Clinical and Experimental Medicine (2020) 20:349–359
https://doi.org/10.1007/s10238-020-00627-2

REVIEW ARTICLE

The current state of MiRNAs as biomarkers and therapeutic tools

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Received: 31 October 2019 / Accepted: 3 April 2020 / Published online: 12 May 2020

Abstract
MicroRNAs (miRNAs) are non-coding RNAs with a length of 18–22 nucleotides that regulate about a third of the human genome at the post-transcriptional level. MiRNAs are involved in almost all biological processes, including cell proliferation, apoptosis, and cell differentiation, but also play a key role in the pathogenesis of many diseases. Most miRNAs are expressed within the cells themselves. Due to various forms of transport from cells like exosomes, circulating miRNAs are stable and can be found in human body fluids, such as blood, saliva, cerebrospinal fluid, and urine. Circulating miRNAs are of great interest as potential noninvasive biomarkers for tumors, lipid disorders, diabetes mellitus, and cardiovascular diseases. However, the possibility of their use in the clinic is limited, and this is associated with a number of problems since currently there are significant differences between the procedures for processing samples, methods of analysis, and especially strategies for standardizing results. Moreover, miRNAs can represent not only potential biomarkers but also become new therapeutic agents and be used in modern clinical practice, which again confirms the need for their study.

Keywords miRNAs · Circulating · Noninvasive biomarkers · Therapy · Tumor · Cardiovascular disease

Introduction

MicroRNAs (miRNAs) are 18–22 nucleotide endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level by interacting with 3′-untranslated regions (3′-UTR) of mRNA targets [1]. The biogenesis of mature miRNA involves a series of biological processes (Fig. 1). A primary miRNA transcript (pre-miRNA) is first transcribed in the nucleus by RNA polymerase II/III, which is subsequently cleaved by Drosha/Pasha (DGCR8) proteins into a precursor miRNA (pre-miRNA). The pre-miRNA is exported from the nucleus to the cytoplasm by Exportin-5 (XPO5) and loaded onto Dicer; the loop is then cleaved, producing a double-stranded structure composed of miRNA and antisense miRNA. The latter is usually degraded, whereas the long mature miRNA strand is incorporated into the miRNA-induced silencing complex (RISC), leading to gene silencing via mRNA cleavage or translational repression depending on the degree of complementarity between the miRNA and mRNA targets transcript [1–3]. Over 60% of human protein-coding genes are predicted to contain miRNA-binding sites in their 3′-UTR. In humans, a single miRNA has several dozens or even hundreds of mRNA targets. It has been proven that miRNAs play a significant role in various biological processes, including the cell cycle, apoptosis, proliferation, differentiation, etc. [1]. Importantly, dysregulation of miRNA is found to be involved in many diseases, such as various tumors and cardiovascular diseases [4, 5]. Furthermore, dysregulation of specific miRNAs can be utilized to identify potential miRNAs candidates for therapeutic intervention [6]. In fact, the emerging miRNA therapeutics with its ability to target multiple pathological target genes may likely yield one of the most exciting breakthroughs in the current treatment options for various diseases, including tumors and cardiovascular disease [7]. Most miRNAs are expressed within the cells themselves. However, in many biological fluids of the human body, such as

