Original Article

Exosomal miR-182-5p is a potential diagnostic marker for malignant pleural effusion

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Background: Biomarkers in pleural fluid are the potential auxiliary diagnostic markers for malignant pleural effusion (MPE). Exosomal microRNAs (miRNAs) represent novel diagnostic markers for various diseases. The diagnostic performance of exosomal miRNAs for MPE remains unclear. Therefore, we examined the exosomal miRNAs profiles of both MPE and benign pleural effusion (BPE), aiming to study diagnostic performance of exosomal miRNAs for MPE.

Methods: We used next-generation sequencing (NGS) technology to analyze the pleural fluid exosomal miRNA profile in five MPE and 15 BPE cases. We analyzed the differentially expressed exosomal miRNAs by reverse transcription polymerase chain reaction (RT-PCR), with cel-miR-39 or snRNA U6 as internal references. We assessed the diagnostic accuracy of exosomal miRNA for MPE with a receiver operating characteristic (ROC) curve. We also analyzed whether exosomal miRNA could improve the diagnostic performance of pleural carcinoembryonic antigen (CEA).

Results: Fifty-eight miRNAs were up-regulated, and 35 miRNAs were down-regulated in MPE. We selected exosomal miR-182-5p for further study and analyzed miR-182-5p in 153 patients with undiagnosed pleural effusion. Exosomal miR-182-5p was undetectable in 32 participants. In the remaining participants with 49 MPE and 72 BPE cases, we found that the areas under the curve (AUCs) and their 95% confidence intervals (95% CIs) for exosomal miR-182-5p were 0.78 (95% CI: 0.69–0.86) when using cel-miR-39 as an internal reference, and 0.80 (95% CI: 0.73–0.88) when using snRNA U6. The combination of exosomal miR-182-5p and CEA can slightly improve the diagnostic accuracy of MPE, with an AUC of 0.91 (95% CI: 0.85–0.97).

Conclusions: Pleural miR-182-5p can assist in the diagnosis of MPE. Its diagnostic performance is slightly affected by internal reference.

Keywords: Malignant pleural effusion (MPE); exosomal miRNA; miR-182-5p; diagnostic accuracy

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Introduction

Pleural effusion is frequently encountered in clinical practice (1). The primary causes of pleural effusion include malignant pleural effusion (MPE), tuberculous pleural effusion (TPE), parapneumonic pleural effusion (PPE), and congestive heart failure (CHF), which collectively account for 27%, 9%, 21%, and 19% of cases, respectively (2). Timely and accurate differentiation between benign pleural effusion (BPE) and MPE is essential for the effective management of this condition.

Effusion cytology is a straightforward and quick method for distinguishing between BPE and MPE. However, its sensitivity is only about 50%, which can vary based on the type of primary tumor and the pathologist's experience (3,4). Pleural biopsy offers high diagnostic accuracy for MPE and is now widely practiced. This procedure can be categorized into imaging-guided (ultrasound or computed tomography) and thoracoscopic biopsy. Despite its effectiveness, both methods are invasive and can lead to complications related to the procedure (5). Additionally, thoracoscopy requires specialized training and equipment, which can limit its use in remote areas (6).

Highlight box

Key findings

- The exosomal microRNAs (miRNAs) profile of patients with undiagnosed pleural effusion is analyzed, revealing that exosomal miR-182-5p is upregulated in patients with malignant pleural effusion (MPE).
- Exosomal miR-182-5p demonstrated moderate diagnostic accuracy for MPE, which was influenced by the internal reference used.
- This miRNA can enhance the diagnostic accuracy of pleural carcinoembryonic antigen.

What is known and what is new?

- Previous studies have shown that miR-182 plays an important role in the development of cancer, and there are differences between benign pleural effusion (BPE) and MPE.
- This study examined the exosomal miRNA profiles of both MPE and BPE, finding that exosomal miR-182-5p levels were elevated in MPE. The miRNA showed an area under the curve of 0.80 when evaluated for MPE. Additionally, the diagnostic accuracy of exosomal miR-182-5p was slightly affected by the internal reference used for measurement.

What is the implication, and what should change now?

- Exosomal miR-182-5p can aid in diagnosing MPE.
- Further research is necessary to standardize the assay for exosomal miR-182-5p in pleural fluid.

