Expert Opinion

informa healthcare

MicroRNA identification in plasma and serum: a new tool to diagnose and monitor diseases

Maria Angelica Cortez & George Adrian Calin[†]
[†]*The University of Texas M. D. Anderson Cancer Center, Department of Experimental Therapeutics, Houston, Texas, USA*

MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate gene expression at the posttranscriptional level by degrading or blocking translation of messenger RNA (mRNA) targets. MiRNAs play important regulatory roles in a variety of cellular functions as well as in several diseases, including cancer. MiRNA-specific expression profiles have been reported for several pathological conditions. Recently, the discovery of miRNAs in serum opens up the possibility of using miRNAs as biomarkers of disease. In this review, we discuss the potential use of miRNAs as clinically diagnostic biomarkers of various cancers and other diseases as well as the approaches used to detect these molecules in serum and plasma.

Keywords: cancer, microRNA, plasma, serum

Expert Opin. Biol. Ther. (2009) 9(6):703-711

1. Introduction

MicroRNAs (miRNAs) are a class of small noncoding RNAs that have central roles in the regulation of gene expression [1-3]. Thousands of human messenger RNAs (mRNAs) are predicted to be miRNA targets [4,5] and a single miRNA may target hundreds of mRNAs [6,7]. MiRNAs are first transcribed in the nucleus as primary transcripts by RNA polymerase II [8,9]. The resulting primary miRNA is processed by a protein complex containing mainly two proteins, the RNAse III endonuclease Drosha and DGCR8, to form the miRNA precursor, also known as pre-miRNA [10]. This ~ 60 – 110-nucleotide (nt), hairpin-structured pre-miRNA is actively transported to the cytoplasm by Ran-GTP and the export receptor Exportin-5 [11,12] and processed by the RNase III endonuclease Dicer, resulting in a small double-stranded RNA structure (~ 22 nt). The single-stranded mature miRNA is loaded into the RNA-induced silencing complex (RISC), which facilitates the interaction of the miRNA with complementary binding sites within the targeted transcript's 3′-untranslated region (UTR) and, thus, regulates gene expression by either translational repression or degradation of the mRNA [13]. MiRNAs play important roles in development, metabolism, cellular differentiation, proliferation, cell-cycle control and cell death [14,15]. In addition, miRNAs have been implicated in a variety of human diseases, such as viral infections [16], cardiovascular disease [17], and cancer [18].

In the past few years, several reports related important aspects of miRNAs biogenesis and function in many cellular processes and diseases. Here, we focused on the potential use of miRNAs as diagnostic biomarkers of diseases in serum-based screening.

10.1517/14712590902932889 © 2009 Informa UK Ltd ISSN 1471-2598

2. miRNAs are differentially expressed in cancer and other diseases

The first evidence for the involvement of miRNAs in cancer came from the study of miR-15a and miR-16a, which are located on chromosome region 13q14, a region that is deleted in more than half of all B-cell chronic lymphocytic leukemia (B-CLL) patients [19]. MiR-15a and miR-16a induce apoptosis by targeting the mRNA of the antiapoptotic gene B-cell leukemia/lymphoma 2 (*BCL2*) [20]. Expression analysis indicated that miR-15a and miR-16a were either absent or downregulated in the majority of B-CLL patients [19]. In addition, several studies have demonstrated, using high-throughput techniques, that miRNA expression profiles display signatures related to tumor classification, diagnosis, and disease progression [21-23], and further evidence has shown that miRNA gene expression is dysregulated in human cancer [24,25]. Since a single miRNA can target hundreds of mRNAs, aberrant miRNA expression is capable of disrupting the expression of several mRNAs and proteins [26]. Disease-specific expression profiles have been reported in many human cancers, including B-CLL [18], breast carcinoma [27], primary glioblastoma [28], hepatocellular carcinoma [29], papillary thyroid carcinoma [30], lung cancer [22], gastric and colon carcinomas [31], and endocrine pancreatic tumors [32]. In this regard, miRNA expression profiles have been useful in tracing the tissue of origin for cancers of unknown primary origin [33].

