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Clinical significance of miR-218-5p and its potential mechanism in acute pulmonary embolism

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Abstract

Introduction and Objective. Acute pulmonary embolism (APE), a life-endangering cardiovascular acute disorder, brings about difficulties in early diagnosis. The aim of the study is to investigate the expression of miR-218-5p, its clinical significance, and mechanism in APE.

Materials and Method. A total of 102 APE patients and 98 healthy controls were recruited, with miR-218-5p levels assayed by qRT-PCR; its diagnostic value was assessed by ROC curves. Pearson correlation and multivariate logistic regression analyzed associations with clinical indicators and independent predictive value. Oxygen-glucose deprivation/reoxygenation (OGD/R)-induced human pulmonary artery endothelial cells (HPAECs) were transfected with miR-218-5p mimic to observe the effects on apoptosis, proliferation, inflammation, and oxidative stress. Bioinformatics and dual-luciferase assays validated miR-218-5p targeting CREB1.

Results. Serum miR-218-5p was significantly downregulated in APE (P<0.0001) with high diagnostic efficacy (AUC=0.893). It positively correlated with D-dimer (r=0.759) and Wells scores (r=0.703; P<0.0001) and was an independent APE risk factor (OR=0.053; P<0.0001). *In vitro,* miR-218-5p overexpression reduced OGD/R-induced HPAEC apoptosis, promoted proliferation, inhibited inflammation (IL-6, IL-1β, TNF-α) and oxidative stress (MDA, ROS), and restored SOD activity (P<0.01). Mechanistically, miR-218-5p directly targeted CREB1 to suppress its expression.

Conclusions. miR-218-5p is downregulated in APE, as a potential diagnostic biomarker. It targets CREB1 to modulate apoptosis, inflammation, and oxidative stress, thereby contributing to APE pathogenesis.

Key words

miR-218-5p, CREB1, Biomarker, Endothelial cells, APE

INTRODUCTION

Acute pulmonary embolism (APE) is a prevalent cardiovascular emergency, with a rising global incidence, ranking third among deaths linked to cardiovascular diseases [1]. APE progresses rapidly and can be life-threatening within the first few hours to days of onset; studies have shown that over 70% of deaths occur during this initial period. As a common emergency, APE can exhibit typical symptoms including dyspnea and pleuritic chest pain, as well as atypical manifestations such as subtle dyspnea or syncope [2]. However, these symptoms are difficult to distinguish from those of other less severe conditions, easily leading to delayed diagnosis [3]. Efficient and early diagnosis of APE remains challenging. Thus, identifying novel biomarkers with high specificity has become a focus of current research.

In recent years, microRNAs (miRNAs) have been shown to participate in various pathophysiological processes through post-transcriptional regulatory mechanisms, and their roles in cardiovascular diseases have attracted increasing attention [4–6]. Multiple studies have shown that specific miRNAs are crucial in thrombus formation, vascular endothelial injury, and inflammatory responses. For instance, miR-34a-3p participates in the pathogenesis of APE by affecting pulmonary vascular function [7], and miR-106b-5p acts on pulmonary artery smooth muscle cells [8]. These miRNAs are involved in APE development through related pathways, providing new insights for diagnosis and treatment. Based on the miRNA expression profiling results reported by Liu Tingwei et al. in the plasma of APE patients, miR-218-5p were identified as a differentially expressed miRNA in APE [9]. Research suggests that miR-218-5p is involved in various physiological and pathological processes by regulating different targets and signaling pathways. MiR-218–5p may affect the differentiation of vascular trophoblasts by regulating inflammatory factors [10], and promote hair regeneration by regulating the β-catenin signaling pathway through skin-derived exosomes [11]. Its downregulation can impair the inhibition of glioma cell proliferation and apoptosis [12]. These studies only preliminarily report the relationship between miR-218-5p and myocardial ischemia-reperfusion injury and atherosclerosis, lacking in-depth exploration of specific regulatory targets and molecular mechanisms in thrombotic cardiovascular disease. It is worth noting that there is currently no systematic study to elucidate the clinical relevance and intrinsic regulatory network of miR-218-5p in APE.

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The aim of the study is to focus on miR-218-5p, which is abnormally expressed in APE, and conduct research through three aspects: clinical sample detection, *in vitro* cell experiments, and molecular mechanism validation. The primary aim is to examine the expression profiles of miR-218-5p in APE patients' serum and its diagnostic value; a secondary aim is to clarify the correlation between its expression level and key disease indicators. The final aim is to verify that miR-218-5p affects APE by targeting *CREB1*.