Biomarkers found in pleural fluid provide a less invasive, cost-effective, and rapid option, making them commonly used as supplementary tests in clinical practice (7,8). Several protein markers in pleural fluid, such as carbohydrate antigen 50 (CA50) (9) and carcinoembryonic antigen (CEA) (10), can aid in diagnosing MPE. However, when used individually, these biomarkers are insufficient for definitively confirming or excluding MPE. Implementing a multi-biomarker approach represents a practical strategy to enhance the accuracy of distinguishing between MPE and BPE (11). Consequently, investigating novel pleural biomarkers is especially valuable, as it is a crucial step toward developing a multi-biomarker approach.

Exosomes are small extracellular vesicles secreted by cells, typically measuring about 100 nm in diameter (12). They play a pivotal role in intercellular communication by containing various bioactive molecules, including nucleic acids, proteins, and lipids (12). Upon uptake by recipient cells, exosomes can modulate cellular functions by releasing these molecular contents (13). Notably, exosomal microRNAs (miRNAs) in bodily fluids exhibit remarkable stability even after prolonged storage or multiple freeze-thaw cycles (14,15), indicating their potential as reliable diagnostic biomarkers for various disorders (16). In addition, body fluid protein markers are typically measured using immunoassays; however, this method is susceptible to non-specific reactions, such as those caused by heterophile antibodies, rheumatoid factors, and human anti-mouse antibodies (17). In contrast, polymerase chain reaction (PCR) is commonly employed to analyze exosomal miRNAs. The use of specific primers for amplification in PCR significantly reduces the risk of non-specific reactions.

A study has investigated the potential of exosomal miRNA in pleural fluid as a diagnostic tool for discriminating BPE and MPE (18). However, the exosomal miRNAs selected for investigation are based on studies investigating the molecular mechanisms underlying the development of cancer. This strategy may miss some valuable exosomal miRNAs. Some studies also describe the exosomal miRNA profile in pleural fluid among patients with undiagnosed pleural effusion (19-21). However, the representativeness of the participants used for exosomal miRNA profile analysis is problematic. In addition, the diagnostic accuracy of exosomal miRNAs has not been investigated in a well-representative cohort (19,22,23). For example, the BPE cases in some studies only encompass patients with CHF (22), TPE (23) or PPE (19). Furthermore, the differentially expressed exosomal miRNAs

reported in these studies have not been widely validated. Furthermore, the internal reference used for determining exosomal miRNAs varied across published studies, and its effect on diagnostic accuracy remains unclear. We examined the exosomal miRNA profile in a representative cohort of patients with undiagnosed pleural effusion and evaluated the diagnostic significance of a differentially expressed miRNA, miR-182-5p, for MPE. We present this article in accordance with the STARD reporting checklist (24) (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1205/rc).

Methods

Specimen collection

We conducted a comprehensive analysis of the exosomal miRNA profiling in pleural fluid specimens collected from participants diagnosed with MPE (n=5), TPE (n=5), PPE (n=5), and CHF (n=5). All cases of MPE were lung cancer. The study population consisted of patients admitted to the Affiliated Hospital of Inner Mongolia Medical University due to unexplained pleural effusion from August 2021 to December 2023. Furthermore, we evaluated the levels of exosomal miR-182-5p in an additional cohort, SIMPLE (24), previously utilized in our earlier research endeavors (25,26). Pleural effusion samples were procured via thoracentesis at the time of admission. Specimens were collected in anticoagulant-free tubes and subjected to centrifugation within four hours. The supernatant was aliquoted and stored at temperatures ranging from –80 to –70 ℃ for future analysis. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study received approval from the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University (Nos. 2021015 and 2018011). Informed consent was duly obtained from all participants or their legal guardians before enrollment.

The inclusion criteria for this study encompassed patients who met the following conditions: (I) diagnosed with undetermined etiology of pleural effusion; (II) underwent diagnostic thoracentesis to ascertain the underlying causes of the pleural effusion. Imaging methods (e.g., chest X-ray, computed tomography, thoracic ultrasound) confirmed the presence of pleural effusion. The exclusion criteria consisted of patients who: (I) developed pleural effusion during hospitalization as a result of medical intervention; (II) were under the age of 18 years; (III) exhibited pleural effusion three months before admission with established etiologies; (IV) provided insufficient pleural fluid specimens for research; (V) were pregnant; or (VI) had pleural effusion resulting from traumatic injury.

