ELSEVIER

Critical Reviews in Oncology/Hematology xxx (2010) xxx–xxx

CRITICAL REVIEWS IN Oncology Hematology Incorporating Geriatric Oncology

www.elsevier.com/locate/critrevonc

Circulating microRNAs: Association with disease and potential use as biomarkers

Glen Reid*, Michaela B. Kirschner, Nico van Zandwijk

Asbestos Diseases Research Institute (ADRI), Bernie Banton Centre, University of Sydney, Gate 3, Hospital Road, Concord, Sydney, NSW 2139, Australia

Accepted 9 November 2010

Contents

Abstract

The control of gene expression by microRNAs influences many cellular processes and has been implicated in the control of many (patho)physiological states. Recently, microRNAs have been detected in serum and plasma, and circulating microRNA profiles have

* Corresponding author.
E-mail addresses: glen.reid@sydney.edu.au (G. Reid), michaela.kirschner@sydney.edu.au (M.B. Kirschner), nico.vanzandwijk@sydney.edu.au (N. van Zandwijk).

1040-8428/$ – see front matter
doi:10.1016/j.critrevonc.2010.11.004

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

now been associated with a range of different tumour types, diseases such as stroke and heart disease, as well as altered physiological states such as pregnancy. Here we review the disease-specific profiles of circulating microRNAs, and the methodologies used for their detection and quantification. We also discuss possible functions of circulating microRNAs and their potential as non-invasive biomarkers.

Keywords: microRNAs; Plasma; Serum; Microvesicles; Exosomes; Biomarkers

1. Introduction

The presence of nucleic acids in the serum has long been recognised, with circulating DNA released from tumours readily detected in the blood. Numerous studies have demonstrated fetal DNA in the maternal blood, leading to a number of clinical tests. Recently microRNAs (miRNAs) circulating in blood have attracted considerable attention. MiRNAs are a newly discovered class of short RNAs, 18–25 nucleotides in length, which regulate gene expression in a post-transcriptional manner, via sequence-specific interaction with target sites in mRNAs [1]. Through partial homology to the 3′UTR in target mRNAs, miRNAs effect control of gene expression via repression of translation as well as reducing mRNA levels directly [2]. Many genes have target sites for regulation by miRNAs and the complexity of this regulatory network is increased by the ability of an individual miRNA to modulate expression of multiple genes [3]. In humans, over 800 miRNAs are known, and expression of many occurs at specific stages of development or in a tissue-specific manner [2].

The expression of characteristic miRNA profiles has been implicated in the control of various developmental and disease states [2]. In cancer, miRNAs can act as tumour suppressor genes or oncogenes [4], as well as controlling various aspects of cancer biology such as chemoresistance [5] and metastasis [6]. The tissue- and disease-specific miRNA expression profiles reported are often more informative and discriminatory than mRNA profiles. This has been exploited in particular in oncology, for which a link between (loss of) miRNA expression and cancer was first demonstrated for chronic lymphocytic leukemia [7]. In a subsequent landmark study, the tissue of origin of poorly differentiated tumours was more accurately determined from miRNA profiles than from mRNA profiles [8]. These early studies have led to tumour-specific miRNA profiles published for many tumour types (reviewed in [2]) and the appearance of diagnostic tests based on the expression of discriminatory miRNA signatures [9,10].

Complementing the informative potential of miRNA expression profiles is the unexpected stability of these short RNAs. Compared with mRNA and other longer RNAs, the short miRNA sequences are extremely stable. This property has enabled the analysis of miRNAs in archival tissue blocks, a source of little utility in mRNA profiling [11]. Recently, cell-free miRNAs have been detected in serum and plasma samples. The stability of miRNAs is similarly high in both fresh and archived serum and plasma, making the miRNAs in these samples potentially useful candidates for diagnostic and other clinical applications.

In this review, we summarise the studies of circulating cell-free miRNAs to date. In addition to commenting on their potential as non-invasive biomarkers for a number of diseases, we describe recent studies shedding light on the source of these miRNAs in the circulation and their possible functions.

2. Circulating miRNAs associated with (patho)physiological states

Circulating miRNAs have been investigated in a wide variety of patient samples and animal models (Table 1). Since the report of an association between circulating miRNAs and lymphoma [12], miRNA profiles have been associated with different tumour types, a range of diseases such as cardiovascular disease, stroke and multiple sclerosis, as well as altered physiological states such as pregnancy and liver injury. We review each of these in the following sections.

2.1. Cancer

Circulating miRNAs have now been associated with different tumour types (Table 1). In these studies, tumour-specific miRNAs have been identified through a combination of rational approaches (quantifying miRNAs known to be overexpressed in tumours) and more traditional discovery methods (profiling circulating miRNAs in patients and controls). The following sections describe studies in which circulating miRNAs have been used to distinguish between cancer patients and controls.

2.1.1. Early studies

The first demonstration of a link between circulating miRNAs and cancer came from studies published in early 2008. In their study investigating sera from 60 patients with diffuse large B cell lymphoma (DLBCL), Lawrie et al. found a significant increase in levels of miR-155, miR-21 and miR-210 compared with controls [12]. Patients with high levels of miR-21 were also found to have a longer relapse-free survival. Shortly thereafter, a study profiling miRNA expression in patients with squamous cell carcinoma of the tongue identified a number of miRNAs either up- or down-regulated compared with matched normal tissue. The miRNA with highest expression in tumour tissue, miR-184, was also present at higher levels in plasma, and these levels decreased following surgery in 22 of 25 patients [13]. Both of these early studies used miRNA profiling in tumour cells to select

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

Table 1
Circulating microRNA studies. Summary of the changes in circulating microRNAs reported to date for various (patho)physiological states.

			miRNA		
Condition	Source	Methods[a]	Increased	Decreased	Associations
DLBCL [2]	Serum	TaqMan	miR-155, miR-210, miR-21		miR-21 associated with RFS
AML [17]	Plasma	Microarray, TaqMan		miR-92a	miR92a/-638 distinguishes pts
Lung cancer [5]	Plasma & serum	Solexa, RT-qPCR	miR-25, miR-233		
NSCLC [7]	Exosomes	Microarray	miR-17-3p, miR-21 miR-106a, miR-146 miR-155, miR-191, miR-192, miR-203, miR-205, miR-212, miR-214		Not detected in control samples
NSCLC [6]	Serum	Solexa, RT-qPCR	miR-486, miR-30d	miR-1, miR-499	Panel associated with OS
CRC [9]	Plasma	RT-qPCR	miR-17-3p, miR-92		Reduced after surgery
CRC [10]	Plasma	RT-qPCR	miR-29a, miR-92a		Dx for advanced CRC
Ovarian cancer [11]	Exosomes	microarray	N.D.	N.D.	miR-21, 141, 200a, 200b, 200c, 203, 205 and 214 higher in cancer than benign disease
Ovarian Cancer [12]	Serum	TLDA, RT-qPCR	miR-21, miR-29a, miR-126, miR-92, miR-93	miR-127 miR-99b miR-155	miR-21, miR-92 and miR-93 higher in pts with normal CA-125
SCC [3]	Plasma	TaqMan	miR-184		miR-184 reduced post-surgery
OSCC [19]	Plasma	TaqMan	miR-24		
OSCC [20]	Plasma	TaqMan	miR-31		Reduced post-surgery
PDAC [13]	Plasma	TaqMan	miR-21, miR-210, miR-155, miR-196a		
PCA [14]	Plasma	TaqMan	miR-210		
Breast Cancer [15]	Serum	RT-qPCR	None found	None found	miR-155 higher in PR-positive patients
Breast [41]	Whole blood	TaqMan	miR-195, let-7a		
Prostate [4]	Plasma	Cloning & sequencing, TLDA, RT-qPCR, 454 sequencing	miR-141		Distinguish PCa from healthy controls
Prostate [55]	Serum	Array	miR-16, miR-92a, miR-92b, miR-103, miR-107, miR-197, miR-34b, miR-328, miR-485-3p, miR-486-5p, miR-574-3p, miR-636, miR-640, miR-766, miR-885-5p		Stage 3 and 4 prostate cancer samples compared with normal donor sera
Gastric cancer [18]	Plasma	TaqMan	miR-17-5p, miR-21, miR-106a, miR-106b	let-7a	
HCC [21]	Serum	TaqMan	miR-500		Only in 3/10 patients; reduced post-surgery
Glioblastoma [48]	Exosomes	TaqMan	miR-21		Increased 40-fold over controls
5 cancers (lung, colon, ovarian, prostate, breast) [55]	Serum	Microarray	N.D.	N.D.	28 miRNA signature distinguished between 5 cancers and controls
Pregnancy [31]	Serum	RT-qPCR	miR-527, miR-520d-5p, miR-526a		Ratio of miRs distinguishes
Pregnancy [30]	Plasma	TaqMan	miR-141, miR-149, miR-299-5p, miR-135b		

