Diagnostic value of microRNAs in asbestos exposure and malignant mesothelioma: systematic review and qualitative meta-analysis

Supplementary Material

Methods

1. Potential biases in the review process

The study was conducted in accordance with the PRISMA checklist [1], a system that improves the clarity and transparency of the systematic review approach. The PRISMA checklist has been developed for randomized trials [2], but it can also be useful as a basis for reporting systematic reviews of other kinds of research [1]. The 11-item AMSTAR (assessment of multiple systematic reviews) tool was also applied, to evaluate the methodological quality of systematic reviews [3]. The PRISMA and AMSTAR checklists are shown in **Supplementary Table 1** and **Supplementary Table 2**, respectively. Even with all these precautions, the possibility of publication bias remains; for example manuscripts that are suitable for publication in scientific journals are more likely to report statistically significant data [4–6]. Due to the small size of studies investigating diagnostic value of circulating miRNAs in MM, we did not evaluated publication bias using a funnel plot.

2. Details about the specially devised vote-counting strategy

Our scoring method was established *a priori* by three investigators. The method considers the direction of deregulation as well as the following 4 features: the number of studies reporting each miRNA as deregulated; the total number of MM samples; the total number of normal samples used; and the number of qRT-PCR series conducted to assess each miRNA. The latter characteristic has been taken into account because some authors have carried out the qRT-PCR analysis in different cohorts of samples (*e.g.* Discovery set, Training set and Validation set [79], see study design in

Table 2) and therefore performing more than one qRT-PCR series to evaluate miRNAs expression in the same study.

A value was attributed to each miRNA by assigning a score for each of the 4 features. We adopted quartiles, which divide an ordered data series into four equal parts, to set up a five-point scale for the number of samples used. In brief, each feature was given a score based on the quartile of the distribution it occupied: 0, the lowest value (Min); 1, the lower quartile (Q1); 2, the median quartile (Q2); 3, the upper quartile (Q3); and 4, the highest value (Max). The quartile rankings of each feature were calculated for (a) and (c) comparison categories, *i.e.* MM tissue *vs*. normal or non-cancer tissue and (c) MM blood samples *vs.* normal blood samples. However, since quartiles are typically calculated for lists with a minimum of 20 items, the approach could be used only for the number of MM or normal samples used. Thus, a 0-4 scale was used to assign a score to the number of qRT-PCR assays performed: 1 qRT-PCR, 0; 2 qRT-PCRs, 1; 4 qRT-PCRs, 2; 5 qRT-PCRs, 3; and 6 qRT-PCRs, 4. Finally, for the number of studies reporting the same miRNA, 1 point was awarded for report in one paper and 2 points for report in two or more papers. The 4 scores were added to obtain a final score for each miRNA, and quartiles were again calculated from their distribution. The following **Tables** (**A** and **B**) display the scoring scheme for tissue miRNAs and circulating miRNAs, respectively. Results are reported as a box-whisker plot, where each dot represents a miRNA (**Figure 2**). The miRNAs belonging to groups Q3 and Max were considered as the most significant.

Table A. Scoring table of tissue MM-miRNAs from qRT-PCR analyses.

Accession number	miRBase ID	miRNAs	No. of qRT-PCR	qRT-PCR score	MM	MM score	H	H score	Ref	Ref score	miRNA Vote
MIMAT0000416	**hsa-miR-1-3p**	miR-1	1	0	25	1	6	0	[75]	1	**2**
MIMAT0000099	**hsa-miR-101-3p**	miR-101	1	0	n.a.	0	n.a.	0	[93]	1	**1**
MIMAT0000445	**hsa-miR-126-3p**	miR-126	5	3	59	3	51	3	[85], [77]	2	**11**
MIMAT0000435	**hsa-miR-143-3p**	miR-143	4	2	32	2	24	3	[77]	1	**8**
MIMAT0000437	**hsa-miR-145-5p**	miR-145	6	4	74	3	74	3	[76], [77]	2	**12**
MIMAT0004658	**hsa-miR-155-3p**	miR-155*	1	0	25	1	6	0	[75]	1	**2**
MIMAT0000068	**hsa-miR-15a-5p**	miR-15a-5p	1	0	60	3	23	2	[94]	1	**6**
MIMAT0000417	**hsa-miR-15b-5p**	miR-15b-5p	1	0	60	3	23	2	[94]	1	**6**
MIMAT0000069	**hsa-miR-16-5p**	miR-16 / miR-16-5p	2	1	78	3	30	3	[90], [94]	2	**9**
MIMAT0000070	**hsa-miR-17-5p**	miR-17-5p	1	0	32	2	24	3	[83]	1	**6**
MIMAT0007890	**miR-1914-3p**	miR-1914-3p	1	0	18	1	7	0	[90]	1	**2**
MIMAT0000222	**hsa-miR-192-5p**	miR-192	2	1	120	4	23	2	[99]	1	**8**
MIMAT0000459	**hsa-miR-193a-3p**	miR-193a-3p	2	1	120	4	23	2	[99]	1	**8**
MIMAT0000461	**hsa-miR-195-5p**	miR-195-5p	1	0	60	3	23	2	[94]	1	**6**
MIMAT0001080	**hsa-miR-196b-5p**	miR-196b	1	0	18	1	7	0	[90]	1	**2**
MIMAT0000318	**hsa-miR-200b-3p**	miR-200b	2	1	120	4	23	2	[99]	1	**8**
MIMAT0000264	**hsa-miR-203a-3p**	miR-203	2	1	120	4	23	2	[99]	1	**8**
MIMAT0000462	**hsa-miR-206**	miR-206	1	0	25	1	6	0	[75]	1	**2**
MIMAT0000278	**hsa-miR-221-3p**	miR-221	1	0	32	2	24	3	[83]	1	**6**
MIMAT0000280	**hsa-miR-223-3p**	miR-223	2	1	17	1	6	0	[98]	1	**3**
MIMAT0000082	**hsa-miR-26a-5p**	miR-26a	1	0	n.a.	0	n.a.	0	[93]	1	**1**
MIMAT0004681	**hsa-miR-26a-2-3p**	miR-26a-2-3p	1	0	18	1	7	0	[90]	1	**2**
MIMAT0004673	**hsa-miR-29c-5p**	miR-29c*	1	0	18	1	7	0	[90]	1	**2**
MIMAT0000089	**hsa-miR-31-5p**	miR-31	1	0	25	1	20	2	[97]	1	**4**
MIMAT0000090	**hsa-miR-32-5p**	miR-32	1	0	27	2	27	3	[85]	1	**6**
MIMAT0000765	**hsa-miR-335-5p**	miR-335	1	0	27	2	27	3	[85]	1	**6**
MIMAT0000255	**hsa-miR-34a-5p**	miR-34a	1	0	47	2	10	2	[101]	1	**5**
MIMAT0004676/ MIMAT0000685	**hsa-miR-34b-3p / hsa-miR-34b-5p**	miR-34b	1	0	47	2	10	2	[101]	1	**5**
MIMAT0000686	**hsa-miR-34c-5p**	miR-34c	1	0	47	2	10	2	[101]	1	**5**
MIMAT0004761	**hsa-miR-483-5p**	miR-483-5p	1	0	25	1	6	0	[75]	1	**2**
MIMAT0004808	**hsa-miR-625-3p**	miR-625-3p	1	0	18	1	7	0	[90]	1	**2**
MIMAT0003322	**hsa-miR-652-3p**	miR-652	4	2	32	2	24	3	[77]	1	**8**