Diagnostic criteria

A participant was classified as having MPE if they met any of the following criteria: (I) a positive result from effusion cytology; (II) a positive result from a pleural biopsy; or (III) in patient with a high probability of MPE who has negative effusion cytology and for whom pleural biopsy is not feasible, MPE was defined as evidence of advanced primary or metastatic cancers, and the possibility of BPE must be excluded.

TPE was defined if a patients meet any of the following criteria: (I) positive *Mtb* culture or Ziehl-Neelsen staining in pleural fluid or sputum specimens; (II) pleural biopsy reveals granuloma with the exclusion of other benign granulomatous diseases; (III) patients with a high probability of TPE [e.g., adenosine deaminase (ADA) >35 U/L, positive nucleic acid amplification tests], and response to anti-tuberculosis treatment.

PPE was diagnosed by the clinical characteristics of the patients (27,28), including (I) positive effusion bacterial culture with the exclusion of contamination; (II) pleural biopsy revealed neutrophil infiltration and negative for tumor cell and granuloma; (III) clinical characteristics are highly suggestive of PPE (e.g., history of pneumonia, imaging, purulence appearance, high serum C-reactive protein), and the patients are responsive to antibiotic treatments.

CHF was diagnosed by clinical characteristics, including (I) transudate; (II) serum N-terminal pro-brain natriuretic peptide (NT-proBNP) >1,500 pg/mL; (III) bilateral pleural effusion; and (IV) response to anti-heart failure treatment.

Two senior clinicians (L.Y. and Z.D.H.) independently made the diagnoses by reviewing the participants' medical records. Any disagreements were resolved by consensus.

Exosome isolation and identification

Exosomes were isolated from the pleural fluid using a Thermo Fisher Scientific isolation reagent kit (Catalog No. 4484453). Transmission electron microscopy was conducted using a Hitachi HT-7700 (Tokyo, Japan) operating at 100 kV for electron microscopy imaging. Particle size analysis was performed with the ZetaVIEW nanoparticle tracking analyzer from PARTIKEL METRIX (Inning am Ammersee, Germany). The proteins TSG101 and HSP7

were quantified by Western blotting, employing a Western blotting solution kit (P0023) obtained from Beyotime (Shanghai, China).

Pleural fluid exosomal miRNA extraction

Exosomal miRNA was extracted using the Tiangen miRcute miRNA extraction kit (DP501). Reverse transcription PCR (RT-PCR) was performed using the TransScript miRNA First-Strand cDNA Synthesis SuperMix kit (Beijing, China; No. AT351). The PerfectStart Green qPCR SuperMix kit (Beijing, China; No. AQ601) was used to detect the level of exosomal miRNA. The internal reference used for qPCR was snRNA U6 or exogenous cel-miR-39.

Next-generation sequencing (NGS)

We used the exoRNeasy Maxi kit (QIGEN, Dusseldorf, Germany) to isolate PE exosomal RNA. Highly sensitive Agilent 2100 pic600 (California, USA) was used to detect RNA's total amount and fragment distribution. Exosomal miRNA sequencing was performed using the Illumina SE50 sequencing platform (California, USA). The differential miRNA screening condition was $P<0.05$ and $|\log_2(\text{fold change})| >1$.

Bioinformatics analysis

We used miRanda (29) and RNAhybrid (30) to predict the targets of differentially expressed miRNAs, and the targets predicted by both were included in the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

Statistical analysis

This study employed the median and interquartile range to describe continuous variables, while categorical data were represented through absolute counts and percentages. Continuous variables between two groups were compared using either the Mann-Whitney *U* test or the *t*-test, contingent upon their distribution characteristics. For the comparison of categorical variables, the Chi-squared test was utilized. We assessed the diagnostic performance of exosomal miR-182-5p for MPE using receiver operating characteristic (ROC) curve analysis. Additionally, we implemented a logistic regression model to integrate CEA levels and miR-182-5p expression, enabling us to estimate the probability of MPE in individual patients. A ROC analysis was subsequently performed on these probabilities to determine the area under the curve (AUC) and its corresponding 95% confidence interval (95% CI). The AUC values were compared using Delong *et al.*'s methodology (31). We applied the Spearman rank correlation coefficient to analyze the correlation between continuous variables. All statistical analyses and graphical representations were conducted using IBM SPSS Statistics version 25.0 and GraphPad Prism version 6.0. A P value of less than 0.05 was considered indicative of statistical significance.

