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REVIEW

MicroRNAs as potential biomarkers in malignant pleural mesothelioma

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Eric Santoni-Rugiu
Morten Andersen
Morten Grauslund

Laboratory of Molecular Pathology, Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Abstract: Malignant pleural mesothelioma (MPM), a highly lethal cancer strictly related to asbestos exposure, is usually characterized by delayed diagnosis, resistance to current therapies, and dismal prognosis. MPM is difficult to distinguish histologically from nonmalignant reactive mesothelial proliferations (RMPs) as there are no clinically validated immunohistochemical markers yet and the main diagnostic criterion remains deep invasion into the pleura and underlying fat tissue, which is often not appreciable in small pleural biopsies. In this regard, microRNAs (miRNAs), given their size and stability, are particularly attractive biomarkers in formalin-fixed paraffin-embedded tissue specimens for routine pathology. Moreover, circulating miRNAs appear to be promising biomarkers for early detection and monitoring of patients with MPM. Here, we review the studies mostly performed by miRNA arrays and reverse transcription-quantitative polymerase chain reaction in formalin-fixed paraffin-embedded or frozen tissue samples, MPM cell lines, and blood/plasma/serum samples that have highlighted the potential of miRNAs as biomarkers in MPM. Certain studies have pointed to the ability of miRNAs to distinguish the different histological MPM subtypes or separate MPM from lung adenocarcinoma, and other investigations have revealed that miRNAs can aid in differentiating MPM from RMP or have prognostic value in predicting the patient outcome. Mechanistic aspects of the involvement of miRNAs in mesothelioma genesis and possible use of miRNAs as future therapeutic targets in MPM are also emphasized. Finally, limitations of the data currently obtained due to the drawbacks of reverse transcription-quantitative polymerase chain reaction, heterogeneity of MPM tissue samples, and differences in methodological platforms as well as in types of specimens utilized in different studies are discussed. Because of these inherent weaknesses of collected data, further studies assessing the expression and distribution of miRNAs by in situ hybridization in combination with the codetection of their respective targets by immunohistochemistry and further validation of miRNAs' targets in vitro are warranted to fully reveal the potential clinical utility of miRNAs in MPM.

Keywords: malignant pleural mesothelioma, miRNAs, cancer biomarkers, diagnosis, prognosis, future therapy

Introduction

Malignant pleural mesothelioma (MPM), a rare, aggressive cancer originating from the mesothelial cells lining the pleura, is in most cases related to long-term exposure to asbestos or asbestos-like fibers. Histologically, MPM is classified as epithelioid malignant pleural mesothelioma (EMPM), sarcomatoid malignant pleural mesothelioma (SMPM), and biphasic malignant pleural mesothelioma (BMPM) subtypes representing 70%, 10%, and 20% of cases, respectively.[1] MPM is most often diagnosed at an advanced stage, where standard treatment is palliative platinum-based chemotherapy.

Correspondence: Eric Santoni-Rugiu
Laboratory of Molecular Pathology, Department of Pathology, Section 5442, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, 2100 Copenhagen, Denmark
Tel +45 3545 5476
Fax +45 3545 5414
Email eric.santoni-rugiu.02@regionh.dk

Consequently, the prognosis remains dismal with a median survival of 12 months after diagnosis.[1] For ~30% of patients judged to be operable at diagnosis, specialized centers offer cytoreductive surgical procedures, such as extrapleural pneumonectomy or pleurectomy/decortication, often combined in a trimodal protocol with neoadjuvant chemotherapy and adjuvant radiotherapy.[2] According to MPM histology, which remains the most significant prognostic factor, operable patients with EMPM, BMPM, and SMPM have a median survival of 19, 13, and 8 months, respectively.[1] MPM typically responds poorly to chemoradiotherapy, and proposed biomarkers for identifying the patients who would most benefit from these treatments need more clinical validation.[3] Recent efforts focused on identifying efficacious molecular targeted therapy and immunotherapy hold promise for the implementation of more personalized protocols in the near future.[4] MPM can be a difficult diagnosis for pathologists. The main diagnostic criterion remains deep invasion into the pleura and underlying fat tissue, which is often not discernable histologically in small pleural biopsies. Effective immunohistochemistry (IHC) algorithms can most often be successfully applied to distinguish MPM from pleural spreading of other cancer types, such as lung cancer.[1] However, it can be quite challenging to distinguish EMPM from nonmalignant reactive mesothelial hyperplasia or SMPM from organizing pleuritis[1] as proposed IHC biomarkers for malignant mesothelial cells have not shown enough specificity and/or sensitivity or require further validation.[5,6] Thus, the identification of new biomarkers that may aid in the problematic diagnosis and/or treatment of MPM is urgently needed. By summarizing the several overlapping general definitions existing in the literature for simplicity, biomarkers can be considered as objectively and reproducibly measurable characteristics of biological (physiological or pathological) processes.

In this respect, a class of noncoding RNAs, known as microRNAs (miRNAs), has shown great potential in other types of cancers as diagnostic, prognostic, and predictive biomarkers (biomarkers that provide information regarding an individual patient's diagnosis, prognosis regardless of treatment, and likelihood to respond to a specific therapy, respectively).[7,8] miRNAs are short RNA strands of 18–24 nucleotides that regulate the gene expression by base pairing to complementary sequences primarily within the 3′-untranslated regions (3′-UTR) of target mRNAs, thereby resulting in mRNA cleavage or translational repression.[9] The latest version of the miRBase (http://www.mirbase.org/) miRNA-registry contains 2,588 entries of mature human miRNAs, which are estimated to target 3′-UTRs in >60% of human mRNAs.[9,10] Each miRNA can modulate the expression of hundreds of target mRNAs, and when its expression is deregulated, it can act as tumor suppressor or oncogene ("oncomiR"), depending on the nature of its targets. Indeed, miRNAs are involved in the regulation of multiple fundamental cellular activities, including proliferation, apoptosis, differentiation, senescence, invasion, motility, stress responses, and metabolism, which are all closely linked to cancer initiation, progression, and treatment response.[11] miRNAs are particularly attractive as biomarkers in tissue samples processed for routine pathology as their short nucleotide sequences remain stable and can be quantified in formalin-fixed paraffin-embedded (FFPE) material by polymerase chain reaction (PCR) and in situ hybridization (ISH) techniques.[12] Similarly, they are detectable in the blood, in which they circulate mostly as exosomal vesicles, thereby representing promising potential noninvasive biomarkers for cancer diagnostics and follow-up.[13] Here, we review the recent discoveries regarding miRNA expression and function in MPM, with special emphasis on the potential clinical use as future biomarkers (Figure 1). Table 1 summarizes experimental details and significant miRNA findings in the studies exploring the biomarker potential of miRNAs in MPM. Table 2 summarizes the studies disclosing predicted and verified target genes of deregulated miRNAs in MPM.

miRNAs with deregulated expression and diagnostic potential in MPM

Several studies, mostly based on initial screening by miRNA microarrays and validation by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), have explored the expression of miRNAs in MPM.[14–20]

The first study demonstrating deregulated miRNA expression in MPM was that by Guled et al,[14] who screened the expression of 723 human miRNAs in total RNA isolated from snap-frozen MPM samples (eleven EMPM, five BMPM, one SMPM) and in commercially available total RNA from normal human pericardium. The MPM tissues displayed overexpression of 12 miRNAs and underexpression of nine miRNAs (Table 1). The study then portrayed the exclusive expression of seven miRNAs in EMPM (miR-135b, -181a-2*, -499-5p, -517b, -519d, -615-5p, -624), five in BMPM (miR-218-2*, -346, -377*, -485-5p, -525-3p), and three in the only SMPM analyzed (miR-301b, -433, -543). Utilizing target-prediction algorithms, the tumor suppressor genes (TSGs), *CDKN2A* and *NF2*, which are commonly inactivated

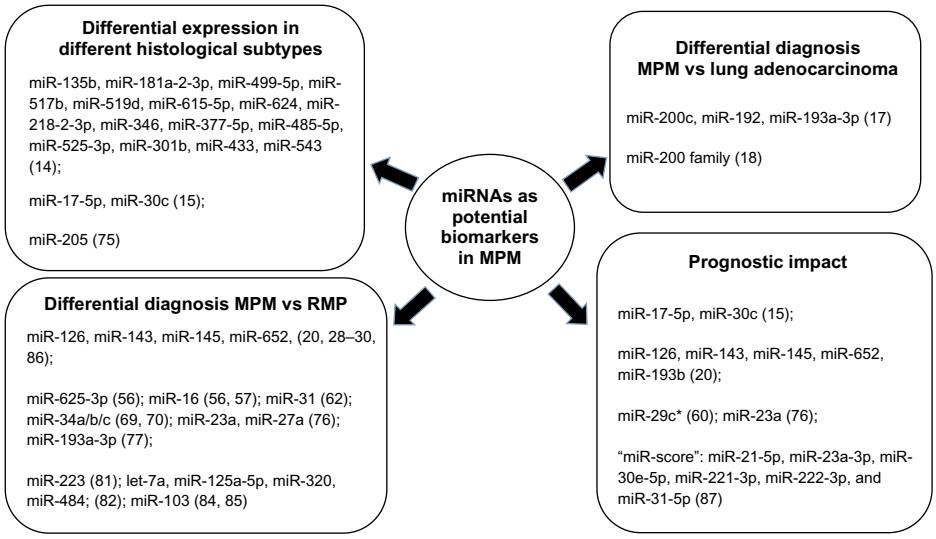

Figure 1 MicroRNAs (miRNAs) with potential as clinical biomarkers in malignant pleural mesothelioma (MPM).
Notes: miRNAs that can distinguish the histological subtypes of MPM, differentiate MPM from lung adenocarcinoma, aid in the differential diagnosis of MPM vs nonmalignant reactive mesothelial proliferation (RMP), or that have displayed prognostic significance for MPM patients are shown. Corresponding references for reported findings on the different miRNAs are shown in brackets (references).