MATERIALS AND METHOD

Study participants. This study consecutively included 102 APE patients treated at the Dalian University Affiliated Xinhua Hospital's between January 2023 – December 2024 as the case group, alongside 98 age- and gender-matched healthy individuals who underwent physical check-ups during the same period as case group. Exclusion criteria: 1) patients complicated with other severe cardiopulmonary diseases (e.g., acute coronary syndrome, heart failure); 2) patients complicated with malignant tumours or immune system diseases; 3) history of recent surgery or trauma; 4) pregnant women. The study was approved by the Ethics Committee of Dalian University Affiliated Xinhua Hospital (Approval No.: 2022–93–01). All participants signed informed consent forms [13].

Sample collection and miRNA detection. Fasting venous blood (5 mL) was collected from the participants. After standing for 30 min, the blood was centrifuged at 3,000 rpm for 15 min to obtain serum. Total serum RNA was extracted using Trizol (Invitrogen, USA). Reverse transcription was performed in accordance with the instructions of the miRNA reverse transcription kit (Takara, Japan) under the conditions of 42 °C for 60 min, followed by 70 °C for 5 min. Quantitative real-time PCR (qRT-PCR) was conducted with SYBR Green qPCR Master Mix (Thermo Fisher, USA) using the reaction protocol: pre-denaturation at 95 °C for 10 min. U6 served as the internal reference gene for miR-218-5p, and *HPRT1* for *CREB1*. Relative expression levels were computed using the $2^{-\Delta\Delta Ct}$ method.

CELL EXPERIMENTS

Cell culture. Human pulmonary artery endothelial cells (HPAEC, ATCC) were cultured in a medium containing 5% foetal bovine serum and 1% endothelial cell growth supplement at 37 °C and 5% CO_2. HPAECs were divided into a control group and an injury group. In the control group, cells were cultured under normal conditions. For the injury group, during the oxygen-glucose deprivation (OGD) phase, glucose-containing complete medium was removed, and the cells were washed twice with glucose-free DMEM medium. Glucose-free DMEM medium was then added, and the cells were placed in a hypoxic incubator (1% O_2, 5% CO_2, 94% N_2) for 4 hours of hypoxic culture. During the re-oxygenation phase, the glucose-free medium was replaced with endothelial cell complete medium containing 5.5 mmol/L glucose. The cells were then transferred to a normoxic incubator (21% O_2, 5% CO_2) for 24 hours of re-oxygenation culture, completing the construction of the endothelial cell injury model.

Cell transfection. When cell confluence reached 60%–70%, human HPAECs were transfected with miR-218-5p mimic or miR-NC using Lipofectamine™ 3000 transfection reagent (Invitrogen, USA) and incubated at room temperature. Six hours after transfection, the medium was replaced with fresh complete medium, and the cells were subsequently cultured under standard conditions.

Cell proliferation assay. Cell proliferation was assessed via the CCK-8 assay (Dojindo, Japan). At 24, 48, and 72 hours after seeding, 10 μL of CCK-8 reagent was added to each well, and the plates were incubated in a humidified incubator at 37 °C for 2 hours. After incubation, the absorbance of each well was measured at 450 nm using a microplate reader (BioTek, USA).

Cell apoptosis assay. Cells were harvested 48 hours after transfection. Apoptosis was assessed using the Annexin V-FITC/PI Apoptosis Detection Kit (BD, USA) according to the manufacturer's instructions, and the apoptosis rate was analyzed using a flow cytometer (BD, USA).

Inflammatory factor measurement. Supernatants were collected 48 h post-transfection, centrifuged at 1200 rpm for 10 min at 4 °C, and stored at -80 °C until assayed. Levels of the inflammatory cytokines TNF-α, IL-1β, and IL-6 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA) according to the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader (BioTek, USA), and cytokine concentrations were calculated based on the corresponding standard curves..

Oxidative stress index detection. ROS levels were measured using DCFH-DA (Beyotime, China). The activity of superoxide dismutase (SOD) in cell lysates was determined by the xanthine oxidase method (Jiancheng Bioengineering, China), while the content of malondialdehyde (MDA) was assessed using the thiobarbituric acid (TBA) method (Jiancheng, China) according to the manufacturer's protocol. Following the specified interventions, cells were cultured for 48 hours and then harvested for the aforementioned analyses.

VALIDATION OF TARGET RELATIONSHIP

Bioinformatics analysis and target gene verification. Potential target genes of miR-218-5p were predicted using TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://www.mirdb.org/), Starbase (https://rnasysu.com/encori/index.php), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/) databases, and intersected with APE-related genes from the Genecard database (https://www.genecards.org/Search). Overlapping genes from the 5 databases underwent GO functional and KEGG pathway enrichment analyses to identify associated genes.

Dual luciferase. DNA fragments containing either the wild-type (WT) or mutant (MUT) version of the putative miR-218-5p binding site in the *CREB1* 3'UTR were synthesized and

cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, USA). Forty-eight hours after transfection, luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega, USA) according to the manufacturer's instructions.