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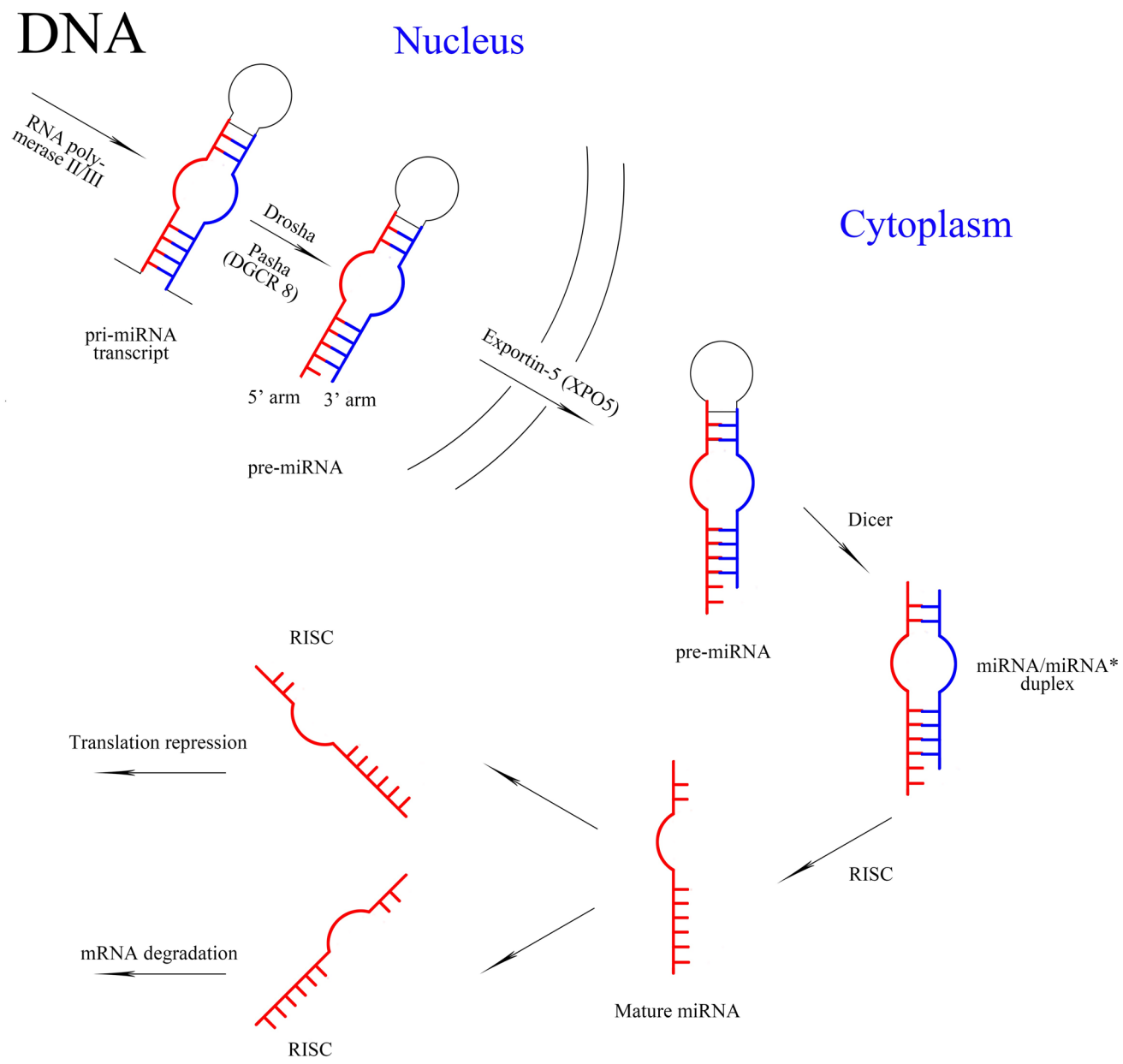

Fig. 1 MiRNA biogenesis

blood, saliva, urine, and cerebrospinal fluid (CSF), numerous miRNAs, called circulating miRNAs, have been found [8]. To date, there are three known ways of secretion of circulating miRNAs: (1) passive secretion from damaged cells due to apoptosis or necrosis; (2) active secretion using extracellular vesicles (EV); and (3) active secretion using an RNA-binding protein-dependent pathway (Fig. 2) [8, 9]. Such miRNAs are resistant to nucleases, which make them attractive as potential biomarkers. Given their biological importance, miRNAs are currently recognized as novel biomarkers and potential therapeutic targets for developing new interventions. This review will focus on those findings that have provided the rationale for the use of miRNAs as noninvasive biomarkers and therapeutic agents. We also present several known limitations of miRNAs exploitation, as well as future challenges and ongoing industrial developments.

Circulating miRNAs as noninvasive biomarkers

Circulating miRNAs have been detected in the peripheral blood (plasma/serum or whole blood) circulation and other body fluids. Owing to their stability and resistance to