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

Table 1 (*Continued*)

			miRNA		
Condition	Source	Methods[a]	Increased	Decreased	Associations
AMI [22]	Plasma	RT-qPCR	miR-1		
Rat AMI model [23]	Plasma	TLDA, RT-qPCR	miR-208		
AMI [24]	Plasma	TaqMan	miR-499		High in all AMI patients
HF [26]	Plasma	RT-qPCR	miR-423-5p		Enriched in HF but not non-HF dyspnea
Sepsis [29]	Serum	RT-qPCR		miR-146a miR-223	
Mouse model of liver injury [27]	Plasma	Microarray, RT-qPCR	miR-122, miR-192		
Stroke [69]	Whole blood	Microarray, RT-qPCR		Various	
Rat stroke model [28]	Plasma		miR-122, miR-133a, miR-124		Increase with brain, liver and muscle injury

[a] Real time RT-qPCR is termed 'TaqMan' when hydrolysis probes were employed, or 'RT-qPCR' when SYBR Green was the dye used.

the miRNAs to be analysed in circulating blood. The first reports of direct profiling of circulating miRNAs followed soon after. Mitchell and colleagues used a combination of cloning and sequencing, RT-qPCR arrays and massively parallel sequencing to analyse plasma from normal individuals, prostate cancer patients and a mouse xenograft model of prostate cancer, identifying miRNAs in circulating blood associated with the presence of tumour [14]. Of the miRNAs found to be present at higher levels in the plasma of prostate cancer patients, miR-141 levels were able to discriminate between prostate cancer patients and controls. A similar study by Chen et al. used Solexa sequencing to catalogue miRNAs present in normal serum from male and female individuals and to compare this with serum from patients with lung or colorectal cancer, or diabetes [15]. Several miRNAs were detected specifically in the sera of patients with each tumour type, with a large number common to both groups.

2.1.2. Lung cancer

In follow-up studies by Chen et al. validating some of their Solexa sequencing findings by RT-qPCR, the levels of miR-25 and miR-223 were increased 5- and 3-fold, respectively, in patients with NSCLC whereas let-7a did not change. In a more recent Solexa-based study by the same group, serum miRNAs in NSCLC patients were used to predict outcome [16]. Eleven miRNAs satisfied the criteria of at least 50 copies in either group, along with greater than 5-fold difference in expression. In subsequent RT-qPCR analysis of a total of 303 patients, higher levels of miR-486 and miR-30d, in combination with lower levels of miR-1 and miR-499, were strongly associated with reduced survival. In an alternative approach, Rabinowits et al. first purified exosomes (see Section 4.1) from the plasma of patients with NSCLC (adenocarcinoma) and controls and found that patients had higher RNA content in these preparations [17]. Twelve NSCLC-associated miRNAs were measured and were found at similar levels in matched tumour tissue and plasma RNA, but were undetectable in control samples. In contrast, a more recent study of vesicle-associated miRNAs found let-7f, miR-20b and miR-30e-3p downregulated in patients, but no significant upregulation of any miRNA was found [18]. Low levels of miR-30e-3p in plasma were associated with longer disease-free survival, whereas a trend towards longer overall survival was found with low let-7f levels.

2.1.3. Colorectal cancer (CRC)

In addition to the sequencing approach applied by Chen and colleagues, two further studies have investigated miRNA profiles in plasma from CRC patients. The first used a RT-qPCR array to identify miRNAs that were at least two-fold higher in both tumour tissue and plasma from 5 CRC patients compared with adjacent normal tissue and plasma from control individuals [19]. Of the 5 miRNAs identified, miR-17-3p and miR-92 were significantly different in plasma from a second set of 25 patients and 20 controls. The levels of these miRNAs were found to be reduced in a third group of CRC patients 7 days after resection. Finally, in a larger validation set, mir-92 differentiated between CRC patients and controls with a sensitivity of 89% and a specificity of 70%. A more recent study aimed to use miRNAs as markers of early CRC [20]. A set of 12 miRNAs previously reported to be associated with CRC were analysed in plasma samples from 20 patients and 20 controls. This case control experiment revealed higher levels of miR-29a and miR-92a in CRC patients. Validation in 100 CRC cases and 59 controls revealed the potential and additive nature of both miRNAs. In addition, plasma levels of both miRNAs were found to be associated with tumour stage and decreased after surgery in most cases [20]. The authors proposed measuring miR-29a and miR-92a within a panel of miRNAs to improve diagnostic accuracy.

2.1.4. Ovarian cancer

Circulating miRNAs in ovarian cancer patients have been the focus of two independent studies. In the first, miRNA pro-

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

files of tumour-derived exosomes isolated from the serum of ovarian cancer patients were compared with tumour cell profiles from the same patient [21]. Most miRNAs were present in both cells and serum, with some of them enriched in one or the other fraction. Eight miRNAs previously reported as highly expressed in ovarian cancer were further analysed in serum, and although only microarray data were presented, all were clearly increased when compared with serum from benign ovarian disease [21]. In a subsequent study, a Taqman-based array was used to profile 365 miRNAs in patient and control serum [22]. There were 21 differentially expressed miRNAs, 10 of which overlapped with previously reported profiles of ovarian cancer. In follow-up studies using sera from 19 patients and 11 controls, miR-21, miR29a, miR-126, miR-92 and miR-93 were found upregulated and miR-127, miR-99b and miR-155 were down-regulated in patients with ovarian cancer. For miR-21, miR-92 and miR-93, levels were high in 3 patients with normal CA-125 levels, a clinical marker of ovarian cancer [22].

2.1.5. Pancreatic cancer

Circulating miRNAs have been investigated as an early detection marker of pancreatic cancer, using those miRNAs previously linked to pancreatic cancer in a proof-of-concept study [23]. All four miRNAs tested – miR-21, miR-210, miR155 and miR-196a – were significantly upregulated in plasma of patients compared with controls, but much of this difference was due to patients with exceptionally high levels [23]. When all 4 miRNAs were analysed together, the combined sensitivity and specificity of the panel increased to 64% and 89%, respectively [23]. In a similar approach, Ho et al. focused on miR-210 and its potential association with hypoxia in pancreatic cancer. Archived plasma samples from 22 patients and 25 controls were used confirming significantly increased levels of miR-210 in patients [24]. However, this observation also appeared to be the consequence of very high miRNA levels in some patients.

2.1.6. Other cancers

In a small-scale study using archived serum from breast cancer patients, miR-16, miR-145 and miR-155 were found at similar levels in patients and controls [25]. A subsequent study quantified 8 miRNAs known to be associated with breast cancer in the (whole) bloods of a larger cohort of breast cancer patients [26]. Here miR-195 and let-7a levels in blood were elevated in patients and could help distinguish cases from controls. Levels of miR-195 were also found to be higher in tumour tissue than in matched control samples. In addition, node-negative disease was associated with higher let-7a levels, and miR-10b and miR-21 were higher in ER-negative patients' blood [26].

A microarray-based approach to analyse plasma from 2 AML patients and 7 controls identified 148 miRNAs in circulation [27]. When the rank order of intensity was calculated, miR-92a was significantly reduced in plasma from leukemia patients. In a larger set (61 patients and 16 controls), the ratio of miR-92/miR-638 expression analysed by RT-qPCR distinguished patients from controls. Unexpectedly, miR-92a expression, as determined by *in situ* hybridisation, was high in leukemic blasts but undetectable in normal blasts.

Tsujiura et al. recently reported an analysis of circulating miRNAs in gastric cancer patients with the aim of identifying markers for diagnosis and disease monitoring [28]. Increased plasma levels of miR-17-5p, miR-21, miR-106a and miR-106b were found along with decreased levels of let-7a. Higher miR-106a and lower let-7a levels were also found when tumour levels were compared with (paired) normal mucosa, while miR-106a plasma levels were found to be decreased one month after surgery. In a large-scale validation study, the ratio of the plasma concentrations of miR-106a and let-7a produced the highest AUC with a sensitivity and a specificity of 85.5% and 80%, respectively.

Two other recent studies identified elevated levels of miR-24 and miR-31 in plasma of patients with squamous cell carcinoma of the oral cavity (OSCC). In the first, miR-24 levels were found to be 2.4-fold higher in pre-surgery plasma samples from the patients with OSCC compared with healthy controls [29]. A prediction of OSCC could be made with 70% sensitivity and 92% specificity. The same group has also reported the potential of miR-31 to act as a marker of OSCC [30]. Regardless of stage, levels of miR-31 were significantly higher in plasma from 43 patients compared with 21 controls (AUC of 0.82). Furthermore, plasma miR-31 levels decreased following tumour resection and interestingly, miR-31 levels in saliva samples were also elevated in patients.

Finally, miR-500 was shown to be present in the sera of patients with hepatocellular carcinoma (HCC) [31]. This miRNA was associated with liver maturation in a mouse model of liver development. Levels tended to be higher in HCC lines and tumour samples when compared with matched normal tissue. However, serum levels were increased in only 3 of 10 HCC patients. Noteworthy is that miR-500 levels in these three patients were dramatically reduced 6 months after surgical resection.

