Plasma Levels of miR-125b-5p and miR-206 in Acute Ischemic Stroke Patients After Recanalization Treatment: A Prospective Observational Study

Xin-Wei He, MD,*,†,[1] Yan-Hui Shi, MD,*,†,[1] Rong Zhao, MD, PhD,*,†,[1] Yi-Sheng Liu, MD,*,† Ge-Fei Li, MD,* Yue Hu, MD,* Wei Chen, MD, PhD,*,† Guo-Hong Cui, MD, PhD,*,† Jing-Jing Su, MD, PhD,*,† and Jian-Ren Liu, MD, PhD*,†

Introduction: Multiple microRNAs (miRNAs) participate in the response to hypoxic/ischemic and ischemia-reperfusion events. However, the expression of these miRNAs in circulation from patients with acute ischemic stroke (AIS) receiving recanalization treatment has not been examined, and whether they are associated with the severity and outcome of stroke is still unknown. *Materials and methods:* In this prospective cohort study, plasma levels of miR-125b-5p, miR-15a-3p, miR-15a-5p, and miR-206 were measured at 24 hours after thrombolysis with or without endovascular treatment in 94 patients with AIS, as determined by qRT-PCR. Stroke severity was assessed based on National Institutes of Health Stroke Scale (NIHSS) score and infarct lesion. Intracranial haemorrhage (ICH) was recorded. An unfavorable outcome was defined as a modified Rankin Scale score greater than 2 at day 90 after stroke. *Results:* miR-125b-5p and miR-206 levels were correlated with NIHSS scores ($P = .014$ and $P = .002$) and cerebral infarction volumes ($P = .025$ and $P = .030$). miR-125b-5p levels were significantly higher in patients with an unfavorable outcome than in patients with a favorable outcome ($P = .002$) and showed good diagnostic accuracy in discriminating the presence of an unfavorable outcome (area under the curve .735, 95% confidence interval .623-.829, $P < .001$). No association was found between different miRNAs and ICH. *Conclusions:* In AIS patients after thrombolysis with or without endovascular treatment, miR-125b-5p is a novel prognostic biomarker highly associated with an unfavorable outcome. miR-125b-5p and miR-206 levels are associated with stroke severity.

Key Words: Ischemic stroke—thrombolysis—microRNA—miR-125b-5p—miR-206

Abbreviations: miRNAs, microRNAs; AIS, acute ischemic stroke; NIHSS, National Institutes of Health Stroke Scale; ICH, intracranial hemorrhage; mRS, modified Rankin Scale; I/R, ischemia-reperfusion; MCAO, middle cerebral artery occlusion; OGD, oxygen and glucose deprivation; rtPA, recombinant tissue type plasminogen activator; CT, computed tomography; DWI, diffusion-weighted imaging; TOAST, Trial of Org 10172 in Acute Stroke Treatment; ROC, receiver-operating characteristic; AUC, area under curve

From the *Department of Neurology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; and †Clinical Research Centre, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Received January 17, 2019; accepted February 16, 2019.

Financial Disclosure: This work was supported by the Biomedicine Key Programme of the Shanghai Municipal Science and Technology Commission [16411953100], the National Natural Science Foundation of China [81271302], a science popularization project and research innovation project of the Shanghai Municipal Science and Technology Commission [18dz2313603 and No. 14JC1404300], the "Prevention and Control of Chronic Diseases Project" of Shanghai Hospital Development Centre [SHDC12015310], a project of Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support [20161422], and the Clinical Research Project of Shanghai Jiao Tong University School of Medicine [DLY201614].

Address correspondence to Jing-Jing Su and Jian-Ren Liu, Department of Neurology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China. E-mails: jingjingsu2000@163.com, liujr021@sjtu.edu.cn.

