs in patients with non-small cell lung cancer and colorectal cancer revealed that most of these miRNAs were involved in general tumorigenesis and cell division or growth (18).

Here, the failure in the expression balance of oncogenic miRNAs (oncomiRs) and tumor-suppressive miRNAs leads to many types of cancers. Overexpression of oncogenic miRNAs has been found in various human cancers, and it significantly induces cancer features such as proliferation, migration, and invasion (19) (20). The overexpression of miR-21 and miR-106 (oncomiRs) in human gastric cancer inhibit tumor-suppressor genes PTEN and Rb, respectively (21). MicroRNA-21 is most common in varied cancer types, including glioblastoma, neuroblastoma, breast, colorectal, lung, pancreas, skin, liver, gastric, cervical, and thyroid, as well as in various lymphatic and hematopoietic cancers. The upregulation of miR-21 can target many crucial tumor-suppressors such as PTEN, PDCD4, RECK, and TPM, which promote cell proliferation, survival, and metastasis (22) (23) (24) (25) (26). On the other hand, cancer can be also caused by the underexpression of tumor-suppressive miRNAs such as miR-34, which is always silent in several tumors and implicated in the p53 pathway (27). Moreover, miR-15 and miR-16 are downregulated in leukemia, which results in an increase of BCL2 (28).

Circulating miRNAs as potential biomarkers for cancer diagnosis

Circulating miRNAs are found free in peripheral blood and other body fluids as well as within lipid microvesicles, exosomes, and apoptotic bodies. Cell-free miRNAs are combined with ribonucleoproteins complexes (such as Argonaute-2) or with high-density lipoprotein. Moreover, microRNAs export into circulatory blood or other body fluids through exocytosis as exosomes (< 100 nM), via budding as microvesicles (100–1000 nM) or as a consequence of necrosis (as Argonaute-2) and apoptosis (as high-density lipoprotein) (29) (30) (31) (32).

Circulating miRNAs in peripheral blood, particularly in serum, are detectable, stable, and resistant to endogenous RNase activity and other harsh conditions. This is useful for strongly proposing them as the diagnostic and prognostic biomarkers for different diseases, including cancers (33) (18).

Most recently, it has been found that serum miRNAs can distinguish patients with specific cancer types from healthy controls as a result of the dysregulation of their expression levels related to cancer. For example, the study of Chen et al. used the Solexa analysis to demonstrate that 63 new miRNAs in the serum of patients with NSCLC (non-small cell lung carcinoma) were not found in normal individuals. Moreover, this study demonstrated that the expression profiling of miR-205, miR-206, and miR-335 is specific for lung cancer, while miR-485-5p, miR-361-3p, miR-326, and miR-487b are specifically related to colorectal cancer (18). Additionally, Heneghan et al. demonstrated that the circulating oncomirs let 7a, miR-10b, and miR-155 were overexpressed in patients with various cancer types, whereas the significant upregulation of miR-195 was specifically associated with breast cancer, which could differentiate patients with breast

cancer from those with other cancers as well as healthy individuals (34). Moreover, a combination of five circulating miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) can discriminate patients with breast cancer from others with different cancer types and controls. Moreover, this panel can also detect breast cancer at an early stage (35). Otherwise, miR-127-3p, miR-148b, miR-409-3p, miR-652, and miR-801 are attractive biomarkers for the early diagnosis of breast cancer and can be detected in stage I or stage II (36). Finally, various recent studies suggest many circulating miRNAs specifically related to cancer types as potential diagnostic and/or prognostic biomarkers. Some of these studies are summarized in Table 1.

MicroRNAs and anticancer therapy

The treatment failure of some cancers results from drug resistance against classical chemotherapy. Therefore, great efforts have been exerted to discover new therapeutic strategies. Hence, miRNAs provide a novel, attractive, and promising approach. MicroRNA-based therapeutics rely on the replacement of tumor-suppressive miRNAs or the suppression of oncomiRNAs expression (67).

Currently, the promising approaches of miRNAs-mediated cancer therapy are based on viral or non-viral (synthetic vectors) miRNA-delivery systems, such as an adeno-associated virus (AAV) (68) as well as non-viral cationic polymer or cationic lipid carrier systems (69)(70). The vectors are necessary to avoid synthetic miRNAs degradation and increase their cellular uptake (71). As a template composed of 71 nucleotides, synthetic or artificial miRNAs are natural double-stranded precursor miRNAs. These precursors undergo, as believed, processing by Dicer within the target cells in order to produce single-stranded mature miRNAs of ~22 nucleotides, in which the stem sequence targets the complementary sequence of the interested gene to selectively inhibit its expression (72).