in MPM,[1] and *RB1* were identified as putative targets of the overexpressed miR-30b*, -32*, -483-3p, -584, and -885-3p.[14] Similarly, the underexpressed miR-9, -7-1*, and -203 were predicted to target the oncogenes *HGF*, *PDGFA*, *EGF*, and *JUN* (Table 2).[14]

A microarray-based miRNA profiling in vitro by Busacca et al displayed upregulation of ten miRNAs and downregulation of 19 miRNAs in two commercially available MPM cell lines as compared to immortalized human mesothelial cells (HMC).[15] Validation by RT-qPCR showed consistent overexpression of miR-17-5p and miR-30c and underexpression of miR-221 and miR-222 in the MPM cell lines. The ten most differentially expressed miRNAs were tested by RT-qPCR in a collection of 24 MPM samples representing each histological subtype of MPM, aiming at uncovering miR signatures that could differentiate these subtypes. However, it is somehow disputable that the expression of the miRNAs was reported relative to a reference sample obtained by pooling an equal amount of RNA from each specimen.[15] The expression of seven of the tested miRNAs was found to be significantly associated with the histological subtypes, and for SMPM, the cases with lower expression of miR-17-5p and miR-30c (below the median) were associated with better prognosis.[15]

By comparing the expression of 470 human and 63 viral miRNAs in primary HMC and five commercially available MPM cell lines, Balatti et al identified eight underexpressed miRNAs and 15 overexpressed miRNAs in the MPM cells.[16] Among the overexpressed miRNAs were seven members of the miR-17-92 cluster (including miR-17-5p, thus, reproducing the upregulation of this miRNA observed by Busacca et al in different MPM cell lines)[15] and its paralogs miR-106a-363 and miR-106b-25. A few of the dysregulated miRNAs were validated. It was also demonstrated that in MPM cells, as in other cancer cell lines, the miR-17-92 cluster targets the tumor suppressor *CDKN1A* gene, which encodes the cyclin-dependent kinase inhibitor p21.[16]

Other comprehensive studies in vivo focused instead on tissue-specific miR signatures that could discriminate MPM from lung adenocarcinoma.[17,18] A diagnostic test based on differentially expressed miRNAs, which allegedly discerns MPM from peripheral adenocarcinomas of the lung with high sensitivity and specificity, is currently being marketed by Rosetta Genomics Ltd (Rehovot, Israel) under the commercial name miRview™ meso. The test was established after comparing the expression of miRNAs in FFPE specimens of 33 MPMs and 210 adenocarcinomas of different organs and detecting the underexpression of seven miRNAs and overexpression of four miRNAs in MPMs.[17] Three of these miRNAs (miR-192, -193a-3p, -200c) were further validated in an expanded set of tissue samples, leading to a diagnostic protocol that by combining the overexpression of miR-200c and -192 in lung adenocarcinomas and overexpression of miR-193a-3p in MPM could distinguish these two malignancies with 100% sensitivity and 94% specificity.[17] This study emphasized the downregulation of the miR-200 family components (miR-141, -200a/b/c, -429) in MPM vs lung adenocarcinoma.[17] Gee et al confirmed the potential value

Table 1 Experimental setup, identified deregulated miRNAs, and main findings of studies exploring the biomarker potential of miRNAs in MPM

Ref	Experimental method	Source of control reference samples	Normalizer	Type of samples (tissue samples or cell lines)	Upregulated miRNAs in MPM	Downregulated miRNAs in MPM	Main findings
Guled et al[14]	Screening of miRNA profile in tumor tissues: miR-microarray Correlation of miRNA expression profile with histological subtypes	Normal human pericardium total RNA	Not specified	17 (11 EMPM, 5 BMPM, 1 SMPM) MPM samples (fresh frozen)	let-7b*, miR-30b*, -32*, -195*, -345, -483-3p, -584, -595, -615-3p, -885-3p, -934, -1228*	let-7e*, miR-7-1*, -9, -34a*, -144*, -203, -340*, -423, -582 (essentially only expressed in the control, not in MPM samples)	Deregulated miRNAs differentiate MPM from control tissue, several are located in chromosomal areas deleted or gained in MPM, such as 8q24, 1p36, and 14q32. Their predicted target genes include genes most frequently affected in MPM (*CDKN2A, NF2, JUN, HGF*, and *PDGFA*). Specific expression of miR-135b, -181a-2*, -499-5p, -517b, -519d, -615-5p, -624 in EMPM; miR-218-2*, -346, -377*, -485-5p, -525-3p in BMPM; miR-301b, -433, -543 in SMPM. Smoking affects miRNA expression in MPM.
Busacca et al[15]	Screening of miRNA profile in cell lines: miR-microarray Validation in cell lines: TaqMan RT-qPCR, miRNA/genes contingency table: cDNA-microarrays and RT-qPCR. Validation in vivo and association with MPM subtypes of ten selected miRNAs: RT-qPCR	Calibrator sample consisting of pooled total RNA from all MPM tissue samples	For screening: global mean For validation: *RNU6-6P* (for miR analysis), *GAPDH* (for gene expression analysis)	MMP-89, REN (MPM cell lines) HMC-TERT (immortalized HMC), 24 (8 EMPM, 8 BMPM, 8 SMPM) MPM samples (FFPE)	miR-17-5p, -21, -30c, -30e-5p, -106a, -143	miR-29a, -31, -221, -222	Screening showed ↑ of 10 miRNAs and ↓ of 19 miRNAs in MPM cell lines vs HMC-TERT, validation showed ↑ of miR-17-5p and -30c and ↓ of miR-221 and -222 in MPM cell lines. Expression of miR-17-5p, -21, -29a, -30c, -30e-5p, -106a, and -143 significantly associated with histological MPM subtypes. Low expression of miR-17-5p and -30c (below median) in SMPM, but not other subtypes, correlated with better patient survival (P=0.005 and 0.006).
Balatti et al[16]	Screening of miRNA profile in cell lines: miR-microarray Validation: RT-qPCR	5 primary HMC lines (4N, 6N, 13N, 16N, and 26N)	For screening: on-gene median normalization For validation: *RNU6-6P*	Five MPM cell lines: MSTO-211H, MPP-89, IST-MES2, NCI-H2052, NCI-H28	miR-7, -17-5p, -18a, -19b, -20a/b, -25, -33, -92a-1/2, -106a/b, -182, -196b, -339	miR-22, -146b, -214, -328, -497, -500, -502, -549	Among the ↑ miRNAs, 7 were members of the miR-17-92 cluster (including miR-17-5p) and its paralogs miR-106a-363 and miR-106b-25. The miR-17-92 cluster targets the tumor suppressor *CDKN1A* gene as in other cancer cell lines.
Benjamin et al[17]	Screening of miRNA profile in tumors: miR-microarray Training set and threshold delineation: RT-qPCR Further validation: RT-qPCR	259 carcinomas of different organs, including 76 lung adenocarcinomas	For screening: median expression levels for each probe across all samples. For training set and validation: *RNU6-6P*	47 (29 EMPM, 6 BMPM, 6 SMPM, 6 unspecified) MPM samples (FFPE)	miR-152, -193a-3p, -193a-5p, -193b	miR-141, -192, -194, -200a/b/c, -429	A validated diagnostic protocol was developed, in which combining ↑ of miR-200c and -192 in lung adenocarcinomas and ↑ of miR-193a-3p in MPM could distinguish these two malignancies with 100% sensitivity and 94% specificity.

Gee et al[18]	Screening of miRNA profile in tumors: miR-microarray Validation: RT-qPCR	32 lung adenocarcinomas	For screening: robust multiarray average procedure For validation: *SNORD44, SNORD48*	100 (39 EMPM, 19 BMPM, 10 SMPM, 32 unspecified) MPM samples (fresh frozen)		miR-141, -200a*/b/c, -203, -205, -429	Significant ↓ of miR-200 family members (miR-200a*/b/c, -141, -429) in MPM vs lung adenocarcinoma. Multiple genes within Wnt signaling pathway, including *MYC* and *JUN* are predicted targets of the ↓ miRNAs.
Andersen et al[19]	TaqMan RT-qPCR (assessment in vivo of cell lines results from Busacca et al[15] and Balatti et al[16])	13 patient-matched NNP	*RNU6-6P*	13 resected EMPMs, 6 patient-matched DBs, 6 independent DBs (FFPE)	miR-221	miR-17-5p	Significant miR-17-5p ↓, miR-221 ↑, and no differential expression of miR-30c and miR-222 in MPM (in contrast to in vitro findings in Busacca et al[15]) = these four miRNAs appear differently regulated in MPM cells in vivo and in vitro. ROC curve analysis demonstrated that miR-17-5p (AUC:0.64, *P*=0.20) and miR-221 (AUC:0.65, *P*=0.18) were not suitable as diagnostic markers.
Andersen et al[20]	qPCR-screening in tumor tissues: miRCURY LNA Universal RT miRNA ready-to-use PCR Validation: TaqMan miRNA assays Expression of known targets: IHC with *H*-score	For screening: five patient-matched NNP For validation: 14 patient-matched NNP, 5 pneumothorax-induced nonneoplastic RMPs	For screening: global mean of all included miRNAs For validation: *SNORD49A*	five resected FFPE EMPM samples, five patient-matched DBs Validation: 40 resected (18 EMPM, 22 BMPM) MPM samples, 12 independent DBs (FFPE)	After screening: miR-193a-3p, -193b, -210, -365a, -378 After validation: miR-193a-3p in EMPM vs BMPM but not in MPM vs controls	Let-7c, miR-99a, -126, -143, -144-5p, -145, -451a, -486-5p, -652 Postvalidation: miR-126, -143, -145, -652	miR-126, -143, -145 and -652 are ↓ in MPM and significantly differentiate MPM from NNP/RMP (AUC:0.78, 0.76, 0.93, and 0.89, respectively). Their combination by binary logistic regression resulted in a 4-miR-classifier that could correctly diagnose MPM with high sensitivity and specificity (AUC:0.96), and overall accuracy (94%). The 4-miR-classifier and ↑ of miR-193b correlated with poor patient survival. The miR-126 target LAT1 was significantly ↑ in MPM and its expression inversely correlated with that of miR-126 and could help distinguishing MPM from RMP (AUC:0.77).
Andersen et al[28]	RT-qPCR, methylation-specific PCR (MSP), pyrosequencing	14 patient-matched NNP, 5 pneumothorax-induced nonneoplastic RMPs	*SNORD49A* (for miR-126 quantification), *GAPDH* (for *EGFL7* quantification) Not applicable for MSP analysis	29 MPM (14 EMPM, 15 BMPM) samples, 5 patient-matched DBs		miR-126 in parallel with ↓ of its host gene *EGFL7*	In MPM, miR-126 and its host gene *EGFL7* are cosilenced by hypermethylation, an event predominantly associated with epithelioid histology and poor prognosis.
Santarelli et al[29]	Screening of miRNA profile in tumor tissues: RT-qPCR Validation: RT-qPCR	Screening: pooled expression of 88 miRs in 5 NNP samples from healthy individuals Validation: serum samples from asbestos-exposed subjects and healthy controls	*RNU6-6P*	Screening: 10 MPM (9 EMPM, 1 SMPM) samples (fresh frozen) Validation: 27 MPM (23 EMPM, 3 BMPM, 1 SMPM) samples (FFPE). Serum samples from patients with MPM		miR-30c, -32, -126, -130a, -181c, -193b, -212, -335 Postvalidation: miR-126	miR-126 ↓ in MPM vs NNP. miR-126's serum levels were ↓ in patients with MPM and inversely correlated with levels of circulating soluble mesothelin-related protein (SMRP), suggesting their possible use in MPM diagnostics.