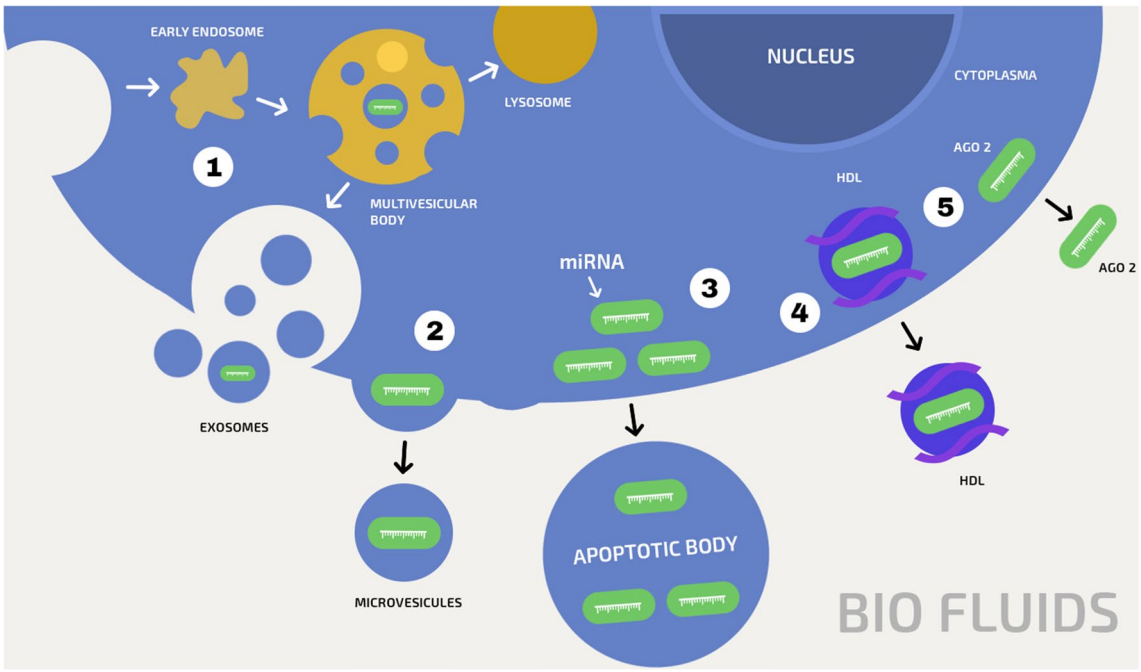

Fig. 2 Pathways of miRNA secretion. Microvesicles (2) are formed after the plasma membrane exits and have a size of 100–1000 nm, while exosomes (1) are much smaller (40–100 nm) and are released after the fusion of the fraction of multivesicular bodies with the plasma membrane. About 90% of circulating miRNAs are in a non-vesicular form, namely they are bound to Ago2 proteins (miRNA-Ago2) (5). MiRNAs were also found in high density lipoproteins (HDL) (4), the size of which varies from 9 to 12 nm. Finally, apoptotic bodies of 1–4 nm in size, which also contains miRNAs (3), can be found in biological fluids

endogenous RNase activity, these miRNAs have been proposed as diagnostic and prognostic biomarkers for diseases, such as tumors and cardiovascular diseases. Table 1 [9–16] and Table 2 [17–24] summarize the circulating miRNAs as novel potential biomarkers in human tumors and cardiovascular diseases. However, after some first promising results, it became apparent that the precise quantification of circulating miRNAs was more complex than expected. The question is why the expression profile of certain circulating miRNAs does not always have specificity for a particular pathology and is it possible to use miRNAs in clinical practice as effective biomarkers. Until now, despite numerous

Table 1 Circulating miRNAs as possible biomarkers for cardiovascular diseases

Disease	miRNA	Sample	Regulation	Diagnostic	Prognostic	Sensitivity (%)	Specificity (%)	AUC	References
AMI	miR-133a	Plasma	Up	Yes	Yes	81.2	92.8	0.87	[9]
AMI	miR-208b	Plasma	Up	No	Yes	/	/	/	[10]
IS	Exosomal miR-134	Serum	Up	Yes	Yes	75.3	72.8	0.83	[11]
ICH	miR-145	Plasma	Up	Yes	No	/	/	0.76	[12]
	miR-181b		Down	Yes	No	/	/	0.78	
AHF	miR-302b-3p	Serum	Up	Yes	No	82.2	92.7	0.87	[13]
CHF	miR-122	Plasma	Up	Yes	Yes	76.0	86.0	0.81	[14]
ACS	miR-21	Serum	Up	Yes	No	74.0	70.0	0.76	[15]
AAA (after endovascular treatment)	miR-191	Whole blood	Down	No	Yes	/	/	/	[16]
	miR-455-3P		Down	No	Yes	/	/	/	

AUC ≥ 0, 75 is considered diagnostically significant for the biomarker; Kaplan–Meier curves and log-rank tests were used in articles to evaluate the prognostic significance of circulating miRNAs in cardiovascular disease;/, not mentioned in article

miRNA, microRNA; AUC, area under ROC curve; AMI, acute myocardial infarction; IS, ischemic stroke; ICH, intracerebral hemorrhage; AHF, acute heart failure; CHF, chronic heart failure; ACS, acute coronary syndrome; AAA, abdominal aortic aneurysm

