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Original Article

Circulating microRNAs as potential biomarkers for unstable angina

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Abstract: Background: Chest pain is a typical presentation in the emergency department (ED), often due to acute coronary syndrome. Accurate and fast identification is crucial. The diagnosis and proper treatment of unstable angina (UA) can reduce the chance of acute myocardial infarction, and reduce high mortality and morbidity rates. However there is a lack of reliable and valid biomarkers in the diagnosis of UA. This study investigated the usefulness of circulating microRNAs (miRNAs) for differentiating UA from non-ischemic chest pain (NICP) in the ED. Methods The expressions of circulating miRNAs in patients with UA were evaluated relative to individuals with NICP (control subjects). Circulating miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451 levels were measured in 98 patients with UA and 95 control subjects in the ED. To investigate the underlying functions of miRNAs in UA, bioinformatic analysis of validated miRNAs was conducted. Results: Circulating miRNAs were upregulated in UA compared with the control group. The combination of the modified HEART score (m-HS) and miR-25 (AUC 0.901, NRI 0.096) could better distinguish UA than m-HS alone. Bioinformatic analysis indicated that miRNAs may take part in platelet activation, cGMP-PKG signaling pathways etc. Conclusion: The circulating levels of miRNAs (miR-21, miR-25, miR-106b, miR-126*) are significantly higher in UA patients compared with patients with NICP, and the addition of the m-HS that combined ECG, age, risk factors and troponin is useful to detect or rule out UA. The associated signaling pathways are involved in the pathogenesis of vulnerable plaque.

Keywords: Acute coronary syndrome, unstable angina, microRNAs

Introduction

Chest pain is a common complaint of patients presenting in the emergency department (ED). Patients with chest pain account for 130 million ED visits annually worldwide, and chest pain is the second most frequent reason for ED visits (5.4%) [1]. Data from the General Practice Research Database indicates that ischemic heart disease is the most common cause of death during the 1-year follow-up , with mortality up to 36% [2]. Acute coronary syndrome (ACS) is a leading cause of cardiac chest pain, which includes acute myocardial infarction (AMI) and unstable angina (UA). Patients with suspected ACS are classified into 3 groups, the first collects patients with ACS, and then admitted, the second group with patients with diagnosis of non-ischemic chest pain (NICP), and finally the group with patients with suspected ACS who require observation. Patients in the latter group account for about 50.2% [3].

The assessment of patients with suspected ACS is based on clinical presentation, 12-lead electrocardiogram (ECG), and cardiac troponin [4]. Cardiac troponin is a specific and sensitive biomarker of cardiomyocyte injury. The rule-out algorithm of AMI needs re-test cardiac troponin sometimes [4]. Accurate diagnosis and effective treatment of UA can reduce the chance of AMI and greatly influence the outcome of the patient in the ED. However there is a lack of reliable and valid biomarkers in the diagnosis of UA.

MicroRNAs (miRNAs) are small non-coding RNAs that can regulate the expression of mRNAs by promoting mRNA degradation or inhibiting their function [5]. It is widely recognized that the expressions of specific miRNAs are associated with various cardiovascular diseases [6]. Some studies show a link between miRNAs and chest pain patients. However, these studies focused on an association be-

Table 1. Baseline characteristics of patients with UA and NICP*

	Total population	NICP	UA	P
Subjects, n	193	95	98	—
Age, y	59.53 ± 17.38	51.01 ± 17.84	68.14 ± 11.82	0.000
Male, %	50.80	50.50	51.00	0.945
SBP, mmHg	143.68 ± 21.51	141.09 ± 22.08	146.09+20.83	0.165
DBP, mmHg	77.67 ± 14.62	79.17 ± 15.39	76.27 ± 13.83	0.237
Heart rate, bpm	79.61 ± 16.31	80.93 ± 13.95	78.30 ± 18.35	0.329
BMI, kg/m^2	24.70 ± 3.65	24.68 ± 3.66	24.71 ± 3.67	0.962
Hypertension, %	57.7	39.2	76.0	0.000
Hyperlipidemia, %	43.6	28.4	58.7	0.000
Diabetes mellitus, %	27.5	13.5	41.3	0.000
Current smoking, %	19.6	16.2	23.0	0.300
White blood cell, ×10^9/L	7.15 ± 2.38	7.11 ± 2.18	7.19 ± 2.57	0.845
Red blood cell, ×10^{12}/L	4.52 ± 0.55	4.63 ± 0.53	4.43 ± 0.56	0.043
Blood platelet, ×10^9/L	225.69 ± 60.81	233.74 ± 55.16	218.47 ± 65.01	0.155
Cr, μmol/L	68.71 ± 19.99	65.33 ± 13.25	70.58 ± 22.76	0.208
eGFR, mL/min/1.73 m^2	88.59 ± 18.92	93.38 ± 18.41	85.93 ± 18.81	0.058
ALT, μ/L	17.00 (13.00, 30.50)	19.50 (13.00, 27.25)	17.00 (12.00, 32.00)	0.865
AST, μ/L	20.00 (17.00, 25.50)	19.50 (16.50, 24.25)	20.00 (17.00, 27.00)	0.602
TC, mmol/L	4.39 ± 1.14	4.78 ± 1.13	4.28 ± 1.13	0.183
TG, mmol/L	1.67 ± 0.94	1.32 ± 0.65	1.77 ± 0.99	0.142
HDL-C, mmol/L	1.19 ± 0.32	1.33 ± 0.28	1.15 ± 0.32	0.099
LDL-C, mmol/L	2.61 ± 0.84	2.94 ± 0.91	2.51 ± 0.80	0.128
D-dimer, ng/mL	105.00 (48.75, 222.25)	97.50 (47.25, 154.25)	118.50 (49.50, 238.75)	0.349
Cardiac troponin, ng/mL	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	1.000
CK-MB, ng/mL	0.80 (0.00, 2.33)	0.00 (0.00, 1.80)	1.30 (0.00, 2.50)	0.052
Myo, ng/mL	46.02 ± 3.10	44.51 ± 2.98	47.21 ± 3.21	0.694