2.2. Heart disease

Specific miRNAs have also been linked to heart disease. After finding levels of miR-1 overexpressed in a rat model, Ai et al. showed that levels of miR-1, but not miR-133, were significantly elevated in acute myocardial infarction (AMI) patients compared with controls, and that these returned to baseline after a period of two weeks [32]. Ji et al. used microarrays to profile tissue-specific miRNAs in the rat, and found miR-208 to be exclusively expressed in heart [33]. Upon induction of myocardial injury, miR-208 increased significantly during the next 3–12 h before returning to (undetectable) baseline levels, whereas levels of control miR-183 and 5S remained constant. This temporary increase of miR-208 was organ specific, as renal infarction did not lead to increased miR-208 levels in the plasma but did increase circulating levels of kidney-derived miR-10a. In a

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

recent follow-up study, the same group found that miR-499 is specifically expressed in human heart, but only low level expression of miR-208a and miR-208b was found [34]. When the levels in plasma were analysed, miR-499 was only detected in AMI patients (not in controls or those with congestive heart failure) and levels returned to normal by the time of hospital discharge.

In a study combining a rat model with clinical observations in AMI patients, miRNAs miR-1, miR-133a, miR-499 and miR-208a were selected as candidates based on their specific expression in skeletal and/or cardiac muscle [35]. All four miRNAs were present at higher levels in AMI patients and miR-208a levels turned out to be the most significantly increased. This miRNA also showed a consistent reduction in plasma of 5 patients followed up after 2 months of treatment. In another group of 20 patients this miRNA appeared earlier than cardiac troponin, suggesting it is a more sensitive earlier marker of AMI [35].

Patients with congestive heart failure (CHF) exhibited a different plasma miRNA profile [36]. Using microarrays to analyse plasma from 12 CHF and 12 healthy controls revealed 108 differentially expressed miRNAs. The best 16 candidates were analysed by RT-qPCR and miR-423-5p appeared to be a good predictor of CHF diagnosis. Interestingly, miR-1 and miR-208, which are predictive of AMI, were not increased in CHF, suggesting a specific miRNA response in blood elicited by different pathologic conditions [36].

2.3. Oxidative liver injury

Changes in plasma miRNAs were also found in a mouse model of drug-induced liver injury [37]. Microarray analysis of liver and plasma samples from acetaminophen-overdosed mice revealed a number of changes in liver and plasma that were for the most part reciprocal. For example, miR-122, and miRs-29a-c all decreased in greater than 5-fold in the liver while increasing 5–500-fold in plasma. Elevated levels of miR-122 and miR-192 in plasma were also more sensitive than ALT levels to acetaminophen dose and appeared more rapidly. A similar study in a rat model was carried out to see whether specific circulating miRNAs were able to monitor tissue injury [38]. Increases in levels of miR-122, miR-133a and miR-124 were found in plasma following injury to liver, muscle or brain, respectively. Moreover, unlike AST and ALT, plasma levels of miR-122 and miR-133a seemed to discriminate between liver and muscle injury.

2.4. Sepsis

A number of miRNAs previously associated with sepsis were analysed in the serum of septic patients and compared with patients with systemic inflammatory response syndrome (SIRS) and controls [39]. Three of these – miR-126, miR-146a and miR-223 – were significantly reduced: miR-126 and miR-146a in the SIRS patients and all three in the septic patients. Reduction in miR-146a was significantly greater in the septic group than in SIRS group, and reduced miR-223 levels showed an AUC of 0.86 and specificity and sensitivity of 100% and 80%, respectively.

2.5. Pregnancy

The presence of specific pregnancy associated miRNAs in the maternal circulation has been investigated in two studies. In the first [40], miRNAs in the placenta, maternal blood cells and plasma were analysed, and candidate miRNAs were selected based on a 10-fold increased concentration in placenta compared with maternal blood cells, and absence in post-delivery plasma. In this way 17 candidate miRNAs were identified, and the 4 miRNAs present at highest concentration were analysed in maternal plasma by RT-qPCR. Both miR-141 and miR-149 were found to be significantly reduced post-delivery, and miR-141 levels increased with pregnancy stage. In a second study a set of 28 miRNAs (consisting of placenta-specific as well as broadly expressed miRNAs) were measured in sera from 20 pregnant (10 in first and 10 in third trimester) and in control sera from 10 non-pregnant women [41]. Twelve miRNAs were present at levels greater than 5-fold increased in the third trimester versus non-pregnant samples. Of these, miR-526 and miR-527 showed a greater than 500-fold increase and together with miR-520d-5p could accurately distinguish pregnant from non-pregnant women. The authors postulated that as many of the miRNAs they analysed are also associated with pre-eclampsia, these could be useful marker for the prediction of this condition. Most recently, placenta-specific miRNAs from the C19MC cluster (miR-517a, -518b, -518e and 524) were found in the plasma of pregnant women only, whereas levels of miR-141 and miR-424 were found in both groups but significantly increased during pregnancy. Although no specific miRNA was upregulated in pregnancies complicated by fetal growth restriction, there was an almost two-fold increase in overall miRNA levels in these pregnancies compared with controls [42].

2.6. Circulating miRNAs in normal individuals

There have been considerable efforts to catalogue the normal spectrum of circulating miRNAs in healthy individuals using different platforms. In the study of Chen et al., all of the small RNAs (<30 nt) isolated from the serum of healthy subjects (10 males and 11 females, pooled separately) were sequenced [15]. Most of the sequences obtained were 21–23 nucleotides in length, consistent with the size of mature miRNAs, and included 190 of the miRNAs known at the time (miRBase V10.0). In RT-qPCR validation experiments there was good correlation, although miRNA species present at less than 10 sequence copies were not reliably amplified and were thus removed from the normal spectrum of circulating miRNAs reported, leaving 101 in the normal profile [15]. This compares with 130 miRNAs found in the serum of a normal individual using a TaqMan low-density RT-qPCR array (TLDA; v1.0) [14], and 148 (detected in 7 normal controls [27]) or 170 (in 4 normal samples; top 40 listed [35]) using

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

microarray analysis. A similar number of miRNAs (105) were detected using TLDA analysis of RNA from microvesicles isolated from peripheral blood [43].

In the Chen study, most species were present at very similar levels in serum from male and female subjects. Interestingly, the miRNA profiles of serum and those of blood cells showed extensive overlap, suggesting that the majority of circulating miRNAs in normal individuals are released into the circulation by these cells [15]. This is consistent with the miRNA content found in microvesicles isolated from peripheral blood, where 71 of 104 miRNAs were also found in peripheral blood mononuclear cells (PBMCs) [43]. In contrast, the overlap between the miRNAs detected in the serum and blood cells of lung cancer patients was far less pronounced, with only 57 of 148 miRNAs detected in both serum and PBMCs from these patients. Furthermore, only 69 of 160 miRNAs were common to serum from lung cancer patients and control subjects, with 63 unique to lung cancer serum. Together, these results suggest that certain circulating miRNAs are derived from the tumour and can be measured in the blood [15]. The observation that patient and control samples will contain many common circulating miRNAs is an important consideration when selecting candidates with diagnostic potential.

If it is assumed that the individuals used as controls in these studies mentioned above were indeed free from disease, it is possible to begin to build a picture of the spectrum of miRNAs found in the circulation in a normal physiological state. Two additional studies have added to this picture, profiling miRNAs using arrays based on more recent versions of miRBase [27,35]. Although identifying many of the more recently catalogued miRNAs that could not be detected with platforms used in the earlier studies, a considerable overlap is still evident. From the 5 studies to date that have investigated samples from normal individuals, over 270 different miRNAs have been detected. The 20 miRNAs identified in at least 4 of these studies are listed in Table 2. Within this group are representatives from well-characterised families such as the miR-17-92 cluster and the miR-15/16 group, as well as the let-7 family. They will be discussed in more detail in the following sections.

Table 2
Most commonly detected miRNAs in circulation of normal individuals.

	Chen [5]	Mitchell [4]	Hunter [33]	Wang [25]	Tanaka [17]
hsa-let-7b					
hsa-miR-16					
hsa-miR-21					
hsa-miR-223					
hsa-miR-24					
hsa-miR-25					
hsa-miR-30d					
hsa-miR-320					
hsa-miR-106b					
hsa-miR-142-3p					
hsa-miR-15a					
hsa-miR-183					
hsa-miR-186					
hsa-miR-19b					
hsa-miR-20a					
hsa-miR-22					
hsa-miR-26a					
hsa-miR-451					
hsa-miR-484					
hsa-miR-92a					

3. Measuring and quantifying circulating miRNAs

In comparing the reports to date, circulating miRNAs have been isolated and detected using different methodologies and from different source materials (Table 1), and quantified employing varying reference gene and normalisation strategies (Table 4). The contribution of these factors to differences in the reported results can be significant and is addressed below.