Table 2 Circulating miRNAs as possible biomarkers for tumors

Tumor type	miRNA	Sample	Regulation	Diagnostic	Prognostic	Sensitivity (%)	Specificity (%)	AUC	References
GBM	miR-203	Serum	Down	Yes	Yes	73.36	85.86	0.86	[17]
NSCLC	miR-182	Serum	Up	Yes	Yes				[18]
	miR-200b		Down	Yes	Yes	64.5 (combination)	74.6 (combination)	0.781 (combination)	
	miR-205		Up	Yes	Yes				
BC	miR-155	Serum	Up	Yes	No	65.0	81.0	0.80	[19]
HCC	Exosomal miR-21	Serum	Up	No	Yes	/	/	/	[20]
PC	miR-141-3p	Serum	Up	Yes	No	/	/	0.831	[21]
	miR-375		Up	Yes	No	/	/	0.906	
	miR-21		Up	Yes	No	/	/	0.856	
CL	Exosomal miR-23a	Serum	Up	Yes	No	/	/	0.90	[22]
	Exosomal miR-301a		Up	Yes	No	/	/	0.84	
GC	miR-25	Serum	Up	Yes	Yes	69.4	81.0	0.76	[23]
Osteosarcoma	miR-195	Serum	Down	Yes	Yes	88.0	83.0	0.89	[24]

AUC ≥ 0, 75 is considered diagnostically significant for the biomarker; Kaplan–Meier curves and log-rank tests were used in articles to evaluate the prognostic significance of circulating miRNAs in tumors; /, not mentioned in article

miRNA, microRNA; AUC, area under ROC curve; GBM, glioblastoma multiforme; NSCLC, non-small-cell lung carcinoma; BC, breast cancer; HCC, hepatocellular carcinoma; PC, prostate cancer; CL, colorectal cancer; GC, gastric cancer

studies over the past decade, their results are not consistent with the use of circulating miRNAs in the clinic [25]. This can be explained by differences in research methods, lack of standard methods for data normalization and inability to distinguish closely related miRNAs [26]. Identification and solution of a number of problems at the pre- and post-analytical stages can have a strong impact on the result of the study. The pre-analytical stage includes sample collection, it is processing, the method of extraction of circulating miRNAs from biological fluids, and quality control. The main problem of the post-analytical stage is data normalization [27].

The use of blood to detect circulating miRNAs is an obvious source, since the collection is minimally invasive and blood samples are regularly taken in clinics. In addition, since sampling is minimally invasive, it allows repeated sampling and can be used as a diagnostic and prognostic tool for monitoring the condition of a disease over a period. However, when profiling the expression of circulating miRNAs in blood samples, several critical points must be taken into account. The venipuncture site itself may induce hemolysis and thereby contamination by platelet-derived miRNAs [28]. In addition, the period between collection and blood processing is also crucial, as this can affect the amount of lysis and thus cellular contamination [28]. For the detection of circulating miRNAs in blood samples, it is important to determine whether to use serum or plasma as the source. The disadvantage of using serum rather than plasma is the release of platelet-derived miRNAs into the serum during clot formation [29]. The time for clot formation for the serum sample may also influence the amount of circulating miRNAs and downstream analyses. If less than 30 min is allowed for clot formation, cellular elements and other contaminating factors are likely to be retained in the specimen, influencing downstream analyses, whereas 60 min of clot formation may induce hemolysis of cells in the clot. Although the use of plasma may eliminate some of the problems associated with hemolysis, a drawback of using plasma is, however, the need for anticoagulants [30]. Commonly used anticoagulants for plasma collection are ethylenediaminetetraacetic acid (EDTA), citrate, or heparin. It should be noted that some anticoagulants, such as heparin and citrate, inhibit enzymes used in the work of the polymerase chain reaction (PCR), such as reverse transcriptase [31]. Glinge et al. found that circulating miRNA expression profiling was better in EDTA-anticoagulated blood compared with blood collected in citrate and lithium heparin [32]. The choice among regularly used anticoagulants should be EDTA.