MiRNA genes are located in chromosomal regions often subject to rearrangements, deletions, and amplifications in cancer cells [34]. For example, changes in the copy number of some miRNA genes may be common to several types of tumor, such as ovarian cancer, breast cancer and melanoma, while other of these genomic changes may be unique to a specific tumor type [35]. Moreover, specific miRNA expression signatures have been associated with specific translocations in hematopoietic malignancies and solid tumors [36-39]. Several miRNAs have been reported to function as either oncogenes or tumor suppressors [40,41]. For instance, members of the miR-34 family are important effectors of p53 activation, which induces apoptosis [42,43], and let-7 negatively regulates expression of the known oncogene *RAS*, which regulates cell-proliferation pathways [44]. On the other hand, oncogenic miRNAs, such as miR-21 and the cluster miR-17-92 (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1) enhance tumorigenicity by targeting important tumor suppressor genes as *PTEN* [45] and *E2F* [46], respectively. Aberrant and specific miRNA expression were also reported in various other diseases, such as heart disease [47], rheumatoid arthritis [48], inflammatory diseases [49], diabetes [50], and muscle disorders [51]. In this regard, the potential usefulness of miRNA expression profiles in diagnosis and disease monitoring has been postulated.

3. Circulating miRNA as a new diagnostic tool

Several studies have established the importance of miRNAs in many cellular processes, mostly focusing on their actions inside the cell [3]. In addition, most studies of miRNA expression profiling have been done using samples from the tissues of origin. However, several studies have indicated the diagnostic and prognostic utility of circulating RNAs (**Table 1**). One of the first studies measuring miRNA levels in serum was reported by Lawrie *et al.* [52], who showed that sera levels of miR-21 were associated with relapse-free survival in patients with diffuse large B-cell lymphoma. Important mRNA targets of miR-21 have been described as phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4), which regulate important cellular processes, including cell growth, proliferation and apoptosis [53,54]. In addition, studies demonstrated upregulation of miR-21 in several types of tumor, indicating an interesting potential use of miR-21 as a diagnostic biomarker. Mitchell *et al.* [55] demonstrated the presence of circulating tumor-derived miRNAs in blood by using a mouse prostate cancer xenograft model system and showed that measurements obtained from plasma were strongly correlated with those obtained from sera, suggesting that both serum and plasma samples would be adequate for measuring specific miRNA levels. Moreover, these investigators found that by measuring serum levels of miR-141 they were able to distinguish patients with prostate cancer from healthy subjects. Interestingly, miR-141 is expressed in a variety of epithelial cancers including breast, lung, colon and prostate [21]. In another study, Chen *et al.* [56] demonstrated that by using serum directly or by extracting RNA from the serum they could identify unique miRNA expression profiles for lung cancer, colorectal cancer and diabetes patients compared with healthy subjects. These expression profiles were performed by using Solexa sequencing and quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) methods. In one example, expression profiles of serum miRNAs found that patients with non-small-cell lung cancer had 28 miRNAs missing and 63 miRNAs added compared with healthy subjects. Interestingly, Chen *et al.* also showed that miRNA expression profiles differed between serum and blood cells of lung cancer patients, while similar miRNA expression profiles were seen in the serum and blood cells of healthy subjects. In a study of ovarian cancer, eight serum miRNAs, among them miR-21, miR-92, miR-93, miR-126 and miR-29a, were significantly overexpressed in patients compared with healthy subjects, as assessed by using qRT-PCR array panels [57]. Circulating miRNAs have also been shown to be predictive of malignancy and survival in renal cell carcinoma patients [58]. In patients with squamous cell carcinoma (SCC) of the tongue, plasma levels of miR-184 were significantly higher than those in healthy individuals, and miR-184 levels were significantly reduced after surgical removal of the primary tumors [59]. Interestingly, *in vitro* inhibition of miR-184 induces apoptosis and increases

Table 1. MiRNAs with potential as biomarkers for cancer or pregnancy.

Overexpressed microRNA	Disease or physiological status	Healthy subjects (*n*)	Patients (*n*)	Statistical significance	Ref.
miRs-21, 155 and 210	Diffuse large B-cell lymphoma	43	60	p = 0.009, 0.02 and 0.04 respectively	Lawrie *et al*. [52]
miR-141	Prostate cancer	25	25	–	Mitchell *et al*. [55]
miRs-205, 206 and 335/miR-485-5p	Lung/colorectal cancer	75	152	–	Chen *et al.* [56]
miRs-21, 141, 200a, 200b, 203, 205 and 214	Ovarian cancer	20	50	–	Taylor *et al*. [79]
miRs-21, 92, 93, 126 and 29a	Ovarian cancer	15	28	p = 0.01	Resnick *et al*. [57]
miR-184	Squamous cell carcinoma	20	20	p = 0.002	Wong *et al.* [59]
miR-21	Glioblastoma	30	30	–	Skog *et al*. [60]
miRs-141 and 149	Pregnacy	5	10	p = 0.002 and 0.002, respectively	Chim *et al.* [65]
miRs-526a, 527 and 520d-5pmiRs-526a, 527 and 520d-5p	Pregnancy	10	20	–	Gilad *et al.* [66]
miR-17-3p and miR-92	Colorectal cancer	50	90	p < 0.0005	Ng *et al*. [61]

