Cell. Mol. Life Sci. (2014) 71:2865–2878
DOI 10.1007/s00018-014-1584-5

Cellular and Molecular Life Sciences

REVIEW

Role of microRNAs in malignant mesothelioma

A. Truini · S. Coco · A. Alama · C. Genova · C. Sini · M. G. Dal Bello · G. Barletta · E. Rijavec · G. Burrafato · F. Boccardo · F. Grossi

Received: 20 November 2013 / Accepted: 4 February 2014 / Published online: 23 February 2014
© Springer Basel 2014

Abstract Malignant mesothelioma (MM) is an aggressive tumor, mainly derived from the pleura, which is predominantly associated with exposure to asbestos fibers. The prognosis of MM patients is particularly severe, with a median survival of approximately 9–12 months and latency between exposure and diagnosis ranging from 20–50 years (median 30 years). Emerging evidence has demonstrated that tumor aggressiveness is associated with genome and gene expression abnormalities; therefore, several studies have recently focused on the role of microRNAs (miRNAs) in MM tumorigenesis. miRNAs are small non-protein coding single-stranded RNAs (17–22 nucleotides) involved in numerous cellular processes that negatively regulate gene expression by modulating the expression of downstream target genes. miRNAs are often deregulated in cancer; in particular, the differential miRNA expression profiles of MM cells compared to unaffected mesothelial cells have suggested potential roles of miRNAs as either oncogenes or tumor suppressor genes in MM oncogenesis. In this review, the mechanism of MM carcinogenesis was evaluated through the analysis of the published miRNA expression data. The roles of miRNAs as diagnostic biomarkers and prognostic factors for potential therapeutic strategies will be presented and discussed.

Keywords Malignant mesothelioma · microRNAs · Prognostic markers · Differential expression

A. Truini (✉) · S. Coco · A. Alama · C. Genova · C. Sini · M. G. Dal Bello · G. Barletta · E. Rijavec · G. Burrafato · F. Grossi
Lung Cancer Unit, IRCCS A.O.U. San Martino IST-Istituto Nazionale per la Ricerca sul Cancro, L.go Rosanna Benzi 10, 16132 Genova, Italy
e-mail: anna.truini@hsanmartino.it

F. Boccardo
Medical Oncology B, IRCCS A.O.U. San Martino IST-Istituto Nazionale per la Ricerca sul Cancro, L.go Rosanna Benzi 10, 16132 Genova, Italy

F. Boccardo
Dipartimento di Medicina Interna e Specialità Mediche (DIMI), Università di Genova, V.le Benedetto XV 6, 16132 Genova, Italy

Abbreviations

MM	Malignant mesothelioma
ADC	Adenocarcinoma
CEA	Carcinoembryonic antigen
EPP	Extrapleural pneumonectomy
P/D	Pleurectomy/decortications
SMRP	Soluble mesothelin-related peptides
miRNAs/miR	MicroRNA
qRT-PCR	Quantitative real-time polymerase chain reaction
FFPE	Formalin-fixed paraffin-embedded
VEGF	Vascular endothelial growth factor
NSCLC	Non-small cell lung cancer
CDKN1	Cyclin-dependent kinase inhibitor 1
RMP	Reactive mesothelial proliferations
PPP6C	Serine/threonine-protein phosphatase 6

Introduction

Generalities, biology, and treatment of pleural mesothelioma

Malignant mesothelioma (MM) is an aggressive and lethal cancer that originates from the mesothelial cells lining the pleural, pericardial, and peritoneal cavities. This severe disease, which has been predominantly linked to asbestos fiber exposure [1], has become more frequent since 1950. The

increased incidence has been associated with many activities involving asbestos exposure in many industrial settings, such as shipyards, heavy or chemical industries, oil refineries, and construction industries [2, 3]. The disease is characterized by a long latency (20–50 years) between the first asbestos fiber exposure and diagnosis [4]; for this reason, it is rare to be diagnosed with MM before 50 years of age, resulting in an incidence peak of approximately 60–70 years. Worldwide, the incidence of MM is expected to increase over the next 20 years in industrialized countries [5].

Approximately 70 % of MM arises from the pleura, with malignant pleural mesothelioma being the most common type of MM [6]. Histologically, MM can be classified into three main subtypes: 50 % of cases present with an epithelial morphology and have a relatively better prognosis, 16 % of cases belong to the sarcomatoid histotype and are extremely aggressive and 34 % of cases are mixed types (epithelioid-sarcomatoid) [3]. The histomorphological similarities to lung adenocarcinoma (ADC) require a complete immunohistochemical investigation to precisely diagnose MM; this involves the characterization of many factors, including calretinin, Wilms' tumor-1, and cytokeratin 5/6, which are particularly expressed in MM; alternatively, lung ADC is characterized by positive staining for MOC31, thyroid transcription factor 1, and carcinoembryonic antigen (CEA), while lacking all of the MM specific markers [7].

MM prognosis is particularly severe, with a median survival of approximately 9–12 months from the time of diagnosis [8, 9]. Intrinsic resistance and poor response rates to conventional anticancer drugs make it difficult to develop appropriate cures for this aggressive malignancy, and even clinical guidelines suffer from the relative lack of strong evidence. Limited therapeutic strategies are available for MM, and current treatments are only partly successful.

MM treatment involves curative and palliative approaches, and generally only early stage epithelioid MM receive curative treatment. Epithelioid MM has a slightly better outcome compared to biphasic-sarcomatoid MM or to advanced stage MM, irrespective of histology [10].

The most common therapeutic approach in the early stage is surgery to completely resect the tumor; the two main surgical approaches are extrapleural pneumonectomy (EPP) and pleurectomy/decortication (P/D), depending on the tumor stage and cardiopulmonary status [11]. Due to the high proliferative property of MM, most tumors are not completely resectable with histologically negative margins; for this reason, surgery can be combined with other approaches, such as neoadjuvant/adjuvant chemotherapy and adjuvant radiotherapy, to eliminate the residual tumor tissue [12, 13]. Of note, surgery should be employed only for MM with an epithelial histology, as it is unable to achieve disease control in biphasic or sarcomatoid MM due to their aggressiveness [14]. EPP is an invasive procedure that is only feasible for resectable disease; this procedure involves the resection of the lung, pleura, pericardium, and diaphragm, as described by Butchart [15]. However, EPP does not provide great advantages in terms of survival [12, 16]. The use of EPP is still controversial, as data from randomized clinical trials are lacking; in the MARS (Mesothelioma And Radical Surgery) feasibility trial, EPP failed to improve the survival of the patients compared to chemotherapy; however, the small number of enrolled patients (50 MM patients) and the notably high surgical mortality should be taken into account when evaluating these results [17].

P/D is a less aggressive method than EPP that consists of the removal of parietal and visceral pleura, leaving the lung in place, and this method has a significant effect on median survival (10–17 months) [3, 18].

Chemotherapy can be used in combination with surgery with curative intent or alone with palliative intent for inoperable MM. Currently, the standard first-line chemotherapy for MM is a combination of cisplatin and pemetrexed or raltitrexed [19, 20]; further treatments include gemcitabine and vinorelbine, although clinical data detailing these treatments are limited [21, 22].

In particular cases, EPP can be followed by preventive radiotherapy, whereas radiotherapy is not recommended after P/D [23]. Radiotherapy is used to prevent the local recurrence and for palliative treatment, and radical radiotherapy is not feasible due to the difficulty of delivering the radiotherapy to the pleural cavity without damaging the heart and lungs [24].

Finally, multimodality approaches involving surgery combined with either chemotherapy or radiotherapy have resulted in some encouraging results, particularly for early stage MM, as described by Sienel [25] and colleagues. The authors reported that neoadjuvant chemotherapy combined with surgery and radiotherapy increased patient survival compared to the unimodal treatment, and this approach prolonged the median survival to 22 months for stage I patients [26].