Statistical analysis. Data statistics and charts were generated using GraphPad Prism and SPSS 26.0 software. Continuous data were expressed as mean±standard deviation (x±s). An independent samples t-test was used for comparisons between 2 groups, and one-way analysis of variance (ANOVA) was used for multiple group comparisons. Pearson correlation analysis was applied to assess correlations. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of miR-218-5p. Multivariate logistic regression was performed to identify independent risk factors for APE.

RESULTS

Clinical data analysis. A comparison of baseline data between 102 APE patients and 98 healthy controls (HC) showed no significant differences in age, BMI, SBP, DBP, TC, TG, and HDL (all $P > 0.05$), indicating well-matched baseline characteristics between groups. Notably, in APE patients, LDL-C (2.90±0.73 mmol/L), WBC count (elevated to 10.12±3.27), D-dimer (4.02±1.96 mg/L), resting heart rate (81.24±11.52 bpm), and Wells score (7.81±2.49 points) were all significantly increased ($P<0.0001$) (Tab. 1).

Table 1. Basic clinical information

Fator	HC (n=98)	APE (n=102)	P
Age (year)	60.77±10.95	60.25±11.38	0.747
BMI (kg/m2)	24.48±2.46	24.12±2.74	0.354
SBP (mmHg)	118±14	122±19	0.07
DBP (mmHg)	75±10	77±13	0.26
TC (mmol/L)	4.23±0.74	4.38±0.96	0.22
TG (mmol/L)	1.45±0.73	1.59±0.74	0.481
HDL (mmol/L)	1.55±0.31	1.48±0.24	0.081
LDL (mmol/L)	2.51±0.61	2.9±0.73	<0.0001
WBC (109/L)	7.31±1.64	10.12±3.27	<0.0001
D-D (mg/L)	0.189±0.1	4.02±1.96	<0.0001
Heart rate (bpm)	74.05±10.68	81.24±11.52	<0.0001
Wells score	2.42±1.71	7.81±2.49	<0.0001
Gender			0.902
male	52 (53.06%)	55 (53.92%)	
female	46 (46.94%)	47 (46.08%)	

$P<0.05$ indicates a significant difference. Data expressed as mean±standard deviation, excluding gender. HC: healthy control; APE – Acute Pulmonary Embolism; BMI – Body Mass Index; SBP – Systolic Blood Pressure; DBP; Diastolic Blood Pressure; TC – Total Cholesterol; TG – Triglyceride; HDL – High-Density Lipoprotein Cholesterol; LDL – Low-Density Lipoprotein Cholesterol; WBC – White Blood Cell Count; D-D – D-Dimer.

Downregulated expression of miR-218-5p. Compared with the healthy control group (0.98 ± 0.29), the relative expression of miR-218-5p in APE patients was significantly reduced (0.55 ± 0.19) (t=12.54; $P<0.0001$ (Fig. 1A). The area under the ROC curve (AUC) for miR-218-5p in APE diagnosis was 0.893, with a sensitivity of 93.1%, specificity of 74.5%, and a 95% confidence interval of 0.848–0.938 (Fig. 1B), indicating its high clinical diagnostic value.

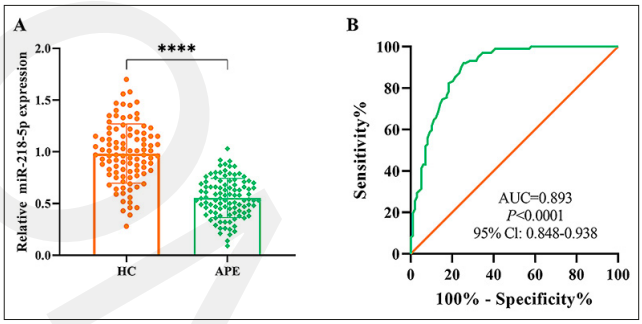

Figure 1. Expression characteristics of miR-218-5p in blood samples from healthy controls and APE patients. (A) Relative expression level of miR-218-5p. (B) ROC curve of miR-218-5p.
HC – healthy controls (n=98); APE – acute pulmonary embolism (n=102); ****$P<0.0001$

Correlation analysis of miR-218-5p. Pearson correlation analysis revealed significant positive correlations between miR-218-5p expression and D-dimer levels (r=0.759; $P<0.0001$) (Fig. 2A) as well as Wells scores (r=0.703; $P<0.0001$) (Fig. 2B), indicating that downregulated miR-218-5p expression is closely associated with APE.