proliferation rates of SCC cells, suggesting a role of miR-181 in SCC tumorigenecity. In another study, mRNA mutants/variants and miRNAs characteristic of gliomas could be detected in serum from glioblastoma patients, suggesting these features' potential for use as diagnostic biomarkers [60]. Recently, Ng *et al.* [61] showed that 5 of 95 analyzed miRNAs are upregulated in colorectal cancer plasma and tissue samples, including miR-17-3p and miR-92. Interestingly, by analyzing an independent set of plasma samples, they demonstrated that miR-92 is differentially expressed in colorectal cancer compared with gastric cancer, inflammatory bowel disease and normal subjects and may be used as potential molecular marker to detect colorectal cancer in plasma samples. MiR-17-92 cluster is frequently reported to be upregulated in many cancers and is positively regulated by Myc, enhancing cell proliferation and inhibiting apoptosis [62]. Wang *et al.* [63] reported a set of miRNAs whose plasma levels are associated with hepatocellular injuries induced by high concentrations of acetaminophen in a mouse model. Interestingly, they demonstrated that selected miRNA species such as mir-22, mir-101b, mir-122 and mir-192 presented dose- and time-dependent changes in plasma. In addition, the data indicate that specific circulating miRNAs, such as mir-122 and mir-192, might be more sensitive biomarkers than certain hepatocellular enzymes used in diagnostic test to detect liver injuries.

Circulating miRNAs have also been useful in monitoring nonpathological conditions. For example, Hunter *et al.* [64] isolated RNA from healthy individuals' plasma and matched mononuclear cells, and demonstrated significant differences in miRNA expression between peripheral blood mononuclear cells and plasma microvesicles. This study also revealed that the predicted gene targets and related biological pathways, which are regulated by the detected miRNAs, were associated with differentiation of blood cells, metabolic pathways, and immune function modulation. In another study, Chim *et al.* [65] demonstrated the existence of placental miRNAs in maternal plasma and the correlation between placental circulating miRNAs and stage of pregnancy. In addition, it has been demonstrated that placental miRNA levels can be used to distinguish pregnant from nonpregnant women [66].

Another avenue of current research is the demonstration of an association between miRNA expression and known serum markers of certain diseases. In one study, there were significant positive correlations between hepatic miR-223 expression levels and serum markers of ischemic injury in mice [67]. However, these results were not observed in colorectal cancer, for which no correlations were found between the miRNA levels analyzed (miR-21, miR-31, miR-143 and miR-145) and serum levels of the biomarker carcinoembryonic antigen [68].

Several studies provide further evidence that miRNAs may be useful as serum biomarkers. Nonetheless, studies in a large population and some aspects of experimental reliability and miRNA stability must be assessed before using serum or plasma miRNAs as biomarkers.

4. Stability of miRNAs in serum

At the time of the first reports decades ago [69,70], most investigators doubted that extracellular RNA could survive in the blood because of the presence of potent ribonucleases [71]. However, several more recent studies have documented the presence of circulating extracellular RNA in serum, and it