Results

Exosomes identification

Exosome isolated from pleural fluid were confirmed through transmission electron microscopy (*Figure 1A*), nanoparticle tracking analysis (NTA) (*Figure 1B*) and Western blot analysis (*Figure 1C*). These findings indicate that the exosomes in the pleural fluid have been successfully isolated.

Sequencing results

Using BPE as a control, we found 58 exosomal miRNAs were up-regulated and 35 miRNAs were down-regulated, as shown in *Figure 2*.

Table 1 shows the top ten miRNAs expressed in the exosome from the pleural fluid of MPE.

Next, we predicted the target genes of these differentially expressed exosomal miRNAs and then performed enrichment analyses. The GO enrichment analysis of the target genes is shown in *Figure 3A*. In the biological process (BP) group, single-organism process (GO: 0044699), single-organism cellular process (GO: 0044763), and regulation of cellular process (GO: 0050794) ranked in the top three. The KEGG enrichment analysis diagram, ranked by P values, in shown in *Figure 3B*. The top 20 pathways are shown in the Table S1.

Validation of differentially expressed miRNA

We evaluated the diagnostic performance of exosomal miR-182-5p in 153 samples from the SIMPLE study (32). However, miR-182-5p was lower than the detection limit in 26 of these samples, and six participants only had cel-miR-39 or snRNA U6 value. To compare the AUCs of snRNA U6 and cel-miR-39 in a head-to-head manner,

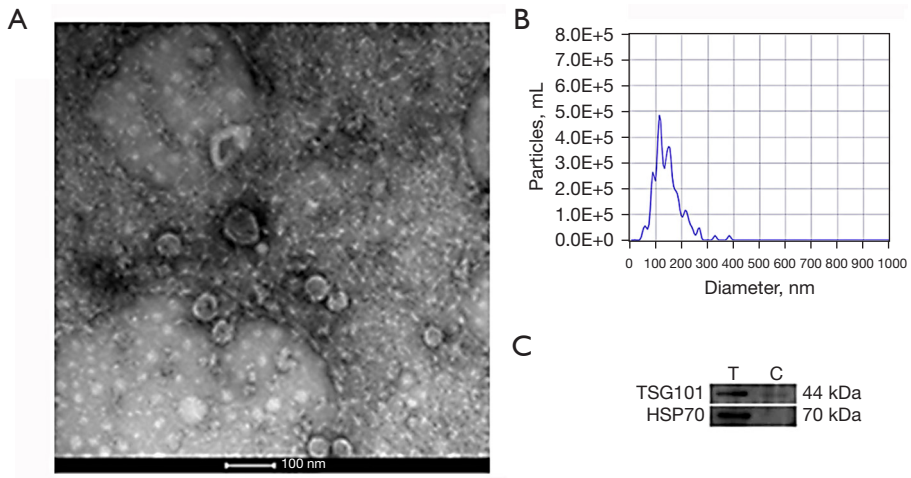

Figure 1 Exosome identification. (A) Transmission electron microscopy. (B) Nanoparticle tracking analysis. The particle size of exosomes in the test samples is 30–300 nm, and most of the particles were concentrated in 30–150 nm. Some larger particles may be related to the adhesion between particles. (C) Western blot analysis. C, control group; T, test group.

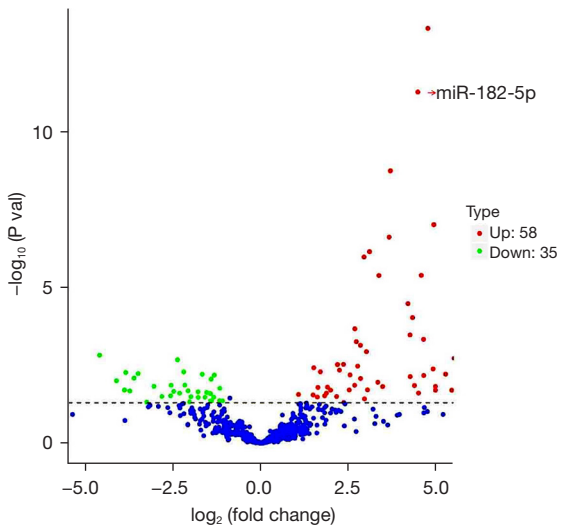

Figure 2 Differentially expressed exosomal miRNA between MPE and BPE. The blue dots represent the exosomal miRNA with no significant difference in the increase or decrease in MPE. BPE, benign pleural effusion; miRNA, microRNA; MPE, malignant pleural effusion.