The level of expression of circulating miRNAs depends on many factors, such as individual variability (sex, ethnicity, and age) and external differences and lifestyles (smoking habits, drug assumption, different treatments, diet, and physical activity) [33]. For example, sex, ethnicity, and age can be adjusted for in the patient selection. However, most

of the external differences are difficult to evaluate and to take into consideration when selecting the patient and control groups. Hence, when evaluating newly identified circulating miRNAs as potential biomarkers for human disease, it is important to discuss the possibility of these being identified due to differences in individual behavior rather than differences in disease states [34]. Therefore, besides the careful selection of a homogenous patient group, it is important to calculate the power of the study, in order to determine the number of samples needed for both patients and control groups to eliminate some of the external differences between individuals and detect the true effects the study aims at.

The method used for the extraction of the circulating miRNAs from the biological fluid sample can also influence the final result. Numerous different protocols and commercially ready-to-use kits, such as miRNeasy Serum/Plasma Kit (Qiagen) and MagMAX mirVana Total RNA Isolation Kit (ThermoFisher Scientific), are available for circulating miRNAs extraction from biological fluids [35]. The low levels of circulating miRNAs in biological fluids also impair quantification of the extracted RNA using a spectrophotometer. It is therefore important to use a fixed volume of input material for all samples in the extraction to eliminate differences detected downstream, as a result of different amounts of starting material. During the circulating miRNA extraction, potential PCR inhibitors may be isolated together with the miRNAs. It is therefore important to determine the purity of the extracted miRNA, by measuring the 260/280 nm ratio for protein contamination and the 260/230 nm ratio for guanidine salts and phenol contaminants [36].

One of the main factors limiting the use of circulating miRNAs as a diagnostic tool, for example, in oncology, is related to the fact that the reported same circulating miRNAs are found in patients with various types of tumors. For example, overexpression of circulating miR-21 was found in the blood of patients with glioma, colorectal cancer, lung cancer, breast cancer, prostate cancer, esophageal cancer, and hepatocellular carcinoma [37]. Circulating miR-141 has been identified as a potential biomarker for at least 6 different tumor types in numerous studies, including prostate cancer, hepatocellular carcinoma, colorectal cancer, bladder cancer, lung cancer, and breast cancer [33]. In addition, the results are not consistent even among very similar studies with the same diseases. The detection of circulating miRNAs may also depend on the biological fluid and even on the forms of miRNA transportation. Shi et al. examined the CSF and serum from patients with recurrent glioma and non-glioma patients as a control group for the levels of cancer-related miRNAs and evaluated the values for prognosis by comparing the measures of CSF-, serum-, and exosome-contained miR-21 levels [38]. The results showed the exosomal miR-21 drastically decreased in CSF after surgical operations. However, no difference was detected in the serum samples pre- or post-surgeries. In this study, they hypothesized that exosomal miR-21 from the CSF of glioma patients may be a reliable, robust, and practical index for the assessment of tumor progression and prognosis. The continuous estimation about the diagnostic value of miR-21, especially in the CSF vesicles, might encompass a complex of microvesicles, apoptotic bodies as well as retrovirus-like particles (RLPs) [39]. By having a clear picture of the circulating miRNAs bio-dynamics, understanding the mechanism through which miRNAs travel in the blood or in the CSF could also improve the diagnostic method. Decreased expression of endogenous miRNAs in tumors may result from genetic changes or mechanisms of epigenetic regulation, but changes in the expression level of circulating miRNAs in biological fluids can occur only if the tumor itself negatively affects the expression of endogenous miRNAs in other healthy cells or reduces the stability of certain circulating miRNAs secreted from healthy cells [40]. In other words, a decrease in the expression level of circulating miRNAs can be attributed to non-specific responses to the presence of a tumor. In addition, the presence of a tumor cannot be correlated with the activation of circulating miRNAs. Due to the anticoagulant system of the blood, only a small number of tumors, for example, late-stage tumors, lead to the excessive expression of specific circulating miRNAs in the blood. Accordingly, it is more likely that any activation of circulating miRNAs is also the result of the response (s) to the presence of a tumor in the body [28].