(*Continued*)

Table 1 (*Continued*)

Ref	Experimental method	Source of control reference samples	Normalizer	Type of samples (tissue samples or cell lines)	Upregulated miRNAs in MPM	Downregulated miRNAs in MPM	Main findings
Tomasetti et al[30]	Quantitative measurement of miR-126 levels in serum: RT-qPCR	56 serum samples from healthy individuals 20 serum samples from patients with NSCLC	Synthetic *C. elegans* cel-miR-39 (exogenous control) *RNU6-6P* (endogenous control)	45 serum samples from patients with MPM		miR-126	Low levels of circulating miR-126 differentiated MPM patients from healthy controls and correlated with poor prognosis.
Cioce et al[45]	Screening in tumors samples and cell lines: miR-microarray Validation: RT-qPCR	For screening: 12 unmatched mesothelial cysts (FFPE), 14 fresh biopsies of NNP, 36 snap-frozen matched samples of peritoneal mesothelium from patients with MPM For validation: human primary mesothelial cells	For screening: quantile normalization For validation: *RNU6-6P, SNORD49*	Screening: 29 MPM (25 EMPM, 3 BMPM, 1 SMPM) samples (FFPE), 6 fresh MPM biopsies, 36 snap-frozen MPM samples. Validation: MPM cell lines: MSTO-211H, NCI-H28, NCI-H2052	miR-96, -181b/d, -193a-5p, -206, -210, -424-3p, -432, -548c-3p, 595, -1228-5p, -1280, -1306	miR-141, -145, -200c, -204, -486-5p, -1287 Postvalidation: miR-145	miR-145 significantly ↓ in MPM tissue samples and cell lines, mainly by promoter hypermethylation. miR-145-mimics negatively modulated some tumorigenic properties of MPM cells and induced their senescence. The EMT-associated transcription factor OCT4 was shown to be a target of miR-145 in MPM cells. OCT4 and miR-145 levels inversely correlated in MPM.
Xu et al[55]	Screening of miRNA profile in tumors: miR-microarray Validation of four of the most significant deregulated miRNAs: RT-qPCR	Six independent samples of parietal pleura from healthy individuals	For screening: quantile-normalization For validation: *SNORD44*	25 MPM (18 EMPM, 4 BMPM, 3 SMPM) samples (fresh frozen)	49 miRNAs Postvalidation: miR-155*	69 miRNAs Postvalidation: miR-1, -206, -483-5p	miR-1 further characterized due to its role as tumor suppressor in other cancer types and the consistent significant ↓ in MPM tissues. miR-1 transfection in MPM cell lines = cell cycle arrest and apoptosis associated with ↑ of p16, p53, p21, and BAX and ↓ BCL2 and survivin (probably via secondary mediators directly targeted by miR-1). In silico miR-1 target prediction yielded 29 candidate genes, 3 of which were part of antiapoptotic pathways (*BDNF*, *DDX3X*, *YWHAZ*).
Kirschner et al[56]	Screening of miRNA profile in serum: miR-microarray Validation: RT-qPCR	For screening: plasma samples of three healthy controls For validation: plasma/serum from 14 healthy individuals, and ten asbestosis patients, as well as 18 matched NNP tissue samples	For screening: 75th percentile with baseline transformation For validation: *RNU6-6P*	Screening: plasma samples of five patients with MPM Validation: plasma/serum from 45 patients with MPM (38 EMPM, 3 BMPM, 2 SMPM, 2 unspecified) as well as 18 MPM tissue samples	Postvalidation: miR-625-3p (after validation of 17 miRNAs significantly ↑ after screening, 15 of which were novel miRNAs)	miR-16, -26a-2-3p, -196b, -1914-3p	After screening and validation the novel miR-625-3p found significantly ↑ in serum of patients with MPM. miR-625-3p discriminated MPM from healthy individuals with 73% sensitivity, 79% specificity, and 82% overall accuracy. miR-652-3p also found ↑ in surgical MPM specimens, corroborating its diagnostic potential.

Reid et al[57]	miRNA profiling in tumor tissues and cell lines: TaqMan RT-qPCR	23 unmatched NNP samples from noncancer patients. MeT-5A mesothelial cell line	*RNU6-6P*	60 MPM samples (FFPE) and MPM cell lines		miR-15a/b, -16, -195 (miR-15 family)	4-22 fold ↓ of miR-15a/b, -16 and -195 in MPM tissues and 2-10 fold in MPM cell lines. Restoring miR-16 expression in MPM cell lines = cell cycle arrest in G0/G1, ↑ apoptosis, and ↓ colony formation (due to cyclin D1 and BCL2 inhibition) as well as sensitization to pemetrexed and gemcitabine. IV administration of miR-16-containing minicells to nude mice xenografted with MPM cell lines = tumor growth inhibition.
Pass et al[60]	Screening (training and testing sets) of prognostic miRNAs: custom miR-microarray with >900 miRs. Validation: RT-qPCR on eight MPM samples with good prognosis and eight with poor prognosis	Mesothelial cell lines: LP9, Met-5A, NYU-590.2	For screening: not specified For validation: mean of 12 miRs quantified with RT-qPCR	129 (81 EMPM, 48 non-epithelioid) MPM samples (fresh frozen) MPM cell lines (functional studies): HP1, HP3, H2373, H2452, H2591, H2595, H2596, H2461	Variable miR-29c* expression, usually ↓. When median expression used as cutoff: two significantly different survival groups of patients with MPM were seen	miR-29c* levels < in SMPM than EMPM	Higher miR-29c* expression correlated with > TTP and OS after cytoreductive surgery for MPM. miR-29c* could separate patients with EMPM into prognostic groups (if ↑ expression = better prognosis). miR-29c* transfection into MPM cell lines ↓ their proliferation, invasiveness, and colony-formation.
Ivanov et al[62]	Screening for miRNAs associated with MPM progression: comparative miR-microarray analysis Endogenous or transfected miR-31 detection: RT-PCR Genome-wide expression of miR-31-regulated genes: gene expression arrays and computational analysis for potential miR-31 targets	Mesothelial cell lines: LP9, Met-5A, NYU-590.2 Array data from seven normal peritoneal samples + 14 matched NNP samples for RT-qPCR validation	Median expression across all samples *PPIA* as normalizer for PPP6C mRNA (as miR-31 target) expression in transfectants and tissue samples. Beta-actin for validation of MCM2 and PPP6C protein levels in transfectants by Western blot	MPM cell lines (cell lines from patients with particular aggressive MPM): HP1, HP3, H2373, H2452, H2591, H2595, H2596, H2461 Array data from 32 MPMs + 14 MPM samples for RT-qPCR validation		miR-31	Homozygous codeletion of *MIR31* and *CDKN2A/B* on 9p21.3 in MPM cell lines. miR-31 reintroduction = ↓ cell proliferation, invasion, migration, and colony formation. Genome-wide target-prediction indicated that miR-31 might regulate multiple genes involved in DNA replication, cell cycle progression, DNA damage response, and cell survival. Two of these targets, MCM2 and PPP6C, validated in miR-31-transfecetd cell lines (= ↓ PPP6C mRNA and MCM2/PPP6C protein expression) and in MPM tissue samples (↑ PPP6C mRNA vs NNP).
Kubo et al[69]	RT-qPCR MSP analysis	Ten NNP samples from patients with lung cancer Two nonmalignant primary mesothelial cell lines	Not specified for miRNA RT-qPCR analysis Not applicable for MSP analysis	47 (32 EMPM, 10 BMPM, 4 SMPM, 1 LMPM) snap-frozen MPM samples. MPM cell lines: NCI-H28, NCI-H290, NCI-H2052, NCI-H2452, HP1, MSTO-211H		miR-34a and -34b/c	miR-34a methylation in 2/6 MPM cell lines and 13/47 MPM tissue samples. miR-34b/c methylation in 6/6 MPM cell lines and 40/47 MPM samples. No methylation of miR-34s in controls. miR-34b/c transfection into MPM cell lines ↓ their proliferation and invasiveness, forced overexpression induced apoptosis.