Table 1 The top 10 upregulated miRNAs in MPE

miRNAs	Log_2fold change	P value
miR-183-5p	4.77	<0.001
miR-182-5p	4.48	<0.001
miR-96-5p	3.70	<0.001
miR-141-3p	4.94	<0.001
miR-200a-5p	3.66	<0.001
miR-203a-3p	3.11	<0.001
miR-203b-5p	3.10	<0.001
miR-200b-3p	2.95	<0.001
miR-1246	4.58	<0.001
miR-200a-3p	3.37	<0.001

miRNA, microRNA; MPE, malignant pleural effusion.

these 32 participants were thus excluded from the study. The clinical characteristics of 121 included and 32 excluded participants are shown in Table S2. There was no significant difference regarding pleural fluid biochemistry and the proportion of MPE and BPE between the participants excluded and included (all P>0.05).

Among 121 participants, 49 were MPE and 72 were BPE. The clinical characteristics of the two groups of patients are shown in *Table 2*. The MPE was composed of lung cancer (n=40), gastric cancer (n=2), mesothelioma (n=4), pulmonary synovial sarcoma (n=1), breast cancer (n=1), and unknown primary cancer (n=1). The BPE was composed of CHF (n=19), TPE (n=17), PPE (n=27), and other types of BPE (n=9).

Since there is no generally accepted internal reference for exosomal miRNA determination (21), we used cel-miRNA-39 and snRNA U6 as internal references. As shown in *Figure 4*, MPE patients had increased exosomal miR-182-5p than BPE (P<0.001), either using cel-miRNA-39 (*Figure 4A*) or snRNA

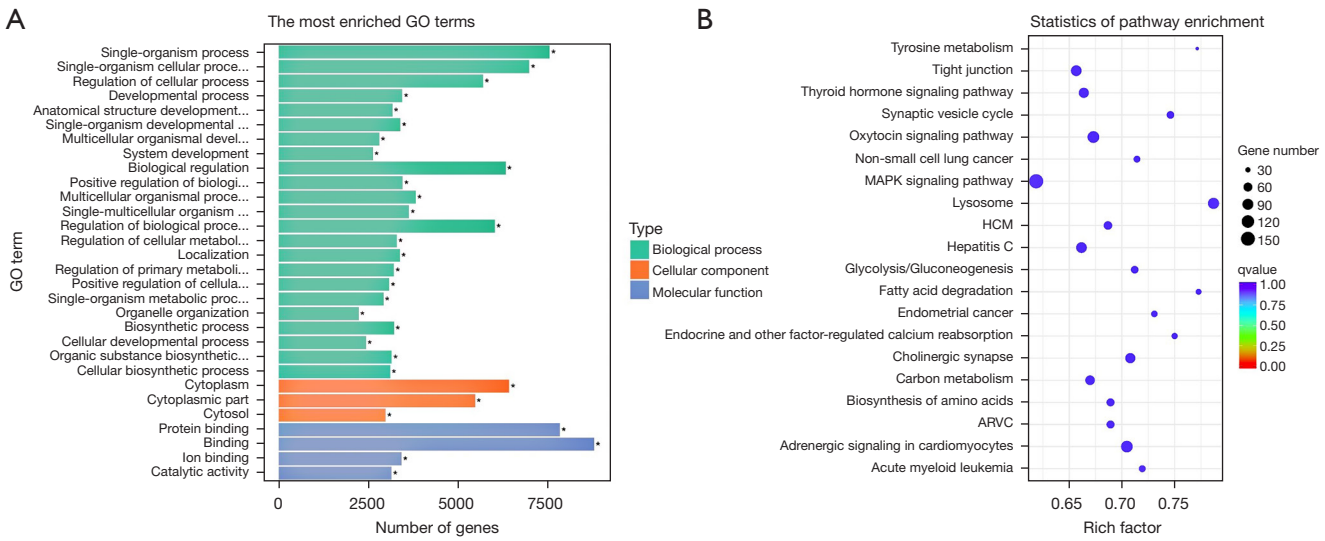

Figure 3 Enrichment analyses with the target gene of the differentially expressed exosomal miRNAs. (A) GO enrichment analysis. *, statistically significant; (B) KEGG enrichment analysis. The size of the dots represents the number of genes related to the pathway, and the color represents the P value. ARVC, arrhythmogenic right ventricular cardiomyopathy; GO, Gene Ontology; HCM, hypertrophic cardiomyopathy; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNA.