Since no universal housekeeping genes were found to normalize the data, a relative quantitative assessment of changes in the expression of circulating miRNAs in biological fluids faced a serious problem. Among these housekeeping genes, RNU6A (U6) or SNORD44 is widely used for normalization. However, they belong to the class of small nuclear RNAs with properties different from miRNAs. In a study to search for suitable housekeeping genes, Xiang et al. analyzed three genes (U6, hsa-miR-16, and hsa-miR-24) in plasma and serum and demonstrated the low efficiency of widely used U6 [41]. In a study to normalize data, Wang et al. analyzed seven common housekeeping genes (5S ribosomal RNA (rRNA), hsa-miR-16, U6, hsa-miR-19b, hsa-miR-24, hsa-miR-15b, let 7i) and demonstrated low efficiency of U6 [42]. Today hsa-miR-16 is very often used to normalize data. It has been described as stable and highly expressed in blood [43–45]. However, among confirmed human miRNAs as reference genes, including miR-16, miR-10b, miR-30a, miR-30d, miR-103, miR-148b, miR-191, and miR-192, differentiated expression in serum or plasma in patients with breast cancer in several works [28]. Therefore, it is important to verify the stability of the expression of putative genes for normalization in a particular pathology. An absolute quantitative assessment of circulating miRNAs

may be useful just for diagnostic purposes, especially taking into account the fact that there is no consensus on the housekeeping gene.

Common methods for analyzing the expression profile of circulating miRNAs are real-time polymerase chain reaction (qRT-PCR), droplet digital polymerase chain reaction (ddPCR), microarrays, and next-generation sequencing (NGS) [46]. Profiling hundreds of differentially expressed circulating miRNAs from biological fluids is a technical problem. Determination of the expression profile of circulating miRNAs using qRT-PCR can be performed by the absolute or relative method. qRT-PCR has been used for several years to analyze the expression profile of circulating miRNAs and is considered the reference method [47]. The advantage is that it can be easily used in everyday practice. In addition, it is sensitive, specific and offers a wide range of measurements. Using this technology, it is possible to profile expression individually for a specific circulating miRNA or in the form of panels of several hundred circulating miRNAs [48]. Microarrays are a miniature hybridization system that allows simultaneous high-level analysis of several hundred circulating microRNAs. This method offers the possibility of conducting combinatorial analysis between circulating miRNAs and gene expression on one sample, which allows us to study the function of miRNAs and target genes [49]. However, the efficiency of miRNA extraction from biological fluids is much lower than from cells or tissues, and microarrays do not actually represent a quantitative analysis method. Therefore, microarrays should be used for initial screening, followed by validation of the circulating miRNAs of interest and their expression profiling using qRT-PCR. Moreover, microarrays have lower sensitivity and specificity than qRT-PCR and are not reliable [48]. NGS is currently one of the best platforms for detecting circulating miRNAs, which is extremely sensitive and provides data on their relative expression in a sample with a large dynamic range compared to microarrays [50]. Compared with other technological approaches for profiling the expression of circulating miRNAs, such as NGS, ddPCR has a large dynamic range (10^6 versus 10^4 and 10^5, respectively) and significantly higher sensitivity [51]. These advantages make ddPCR also an attractive method for use both in studies devoted to the fundamental issues of regulation of gene expression of miRNAs and, in the future, in clinical diagnostic laboratories for routine assessment of the expression level of circulating miRNAs in patients with different pathologies.

In summary, each technology has its advantages and limitations. The platform should therefore be selected based on the particular study requirements and questions addressed (Table 3).

MiRNAs as potential therapeutic tools

As miRNAs are important for various cellular homeostasis functions, their role extends to a number of disease manifestations beyond tumors like cardiovascular disease. However, there are two main strategies for using miRNAs as therapeutic agents: (1) restoring the downregulated miRNA target or (2) inhibiting the overexpressed miRNA target [52]. Restoring the downregulated miRNA target can be achieved with miRNA mimics, which are synthetic double-stranded RNA molecules with an identical sequence as natural miRNAs that are able to integrate into the RISC and perform the function of the missing miRNA [53]. Several preclinical studies using miR-34 mimics have demonstrated their potential as antitumor therapeutics. For example, lipid nanoparticle-encapsulated miR-34 mimics showed promising activity of liver and lung cancer in vivo [54, 55]. Bejerano et al. present a new therapeutic strategy to manipulate macrophage phenotype using nanoparticle delivery of miR-21 mimic with a potential for use to attenuate post-myocardial infarction remodeling and heart failure [56].