(*Continued*)

Table 1 (*Continued*)

Ref	Experimental method	Source of control reference samples	Normalizer	Type of samples (tissue samples or cell lines)	Upregulated miRNAs in MPM	Downregulated miRNAs in MPM	Main findings
Muraoka et al[70]	Digital MSP analysis	Serum fractions of peripheral blood samples from 21 patients with benign asbestosis pleurisy and 41 healthy volunteers	Not applicable for MSP analysis	Serum fraction of peripheral blood from 48 MPM (36 EMPM, 8 BMPM, 4 SMPM) patients		miR-34b/c	*MIR34B/C* methylation > in the serum of MPM patients than control groups, > in advanced stage of MPM, and could differentiate MPM from benign cases with 67% sensitivity, 77%, specificity and AUC:0.77.
Menges et al[73]	TaqMan RT-qPCR	Malignant mesothelioma cells in wild-type mice	*Murine peptidylprolyl isomerase D*	Malignant mesothelioma cells in *Nf2*$^{+/-}$; *Cdkn2a*$^{+/-}$ mice		miR-34a	Malignant mesotheliomas from *Nf2*$^{+/-}$; *Cdkn2a*$^{+/-}$ mice harbor invasive cancer stem cell populations with c-Met activation due to ↓ of miR-34a expression and disruption of p53/miR-34a-dependent control of c-Met expression.
Fassina et al[75]	RT-qPCR, ISH, and IHC	In vivo: not specified In vitro: MeT-5A mesothelial cell line	*β-2-microglobulin* (for quantification of epithelial and mesenchymal markers' mRNAs) *RNU6-6P* (for miR-205 quantification)	109 pleural or peritoneal mesotheliomas (58 EMPMs, 26 BMPMs and 25 SMPMs). MPM cell lines: H2452, MSTO-211H		miR-205	BMPM and SMPM show miR-205 ↓ and a switch from epithelial to mesenchymal markers as compared to EMPM. Ectopic miR-205 in nonmalignant mesothelial cells and MPM cell lines significantly ↓ the mesenchymal transcription factors ZEB1 and ZEB2, ↑ epithelial markers and impaired invasiveness.
Cheng et al[76]	miR-microarray RT-qPCR for miR-23a and -27a validation	MeT-5A mesothelial cell line	For screening: 75th percentile with baseline transformation For validation: *RNU6-6P*	MPM cell lines: NCI-H2052, NCI-H2452, NCI-H28, NCI-H226, MSTO-211H. 24 MPM samples (FFPE)	miR-23a, -27a		*ZIC1* TSG ↓ by methylation in MPM cell lines and 16/24 tissue samples resulting in ↑ of miR-23a and -27a. Overexpression of miR-23a was a negative prognostic factor in MPM. Restoring ZIC1 expression in MPM cells ↓ miR-23a and miR-27a expression and proliferation and invasion.
Williams et al[77]	RT-qPCR	23 NNP tissue samples MeT-5A mesothelial cell line	*RNU6-6P*	120 MPM tissue samples (72 EMPMs, 39 BMPMs, 9 SMPMs) from EPP or P/D 12 MPM cell lines		miR-192, -193a-3p	↓ of miR-192 and -193a-3p in MPM tissue samples and cell lines (not due to methylation). Transfection with miR-193a-mimics ↓ *MCL1* expression and caused apoptosis of MPM cells. Targeted delivery of miR-193a-3p ↓ MPM xenograft growth by inducing apoptosis.
Birnie et al[81]	Screening of miRNA profile in MPM cell lines: RT2 miRNA PCR array Validation of miR-223: TaqMan RT-qPCR Stathmin1 (STMN1) expression: RT-qPCR, Western blot	Six control pericardial mesothelium samples from unmatched patients without cancer undergoing cardiac or aortic surgery. Ten pleural effusions from benign pleural diseases.	*RNU6-6P* for miR-223 expression in tumor samples and *RNU48, RNU44,* or *SNOR202* in cells *18S ribosomal RNA* for STMN1 mRNA expression, α-tubulin for STMN1 protein expression	17 MPM tissue samples (FFPE). 26 pleural effusions from patients with MPM. Eight MPM cell lines (five human, three murine)		miR-223 (the only miR consistently ↓ after screening in MPM cell lines and validation in MPM cell lines and tissue samples)	miR-223 is ↓ in MPM cell lines, tissue samples, and effusions, resulting in ↑ of its target, the microtubule-regulator STMN1. miR-223 is inducible by JNK-signaling and its overexpression in MPM cell lines ↓ STMN1 levels, cell motility, and proliferation.

		Primary mesothelial cells (murine from the omentum, human from pericardial fluid); Met-5A mesothelial cell line					
Ak et al[82]	miRNA and mRNA expression profiling: TaqMan RT-qPCR and cDNA-microarrays.	Six benign asbestos-related pleural effusion (BAPE)	*RNU6-6P* (for miRNAs) and "gcrma" in the R package @ http://www.bioconductor.org/ (for normalization of cDNA-microarray data based on Log2 intensity values > or < signal to noise ratio for differentially expressed genes in MPM vs BAPE)	18 frozen MPM tissue samples (10 EMPMs, 4 BMPMs, 4 SMPMs)	let-7a, miR-125a-5p, -320, -484		↑ of let-7a, miR-125a-5p, -320, -484 could distinguish MPM from BAPE with AUC: ≥0.90 and sensitivity/specificity 94%/83%; 89%/100%, 78%/100%, and 100%/100%, respectively.
Weber et al[84]	Screening of miRNA profile in cellular fraction of peripheral blood: miR-microarray Validation: TaqMan RT-qPCR analysis	Cellular fraction of peripheral blood from 17 asbestos-exposed individuals and 25 volunteers from general population	Normalizer not specified (data transformation measurements <0.01 set to 0.01 and normalization per microarray performed using the 50th percentile. For altered miRNA expression, a fold change of 3.0 was used as threshold). miR-125a (most stable miR) = reference to normalize raw Ct values of miRs obtained in the RT-qPCR analysis.	Cellular fraction of blood from 23 (12 EMPMs, 7 BMPMs, 1 SMPMs, 3 unspecified) patients with MPM		miR-103 (after validation of 49 deregulated miRNAs after screening)	Significant ↓ of miR-103 in the cell fraction of peripheral blood from patients with MPM that could discriminate them from asbestos-exposed healthy individuals with AUC: 0.76, 83% sensitivity, 71% specificity and from the general population with AUC: 0.87, 78% sensitivity, 76% specificity.
Weber et al[85]	TaqMan RT-qPCR analysis Plasma mesothelin measured by ELISA	Cellular fraction of blood from 52 asbestos-exposed individuals	miR-125a (reference miR due to its stable expression)	Cellular fraction of blood from 43 (28 EMPMs, 6 BMPMs, 5 SMPMs, 4 unspecified) patients with MPM		miR-103a-3p	Combining mesothelin and miR-103a-3p improved the diagnostic performance of either marker alone by ↑ sensitivity and specificity to detect MPM (AUC: 0.93 after exclusion of SMPMs due to their high rate of false-negative results).
Santarelli et al[86]	TaqMan RT-qPCR for miR-126; real-time qMSP for methylated thrombomodulin promoter (Met-TM); ELISA for SMRPs	Serum of 99 asbestos-exposed subjects, and 44 healthy controls Validation cohort: serum of 20 healthy controls, 50 asbestos-exposed subjects	Endogenous control for miR-126: *RNU6-6P.* Exogenous control: *C. elegans* cel-miR-39. Not applicable for MSP	Serum of 45 patients with MPM (33 EMPMs, 3 BMPMs, 9 SMPMs) Validation cohort: serum of 18 patients with MPM, 42 patients affected by lung cancer		miR-126 (together with ↑ SMRPs and Met-TM)	The triple combination of serum levels of miR-126 and Met-TM with SMRPs significantly ↑ the performance of SMRPs alone in distinguishing patients with MPM from control groups, thereby overcoming the sensitivity limitations of SMRPs alone. The 3-biomarker combination distinguished MPM from lung cancer.

(*Continued*)

Table 1 (*Continued*)

Ref	Experimental method	Source of control reference samples	Normalizer	Type of samples (tissue samples or cell lines)	Upregulated miRNAs in MPM	Downregulated miRNAs in MPM	Main findings
Kirschner et al[87]	miRNA microarray analysis of MPM samples from eight long and eight short survivors following EPP (discovery set). Validation of candidate miRNAs: RT-qPCR		Processing of miRNA microarray data from discovery set involved thresholding of signal values to 1, transformation into Log2 and normalization by shift to the 90th percentile. *RNU6-6P* for RT-qPCR analysis of 13 selected miRNA candidates on training set. Binary logistic regression used to construct a miR signature ("miR-score") predicting OS of ≥20 months. Performance of miR-score evaluated by ROC curve analysis in the validation set	FFPE tissue samples from 64 MPM patients operated by EPP divided in: a discovery set of eight long + eight short survivors, (median OS 57.7 and 6.4 months, respectively, all EMPMs) and training set of 48 patients (31 EMPMs, 17 BMPMs). Independent validation set of 43 patients with MPM who underwent P/D (25 EMPMs, 13 BMPMs, 5 SMPMs)		miR-21-5p, -23a-3p, -30e-5p, -31-5p, -221-3p, -222-3p = miR-score (after validation of candidate miRNAs on training set and independent validation set with 20 months OS as cutoff)	The miR-score enabled prediction of prolonged postsurgical survival with an accuracy of 92.3% for EPP and 71.9% for palliative P/D. By adding the miR-score, the prognostic accuracy of clinical parameters (histology, age, sex) ↑ from 76.3% to 87.3% in the independent validation set.