3.1. Detection: arrays, qPCR, sequencing

The variation in detection of miRNAs by different platforms is well known in the field and has been described in detail elsewhere [44–48]. In terms of circulating miRNAs, this is perhaps best illustrated when comparing miRNAs detected in normal individuals both between and within different studies. The miRNAs most commonly detected in the plasma or serum of normal individuals are listed in Table 2. Three early studies catalogued the normal circulating miRNA profile, one using Solexa deep sequencing, a second by RT-qPCR and the third applying a cloning and sequencing approach as well as a RT-qPCR based array detection system. Being carried out at a similar time using platforms based on the same version of miRBase (V10.0), these studies are suitable for comparison between the different profiling approaches. The studies detected many common miRNAs (two newer studies have used more recent miRBase updates and consequently identified many of the more recently identified miRNAs [27,35]), but clear differences are apparent when the relative levels of miRNAs are considered. Although the data were obtained using different platforms, normalising the data based on rank order allows the relative abundance of the 20 most abundant miRNAs in each study to be compared (Table 3). This approach reveals that the two RT-qPCR based studies both find miR-223 at a level of at least 5-fold higher than all others. Interestingly, this miRNA is also detected within the sequences cloned by Mitchell, but the abundance

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

Table 3
Relative abundance of the most commonly detected miRNAs in independent studies.

	Chen		Mitchell		Hunter		Mitchell	
	miRNA	A[a]	miRNA	A		A	miRNA	A
1	hsa-miR-451	106	hsa-miR-223	100	hsa-miR-223	338	hsa-let-7f	16
2	hsa-miR-16	12	hsa-miR-16	20	hsa-miR-484	11	hsa-miR-223	11
3	hsa-miR-486-5p	8	hsa-miR-126	7	hsa-miR-191	10	hsa-miR-21	9
4	hsa-miR-101	5	hsa-miR-26a	6	hsa-miR-146a	8	hsa-let-7a	6
5	hsa-let-7g	4	hsa-miR-24	6	hsa-miR-16	5	hsa-miR-103	4
6	hsa-let-7f	4	hsa-miR-19b	6	hsa-miR-26a	5	hsa-miR-101	3
7	hsa-let-7a	4	hsa-miR-142-3p	5	hsa-miR-222	5	hsa-miR-16	3
8	hsa-miR-185	3	hsa-miR-9[a]	4	hsa-miR-24	5	hsa-miR-24	3
9	hsa-miR-20a	3	hsa-miR-26b	4	hsa-miR-126	4	hsa-miR-26a	3
10	hsa-miR-106b	3	hsa-miR-191	3	hsa-miR-32	3	hsa-let-7g	2
11	hsa-let-7i	2	hsa-miR-20a-	2	hsa-miR-486	3	hsa-miR-185	2
12	hsa-miR-103	2	hsa-miR-146a	2	hsa-miR-20a	2	hsa-miR-30d	2
13	hsa-miR-21	2	hsa-miR-484	2	hsa-miR-19b	2	hsa-miR-451	2
14	hsa-miR-25	1	hsa-miR-222	2	hsa-miR-150	2	hsa-let-7e	1
15	hsa-let-7b	1	hsa-miR-92a	2	hsa-miR-574	1	hsa-let-7i	1
16	hsa-miR-192	1	hsa-miR-486	1	hsa-miR-92	1	hsa-miR-125a-5p	1
17	hsa-miR-191	1	hsa-miR-186	1	hsa-miR-93	1	hsa-miR-126	1
18	hsa-miR-17	1	hsa-miR-126[a]	1	hsa-miR-342	1	hsa-miR-140-3p	1
19	hsa-miR-26a	1	hsa-miR-30b	1	hsa-miR-197	1	hsa-miR-146a	1
20	hsa-miR-142-5p	1	hsa-miR-15b	1	hsa-miR-328	1	hsa-miR-148b	1

[a] *A* = relative abundance of the twenty most commonly detected miRNAs in each study, calculated in relation to the 20th most common miRNA.

is an order of magnitude lower than in the same study when using the RT-qPCR. Furthermore, miR-223 is not detected in the sequencing approach of the Chen study.

Comparing the two RT-qPCR based studies also reveals that they have only 9 of the 20 most abundant miRNAs in common and that their abundance shows striking differences. The same is true when comparing the two cloning and sequencing approaches. In terms of the miRNAs detected at highest abundance, in contrast to the TLDA studies, the sequencing approach used by Chen et al. identifies miR-451 as the most abundant miRNA found in the circulation of healthy subjects. Similar to the results for miR-223, this miRNA shows a 5-fold lower abundance in the cloning and sequencing approach used by Mitchell et al. and does not appear in either RT-qPCR study. Interestingly, a more recent study of AMI that included some data on normal circulating miRNA profiles found miR-451 to be the most abundant when analysed by microarray [35]. This further suggests that the platform with which the miRNAs are analysed can influence results. As the two studies identifying miR-451 were based on Chinese cohorts, this also raises the possibility of ethnic differences in normal profiles. Underlining the differences in results obtained using different approaches are the results of a plasma miRNA study by Tijsen et al. The authors analysed plasma miRNA content in 12 HF patients and 12 controls using microarray and identified 16 candidate miRNAs. Upon validation by RT-qPCR, only one, miR-423-5p, exhibited a similar fold-change [36].

As described above, a number of studies have reported the detection of miRNAs in the blood, in various cell-free compartments. These vary in purity from serum to plasma, to purified microvesicles and exosomes (Table 4). Although no differences are detected in the levels of miRNAs found in serum or plasma within studies [14,41], this variation makes comparison between independent studies difficult, even when the same tumour or altered physiological state is considered. For example, there is only partial overlap between the circulating miRNA species identified in the three studies of CRC to date, two of which used RT-qPCR analysis of plasma [19,20], with the other relying on Solexa sequencing of serum [15]. In fact, although both RT-qPCR studies used similar RNA extraction methods and the same RT-qPCR approach, one of the studies found miR-17-3p to be higher in patient plasma [19], whereas the other reported plasma levels too low to accurately quantify [20].

The identification of miRNAs associated with pregnancy also led to different results depending on whether serum [41] or plasma [40] samples were analysed. Further complicating this comparison is that although both studies used RT-qPCR to determine miRNA levels, one of them applied a stem-loop RT reaction combined with TaqMan qPCR, while the other used a polyadenylation together with TaqMan qPCR. A further example of the different conclusions that can be drawn from these types of studies is seen when the two studies investigating circulating miRNAs in ovarian cancer are compared [21,22]. In the first, the miRNA content of RNA isolated from exosomes derived from patient blood was compared to the miRNA profile of tumour tissue from the same patient using microarrays [21]. These were predominantly detected at similar levels between tumour tissue and exosomes with only a few exceptions. Six of 8 miRNAs identified in the TLDA analyses of serum samples in the second study [22] were also found in the former, but interestingly none of these were found in the exosome-enriched fraction.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

Table 4
RT-qPCR quantification strategies used to measure circulating microRNAs.

	Source	Template	Quantification	Reference gene
Lawrie [12]	Serum	10 ng total RNA	$2^{-\Delta\Delta C_T}$ (tumour-ctrl)	miR-16; U6 and 5S not detected
Wong [13]	Plasma	NR	$2^{-\Delta\Delta C_T}$ (tumour-ctrl)	miR-16
Mitchell [14]	Plasma	Fixed volume RNA	Absolute; copies/μl normalised to Cel-miRs	Spiked Cel-miRs
Chen [15]	Serum	10 μl purified serum	Raw C_T, relative expression, $2^{-\Delta C_T}$	Total RNA; miR-16 inconsistent, U6 and 5S degraded
Ng [19]	Plasma	40 ng total RNA	$2^{-\Delta\Delta C_T}$ (tumour-ctrl)	RNU6B
Tanaka [27]	Plasma	20 ng total RNA	Ratio 92a/638	miR-638
Zhu [25]	Serum	Fixed volume RNA	C_T normalised to 18S	18S
Huang [20]	Plasma	Fixed volume/fixed RNA quantity	$2^{-\Delta\Delta C_T}$	miR-16; RNU6B unstable[a]
Resnick [22]	Serum	1 ng total RNA; 50 ng (array)	$2^{-\Delta\Delta C_T}$	miR-142-3p
Wang, K [37]	Plasma	50 ng	$2^{-\Delta C_T}$, [control-treated]	miSpike for recovery;
Wang, J [23]	Plasma	10 ng	$2^{-\Delta\Delta C_T}$	miR-16
Lin [29]	Plasma	NR	$2^{-\Delta\Delta C_T}$	RNU6B, let-7a
Liu [30]	Plasma	Fixed volume	$2^{-\Delta\Delta C_T}$	miR-16
Tsujiura [28]	Plasma	Fixed volume	amol/μl based on std curves	RNU6B
Ho [24]	Plasma	Fixed volume boiled plasma	$2^{-\Delta\Delta C_T}$	Cel-miR-54
Hu [16]	Serum	10 μl serum	$2^{-\Delta C_T}$ (patient-ctrl)	None
Hunter [43]	Microvesicles	500 ng RNA	C_T normalised to median	18S, 5S, RNU38B, RNU43 and RNU6 all variable[b]
Chim [40]	Plasma	2.5 ng OR Fixed volume	Copies/ml based on std curves	None
Gilad [41]	Plasma	NR	Raw C_T; 50-(C_T target-C_T ave of 6)	Mean of 6 miRNAs; spike-ins for normalising recovery
Ji [33]	Plasma	Fixed volume	Absolute (copies/μl); relative ($2^{-35\text{-}CT}$)	5S for recovery; standard curves
Ai [32]	Plasma	NR	Normalised Ct	U6
Adachi [34]	Plasma	Fixed volume	Copies/100 μl based on std curve	internal reference RNA
Yamamoto [31]	Serum	14 ng	Ratio miR-500/miR-16 set to 1 before treatment	miR-16
Tijsen [36]	Plasma	Fixed volume	$2^{-\Delta\Delta C_T}$	miR-1249
Heneghan [26]	Whole blood	100 ng total RNA	$2^{-\Delta\Delta C_T}$	miR-16
Wang, J-F [39]	Serum	NR	$2^{-\Delta\Delta C_T}$	mmu-miR-295