To improve early detection, diagnosis, and treatment of MM, novel detection methods are required, and a number of clinical prognostic factors have already been identified. Additionally, novel biomarkers are now being considered to predict the evolution of this malignancy.

Recently, serum and pleural effusion markers have been evaluated to help diagnose MM; for instance, MM can be excluded from a differential diagnosis due to the high concentrations of CEA and cytokeratin 19 fragment [27, 28]. Over the last few years, new tumor markers have been reported, such as soluble mesothelin-related peptide (SMRP), megakaryocyte potentiation factor, and osteopontin, but their roles are still unclear [29–31].

To date, no specific biomarkers for the detection and diagnosis of MM have been validated, and many efforts have been made in this direction. Indeed, the identification of novel tumor markers for MM may greatly contribute to the early detection, diagnosis, and treatment of this cancer. For this reason, much interest has been focused on a novel class of markers called microRNAs (miRNAs).

Biogenesis and function of miRNAs

miRNAs are short non-coding, single-stranded RNAs (19–22 nucleotides long) that act as a novel class of gene regulators [32] by binding to the 3′ untranslated regions of their target mRNA genes, resulting in post-transcriptional gene expression regulation. The inhibition of mRNA translation into protein occurs in the presence of a partial complementary binding between the miRNA and its target; alternatively, in the case of complete complementary binding, the mRNA is degraded. One single miRNA can regulate hundreds of downstream targets. Therefore, these molecules play important roles in various cellular processes, including proliferation, development, differentiation, apoptosis, and stress response, but they are also aberrantly expressed in several cancers, thus playing a key role in oncogenesis.

Recent studies have reported that miRNAs are noninvasive biomarkers that can open new avenues for the early detection, diagnosis, and treatment of several neoplasms due to their tumor-specific expression profiles and their presence in the blood of patients. These studies have shown that miRNAs can play the role of a tumor suppressor gene or an oncogene during oncogenesis [33–35]. Additionally, due to their tissue specificity, miRNAs can be studied to identify the tissue source of the metastatic cancer [36, 37].

Because of their short sequences that result in more stability and decreased degradation by endogenous RNases, the presence of miRNAs has been observed not only in the primary tumor but also in all human bodily fluids, such as serum, plasma, saliva, and urine. Their presence in human fluids helps predict clinical outcome and cancer treatment response [38–41]. The high tissue specificity together with short sequence of miRNAs that prevent degradation by RNases and their presence in all human bodily fluids made miRNAs analyses more reliable and reproducible than mRNA expression studies opening new avenues in the genomic field. Furthermore, recent in vitro experiments mimicking or silencing candidate miRNAs have resulted in the modulation of miRNA expression in different types of cancers, showing their potential use as anti-cancer therapeutics [42].

To date, only a small number of papers have reported the roles of miRNAs as prognostic markers in a variety of different primary neoplasms, including leukemia, breast, pancreatic, ovarian, and lung cancer [43–49].

Due to these findings and the lack of tumor biomarkers for MM diagnosis, attention has focused on miRNAs. Moreover, miRNA expression profiles are more reliable for the classification of different tumor subtypes than mRNA gene expression profiles [50, 51].

Recently, the significance of miRNAs has raised particular attention in the biological/clinical setting, and only a few papers have reported their role in MM development.

The most commonly used techniques to investigate miRNA expression are microarray analyses and quantitative real-time polymerase chain reaction (qRT-PCR). Microarray profiling is a high-throughput tool based on the hybridization of labeled miRNA to probes that have been previously spotted on an array [52]. A specific and precise probes design on the microarray platform must be taken into account to distinguish between the precursor and mature miRNA. The second approach is qRT-PCR, a more sensitive and quantitative profiling tool to investigate the expression levels of a single transcript, which allows real-time amplification using primers combined with specific assays [53]. These two techniques can be used to make stronger and more reliable data even though some of the presented studies utilized only one.

Role of miRNA in MM

To date, a few studies have evaluated the differential expression of miRNAs using different sample sources in the hopes of improving MM diagnosis and treatment.

One of the first studies was carried out by Guled and colleagues in 2009. For the first time, a miRNA microarray was used to analyze the miRNA expression profiles of 17 freshly frozen surgical MM samples and normal human pericardium. A subset of miRNAs was differentially expressed between the neoplastic and unaffected tissue (Fig. 1; Tables 1, 2). Additionally, specific miRNAs for each histopathological subtype of MM were identified. Epithelioid MM expressed miR-135b, miR-181a-2*, miR-499-5p, miR-517b, miR-519d, miR-615-5p, and miR-624, whereas biphasic MM expressed miR-218-2*, miR-346, miR-377*, miR-485-5p, and miR-525-3p; sarcomatoid MM expressed miR-301b, miR-433 and miR-543.

In this study, the smoking status and asbestos exposure of the patients were also considered, and some miRNAs were identified in smokers compared to nonsmokers (miR-379, miR-301a, miR-299-3p, miR-455-3p, and miR-127-3p). Conversely, no differentially expressed miRNA was detected in the asbestos-exposed samples compared to the non-exposed subjects, but this could be correlated to the different method of asbestos exposure assessment.

Further analysis reported that these miRNAs target genes included the most frequently affected genes in MM

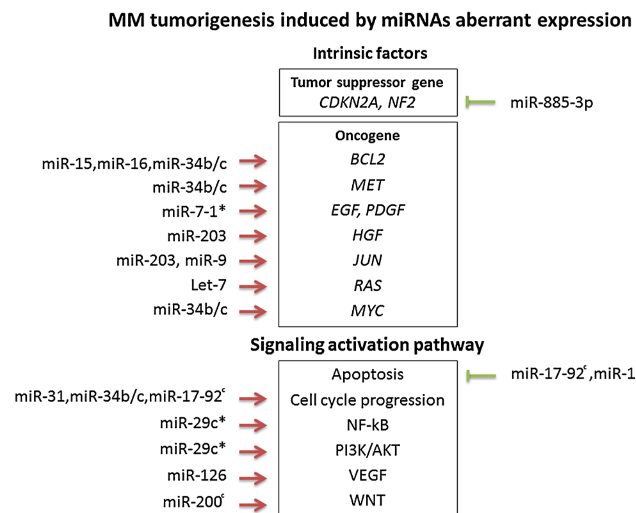

Fig. 1 Schematic diagram reporting the interaction of miRNAs and downstream signaling pathway genes in MM carcinogenesis. *Red arrow* indicates gene/pathway activation, whereas the *green symbol* shows gene/pathway inhibition. [c] miRNA cluster

(e.g., *CDKN2A*, *NF2*, *JUN*, *HGF*, and *PDGFA*), and the majority of these miRNAs mapped to chromosomal areas that are known to be altered in MM, such as gain of 8q24 and deletion of 1p36, and 14q32 [54].

This pioneering study open a new research avenue in this field, and since then, a number of investigators have explored the role of miRNA in MM in more detail.

miRNA as biomarker for early detection in MM

The importance of new potential biomarkers to identify MM is increasing, and different studies have been performed to evaluate the accuracy, feasibility, and specificity of miRNAs as clinical biomarkers.

Santarelli et al. revealed the importance of miRNAs as tools for the early detection of MM. According to previous studies reporting abnormal levels of miRNAs in different tumors, the authors hypothesized that examining the expression of a panel of miRNAs involved in MM tumorigenesis could be used to monitor MM development and progression. Ten freshly frozen MM biopsies were surgically excised from patients who received no further adjuvant chemotherapy or radiotherapy, and the expression of the 88 miRNAs most frequently involved in oncogenesis were assayed in these samples using PCR. Three miRNAs were significantly down-regulated (miR-335, miR-126, and miR-32) and were further validated in another cohort of 27 formalin-fixed paraffin-embedded (FFPE) samples using qRT-PCR analysis. Only miR-126 was down-regulated in the malignant tissue compared to the normal pleura.