Table 2 Comparison of clinical characteristics of participants included in the study

Characteristics	MPE (n=49)	BPE (n=72)	P
Age, years	73 [66–80]	72 [65–76]	0.25
Sex, M/F, n	32/17	48/24	0.02
WBC, 10^6/mL	925 [702–1,516]	780 [362.5–1,896]	0.21
Glucose, mmol/L	5.8 [4.6–6.5]	5.8 [4.7–7.0]	0.53
LDH, U/L	229 [185–447]	179.5 [94–367.5]	0.01
ADA, g/L	8.1 [5.3–11.8]	9.7 [3.9–24.9]	0.33
Protein, g/L	37.7 [34.3–43.2]	29.8 [16.9–41.0]	<0.001
CEA, g/L	49.62 [5.91–301.94]	1.17 [0.72–2.28]	<0.001

Continuous data were expressed as median [25–75%] and compared using the Mann-Whitney *U* test. Categorical variables were compared using the Chi-squared test. ADA, adenosine deaminase; BPE, benign pleural effusion; CEA, carcinoembryonic antigen; F, female; LDH, lactate dehydrogenase; M, male; MPE, malignant pleural effusion; WBC, white blood cell.

U6 as an internal reference (*Figure 4B*). Besides, we also compared exosomal miR-182-5p among all types of BPE and found the it was lower in CHF patients (Figure S1).

The diagnostic performance of exosomal miR-182-5p for MPE

Figure 5 is the ROC curve of pleural fluid exosomal miR-182-5p, CEA, and their combination for diagnosing MPE. With cel-miRNA-39 as an internal reference, the AUC of exosomal miR-182-5p was 0.78 (95% CI: 0.69–0.86). The AUC of exosomal miR-182-5p was 0.80 (95% CI: 0.73–0.88) when snRNA U6 was used as an internal reference. The AUC of pleural CEA was 0.90 (95% CI: 0.84–0.97). We used a logistic regression model to combine CEA and exosomal miR-182-5p (with U6 as an internal reference) and calculate the probability of MPE for each participant. The AUC of this probability was 0.91 (95% CI: 0.85–0.97).

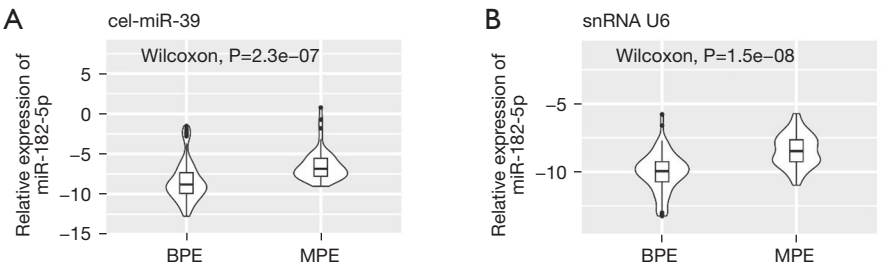

Figure 4 Relative expression of exosomal miR-182-5p in MPE and BPE. (A) Relative expression of exosomal miR-182-5p using cel-miRNA-39 as an internal reference in MPE and BPE; (B) relative expression of exosomal miR-182-5p using snRNA U6 as an internal reference in MPE and BPE. BPE, benign pleural effusion; MPE, malignant pleural effusion.

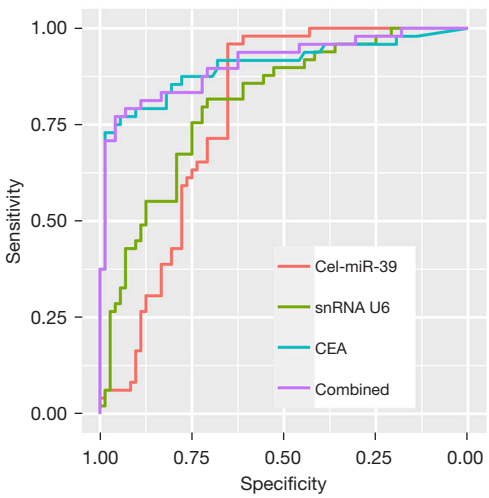

Figure 5 The ROC curves of miR-182-5p, CEA and their combination. CEA, carcinoembryonic antigen; ROC, receiver operating characteristic.