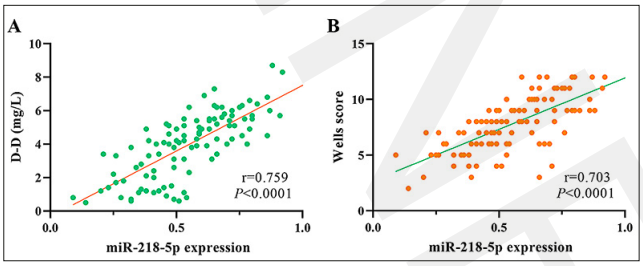

Figure 2. Correlation of miR-218-5p with APE diagnostic indicators, n=102. (A) Correlation with D-dimer (mg/L). D-D: D-dimer. (B) Correlation with Wells score

miR-218-5p as a risk factor for APE. Multivariate logistic regression analysis revealed that following adjustment for traditional risk factors (age, gender, BMI, blood lipids, blood pressure), the OR for serum miR-218-5p was 0.053 (95% CI: 0.023–0.124; $P<0.0001$). In comparison with WBC (OR=0.266, 95% CI: 0.115–0.615; $P=0.002$), miR-218-5p exhibited a 5.28-fold lower OR, demonstrating better predictive performance than traditional risk factors and greater negative predictive ability for APE (Tab. 2).

Effect of miR-218-5p on OGD/R-induced HPAECs. The expression level of miR-218-5p was significantly downregulated under OGD/R induction ($P<0.0001$), and significantly upregulated after transfection with miR-218-5p mimic (Fig. 3A; $P<0.01$). In addition, OGD/R significantly increased cell apoptosis (Fig. 3B) and inhibited cell proliferation (Fig. 3C). Interestingly, miR-218-5p could significantly alleviate the damage of OGD/R to HPAECs cells.

Effect of miR-218-5p on OGD/R-induced inflammation and oxidative stress in HPAECs. OGD/R induced significant increases in the inflammatory cytokines IL-6 (Figure. 4A; $P<0.0001$), IL-1β (Fig.. 4B; $P<0.01$), and TNF-α (Fig. 4C; $P<0.01$), which were significantly reduced after miR-218-5p mimic treatment ($P<0.01$). In addition, OGD/R led to elevated MDA (Fig. 4D) and ROS (Fig. 4F) levels, along with a significant decrease in SOD (Fig. 4E; $P<0.0001$); these changes were alleviated following miR-218-5p mimic treatment.

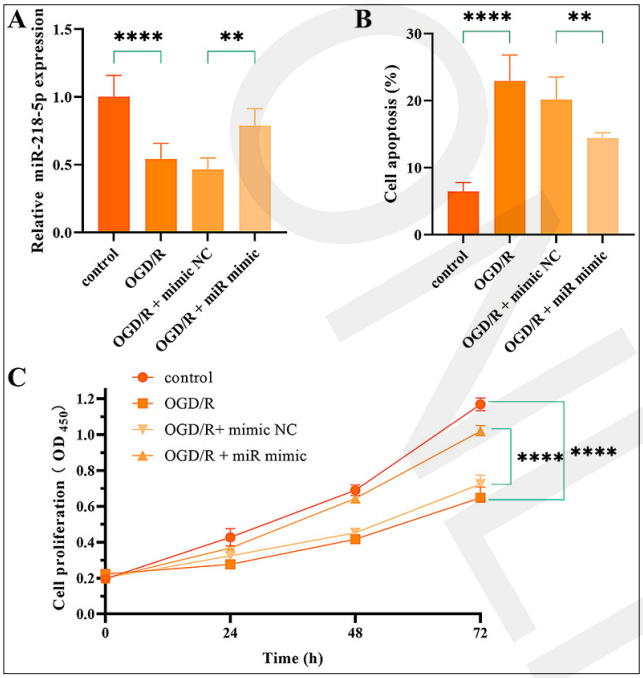

Figure 3. Effect of miR-218-5p on OGD/R-induced cellular functions. (A) miR-218-5p expression under different treatments. (B) Cell apoptosis under different treatments. (C) Cell proliferation from 0–72 hours. Control: Untreated blank control group. OGD/R – Cells subjected to oxygen-glucose deprivation/reoxygenation treatment; miR-NC – Cells transfected with miR-218-5p negative control mimics; miR-mimic: Cells transfected with miR-218-5p mimics (n=3). ****P<0.0001; ***P<0.001, **P<0.01