[a] Quantity of RNU6B decreased rapidly upon incubation at RT, and polyadenylation step of RT occurred at different sites as determined by sequence analysis.
[b] Compared with data obtained from blood cells (PBMCs).

3.2. Source material

As described above, plasma, serum and isolated exosomes or microvesicles have been used as the source of RNA from which miRNA profiles have been generated. Each approach has advantages and disadvantages. Plasma and serum are readily available and the extraction process is straightforward, notwithstanding the difficulties associated with isolating RNA from dilute solutions with high protein content [49]. In contrast, miRNA isolation from exosomes or microvesicles requires extensive sample preparation via a range of techniques [50]. Nevertheless, these labour-intensive preparations can, depending on the method employed, yield specifically isolated vesicle populations [50]. In the case of samples from cancer patients, this may enrich for tumour-specific miRNAs that would otherwise be difficult to detect in the background of miRNA circulating in plasma of normal individuals [21,51].

More recently, RNA extracted from whole blood has been used as starting material for profiling miRNAs in cancer [26,52,53], stroke [54] and multiple sclerosis [55]. While these studies have each identified candidate miRNAs linked to disease, it is difficult to determine whether these changes represent differences in cell-free circulating miRNAs, or derive from changes in miRNA profiles of white blood cells, as was found in the case of upregulated miR-146a in the PBMCs of rheumatoid arthritis patients [56]. In the case of cancer, circulating tumour cells may in addition contribute to the profile from whole blood of patients; this possibility has been explored in an attempt to detect the presence of gastric cancer cells in blood via measurements of miR-106a and miR-17 [57].

3.3. Normalisation, quantification and reference genes

The many steps involved in quantifying circulating miRNAs each have associated error. The extraction protocols used have varied, and have used different starting material (whole blood, serum, plasma, or exosomes; Table 4). Added to the relatively low concentration of RNA in the circulation [49], this can have considerable impact on the quantification. Also, the choice of methodology for RNA extraction has an influence on RNA yield. The high protein content of blood complicates extraction, and this is evidenced by most reports having used modified extraction protocols. But even in cases where isolation procedures were carefully modified, not all

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

samples yield enough RNA of sufficient quality for analysis [23]. Furthermore, as the RNA (and protein) concentration in plasma or serum can vary between individuals, and this has been shown to increase for a number of diseases, there is still debate on whether circulating miRNAs should be quantified as copies/μl or copies/ng total RNA. Therefore, to date there is no convention regarding the quantification of miRNAs in plasma or serum.

In the studies published to date, quantities of miRNA detected in serum/plasma have been reported in various ways, ranging from raw Ct, 50-ΔCt or $2^{-\Delta\Delta Ct}$ ratios, to copies per ng RNA or copies per μl serum/plasma (Table 4). There are further studies in which measurements of absolute quantities are eschewed in favour of ratios from one (disease) state to another. Obviously this wide range of units makes comparison between studies difficult. Nevertheless, and despite these ranges, most values have been (or can be) converted to copies (or Ct) per μl serum or per ng total RNA by the use of standard curves generated using synthetic oligos corresponding in sequence to the mature miRNA of interest. The main argument for adopting a conversion into copies per μl starting material over copies per ng extracted RNA is that RNA concentration can vary both with disease state as well as between individuals, therefore making comparisons between individuals and disease states less reliable. A second factor is that expressing levels as of copies per μl is likely to be preferable in terms of a diagnostic test.

The selection of a reference gene is a further confounding factor (Table 4). There has been frequent use of miR-16, shown to be expressed at similar levels in most tissues [58], and a number of groups have reported that the levels in circulation do not vary considerably between individuals [12,13,20,23,30,31,59]. However, this has been questioned by others, and has yet to be accepted as the optimal reference. Resnick et al. found miR-142-3p and miR-16 consistent across all patients and controls, but used only miR-142-3p as reference. Besides miR-16, other genes such as RNU6B or 18S, have been used to normalise data in some studies [19,28,29,32,33], but have been shown to be highly variable or prone to degradation in others. For example, Zhu et al. did not detect RNU44, 48 or 66, and GAPDH only at high Ct and therefore used 18S as reference gene [25]. While Ng [19], Ai [32] and Ji [33] used U6 as a single reference gene, Lin used both RNU6B and let-7a to normalise their data. Finally, Hunter et al. showed in their study that although (sno) RNA U38B, (sno) RNA U43, (sn) RNA U6 and the rRNAs 5S and 18S are readily detectable in most samples, their expression is quite variable [43]. For this reason they decided to use a median normalisation procedure instead of a reference gene. Not relying on a reference gene at all, Mitchell et al. pioneered a method in which synthetic *C. elegans* miRNAs – Cel-miRs-39, -54, and -238 – are spiked into the RNA isolation process to act as normalisers for differences in recovery between samples [14]. Since then, a number of groups have adopted a similar strategy [20,24,25,34,39,41], including mmu-miR-295, a mouse miRNA not detectable in human serum [39].

The final point of difference in the studies published to date is the type of normalisation and quantification strategy used (Table 4). This is highly dependent on the choice of either using a reference gene or a spike-in control and the methods used range from raw Ct to comparative Ct, to quantification based on limits of detection or absolute quantification based on standard curves. In most cases were a reference gene is used, the quantification of data is then carried out using a comparative Ct method. Nevertheless, as some groups question the use of reference genes as being a reliable normalisation procedure, other methods like absolute quantification based on standard curves are also quite common. As mentioned earlier Mitchell et al. pioneered the use of synthetic spike-in controls to normalise for the extraction procedure. But again, many differences between the different approaches can be seen. While Mitchell et al. used a combination of three different spike-in controls to normalise before calculating absolute copy numbers based on standard curves [14], others like Ho [24] or Wang [39] only used one spike-in control.

The wide range of platforms employed for miRNA isolation and quantification in the studies of circulating miRNA to date makes comparing the findings in these studies difficult. In addition to methodological differences, there is also a lack of consistency regarding optimal normalisation and quantification strategies. Further research is needed to address these important points and to bring consensus to this rapidly expanding field.

4. Remaining questions

Despite the unarguable potential for circulating miRNAs to act as non-invasive biomarkers, studies to date have raised a number of questions that remain to be answered.

4.1. Why are circulating miRNAs so stable?

The blood contains high levels of RNase activity that degrade exogenously added mRNA within seconds [60] indicating that miRNAs are unlikely to exist in a free (unprotected) state. This has been addressed in various ways in a number of studies. Serum or plasma miRNA levels do not appear to be affected by incubation of the serum or plasma samples at 4 °C [21] or room temperature [14,20,24,41], multiple freeze–thaw cycles [14,15,24,41], long-term storage [15] or even boiling [15,24] or treatment with acid or base [15]. Synthetic RNA oligos (corresponding to mature miRNA sequences) spiked into serum or plasma were rapidly lost [14], albeit at a rate slower than mRNA degradation [14,15], and detection of spiked-in miRNAs was RNase sensitive [40]. Filtration of serum or plasma samples has been used to remove subcellular particles [14,40], which removed placental mRNAs but not miR-141 [40]. Finally, treatment of plasma or serum samples with RNase before extraction had little effect on endogenous miRNA levels [14,15], suggesting