*Data are shown as mean ± standard deviation, percentages (%) or median (interquartile range). Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; NICP, non-ischemic chest pain; SBP, systolic blood pressure; UA, unstable angina.

tween miRNAs and AMI patients with chest pain.

This study investigated the usefulness of circulating miRNAs for detecting UA.

Materials and methods

Study population

This study was a prospective observational study, with no intervention or treatment changes. The Ethics Committee of Peking University People's Hospital, Beijing, China approved the research protocol. Informed consent was obtained from participating patients.

Between October 2015 and July 2016, 193 patients presented with chest pain to the ED of Peking University People's Hospital and were eligible for the study. To be included in the study, patients were aged ≥ 18 years and agreed to medical examination and blood sample collection. Patients with any of the following were excluded: liver or renal impairment; anemia; stable angina; elevation of troponin and previous history of hematonosis; malignant tumor; heart failure; or autoimmune diseases.

All patients were apportioned to either a UA (n = 98) or NICP (n = 95) control group. UA was defined as typical chest pain (angina occurring at rest and prolonged usually > 20 min; new-onset angina within 1 month; increasing angina of longer duration, more frequent, or lower threshold), without elevation in troponin and with or without ECG changes indicative of ischemia. The NICP patients had chest pain, but not related to myocardial ischemia, and therefore

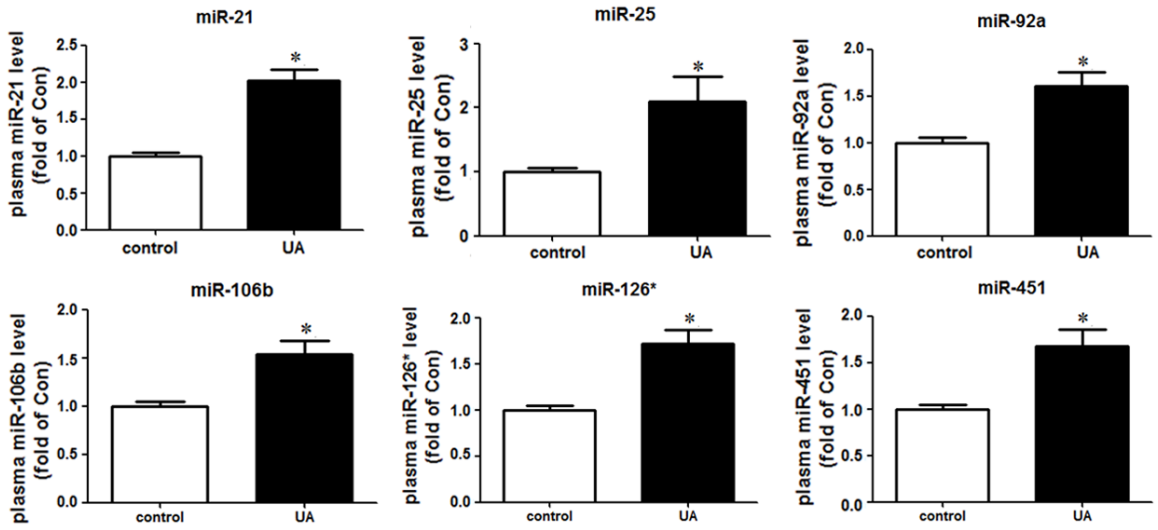

Figure 1. Plasma miRNAs levels in the UA and control group (NICP). The histograms show the expression levels of miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451 measured by real-time PCR. UA, unstable angina; NICP, non-ischemic chest pain; *P < 0.05.