Table (1): Circulating miRNAs as diagnostic and/or prognostic biomarkers for specific cancer type

Cancer type	Sample	miRNA biomarkers	Expression level	Type of biomarkers	Method	Reference
Glioblastoma	Serum	miR-15b, -23a, -133a, -150, -197, -497	Up	Diagnostic	Solexa sequencing , qRT-PCR	(37)
Breast cancer	Serum	miR-373, -155	Up	Diagnostic	qRT-PCR	(38)
		miR-34a, -17	Down	Diagnostic	qRT-PCR	(38)
		miR-222, -103, -23a, -29a, -23b, -24, -25	Up	Diagnostic	NGS -SOLiD sequencing, Real-time PCR	(39)
	Plasma	miR-148b, -376c, -409-3p, -801	Up	Diagnostic	TaqMan low-density arrays, RT-qPCR	(40)
Lung cancer	Serum	miR-21-3p, -205-5p, -205-3p, -141, -200c	Up	Prognostic	Microarray, qRT-PCR	(41)
		miR-21, -24, -205, -30d	Up	Diagnostic	qRT-PCR	(42)
Liver cancer	Serum	miR-122	Up	Diagnostic	Real-time PCR	(43)
		miR-21, -122, -223	Up	Diagnostic	qRT-PCR	(44)
		miR-16	Down	Diagnostic	qRT-PCR	(45)
Gastric cancer	Serum	miR-221, -376c, -744	Up	Early diagnostic	TaqMan low-density array, qRT-PCR	(46)
		miR-1, -20a, -27a, -34, -423-5p	Up	Diagnostic	Solexa sequencing, qRT-PCR	(47)
	Plasma	miR-106b, -20a, -221	Up	Early diagnostic	qRT-PCR	(48)
		miR-21, -223	Up	Early diagnostic	qRT-PCR	(49)
		miR-218	Down	Diagnostic	qRT-PCR	(49)
		miR-451, -486	Down	Diagnostic	Microarray, qRT-PCR	(50)
Pancreatic cancer	Plasma	miR-16, 21, 155, 181a, 181b, 196a and 210	Up	Early diagnostic	Real-time PCR	(51)
		miR-21, -210, -155, -196a	Up	Early diagnostic	Real-time PCR	(52)
Colorectal cancer	Serum	miR-29a	Up	Early diagnostic	Real-time PCR	(53)
	Plasma	miR-18a, -20a, -21, -29a, -92a, -106b, -133a, -143, -145	Up	Early diagnostic	Microarray, qRT-PCR	(54)
		miR-17-3p, -92	Up	Diagnostic	Real-time PCR	(55)
		miR-92a, -29a	Up	Early diagnostic	Real-time PCR	(56)
		miR-221	Up	Diagnostic and prognostic	qRT-PCR	(57)
Ovarian cancer	Serum	miR-21, -92, -93, -126, -29a	Up	Diagnostic	Real-time PCR	(58)
		miR-155, -127, -99b	Down	Diagnostic	Real-time PCR	(58)
	Plasma	miR-205	Up	Diagnostic and prognostic	TaqMan low-density array, qRT-PCR	(59)
		let-7f	Down	Diagnostic and prognostic	TaqMan low-density array, qRT-PCR	(59)
Prostate cancer	Serum	miR-16, -195, -26a, let7i	Up	Diagnostic	Real-time PCR	(60)
		miR-20b, -874, -1274a, -1207-5p, -93, -106a	Up	Diagnostic and prognostic	multiplex qRT-PCR	(61)
		miR-223, -26b, -30c, -24	Down	Diagnostic and prognostic	multiplex qRT-PCR	(61)
Bladder cancer	Blood	miR-26b-5p, -144-5p, -374-5p	Up	Diagnostic	Microarray, qRT-PCR	(62)
Large B-cell lymphoma	Serum	miR-21, -155, -210	Up	Diagnostic	qRT-PCR	(63)
		miR-15a, -16-1, -29c, -155	Up	Diagnostic	qRT-PCR	(64)
		miR-34a	Down	Diagnostic	qRT-PCR	(64)
Leukemia	Serum	miR-181b-5p, -10a-5p, -93-5p, -129-5p, -155-5p, -320d	Up	Diagnostic	Solexa sequencing, qRT- PCR	(65)
		miR-29c, -223	Down	Prognostic	qRT-PCR	(66)

