Minireview

MicroRNAs as biomarkers for clinical studies

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Impact statement

This review summarizes the current knowledge on the role of circulating miRNAs as clinical diagnostic biomarkers and highlights the challenges that need to be addressed in future studies for a successful translation of circulating miRNAs into a novel diagnostic tool.

Abstract

The development of better diagnostic and prognostic non-invasive biomarkers holds an enormous potential to improve the ability to diagnose and individualize treatment of a great number of human diseases and substantially reduce health care cost. The discovery of a fundamental role of microRNAs in the disease pathogenesis and their presence and stability in biological fluids has led to extensive investigation of the role of microRNAs as potential non-invasive biomarkers for disease diagnosis and prognosis. The result of this research has suggested that alterations of microRNAs may be sensitive indicators of various pathologies; however, despite the indisputable progress in this field, the diagnostic promise of microRNAs has remained a work in progress, and circulating microRNAs have not entered the field of clinical medicine yet. Commonly reported microRNAs as disease biomarkers are largely not disease-specific and the results are often contradicting in independent studies. This review summarizes the current knowledge on the role of microRNAs as disease indicators and emphasizes the current gaps, challenges, and questions that need to be addressed in future well-designed and well-controlled studies for a successful translation of microRNA profiling into clinically meaningful tests.

Keywords: Biomarkers, blood, clinical, diseases, MicroRNA, monitoring

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Introduction

The search for reliable minimally invasive or non-invasive tests for the diagnosis of disease, monitoring its progression, and response to therapy continues to be of great importance for clinical medicine. Remarkable technological advances have substantially increased diagnostic accuracy; however, there are still substantial gaps related to disease diagnosis in a general human population setting.

The discovery and elucidation of a fundamental role of microRNAs (miRNAs) in the pathogenesis of a broad range of human oncological and non-oncological diseases, and the discovery of the presence of cell-free miRNAs in the blood stream inclined researchers to investigate the role of miRNAs as potential non-invasive biomarkers. The result of this research has documented an existence of quantitative and qualitative differences in the levels of circulating miRNAs between healthy and diseased individual suggesting that miRNA alterations may be sensitive indicators of various pathologies. Remarkably, it has been suggested that miRNAs may be used as several categories of biomarkers, including diagnostic, prognostic, monitoring, risk, and safety biomarkers.[1] However, despite of an extensive research, circulating miRNAs have not entered yet the field of clinical medicine, and many questions related to the significance of the observed miRNA alterations and challenges in translating the potential of circulating miRNAs into a novel diagnostic tool still remain.

Circulating miRNAs: A brief overview

The discovery of miRNAs in 1993 by Lee *et al*.[2,3] and rapid expansion of research on miRNAs convincingly established the significance of miRNAs as major negative regulators of gene expression of hundreds of protein-coding genes in diverse physiologic processes.[2,3] miRNAs are a class of small evolutionary conserved endogenous single-stranded, non-coding RNAs, 18–24 nucleotides in length.[4] The primary function of miRNAs is regulating gene expression at the post-transcriptional level by degrading or blocking translation of messenger RNAs (mRNAs) through sequence-specific binding. miRNA-encoding genes are transcribed in the nucleus by the RNA polymerases II and III as long primary transcripts (pri-miRNAs), 100–1000 nucleotides in length, that are later cleaved to precursor miRNAs (pre-miRNAs). The pre-miRNAs are exported to the cytoplasm by Exportin 5. In the cytoplasm, pre-miRNAs are processed by the double-stranded RNA (dsRNA)-specific RNAse III Dicer first to miRNA/miRNA duplexes, and then to mature single-stranded miRNAs that are

incorporated into RNA-induced silencing complexes (RISC). The portions of miRNAs are released from cells into the extracellular environment as (i) multi-vesicular bodies, secreted as exosomes or (ii) associated with protein complexes, e.g. miR-high-density lipoproteins (miR-HDL) and miR-Argonaute 2 (miR-Ago2) complexes.

Currently, it is established that miRNAs are actively involved and altered in various pathologies, and numbers of aberrantly expressed disease-associated miRNAs continue to expand rapidly. An extensive list of abnormally expressed disease-specific miRNAs has led to the suggestion that altered levels of miRNAs may be useful as a biomarker of disease. Indeed, numbers of studies have demonstrated that normal and pathological tissues can be distinguished by altered miRNA profiles; however, while this may provide valuable inside into the underlying mechanisms and pathogenesis of disease, it has limited practical application for the diagnostic or prognostic purpose due to their invasive nature. This substantially changed in 2008, when three independent studies reported that tissue- and organ-specific miRNAs can be released into the blood circulation, and cell-free miRNAs are present in human plasma and serum in a remarkably stable form.[5–7] Additionally, cell-free miRNAs have also been discovered in a variety of other biological fluids, including cerebrospinal fluid, urine, and saliva.

Circulating miRNAs as diagnostic biomarkers

miRNAs in cancer diagnosis

Despite a substantial effort and tremendous progress in a fight against cancer, it continues to be an enormous public health challenge worldwide due to increasing incidence and rising cancer-related death,[8] including adolescent cancer.[9] It is predicted that the number of numbers deaths will increase among men and women, and cancer will become the leading cause of death by 2020 in the United States.[10] This signifies the importance of the research focusing on uncovering the underlying mechanisms of cancer initiation and progression and discovery of novel diagnostic approaches for an early cancer detection.