Abbreviations: miRNA, microRNA; MPM, malignant pleural mesothelioma; EMPM, epithelioid malignant pleural mesothelioma; BMPM, biphasic malignant pleural mesothelioma; SMPM, sarcomatoid malignant pleural mesothelioma; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *EGFL7*, epidermal growth factor-like 7; FFPE, formalin-fixed paraffin-embedded; TTP, time to progression; OS, overall survival; ROC, receiver operating characteristic; EPP, extrapleural pneumonectomy; P/D, pleurectomy/decortication; AUC, area under the curve; IHC, immunohistochemistry; NNP, nonneoplastic pleura; DBs, diagnostic biopsies; RMPs, reactive mesothelial proliferations; HMC, human mesothelial cells; EMT, epithelial-to-mesenchymal transition; ISH, in situ hybridization; mRNA, messenger RNA; Ref, reference; *C. elegans, Caenorhabditis elegans*; ELISA, enzyme-linked immunosorbent assay; TSG, tumor suppressor gene.

of miR-200 family members in the differential diagnosis between MPM and pulmonary adenocarcinoma.[18] Using target prediction algorithms, furthermore, they identified multiple genes associated with the Wnt signaling pathway (eg, *MYC* and *JUN*) as putative targets of the downregulated miRNAs.[18] However, with the exception of miR-17-5p and miR-106a overexpression in vitro, the aforementioned studies reported deregulation of different miRNAs in MPM cells and were difficult to compare. This is likely due to the differences in experimental setups, reference samples, and sources of specimens employed (MPM tissue samples vs cell lines) as well as size and goals of the studies (Table 1). Although the miRNA signatures obtained in these studies could apparently distinguish the different histological subtypes of MPM or discriminate between MPM cell lines and HMC or between MPM and other cancer types, it remained unclear whether the reported miRNAs could be useful as candidate biomarkers for the challenging differential diagnosis between MPM and reactive mesothelial proliferation (RMP) in patient samples.

Thus, we assessed whether the deregulation of miR-17-5p, -30c, -221, and -222 observed in vitro may be useful in differentiating MPM from RMP in vivo.[15,16,19] We quantified the expression of these four miRNAs by RT-PCR-based TaqMan® assays in a set of patient-matched surgical FFPE samples of EMPM (stages I–IV) and nonneoplastic pleura (NNP) tissue from patients treated with trimodal protocol.[19] We also included diagnostic biopsies from chemotherapy-naïve patients with MPM and found no significant differential expression of these miRNAs in the diagnostic biopsies vs the surgical samples, suggesting that their expression was not affected by chemotherapy. Moreover, in contrast to the finding of Busacca et al in vitro,[15] we found significant downregulation of miR-17-5p, upregulation of miR-221, and no differential expression of miR-30c and miR-222 in MPM when compared to NNP specimens.[19] Thus, these four miRNAs appear differently regulated in MPM cells in vivo and in vitro. Suitable MPM marker should have a sensitivity or specificity of >80%;[1] however, receiver operating characteristic curve analysis revealed that miR-17-5p and miR-221 did not fulfill these criteria (area under the curve [AUC] values of 0.64 and 0.65, respectively).[19] Therefore, despite being reportedly specific for MPM subtypes or differentially expressed in MPM cell lines vs HMC, miR-17-5p, miR-30c, and miR-221/222 do not seem to be the suitable biomarkers for accurately discriminating MPM from RMP.

Hence, to detect more specific miRNAs that could fulfill this task, we recently performed an RT-qPCR screening of 742 miRNAs and validated the 14 identified differentially expressed miRNAs with diagnostic potential in different cohorts of preoperative chemotherapy-naïve FFPE tumor biopsies, chemotherapy-treated surgically resected MPM specimens, and corresponding patient-matched NNP samples as well independent pleural samples from pneumothorax-induced RMPs.[20] We discovered that miR-126, -143, -145, and -652 were consistently downregulated in MPM resections and tumor biopsies and were the best candidate miRNAs to diagnose MPM. Not only these four miRNAs could significantly distinguish MPM from NNP/RMP samples but combining by binary logistic regression analysis, the four miRNAs' expression data resulted in a 4-miR classifier with enhanced diagnostic performance that could correctly diagnose MPM with high sensitivity, specificity (AUC 0.96), and overall accuracy (94%). Moreover, we showed that the 4-miR signature and high expression levels of miR-193b in MPM samples were associated with poor patient survival, suggesting that these miRNAs also possessed prognostic value.[20] We also observed that miR-126 expression in MPM was inversely correlated with the levels of one of its known targets, the large neutral amino acid transporter, small subunit 1 (LAT1), which was significantly overexpressed in MPM samples in comparison to the NNP and pneumothorax samples.[20,21] Interestingly, our data on miR-126 downregulation's negative prognostic value and inverse correlation with LAT1 expression are consistent with the previously reported association between reduced survival and high LAT1 expression in MPM.[22]

miRNAs in the diagnostic signature discriminating MPM from RMPs

MicroRNA-126

This miRNA has been reported downregulated in different malignancies.[23–25] *MIR126* is located within intron 7 of the *EGFL7* gene, which encodes for epidermal growth factor-like 7 (EGFL7), an endothelial cell-secreted protein that contributes to normal and pathological vasculogenesis.[26,27] *EGFL7* contains eleven introns generating three different transcripts through three independent transcriptional initiation sites. *MIR126* is coexpressed with one of these alternative transcripts which has transcription initiation site positioned at a CpG island in intron 2 of *EGFL7*.[27] Aiming at elucidating the molecular mechanisms of the downregulation of miR-126 in MPM, we recently discovered that miR-126 and its host *EGFL7* gene were cosilenced in MPM by hypermethylation of CpG islands flanking *EGFL7*'s transcription-start-site in intron 2. This hypermethylation of *EGFL* and *MIR126* was

Table 2 Predicted or verified target genes of deregulated miRNAs in MPM

Ref	miRNA(s)	Target gene(s)	Evidence
Guled et al[14]	miR-885-3p	*CDKN2A, NF2*	Computational
Guled et al[14]	miR-7-1*	*EGF, PDGFA*	Computational
Guled et al[14]	miR-30b*, -32*, -483-3p, -584	*RB1*	Computational
Guled et al[14] Gee et al[18]	miR-203	*HGF, CTNNBIP1, DKK1, DKK4, DVL3, EGR1, FZD2, NLK, WNT8A*	Computational
Guled et al[14] Gee et al[18]	miR-9, -141, -200a*/b/c, -203, -429 (certain targets are predicted to be regulated by -200a*/141 and -200b/c/429. These possibilities also concern the other putative targets underneath)	*JUN*	Computational
Busacca et al[15]	miR-17-5p, -106a, -143	*BTG1*	Computational
Balatti et al[16]	miR-17-5p, -20a, -92	*CDKN1A*	Western blot
Gee et al[18]	miR-203, -205	*APC*	Computational
Gee et al[18]	miR141, -200a*	*CTNNB1, DKK3*	Computational
Gee et al[18]	miR-141, 200a*/b/c, -203, -429	*DIXDC1*	Computational
Gee et al[18]	miR141, -200a*, -203	*DKK2*	Computational
Gee et al[18]	miR-141, -200a*	*SOX17*	Computational
Gee et al[18]	miR-200b/c, -429	*EP300*	Computational
Gee et al[18]	miR-141, -200a*/b/c, -429	*FBXW2, FBXW11*	Computational
Gee et al[18]	miR-200a*	*FGF4*	Computational
Gee et al[18]	miR-141, -200a*, -203, -205	*FSHB*	Computational
Gee et al[18]	miR-200a*, -203, -205	*FZD1*	Computational
Gee et al[18]	miR-141, -200a*/b/c/, -203, -429	*FZD4*	Computational
Gee et al[18]	miR-200b/c, -203, -429	*FZD5, FZD6*	Computational
Gee et al[18]	miR -141, -200a*	*FZD8, SOX17*	Computational
Gee et al[18]	miR-141, -200a*, -205	*LRP6*	Computational
Gee et al[18]	miR-200b/c, -203, -429	*MYC*	Computational
Gee et al[18]	miR-200b/c, -205, -429	*NKD1*	Computational
Gee et al[18]	miR-141, -200a*, -203, -205	*PLCB1*	Computational
Gee et al[18]	miR-141, -200a*/b/c, -429	*PPP2CA*	Computational
Gee et al[18]	miR-141, -200a*/b/c/, -203, -429	*PPP2R5C, PPP2R5E*	Computational
Gee et al[18]	miR -200b/c, -429	*RHOA, ROCK2*	Computational
Gee et al[18]	miR-141, -200a*/b/c/, -203, -205, -429	*TCF4*	Computational
Gee et al[18]	miR-141, -200a*/b/c/, -203, -429	*VANGL1*	Computational
Gee et al[18]	miR-141, -200b/c/, -203, -205, -429	*VEGFA*	Computational
Gee et al[18]	miR-200b/c, -203, -429	*WIF1, WNT4*	Computational
Gee et al[18]	miR-205	*WNT3A, WNT5B*	Computational
Gee et al[18]	miR-141, -200a*/b/c, -429	*WNT5A, WNT16*	Computational
Andersen et al[20]	miR-126	*LAT1*	IHC with *H*-score, Spearman test for correlation between miR-126 and LAT1 expression
Bao et al[34]	miR-126	*IRS1*	Luciferase assay, transfection with *MIR126* expression plasmid and pGL3-promoter plasmid containing *IRS1* 3′-UTR
Cioce et al[45]	miR-145	*OCT4*	Computational, luciferase activity, Western blot, miR-145-mimic transfection
Xu et al[55]	miR-1	*ANKIB1, ARID2, AZIN1, BCL11A, BDNF, CALM2, CLTC, DDX3X, EFNB2, FBXW7, FNDC3A, FNDC3B, HAND2, HNRNPU, HS3ST3B1, JARID2, MIPOLI, MON2, NCL, NR4A2, PICALM, PTMA, RNF165, TBC1D15, TMSL3, VEZF1, YWHAZ, ZFHX4, ZNF827*	Computational
Reid et al[57]	miR-16	Many predicted and qPCR-validated targets, of these *CCND1* and *BCL2* also validated at protein level	miR-16-mimic transfection, RT-qPCR, Western blot
Pass et al[60]	miR-29c*	*ADIPOQ, CTRP8, CTRP1, DNMT3A*	Transfection of miR-29c*-mimic, RT-qPCR