has also been shown that this RNA is protected from plasma RNase activity [71,72]. Chen *et al.* [56] showed that serum and plasma contain a large number of miRNAs and that serum miRNAs remained stable after being subjected to severe conditions, such as boiling, very low or high pH, extended storage, and 10 freeze-thaw cycles, conditions that would normally degrade most RNAs. Many theories have attempted to explain the possible mechanisms by which RNA is protected from plasma RNase digestion. One theory suggested that RNAs may annel with DNA, which would render them resistant to both RNase and DNase activity [73]. However, evidence from El-Hefnawy *et al.* [71] showed that RNA present in plasma is protected from degradation not by binding to DNA, but probably by inclusion in lipid or lipoprotein complexes. Studies have reported the genetic exchange of mRNA and miRNA across cell membranes either in microvesicles (up to 1 μm) or in small membrane vesicles of endocytic origin called exosomes (50 – 90 nm) [74,75]. Additionally, it has been suggested that miRNAs, mRNAs and proteins are transferred by exosomal signaling in the nervous system [76] and in embryonic stem cells microvesicles *in vitro* [75,77]. Evidence of the presence of tumor-derived exosomes in the peripheral circulation was presented decades ago in a study by Taylor *et al.* [78]. Later, the group also demonstrated that miRNA contained in tumor exosomes is functional and can suppress the mRNA that encodes signal transduction components within T-cells [79]. In the same study, the authors reported an miRNA signature of circulating ovarian cancer exosomes that had a high correlation with primary tumor miRNA expression. Moreover, these miRNAs were identified at lower levels in exosomes from women with benign disease and were not identified in normal controls. In addition, Rabinowits *et al.* [80] demonstrated a significant difference in exosomal miRNA levels between patients with lung adenocarcinoma and patients without this disease. Also, they showed a similarity in miRNA signatures between circulating exosomal miRNA and originating tumor cells. Moreover, Skog *et al.* [60] showed that exosomes released by glioblastoma cells containing mRNA, miRNA, and angiogenic proteins are taken up by normal recipient cells, such as brain microvascular endothelial cells. They showed that messages delivered by tumor-derived exosomes are translated by recipient cells and stimulate proliferation of a human glioma cell line. In the same study, the tumor-specific epidermal growth factor receptor vIII was detected in serum exosomes from 7 of 25 glioblastoma patients. Moreover, miR-21, known to be overexpressed in glioblastoma tumors [81], was elevated in serum microvesicles from glioblastoma patients. Consistent with these observations, these results indicate that cancer patients present elevated levels of tumor-derived exosomes in plasma compared with controls. Although normal cells within the peripheral circulation can contribute to exosome population, the primary source of circulating exosomes in cancer patients is the tumor [57]. Moreover, mRNA and miRNA containing tumor-derived exosomes can affect biological processes inside of recipient cells. Nevertheless, little is known about the mechanisms in which miRNAs are generated in plasma and the biological impact of these molecules in distant sites of the body. Studies suggest that RNA molecules associated to specific types of exosomes can be released in the circulating compartment on fusion of multivesicular bodies (MVB) with the plasma membrane and may be internalized by recipient cells by endocytosis (**Figure 1**) [74,82]. In addition, studies demonstrated the possibility to analyze miRNA expression using serum and plasma directly without any RNA extraction or serum filtration procedure [56]. Therefore, lysed cells might contribute to the composition of miRNAs in the plasma. Nonetheless, additional studies are necessary to elucidate the mechanism in which miRNAs reach the bloodstream and the physiological impact of exosomal miRNA in global cellular processes.

Although the presence of miRNAs in exosomes could explain their stability in serum, other possibilities include protection by chemical modifications or association with proteins complex. Nevertheless, there is a lack of an established endogenous miRNA control to normalize for plasma or serum miRNA levels measured by commonly used techniques as qRT-PCR. Usually, qRT-PCR data are normalized to an endogenous control gene, which is ideally stably expressed across the analyzed samples to reduce measurement errors, which may be due to technical variations [83,84]. To date, there are very few reports of validated controls that are used to normalize miRNA levels measured in serum or plasma. U6 small nuclear RNA (RNU6B), a control commonly used to normalize miRNA qRT-PCR data [85,86] was found to be less stably expressed than miR-93, miR-106a, miR-17 – 5p, and miR-25 in serum [84]. Moreover, another study reported that RNAU6B and 5S ribosomal RNA were degraded in serum samples [56]. In another study to identify stable controls for normalization, Resnick *et al.* [57] identified 2 of 21 miRNAs (miR-142-3p and miR-16) from a previous expression profile study with cycle threshold (Ct) differences of four cycles or greater between ovarian cancer patients and healthy controls. In another study, a robust normalization protocol was identified by using synthetic versions of *Caenorhabditis elegans* miRNAs [55]. Three *C. elegans* miRNAs were chosen because they did not hybridize to human miRNA probes on miRNA microarrays and produced Ct values comparable to those of miR-16, a moderately abundant miRNA measured in blood plasma from healthy donors. However, more studies are necessary for the identification of an accurate normalization protocol, and empirical validation of stable endogenous control miRNAs for each type of cell or tissue studied is also necessary.

5. Serum miRNAs as potential targets for therapy

One important aspect of circulating miRNAs is the possibility that these molecules affect cellular systems elsewhere in the body and produce conditions favorable for disease progression. In this regard, therapeutic strategies that involve serum miRNAs