Since the first report in 2002 on the down-regulation of miR-15 and miR-16 in chronic lymphocytic leukemia,[11] extensive data have demonstrated that the expression of miRNAs is profoundly altered in all types of human cancers.[12] In tumors, miRNAs can act as oncogenes or tumor suppressors, and altered expression of miRNAs has been associated with every aspect of tumor biology.[12] This suggests that altered expression of miRNAs may not only be a key pathophysiological component associated with tumor development, but also as a useful biomarker for cancer detection. Typically, cancer diagnosis is based on imaging, biopsy, and a few serological cancer biomarkers; however, biopsy poses a substantial danger to a patient, and blood-based biomarkers exhibit low specificity and sensitivity. Additionally, all of these approaches are not always cancer type or subtype oriented. In contrast, the analysis of circulating tumor cells and circulating cell-free tumor DNA and RNA, including miRNAs, in cancer patient blood, termed ''liquid biopsy'' has shown immense diagnostic potential.[13] Over the past several years, numerous clinical studies have focused on the discovery and identification of circulating miRNAs associated with various types of cancer.[14] The diagnostic value of circulating miRNAs has been reported for chronic and acute leukemia (e.g. miR-26a, miR-29, miR-34a, miR-150, miR-155, miR-181b-5p, miR-210, miR-221, miR-222, miR-223, miR-328, miR-339, miR-342, miR-375, miR-511, and miR-523),[15] breast cancer (e.g. miR-155 and miR-181a-5p),[16,17] non-small-cell lung cancer (e.g. miR-7, miR-25, miR-193a-3p, miR-214, and miR-483-5p),[18] and other human cancers. In general, miRNAs, detected in plasma and urine, may have a greater sensitivity as compared to other diagnostic markers. Indeed, Lai *et al.*[19] have reported a superior effectiveness of circulating miRNAs in diagnosing pancreatic ductal adenocarcinoma than glypican-1 and cancer antigen 19-9 (CA 19-9), the only U.S. Food and Drug Administration (FDA)-approved biomarker of pancreatic ductal adenocarcinoma. However, one of the major obstacles limiting the use of miRNAs as a diagnostic tool in the clinic setting is associated with the fact that altered level of the same miRNA is detected in patients with different tumor types. For example, the increased serum level of miR-21, one of the most studied miRNAs in human cancers, has been found in patients with colorectal,[20] lung,[21] breast,[22] prostate,[23] liver,[24] esophageal,[25] and endometrial[26] cancers. In contrast, it has been suggested that a panel of selected miRNAs that contains tissue-specific miRNAs may have a greater target-organ specificity and better diagnostic value than a single miRNA or well-established clinical biomarker. This can be illustrated by the report of Lin *et al.*[27] who have demonstrated a greater sensitivity of a serum miRNA classifier panel containing of seven miRNAs, miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505, to detect hepatocellular carcinoma, especially small size or early-stage hepatocellular carcinomas, than α-fetoprotein. Likewise, a panel of circulating miRNAs, miR-15b, miR-17, miR-21, miR-26b, and miR-145, has a greater value as diagnostic biomarker for colorectal cancer, than single miRNA.[28] Additionally, circulating miRNAs can be used not only for cancer detection, but also for diagnosis of cancer stratification. For example, Toiyama *et al.*[29] have demonstrated that the level of miR-200c in serum in patients with stage IV colorectal cancer was significantly greater than in patients with stage I-III.

miRNAs in cardiovascular disease diagnosis

Despite a decrease in the number of heart disease-associated deaths from 1968 to the present the United States,[10] cardiovascular disease, especially acute coronary syndrome (ACS), remains one of the leading causes of mortality. Evidence accumulated over the past decade has established a key role of miRNAs in the pathogenesis of a number of cardiovascular diseases.[30,31] In clinical setting, early diagnosis and differential diagnosis of ACS, which consists of ST-elevated myocardial infarction (STEMI), non-ST-elevated myocardial infarction (NSTEMI), and unstable angina pectoris (UA), is critical for the determination of optimal treatment tactic.[32] The clinical diagnosis of ACS is

based on (i) clinical symptoms; (ii) ST-segment elevation and Q-wave abnormalities on the electrocardiogram; and (iii) changes in blood biomarkers, especially in cardiac troponin levels.[32] In light of this, the evaluation of circulating cardiomyocyte-specific miRNAs has been proposed as novel attractive diagnostic markers. An extensive systematic review of 52 independent studies investigating the potential diagnostic utility of circulating miRNAs in cardiology has identified that individual circulating cardiomyocyte-enriched miRNAs, miR-1, miR-133a, miR-133b, miR-145, miR-208a, miR-208b, and miR-499, may have diagnostic potential for coronary heart disease[33]; however, it is still lower as compared to that in cardiac troponin.[34] Furthermore, the diagnostic accuracy of miRNAs in diagnosis of ACS and differential diagnosis of ACS pathological forms (e.g. STEMI, NSTEMI, and UA) is contradictory and requires future investigation.[35,36] Additionally, it has been suggested that new miRNA biomarker searches should focus not on areas where well-performing, established diagnostic markers exist,[30] but on unmet clinical needs, such as prediction of myocardial infarction. For example, in a prospective study on circulating miRNAs and risk of myocardial infarction, Zampetaki *et al*.[37] have demonstrated that an addition of miR-126, miR-197, and miR-197 to a base model of the Framingham Risk Score for hard coronary heart disease substantially improved the predictive power. Importantly, this report, similar to study by Lin *et al*.[27] on hepatocellular carcinoma, emphasized that a panel of circulating miRNAs has much greater diagnostic value than a single miRNA.