The anti-miRNAs, which are oligonucleotides with complementary sequences to miRNA, can inhibit the function of the specific miRNAs and the inhibition effect of anti-miRNAs on the growth of tumor cells or reduce the inflammatory process [57]. Yang et al. designed a tumor-targeting anti-miR-155 delivery system based on biodegradable poly(ester amine) and hyaluronic acid shielding (PEA/anti-miR-155/HA–peptide complexes) for lung cancer therapy. The PEA/anti-miR-155/HA–peptide complexes could

Table 3 Selection of miRNA profiling platform

miRNA profiling platform	Sensitivity	Specificity	Accuracy	Reproducibility	Throughput	Flexibility	Standardization	Dynamic range
qRT-PCR	+++	+++	+++	++/+++	+/++	+++	+++	+++
Microarray	+/++	++	+	+++	+++	++	+++	+/++
NGS	+++	++/+++	++	++/+++	+++	+++	+/++	++/+++
ddPCR	+++	++/+++	+++	+++	++	++/+++	+++	+++

Sensitivity, specificity, accuracy, reproducibility, throughput, flexibility (refers to ease of customization), standardization, and dynamic range are classified as follows: +++ (very high); ++ (moderate); + (low); ++/+++ (very high to moderate); and +/++ (moderate to low)

deliver anti-miR-155 into lung cancer cells and played an active role in tumor growth inhibition [58].

The potential application of anti-miRs and miRNAs mimics as therapeutic agents in vivo has been challenging owing to many limitations including rapid degradation in blood conditions and the lack of an effective delivery vector [59]. Therefore, some synthetic cationic materials can be used as effective gene delivery vectors, such as liposomes, polyethylenimine (PEI), and other non-viral polymers, which have been used to study the delivery capacity of miRNA [54–56, 58]. Although future studies are needed to address long-term efficacy and safety, these data provide proof of concept for the systemic delivery of a miRNAs mimic and anti-miRNAs, obviating obstacles associated with viral-based miRNA delivery and facilitating a rapid route for miRNA replacement therapy into the clinic.

A clear idea of target miRNAs in tumor therapy, which determines the number of oncogenes or tumor suppressors that a particular miRNA is aimed at, is still in doubt. The ability of microRNAs to target multiple genes is attractive because this feature may facilitate targeting to multiple compensatory pathways [60]. However, a particular miRNA target may include both oncogenes and tumor suppressors, as well as a number of targets not involved in oncogenesis, which complicates the development of selective miRNA-based therapy [61]. In addition, by decreasing or increasing the level of target miRNA expression, especially at non-physiological concentrations, they may have unknown to us the genes of normal cell homeostasis regulated by them, which could potentially lead to adverse effects [62].

A problem that continues to interfere with existing treatment options in modern clinical practice is drug resistance to chemotherapy in patients with tumors. A number of published reports show that manipulating the expression of specific miRNAs may alter chemotherapeutic drug sensitivity or that miRNAs themselves are biologically involved in the body's response to resistance [63, 64]. Many of the standard therapies for glioma, such as temozolomide and demethoxycurcumin, are all associated with deregulated miRNAs, which may modulate resistance to the chemotherapeutic drug [65, 66]. Evidence suggests that select miRNAs may even participate in the treatment of malignant gliomas, such as glioblastoma multiforme (GBM), which is highly resistant to chemotherapy [66]. Accumulating evidence shows that the ATP-binding cassette (ABC) transporter family of proteins that activate drug resistance are regulated by miRNAs [67, 68]. Lv et al. showed that miR-155 is highly expressed in doxorubicin-resistant non-small-cell lung carcinoma (NSCLC) cells, and inhibition of miR-155 by antisense oligonucleotides reversed doxorubicin resistance and decreased the ABC transporters breast cancer resistance protein (BCRP), P-glycoprotein, and multidrug resistance-associated proteins 1 (MRP1), in the doxorubicin-resistant cell line NSCLC [68]. The mechanisms of chemotherapeutic drug resistance mediated by miRNAs are under ongoing investigation. It is possible that co-administration of standard chemotherapeutic drugs with the selected miRNA (miRNA mimic or anti-miRNA) can help in some cases limit drug resistance by suppressing key genes that directly contribute to the low bioavailability of the drug, or through alternative signaling pathways, such as miR-155, for lung cancer.

Table 4 presents information about miRNA-based therapy tumors and cardiovascular disease [69–79].

Conclusion

Circulating miRNAs are considered today as potential biomarkers of human pathologies. The success of their implementation in personalized medicine will largely depend on the availability of a method that allows for the efficient verification and validation of promising biomarkers based on circulating miRNAs. Focusing on certain forms of transportation (exosomes), improving sampling and extraction methods combined with absolute quantification without using the housekeeping gene (s) may lead to the practical use of circulating miRNAs as biomarkers in the future. You should know which biological fluid of the human body and in what pathology this biological fluid is best suited for measuring the level of expression of circulating miRNAs. In addition, the discovery of new circulating miRNAs should be further confirmed in independent studies. The use of a panel of two or more selected circulating miRNAs can be more effective and guarantee specificity for one or another pathology, such as a tumor.