[1]These authors contributed equally to this work.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.02.026

Introduction

Acute ischemic stroke (AIS) is a common cause of disability and mortality worldwide and imposes a heavy burden on society.[1] AIS contributes to a loss of brain function, mainly due to a reduction in cerebral blood flow in an area of the brain. As new technologies were developed, recanalization by intravenous thrombolysis and/or combined with endovascular treatment grew to be the most effective strategy for AIS in clinics worldwide.[2,3] However, there are also possible complications after recanalization, such as intracerebral hemorrhage (ICH) and brain edema. Cerebral ischemia-reperfusion (I/R) injury is proposed to be one of the most common complications following recanalization.[2,4]

MicroRNAs (miRNAs) are endogenous approximately 23 nucleotides conserved RNAs that play important gene-regulatory roles, which may serve as diagnostic and prognostic markers due to their easy detection and stability in peripheral blood.[5]

Evidence from experimental studies implicated multiple miRNAs participate in the response to I/R injury and hypoxic/ischemic events.[6] For example, miR-125b-5p is increased in the middle cerebral artery occlusion model, and overexpression of miR-125b-5p exacerbates the injury induced by oxygen and glucose deprivation (OGD).[7] In addition, miR-125b-5p is involved in the acute response to myocardial I/R injury and myocardial infarction.[8,9] miR-15a is increased in the brain after I/R injury or transient ischemia.[10-12] Genetic deletion or pharmacological inhibition of the miR-15a/16-1 cluster significantly reduced I/R brain injury.[13] Similarly, inhibition of miR-15 has been shown to protect against myocardial I/R injury.[14] miR-206 is involved in I/R injury in cerebral ischemia,[15] and myocardial infarction.[16]

However, these studies were performed primarily in animal models or in vitro, and few studies have investigated whether these I/R injury-related miRNAs in circulation are associated with stroke in humans, particularly after recanalization treatment, which is more likely to be linked to I/R injury. Thus, the aim of this study was to comprehensively investigate whether the plasma levels of candidate I/R- and hypoxic/ischemic-related miRNAs, including miR-125b-5p, miR-15a-3p, miR-15a-5p and miR-206, can be effective indicators of stroke severity and functional outcome in AIS patients receiving recanalization treatment.

Materials and Methods

Study Participants

Patients with AIS receiving recombinant tissue-type plasminogen activator (rt-PA) within 4.5 hours of their symptom onset were consecutively recruited from February 2015 to April 2018 in the department of neurology of Shanghai Ninth People's Hospital. Inclusion and exclusion criteria for intravenous rt-PA were used according to the 2013 Guidelines for the early management of patients with acute ischemic stroke.[17] The dose of rt-PA (Actilyse, Boehringer Ingelheim, Germany) is .9 mg/kg (an upper limit of 90 mg), with 10% of the total dosage as a bolus within 1 minute and the rest as a continuous intravenous infusion over a period of 60 minutes.

In addition, subjects with severe renal, liver or thyroid failure; acute infectious disease; rheumatic immune or hematologic disease; or cancer were excluded, as were patients who were unable to cooperate with most clinical examinations or whose samples might be hemolyzed were also excluded. A flow chart of the present study is provided in Figure 1.

The study was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. All procedures were performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. All patients or their next of kin provided written informed consent for the collection of data, blood samples, and subsequent analyses.

Clinical Protocol

On arrival at the emergency department, patients underwent standard neurological examinations, routine blood and biochemistry, and noncontrast computed tomography (CT). Stroke onset was defined as the last time the patient was known to be without any neurological deficit. Neurological deficit was assessed by the National Institutes of Health Stroke Scale (NIHSS).[18] All patients underwent a CT scan at 24 hours or whenever neurological worsening occurred to evaluate the presence of intracranial hemorrhage (ICH). Additionally, magnetic