Table 2. Expression levels of plasma miRNAs in UA relative to control

	UA/control ratio	P
miR-21	2.020	0.000
miR-25	2.097	0.006
miR-92a	1.611	0.000
miR-106b	1.540	0.000
miR-126-5p	1.716	0.000
miR-451	1.678	0.000

no evidence of ACS, no elevation in troponin, and no ECG ST-elevation or depression.

Data collection

Each patient's past medical history and chief complaint were collected from the patient and from the electronic medical record. Data regarding age, gender, blood pressure (BP), heart rate (HR), cardiovascular risk factors, blood sample, and ECG results were recorded for all patients.

Visser et al.'s [7] research used the HEART score (history, ECG, age, risk factors, and troponin) for diagnosing ACS in patients with chest pain presenting in the ED. In the present study, a modified HEART score (m-HS) was calculated for each patient. The m-HS consisted of the following components: ECG, age, risk factors, and troponin. The risk factors of a high m-HS score include hypertension, hypercholesterolemia, diabetes mellitus, obesity, cigarette smoking, and positive family history.

Methods

Peripheral venous plasma samples were collected during patient admission for detection of miRNAs (< 12 hours after symptom onset). The expressions of miRNAs were quantified in individual plasma samples from all patients using real-time PCR. If a patient was initially considered to have UA but experienced increased troponin later, the patient was excluded from the study.

Extraction of total RNA from plasma

The total RNA was extracted from plasma using a miRNeasy Mini Kit (Qiagen) in accordance with the manufacturer's instructions. The RNA was used for reverse transcription (RT) reactions.

MiRNA measurements

The cDNA (i.e., the RT reaction products) was prepared using a TaqMan MicroRNA Reverse Transcription Kit in accordance with the introduction of the recommended protocol. The RT reaction was performed at 16°C for 30 min, and then 42°C for 30 min and 85°C for 5 min.

The RT reaction products were combined with the TagMan Universal PCR Master Mix in accor-

Table 3. Spearman's correlation analysis between the miRNAs and cardiac risk factors

	miR-21	miR-25	miR-92a	miR-106b	miR-126*	miR-451
miR-21	—	—	—	—	—	—
miR-25	0.758*	—	—	—	—	—
miR-92a	0.761*	0.952*	—	—	—	—
miR-106b	0.717*	0.881*	0.864*	—	—	—
miR-126*	0.867*	0.680*	0.699*	0.742*	—	—
miR-451	0.647*	0.856*	0.879*	0.899*	0.643*	—
Age	0.163	0.050	0.023	-0.067	0.074	-0.022
Hypertension	0.125	0.015	0.009	-0.008	0.063	0.008
Hyperlipidemia	0.050	0.097	0.096	0.015	0.111	0.044
Diabetes mellitus	0.094	0.084	0.068	0.030	0.055	0.115
Current smoking	0.008	0.060	0.036	0.090	0.021	0.054

*P < 0.05. r = Spearman's correlation coefficient.

Table 4. Multivariate logistic regression analysis for the risk of UA*

	B	SE	Wald	*P* value	OR	95% CI
miR-21	1.849	0.504	13.436	0.000	6.354	2.364-17.080
miR-25	0.889	0.318	7.784	0.005	2.432	1.303-4.539
miR-92a	0.748	0.297	6.326	0.012	2.112	1.179-3.781
miR-106b	0.848	0.285	8.873	0.003	2.334	1.336-4.077
miR-126*	1.004	0.373	7.237	0.007	2.728	1.313-5.669
miR-451	0.711	0.277	6.585	0.010	2.036	1.183-3.503

*The model included age, hypertension, hyperlipidemia, and diabetes. OR = odds ratio, CI = confidence interval.

Table 5. ROC curve analysis of the miRNAs

	AUC	P	95% CI	Cut-off	Sensitivity	Specificity
miR-21	0.744	0.000	0.666-0.822	1.62	0.506	0.960
miR-25	0.649	0.000	0.571-0.727	1.64	0.412	0.872
miR-92a	0.620	0.004	0.541-0.699	1.91	0.286	0.958
miR-106b	0.573	0.081	0.490-0.655	2.18	0.265	0.989
miR-126-5p	0.653	0.000	0.574-0.732	2.03	0.298	1.000
miR-451	0.581	0.055	0.498-0.663	2.03	0.278	0.989

dance with the instructions for the measurement of miRNAs. Subsequently, miRNAs were measured by real-time PCR using the cycling conditions 95°C for 10 min; and 40 cycles of 95°C for 15 s and 60°C for 1 min. Cel-miR-39 was used for normalization. The level of miRNA was expressed as $2^{-(CT[miRNA]-CT[miR-39])}$, and CT is the cycle threshold.