Table S1. GO function enrichment of miR-218-5p target genes

GO function		Enriched'genes	P-value
CC	cytosol	SOCS3, NRAS, CREB1, SERP1, RPS6KB1, PLCG1	0.21
	cell surface	RPS6KB1, CDH2, FGFR2	0.05
	cytoplasm	RPS6KB1, CDH2, ZBTB20, PLCG1, FGFR2	0.47
	Golgi apparatus	NRAS, EXTL3, FGFR2	0.13
	plasma membrane	NRAS, CDH2, ADRB1, PLCG1, EXTL3, FGFR2	0.20
	nucleus	CREB1, ZBTB20, AMMECR1, EXTL3, FGFR2	0.28
	nucleoplasm	CREB1, RPS6KB1, ZBTB20, AMMECR1	0.43
	signal transduction	NRAS, CREB1, RPS6KB1	0.17
	negative regulation of apoptotic process	SOCS3, CREB1, RPS6KB1	0.03
	positive regulation of MAPK cascade	CDH2, ADRB1, FGFR2	0.01
BP	signal transduction	CREB1, RPS6KB1, CDH2, FGFR2	0.09
	negative regulation of apoptotic process	SOCS3, NRAS, CREB1, SERP1, RPS6KB1, PLCG1	0.21
	positive regulation of MAPK cascade	RPS6KB1, CDH2, FGFR2	0.05
MF	identical protein binding	CDH2, ZBTB20, PLCG1, FGFR2	0.47

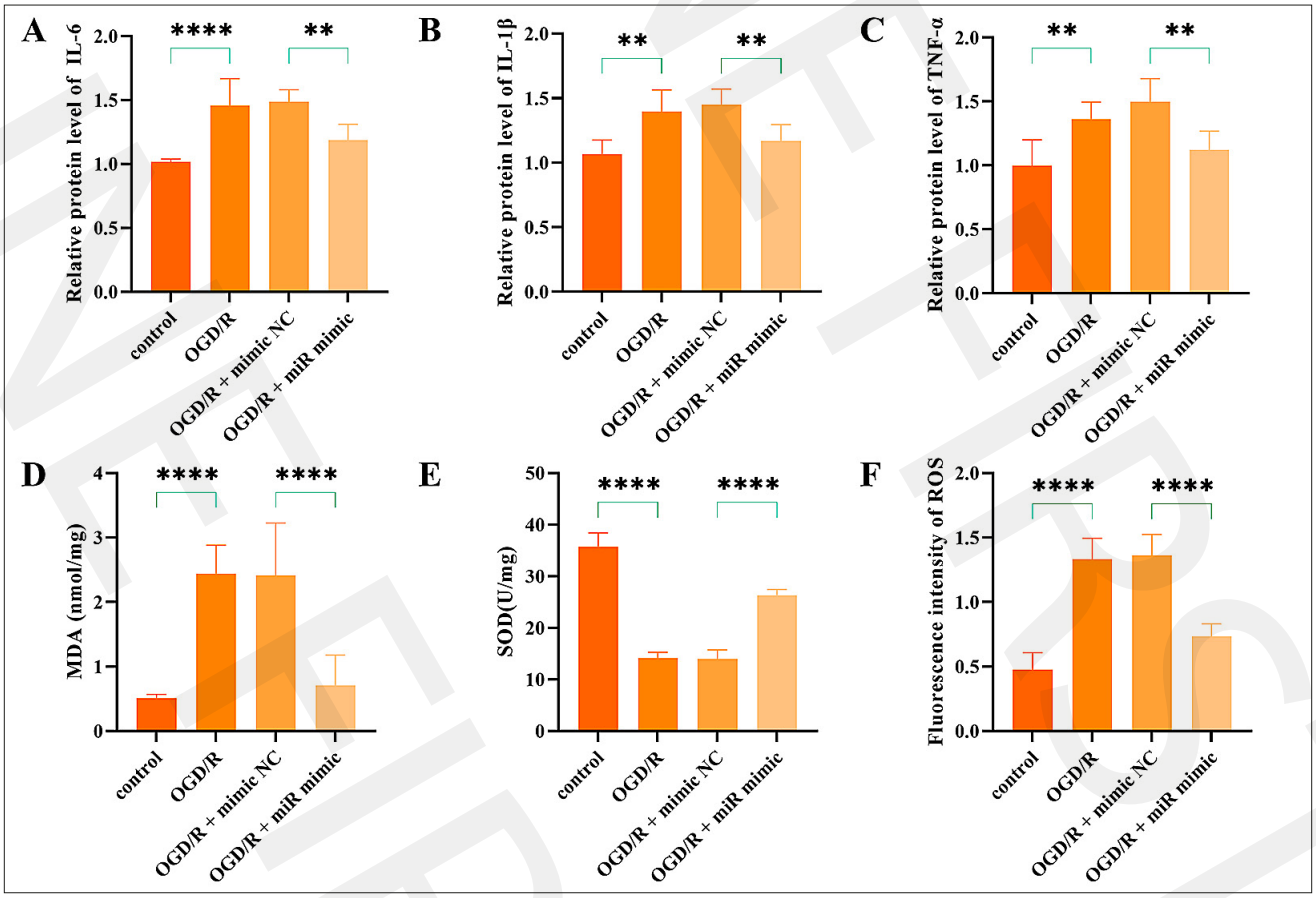

Figure 4. Effect of miR-218-5p on OGD/R-induced inflammation and oxidative stress. (A) Levels of inflammatory factors IL-6 under different treatments. (B) IL-1β levels under different treatments. (C) TNF-α levels under different treatments. (D) Oxidative index MDA levels under different treatments. (E) SOD levels under different treatments. (F) ROS levels under different treatments. Control – Untreated blank control group;
OGD/R – Cells subjected to oxygen-glucose deprivation/reoxygenation treatment; miR-NC – Cells transfected with miR-218-5p negative control mimics; miR-mimic – Cells transfected with miR-218-5p mimics (n=3); ****P<0.0001; **P<0.01.