Notably, one target of miR-126 is vascular endothelial growth factor (*VEGF*) mRNA, which encodes a protein that stimulates angiogenesis, and MM patients exhibit high amounts of VEGF in their serum [55].

Additionally, in support of these data, miR-126 expression was found to be down-regulated in human lung cancer cells that exhibit high levels of VEGF, suggesting that this miRNA plays a tumor suppressor role, as reported by Liu et al. [56]. Furthermore, a correlation between the levels of miR-126 in the serum and SMRP, a specific marker of MM, was reported in subjects that had a high risk of developing MM, suggesting that the association between miR-126 and SMRP could be used as a marker for the early detection of MM [55].

More recently, the role of miR-126 in the carcinogenesis of MM has been investigated in 45 untreated MM patients compared to 20 untreated non-small cell lung cancer (NSCLC) patients using qRT-PCR analysis. Low-levels of circulating miR-126 were observed in MM patients, which significantly differentiated the MM patients from the NSCLC samples and from the healthy controls. Additionally, Kaplan–Meier analysis showed a strong association between low levels of circulating miR-126 and a worse prognosis in MM patients. Although miR-126 can be considered to be a sensitivity marker for the disease, it lacks tumor specificity, as it is expressed at a low level in other neoplasms, which suggests that down-modulation of miR-126 would have be used only in combination with other MM-specific biomarkers, such as mesothelin [57].

Taken together, these results support the idea of using miR-126 as a biomarker for early detection, as it has been validated in different cohorts of samples, including serum, fresh tissue, and FFPE samples. The possibility of finding a useful biomarker is very important for the early diagnosis of MM and can also be used to monitor the serum of individuals exposed to asbestos fibers.

miRNA in diagnosis

Because of the difficulty in discriminating MM from lung ADC and other metastatic epithelial cancers and because of the absence of accurate markers, miRNA expression analysis represents an interesting option for differential diagnosis due to their tissue-specificities, which are highly useful for identifying the tumor origin.

Using a standardized in vivo miRNA-based assay, Benjamin and colleagues identified a panel of differentially expressed miRNAs in FFPE samples from large resection specimens MM and various carcinomas. The authors observed the over-expression of miR-193-3p in MM and the over-expression of miR-192 and miR-200c, which belongs to the miR-200 family, in peripheral lung ADC and carcinomas that usually metastasize to the lung pleura.

Table 1 Source and amount of tumor samples and control and technique used in each study

Reference	Source of tumor samples	Tumor samples number	Histology			Source of control samples	Control samples number	Technique	
Guled et al. [54]	Fresh tissue	17	epith	sarc	mix	Fresh tissue (pericardium)	1		Microarray
			11	1	5				
Santarelli et al. [55]	Fresh tissue	10	epith	sarc		Fresh tissue (normal pleura)	5	qRT-PCR	
			9	1		FFPE tissue (normal pleura)	27		
	FFPE tissue	27	epith	sarc	mix	Serum (healthy subjects)	50		
			23	1	3	Serum (asbestos-exposed subjects)	196		
	Serum	44	epith	sarc	mix				
			30	6	8				
Tomasetti et al. [57]	Serum	45	n.a.			Serum (NSCLC patients)	20	qRT-PCR	
						Serum (healthy subjects)	56		
Benjamin et al. [58]	FFPE tissue	47	epith	sarc	mix	FFPE tissue (carcinomas non-MM)	259	qRT-PCR	Microarray
			29	6	6				
			n.a. 6						
Gee et al. [59]	Fresh tissue	115	epith	sarc	mix	Fresh tissue (ADC patients)	42	qRT-PCR	Microarray
			39	10	19	Fresh tissue (normal lung tissue)	4		
			n.a. 47						
Busacca et al. [61]	Cell line FFPE tissue	2	epith			Cell line (immortalized mesothelial cell line)	1	qRT-PCR	Microarray
			2						
		24	epith	sarc	mix				
			8	8	8				
Andersen et al. [62]	FFPE tissue	13	epith			FFPE tissue (normal pleura)	13	qRT-PCR	
			13						
Kirschner et al. [65]	Plasma/serum	45	epith	sarc	mix	Plasma/serum (healthy subjects)	14	qRT-PCR	Microarray
			38	2	3				
			n.a. 2			Plasma/serum (asbestosis)	10		
	FFPE tissue	18	epith		mix	FFPE tissue (pericardium)	7		
			15		3				
Weber et al. [66]	Cellular fraction of peripheral blood	23	epith	sarc	mix	Cellular fraction of PB (asbestos-exposed subjects) Cellular fraction of PB (healthy subjects)	17	qRT-PCR	Microarray
			12	1	7				
			n.a. 3				25		
Pass et al. [64]	Fresh tissue	129	epith	non epith		Cell line (immortalized mesothelial cell line)	1	qRT-PCR	Microarray
			81	48					
	Cell line	8	epith	sarc		Cell line (primary mesothelial cell culture)	1		
			5	2					
			n.a. 1			Cell line (SV-40 transformed mesothelial cell line)	1		

Table 1 continued

Reference	Source of tumor samples	Tumor samples number	Histology			Source of control samples	Control samples number	Technique	
Balatti et al. [63]	Cell line	5	epith 3	sarc 1	mix 1	Cell line (primary mesothelial cell culture)	5	qRT-PCR	Microarray
Ivanov et al. [71]	Cell line	8	epith 5	sarc 2		Cell line (immortalized mesothelial cell line)	1	qRT-PCR	Microarray
						Cell line (primary mesothelial cell culture)	1		
			n.a. 1			Cell line (SV-40 transformed mesothelial cell line)	1		
Kubo et al. [72]	Fresh tissue	47	epith 32 1 lym	sarc 4	mix 10	Fresh tissue (normal pleura)	10	qRT-PCR Methylation PCR	
						Cell line (primary mesothelial cell culture)	2		
	Cell line	6	epith 2	sarc 1	mix 3				
Maki et al. [73]	Cell line	2		sarc 2		Cell line (WT MM cell line)	2	qRT-PCR	
Tanaka et al. [74]	Cell line	4	Non-neoplastic			Cell line (mesothelial cell line)	1	qRT-PCR	
						Cell line (primary mesothelial cells culture)	3		
Muraoka et al. [75]	Serum	48	epith 36	sarc 4	mix 8	Serum (benign asbestos pleurisy)	21	Methylation PCR	
						Serum (healthy subjects)	41		
						Cell line (MM heavy methylated cell line)	1		
						Cell line (peritoneal mesothelial cell line)	1		
Khodayari et al. [76]	Cell line	2	epith 1		mix 1	Cell line (WT MM cell line)	2	qRT-PCR	
Goparaju et al. [77]	Cell line	3	epith 2	sarc 1		Cell line (WT MM cell line)	3	qRT-PCR	
Xu et al. [80]	Fresh tissue	25	epith 18	sarc 3	mix 4	Fresh tissue (normal pleura)	6	qRT-PCR	Microarray
	Cell line	2	epith 1	sarc 1					
Reid et al. [85]	FFPE tissue	60	epith 46		mix 16	FFPE tissue (normal pleura)	23	qRT-PCR	
	Cell line	6	epith 2	sarc 2	mix 2	Cell line (mesothelial cell line)	1		

(*epith* epithelioid, *sarc* sarcomatoid, *mix* epithelioid-sarcomatoid, *n.a.* not available, *lym* lymphohistiocytic, *non epith* sarcomatoid and mixed, *PB* peripheral blood, *WT* non-transfected MM cell line)

Table 2 Deregulated miRNAs: up-regulated and down-regulated vs. control samples expression