Note: MiRNAs were ranked based on the total number of tumor and healthy samples involved and on the number of qRT-PCR assays performed. Accession number and miRNA unique identifier (ID) are reported for each miRNA according to the last miRBase release (miRBase v21).

No. of qRT-PCR: total number of additional qRT-PCR assays described in the same paper and/or in different papers and correspondig score (**qRT-PCR score**); **MM:** number of MM samples used in qRT-PCR analyses and correspondig score (**MM score**); **H:** number of non-cancer controls samples used in qRT-PCR analyses and correspondig score (**H score**); **Ref**: number of references for each miRNAs and corresponding score (**Ref. score**), references in parentheses are numbered according to the reference list; **miRNA Vote:** final score for each miRNA obtained by adding up the scores of the 4 features.

Table B. Scoring table of circulating MM-miRNAs from qRT-PCR analyses.

Accession number	miRBase ID	miRNAs	No. of qRT-PCR	qRT-PCR score	MM	MM score	H	H score	Ref	Ref score	miRNA Vote
MIMAT0000099	hsa-miR-101-3p	miR-101	1	0	14	0	10	2	[91]	1	**3**
MIMAT0000101	hsa-miR-103a-3p	miR-103 / miR-103a-3p	2	1	66	3	77	3	[88], [89]	2	**9**
MIMAT0000445	hsa-miR-126-3p	miR-126	2	1	89	3	106	4	[85], [86]	2	**10**
MIMAT0000440	hsa-miR-191-5p	miR-191	1	0	14	0	10	2	[91]	1	**3**
MIMAT0000075	hsa-miR-20a-5p	miR-20a	1	0	23	1	25	3	[88]	1	**5**
MIMAT0000280	hsa-miR-223-3p	miR-223	1	0	14	0	10	2	[91]	1	**3**
MIMAT0000081	hsa-miR-25-3p	miR-25	1	0	14	0	10	2	[91]	1	**3**
MIMAT0000083	hsa-miR-26b-5p	miR-26b	1	0	14	0	10	2	[91]	1	**3**
MIMAT0000086	hsa-miR-29a-3p	miR-29a	1	0	14	0	10	2	[91]	1	**3**
MIMAT0004673	hsa-miR-29c-5p	miR-29c*	1	0	45	2	24	3	[90]	1	**6**
MIMAT0000765	hsa-miR-335-5p	miR-335	1	0	14	0	10	2	[91]	1	**3**
MIMAT0001627	hsa-miR-433-3p	miR-433	1	0	14	0	10	2	[91]	1	**3**
MIMAT0006778	hsa-miR-516a-3p (unclear)	miR-516	1	0	14	0	10	2	[91]	1	**3**
MIMAT0004808	hsa-miR-625-3p	miR-625-3p	2	1	45	2	24	3	[90]	1	**7**
MIMAT0000092	hsa-miR-92a-3p	miR-92a	1	0	45	2	24	3	[90]	1	**6**

Note: MiRNAs were ranked based on the total number of tumor and healthy samples involved and on the number of qRT-PCR assays performed. Accession number and miRNA unique identifier (ID) are reported for each miRNA according to the last miRBase release (miRBase v21).

No. of qRT-PCR: total number of additional qRT-PCR assays described in the same paper and/or in different papers and correspondig score (**qRT-PCR score**); **MM:** number of MM samples used in qRT-PCR analyses and correspondig score (**MM score**); **H:** number of non-cancer controls samples used in qRT-PCR analyses and correspondig score (**H score**); **Ref:** number of references for each miRNAs and corresponding score (**Ref. score**), references in parentheses are numbered according to the reference list; **miRNA Vote:** final score for each miRNA obtained by adding up the scores of the 4 features.

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