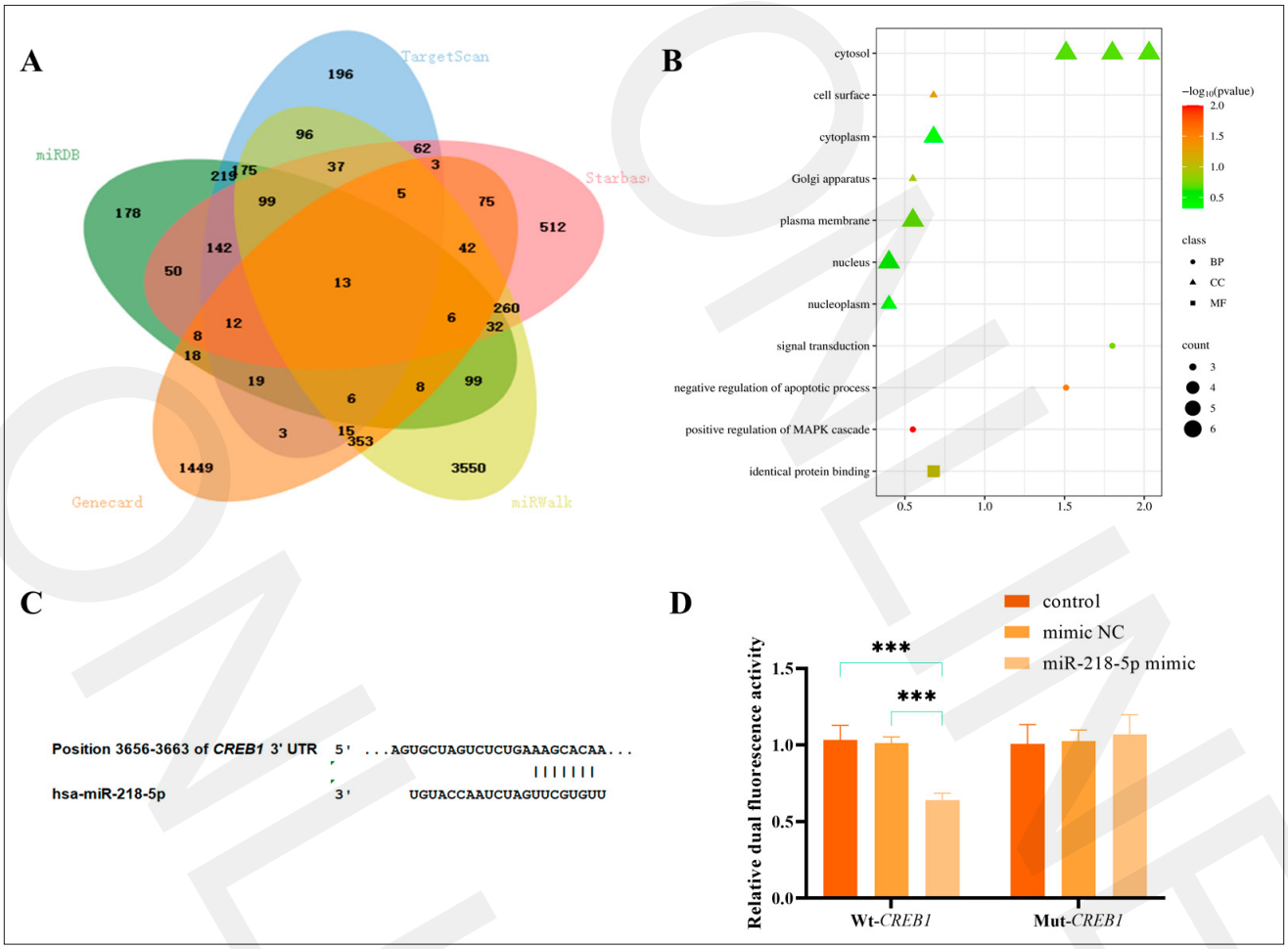

Figure 5. Validation of miR-218-5p targeting *CREB1*. (A) Venn diagram of predicted downstream target genes of miR-218-5p and APE-related genes. (B) GO pathway enrichment of screened genes. (C) Predicted miR-218-5p binding site in *CREB1* and dual-luciferase validation. (D) Dual-luciferase assay for miR-218-5p and *CREB1* (n=3). NC – negative control; WT – wild-type; MUT – mutant; *** $P<0.001$