Reference	Source of tumor samples	Up-regulated miRNA	Down-regulated miRNA
Guled et al. [54]	Fresh tissue	let-7b*, miR-1228*, miR-195*, miR-30b*, miR-32*, miR-345, miR-483-3p, miR-584, miR-595, miR-615-3p, and miR-885-3p	let-7e*, miR-144*, miR-203, miR-340*, miR-34a*, miR-423, miR-582, miR-7-1*, and miR-9
Santarelli et al. [55]	Fresh tissue FFPE tissue		miR-126
Tomasetti et al. [57]	Serum		miR-126
Benjamin et al. [58]	FFPE tissue	miR-193-3p	
Gee et al. [59]	Fresh tissue		miR-200a*, miR-200b, miR-200c, miR-141, miR-203, miR-205, miR-429
Busacca et al. [61]	Cell line FFPE tissue	miR17-3p, miR-17-5p, miR-18a, miR-20a, miR-21, miR-106a, miR-143, miR-30c	miR-31, miR-221, miR-222
Andersen et al. [62]	FFPE tissue	miR-221	miR-17-5p
Kirschner et al. [65]	Plasma/serum FFPE tissue	miR-625-3p	
Weber et al. [66]	Cellular fraction of peripheral blood		miR-103
Pass et al. [64]	Fresh tissue Cell line		miR-29c*
Balatti et al. [63]	Cell line	miR-17-5p, miR-18a, miR-19b, miR-20a, miR-20b, miR-25, miR-92, miR-106a, miR-106b, miR-7, miR-182, miR-339, miR-196b, miR-33	miR-214, miR-497, miR-500, miR-549, miR-22, miR-146b, miR-502, miR-328
Ivanov et al. [71]	Cell line		miR-31
Kubo et al. [72]	Fresh tissue Cell line		miR-34b/c
Maki et al. [73]	Cell line		miR-34b/c
Tanaka et al. [74]	Cell line		miR-34b/c
Muraoka et al. [75]	Serum		miR-34b/c
Khodayari et al. [76]	Cell line		let-7
Goparaju et al. [77]	Cell line	miR-30c	miR-17*
Xu et al. [80]	Fresh tissue Cell line		miR-1
Reid et al. [85]	FFPE tissue Cell line		miR-15, miR-16

Further analysis by qRT-PCR confirmed that these miRNA expressions patterns alone were able to differentially diagnose and distinguish MM from ADC and other malignancies involving the lung and pleura.

Similarly, Gee et al. performed an in vivo analysis using pleural biopsies to distinguish 15 non-histologically defined MM from ten lung ADC using miRNA microarrays; this study also further validated these results in 100 MM and 32 lung ADC surgical resected samples using qRT-PCR to study the molecular differences between these two diseases. The analysis revealed a strong down-regulation of miRNAs, specifically of miR-141, miR-200a*, miR-200b, miR-200c, miR-203, miR-205, and miR-429, in the MM samples compared to the lung ADC samples, and these changes range from 6- to 42-fold reductions in expression.

Interestingly, both papers found a strong expression of the miR-200 family in the ADC samples but minimal expression in MM, confirming the important role of the miR-200 gene family in ADC disease.

The above in vivo studies were the first studies aimed at comparing the miRNA expression profiles in MM and carcinomas infiltrating the lung and pleura.

This diagnostic assay based on the decreased expression of specific miRNAs could provide a useful tool and a novel approach in the differential and rapid diagnosis of MM from ADC and other malignancies in the pleura [58, 59].

A number of other investigations also reported the relevance of miRNAs as diagnostic markers of MM. One of the first studies was conducted by Busacca and colleagues, who examined the miRNA expression profiles of MM cell cultures compared to normal mesothelial cell lines using

microarray and qRT-PCR analyses; markedly deregulated miRNAs were further evaluated by in vivo miRNA signatures from MM primary tumor tissues that represented all three histological types.

miRNA belonging to the onco-miR cluster miR-17-92 appeared to be up-regulated in MM cells, and miRNAs previously reported to be down-regulated in several tumors (miR-21, miR-29a, miR-30b, and miR-106a) were also deregulated [60].

Furthermore, marked up-regulation of miR-143 and miR-30c was observed in the MM cell lines, whereas miR-31, miR-221, and miR-222 were down-regulated both in the MM cells and MM samples [61]. Because both miR-221 and miR-222 are known to be negative regulators of the cyclin-dependent kinase inhibitor (*CDKI*) p27 and the *PTEN* suppressor gene and are usually associated with poor MM prognosis, the down-regulation of these miRNAs in the MM cell lines is somewhat surprising [62].

Additionally, several targets of these miRNAs have been associated with MM oncogenesis, such as *CDKI*, which encodes p27Kip, B cell translocation gene 1, hepatocyte growth factor, and a methyl-CpG binding protein involved in transcription repression.

The down-regulation of miR-17-5p and miR-30c, which are differentially expressed according to histotype and are associated with other malignancies, were correlated with a better outcome in sarcomatoid MM, suggesting their potential role as diagnostic and prognostic markers [61].

Recently, the expression levels of mir-17-5p, mirR-30c, miR-221, and miR-222 were evaluated in vivo between MM and reactive mesothelial proliferations (RMP) using FFPE epithelioid MM and non-neoplastic pleura from EPP tissues of patients previously treated with chemotherapy. Significant down-regulation of miR-17-5p, up-regulation of miR-221 and no differential expression of miR-30c and miR-222 were observed in MM compared to the non-neoplastic pleura specimens.

Surprisingly, Andersen et al. found the opposite expression pattern for both miR-17-5p and miR-221 compared to results previously reported by Balatti et al. and Gee et al. in MM cell lines; this suggests that comparing in vitro and in vivo results is currently not feasible. This difference was not due to neoadjuvant chemotherapy as they compared miRNA expression in MM tissue from patients prior to any treatment.

Receiver operating characteristic (ROC) curves indicated that although miR-17-5p, miR-30, miR-221, and miR-222 were specific for MM subtypes and were differentially expressed in MM cell lines vs. normal mesothelial cells [58, 59, 61, 63], they could not be considered diagnostic markers for differentiating MM from RMP.

Therefore, for the identification of miRNAs candidates that can be used in the differential diagnosis of MM and RMP, a broad, comparative miRNA expression analysis using qRT-PCR in surgical samples from MM and non-neoplastic pleura, followed by direct validation on tissue sections by in situ hybridization, was suggested to fully investigate their histopathological diagnostic value and pathogenic roles [62].

The predictive role of miRNAs in plasma and serum as diagnostic biomarkers for MM has been recently proposed by Kirschner et al. Based on 90 miRNAs previously reported to be associated with MM [54, 55, 58, 59, 61, 63, 64], the authors identified the novel miR-625-3p, which was differentially expressed in MM patients compared to the controls. miRNA microarray analysis of the plasma of MM patients, followed by further validation by qRT-PCR analysis and examination of FFPE MM tumor blocks that underwent EPP, confirmed the up-regulation of miR-625-3p in the plasma of MM patients. The increased expression of this miRNA in two independent cohorts of samples, both plasma/serum and FFPE blocks, led to the hypothesis that miR-625-3p may be a promising novel diagnostic marker [65].