miRNAs in non-alcoholic fatty liver disease diagnosis

Nonalcoholic fatty liver disease (NAFLD), a liver component of the metabolic syndrome, is the most common cause of chronic liver diseases and a major health problem in the United States and Western countries.[38,39] NAFLD is represented by several related liver disorders ranging from simple non-alcoholic fatty liver (hepatic steatosis) to non-alcoholic steatohepatitis (NASH) and cirrhosis.[38,40] Uncomplicated hepatic steatosis is generally considered to be a benign form of NAFLD; however, approximately 30% of NAFLD individuals diagnosed with simple fatty liver will develop NASH, an advanced form of NAFLD,[39] which is associated with increased morbidity and mortality, and is the second cause of liver transplantation in the United States.[41] Additionally, available treatment options for NASH are limited and there is no drug approved by the FDA for NASH treatment.[38] This indicates a significance of the early identification of individuals with a high risk of developing NASH. Currently, liver biopsy remains ''the gold standard method'' for detecting NASH and evaluating the degree of liver injury; however, non-invasive serum diagnostic biomarkers have been also developed. Several studies have shown a marked increase in the levels of miR-21, miR-34a, miR-122, miR-192, miR-223, and miR-375 in serum of patients with NAFLD and NASH,[42–44] among which the levels of circulating miR-34a, miR-122, miR-192, and miR-375 in NASH patients were substantially greater as compared to NAFLD patients.[42–44] This illustrates the potential value of circulating miRNAs not only for the detection of NAFLD but also for the differential diagnosis between NAFLD and NASH.

miRNAs in neurodegenerative disease diagnosis

The current diagnosis of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis is mainly based on the clinical analysis of cognitive and motor features. Accumulated evidence has documented a great number of altered circulating miRNAs in neurodegenerative disease patients, indicating their potential diagnostic significance; however, a much smaller number of miRNAs retained their diagnostic value in independent studies.[45,46] In particular, in a recent review article, Kumar and Reddy[46] analyzed the results of 12 studies that investigated the role of circulating miRNAs as biomarkers for Alzheimer's disease. Among 100 altered miRNAs, only six miRNAs, miR-9, miR-125b, miR-146a, miR-181c, let-7g-5, and miR-191-5p, were uniformly altered in multiple studies.[46] A study by Dong *et al*.[47] has illustrated a further significance of circulating miRNAs in diagnosis of neurodegenerative diseases. In particular, it has been shown that a panel of four decreased serum miRNAs, miR-141, miR-146b-5p, miR-193a-3p, and miR-214, permitted to detect Parkinson's disease at early stages of the disease development.[47]

miRNAs in other disease diagnosis

There is a fast-growing list of the original reports and review articles aimed to address the clinical value of circulating miRNAs in diagnosis of virtually all major human diseases, including infectious diseases,[48] diabetes mellitus and obesity,[49,50] chronic kidney disease,[51] and autoimmune diseases.[52] The number of these studies is expected to grow. This indicates a great interest and substantial potential benefits of circulating miRNAs in clinical medicine, but it also illustrates an existence of an insufficient knowledge and a lack of conclusive information to clarify the role of circulating miRNAs in the disease diagnosis process.

miRNAs as indicators of drug toxicity

Safe medical treatment and drug toxicity represent an important and growing public health problem worldwide. This is evidenced by the fact that nearly one-third of drug candidates fail due to toxicity issues, and that adverse drug reactions, especially hepatotoxicity and cardiotoxicity, cause a substantial rate of hospitalizations, serious financial burden, and are the major cause for withdrawing drugs from the market.[53,54] In a clinical setting, the diagnosis of drug-induced organ toxicity is based on clinical symptoms and well-established diagnostic tests. For example, the standard clinical biomarkers for the assessment of hepatotoxicity are serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and bilirubin[55]; however, despite the well-accepted clinical value of these biomarkers, they appear, at times, only when tissue damage is irreversible, provide little or no insight into mechanisms of hepatotoxicity, and, in some cases, have limited prognostic value.