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

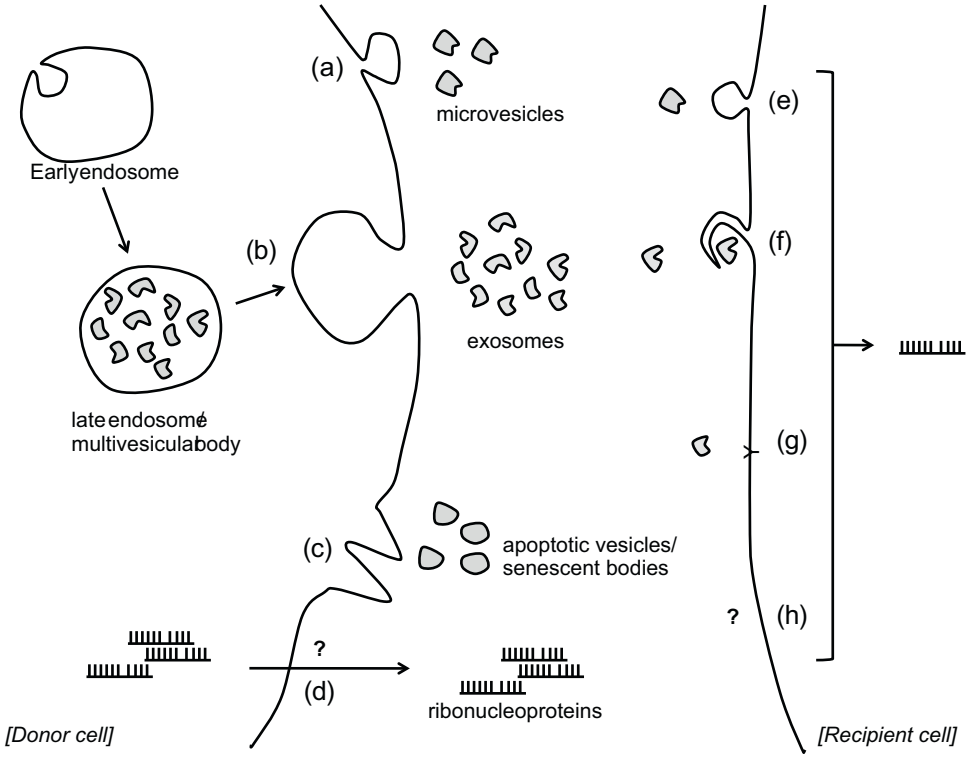

Fig. 1. Extracellular miRNAs in the circulation. Numerous studies have now demonstrated the presence of miRNAs (and other RNAs) in the extracellular space following release from cells. These miRNAs may be contained within vesicles, including: microvesicles released by exocytosis (a); exosomes (formed via invagination of the early endosome) and released upon fusion of late endosomes with the plasma membrane (b); apoptotic vesicles and/or senescent bodies (c). There are also reports of extracellular miRNAs that are not associated with vesicles, but rather are released via an unknown pathway (d), which are protected by proteins in ribonucleoprotein complexes. Once present outside of the donor cell, extracellular miRNAs can potentially interact with recipient cells via a number of different processes, including: direct fusion (e); internalisation (f); receptor-mediated interactions. There are likely to be other mechanisms, especially for vesicle-free miRNAs (h), but all await further investigation to provide convincing evidence of their involvement in inter-cellular (mi)RNA exchange. Cellular release (and/or uptake) of miRNAs depicted here as the mature form, may also include pre-miRs. See Refs. [49–51] for a more detailed discussion of exosomes and other microRNAs-containing vesicles.

that they exist in a compartment that is not accessible to the action of RNases.

Several studies have demonstrated the association of circulating miRNA with secreted membrane vesicles [17,21,43,51,61–65]. An in-depth description of the many classes of microvesicle and their biogenesis, recently reviewed elsewhere [66–68], is beyond the scope of this review but is briefly summarised in Fig. 1. These membrane vesicles are secreted by a number of cell types and are produced by various organs as well as tumours. The lack of consensus regarding nomenclature [68] of these vesicles is a confounding factor for a number of studies as, depending on the method of isolation, different populations of vesicles are recovered. For the purposes of this review microvesicle refers to actively secreted vesicles as well as those released by cells undergoing senescence and apoptosis, whereas exosomes refers to vesicles of endosomal origin derived from multivesicular bodies that fuse with the plasma membrane to release their contents [68].

Exosomes from various cell types contain discrete subsets of miRNAs, with the first evidence coming from a study of mouse and human mast cell lines [64]. When compared with cellular miRNA content, the exosomal concentration of several miRNAs was found to be much higher and derived from a particular subset of genes, suggesting that miRNAs are packaged selectively into exosomes [64]. In a study of cells cultured from glioblastoma patients, the exosomes produced contained miRNAs abundant in glioblastoma [51]. Furthermore, miR-21, overexpressed in these tumours, was detected at 40-fold elevated levels in the exosomes isolated from serum of patients compared with controls [51]. Similarly, the miRNA content of exosomes from ovarian cancer patients revealed an increased concentration of eight miRNAs that distinguished between benign ovarian disease and cancer [21]. Microvesicles are also released from normal cells, especially those of haematopoietic lineages, and the miRNA content of these has been isolated and characterised [43]. MiRNAs were also detected in the microvesicles released from mesenchymal stem cells, with a number enriched compared with levels in the cells [69]. Even in studies where miRNAs were detected directly from plasma or serum without first purifying exosomes or microvesicles, the stability of the miRNAs detected is often attributed to their encapsulation in vesicles.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

As RNA is extremely labile in serum, microvesicles are believed by many to be the only source of tumour-derived miRNAs detected in serum or plasma. However, the different techniques used to isolate exosomes (immunopurification versus ultracentrifugation) are expected to yield different vesicle populations fractions [50]. Of note apoptotic bodies (ABs), membrane vesicles released by dying and apoptotic cells, have been shown to contain an RNA component [62]. ABs from endothelial cells were also observed to modulate function of recipient endothelial progenitor cells *in vitro* [70]. The rapid appearance of miRNAs within 3 h following tissue injury in animal models [33] (and human trials) is also poorly understood – these may also be in exosomes, but could also be derived from lysed or necrotic cells; Lodes suggests lysis accounts for much of the miRNA [71]. Irradiation also induced exosome release (senescence-associated exosomes) by cancer cells, but this was slower and they accumulate in days rather than hours via two mechanisms – immediate versus delayed [62,72]. Further complicating matters is the observation that the majority of circulating vesicles under normal physiological conditions are derived from platelets [69].

In an AMI model it was suggested that the increased plasma miRNA levels results from cell damage in the myocardium leading to 'leakage', and it is not yet clear whether these AMI-specific increases in miRNA levels are exosome/microvesicle associated [35]. This hypothesis was also put forward by Ai et al., who suggested release from necrotic myocytes, although this does not rule out the possibility that damaged cells release exosomes [32]. Similarly, a decrease in the hepatic levels of a number of liver-specific miRNAs in a model of drug-induced liver damage was reciprocated by their increased appearance in plasma, which could be explained by cell injury or active release [37]. The opposite observation – that upon drug treatment some miRNAs decrease in plasma and increase in liver – is more difficult to explain. This phenomenon was also observed by Tanaka et al. who found decreased levels of miR-92a in plasma of AML/ALL patients, but increased levels in the leukemic versus normal blasts, a result that they postulate is explained by active uptake by the cells. The complete picture of the location of miRNAs in the circulation awaits further study.

Despite the frequent association of miRNAs with microvesicles, it is still unknown whether miRNAs are present in other forms, for example associated with serum (or other cell-derived) proteins that confer RNase stability. A clue was provided by a study in which miRNAs secreted by serum-deprived cells in culture were found to export substantial miRNAs into the medium [73]. These were only partially contained within the vesicle pellets derived from the conditioned medium, with the remainder extravesicular and associated with proteins. Among these were RNA-binding proteins including NPM1, which was able to protect miRNAs from degradation [73]. Whether these miRNA-containing nucleoprotein complexes exist *in vivo* and whether they can be taken up by cells remains to be seen. Nevertheless, these observations suggest that cells have various ways of exporting miRNAs (Fig. 1), and the relative importance of these mechanisms awaits further investigation.

4.2. Inter- and intra-individual variations

So far, with the exception of differences associated with pregnancy [40,41], differences in circulating miRNAs between males and females have not been found. Studies of serum [15] (10 males and 11 females, separately pooled and sequences) or microvesicles [43] (27 males and 24 females, analysed individually) found no significant differences between male and female individuals. The latter study further failed to detect any differences between age groups when comparing the miRNAs in microvesicles from individuals in the upper quartile range with those from the lower quartile range. Nevertheless, no study has extensively profiled the miRNA content of the same individual over time. As the microvesicle and miRNA content can vary with physiological condition or disease state [17,21], it is also possible that miRNAs may vary within individuals at different times.