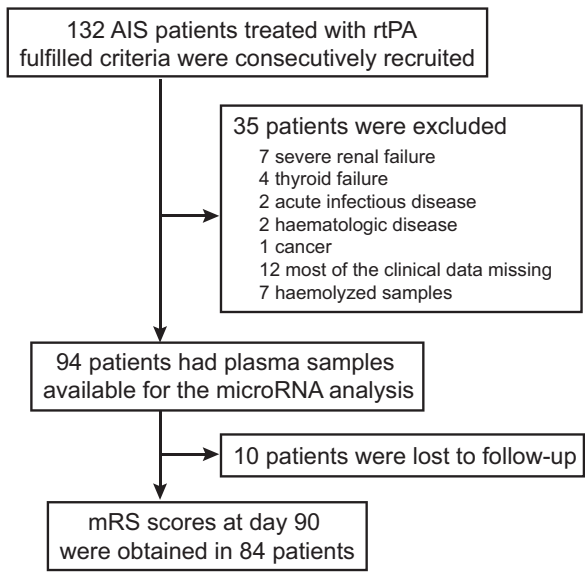

Figure 1. ***Study flow chart****. Abbreviations: AIS, acute ischemic stroke; mRS, modified Rankin Scale; rt-PA, recombinant tissue-type plasminogen activator.*

resonance imaging with diffusion-weighted imaging and/or fluid-attenuated inversion recovery imaging was performed in the following days. Stroke etiology was determined according to the criteria of the Trial of Org 10172 in Acute Stroke Treatment criteria.[19]

Determination of Severity and Prognosis

Stroke severity was assessed based on NIHSS score and infarct lesion. Mild stroke was defined as an NIHSS score <8 on admission, and moderate-to-severe stroke weres defined as an NIHSS score ≥8 on admission, consistent with the existing literature.[20,21] The infarct volume was quantified on the diffusion-weighted imaging and/or fluid-attenuated inversion recovery (in 65 patients). The modality with the largest infarct size was used for volumetry, calculated as the sum of the infarcted area of every slice multiplied by the slice thickness, based on several previous studies.[22] Symptomatic ICH was defined according to the European Cooperative Acute Stroke Study (ECASS) II criteria.[23] Functional outcome evaluation was performed at day 90 after stroke using the modified Rankin Scale (mRS),[24] with a structured follow-up telephone interview conducted by a trained medical investigator who was unaware of the patients' baseline parameters. Outcome was dichotomized as favorable outcome (a score of 0-2) or unfavorable outcome (a score of 3-6), respectively.

Collection and Definition of Clinical Data

The following data were collected, including vital signs, related previous medical history, cardiovascular risk factors, associated laboratory tests, and imaging information. Risk factors were defined as follows: hypertension (systolic blood pressure and average diastolic blood pressure ≥140/90 mmHg or on antihypertensive treatment), diabetes mellitus (fasting blood glucose level ≥7.0 mmol/L or use of antidiabetic medications or insulin), dyslipidemia (serum triglycerides >1.7 mmol/L, low-density lipoprotein >3.4 mmol/L, high-density lipoprotein cholesterol <.8 mmol/L, or use of lipid-lowering drugs), smoking (smoked at the time of stroke or had quit smoking within 1 year of the stroke), and alcohol consumption (>2 standard alcoholic beverages consumed per day).

Blood Samples Collection and miRNA Assessment

Blood samples were collected in tubes with Ethylenediaminetetraacetic acid anticoagulant at 24 hours after thrombolytic treatment. After centrifugation at 1600 g at 4°C for 10 minutes, plasma was aliquoted and frozen at −80°C until analysis. Each sample was thawed only once prior to use.