In order to overcome these limitations, a number of studies investigated the role of circulating miRNAs as biomarkers of drug-induced organ injury, especially liver, in the clinical setting. Acetaminophen, a widely used over-the-counter analgesic and antipyretic drug, is the most common cause of liver toxicity. Among approximately 2000 patients who experience acute liver failure in the United States each year, acetaminophen is responsible for nearly 45% of cases.[56] In a pioneering study, Wang *et al*.[57] using acetaminophen overdose-induced liver injury in mice have demonstrated a significance of circulating liver-enriched miR-122 and miR-192 in the diagnosis of drug-induced hepatotoxicity. This was evidenced by dose-dependent alterations in the levels of miR-122 and miR-192 in plasma that paralleled with the serum ALT level and the extent of liver histopathology. Several subsequent clinical studies have investigated the diagnostic potential of circulating miRNAs, especially liver-enriched miR-122 and miR-192, as biomarkers of acetaminophen hepatotoxicity in humans.[58,59] Starkey Lewis *et al*.[58] have reported that the levels of plasma miR-122 and miR-192 were increased in 53 adult patients with acute liver injury induced by acetaminophen,[58] and Antoine *et al*.[59] demonstrated that plasma miR-122 is a more sensitive indicator than ALT in identifying acetaminophen-induced acute liver injury in humans. These findings were confirmed in a later study demonstrating that an elevation of circulating miR-122 in patients with acetaminophen overdoses preceded changes in the standard clinical biomarkers and was more sensitive than ALT at hospital presentation.[60] However, an increase of plasma miR-122 accompanied by ALT and AST elevation has been reported also in individuals after the treatment with non-hepatotoxic drugs, e.g. a cholesterol lowering drug cholestyramine and heparins.[61,62] In light of this, a panel of miRNAs may have a greater specificity. Indeed, Ward *et al*.[63] have reported that a panel of serum classifier miRNAs consisting of miR-122, miR-27b, and miR-21 exhibited a greater diagnostic value as compared to a single miRNA, preceded ALT rise, and differentiated acetaminophen hepatotoxicity from ischemic hepatitis. Likewise, in a recent separate study, Krauskopf *et al*.[64] demonstrated a minimal overlap in unique circulating miRNA signatures between several liver pathological states, including acetaminophen-induced liver injury, hepatitis B infection, liver cirrhosis, and type 2 diabetes, while the level of ALT was elevated uniformly in all conditions.

In addition to drug-induced hepatotoxicity, there is a fast growing number of studies aimed to address the clinical value of circulating miRNAs in the diagnosis of drug-induced injury of other organs, including kidney toxicity and cardiotoxicity[65,66]; however, these studies are still in their infancy.

Circulating miRNAs as prognostic biomarkers

In addition to the diagnostic potential, circulating miRNAs exhibit much greater value as monitoring and prognostic biomarker. Several studies have investigated the potential significance of circulating miRNAs a prognostic biomarker in several major human cancers. Specifically, it has been demonstrated that high serum miR-200c and miR-885 positively correlated with lymph node and distant metastasis and independently predicted prognosis in patients with colorectal cancer.[29,67] In addition to miR-200c, Maierthaler *et al*.[68] have reported that an increased level of miR-122 in colorectal cancer patients was associated with a metastatic colorectal cancer subtype, shorter relapse-free survival, and overall survival for non-metastatic and metastatic cancer. Similar finding of the potential prognostic value of circulating miRNAs has been demonstrated in patients with several major human cancer types, including leukemia, lung, breast, prostate cancers, and multiple myeloma.[15,69–72] In particular, Madhavan *et al*.[71] have reported that the levels of miR-200a, miR-200b, miR-200c, miR-210, miR-215, and miR-486 were associated with an overall survival and highly associated with an onset of metastasis up to two years prior to a clinical diagnosis in breast cancer patients. Additionally, there is growing evidence that circulating miRNAs can be used not only as prognostic biomarkers, but also as indicators of therapeutic efficacy.

Similarly to oncological diseases, several reports have demonstrated a great value of miRNAs as monitoring and prognostic biomarkers for non-neoplastic diseases, especially cardiovascular disease.[73–76] In a recent study, Karakas *et al*.[73] have reported that circulating miRNAs miR-132, miR-140-3p, and miR-210 in a cohort of 1112 patients with documented coronary artery disease consisting of 430 patients with ACS and 682 patients with stable angina pectoris, during a median follow-up of 4.0 years, precisely predicted cardiovascular death in ACS patients. In three separate studies, it has been demonstrated that circulating miRNAs improved the prediction of adverse cardiovascular outcome in patients after acute myocardial infarction (miR-16, miR-27a, miR-101 and miR-150),[74] STEMI (miR-26b-59, miR-320a, and miR-660-5p),[75] and acute heart failure (miR-423-5p).[76]

Conclusions, challenges, and perspectives

Accumulated up-to-date extensive evidence has established the importance of miRNAs in the pathogenesis of many major human diseases and has indicated the potential significance of evaluation of circulating miRNA profiling diagnosing disease, monitoring disease progression, and evaluating treatment efficiency. The discovery in 2008 that miRNAs are present in human plasma and serum in stable form resulted in intensive research on the usefulness of circulating miRNAs for diagnosis of various human diseases, especially cancer. However, despite the research effort, at present, circulating miRNA research is still in its infancy and has not resulted in highly specific and validated disease markers.[77–79] Currently, fulfilling the diagnostic promise of stable miRNAs in circulation has remained a work in progress, in both cancer and non-neoplastic disease studies, and circulating miRNAs have not yet entered the field of clinical medicine. Commonly reported miRNAs as disease biomarkers are largely not disease-specific and the results are often contradicting in independent studies. A limited overlap has been observed between the findings of even very similar studies of the

same disease. This is largely due to flaws in study design, a small number of participants, or unstandardized (e.g. different age, sex, length of disease, etc.) participants, a wide range of confounding factors, conditions and outcomes, and methodological issues. To improve the diagnostic value of circulating miRNAs, the following several major issues need to be addressed.