(*Continued*)

Table 2 (*Continued*)

Ref	miRNA(s)	Target gene(s)	Evidence
Ivanov et al[62]	miR-31	Computational target prediction: multiple genes, of which *CCNB2, CDC2, CDC6, CHEK1*, *SKP2* most ↑ in MPM. Predicted and validated: *MCM2, PPP6C*	miR-31 transfection, gene expression array, computational search, RT-qPCR in miR-31-transfected MPM cell lines and in MPM tissue samples, Western blot in transfectants
Kubo et al,[69] Maki et al[71]	miR-34b, -34c	*BCL2, CCND1, CCNE2, CDK4, CDK6, E2F3, MET, MYC*	miR-34b/c transfection in MPM cell lines, Western blot
Tanaka et al[72]	miR-34a, -34b, -34c	*BCL2*, *MET*	Transfection of miR-34 inhibitors in HMC, Western blot
Menges et al[73]	miR-34a	*MET*	siRNA silencing of *miR34* in *Nf2*$^{+/-}$; *Cdkn2a*$^{+/-}$ mice
Fassina et al[75]	miR-205	*ZEB1, ZEB2*	miR-205 transfection in nonmalignant mesothelial cells and MPM cell lines, RT-qPCR
Williams et al[77]	miR-193a-3p	*E2F1, MCL1, TYMS*	RT-qPCR, Western blot
Birnie et al[81]	miR-223	*STMN1*	RT-qPCR, Western blot, immunoprecipitation
Ak et al[82]	miR-449a	*CCNE2, MET*	Integrated analysis of ↑ mRNAs and target genes of ↓ miRNAs
Kirschner et al[87]	miR-21-5p, -23a-3p, -30e-5p, -31-5p, -221-3p, -222-3p (prognostic 6 miR-signature = "miR-score")	Multiple genes associated to pathways implicated in MPM development (Wnt-, Hippo-, FAK-, PI3K/Akt-, ErBb-signaling, etc)	Computational pathway enrichment analysis of miR-score's miRNA target genes

Abbreviations: miRNA, microRNA; mRNAs, messenger RNAs; MPM, malignant pleural mesothelioma; IHC, immunohistochemistry; *IRS1*, insulin receptor substrate-1; *CCND1*, cyclin D1-encoding gene; miR-126, MicroRNA-126; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; HMC, human mesothelial cell; Ref, reference.

predominantly associated with epithelioid histology and correlated with poor clinical outcome.[28] These results suggest that demethylating strategies reestablishing *EGFL7* and miR-126 expression might provide future therapeutic options for MPM. Moreover, considering that miR-126 has promising diagnostic potential in MPM,[20] concomitant silencing of *EGFL7* might represent an additional event that could be used in the difficult differential diagnosis between MPM and RMPs.

Downregulation of miR-126 was first reported in MPM by Santarelli et al after expression screening of 88 cancer-related miRNAs in an independent set of fresh-frozen MPM biopsies, surgical FFPE MPM samples, and adjacent NNP.[29] These authors also reported that the serum levels of miR-126 were reduced in patients with MPM and subjects who are at high risk to develop MPM and were inversely correlated with the circulating soluble mesothelin-related protein (SMRP) concentration. Thus, circulating miR-126 and SMRP were proposed as valuable biomarkers for the early diagnosis of MPM.[29] Confirming the relevance of these findings, low levels of circulating miR-126 differentiated patients with MPM from healthy control subjects and correlated with poor prognosis.[30] By targeting genes such as *LAT1*, vascular endothelial growth factor-A (*VEGF-A*), *EGFL7* itself (as a feed back), insulin receptor substrate-1 (*IRS1*), phosphoinositide-3-kinase, regulatory subunit 2 (*PI3KR2*), and Kirsten rat sarcoma viral oncogene homolog (*KRAS*), miR-126 has been linked to vascular integrity and tumor suppression.[20,21,31–33] However, the mechanisms by which miR-126 suppresses mesothelioma genesis remain poorly understood. Recently, Tomasetti et al showed that ectopic miR-126 modified the response of the MPM cell line NCI-H28 to hypoxic oxidative stress through the inhibition of IRS1-PI3K-Akt signaling and activation of the Forkhead-box-O1 (FoxO1) transcription factor, resulting in mitochondrial respiration repression, increased glycolysis, and FoxO1-induced gluconeogenesis and oxidative stress defense.[34] The authors also observed concomitant activation of the hypoxia-inducible factor-1α, which controls the adaptation of malignant cells to hypoxic microenvironments and the expression of glucose transporters, glycolytic enzymes, angiogenic growth factors, and apoptosis regulators.[34,35] Moreover, the expression of miR-126 in NCI-H28 cells suppressed their proliferation, colony formation, and tumor formation in nude mice.[34] Thus, miR-126 appears to be tumor suppressive in MPM at least in part through pleiotropic metabolic effects linked to the inhibition of the IRS1-PI3K-Akt signaling pathway.

It has also been shown that miR-126 targets certain chemokines and related receptors, which are highly expressed in MPM and promote the accumulation of lymphocytes and mesenchymal stem cells. Thus, changes in the tumor microenvironment due to miR-126 downregulation may in turn favor cancer progression by enhancing invasiveness and metastatic potential of neoplastic cells.[36–38]

MicroRNA-143 and -145

miR-145 is clustered with miR-143 and often lost in several cancer types.[39–41] The expression of miR-143/-145 cluster is regulated by p53, and the oncogene *MDM2* is a direct target of miR-143/-145; thus, deregulation of miR-143/-145 in malignant cells impairs the MDM2-p53 feedback loop.[42] Additionally, miR-143 and miR-145 are also considered tumor suppressors because they target other oncogenes.[40–44] Further studies assessing the expression of miR-143 and miR-145 together with that of some of their targets will clarify whether these regulatory loops are also relevant for MPM.

A recent study comparing the expression of 887 miRNAs in FFPE MPM-specimens and a control group of unmatched mesothelial cysts showed that miR-145 was the most deregulated among 19 differentially expressed miRNAs.[45] The downregulation of miR-145 in malignant mesothelial cells was validated in independent sets of unmatched MPM and NNP biopsies, patient-matched peritoneal mesothelioma and normal mesothelium, and MPM cell lines as compared to HMC. These results, together with our findings,[20] indicate that the expression levels of miR-145 can be potentially useful for differentiating benign from malignant mesothelial proliferations. Moreover, downregulation of miR-145 in malignant mesothelial cells was in large part due to *MIR145*-promoter hypermethylation.[45] Similarly, we recently found that, in contrast to patient-matched NNP samples, MPM specimens display methylated or hemimethylated *MIR145* gene promoter (Aslan et al, unpublished data, 2015).

From a mechanistic point of view, forced expression of miR-145-agonists in MPM cell lines impaired their proliferation, colony formation, migration, and resistance to pemetrexed treatment, leading to replicative senescence and hampering the ability of cells to form tumors after transplantation into SCID mice.[45] Furthermore, miR-145 targets the transcription factor OCT4, which regulates the epithelial-to-mesenchymal transition (EMT), and has been associated with acquired resistance to pemetrexed in MPM.[45,46] Accordingly, ectopic miR-145 downregulated OCT4 in MPM cell lines, while reintroduction of OCT4 into these cells counteracted miR-145-induced replicative senescence. Consistent with these observations, an inverse correlation between the levels of miR-145 and OCT4 was observed in MPM samples.[45] Collectively, these data imply that miR-145 acts as mesothelial tumor suppressor at least in part by targeting OCT4.

MicroRNA-652

In contrast to the tumor-suppressive miR-126, -143, and -145, miR-652 is poorly characterized. Single reports have described deregulated miR-652 expression in other tumor types.[47,48] Diagnostic and prognostic potentials of miR-652 deregulation have been reported in breast cancer.[49,50] However, the knowledge on validated targets and mechanism of action for miR-652 is scant. This miRNA was recently found upregulated in the dysfunctional heart, and the hematopoiesis-related Notch1-ligand, Jagged1, was identified and validated as miR-652 target in a mouse cardiac hypertrophy model.[51] Notably, Jagged1 was previously found overexpressed in MPM cell lines.[52] To learn more about potential miR-652 targets and determine specific pathways possibly regulated by miR-652, we recently performed in silico computational analysis combining seven different target prediction algorithms and functional annotation tools. Preliminary unpublished data show that miR-652 may target genes encoding proteins of cellular membranes involved in the regulation of transport activities of ion channels or acting as receptors. Interestingly, among these putative miR-652 targets, we found the *BSG* gene encoding basigin/CD147, a membrane protein that has been related to metastatic potential, drug resistance, and poor prognosis in different cancer types and has been proposed as IHC-marker for distinguishing benign RMP cells from MPM.[53,54] Further investigations are needed to corroborate these data and expand the knowledge of miR-652 function in MPM.

Role of other deregulated miRNAs with diagnostic or prognostic value in MPM

Additional studies have shed light on the role of pathophysiologic, diagnostic, prognostic as well as therapeutic relevance of individual miRNAs or families of miRNAs in MPM.