Another potential biomarker for the diagnosis of MM, miR-103, was identified by Weber et al. in the cellular fraction of human peripheral blood. An oligonucleotide microarray was used to detect the miRNA levels in the cellular fraction of peripheral blood of epithelioid and biphasic MM patients who were not treated with surgery, chemotherapy, or radiation as well as to compare asbestos-exposed and control individuals. qRT-PCR analysis was performed to validate the novel potential biomarker miR-103, confirming its down-regulation in both MM subtypes compared to the asbestos-exposed and controls. This encouraging result supports the idea of using the cellular fraction of human blood for biomarker detection, whether the evaluation of miR-103 alone or in combination with other biomarkers, in the diagnosis of MM, needs to be further established in larger prospective studies [66].

miRNA as a prognostic factor in MM

miRNAs have also been implicated in MM prognosis, as described by Pass and colleagues; the aim of their study was to distribute MM patients into good or poor prognosis groups depending on the expression of specific miRNAs. One hundred and twenty-nine surgically treated snap-frozen MM samples, nine MM cell lines, and three normal mesothelial cell lines were collected and tested using a custom miRNA platform, followed by further validation by qRT-PCR. These analyses revealed the independent prognostic significance of miR-29c*, in terms of time to recurrence and survival after surgical cytoreduction; its expression was increased in the epithelial vs. the non-epithelial MM. Moreover, in the epithelial histotype group,

higher miR-29c* expression predicted a more favorable prognosis compared to the group characterized by lower miR-29 expression (21.6 vs. 9.1 months median survival, respectively).

This result was also supported by in vitro data showing the inhibition of proliferation, migration, invasion, and colony formation when miR-29c* was over-expressed in MM cell lines. Because the decreased expression of miR-29c* has already been reported in MM by Busacca et al. and in several cancers, it can be assumed that this miRNA plays a tumor suppressor role [67–69].

Busacca et al. reported that the expression levels of miR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143 were associated with different MM histologies, and down-regulation of miR-17-5p and miR-30c correlated with a more favorable outcome in sarcomatoid MM patients. Similar to Guled's paper, informatics algorithms were employed to investigate the downstream targets of the potential prognostic miRNAs and showed that miR-29c* was involved in epigenetic regulation, resulting in the up-regulation of de-methylating genes and a decrease in the expression of DNA methyltransferases; methylation is an important step for genetic alteration caused by asbestos exposure [64].

Interestingly, Ivanov et al. [70, 71] analyzed the prognostic role of miR-31 in 8 MM cell lines, showing that a marked decrease in miR-31 was associated with a worse prognosis and a shorter time to tumor recurrence in MM. Strong miR-31 down-regulation was linked to the previously described deletion of the 9p21.3 chromosome in MM patients, thus demonstrating the homozygous deletion of the miR-31 gene, which resides in that area. The oncogenic nature of this genetic alteration has been associated with the loss of two tumor suppressor genes that map in this area named *CDKN2A* and *CDKN2B*; this deletion has been frequently reported in MM as well as in other cancers. In this study, microarray analysis showed that 8 MM cell lines derived from patients with more aggressive disease failed to express miR-31, a miRNA that has been shown to be involved in the suppression of breast cancer metastases.

miRNAs as MM markers and potential targets for anti-cancer therapy

The importance of miRNAs as MM biomarkers has been reported by Balatti [63] and colleagues that performed in vitro analysis to compare the miRNA expression in normal pleural mesothelial cell cultures and MM cell lines using microarray and qRT-PCR analysis. In addition, the protein expression of some genes was validated using Western-blot analysis. The results showed different miRNA expression patterns between the MM and mesothelial cell cultures. Specific members of the onco-miR miR-17-92 cluster (miR 17-5p, 18a, 19b, 20a, 20b, 25, 92, 106a, 106b) were strongly up-regulated, as already reported by Busacca et al., whereas other miRNAs exhibited deregulation in MM. Notably, genes involved in cell cycle regulation are also targets of these miRNAs. These findings may suggest novel miRNA as potential targets for new therapeutic interventions involving anti-miR oligonucleotides or the forced expression of the absent miRNA.

In particular, specific miRNAs such as miR-31 have been shown to play important roles in MM therapeutic approaches. Indeed, as reported by Ivanov and colleagues, an association has been found between low expression of miR-31 and a short time to tumor recurrence in MM. Functional studies involving the forced re-expression of miR-31 resulted in cell cycle suppression and the inhibition of several important factors implicated in DNA replication and cell cycle progression, such as pro-survival serine/threonine-protein phosphatase 6 (*PPP6C*); *PPP6C* has been previously associated with chemotherapy and radiation therapy resistance and the maintenance of chromosomal stability. Furthermore, in an attempt to identify the targets of miR-31, it was shown that *PPP6C* mRNA contained 3 miR-31-binding sites playing an important role in proliferation, migration, invasion, and clonogenicity attenuation of the MM cell lines.

These data indicate that miR-31 possesses a tumor-suppressive property, suggesting the possibility of developing novel therapeutic agents against MM and other cancers that exhibit 9p21.3 chromosome loss [71].

More recently, Kubo et al. [72] investigated the alterations of a direct transcriptional target of *TP53*, miR-34s (miR-34a/b/c), and its involvement in MM tumorigenesis obtaining very impressive results. Epigenetic modification by methylation results in miR-34b/c gene silencing, and the silencing of this miRNA plays an important role in the pathogenesis of MM; miR-34b/c restoration was able to suppress the oncologic features. The miR-34s methylation status and expression were examined in MM cell lines as well as in 47 surgically resected MM samples; aberrant methylation was equally distributed in the cell lines and tumors. However, because this epigenetic silencing involved miR-34b/c more frequently than miR-34a, the study focused on the role of miR-34b/c in MM. Forced miR-34b/c over-expression in transfected MM cell lines resulted in a marked antitumor effect due to cell cycle arrest as well as the suppression of migration, invasion, and motility. Taken together, these data indicated that the restoration of miR-34b/c expression may have potential therapeutic implications for the treatment of MM due to its relevant tumor suppressor properties. The role of miR-34b/c was further investigated by Maki and colleagues: they studied the effect of miR-34b/c restoration on the radiosensitivity of two MM cells. The impact of miR-34b/c transfection

was examined by in vitro analysis such as cell cycle analysis, and clonogenic and apoptosis assays revealing that miR-34b/c transfection radiosensitized MM cells by promoting radiation-induced apoptosis. In order to improve MM treatment, this miRNA might be an important therapeutic molecule to enhance radiotherapy in MM patients [73].

The investigation of miR-34s methylation and silencing continued thanks to Tanaka [74] and colleagues who recently examined the cellular effect of miR-34s knockdown in human mesothelial cell lines. Cell proliferation, migration, and invasion increased transfecting non-malignant cell lines with miR-34s inhibitors compared with the control. The effect was also evaluated at the protein level, confirming the up-regulation proteins involved in carcinogenesis such as phospho-c-MET and bcl-2. This recent study highly suggests the importance of the miR-34 family in the oncogenic transformation of normal mesothelial cells in MM cells.

Additionally, Muraoka et al. [75] investigated the degree of miR-34b/c methylation in serum circulating DNA, as DNA methylation occurs in about 90 % of MM samples. They used a real-time methylation-specific PCR assay to examine the methylation rate of circulating DNA in the serum of MM patients in comparison with controls. Patient characteristics such as sex, age, histotype, and smoking habit did not disclose any significant discrepancies. On the other hand, high methylation grade correlated with advanced stage of disease compared to early stage. This assay showed high sensitivity for miR-34b/c methylation status in serum-circulating DNA and it revealed a marked difference between MM cases and healthy individuals, suggesting that this assay might be a new detection approach for MM patients. All of these results strongly show the importance of the miR-34 family contributing to MM carcinogenesis and its role as a tumor suppressor.

Khodayari et al. reported that the activation of the Ephrin type-A receptor by its ligand EphrinA1 leads to the up-regulation of the let-7 miRNA in 2 MM cell lines, and this up-regulation contributes to the suppression of RAS family proto-oncogenes (*H-RAS*, *K-RAS*, *N-RAS*), resulting in a decrease in MM tumor growth.