Pre-analytical study design

One of the major shortcomings in the majority of up-to-date studies is a relatively small number of participants, which causes a substantial variation and inconsistency in results. In light of this, well-designed and controlled multicenter studies of the same disease are in a better position to improve the diagnostic value of circulating miRNAs over studies conducted by individual investigators. Specifically, the multicenter studies allow recruiting a much greater number of participants that allows assembling them better according to age, sex, specific stage and length of disease, and minimize the influence of uncontrollable confounding factors, not directly related to specific disease, e.g. lifestyle factors, presence of other pathologies, and existing patient medications. Additionally, this approach will have great positive effect on optimization and standardization procedure of collecting and handling biological samples, their storage, sample processing, and subsequent analysis as highlighted in great details by Khan *et al*.[80]

miRNA classifiers versus single miRNA

In general, the diagnostic value of miRNA panel may be higher than of a single miRNA, because the same single miRNA might be altered in different unrelated diseases. However, investigations focusing on the discovery of unique disease-specific miRNAs should not be disregarded. For example, it has been demonstrated that elevated circulating miR-195 differentiated breast cancer from control and other types of cancer, including prostate, colon, and renal cancer and melanoma, with great sensitivity and specificity.[81]

Analytical challenges

There is a lack of consensus on a number of miRNA analysis-related methodological questions, including extraction of miRNAs, detection, and normalization methods. Currently, at least three different detection approaches, including PCR amplification-based methods, miRNA microarrays, and miRNA-Seq, are employed for analysis of circulating miRNAs. While miRNA microarrays and miRNA-Seq methodologies provide ''hypothesis-free'' identification of small non-coding RNA species, including miRNAs, piRNAs, snoRNAs, and tRNA-derived fragments, and pose a great potential in the discovery of new disease-related miRNAs in the experimental setting, it is highly unlikely, at least for now, that these techniques can be used in clinics because of time-, cost-, and labor-related issues. In contrast, quantitative RT-PCR amplification-based methods, which are already routinely used in clinics, represent a balance of cost, precision, and sample size in miRNA profiling and validating the results obtained by other methods, and have a greater potential for analysis of circulating miRNAs in a clinical setting. Currently, several different miRNA platforms and methodologies are commercially available for routine analysis of circulating miRNAs. Experimental work focusing on the investigation of reproducibility, comparability, and reliability of these RT-PCR amplification-based methods for analysis of circulating miRNAs is needed to advance the use of miRNA evaluation in the disease diagnostic process. Additionally, normalization of the data and absolute, not relative, quantification of the amount of altered miRNAs are other key issues hammering the translation of basic research of circulating miRNAs into the clinic.

Circulating miRNAs hold great promise as cancer and non-neoplastic disease diagnostic and prognostic biomarkers. However, as has been discussed in this review, the development and the application of standard operating procedures related to all aspects of circulating miRNAs analysis, their proper standardization and validation are required before translation into clinical practice and successful translation of miRNA profiling into clinically meaningful tests. One of the approaches that hold a huge diagnostic potential in the enhancing disease diagnostic is the integrative analysis of circulating miRNAs with already verified and established diagnostic markers.

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REFERENCES

1. FDA-NIH Biomarker Working Group. *BEST (biomarkers, endpoints, and other tools) resource*. Maryland: Food and Drug Administration (US)/ National Institutes of Health (US), 2016
2. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993;**75**:843–54
3. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 2010;**466**:835–40
4. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;**136**:215–33
5. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CSR, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* 2008;**141**:672–5
6. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang C-Y. Characterization microRNAs in serum: a novel class