Using the miRNA-anti-sense/plasmid/mimic delivery approaches to replace downregulated tumor-suppressive miRNAs, certain studies conducted on mice with aggressive cancer achieved success in the miRNA-based therapies (i.e. *miR-145*, *miR-107*, *miR-34a)* (69) (73) (74) (75). A novel anionic lipopolyplex nanocarrier system was used by Huang et al. to deliver synthetic miRNA mimic molecules to the cells of acute myeloid leukemia (AML) for therapeutic increase in miR-29b, which is underexpressed in these cells. It is noteworthy that the overexpression of miR-29b was revealed after classical chemotherapy in the patients with AML and was associated with longer survival, which indicates the anti-leukemic activity of this miRNA. Thus, there is clinical benefit in raising the expression level of miR-29b in AML cells (71).

The second miRNA-based therapeutic approach is the repression of the oncogenic miRNAs (oncomiRNAs) expression using anti-miRNA oligonucleotides (antagomirs). These synthetic antisense oligonucleotides are single-stranded RNA molecules that competitively target the complementary sequence of endogenous mature miRNA in order to prevent its interaction with target genes (76)(77). Other targets of synthetic oligonucleotides are primary and precursor miRNAs. Velagapudi et al. designed a dimeric small molecule named Targaprimir-96 in order to target primary miR-96 and inhibit Drosha processing. MicroRNA-96 is an oncogenic that is overexpressed in breast cancer and inhibits the apoptosis by repressing the synthesis of pro-apoptotic forkhead box O1 (FOXO1) protein in cancer cells (78). However, in comparison to the delivery of synthetic tumor-suppressive miRNA molecules, few studies used nanotechnological antagomir delivery (as polymers) and drugs to suppress the overexpressed oncogenic miRNAs *in vitro* and *in vivo*. The delivered antisense miRNA molecules used in these studies are costly, highly active, and probably lead to side effects on target cells. In addition, there is a limit to the use of viral vectors or plasmids in laboratory studies. Otherwise, the synthetic DNA nanostructures carrying DNA sequences that are complementary to the oncogenic miRNAs are considered promising anticancer therapy. They can effectively inhibit the high levels of oncomiRNAs by competing with mature miRNA duplexes or miRNA-induced silencing complexes and, subsequently, suppress cancer cell proliferation (79).

MicroRNAs detection

Early detection of cancer based on protein indicators of serum is limited and remains labour-intensive. Currently, the best available blood test is a carcinoembryonic antigen, but it shows low sensitivity and specificity, particularly in the early stages of cancer. Recently, a proteomic analysis nominated a set of differentially expressed proteins as cancer markers for significant and accurate diagnosis, but its protein assay procedure is complicated for application in clinical diagnosis (80) (81).

However, expression profiling of miRNAs has a great benefit in clinical application by providing a panel of miRNAs rather than a single miRNA that can be used as a promising molecular diagnostic and prognostic biomarkers for diverse tumors even in early stages or for discriminating between cancer patients and healthy individuals (82).

Interestingly, extraction of high-quality miRNAs is possible from a broad range of cells, tissues, and body fluids, such as cell lines, fresh tissues, formalin-fixed paraffin-embedded tissues, plasma, serum, urine, and others. For miRNAs isolation, the products are commercially available such as miRNeasy (Qiagen), *mir*Vana™ (Ambion), and PureLink™ (Invitrogen) miRNA isolation kits (83). It is important to assess the miRNA quantity and quality using NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer (84).

MicroRNA profiling is currently performed using three major approaches, including hybridisation-based methods (e.g., DNA microarrays), high-throughput sequencing (e.g., RNA-seq), and validation with real-time quantitative reverse transcription (e.g., qRT-PCR) (82) (83). Importantly, qRT-PCR is widely used with a tiny amount of RNA from clinical samples and considered an important method to easily detect a defined set of miRNAs because it is more sensitive, specific, and reliable as well as less costly than other methods (85) (29).

Conclusion

In conclusion, there are two types of microRNAs involved in cancer: oncogenic and tumor-suppressive miRNAs. During cancer, the oncogenic miRNAs are upregulated, while others are downregulated. A change of their expression profiling is specifically related to cancer type. Each type of cancer correlates with a different set of miRNAs that serves to introduce them as novel potential targets for anti-cancer therapies and as promising diagnostic and

predictive biomarkers. Furthermore, it is possible to detect the expression levels of miRNAs in peripheral blood using an available, easy, sensitive, specific, and rapid method (e.g., qRT-PCR) that can use a panel or cluster of miRNA biomarkers (no single or in couple) as a non-invasive significant diagnostic and prognostic tool for the early detection of different tumors.

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