4.3. Which are the forms of miRNA found in the circulation?

Almost all of the studies reviewed here have looked exclusively at the mature form of miRNA, the short single-stranded RNA contained within the RISC complex [1]. In addition to mRNAs and mature miRNAs [51,64], the presence of primary miRNAs in microvesicles was recently reported [61]. In this study, conditioned medium (CM) from mesenchymal stem cells (MSCs) was shown to contain small RNAs encapsulated within lipid vesicles. The RNA content of the CM was predominantly made up of small (<300 nt) RNA species, with undetectable 18S and 28S. Microarray analysis of MSCs and CM revealed 60 miRNAs in the CM of which 15 were not detected in the cells, suggesting a selective loading of vesicles. Furthermore, RT-qPCR analysis of let-7b and let-7g showed a high pre/mature miRNA ratio that was reduced following RNaseIII treatment. HPLC purification of exosomal fractions revealed a further increase in the pre/mature miRNA ratio. Interestingly, neither Ago2 nor Dicer, two major protein components of the RISC machinery, was detected in the CM. This led the authors to postulate that the mature miRNAs in the vesicles are unlikely to be functional as they can no longer be loaded into the RISC. In contrast, Collino et al. found Ago2 and other RNA binding proteins in the microvesicles (MVs) from MSCs [69]. Whether selective loading of pre-miRNAs and absence of RISC components is a specific feature of exosomes from MSCs, or whether it is a more general phenomenon, remains to be determined.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

4.4. What are the functions of circulating RNAs?

Circulating microvesicles have been recognised for many years, but their generation and physiological roles are still incompletely understood. Suggested roles include hematostasis, involvement in antigen presentation to T cells, and the development of tolerance, for example the immune suppression directed by the placenta during pregnancy [68]. Their importance is underlined by the number of disorders associated with dysregulation of microvesicle levels (reviewed in [74]). A number of studies have demonstrated the presence of mRNA in various microvesicles [51,64,75], and when exosomal mRNAs were analysed bioinformatically, their key functions included cellular development, protein synthesis and RNA post-transcriptional modification [64]. Some of these were transferred to recipient cells and translated [64], as was mRNA from the Gluc reporter gene packaged into exosomes by transduced glioblastoma cells [51]. Other studies have measured the effects on gene expression *in vitro* of exposing cells to purified exosomes, but have done so without demonstrating transfer (and translation) of exosomal mRNAs.

The observation that miRNAs are present in the circulation is a more recent one. Based on the known function of the miRNAs commonly found circulating in normal healthy controls, many authors have proposed a role for maintaining homeostasis of the circulatory system. Further supporting this suggestion is the observation that many of these appear to derive from PBMCs and other blood cells [15,40,43]. As an example, miR-223 (the circulating miRNA found at highest levels in the studies of Mitchell [14] and Hunter [43]) is implicated in regulation of the differentiation of various blood cell lineages, as well as that of hematopoietic stem cells [43]. Interestingly, miR-223 is lost in AML [76]. MiR-16 regulates lymphoid development and red cell development together with miR-24, both of which are also readily found in normal circulation [43]. MiR-24 also suppresses p16(INK4a) [77].

Circulating levels of miR-451 were highest in the studies of Chen and Wang, and this miRNA is robustly expressed in erythrocytes [78]. Reduced platelet vesicle production causes a bleeding disorder (Scott syndrome) and increases are associated with a number of other disorders [65], but whether this is related to miRNA content is unknown. Of two further miRNAs found at high levels, miR-146a is closely linked to immune and lymphoid function, and miR-486, the highest differentially expressed in microvesicles compared with PBMCs, is predicted to regulate a number of metabolic pathways [43]. An absence of long(er) mRNAs and predominance of short (mi)RNAs has been suggested by a number of authors as an indication of a regulatory role [15,51,64,65,79]. Consistent with this idea, miRNAs in MVs derived from MSCs target genes involved in proliferation/differentiation, cell cycle and cell death. Furthermore, miRNAs enriched in MVs are involved in metabolism and organ development [69]. The treatment of normal human blood or THP-1 cells with various stimuli also led to increased levels of selected miRNAs in MVs, with miR-150 especially sensitive to LPS stimulation [80].

Table 5
MicroRNAs commonly found in the circulation of cancer patients.

microRNA	Tumour type
miR-21	Diffuse large B-cell lymphoma, gastric cancer, ovarian carcinoma, pancreatic ductal adenocarcinoma, non-small cell lung cancer, glioblastoma
miR-29a	Colorectal cancer, ovarian carcinoma
miR-92	Colorectal cancer, ovarian carcinoma, prostate cancer
miR-106a	gastric cancer, non-small cell lung cancer
miR-155	Diffuse large B-cell lymphoma, PDC
miR-210	Diffuse large B-cell lymphoma, pancreatic ductal adenocarcinoma
miR-203/205/214	ovarian carcinoma, non-small cell lung cancer (exosomes)

The role played by miRNAs released by tumours is also poorly understood. Some miRNAs are released by a number of different tumour types (Table 5). For example, miR-21 is upregulated in a variety of solid tumours [81] and is commonly found at high levels in the circulation of patients with these tumour types. Likewise, miR-155 has oncogenic properties and is upregulated in a number of cancers [82]. It is both an early marker and predictor of poor survival in pancreatic cancer, as well as being implicated in DLBCL [12,23]. It is tempting to speculate that circulating miRNAs are secreted to promote angiogenesis or as a means of avoiding tumour surveillance. Supporting this idea, the function of those circulating miRNAs specific to NSCLC & CRC appears to be involved in tumorigenesis and cell growth [15]. The appearance of these overexpressed miRNAs in the circulation may be a consequence of tumour growth and cell lysis [71], or alternatively, cancer-specific miRNAs may derive from cells infiltrating the tumour [12]. Interestingly, Tanaka et al. found high miR-92a in leukemic blasts, but low levels in plasma [27]. They suggest leukemic cells may actively take up exosomes containing miRNAs thereby reducing miR-92 in the circulation.

4.5. Which cells are targeted by circulating miRNAs?

While many of these suggested roles for circulating miRNAs are plausible, elucidation of the functional role requires demonstration of a gene regulatory effect of miRNAs transferred to recipient cells. As exosomes and microvesicles bear the same surface proteins and ligands as their parent cells, receptor mediated interactions could potentially occur with specific recipient cells (Fig. 1). Valadi et al. showed that mast cell-derived exosomes transferred labeled RNA to other mast cells, and the mRNA component was translated. As this RNA transfer was to mast cells only and did not occur to $CD4^+$ cells, this provides evidence that the exosome exchange represents a controlled communication pathway [64]. The transfer of some miRNAs contained within embryonic stem

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

cell microvesicles to mouse embryonic fibroblasts (MEFs) was demonstrated by RT-qPCR [65]. This transfer appeared specific to vesicle-enriched miRNAs, but could be due to the abundance in recipient cells. For example, the high levels of miR-16 make it more difficult to detect changes in overall levels owing to delivery of a few copies, whereas delivery of a few copies in a background of absence in MEFs are more easily detectable.

In contrast to studies of functional transfer of mRNAs, demonstrating that circulating miRNAs are transferred to (and have effects on gene expression in) recipient cells is more difficult owing to their more modest effects on gene expression. Several recent studies have addressed this, showing effects of horizontally transferred miRNAs on recipient cell gene expression [79,83]. Exosomes from EBV-transformed B cells containing EBV-specific miRNAs were transferred to HeLa cells and primary monocyte-derived dendritic cells (DCs). In both cases this led to repression of an exogenously expressed luciferase reporter engineered to contain the 3′UTR of the *CXCL11* gene, a target of EBV miRNAs [79]. The authors suggested that detection of transferred miRNAs is physiologically relevant because as few as 100 copies are sufficient to repress target genes [84]. Moreover, the exosomes taken up by the DCs appear in late endosomes, the site associated with mRNA recognition and miRNA-mediated gene silencing [85]. In a second study, HEK293 cells transfected with pri-miRNA or shRNA-encoding plasmids secreted exosomes into the medium that contained the mature miRNA or siRNA [83]. When added to luciferase-reporter expressing cells, the exosomes containing the luciferase-specific siRNA reduced luciferase expression in the recipient cells. Similarly, cells transfected with an engineered luciferase reporter with miR-146a target site produced less luciferase when treated with miR-146a-containing exosomes. In the study of Collino et al., MVs from MSCs transferred highly abundant miRs that led to downregulation of the proteins of known target genes of these miRs [69]. Similarly, MVs from THP-1 cells containing miR-150 could transfer this to HMEC-1 cells and reduce c-Myb protein levels, resulting in reduced migration of the recipient cells. These MVs also transferred miR-150 to mouse blood vessels *in vivo* [80]. ABs from HUVECs contained miR-126 and this led to CXCL12 expression in recipient HUVECs via inhibition of RGS16 [70]. Administration of these ABs to mice was able to protect them from atherosclerosis, thereby representing the first demonstration of horizontal transfer of miRNAs by extracellular vesicles leading to functional consequence *in vivo* [70].

5. Conclusion and perspectives

Over the last two years the presence of circulating miRNAs has now been detected in a variety of conditions. These miRNAs are extremely stable, often found in association with exosomes, and represent potentially informative biomarkers for a range of diseases. As with any field in its infancy, methodologies for the detection and quantification of circulating miRNAs suffer from a lack of convention, similar to the problems that were associated with the rapid early adoption of microarrays in gene expression profiling research. Once common methods are used in these studies, it will become possible to compare results produced by different laboratories. Nevertheless, the promise of using miRNAs as a readily detectable and measurable component of the blood remains great and has the potential to aid in the diagnosis and treatment of cancer and other disorders.