of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008;**18**:997–1006
7. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008;**105**:10513–18
8. American Association for Cancer Research. AACR Cancer Progress Report 2016. *Clin Cancer Res* 2016;**22**:S1–S137
9. Burkhamer J, Kriebel D, Clapp R. The increasing toll of adolescent cancer incidence in the US. *PLoS One* 2017;**12**:e0172986
10. Weir HK, Anderson RN, Coleman King SM, Soman A, Thompson TD, Hong Y, Moller B, Leadbetter S. Heart disease and cancer deaths – trends and projections in the United States, 1969-2020. *Prev Chron Dis* 2016;**13**:160211
11. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002;**99**:15524–9
12. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. *Annu Rev Pathol* 2014;**9**:287–314
13. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov* 2016;**6**:479–91
14. Wittmann J, Jäck H-M. Serum microRNAs as powerful cancer biomarkers. *Biochim Biophys Acta* 2010;**1806**:200–7
15. Yeh C-H, Moles R, Nicot C. Clinical significance of microRNAs in chronic and acute human leukemia. *Mol Cancer* 2016;**15**:37
16. Wang F, Hou J, Jin W, Li J, Yue Y, Jin H, Wang X. Increased circulating microRNA-155 as a potential biomarker for breast cancer screening: a meta-analysis. *Molecules* 2014;**19**:6282–93
17. Ferracin M, Lupini L, Salamon I, Saccenti E, Zanzi MV, Rocchi A, Da Ross L, Zagatti B, Musa G, Bassi C, Mangolini A, Cavallesco G, Frassoldati A, Volpano S, Carcoforo P, Hollingsworth AB, Negrini M. Absolute quantification of cell-free microRNAs in cancer patients. *Oncotarget* 2015;**6**:14545–55
18. Wang C, Ding M, Xia M, Chen S, Van Le A, Soto-Gil R, Shen Y, Wang N, Wang J, Gu W, Wang X, Zhang Y, Zen K, Chen X, Zhang C, Zhang C-Y. A five-miRNA panel identified from a multicentric case-control study serves as a novel diagnostic tool for ethnically diverse non-small-cell lung cancer patients. *EBioMedicine* 2015;**2**:1377–85
19. Lai X, Wang M, McElyea SD, Sherman S, House M, Korc M. A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer. *Cancer Lett* 2017;**393**:86–93
20. Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A. Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. *J Natl Cancer Inst* 2013;**105**:849–59
21. Yang X, Guo Y, Du Y, Yang J, Li S, Liu S, Li K, Zhang D. Serum microRNA-21 as a diagnostic marker for lung carcinoma: a systematic review and meta-analysis. *PLoS One* 2014;**9**:e97460
22. Li S, Yang X, Yang J, Zhen J, Zhang D. Serum microRNA-21 as a potential diagnostic biomarker for breast cancer: a systematic review and meta-analysis. *Clin Exp Med* 2016;**16**:29–35
23. Zhang H-L, Yang L-F, Zhu Y, Yao X-D, Zhang S-L, Dai B, Zhu Y-P, Shen Y-J, Shi G-H, Ye D-W. Serum miRNA-21: elevated levels in patients with metastatic hormone-refractory prostate cancer and potential predictive factor for the efficacy of docetaxel-based chemotherapy. *Prostate* 2011;**71**:326–31
24. Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. *Biomed Res Int* 2015;**2014**:864894
25. Tanaka Y, Kamohara H, Kinoshita K, Kurashige J, Ishimoto T, Iwatsuki M, Watanabe M, Baba H. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. *Cancer* 2013;**119**:1159–67
26. Gao Y, Dai M, Liu H, He W, Lin S, Yuan T, Chen H, Dai S. Diagnostic value of circulating miR-21: an update meta-analysis in various cancers and validation in endometrial cancer. *Oncotarget* 2016;**7**:68894–908
27. Lin X-J, Chong Y, Guo Z-W, Xie C, Yang X-J, Zhang Q, Li S-P, Xiong Y, Yuan Y, Min J, Jia W-H, Jie Y, Chen M-S, Chen M-X, Fang J-H, Zeng C, Zhang Y, Guo R-P, Wu Y, Lin G, Zheng L, Zhuang S-M. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicenter, retrospective, longitudinal biomarker identification study with nested case-control study. *Lancet Oncol* 2015;**16**:804–15
28. Pan C, Yan X, Li H, Huang L, Yin M, Yang Y, Gao R, Hong L, Ma Y, Shi C, Qin H, Zhang P. Systematic literature review and clinical validation of circulating microRNAs as diagnostic biomarkers for colorectal cancer. *Oncotarget* 2017 (in press).
29. Toiyama Y, Hur K, Tanaka K, Inoue Y, Kusunoki M, Boland CR, Goel A. Serum miR-200c is a novel prognostic and metatstasis-predictive biomarker in patients with colorectal cancer. *Ann Surg* 2014;**259**:735–43
30. Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. *J Am Coll Cardiol* 2016;**68**:2577–84
31. Cavarretta E, Frati G. MicroRNAs in coronary disease: ready to enter the clinical arena? *Biomed Res Int* 2016;**2016**:2150763
32. Deddens JC, Colijn JM, Oerlemans MIFJ, Pasterkamp G, Chamuleau SA, Doevendans PA, Sluijter JPG. Circulating microRNAs as novel biomarkers for the early diagnosis of acute coronary syndrome. *J Cardiovasc Trans Res* 2013;**6**:884–98
33. Navickas R, Gal D, Laucevičius A, Taparauskaitė A, Zdanytė M, Holvoet P. Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systemic review. *Cardiovasc Res* 2016;**111**:322–47
34. Devaux Y, Mueller M, Haaf P, Goretti E, Twerenbold R, Zangrando J, Vausort M, Reichlin T, Wildi K, Moering B, Wagner DR, Mueller C. Diagnostic and prognostic value of circulating microRNAs in patients with acute chest pain. *J Intern Med* 2015;**277**:260–71
35. Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, Kempf T, Wollert KC, Thum T. Dignostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. *J Mol Cell Cardiol* 2011;**51**:872–5
36. Jia K, Shi P, Han X, Chen T, Tang H, Wang J. Diagnostic value of miR-30d-5- and miR-125b-5p in acute myocardial infarction. *Mol Med Rep* 2016;**14**:184–94
37. Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard J-M, Mayr A, Weger S, Schett G, Shah A, Boulanger CM, Willeit J, Chowienczyk PJ, Kiechl S, Mayr M. Prospective study on circulating MicroRNAs and risk of myocardial infarction. *J Am Coll Cardiol* 2012;**60**:290–9
38. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015;**313**:2263–73
39. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease–meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;**64**:73–84
40. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease–meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;**64**:73–84
41. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015;**148**:547–555
42. Pirola CJ, Fernández-Gianotti T, Castaño GO, Mallardi P, San Marino J, Mora Gonzales Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2015;**64**:800–12
43. Becker PP, Rau M, Schmitt J, Malsch C, Hammer C, Bantel H, Müllhaupt B, Geier A. Performance of serum microRNAs -122, -192,