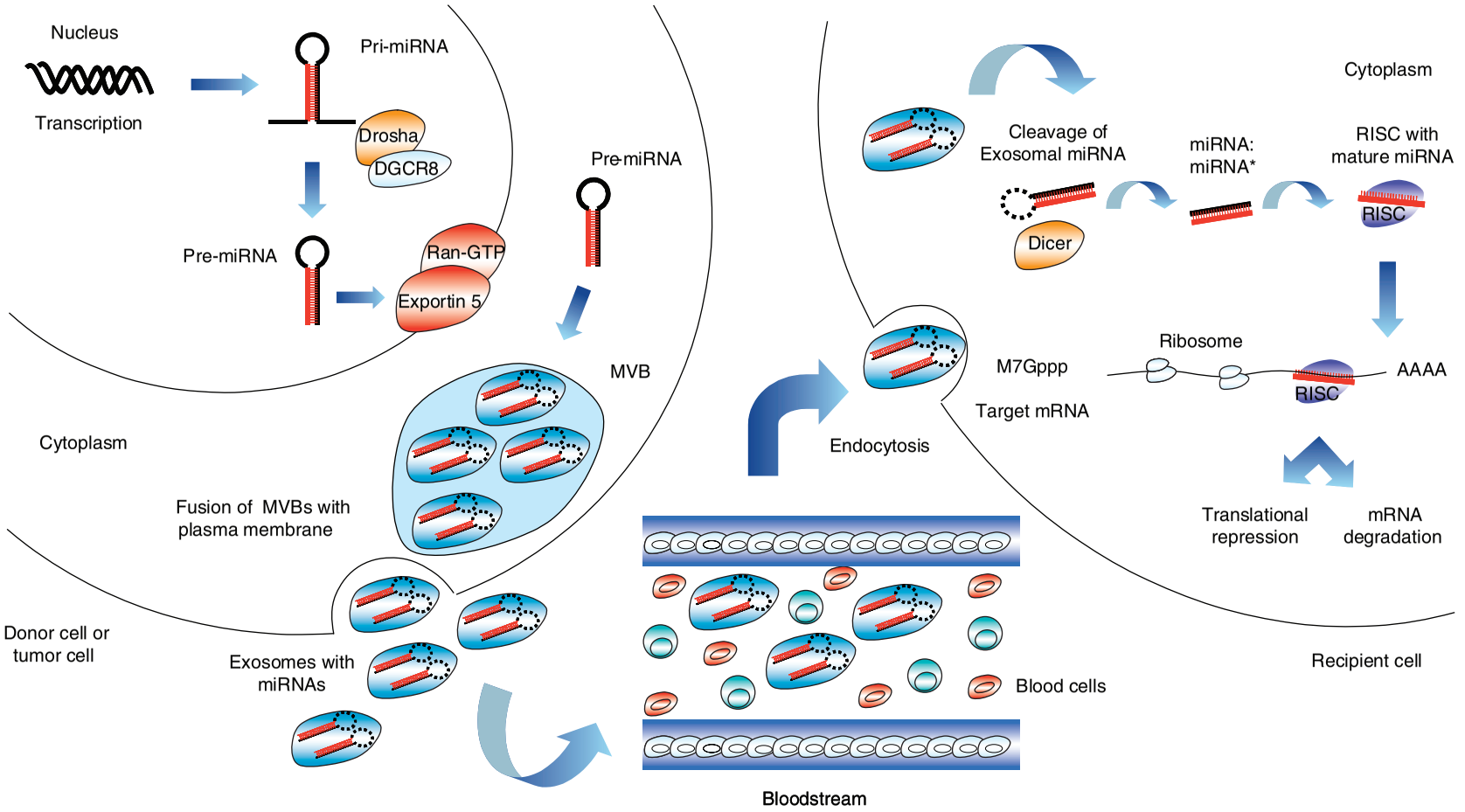

Figure 1. Hypothetical way in which exosomal miRNAs are generate in plasma. MiRNAs are first transcribed in the nucleus as primary transcripts (pri-miRNA) which are processed by Drosha and DGCR8, to form the miRNA precursors (pre-miRNA) in donor cells. Pre-miRNA is actively transported to the cytoplasm by Ran-GTP and the export receptor Exportin-5. Pre-miRNA molecules can bind to multivesicular bodies (MVB) and then associate with exosomes. After fusion with plasma membrane, MVBs are able to release exosomes to the circulating compartments and bloodstream. These exosomes can donate their miRNAs to another cell (recipient cell) by endocytosis process. Exosomal miRNAs are processed by Dicer, resulting in a small double-stranded RNA structure (miRNA:*miRNA). The single-stranded mature miRNA is loaded into the RNA-induced silencing complex (RISC), which facilitates the interaction of the miRNA with complementary binding sites within the target mRNA and, thus, regulates gene expression by either translational repression or degradation of the mRNA. Although we suggest here that pre-miRNAs bind to MVBs, it remains to be elucidated if pre-miRNAs or mature miRNAs are involved in this process.