Bioinformatic analysis of miRNAs

The target genes of the miRNAs were assessed using three databases (miRanda, miRWalk and TargetScan). Enrichment analysis was performed to find the signaling pathways that were associated with the target genes, using DAVID (Database for Annotation, Visualization, and Integrated Discovery) bioinformatics resources and KEGG (Kyoto Encyclopedia of Genes and Genomes). Interactions between the miRNAs and signaling pathways were visualized ($P < 0.05$) using Cytoscape software (Version 3.4.0).

Statistical analysis

Continuous variables that were normally distributed are presented as mean ± standard deviation, and comparisons were performed with Student's t-test. Continuous variables that were not normally distributed are presented as median ± interquartile range, and comparisons between groups employed the Wilcoxon rank-sum test. Categorical variables are presented as percentages, and the chi-squared test was used for the comparisons.

Spearman's correlation analysis was performed to investigate associations between miRNAs and risk factors. Multivariate logistic regression analysis was used to study the miRNA expressions after adjustments for risk factors. The receiver operating characteristic (ROC) curve was analyzed to evaluate the ability of miRNAs and the m-HS to detect UA. The net reclassification improvement (NRI) index was evaluated to determine the incremental influence of miRNAs for detecting UA.

Results

Characteristics of study participants

The study included 193 patients with an average age of 59.53 years, and 50.8% were men (**Table 1**). All participants were apportioned to

Table 6. ROC curve analysis of the combinations of miRNAs

		AUC	P	95% CI	Cut-off	Sensitivity	Specificity
miR-21		0.744	0.000	0.666-0.822	1.62	0.506	0.960
	+miR-25	0.747	0.000	0.669-0.826	1.60	0.513	0.973
	+miR-92a	0.749	0.000	0.671-0.826	1.49	0.494	0.973
	+miR-106b	0.759	0.000	0.684-0.835	1.30	0.506	0.960
	+miR-126-5p	0.738	0.000	0.657-0.818	1.31	0.480	0.972
	+miR-451	0.742	0.000	0.663-0.821	1.63	0.513	0.946
miR-25		0.649	0.000	0.571-0.727	1.64	0.412	0.872
	+miR-92a	0.654	0.000	0.576-0.732	0.91	0.412	0.915
	+miR-106b	0.655	0.000	0.578-0.733	1.39	0.299	0.979
	+miR-126-5p	0.674	0.000	0.597-0.752	4.20	0.538	0.780
	+miR-451	0.655	0.000	0.577-0.733	1.60	0.344	0.946
miR-92a		0.620	0.004	0.541-0.699	1.91	0.286	0.958
	+miR-106b	0.616	0.005	0.536-0.695	1.80	0.408	0.863
	+miR-126-5p	0.656	0.000	0.578-0.734	11.11	0.298	0.989
	+miR-451	0.614	0.006	0.535-0.694	2.33	0.316	0.947
miR-106b		0.573	0.081	0.490-0.655	2.18	0.265	0.989
	+miR-126-5p	0.657	0.000	0.579-0.736	1.61	0.319	0.989
	+miR-451	0.586	0.040	0.504-0.668	5.78	0.289	0.989
miR-126-5p		0.653	0.000	0.574-0.732	2.03	0.298	1.000
	+miR-451	0.656	0.000	0.577-0.735	2.08	0.298	1.000
miR-451		0.581	0.055	0.498-0.663	2.03	0.278	0.989

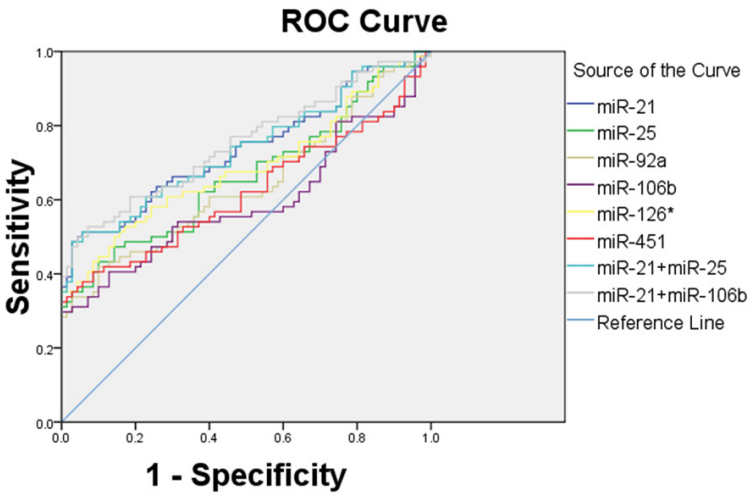

Figure 2. ROC curve of miRNAs. AUCs: miR-21, 0.744 (0.666-0.822); miR-25, 0.649 (0.571-0.727); miR-106b, 0.573 (0.490-0.655); miR-21+miR-25, 0.747 (0.669-0.826); miR-21+miR-106b, 0.759 (0.684-0.835).

either a UA group (n = 98) or a control group (NICP group, n = 95). Between the 2 groups there were differences in age, hypertension, hyperlipidemia, diabetes mellitus, and red blood cells. The red blood cell levels were within the normal range in the participants of both two groups.