In addition, we separately analyzed the diagnostic accuracy of exosomal miR-182-5p in participants with negative cytology. This group included individuals diagnosed with BPE and cytology-negative MPE. Notably, the AUC for exosomal miR-182-5p in this group was determined to be 0.82 (95% CI: 0.71–0.94), irrespective of whether cel-miR-39 or snRNA U6 was employed as the internal control.

Table 3 shows the sensitivity, specificity, and positive and negative likelihood ratios of miR-182-5p, CEA, and their combination. We determined the cutoff value based on the maximum Youden index.

Discussion

This study examined the profile of exosomal miRNAs in pleural fluid from MPE and BPE using NGS. We identified 58 miRNAs that were up-regulated and 35 that were down-regulated in MPE. We selected miR-182-5p for further investigation and found that its expression was significantly higher in MPE compared to BPE. The ROC curve analysis showed that miR-182-5p achieved an AUC of 0.78 when using cel-miRNA-39 as an internal reference. Additionally, when using snRNA U6 as the internal reference, the AUC for miR-182-5p increased to 0.80. These results suggest that exosomal miR-182-5p can aid in diagnosing MPE, and the choice of internal reference slightly influences its accuracy. Compared to previous studies that analyzed the exosomal miRNA profile and evaluated its diagnostic accuracy (20,23,33), the strengths of our study include a prospective design, well-representative participants, and the use of two internal references.

We conducted a comparative analysis of the diagnostic accuracy of miR-182-5p alongside CEA and determined that their accuracies were statistically comparable. Notably, the combination of miR-182-5p and CEA yielded an AUC of 0.91, suggesting that miR-182-5p may enhance the diagnostic efficacy of CEA. Additionally, we performed bioinformatics analyses on the targets of differentially expressed exosomal miRNAs, which provided novel insights into the pathogenesis of MPE. It's important to note that the functions of these exosomal miRNAs vary among different cellular types, such as pleural cells and immune cells, depending on which cells receive these exosomal miRNAs. Previous studies have established that miR-182-5p plays a pivotal role in the onset and progression of lung cancer (34,35). Specifically, miR-182-5p is found to be upregulated in lung cancer tissues, where it promotes cellular proliferation, inhibits apoptosis, and influences cell cycle progression and colony formation through the targeting of F-box and WD repeat domain containing 11

Table 3 The diagnostic accuracy of miR-182-5p, CEA and their combination

Diagnostic metrics	miR-182-5p (cel-miRNA-39)	miR-182-5p (snRNA U6)	miR-182-5p (cel-miRNA-39) + CEA
Cut off	2.4×10^{-4}	8.88×10^{-5}	0.82
AUC (95% CI)	0.78 (0.69–0.86)	0.80 (0.73–0.88)	0.91 (0.85–0.97)
Sensitivity (95% CI)	0.65 (0.54–0.76)	0.71 (0.61–0.81)	0.96 (0.90–1)
Specificity (95% CI)	0.96 (0.90–1.00)	0.82 (0.71–0.92)	0.77 (0.65–0.88)
PLR (95% CI)	16.25 (5.40–76)	3.94 (2.10–10.13)	4.17 (2.57–8.33)
NLR (95% CI)	0.36 (0.24–0.51)	0.35 (0.21–0.55)	0.05 (0–0.15)

AUC, the area under the curve; CEA, carcinoembryonic antigen; CI, confidence interval; NLR, negative likelihood ratio; PLR, positive likelihood ratio.

(FBXW11) and F-box and WD repeat domain containing 7 (FBXW7) (35). Furthermore, miR-182-5p has been shown to attenuate lung cancer metastasis by targeting the mesenchymal-epithelial transition (MET) factor (34). Given these findings, we hypothesize that exosomal miR-182-5p in the pleural fluid of MPE may originate from lung cancer cells that have metastasized to the pleura, potentially facilitating the progression of MPE. Consequently, we advocate for further bioinformatics analyses to elucidate the pathways and mechanisms by which exosomal miRNAs contribute to the development of MPE.