Table S2. KEGG enrichment of miR-218-5p target genes

KEGG Pathwaye	Enriched'genes	P-value
Growth hormone synthesis, secretion and action	SOCS3, NRAS, CREB1, PLCG1	1.514
Kaposi sarcoma-associated herpesvirus infection	NRAS, CREB1, PLCG1	0.013
PI3K-Akt signaling pathway	NRAS, CREB1, RPS6KB1, FGFR2	0.003
EGFR tyrosine kinase inhibitor resistance	NRAS, RPS6KB1, PLCG1, FGFR2	4.294
Chemical carcinogenesis - receptor activation	NRAS, CREB1, RPS6KB1, ADRB1	8.041
Longevity regulating pathway	NRAS, CREB1, RPS6KB1	0.002
Human cytomegalovirus infection	NRAS, CREB1, RPS6KB1	0.017

***CREB1* as a target of miR-218-5p in APE.** Thirteen target genes, including *CREB1*, *SERP1*, *ADRB1*, and *ZFYVE26*, were identified by intersecting 4 miRNA target databases with APE-related gene databases (Fig. 5A). Combining the GO enrichment (Tab. S1) and KEGG pathway (Tab. S2) results, *CREB1* appeared with the highest frequency in repeated terms. Furthermore, according to the bubble plot results, *CREB1* was identified as one of the key functional genes (Fig. 5B). Therefore, *CREB1* was chosen for further research. The predicted miR-218-5p binding site in *CREB1* was located at 3656–3663bp (Fig. 5C). In addition, compared with the NC group, co- transfection of miR-218-5p with *CREB1* 3'UTR-WT reporter vector significantly reduced luciferase activity in the vector; and this phenomenon, however, disappeared when its binding site was mutated (Fig. 5D).

DISCUSSION

APE is a common life-threatening cardiovascular emergency which still poses challenges for early diagnosis based on initial symptoms and subsequent effective treatment. Thus, exploring early accurate diagnosis and clarifying underlying mechanisms is crucial for improving prognosis [2, 14]. The cirrent study demonstrates the significant clinical diagnostic value of miR-218-5p in APE based on its downregulated expression in APE patients, diagnostic efficacy, and correlation with clinical indicators. By constructing an OGD/R cell model and validating its target, it was confirmed that miR-218-5p targets *CREB1*, alleviates OGD/R-induced apoptosis in HPAECs, and inhibits inflammation and oxidative stress, thereby exerting a protective effect on endothelial function. These findings provide new insights for identifying novel biomarkers and therapeutic targets for APE diagnosis.

An increasing number of miRNAs have been recognized as key players in disease diagnosis, particularly in haemostasis

and thrombosis, yet the potential role of miRNAs in APE diagnosis remains to be fully explored [15, 16]. The expression of miR-218-5p was validated based on the miRNA expression profile of APE patients. Compared with the healthy control group, the expression level of serum miR-218-5p in APE patients was significantly lower, which aligns with the earlier findings of Liu Tingwei et al. [9]. This study further confirms the aberrant expression of this miRNA in APE. Liu Yu and colleagues identified miRNA as a potential diagnostic tool and established miR-134 as a novel biomarker for APE diagnosis [17], while miR-1233 has been shown to differentiate acute PE, acute NSTEMI, and healthy subjects with high specificity and sensitivity [18]. Interestingly, ROC results confirmed that the diagnostic accuracy of miR-218-5p in this study is superior to that of other miRNAs (AUC=0.86).

D-dimer levels [19] and Wells score [20] have been reported as common diagnostic criteria for APE. However, the high sensitivity but low specificity of D-dimer, along with concerns regarding the accuracy of the Wells score system [21], highlight the urgency in exploring effective biomarkers for the diagnosis and exclusion of APE. Pearson correlation analysis revealed significant positive correlations between miR-218-5p expression and D-dimer levels (r=0.759) as well as Wells score (r=0.703), suggesting that lower miR-218-5p expression is associated with higher thrombotic burden and greater clinical severity. As a marker of fibrinolysis activation, elevated D-dimer reflects the hypercoagulable state in APE patients, while the Wells score integrates clinical symptoms and risk factors [22]. Their positive correlation with miR-218-5p indicates that this miRNA may function by inhibiting pathways associated with endothelial injury and thrombogenesis.

Notably, multivariate logistic regression analysis showed that the OR for miR-218-5p was 0.05, significantly lower than that for the traditional risk factor white blood cells, indicating stronger negative predictive power for APE and its potential as a core risk indicator independent of age, gender, and lipid levels [23]. Therefore, miR-218-5p holds high diagnostic value as a risk factor for APE and is more advantageous than traditional diagnostic criteria for early and specific diagnosis. However, future large-scale prospective studies are still needed to directly compare the diagnostic performance of different biomarkers.