Levels of candidate miRNAs

Six miRNAs (miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451) were selected for validation, based on relevant literature. Real-time PCR was applied to evaluate the levels of miRNAs. The plasma levels of miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451 were higher in the UA group than in the control group (**Figure 1**; **Table 2**).

Associations between the miRNAs and cardiovascular risk factors

To identify whether the miRNAs were associated with the known cardiovascular risk factors (age, hypertension, hyperlipidemia, diabetes and smoking), Spearman's rank correlation coefficients between the levels of miRNAs and risk factors were calculated (**Table 3**). The results indicated that miRNAs correlated with each other, while the miRNAs were independent from other risk

Table 7. Reclassification for the presence of UA after addition of miRNAs to the m-HS

		AUC	*P*	95% CI	Cut-off	Sensitivity	Specificity	NRI
m-HS		0.874	0.000	0.817-0.930	3.50	0.803	0.860	—
	+miR-21	0.928	0.000	0.885-0.970	5.43	0.884	0.829	-0.120
	+miR-25	0.901	0.000	0.853-0.949	4.24	0.920	0.800	0.096
	+miR-92a	0.895	0.000	0.846-0.945	4.12	0.882	0.814	0.065
	+miR-106b	0.900	0.000	0.852-0.948	4.11	0.895	0.837	0.138
	+miR-126-5p	0.911	0.000	0.866-0.955	4.70	0.861	0.869	0.079
	+miR-451	0.900	0.000	0.851-0.949	4.09	0.867	0.847	0.084
	+miR-21+miR-25	0.914	0.000	0.866-0.962	5.46	0.870	0.826	-0.132
	+miR-21+miR-92a	0.928	0.000	0.886-0.970	5.57	0.870	0.871	-0.050
	+miR-21+miR-106b	0.928	0.000	0.885-0.970	5.53	0.853	0.870	-0.088

UA, unstable angina; m-HS, modified HEART score; NRI, net reclassification improvement.

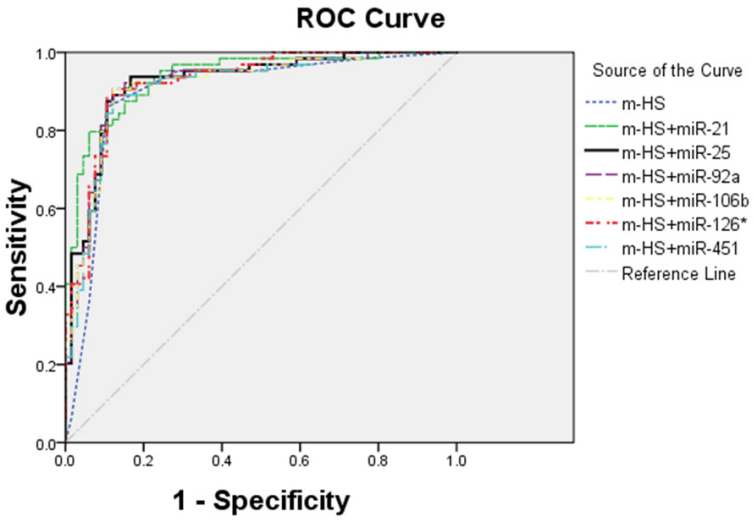

Figure 3. ROC curves of m-HS. AUCs: m-HS, 0.874 (0.817-0.930); m-HS+miR-25, 0.901 (0.853-0.949); m-HS+miR-126*, 0.911 (0.866-0.955).

factors such as age, hypertension, hyperlipidemia, diabetes and smoking.

Multivariate logistic regression analysis

Multivariate logistic regression analysis was adjusted for age, hypertension, hyperlipidemia, and diabetes (**Table 4**). After the adjustment, the levels of miRNA (miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451) were still independently associated with UA. The odds ratios for the six miRNAs ranged from 2.036 to 6.354 after adjustment.

The diagnostic accuracy of the miRNAs

ROC curve analyses were performed to evaluate the diagnostic accuracy of the miRNAs (**Tables 5**, **6**). The results showed that the area under the ROC curve (AUC) for miR-21 was 0.744 ($P < 0.05$). Combining miR-21 and miR-106b increased the power, with an AUC of 0.759 (**Figure 2**).