Indeed, EphrinA1 activation induces the expression of the let-7 miRNA family members by several fold in MM cells, resulting in the silencing of *H-RAS*, *K-RAS*, and *N-RAS* expression and, in turn, the inhibition of MM cell proliferation and the consequent decrease in tumor growth in MM cell lines.

To validate these data, MM cell lines were transfected with let-7 miRNA antisense oligonucleotides, and EphrinA1 failed to inhibit proliferation under these conditions. On the other hand, the introduction of the let-7 miR precursor into MM cells inhibited the expression of the RAS family proto-oncogene, resulting in tumor growth attenuation [76].

Another study by Goparaju et al. [77] investigated the roles of miR-17* and miR-30c in MM using 3 MM cell lines treated with the chemotherapeutic agent ranpirnase (Onconase), a single peptide chain that induces intracellular RNA degradation. Notably, Onconase treatment induced a marked increase in miR-17* expression and a significant down-regulation of miR-30c expression in all cell lines, as well as down-regulation of *NF-κB* and its downstream targets, thus resulting in cell apoptosis and reduced aggressive behavior. Functional studies, such as cell proliferation assays, Matrigel invasion assays, and soft agar colony formation, were performed to determine the efficacy of Onconase treatment, and the results indicated the direct involvement of specific miRNAs in the antitumor effect, suggesting a possible application in MM treatment.

In a recent paper, Xu et al. reported that miR-1 is also one of the MM-associated miRNAs due to their discovery of its potential role as a therapeutic target. They examined a total of 25 MM freshly frozen tissues and normal pleura by microarray analysis, confirming their findings using qRT-PCR: miR-1 was down-regulated compared to unaffected mesothelium; this result was in agreement with previous studies reporting miR-1 down-regulation in hepatocellular carcinoma and bladder cancer [78, 79]. To prove this result, they tested the effect of miR-1 by in vitro assays on 2 MM cell lines: its over-expression induced inhibition of proliferation and apoptosis. In silico analysis revealed miR-1 involvement on apoptosis pathways. These findings suggest a possible role of miR-1 as a tumor suppressor in MM therapy [80].

Very recently, Reid et al. analyzed FFPE tissue from 60 MM patients who underwent EPP and 6 MM cell lines by qRT-PCR, reporting a marked reduced expression of miR-15 and miR-16 (range from 4- to 22-fold down-regulation). Forced expression of miR-15 and miR-16 resulted in inhibition of growth in MM cell lines. Interestingly, in vivo experiments showed that the intravenous administration of miR-16 targeted with epidermal growth factor receptor (*EGFR*) specific antibodies led to marked and dose-dependent growth inhibition. Growth inhibition resulted in low expression of target oncogenes such as Bcl-2 and CCND. This important paper underlines the tumor suppressor function for miR-15 and miR-16 in line with previous studies in prostate, lung, colon, and ovary cancer [81–84]. In addition, the ability to replace miRNAs in vivo opens new important avenues for therapeutic options in MM patients [85]. These observations suggest that targeting miRNAs expression may represent a novel therapeutic strategy in the treatment of MM patients.

Conclusions and future directions

All of the presented studies reveal the increasing importance of miRNAs in MM due to their potential roles as both diagnostic/prognostic markers and as anti-cancer agents in the treatment of this disease.

To date, a limited number of investigations have focused on miRNA expression in MM; therefore, it is not surprising that some results are not in agreement due to the different histological subtypes, sample sources (freshly frozen, FFPE tumor, cell lines, and serum) used in the in vitro and ex vivo studies (data from cell line do not always correlated in a biologically relevant manner with data from human tumors), control groups, approaches, and normalization techniques, as well as qRT-PCR and microarray analyses.

To overcome some conflicting results, larger samples should be enrolled to minimize fault-positive findings and micro or macro dissection of MM clinical samples should be performed before RNA extraction to increase analysis precision due to high tissue specificity of miRNAs. Furthermore, to identify miRNAs as novel biomarkers, the application of two techniques in the initial screening step would increase accuracy and reliability.

Additionally, some studies have focused on malignant pleural mesothelioma only, whereas others have studied all types of MM. Further validations are needed to define strong miRNA signature that can be used to discriminate MM patients from ADC patients and from healthy controls.

Based on these results, it is possible to determine miRNA expression from the serum of individuals with a personal history of exposure to asbestos fibers or who live in urban risk areas, and this information may be used to diagnose MM in its early stages.

In conclusion, all reported data clearly demonstrate the relevance of miRNAs in the diagnosis, prognosis, and treatment of MM. All data should be validated in a uniform manner to identify potential miRNAs as predictive and prognostic markers. Finally, the recent advances in the delivery of miRNAs mimic/inhibitor offer novel therapeutic opportunity in the treatment of this deadly disease.

References

1. Wagner JC, Sleggs CA, Marchand P (1960) Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 17:260–271
2. Albin M, Magnani C, Krstev S, Rapiti E, Sheferl I (1999) Asbestos and cancer: an overview of current trends in Europe. Environ Health Perspect 107(Suppl 2):289–298
3. Ismail-Khan R, Robinson LA, Williams CC Jr, Garrett CR, Bepler G, Simon GR (2006) Malignant pleural mesothelioma: a comprehensive review. Cancer Control 13:255–263
4. Carbone M, Kratzke RA, Testa JR (2002) The pathogenesis of mesothelioma. Semin Oncol 29:2–17
5. Kaufman AJ, Pass HI (2008) Current concepts in malignant pleural mesothelioma. Expert Rev Anticancer Ther 8:293–303
6. Bridda A, Padoan I, Mencarelli R, Frego M (2007) Peritoneal mesothelioma: a review. MedGenMed 9:32
7. Husain AN, Colby T, Ordonez N, Krausz T, Attanoos R, Beasley MB, Borczuk AC, Butnor K, Cagle PT, Chirieac LR, Churg A, Dacic S, Fraire A, Galateau-Salle F, Gibbs A, Gown A, Hammar S, Litzky L, Marchevsky AM, Nicholson AG, Roggli V, Travis WD, Wick M, International Mesothelioma Interest Group (2013) Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med 137:647–667
8. Robinson BW, Musk AW, Lake RA (2005) Malignant mesothelioma. Lancet 366:397–408
9. Grondin SC, Sugarbaker DJ (1999) Malignant mesothelioma of the pleural space. Oncology (Williston Park) 13:919–926 (discussion 926, 931–932)
10. Neumann V, Löseke S, Nowak D, Herth FJ, Tannapfel A (2013) Malignant pleural mesothelioma: incidence, etiology, diagnosis, treatment, and occupational health. Dtsch Arztebl Int 110:319–326
11. Bölükbas S, Schirren J (2013) Malignant pleural mesothelioma : Comparison of radical pleurectomy und extrapleural pneumonectomy. Chirurg 84:487–491
12. Rice D (2011) Surgical therapy of mesothelioma. Recent Results Cancer Res 189:97–125
13. Cao C, Tian D, Manganas C, Matthews P, Yan TD (2012) Systematic review of trimodality therapy for patients with malignant pleural mesothelioma. Ann Cardiothorac Surg 1:428–437
14. Kaufman AJ, Flores RM (2011) Surgical treatment of malignant pleural mesothelioma. Curr Treat Options Oncol 12:201–216
15. Butchart EG, Ashcroft T, Barnsley WC, Holden MP (1976) Pleuropneumonectomy in the management of diffuse malignant mesothelioma of the pleura. Experience with 29 patients. Thorax 31:15–24
16. Stewart DJ, Martin-Ucar A, Pilling JE, Edwards JG, O'Byrne KJ, Waller DA (2004) The effect of extent of local resection on patterns of disease progression in malignant mesothelioma. Ann Thorac Surg 78:245–252
17. Treasure T, Lang-Lazdunski L, Waller D, Bliss JM, Tan C, Entwisle J, Snee M, O'Brien M, Thomas G, Senan S, O'Byrne K, Kilburn LS, Spicer J, Landau D, Edwards J, Coombes G, Darlison L, Peto J, MARS trialists (2011) Extra-pleural pneumonectomy versus no extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: clinical outcomes of the Mesothelioma and Radical Surgery (MARS) randomised feasibility study. Lancet Oncol 12:763–772
18. Soysal O, Karaoğlanoğlu N, Demiracan S, Topçu S, Taştepe I, Kaya S, Unlü M, Celtin G (1997) Pleurectomy/decortication for palliation in malignant pleural mesothelioma: results of surgery. Eur J Cardiothorac Surg 11:210–213
19. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C, Paoletti P (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21:2636–2644
20. van Meerbeeck JP, Gaafar R, Manegold C, Klaveren RJ, Van Marck EA, Vincent M, Legrand C, Bottomley A, Debruyne C, Giaccone G, European Organisation for Research and Treatment of Cancer Lung Cancer Group; National Cancer Institute of Canada (2005) Randomized phase III study of cisplatin with or without raltitrexed in patients with malignant pleural mesothelioma: an intergroup study of the European Organisation for Research and Treatment of Cancer Lung Cancer Group and the National Cancer Institute of Canada. J Clin Oncol 23:6881–6889