emerge as an interesting field to be explored [87]. Although only few miRNA targets have been validated [88], several reports of specific miRNA expression profiles associated with diseases have been published as discussed previously. Thus, using this knowledge, some research groups are now exploring miRNA antagonists (antagomirs) as potential therapeutic molecules. In one study, intravenous administration of antagomirs against miR-16, miR-122, miR-192 and miR-194 resulted in a reduction of the corresponding miRNA levels in liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenals of mice [89]. Esau *et al.* [90], using the 2′-O-methoxyethyl phosphorothioate antisense oligonucleotide approach inhibited liver-specific miR-122 in the livers of adult mice. MiR-122 inhibition in normal mice resulted in reduced plasma cholesterol levels, increased hepatic fatty acid oxidation, and a decrease in hepatic fatty acid and cholesterol synthesis rates. Interestingly, miR-122 inhibition in a mouse model of diet-induced obesity resulted in decreased plasma cholesterol levels and a significant improvement in liver steatosis, accompanied by reductions in several lipogenic genes. Similarly, two other groups systemically administered locked nucleic acid oligonucleotides or antisense oligonucleotides, which specifically inhibit miR-122, to mice and African green monkeys by intravenous injections, and they found a reduction in plasma cholesterol levels in both types of treated animal [91,92]. Interestingly, in another study, mice bearing subcutaneous prostate carcinoma xenografts were treated with anti-miR-221/222, which significantly reduced the tumor sizes relative to control treatment [93]. However, before this strategy can be assessed in humans, more studies are necessary to elucidate the role of target miRNAs in each type of disease, to provide biological validation of potential miRNA targets and to measure pharmacokinetic features such as biodistribution and dose response.

6. Conclusion and perspectives

MiRNA expression profiling is of increasing importance as a new diagnostic and prognostic tool. The use of miRNAs as novel biomarkers in serum or plasma represents a new approach to diagnostic screening of blood. Given that most current approaches to cancer screening are invasive and unable to detect early-stage disease, it will be important to determine at which point in a disease's evolution tumor-related circulating miRNAs can be detected in the bloodstream. Certainly, more studies are necessary to establish miRNAs as useful biomarkers, but it should be possible to detect tumor-related miRNAs rapidly in serum and plasma in the near future, which should prove useful in the diagnosis and prediction of treatment outcomes in cancer.

7. Expert opinion

MiRNAs are a class of small noncoding RNAs that play a role in virtually all cellular processes. Dysregulation of miRNA expression can lead to alterations in the expression of hundreds of mRNAs and proteins. Several studies have demonstrated aberrant expression of miRNAs in patient tissue samples from a variety of cancers and diseases compared with samples from normal tissues. In tests of these expression differences, specific miRNA expression profiles have been associated with several pathological conditions, including some cancers. Since the discovery of miRNAs in serum, several studies have raised the possibility of finding a connection between specific miRNA levels in serum and various disease states. Indeed, the existence of tumor- and disease-related miRNAs in serum has been demonstrated in several studies. These findings indicate the potential usefulness of miRNAs as serum biomarkers, since some commonly used tumor markers are not detectable in the early disease stages.

Even though diverse studies have reported the stability of miRNAs in serum even under severe denaturing/degrading conditions, several topics still need to be refined further. First, we are missing large studies reporting miRNA levels in plasma and serum from hundreds of normal individuals of both genders and various ages, so we do not know if specific miRNA levels are the same in young women and old men, for example. Second, some studies have demonstrated that the endogenous controls used at present, such as RNU6B, in miRNA expression studies are degraded in serum; therefore, to obtain accurate and reproducible results, we need new, more robust standardization methods. Finally, we need to gain a better understanding of the mechanisms by which miRNAs are released in plasma/serum. That is, does this occur by cellular destruction or does an active secretory mechanism really exist? Certainly the future will bring more details, which will allow us to solve the puzzle of miRNA detection in various types of body fluids; and this will bring a new era to the field of diagnostic markers for human diseases.

Declaration of interest

Dr Calin is supported as a fellow of The University of Texas M. D. Anderson Research Trust, as a University of Texas System Regents Research Scholar and by the Ladjevardian Regents Research Scholar Fund. This study was partly funded also by an Institutional Research Grant and by a CCSG (New Faculty Award) to Dr Calin.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (• •) to readers.