In the ROC curve analysis for discriminating the UA patients, the AUC for the m-HS alone was 0.874 (95% CI 0.817-0.930). When miR-25 was added to the m-HS, the AUC increased to 0.901 (95% CI 0.853-0.949), and the NRI was positive (0.096). A significant positive NRI (0.079) was also observed by adding miR-126* to m-HS (**Table 7**; **Figure 3**).

Bioinformatic analysis of miRNAs

Five miRNAs (miR-21, miR-25, miR-92a, miR-106b and miR-126*) had common target genes in the 3 databases (miRanda, miRWalk and TargetScan). Consequently, using the DAVID bioinformatics resource, signaling pathways ($P < 0.05$) associated with target genes in the KEGG database were found, and combined with the function of the signaling pathways, we found that the targeted genes take part in platelet activation, cGMP-PKG, AMPK, PI3K-Akt, FoxO, and MAPK pathways (**Table 8**). Finally, the Cytoscape software revealed miRNA-target gene (**Figure 4**) and miRNA-target gene-signaling pathway interaction networks (**Figure 5**).

Table 8. Enriched KEGG pathways associated with target genes of miRNAs

	Target gene	Signaling pathway	P
miR-21	ATP2B4, CREB5, GTF2I, etc.	cGMP-PKG	0.0026
	FASLG, GNG12, MKNK2, etc.	MAPK	0.0024
	ATM, FASLG, SOS2, etc.	FoxO	0.00042
	HMGCR, PFKFB2, CREB5, etc.	AMPK	0.028
miR-25	ADCY3, ADCY9, COL1A2, etc.	Platelet activation	0.029
	ATP1B2, ATP2B4, ATP2A2, etc.	cGMP-PKG	0.003
	MCL1, BCL2, FASLG, etc.	PI3K-Akt	0.0042
	HMGCR, RAB14, CREB1, etc.	AMPK	0.0079
	ATP1B2, ATP2B4, BCL2, etc.	Adrenergic signaling in cardiomyocytes	0.0037
miR-92a	ATP1B2, ATP2B1, IRS2, etc.	cGMP-PKG	0.00058
	MCL1, BCL2L11, BCL2, etc.	PI3K-Akt	0.0042
	HMGCR, PFKFB2, INSR, etc.	AMPK	0.0017
	BCL2L11, FASLG, KLF2, etc.	FoxO	0.0026
	ATP1B2, ATP2B1, ATP2B4, etc.	Adrenergic in cardiomyocytes	0.028
miR-106b	PDPK1, AKT3, ATM, etc.	FoxO	0.000079
miR-126-5p	PDPK1, AKT3, MCL1, etc.	PI3K-Akt	0.000059