21. van Meerbeeck JP, Baas P, Debruyne C, Groen HJ, Manegold C, Ardizzoni A, Gridelli C, van Marck EA, Lentz M, Giaccone G (1999) A phase II study of gemcitabine in patients with malignant pleural mesothelioma. European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group. Cancer 85:2577–2582
22. Stebbing J, Powles T, McPherson K, Shamash J, Wells P, Sheaff MT, Slater S, Rudd RM, Fennell D, Steele JP (2009) The efficacy and safety of weekly vinorelbine in relapsed malignant pleural mesothelioma. Lung Cancer 63:94–97
23. Scherpereel A, Astoul P, Baas P, Berghmans T, Clayson H, de Vuyst P, Galateau-Salle F, Hennequin C, Hillerdal G, Le Péchoux C, Mutti L, Pairon JC, Stahel R, van Houtte P, van Meerbeeck J, Waller D, Weder W, European Respiratory Society/European Society of Thoracic Surgeons Task Force (2010) Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleura mesothelioma. Eur Respir J 35:479–495
24. Dhalluin X, Scherpereel A (2011) Chemotherapy and radiotherapy for mesothelioma. Recent Results Cancer Res 189:127–147
25. Sienel W, Kirschbaum A, Passlick B (2008) Multimodal therapy for malignant pleural mesothelioma including extrapleural pneumonectomy. Zentralbl Chir 133:231–237
26. Sugarbaker DJ, Garcia JP, Richards WG, Harpole DH Jr, Healy-Baldini E, DeCamp MM Jr, Mentzer SJ, Liptay MJ, Strauss GM, Swanson SJ (1996) Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma. Results in 120 consecutive patients. Ann Surg 224:288–294 (discussion 294–296)
27. Klebe S, Henderson DW (2011) Early stages of mesothelioma, screening and biomarkers. Recent Results Cancer Res 189:169–193
28. Paganuzzi Onetto M, Marroni P, Filiberti R, Tassara E, Parodi S, Felletti R (2011) Diagnostic value of CYFRA 21-1 tumor marker and CEA in pleural effusion due to mesothelioma. Chest 119:1138–1142
29. Onda M, Nagata S, Ho M, Bera TK, Hassan R, Alexander RH, Pastan I (2006) Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. Clin Cancer Res 12:4225–4423
30. Pass HI, Lott D, Lonardo F, Harbut M, Liu Z, Tang N, Carbone M, Webb C, Wali A (2005) Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 353:1564–1573
31. Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I (2003) Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 362:1612–1616
32. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297
33. Esquela-Kerscher A, Slack FJ (2006) Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 6:259–269
34. Bentwich I (2005) Prediction and validation of microRNAs and their targets. FEBS Lett 579:5904–5910
35. Jean D, Daubriac J, Le Pimpec-Barthes F, Galateau-Salle F, Jaurand MC (2012) Molecular changes in mesothelioma with an impact on prognosis and treatment. Arch Pathol Lab Med 136:277–293
36. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, Benjamin H, Shabes N, Tabak S, Levy A, Lebanony D, Goren Y, Silberschein E, Targan N, Ben-Ari A, Gilad S, Sion-Vardy N, Tobar A, Feinmesser M, Kharenko O, Nativ O, Nass D, Perelman M, Yosepovich A, Shalmon B, Polak-Charcon S, Fridman E, Avniel A, Bentwich I, Bentwich Z, Cohen D, Chajut A, Barshack I (2008) MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol 26:462–469
37. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A (2008) Serum microRNAs are promising novel biomarkers. PLoS One 3:e3148
38. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K (2010) The microRNA spectrum in 12 body fluids. Clin Chem 56:1733–1741
39. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 105:10513–10518
40. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY (2008) Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 18:997–1006
41. McDonald JS, Milosevic D, Reddi HV, Grebe SK, Algeciras-Schimnich A (2011) Analysis of circulating microRNA: preanalytical and analytical challenges. Clin Chem 57:833–840
42. Fabbri M (2013) MicroRNAs and cancer: towards a personalized medicine. Curr Mol Med 13:751–756
43. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM (2002) Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99:15524–15529
44. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Kerin MJ (2009) MicroRNAs as novel biomarkers for breast cancer. J Oncol 2009:950201
45. Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, Calin GA, Volinia S, Liu CG, Scarpa A, Croce CM (2006) MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol 24:4677–4684
46. Xu YZ, Xi QH, Ge WL, Zhang XQ (2013) Identification of serum microRNA-21 as a biomarker for early detection and prognosis in human epithelial ovarian cancer. Asian Pac J Cancer Prev 14:1057–1060
47. Raponi M, Dossey L, Jatkoe T, Wu X, Chen G, Fan H, Beer DG (2009) MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res 69:5776–5783
48. Pass HI, Beer DG, Joseph S, Massion P (2013) Biomarkers and molecular testing for early detection, diagnosis, and therapeutic prediction of lung cancer. Thorac Surg Clin 23:211–224
49. Nadal E, Chen G, Gallegos M, Lin L, Ferrer-Torres D, Truini A, Wang Z, Lin J, Reddy RM, Llatjos R, Escobar I, Moya J, Chang AC, Cardenal F, Capella G, Beer DG (2013) Epigenetic inactivation of microRNA-34b/c predicts poor disease-free survival in early stage lung adenocarcinoma. Clin Cancer Res 19:6842–6852
50. Minoia C, Sturchio E, Porro B, Ficociello B, Zambelli A, Imbriani M (2011) MicroRNAs as biological indicators of environmental and occupational exposure to asbestos. G Ital Med Lav Ergon 33:420–434
51. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866
52. Yin JQ, Zhao RC, Morris KV (2008) Profiling microRNA expression with microarrays. Trends Biotechnol 26:70–76
53. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ (2005) Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res 33:e179
54. Guled M, Lahti L, Lindholm PM, Salmenkivi K, Bagwan I, Nicholson AG, Knuutila S (2009) CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant

mesothelioma—a miRNA microarray analysis. Genes Chromosomes Cancer 48:615–623

55. Santarelli L, Strafella E, Staffolani S, Amati M, Emanuelli M, Sartini D, Pozzi V, Carbonari D, Bracci M, Pignotti E, Mazzanti P, Sabbatini A, Ranaldi R, Gasparini S, Neuzil J, Tomasetti M (2011) Association of MiR-126 with soluble mesothelin-related peptides, a marker for malignant mesothelioma. PLoS One 6:e18232
56. Liu B, Peng XC, Zheng XL, Wang J, Qin YW (2009) MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. Lung Cancer 66:169–175
57. Tomasetti M, Staffolani S, Nocchi L, Neuzil J, Strafella E, Manzella N, Mariotti L, Bracci M, Valentino M, Amati M, Santarelli L (2012) Clinical significance of circulating miR-126 quantification in malignant mesothelioma patients. Clin Biochem 45:575–581
58. Benjamin H, Lebanony D, Rosenwald S, Cohen L, Gibori H, Barabash N, Ashkenazi K, Goren E, Meiri E, Morgenstern S, Perelman M, Barshack I, Goren Y, Edmonston TB, Chajut A, Aharonov R, Bentwich Z, Rosenfeld N, Cohen D (2010) A diagnostic assay based on microRNA expression accurately identifies malignant pleural mesothelioma. J Mol Diagn 12:771–779
59. Gee GV, Koestler DC, Christensen BC, Sugarbaker DJ, Ugolini D, Ivaldi GP, Resnick MB, Houseman EA, Kelsey KT, Marsit CJ (2010) Downregulated microRNAs in the differential diagnosis of malignant pleural mesothelioma. Int J Cancer 127:2859–2869
60. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103:2257–2261
61. Busacca S, Germano S, De Cecco L, Rinaldi M, Comoglio F, Favero F, Murer B, Mutti L, Pierotti M, Gaudino G (2010) MicroRNA signature of malignant mesothelioma with potential diagnostic and prognostic implications. Am J Respir Cell Mol Biol 42:312–319
62. Andersen M, Grauslund M, Muhammad-Ali M, Ravn J, Sørensen JB, Andersen CB, Santoni-Rugiu E (2012) Are differentially expressed microRNAs useful in the diagnostics of malignant pleural mesothelioma? APMIS 120:767–769
63. Balatti V, Maniero S, Ferracin M, Veronese A, Negrini M, Ferrocci G, Martini F, Tognon MG (2011) MicroRNAs dysregulation in human malignant pleural mesothelioma. J Thorac Oncol 6:844–851
64. Pass HI, Goparaju C, Ivanov S, Donington J, Carbone M, Hoshen M, Cohen D, Chajut A, Rosenwald S, Dan H, Benjamin S, Aharonov R (2010) hsa-miR-29c* is linked to the prognosis of malignant pleural mesothelioma. Cancer Res 70:1916–1924
65. Kirschner MB, Cheng YY, Badrian B, Kao SC, Creaney J, Edelman JJ, Armstrong NJ, Vallely MP, Musk AW, Robinson BW, McCaughan BC, Klebe S, Mutsaers SE, van Zandwijk N, Reid G (2012) Increased circulating miR-625-3p: a potential biomarker for patients with malignant pleural mesothelioma. J Thorac Oncol 7:1184–1191
66. Weber DG, Johnen G, Bryk O, Jöckel KH, Brüning T (2012) Identification of miRNA-103 in the cellular fraction of human peripheral blood as a potential biomarker for malignant mesothelioma—a pilot study. PLoS ONE 7:e30221
67. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA 104:15805–15810
68. Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, Calin GA, Hagan JP, Kipps T, Croce CM (2006) Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. Cancer Res 66:11590–11593
69. Gebeshuber CA, Zatloukal K, Martinez J (2009) miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. EMBO Rep 10:400–405
70. Ivanov SV, Miller J, Lucito R, Tang C, Ivanova AV, Pei J, Carbone M, Cruz C, Beck A, Webb C, Nonaka D, Testa JR, Pass HI (2009) Genomic events associated with progression of pleural malignant mesothelioma. Int J Cancer 124:589–599
71. Ivanov SV, Goparaju CM, Lopez P, Zavadil J, Toren-Haritan G, Rosenwald S, Hoshen M, Chajut A, Cohen D, Pass HI (2010) Pro-tumorigenic effects of miR-31 loss in mesothelioma. J Biol Chem 285:22809–22817
72. Kubo T, Toyooka S, Tsukuda K, Sakaguchi M, Fukazawa T, Soh J, Asano H, Ueno T, Muraoka T, Yamamoto H, Nasu Y, Kishimoto T, Pass HI, Matsui H, Huh NH, Miyoshi S (2011) Epigenetic silencing of microRNA-34b/c plays an important role in the pathogenesis of malignant pleural mesothelioma. Clin Cancer Res 17:4965–4974
73. Maki Y, Asano H, Toyooka S, Soh J, Kubo T, Katsui K, Ueno T, Shien K, Muraoka T, Tanaka N, Yamamoto H, Tsukuda K, Kishimoto T, Kanazawa S, Miyoshi S (2012) MicroRNA miR-34b/c enhances cellular radiosensitivity of malignant pleural mesothelioma cells. Anticancer Res 32:4871–4875
74. Tanaka N, Toyooka S, Soh J, Tsukuda K, Shien K, Furukawa M, Muraoka T, Maki Y, Ueno T, Yamamoto H, Asano H, Otsuki T, Miyoshi S (2013) Downregulation of microRNA-34 induces cell proliferation and invasion of human mesothelial cells. Oncol Rep 29:2169–2174
75. Muraoka T, Soh J, Toyooka S, Aoe K, Fujimoto N, Hashida S, Maki Y, Tanaka N, Shien K, Furukawa M, Yamamoto H, Asano H, Tsukuda K, Kishimoto T, Otsuki T, Miyoshi S (2013) The degree of microRNA-34b/c methylation in serum-circulating DNA is associated with malignant pleural mesothelioma. Lung Cancer [Epub ahead of print]
76. Khodayari N, Mohammed KA, Goldberg EP, Nasreen N (2011) EphrinA1 inhibits malignant mesothelioma tumor growth via let-7 microRNA-mediated repression of the RAS oncogene. Cancer Gene Ther 18:806–816
77. Goparaju CM, Blasberg JD, Volinia S, Palatini J, Ivanov S, Donington JS, Croce C, Carbone M, Yang H, Pass HI (2011) ONCONASE-mediated NFKβ downregulation in malignant pleural mesothelioma. Oncogene 30:2767–2777
78. Datta J, Kutay H, Nasser MW, Nuovo GJ, Wang B, Majumder S, Liu CG, Volinia S, Croce CM, Schmittgen TD, Ghoshal K, Jacob ST (2008) Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Res 68:5049–5058
79. Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, Nohata N, Seki N, Nakagawa M (2011) The tumour-suppressive function of miR-1 and miR-133a targeting TAGLN2 in bladder cancer. Br J Cancer 104:808–818
80. Xu Y, Zheng M, Merritt RE, Shrager JB, Wakelee H, Kratzke RA, Hoang CD (2013) miR-1 induces growth arrest and apoptosis in malignant mesothelioma. Chest 144:1632–1643
81. Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, Bartucci M, Muto G, Peschle C, De Maria R (2008) The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 14:1271–1277
82. Bandi N, Zbinden S, Gugger M, Arnold M, Kocher V, Hasan L, Kappeler A, Brunner T, Vassella E (2009) miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. Cancer Res 69:5553–5559

83. Wang X, Wang J, Ma H, Zhang J, Zhou X (2012) Downregulation of miR-195 correlates with lymph node metastasis and poor prognosis in colorectal cancer. Med Oncol 29:919–927
84. Bhattacharya R, Nicoloso M, Arvizo R, Wang E, Cortez A, Rossi S, Calin GA, Mukherjee P (2009) MiR-15a and MiR-16 control Bmi-1 expression in ovarian cancer. Cancer Res 69:9090–9095
85. Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J, Williams M, Wright C, Edelman JJ, Vallely MP, McCaughan BC, Klebe S, Brahmbhatt H, Macdiarmid JA, van Zandwijk N (2013) Restoring expression of miR-16: a novel approach to therapy for malignant pleural mesothelioma. Ann Oncol 24:3128–3135