1. Ambros V. The functions of animal microRNAs. Nature 2004;431:350-5
- **An important review on miRNA functions.**
2. Zamore PD, Haley B. Ribo-genome: the big world of small RNAs. Science 2005;309:1519-24
3. Meister G. miRNAs get an early start on translational silencing. Cell 2007;131:25-8
4. Lewis BP, Shih IH, Jones-Rhoades MW, et al. Prediction of mammalian microRNA targets. Cell 2003;115:787-98
5. Xie X, Mikkelsen TS, Gnirke A, et al. Systematic discovery of regulatory motifs in conserved regions of the human genome, including thousands of CTCF insulator sites. Proc Natl Acad Sci USA 2007;104:7145-50
6. Krek A, Grun D, Poy MN, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37:495-500
7. Lall S, Grun D, Krek A, et al. A genome-wide map of conserved microRNA targets in C. elegans. Curr Biol 2006;16:460-71
8. Lee Y, Jeon K, Lee JT, et al. MicroRNA maturation: stepwise processing and subcellular localization. EMBO J 2002;21:4663-70
9. Zeng Y, Yi R, Cullen BR. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci USA 2003;100:9779-84
10. Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003;425:415-19
11. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 2003;17:3011-16
12. Lund E, Guttinger S, Calado A, et al. Nuclear export of microRNA precursors. Science 2004;303:95-8
13. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004;10:185-91
14. Miska EA. How microRNAs control cell division, differentiation and death. Curr Opin Genet Dev 2005;15:563-8
15. Jannot G, Simard MJ. Tumour-related microRNA functions in Caenorhabditis elegans. Oncogene 2006;25:6197-201
16. Umbach JL, Kramer MF, Jurak I, et al. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 2008;454:780-3
17. Thum T, Catalucci D, Bauersachs J. MicroRNAs: novel regulators in cardiac development and disease. Cardiovasc Res 2008;79:562-70
18. Calin GA, Liu CG, Sevignani C, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 2004;101:11755-60
19. Calin GA, Dumitru CD, Shimizu M, et al. 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
- **The first report of miRNA in cancer.**
20. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 2005;102:13944-9
21. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-8
- **The first report of bead-based miRNA profiling.**
22. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006;9:189-98
23. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857-66
24. McManus MT. MicroRNAs and cancer. Semin Cancer Biol 2003;13:253-8
25. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell 2005;122:6-7
26. Chin LJ, Slack FJ. A truth serum for cancer – microRNAs have major potential as cancer biomarkers. Cell Res 2008;18:983-4
27. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065-70
28. Ciafre SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun 2005;334:1351-8
29. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25:2537-45
30. He H, Jazdzewski K, Li W, et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA 2005;102:19075-80
31. Michael MZ, O'Connor SM, van Holst Pellekaan NG, et al. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882-91
32. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103:2257-61
33. Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol 2008;26:462-9
34. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 2004;101:2999-3004
35. Zhang L, Huang J, Yang N, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA 2006;103:9136-41
36. Garzon R, Croce CM. MicroRNAs in normal and malignant hematopoiesis. Curr Opin Hematol 2008;15:352-8
37. Jongen-Lavrencic M, Sun SM, Dijkstra MK, et al. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood 2008;111:5078-85
38. Dixon-McIver A, East P, Mein CA, et al. Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. PLoS ONE 2008;3:e2141
39. Varambally S, Cao Q, Mani RS, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 2008;322:1695-9
40. Esquela-Kerscher A, Slack FJ. Oncomirs – microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-69
- **An interesting review about miRNA functions in human cancer.**

41. He L, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network – another piece in the tumour-suppression puzzle. Nat Rev Cancer 2007;7:819-22

42. Bommer GT, Gerin I, Feng Y, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol 2007;17:1298-307

43. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 2007;26:745-52

44. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635-47

45. Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647-58

46. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell 2008;133:217-22

47. Ikeda S, Kong SW, Lu J, et al. Altered microRNA expression in human heart disease. Physiol Genomics 2007;31:367-73

48. Pauley KM, Satoh M, Chan AL, et al. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther 2008;10:R101

49. Sheedy FJ, O'neill LA. Adding fuel to fire: microRNAs as a new class of mediators of inflammation. Ann Rheum Dis 2008;67(Suppl 3):iii50-5

50. Tang X, Tang G, Ozcan S. Role of microRNAs in diabetes. Biochim Biophys Acta 2008;1779:697-701

51. Chen JF, Callis TE, Wang DZ. microRNAs and muscle disorders. J Cell Sci 2009;122:13-20

52. Lawrie CH, Gal S, Dunlop HM, et 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

• **One of the first studies of miRNA detection in cancer patients' serum.**

53. Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647-58

54. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008;27:2128-36

55. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008;105:10513-18

56. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997-1006

57. Resnick KE, Alder H, Hagan JP, et al. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009;112:55-9

58. Feng G, Li G, Gentil-Perret A, et al. Elevated serum-circulating RNA in patients with conventional renal cell cancer. Anticancer Res 2008;28:321-6