MicroRNA-1

Xu et al reported significant downregulation of miR-1 in 25 MPM-specimens (18 EMPM, four BMPM, and three SMPM), as compared to six pleura samples from patients with out cancer.[55] miR-1 transfection in MPM cell lines caused cell cycle arrest and apoptosis associated with induction of the tumor suppressors p53, BCL2-associated X protein, and cyclin-dependent kinase inhibitors p16 and p21 as well as repression of BCL2 and survivin, possibly through secondary mediators directly targeted by miR-1.[55] Further corroboration in vivo is required in order to completely evaluate pathogenetic and therapeutic significance of miR-1 in MPM.

MicroRNA-16

The underexpression of miR-16, a member of the miR-15 family, which according to the miRBase comprises

miR-15a, -15b, -16, and -195,[10] was first reported in MPM by Kirschner et al.[56] Subsequently, Reid et al found marked underexpression of the entire miR-15 family (fourfold to 22-fold) when they compared a set of 60 FFPE MPM specimens to a reference cohort of 23 NNP specimens from patients undergoing cardiac or aortic surgery.[57] Similar downregulation (twofold to tenfold) of miR-15 family was detected in MPM cell lines.[57] Restoring miR-16 expression in these cell lines resulted in cell cycle arrest in G0/G1, increased apoptosis, and reduced colony formation, consistent with the cyclin D1-encoding gene (*CCND1*) and the antiapoptotic gene *BCL2* being known miR-16 targets.[57–59] Transfecting varying doses of miR-16-mimics resulted also in dose-dependent sensitization to pemetrexed and gemcitabine, while IV administration of miR-16-containing minicells to nude mice xenografted with MPM cell lines led to significant dose-dependent tumor growth inhibition.[57] Together, these results imply that miR-16 may have diagnostic potential and reestablishing its expression may represent a potential future therapeutic tool for MPM.

MicroRNA-29c*

Investigating the prognostic impact of specific miRNAs in a cohort of surgically treated patients with MPM, Pass et al discovered that the expression of miR-29c* correlated with time-to-progression and overall survival after cytoreductive surgery.[60] The levels of this miRNA were higher in EMPM than in SMPM and could be used to separate patients with epithelioid histology into prognostic groups, as increased expression was associated with more favorable prognosis.[60] miR-29c* transfection of MPM cell lines decreased their proliferation, invasiveness, and colony formation, suggesting an antitumorigenic function of this miRNA as well. Moreover, the levels of DNA methyltransferases in MPM were found to be more elevated than in corresponding NNP, while the miR-29c*-transfected cell lines displayed significant downregulation of these enzymes and reexpression of specific methylated genes.[60] This is consistent with previous data showing that the DNA-(cytosine-5)-methyltransferases DNMT3A and DNMT3B are targets of the miR-29 family members (miR-29a/b/c).[61]

MicroRNA-31

Deletion of the 9p21.3 chromosomal region, including the TSGs *CDKN2A/2B*, is frequent in MPM and is associated with poor prognosis.[1] Intriguingly, the 9p21.3 region harbors the *MIR31* gene, which is frequently codeleted with the *CDKN2* genes in MPM cell lines derived from rapidly progressing MPMs. Moreover, reintroduction of miR-31 inhibits the proliferation, invasion, migration, and clonogenicity of these cells.[62] A genome-wide search using target-prediction algorithms indicated that miR-31 might regulate multiple genes involved in DNA replication, cell cycle progression, DNA damage response, and cell survival (Table 2).[62] One particularly interesting putative miR-31 target is the mRNA for the catalytic subunit of the prosurvival protein phosphatase 6 (PPP6C). This transcript, which contains three binding sites for miR-31 in its 3′-UTR region, was repressed in miR-31-transfected MPM cells and overexpressed in miR-31-deleted MPM specimens.[62] In addition to providing survival advantages, PPP6C is involved in the maintenance of DNA replication, chromosomal segregation, and cell cycle progression as well as resistance to chemoradiotherapy; thus, its overexpression in MPM may result in aberrant control of these fundamental cellular processes.[62,63] Together, these results suggest that the deletion of miR-31 and overexpression of PPP6C may be potential diagnostic biomarkers for MPM. Moreover, the reintroduction of miR-31 or the inhibition of its targets might help to recoordinate cell division with DNA repair and chromosomal stability, thereby representing possible future therapeutic avenues to pursue in MPM.

Another group recently reported significant downregulation of miR-31 in FFPE MPM vs RMP samples.[64] However, four out of the five cases with higher miR-31 levels were BMPM or SMPM and associated with worse prognosis when compared to BMPM or SMPM with lower miR-31 expression.[64] Although the data support a possible role for miR-31 in distinguishing MPM from benign RMPs, its value in differentiating the histological subtypes of MPM and its prognostic impact require analysis of larger cohorts.

The microRNA-34 family

This miRNA family includes miR-34a, encoded by the *MIR34A* gene on chromosome 1p36.22 and miR-34b/-34c, which derive from a single primary transcript of *MIR34B/MIR34C* on chromosome 11q23.1. The miR-34s are p53 transcriptional targets; mediate some of tumor-suppressive p53-functions; and regulate cell proliferation, survival, invasion, and angiogenesis.[65–68] Epigenetic silencing of miR-34s appears to be involved in mesothelioma genesis. Kubo et al detected the methylation of the *MIR34A* promoter region in two of the six tested MPM cell lines and 13 of 47 resected MPM samples (of different histotypes), whereas the *MIR34B/C* promoter was methylated in all six cell lines and in 40 of 47 tumor samples.[69] Methylation correlated with the reduced expression of miR-34s. In contrast, no methylation of *MIR34A/B/C* was detected in two nonmalignant mesothelial cell cultures or ten NNP samples surgically removed from patients with lung cancer.[69] Reestablishing the

physiologic miR-34b/c expression levels in MPM cell lines by stable transfection suppressed cell proliferation, -migration, and -invasion, while the overexperssion of miR-34b/c by adenoviral vectors led to apoptotic cell death.[69]

In keeping with these results, circulating *MIR34B/C*-DNA was observed to be significantly more methylated in the serum of 48 patients with MPM than 21 patients with benign asbestos pleurisy and 41 healthy volunteers.[70] Advanced MPM cases displayed more methylation than early cases, and receiver operating characteristic curve analysis showed that *MIR34B/C* methylation could differentiate MPM from benign cases with sensitivity of 67%, specificity of 77%, and AUC of 0.77.[70]

Furthermore, miR-34b/c enhanced the radiosensitivity of MPM cell lines by impairing DNA repair potential after X-ray irradiation.[71] In contrast, inhibiting miR-34a/b/c expression with complementary RNA oligonucleotides in primary HMC significantly increased their proliferation, migration, and colony formation.[72] Upregulation of the c-Met tyrosine-kinase receptor and the antiapoptotic effector BCL2 was detected in the oligonucleotide-transfected normal mesothelial cells,[72] consistent with their reported pathogenetic role as potential miR-34b/c targets in MPM (Table 2).[69]

The impact of miR-34-mediated c-Met-regulation was further confirmed in vivo. Asbestos-exposed mice with heterozygous deletion of the TSGs *NF2* and *CDKN2A* showed accelerated mesothelioma genesis characterized by highly invasive and metastatic cells, including populations of cancer stem cells with c-Met activation.[73] Notably, this c-Met activation was partly dependent on disrupted p53-mediated miR-34a induction. Thus, the inactivation of the p53/miR-34a-dependent c-Met-modulation appears to be an important step in the development of MPM associated with the loss of *NF2* and *CDKN2A*.[73]

Collectively, the aforementioned studies suggest that the methylation and downregulation of miR-34s may have diagnostic potential in MPM and restoring their expression could represent a future appealing therapeutic option in this dismal disease. Evaluating this concept preclinically, Ueno et al subcutaneously transplanted the miR-34-methylated MPM cell line NCI-H290 into BALB/C mice and observed that the resulting tumor growth was significantly inhibited by injecting a miR-34b/c-expressing adenoviral vector.[74] Thus, miR-34b/c can inhibit MPM cells in vivo, and MPM therapy using miR-34b/c could be a future possibility.

MicroRNA-205

miR-205 appears to be involved in counteracting the EMT that MPM cells undergo when they acquire a sarcomatoid phenotype,[75] a feature known to be associated with worse prognosis.[1] The investigation of 58 EMPMs, 25 BMPMs, and 26 SMPMs identified underexpression of miR-205 and a switch from epithelial to mesenchymal markers in BMPM and SMPM as compared to EMPM. Furthermore, ectopic expression of miR-205 in nonmalignant mesothelial cells and MPM cell lines resulted in significant downregulation of the mesenchymal transcription factors ZEB1 and ZEB2, consequent upregulation of E-cadherin expression, and impaired migration and invasion.[75] These results imply that miR-205 downregulation is an important event in the EMT of MPM cells and acquisition of a particularly aggressive behavior, which could be exploited for diagnostics, prognostics, and treatment of BMPM and SMPM.

Additional miRNAs with biomarker potential in MPM

Recently, additional studies highlighting the many-sided roles of miRNAs in MPM pathogenesis were published.

Methylation-induced silencing of the putative TSG *ZIC1* leading to the overexpression of its targets miR-23a and miR-27a was recently described in MPM specimens and cell lines.[76] Overexpression of miR-23a was a negative prognostic factor for patients with MPM, and reexpressing *ZIC1* in MPM cells inhibited miR-23a and miR-27a expression and suppressed proliferation and invasion.[76] Therefore, these two miRNAs, which have shown tumorigenic properties in other cancer types, seem to contribute to the development of MPM too. If further studies can confirm that these miRNAs act as oncomiRs in MPM, they could represent diagnostic markers and attractive therapeutic targets via miR antagonists.