Reviewers

Graeme Doran, M.D., Ph.D., Massachusetts Institute of Technology, Center for Cancer Research, Cambridge, MA, United States.

Marcel Dinger, M.D., Ph.D., The University of Queensland, Institute of Molecular Bioscience, 306 Carmody Road, St Lucia, Brisbane, QLD 4072, Australia.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

We thank our colleagues for helpful discussions and reviewing the manuscript.

References

[1] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[2] Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009;4:199–227.
[3] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15–20.
[4] Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annu Rev Med 2009;60:167–79.
[5] Ma J, Dong C, Ji C. MicroRNA and drug resistance. Cancer Gene Ther 2010;17:523–31.
[6] Hurst DR, Edmonds MD, Welch DR. Metastamir: the field of metastasis-regulatory microRNA is spreading. Cancer Res 2009;69:7495–8.
[7] 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.
[8] Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
[9] Benjamin H, Lebanony D, Rosenwald S, et al. A diagnostic assay based on microRNA expression accurately identifies malignant pleural mesothelioma. J Mol Diagn 2010.
[10] Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol 2010;23:814–23.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

[11] Szafranska AE, Davison TS, Shingara J, et al. Accurate molecular characterization of formalin-fixed, paraffin-embedded tissues by microRNA expression profiling. J Mol Diagn 2008;10:415–23.
[12] 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.
[13] Wong TS, Liu XB, Wong BY, Ng RW, Yuen AP, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res 2008;14:2588–92.
[14] Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008;105:10513–8.
[15] 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.
[16] Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol 2010;28:1721–6.
[17] Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009;10:42–6.
[18] Silva J, Garcia V, Zaballos A, et al. Vesicle-related microRNAs in plasma of NSCLC patients and correlation with survival. Eur Respir J 2010.
[19] Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009;58:1375–81.
[20] Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 2009.
[21] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13–21.
[22] Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. 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.
[23] Wang J, Chen J, Chang P, et al. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res (Phila Pa) 2009;2:807–13.
[24] Ho AS, Huang X, Cao H, et al. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. Transl Oncol 2010;3:109–13.
[25] Zhu W, Qin W, Atasoy U, Sauter ER. Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes 2009;2:89.
[26] Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 2010;251:499–505.
[27] Tanaka M, Oikawa K, Takanashi M, et al. Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. PLoS One 2009;4:e5532.
[28] Tsujiura M, Ichikawa D, Komatsu S, et al. Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer 2010;102:1174–9.
[29] Lin SC, Liu CJ, Lin JA, Chiang WF, Hung PS, Chang KW. miR-24 up-regulation in oral carcinoma: positive association from clinical and in vitro analysis. Oral Oncol 2010;46:204–8.
[30] Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. Oral Dis 2010.
[31] Yamamoto Y, Kosaka N, Tanaka M, et al. MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. Biomarkers 2009;14:529–38.
[32] Ai J, Zhang R, Li Y, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem Biophys Res Commun 2010;391:73–7.
[33] Ji X, Takahashi R, Hiura Y, Hirokawa G, Fukushima Y, Iwai N. Plasma miR-208 as a biomarker of myocardial injury. Clin Chem 2009;55:1944–9.
[34] Adachi T, Nakanishi M, Otsuka Y, et al. Plasma MicroRNA 499 as a Biomarker of Acute Myocardial Infarction. Clin Chem 2010.
[35] Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J 2010;31:659–66.
[36] Tijsen AJ, Creemers EE, Moerland PD, et al. MiR423-5p as a circulating biomarker for heart failure. Circ Res 2010;106:1035–9.
[37] 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.
[38] Laterza OF, Lim L, Garrett-Engele PW, et al. Plasma microRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem 2009;55:1977–83.
[39] Wang JF, Yu ML, Yu G, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun 2010;394:184–8.
[40] Chim SS, Shing TK, Hung EC, et al. Detection and characterization of placental microRNAs in maternal plasma. Clin Chem 2008;54:482–90.
[41] Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. PLoS One 2008;3:e3148.
[42] Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT, Sadovsky Y. The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta 2010.
[43] Hunter MP, Ismail N, Zhang X, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008;3:e3694.
[44] Git A, Dvinge H, Salmon-Divon M, et al. Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA 2010;16:991–1006.
[45] Willenbrock H, Salomon J, Sokilde R, et al. Quantitative miRNA expression analysis: comparing microarrays with next-generation sequencing. RNA 2009;15:2028–34.
[46] Koshiol J, Wang E, Zhao Y, Marincola F, Landi MT. Strengths and limitations of laboratory procedures for microRNA detection. Cancer Epidemiol Biomarkers Prev 2010;19:907–11.
[47] Nelson PT, Wang WX, Wilfred BR, Tang G. Technical variables in high-throughput miRNA expression profiling: much work remains to be done. Biochim Biophys Acta 2008;1779:758–65.
[48] Chen Y, Gelfond JA, McManus LM, Shireman PK. Reproducibility of quantitative RT-PCR array in miRNA expression profiling and comparison with microarray analysis. BMC Genomics 2009;10:407.
[49] Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 2010;50:298–301.
[50] Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. Proteomics 2008;8:4083–99.
[51] Skog J, Wurdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008;10:1470–6.
[52] Hausler SF, Keller A, Chandran PA, et al. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer 2010;103:693–700.
[53] Keller A, Leidinger P, Borries A, et al. miRNAs in lung cancer – studying complex fingerprints in patient's blood cells by microarray experiments. BMC Cancer 2009;9:353.
[54] Tan KS, Armugam A, Sepramaniam S, et al. Expression profile of MicroRNAs in young stroke patients. PLoS One 2009;4:e7689.
[55] Keller A, Leidinger P, Lange J, et al. Multiple sclerosis: microRNA expression profiles accurately differentiate patients with relapsing-remitting disease from healthy controls. PLoS One 2009;4:e7440.
[56] Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH, Chan EK. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther 2008;10:R101.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004

[57] Zhou H, Guo JM, Lou YR, et al. Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. J Mol Med 2010;88:709–17.
[58] Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics 2007;8:166.
[59] Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Kerin MJ. MicroRNAs as novel biomarkers for breast cancer. J Oncol 2009;2009:950201.
[60] Tsui NB, Ng EK, Lo YM. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem 2002;48:1647–53.
[61] Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res 2010;38:215–24.
[62] Lehmann BD, Paine MS, Brooks AM, et al. Senescence-associated exosome release from human prostate cancer cells. Cancer Res 2008;68:7864–71.
[63] Luo SS, Ishibashi O, Ishikawa G, et al. Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod 2009;81:717–29.
[64] Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9.
[65] Yuan A, Farber EL, Rapoport AL, et al. Transfer of microRNAs by embryonic stem cell microvesicles. PLoS One 2009;4:e4722.
[66] Simons M, Raposo G. Exosomes—vesicular carriers for intercellular communication. Curr Opin Cell Biol 2009;21:575–81.
[67] Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics 2009;6:267–83.
[68] Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009;9:581–93.
[69] Collino F, Deregibus MC, Bruno S, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. PLoS One 2010;5:e11803.
[70] Zernecke A, Bidzhekov K, Noels H, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal 2009;2:ra81.
[71] Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One 2009;4:e6229.
[72] Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol 2009;19:43–51.
[73] Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res 2010.
[74] Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. Hypertension 2006;48:180–6.
[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] Mi S, Lu J, Sun M, et al. MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci USA 2007;104:19971–6.
[77] Lal A, Navarro F, Maher CA, et al. miR-24 Inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to "seedless" 3'UTR microRNA recognition elements. Mol Cell 2009;35:610–25.
[78] Merkerova M, Belickova M, Bruchova H. Differential expression of microRNAs in hematopoietic cell lineages. Eur J Haematol 2008;81:304–10.
[79] Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, et al. Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci USA 2010;107:6328–33.
[80] Zhang Y, Liu D, Chen X, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell 2010;39:133–44.
[81] Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. J Cell Mol Med 2009;13:39–53.
[82] Faraoni I, Antonetti FR, Cardone J. Bonmassar E. miR-155 gene: a typical multifunctional microRNA. Biochim Biophys Acta 2009;1792:497–505.
[83] Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem 2010.
[84] Brown BD, Gentner B, Cantore A, et al. Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. Nat Biotechnol 2007;25:1457–67.
[85] Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 2009;11:1143–9.

Biography

Glen Reid graduated from Georg-August-Universität Göttingen in 2000. He worked as a postdoc in the group of Piet Borst at the Netherlands Cancer Institute in Amsterdam, studying multidrug resistance in cancer. More recently he was Principal Investigator at Genesis Research & Development a biotech company in Auckland, where his focus was the development of siRNA as a therapeutic. He is currently Senior Research Scientist at the ADRI, and is investigating the role of microRNAs in malignant mesothelioma.

Please cite this article in press as: Reid G, et al. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol/Hematol (2010), doi:10.1016/j.critrevonc.2010.11.004