59. Wong TS, Liu XB, Wong BY, et al. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res 2008;14:2588-92

60. Skog J, Wurdinger T, van RS, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008;10:1470-6

61. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of colorectal cancer patients: a potential marker for colorectal cancer screening. Gut 2009. [Epub 6 Feb 2009; doi: 10.1136/gut.2008.167817]

62. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839-43

63. Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci USA 2009;106:4402-7

64. Hunter MP, Ismail N, Zhang X, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS ONE 2008;3:e3694

65. Chim SS, Shing TK, Hung EC, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem 2008;54:482-90

• **An interesting report of miRNA in pregnancy, and one of the first reports of placental miRNA detection in serum.**

66. Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. PLoS ONE 2008;3:e3148

67. Yu CH, Xu CF, Li YM. Association of microRNA-223 expression with hepatic ischemia/reperfusion injury in mice. Dig Dis Sci 2008 [doi: 10.1007/s10620-008-0629-8]

68. Slaby O, Svoboda M, Fabian P, et al. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 2007;72:397-402

69. Kamm RC, Smith AG. Nucleic acid concentrations in normal human plasma. Clin Chem 1972;18:519-22

70. Stroun M, Anker P, Maurice P, Gahan PB. Circulating nucleic acids in higher organisms. Int Rev Cytol 1977;51:1-48

71. El-Hefnawy T, Raja S, Kelly L, et al. Characterization of amplifiable, circulating RNA in plasma and its potential as a tool for cancer diagnostics. Clin Chem 2004;50:564-73

72. Tsui NB, Ng EK, Lo YM. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem 2002;48:1647-53

73. Sisco KL. Is RNA in serum bound to nucleoprotein complexes? Clin Chem 2001;47:1744-5

74. Valadi H, Ekstrom K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654-9

75. Ratajczak J, Miekus K, Kucia M, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia 2006;20:847-56

76. Smalheiser NR. Exosomal transfer of proteins and RNAs at synapses in the nervous system. Biol Direct 2007;2:35-49

77. Yuan A, Farber EL, Rapoport AL, et al. Transfer of microRNAs by embryonic stem cell microvesicles. PLoS ONE 2009;4:e4722

78. Taylor DD, Homesley HD, Doellgast GJ. Binding of specific peroxidase-labeled antibody to placental-type phosphatase on tumor-derived membrane fragments. Cancer Res 1980;40:4064-9

79. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13-21

80. Rabinowits G, Gercel-Taylor C, Day JM, et al. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009;10:42-6

81. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029-33

82. Lotvall J, Valadi H. Cell to cell signalling via exosomes through esRNA. Cell Adh Migr 2007;1:156-8

83. Davoren PA, McNeill RE, Lowery AJ, et al. Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol 2008;9:76-86

84. Peltier HJ, Latham GJ. Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA 2008;14:844-52

85. Shell S, Park SM, Radjabi AR, et al. Let-7 expression defines two differentiation stages of cancer. Proc Natl Acad Sci USA 2007;104:11400-5

86. Corney DC, Flesken-Nikitin A, Godwin AK, et al. microRNA-34b and microRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res 2007;67:8433-8

87. Jackson DB. Serum-based microRNAs: are we blinded by potential? Proc Natl Acad Sci USA 2008;106:E5

88. Sethupathy P, Megraw M, Hatzigeorgiou AG. A guide through present computational approaches for the identification of mammalian microRNA targets. Nat Methods 2006;3:881-6

89. Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005;438:685-9

90. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3:87-98

91. Elmen J, Lindow M, Silahtaroglu A, et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 2008;36:1153-62

92. Bhat B, Esau C, Davis S, et al. 2′-O-methoxyethyl/2′-fluoro modified oligonucleotides result in more potent inhibition of microRNA-122 in vivo: a target implicated in HCV replication. Nucleic Acids Symp Ser (Oxf) 2008;52-69

93. Mercatelli N, Coppola V, Bonci D, et al. The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. PLoS ONE 2008;3:e4029

Affiliation

Maria Angelica Cortez[1] MSc &
George Adrian Calin[†2] MD PhD
[†]Author for correspondence
[1]Faculty of Medicine of Ribeirão Preto,
Department of Genetics,
Sao Paulo, Brazil
[2]Associate Professor
The University of Texas
M. D. Anderson Cancer Center,
Department of Experimental Therapeutics,
Unit 422,
1515 Holcombe Blvd,
Houston, Texas 77030, USA
Tel: +1 713 792 5461; Fax: +1 713 745 4528;
E-mail: gcalin@mdanderson.org