As mentioned earlier, higher miR-193a-3p levels in MPM can help to differentiate this cancer from pulmonary adenocarcinoma;[17] however, this miRNA was newly reported to be significantly downregulated in MPM when compared to normal pleura.[77] Transfecting MPM cell lines with miR-193a-3p-mimics decreased the expression of antiapoptotic *MCL-1* gene and induced cell death, whereas targeted delivery of miR-193a-3p-containing nanocells induced apoptosis and suppressed the growth of subcutaneous MPM xenografts in nude mice.[77] Thus, miR-193a-3p acts as a tumor suppressor in MPM and may potentially be useful in its diagnostics and targeted therapy, utilizing the same delivery approach currently being tested with promising preliminary results in a Phase I clinical trial for a miR-15/16-derived mimic.[78]

miR-223 has shown both stimulatory and inhibitory effects on inflammation and carcinogenesis in different organs.[79,80] Recently, diminished levels of miR-223 in MPM

cell lines, surgical specimens, and cells from pleural effusions were observed when compared with respective nonmalignant controls.[81] The authors also showed that miR-223 can be induced by c-Jun N-terminal kinase signaling and inhibits the proliferation and motility of MPM cell lines by repressing the microtubule regulator stathmin.[81] Thus, if validated, both miR-223 and stathmin could be future MPM biomarkers and, being modulated by the c-Jun N-terminal kinase signaling pathway, they could be potential therapeutic targets.

An additional study comparing the expression of miRNAs and mRNAs in 18 snap-frozen MPM specimens and six benign asbestos-induced pleural effusions found significant upregulation of let-7a, miR-125a-5p, miR-320, and miR-484 as well as the overexpression of c-Met transcript in the former group.[82] Moreover, integrated analysis of miRNA–mRNA interactions revealed multiple altered targets within the Notch signaling pathway. The pathogenetic and diagnostic values of these findings await validation in larger, more homogeneous, and possibly patient-matched cohorts of MPM/NNP samples.

Recent bioinformatics and interactome network analysis of differentially expressed miRNAs in chronic pleuritis, atypical mesothelial hyperplasia, and MPM showed that these three interconnected pathological processes share downregulation of certain cancer-related miRNAs and their associated biological networks.[83] Most of the downregulated miRNAs in MPM targeted the signaling activation molecule MAPK1, the transcription factor ETS1, the EMT-associated molecule FZDA, and the proinflammatory enzyme COX-2, and these were among the overlapping miRNA targets in atypical hyperplasia and chronic pleuritis.[83] This network analysis supports the notion of a potential combinatory effect of deregulated miRNAs in MPM pathogenesis and indicates potential molecular mechanistic links of pleural inflammation and atypical hyperplasia with the development of MPM.

Circulating miRNAs as diagnostic biomarkers of MPM

Tumor-specific profiles of circulating miRNAs as minimally invasive diagnostic, prognostic, or predictive biomarkers have been proposed in several types of cancer. However, only few studies on circulating miRNAs in MPM have been performed. Those concerning miR-126 and miR-34 have been mentioned earlier.[29,30,70]

Significant underexpression of miR-103 was reported in the cell fraction of peripheral blood from patients with MPM, when compared to asbestos-exposed healthy individuals and controls from the general population.[84] Evaluating the diagnostic performance, miR-103 discriminated patients with MPM from asbestos-exposed individuals with a sensitivity of 83% and a specificity of 71% and from the general population with a sensitivity of 78% and a specificity of 76%.[84]

The ability of miRNAs in the cell-free fraction to serve as diagnostic biomarkers of MPM was also explored. Kirschner et al profiled plasma samples from five patients with MPM and three healthy individuals, revealing that the levels of two previously MPM-associated miRNAs (miR-29c* and -92a) and 15 novel miRNAs were increased in the former.[56] Further validation by RT-qPCR in an expanded group of 45 plasma samples disclosed increased expression of the novel miR-625-3p as a potential diagnostic biomarker discriminating MPM from healthy individuals with a sensitivity of 73%, a specificity of 79%, and an overall accuracy of 82%.[56] Notably, miR-625-3p upregulation was also detected in surgical MPM specimens, corroborating the diagnostic potential of circulating miR-625-3p.

Interestingly, miRNAs seem capable of successfully complementing soluble proteins, such as mesothelin, possessing high specificity but low sensitivity of 60% for early detection of MPM in serum or pleural exudates, thereby improving the accuracy of MPM diagnosis.[29] The combination of mesothelin and the aforementioned miR-103 (now with the new miRBase ID: miR-103a-3p) as blood-based biomarkers could discriminate EMPM and BMPM from asbestos-exposed controls with improved specificity and sensitivity as compared to either individual marker alone.[85] Moreover, the combined triple measurement of SMRPs with methylated thrombomodulin promoter and miR-126 in the serum was reported to significantly enhance the performance of serum SMRPs in distinguishing patients with MPM from asbestos-exposed subjects and healthy controls.[86]

Although the possibility of measuring serum levels of miRNAs for diagnostic purposes needs to be analyzed prospectively, these results support the notion that circulating miRNAs may represent promising and less "invasive" biomarkers in MPM.

Prognostic impact of miRNAs in MPM

As noticed earlier, miRNA expression profiles in MPM could also aid in predicting the patient outcome.[15,20,29,59,63] Recently, miRNA profiling in tissue specimens from patients with MPM with short- and long-term postoperative survival was combined with binary logistic regression to identify a 6-miR signature (miR-21-5p, -23a-3p, -30e-5p, -221-3p, -222-3p, and -31-5p) called miR-score, which was capable of predicting a survival of ≥20 months with an accuracy of 92.3% for

extrapleural pneumonectomy and 71.9% for pleurectomy/decortication.[87] When the miR-score was associated with clinical prognostic parameters (histology, age, and sex), the predictive accuracy in a validation set was improved from 76.3% to 87.3%. Therefore, the authors proposed the miR-score as novel accurate prognostic biomarker in MPM.[87]

Current limitations and possible new applications of miRNAs in MPM diagnostics

As mentioned earlier, one limitation of the reported studies is that they have used different methodological platforms of miRNA microarrays and/or RT-qPCR and types of specimens, thereby generating different miR signatures in MPM cells that are difficult to compare. A common problem to studies on MPM tissue specimens is the difficult acquisition of patient-matched NNP samples due to the diffused growth of MPM. Moreover, MPM is a heterogeneous and poorly circumscribed tumor tissue containing a mixture of malignant cells, inflammatory cells, and reactive stromal structures. Thus, RT-qPCR-based analyses (miRNA arrays or TaqMan® assays) of RNA extracts may provide results that are not always reliable for discriminating miRNA changes occurring within MPM cells from those associated with nonneoplastic components.[88] To compensate for these confounding factors, the use of tissue samples enriched with cancer cells (representing at least 50% of cells in the tissue) is recommendable, though it does not eliminate the risk of drawing deceptive conclusions. Tumor content enrichment by laser capture microdissection followed by accurate quality verification of intact miRNA fraction has also been proposed.[87]

A possible solution to avoid these drawbacks and enhance the feasibility and accuracy of routinely testing miRNAs in FFPE MPM specimens is to investigate them directly on tissue sections by ISH with specific locked nucleic acid-modified probes.[89] ISH provides information regarding contextual tissue distribution and cellular origin of candidate miR biomarkers and better interpretation of their functions and interactions in complex multicellular tissue samples. Furthermore, the codetection of miRNA targets by IHC can be employed to further improve the knowledge of the biological function of miRNAs and colocalization within the tumor.[88,90] A diagnostic assay combining ISH of deregulated miRNAs and IHC-based detection of related targets in tissue biopsies could further improve the differential diagnosis of RMP vs MPM, by also enabling the analysis of samples with low tumor cell content and by helping to monitor the changes of potential miRNA targets in a therapeutic setting.[90]

However, to construct a combined ISH-IHC assay, the related targets of candidate miR biomarkers need to be identified in MPM and their diagnostic potential assessed. In this respect, the number of miRNA targets in MPM that have been validated by in vitro experiments is currently small. To date, direct physical miRNA–mRNA interaction has only been evidenced in MPM for miR-126-IRS1 and miR-145-OCT4 (Table 2). Some evidence has been provided for *LAT1* as target of miR-126, *BCL2* and *CCND1* of miR-16, *PPP6C* of miR-31, *MCL1* of miR-193-3p, *STMN1* of miR-223, and for few additional targets of other miRNAs.[16,20,34,45,57,60,62,77,81] The detection of biologically active miRNA targets is challenging and initially involves the use of computational target prediction, although some algorithms may not be accurate enough and require caution in interpreting the obtained results. Combining the predictions of several algorithms may improve the accuracy, yet this approach does not consider possible cell type-specific obstruction of the target site by RNA-binding proteins. Given that miRNAs primarily act to repress target mRNAs, one method to achieve better prediction accuracy is to compare changes in gene expression and miRNA levels of patient-matched MPM/NNP specimens.

General pitfalls concerning circulating miRNAs as biomarkers, not least the need of more validation and the contribution to miRNA secretion by blood cells, together with possible solutions, have been extensively reviewed elsewhere.[91]

Conclusion

In summary, despite several aspects of the involvement of miRNAs in mesothelioma genesis remain contentious, the aforementioned results illustrate that miRNAs have a great potential as diagnostic and prognostic biomarkers in MPM. Because of the inherent weaknesses of RT-qPCR-based detection methods, further studies assessing the expression and distribution of miRNAs by ISH and codetection of their respective targets by IHC, as well as validation of miRNA targets in vitro are necessary to fully reveal the potential clinical utility of miRNAs and their related targets. Moreover, circulating miRNAs are attractive, though not yet optimized, "noninvasive" biomarkers for diagnostics and monitoring of patients with MPM.

Disclosure

The authors report no conflicts of interest in this work.

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