The detection of more miRNAs associated with pathogenesis of diseases can lead to the development of combination therapy, including regulation of miRNA expression and introduction into the clinic. Some caution should be exercised with respect to possible side effects of miRNAs in human trials, with particular emphasis on the safety, tolerability, and effectiveness of treatment. The optimal dose and time of therapeutic intervention with specific inhibitors or mimicry of miRNAs should be carefully determined to avoid any destructive intervention in the process of natural regeneration. This approach in such therapy should be based on knowledge of the specific function of miRNAs, including its effect on the viability, proliferation, and differentiation of the cellular component. The degree of expression of specific miRNAs should also be considered. A moderate decrease or increase in miRNA expression may be the best choice, in order to avoid secondary non-specific side effects.

Table 4 Clinical trials using miRNA therapy in cardiovascular disease and tumors

Company	Drug name	miRNA target	Therapy type	Disease	Effect	Stage of development	References
miRagen therapeutics	MRG-110	miR-92a	Anti-miR	Myocardial infarction, limb ischemia, vascular injury, and wound healing in rodents	Induces angiogenesis and tissue repair	Phase I	[69]
MiRNA therapeutics	MRX34	miR-34	Mimic	NSCLC, primary liver cancer, lymphoma, melanoma, multiple myeloma, renal cell carcinoma	Reduction in the expression of oncogenes (MET, MEK1, MYC, PDGFR-α, CDK4/6, BCL2, WNT 1/3, NOTCH1, CD44), tumor regression, enhanced the survival, and inhibited the growth	Phase I	[70, 71]
EnGeneIC	MesomiR-1	miR-16	Mimic	NSCLC, MPM	Inhibition of tumor growth	Phase I	[72]
miRagen therapeutics	Cobomarsen (MRG-106)	CTCL	Anti-miR	CTCL, mycosis fungoides subtype	Inhibits cell proliferation and induces apoptosis	Phase II	[73, 74]
miRagen Therapeutics	MGN-1374	miR-15 and miR-195	Anti-miR	Heart failure; Post-MI	Improvement in the proliferation of cardiomyocytes	Preclinical stage	[73]
miRagen Therapeutics	MGN-2677	miR-143 and miR-145	Mimic	Hypertension	Increase under shear stress	Preclinical stage	[75]
miRagen Therapeutics	MGN-4220	miR-29	Mimic	Cardiac fibrosis	Targets multiple components of the fibrosis pathway	Preclinical stage	[76]
miRagen Therapeutics	MGN-6114	miR-92	Anti-miR	Peripheral arterial disease	Improves re-endothelialization	Preclinical stage	[76]
miRagen Therapeutics	MGN-9103	miR-208 and miR-499	Anti-miR	CHF	Not specified	Preclinical stage	[77]
Rosetta Genomics	MiR-34a mimetic	miR-34a	Anti-miR	Cancer	Tumor suppressor protein p53 modulators	Preclinical stage	[78]
Regulus therapeutics	Not specified	miR-350 and miR-10b	Anti-miR	Neuroblastoma	Tumor suppressor protein p53 modulators	Preclinical stage	[74, 79]

miRNA, microRNA; NSCLC, non-small-cell lung carcinoma; MPM, malignant pleural mesothelioma; CTCL, cutaneous T cell lymphoma; MET, MET proto-oncogene, receptor tyrosine kinase; MEK1, dual-specificity mitogen-activated protein kinase kinase 1; PDGFR-α, platelet-derived growth factor receptor alpha; CDK4/6, cyclin-dependent kinase 4 and 6; BCL2, B cell lymphoma 2; NOTCH1, notch homolog 1, translocation-associated; MI, myocardial infarction; CHF, chronic heart failure

Authors contribution IG, OB, and GY contributed to conception and design. VP, AI, and JS helped in the acquisition of data. IG and OB performed analysis and interpretation of data. IG drafted the article. GY and VP critically revised the article. SZsupervised the study and approved the final version of the manuscript on behalf of all authors.

Funding This study was funded by Grant of the Republic of Bashkortostan to young scientists of February 7, 2020 No. CD-43.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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