The quantification of exosomal miRNAs in body fluids presents a promising diagnostic avenue for various diseases. Nevertheless, this field faces a significant challenge due to the absence of a universally accepted reference gene for assessing exosomal miRNAs (21). While certain internal references have been employed in previous research, consistency across studies is lacking (36). An ideal internal reference should exhibit stable expression in exosomes irrespective of the disease state; however, variations in the source and compositional ratios of exosomes in body fluids under different pathological conditions complicate this endeavor. For instance, exosomes derived from pleural effusion may originate from pleural epithelial cells, immune cells, tumor cells, and vascular endothelial cells and may also infiltrate the thoracic cavity via the bloodstream. The heterogeneity of exosomal sources underscores the difficulty of identifying a uniform internal reference (36-38). In alignment with prior studies, we adopted two internal references, cel-miRNA-39 and snRNA U6, to quantify the expression levels of exosomal miR-182-5p. SnRNA U6, recognized as a housekeeping gene, is widely utilized for the relative quantification of miRNAs. However, its constant expression within exosomes has yet to be established. On the other hand, cel-miRNA-39 serves as an external reference commonly used in the assessment of circulating miRNAs (39). Its utility is primarily limited to correcting for technical variations following exosome isolation, with inherent technical variations during the isolation process remaining unaddressed. Our findings indicate that the choice of internal reference slightly influences the AUC for miR-182-5p. Moreover, the correlation between the expression levels of miR-182-5p as normalized by snRNA U6 and cel-miRNA-39 is not robust, necessitating further investigations to identify a more suitable internal reference. This highlights the urgent need for standardized protocols in quantifying exosomal miRNAs to ensure reliable diagnostic outcomes.

The current assay for exosomal miRNA is both time-consuming and labor-intensive, involving several steps: exosome isolation, miRNA extraction, reverse transcription, and PCR reaction. This multi-step process presents two main disadvantages: high costs and decreased precision. These issues highlight the need for standardization in the determination of exosomal miRNA.

Our study found that miR-182-5p levels in pleural effusion exosomes were undetectable in 21% (32 out of 153) of patients with undiagnosed pleural effusion, suggesting that the methods for isolating exosomes from pleural fluid and amplifying exosomal miRNA need improvement. We also compared the clinical characteristics of participants included in the data analysis with those excluded. Our comparison showed no significant differences between the two groups, indicating that excluding some participants has minimal impact on the overall representativeness of the studied cohort.

This study found that the AUC of miR-182-5p for diagnosing MPE was about 0.80, lower than that of CEA.

However, the combination of miR-182-5p and CEA can improve the AUC to 0.91, implying that miR-182-5p can enhance the diagnostic accuracy of CEA. Owing to the limited sample size, the statistical power of this study is insufficient, indicating that larger studies are necessary to draw more definitive conclusions. When used alone, the diagnostic accuracy of any pleural effusion tumor marker is inadequate for ruling in or ruling out MPE (8), and combinational using of tumor markers represents a potential strategy to improve the diagnostic accuracy of tumor markers (40). Therefore, we proposed that exosomal miR-182-5p and pleural fluid CEA could be detected simultaneously to improve the diagnostic accuracy of MPE. Moreover, the use of multiple exosomal miRNAs presents a potential method for diagnosing MPE, and a combinatorial assay incorporating various miRNAs could be intriguing.

There are several limitations in this study. First, this is a monocenter study, and the general ability of our findings needs to be validated by other centers. Second, we used the stored pleural fluid specimen to determine exosomal miR-182-5p, but the long-term stability of exosomal miRNAs in the pleural fluid remains unknown. Third, the limited volume of the effusion specimen (550 mL) resulted in a low concentration of exosomal miRNA, which led to undetectable levels of exosomal miR-182-5p in some participants. Fourth, the sample size in NGS is small, which increases the likelihood of chance findings. Therefore, qPCR is necessary to validate the NGS results when assessing the diagnostic value of exosomal miRNAs revealed by this study.

Conclusions

Exosomal miR-182-5p found in pleural fluid demonstrates moderate diagnostic accuracy for MPE. Both miR-182-5p and other exosomal miRNAs show promise as diagnostic tools for MPE. However, these findings are preliminary, and further studies are required to evaluate their diagnostic accuracy. The accuracy of exosomal miR-182-5p can be slightly influenced by the internal reference used in the analysis. Moreover, as our study was conducted at a single center with a small sample size and utilized frozen specimens, validation through future research is necessary.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1205/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study received approval from the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University (Nos. 2021015 and 2018011). Informed consent was duly obtained from all participants or their legal guardians before enrollment.

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