and -21 as biomarkers in patients with non-alcoholic steatohepatitis. *PLoS One* 2015;**10**:e0142661
44. Liu X-L, Pan Q, Zhang R-N, Shen F, Yan S-Y, Sun C, Xu Z-J, Chen Y-W, Fan J-G. Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population. *World J Gastroenterol* 2016;**22**:9844–52
45. Grasso M, Piscopo P, Confaloni A, Denti MA. Circulating miRNAs as biomarkers for neurodegenerative disorders. *Molecules* 2014;**19**:6891–910
46. Kumar S, Reddy PH. Are circulating microRNAs peripheral biomarkers for Alzheimer's disease? *Biochim Biophys Acta* 2016;**1862**:1617–27
47. Dong H, Wang C, Lu S, Yu C, Huang L, Feng W, Xu H, Chen X, Zen K, Yan Q, Liu W, Zhang C, Zhang C-Y. A panel of four decreased serum microRNAs as a novel biomarker for early Parkinson's disease. *Biomarkers* 2016;**21**:129–37
48. Correia CN, Nalpas NC, McLoughlin KE, Browne JA, Gordon SV, MacHugh DE, Shaughnessy RG. Circulating miRNAs as potential biomarkers of infectious disease. *Front Immunol* 2017;**8**:118
49. Villard A, Marchand L, Thivolet C, Rome S. Diagnostic value of cell-free circulating microRNAs for obesity and type 2 diabetes: a meta-analysis. *J Mol Biomark Diagn* 2015;**6**:pii251
50. Sebastiani G, Nigi L, Grieco GE, Mancarella F, Ventriglia G, Dotta F. Circulating microRNAs and diabetes mellitus: a novel tool for diseases prediction, diagnosis, and staging? *J Edocrinol Invest* 2017;**40**:591–610
51. Brigant B, Metzinger-Le Meuth V, Massy ZA, McKay N, Liabeuf S, Pelletier M, Sallée M, M'Baya-Moutoula E, Paul P, Drueke TB, Burtey S, Metzinger L. Serum microRNAs are altered in various stages of chronic kidney disease: a preliminary study. *Clin Kidney J* 2017;**10**:30–37
52. Zeng L, Cui J, Wu H, Lu Q. The emerging role of circulating microRNAs as biomarkers in autoimmune diseases. *Autoimmunity* 2014;**47**:419–29
53. Qureshi ZP, Seoane-Vazquez E, Rodriguez-Monguio R, Stevenson KB, Szeinbach SL. Market withdrawal of new molecular entities approved in the United States from 1980 to 2009. *Pharmacoepidemiol Drug Saf* 2011;**20**:772–7
54. Marrone AK, Beland FA, Pogribny IP. The role of microRNAs in drug toxicity and in safety assessment. *Expert Opin Drug Metab Toxicol* 2015;**11**:601–11
55. Amacher DE, Schomaker SJ, Aubrecht J. Development of blood biomarkers for drug-induced liver injury: an evaluation of their potential for risk assessment and diagnostics. *Mol Dign Ther* 2013;**17**:343–54
56. Lee WM. Drug-induced acute liver failure. *Clin Liver Dis* 2013;**17**:575–86
57. Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, Hood LE, Galas DJ. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci USA* 2009;**106**:4402–7
58. Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DGN, Antoine DJ, French NS, Dhaun N, Webb DJ, Costello EM, Neoptolemos JP, Moggs J, Goldring CE, Park BK. Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology* 2011;**54**:1767–76
59. Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, Masson M, Thanacoody RH, Gray AJ, Webb DJ, Moggs JG, Bateman DN, Goldring CE, Park BK. Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. *Hepatology* 2013;**58**:777–87
60. Vliegenthart ADB, Shaffer JM, Clarke JI, Peeters LEJ, Caporali A, Bateman DN, Wood DM, Dargan PI, Craig DG, Moore JK, Thompson AI, Henderson NC, Webb DJ, Sharkey J, Antoine DJ, Park BK, Bailey MA, Lader E, Simpson KJ, Dear JW. Comprehensive microRNA profiling in acetaminophen toxicity identifies novel circulating biomarkers for human liver and kidney injury. *Sci Rep* 2015;**5**:15501
61. Harrill AH, Roach J, Fier I, Eaddy JS, Kurtz CL, Antoine DJ, Spencer DM, Kishimoto TK, Pisetsky DS, Park BK, Watkins PB. The effects of heparins on the liver: application of mechanistic serum biomarkers in a randomized study in healthy volunteers. *Clin Pharmacol Ther* 2012;**92**:214–20
62. Singhal R, Harrill AH, Menguy-Vacheron F, Jayyosi Z, Benzerdjeb H, Watkins PB. Benign elevations in serum aminotransferases and biomarkers of hepatotoxicity in healthy volunteers treated with cholestyramine. *BMC Pharmacol Toxicol* 2014;**15**:42
63. Ward J, Kanchagar C, Veksler-Lublinsky I, Lee RC, McGill MR, Jaeschke H, Curry SC, Ambros VR. Circulating microRNA profiles in human patients with acetaminophen hetatotoxicity or ischemic hepatitis. *Proc Natl Acad Sci USA* 2014;**111**:12169–74