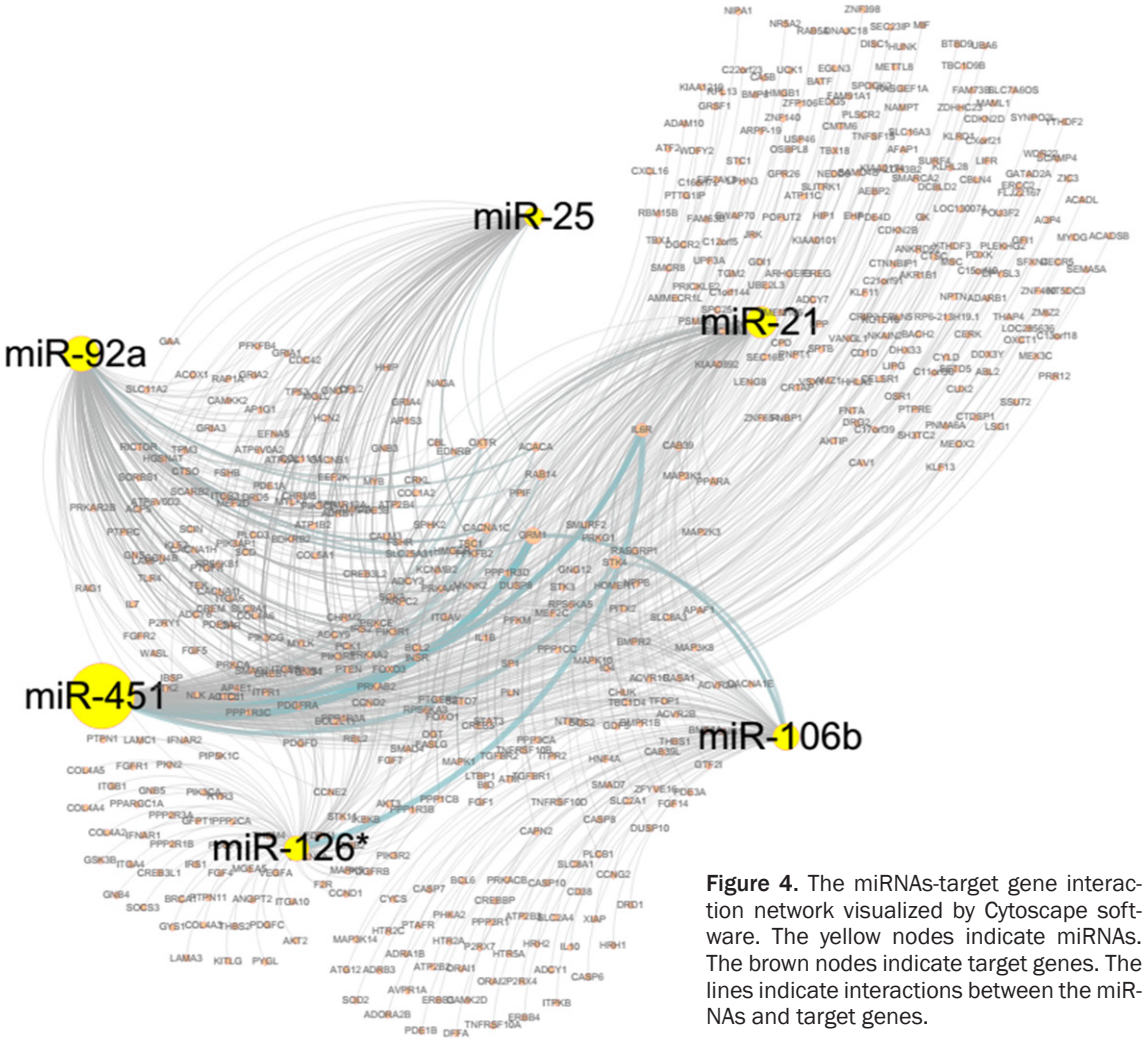

Figure 4. The miRNAs-target gene interaction network visualized by Cytoscape software. The yellow nodes indicate miRNAs. The brown nodes indicate target genes. The lines indicate interactions between the miRNAs and target genes.

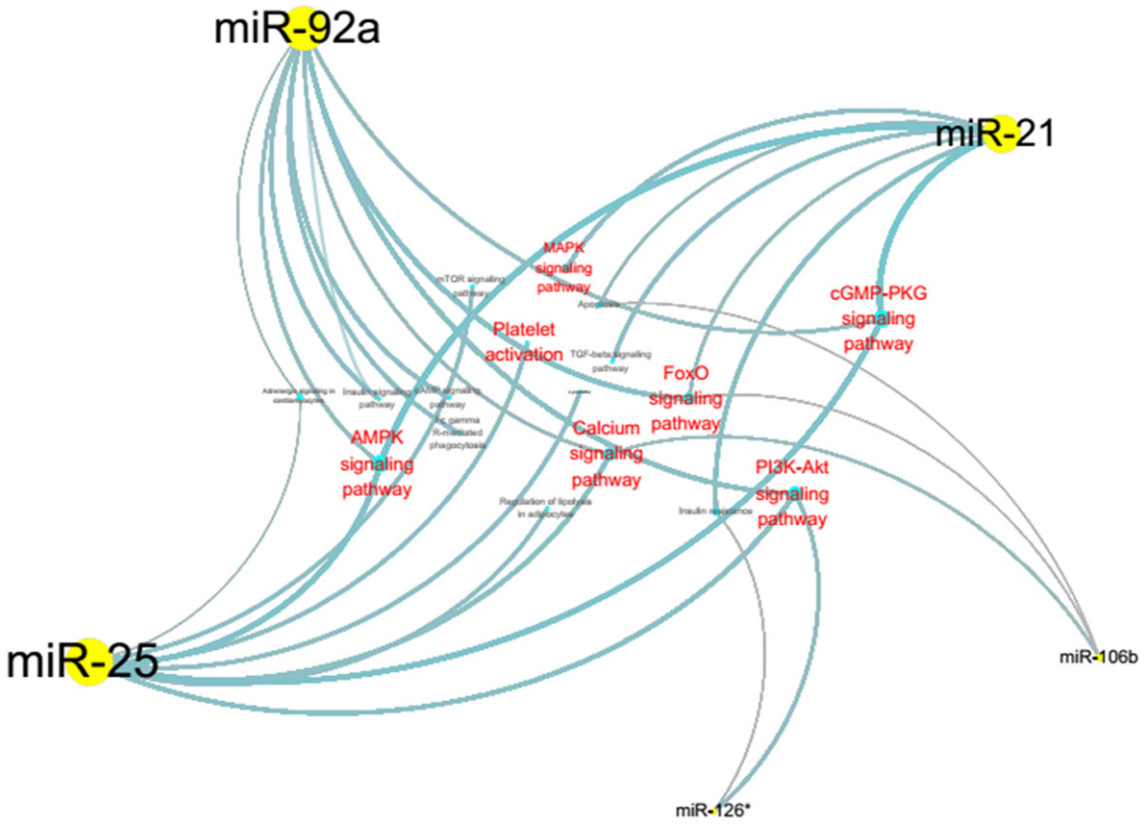

Figure 5. The miRNAs-signaling pathway interaction network visualized by Cytoscape software. The yellow nodes indicate miRNAs. The blue nodes indicate signaling pathways. The lines indicate target genes. The target genes establish linkages between miRNAs and signaling pathways.