It has been reported that HPAECs can effectively reflect APE-induced inflammatory responses under adverse stimuli [24]. Therefore, this study utilized an *in vitro* HPAEC model to further demonstrate that over-expression of miR-218-5p significantly reduced OGD/R-induced HPAEC apoptosis, promoted cell proliferation, suppressed the increase in inflammatory factors (IL-6, IL-1β, TNF-α) and oxidative stress indicators (MDA, ROS), while restoring SOD activity. This is consistent with studies indicating that miR-218-5p participates in regulating the anti-apoptotic, anti-inflammatory, and antioxidant stress capacities of endothelial cells [25–27]. However, the current study is the first to propose that miR-218-5p alleviates APE-related vascular endothelial damage through these responses. Furthermore, through prediction using multiple databases and experimental validation, this study is also the first to confirm that *CREB1* is a direct target of miR-218-5p in APE. Dual-luciferase reporter assays showed that miR-218-5p mimics significantly inhibited the activity of the *CREB1*-WT reporter gene. Existing studies have confirmed that *CREB1* is abnormally activated in diseases such as deep vein thrombosis and lung injury, exacerbating vascular damage by promoting the release of inflammatory factors and endothelial cell apoptosis [28, 29]. At the mechanistic level, this study is the first to reveal the miR-218-5p/*CREB1* axis, providing a new perspective for understanding APE-related endothelial injury.

In recent years, it has been reported that miR-150–5p mitigates endothelial inflammation by targeting the regulation of endothelial cell proliferation [30], and miR-22–5p alleviates endothelial inflammation via its pathway [31]. Within the known regulatory mechanisms of miR-218-5p, it participates in the pathogenesis of diseases such as psoriasis and glioma through its roles in proliferation and inflammation [32, 33]. Unlike the studies cited above, the current study is the first to report that miR-218-5p, by targeting *CREB1*, regulates endothelial cell proliferation, apoptosis, and inflammatory responses, suggesting it may play a broader protective role in maintaining endothelial homeostasis and providing a novel multi-effect target for intervening in APE vascular endothelial injury. Notably, endothelial injury is a key pathological basis for APE development, and endothelial dysfunction caused by thrombin and hypoxia can trigger a thrombotic cascade [34, 35]. Therefore, the protective effect of miR-218-5p may be a critical link in blocking this process. Additionally, *CREB1* promotes the activation and apoptosis of the NF-κB pathway in lung epithelial cells through LTBR-mediated transcriptional activation [36]. Thus, miR-218-5p inhibits *CREB1* expression, thereby affecting cell apoptosis, inflammation, and oxidative stress responses, alleviating endothelial injury, and participating in the pathogenesis of APE. This mechanism complements earlier studies showing that miR-218-5p targets TGF-β/SMAD2 and influences inflammatory responses [10], indicating that this miRNA may function through a multi-target network.

The findings of the current study reveal that miR-218-5p, via *CREB1*, may converge or regulate more upstream signaling nodes, thereby broadly influencing downstream apoptotic and inflammatory networks, providing a potential hub target for intervention. This not only maintains mechanistic consistency, but also expands the specific regulatory mode of this pathway in APE, strengthening the reliability and innovativeness of the mechanisms proposed in this study..

Finally, this study lays an important experimental foundation for the potential application of miR-218-5p as a therapeutic target. Based on the molecular mechanisms and *in vitro* experimental results, miR-218-5p holds promise as a novel therapeutic target for APE. Potential strategies include developing miR-218-5p mimics or agonists as replacement therapies, designing drugs that target *CREB1* or its downstream effectors, or utilizing miR-218-5p expression levels for patient stratification to guide personalized treatment.

Limitations of the study. The study has several limitations: 1) clinical samples were obtained from a single centre, and the overall sample size was relatively limited, which may have affected the generalizability of the research results. 2) in addition, due to cost constraints and strict technical requirements that hindered the successful construction of *in vivo* animal models, the study relied entirely on *in vitro* cell models. 3) the downstream pathway of miR-218-5p/*CREB1* has not been extensively explored. In future research, it is planned that: 1) multicentre, large sample prospective cohort

studies will be conducted to further confirm the diagnostic and prognostic value of miR-218-5p; 2) an APE mouse model will be established to verify the regulatory effects of miR-218-5p mimetics on thrombosis and endothelial injury; 3) a thorough analysis will be carried out of the complete regulatory cascade of the downstream pathways NF-κB/ apoptosis pathway.

CONCLUSION

This study is the first to systematically clarify the expression traits, clinical value, and molecular mechanism of miR-218-5p in APE. miR-218-5p regulates endothelial function by targeting *CREB1*; its low expression is closely linked to APE onset and development, with potential as an early diagnostic biomarker and therapeutic target.

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