64. Krauskopf J, de Kok TM, Schomaker SJ, Gosink M, Burt DA, Chandler P, Warner RL, Johnson KJ, Caiment F, Kleinjans JC, Aubrecht J. Serum microRNA signatures as ''liquid biopsies'' for interrogating hepatotoxic mechanisms and liver pathogenesis in human. *PLoS One* 2017;**12**:e0177928
65. Lorenzen JM, Kielstein JT, Hafer C, Gupta SK, Kümpers P, Faulhaber-Walter R, Haller H, Fliser D, Thum T. Circulating miR-210 predicts survival in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol* 2011;**6**:1540–6
66. Zhao Z, He J, Zhang J, Liu M, Yang S, Li N, Li X. Dysregulated miR1254 and miR579 for cardiotoxicity in patients treated with bevacizumab in colorectal cancer. *Tumour Biol* 2014;**35**:5227–35
67. Hur K, Toiyama Y, Schetter AJ, Okugawa Y, Harris CC, Boland CR, Goel A. Identification of a metastasis-specific microRNA signature in human colorectal cancer. *J Natl Cancer Inst* 2015;**107**:dju:492
68. Maierthaler M, Benner A, Hoffmeister M, Surowy H, Jansen L, Knebel P, Chang-Claude J, Brenner H, Burwinkel B. Plasma miR-122 and miR-200 family are prognostic markers in colorectal cancer. *Int J Cancer* 2017;**140**:176–87
69. Kaduthanam S, Gade S, Meister M, Brase JC, Johannes M, Dienemann H, Warth A, Schnabel PA, Herth FJ, Sültmann H, Muley T, Kuner R. Serum miR-142-3p is associated with early relapse in operable lung adenocarcinoma patients. *Lung Cancer* 2013;**80**:223–7
70. Selth LA, Towmley SL, Bert AG, Stricker PD, Sutherland PD, Horvath LG, Goodall GJ, Butler LM, Tilley WD. Circulating microRNAs predict biochemical recurrence in prostate cancer patients. *Br J Cancer* 2013;**109**:641–50
71. Madhavan D, Peng C, Wallwiener M, Zucknick M, Nees J, Schott S, Rudolph A, Riethdorf S, Trumpp A, Pantel K, Sohn C, Chang-Claude J, Schneeweiss A, Burwinkel B. Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis. *Carcinogenesis* 2016;**37**:461–70
72. Hao M, Zang M, Wendlandt E, Xu Y, An G, Gong D, Li F, Qi F, Zhang Y, Yang Y, Zhan F, Qiu L. Low serum miR-19a expression as a novel poor prognostic indicator in multiple myeloma. *Int J Cancer* 2015;**136**:1835–44
73. Karakas M, Schulte C, Appelbaum S, Ojeda F, Lackner KJ, Münzel T, Schnabel RB, Blankenberg S, Zeller T. Circulating microRNAs strongly predict cardiovascular death in patients with coronary artery diseases-results from large AtheroGene study. *Eur Heart J* 2017;**38**:516–23
74. Devaux Y, Vausort M, McCann GP, Kelly D, Collignon O, Ng LL, Wagner DR, Squire IB. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. *PLoS One* 2013;**8**:e70644
75. Jakob P, Kacprowski T, Briand-Schumacher S, Heg D, Klingenberg R, Stähli BE, Jaguszewski M, Rodondi N, Nanchen D, Räber L, Vogt P, Mach F, Windecker S, Völker U, Matter CM, Lüscher TF, Landmesser U. Profiling and validation of circulating microRnas for cardiovascular events in patients presenting with ST-segment elevation myocardial infarction. *Eur Heart J* 2017;**38**:511–5
76. Seronde MF, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, Vodovar N, Sadoune M, Samuel JL, Thum T, Solal AC, Laribi S, Plaisance P, Wagner DR, Mebazaa A, Devaux Y, GREAT network. Circulating microRNAs and outcome in patients with acute heart failure. *PLoS One* 2015;**10**:e0142237
77. Jarry J, Schadendorf D, Green wood C, Spatz A, van Kempen LC. The validity of circulating microRNAs in oncology: five years of challenges and contradictions. *Mol Oncol* 2014;**8**:819–29
78. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. *Clin Chem* 2015;**61**:56–63

79. Tiberio P, Callari M, Angeloni V, Daidone MG, Appierto V. Challenges in using circulating miRNAs as cancer biomarkers. *Biomed Res Int* 2015;**2015**:731479
80. Khan J, Lieberman JA, Lockwood CM. Variability in, variability out: best practice recommendations to standardize pre-analytical variables in the detection of circulating and tissue microRNAs. *Clin Chem Lab Med* 2017;**55**:608–21
81. Heneghan HM, Miller N, Kelly R, Newell J, Kerin MJ. Systematic miRNA-195 differentiates breast cancer from other malignancies and is a potential biomarker for detecting noninvasive and early stage disease. *Oncologist* 2010;**15**:673–82