Discussion

Early detection of ACS in the ED is crucial for improving the quality of patient care and reducing medical costs. For this reason, an association between the overexpression of some miRNAs and myocardial infarction has been the subject of several studies. Some research has indicated a link between miRNAs and patients with cardiogenic chest pain in the ED [8]. For example, levels of miR-221-3p were elevated in AMI patients who were admitted to the ED with chest pain [9], and miR-423-5p and miR-30d levels were also elevated in ST-elevation myocardial infarction (STEMI) patients with chest pain on presentation to the ED [10]. Circulating miR-133a was upregulated in UA and AMI patients with chest pain admitted to the ED [8]. In UA and MI patients, miR-486 and miR-92a prevailed in cases with high levels of high-density lipoprotein cholesterol, and their levels discriminate between vulnerable and stable coronary artery disease patients [11]. Serum miRNA-210 and miRNA-499 were associated with non-STEMI and UA patients with chest pain in the ED [12]; miR-122-5p may be a useful biomarker for diagnosis of AMI patients [13]. Levels of miR-208b, miR-320a, and miR-499 were significantly higher in AMI patients [14].

There has been less interest and research that focused on miRNAs in UA, although early diagnosis and treatment is essential to avoid myocardial infarction and related complications. The present study draws attention to the role of six miRNAs and their elevation in UA. This may be essential in distinguishing patients with UA from those with NICP.

Previous studies have confirmed that miRNAs are involved in the different pathologies of coronary heart disease [6, 15]. The expression of miR-21 is upregulated after injury of large vessels, and recovery to normal levels can prevent restenosis [16]. Overexpression of miR-25 can protect cardiomyocytes from oxidative damage [17]. The miR-106b~25 cluster participates in

the regulation of post-ischemic neovascularization [18]. Hypoxia can activate of miR-92a in endothelial cells that can block angiogenesis [19]. MiR-126 is also rich in endothelial cells that can influence susceptibility to atherosclerosis by regulating endothelial cell function [20]. miR-451 can regulate the proliferation and differentiation of erythrocytes under oxidative stress [21].

In the present study, to evaluate the underlying function of miRNAs in UA, a complete bioinformatic analysis was performed of validated miRNAs. It may be that miRNAs are involved in multiple signaling pathways by regulating multiple target genes, and the interrelations of miRNAs and signaling pathways are complex. MiRNAs may take part in platelet activation, cGMP-PKG, AMPK, PI3K-Akt, FoxO, and the MAPK signaling pathways. The cGMP-PKG pathway has a cardioprotective role in ischemia and reperfusion injury [22]. Nitrous oxide and its secondary messenger cGMP are important in the process [22]. The AMPK signaling pathway can promote cardiac protection against ischemia [23, 24]. The PI3K-Akt signaling pathway may underlie thrombosis in ACS [25]. The activity of FoxO is important in maintaining endothelial quiescence [26].

Results of the present study show that levels of miR-21, miR-25, miR-92a, miR-106b, miR-126* and miR-451 are elevated in patients with UA. Compared with the NICP control group, the levels of the six miRNAs remained higher after adjustment for the risk factors (age, hypertension, hyperlipidemia, and diabetes). Moreover, this study highlights how the combination of these miRNAs and m-HS can increase the specificity and sensitivity for detecting patients with UA. The HEART score has been used to estimate the risk of patients with chest pain in the ED [27], and the HEART score can facilitate accurate diagnosis and improve therapeutic choices [7] [28]. The score system has five components: past medical history, ECG, age, risk factors, and troponin [7]. In the present study, objective index (ECG, age, risk factors, and troponin) were selected to define an m-HS. The AUC of m-HS and miR-25 were 0.874 and 0.649 respectively. The combination of miR-25 and m-HS (AUC 0.901, NRI 0.096) can better differentiate UA than m-HS alone.

In summary, this study provides evidence that the expression profiles of circulating miRNAs may have an essential role in the diagnosis of UA among patients with chest pain in the ED. This evidence, which is stronger in association with m-HS, is an interesting starting point that needs further research and validation, and is an important advancement toward providing better management of chest pain of patients in the ED.

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Disclosure of conflict of interest

None.

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