Plasma total RNA was extracted and purified from plasma samples using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany; Catalogue number 217184). The synthetic *C. elegans* miR-39 (cel-miR-39, Qiagen; 219610) was added to plasma after lysis as an external reference, which is devoid of sequence homology to human miRNAs. RNA was reverse transcribed with the miScript reverse transcription kit (Qiagen; 218161). The expression of miRNA was tested by real-time PCR using the miScript SYBR Green PCR kit (Qiagen; 218073). Specific miRNA primers were obtained from TIANGEN biotech CO., LTD (Beijing, China). The experimental process is operated mainly according to the manufacturer's instructions. Each test was repeated 3 times. The expression levels of different miRNAs were normalized with cel-miR-39 according to the formula $2\exp[-(C_T\text{microRNA} - C_T\text{cel-miR-39})]$ and compared as previously described.[25]

Statistical Analyses

Continuous variables were described as the means ± standard deviation (SD) or medians and interquartile range. The normality of the data was evaluated by the Shapiro-Wilk test. For miRNAs, the distribution of raw data was skewed. The distribution of these data approximated a normal distribution after $\log_{10}$ transformation. In 2-group comparisons, unpaired Student's *t*-test or Mann-Whitney *U* test was used, as appropriate. Categorical variables were expressed as frequency (percentage) and were compared using Pearson's chi-squared statistic or Fisher exact test. Spearman's rank correlation test was used to determine the strength of correlation. A receiver operating characteristic (ROC) curve analysis was applied to determine diagnostic accuracy with the area under curve (AUC), and the cut-off point was calculated according to the Youden index. Multivariate analysis was performed using a logistic regression model, adjusted for potential influenced factors, selected based on previous literature and the results of univariate and correlation analyses. The results were expressed as adjusted odds ratios along with the corresponding 95% confidence intervals (95% CIs). Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, USA) and MedCalc 12.5 (MedCalc Software, Ostend, Belgium). A P value <.05 was considered statistically significant.

Results

Baseline Characteristics of Patients

A total of 94 patients were enrolled in the present study. The baseline demographic and stroke characteristics of the patients are shown in Table 1. The average age of the present cohort was 68.1 ± 12.2 years, and 70.2% of the patients were male. The median NIHSS on admission was 5 (interquartile range 2-12). The median time to treatment with rt-PA was 141 (107, 187) minutes. A total of 21.3% patients received subsequent endovascular treatment (19 embolectomy and 1 balloon dilatation). According to the Trial of Org 10172 in Acute Stroke Treatment criteria, 34.0% of

Table 1. *Baseline characteristics of all patients in the aggregate as well as stratified by mRS*

	All patients (n = 94)	mRS ≤2 (n = 61)	mRS > 2 (n = 23)	*P* value
Demographic data				
Age, years	68.1 ± 12.2	66.0 ± 11.7	74.7 ± 13.0	.004
Male, n (%)	66 (70.2)	43 (70.5)	16 (69.6)	.934
Stroke risk factors, n (%)				
Hypertension	78 (83.0)	52 (85.2)	18 (78.3)	.515
Diabetes mellitus	34 (36.2)	22 (36.1)	9 (39.1)	.795
Dyslipidemia	61 (64.9)	43 (70.5)	13 (56.5)	.226
History of stroke	15 (16.0)	5 (8.2)	7 (30.4)	.015
Coronary heart disease	20 (21.3)	13 (21.3)	6 (26.1)	.641
Atrial fibrillation	21 (22.3)	12 (19.7)	8 (34.8)	.147
Smoking	37 (39.4)	28 (45.9)	4 (17.4)	.016
Alcohol consumers	26 (27.7)	21 (34.4)	4 (17.4)	.128
Medication uses, n (%)				
Hypertension medication use	54 (57.4)	37 (60.7)	13 (56.5)	.731
Diabetes medication use	18 (19.1)	15 (24.6)	3 (13.0)	.373
Lipid-lowering medication use	28 (29.8)	23 (37.7)	2 (8.7)	.010
Laboratory values				
HbA1c, %	6.0 (5.6, 6.7)	6.0 (5.6, 7.7)	6.3 (5.5, 6.7)	.689
Homocysteine, μmol/L	12.7 (9.5, 16.2)	12.9 (9.9, 16.3)	12.5 (9.1, 18.1)	.950
CRP, mg/L	3.3 (3.0, 7.2)	3.3 (3.0, 6.4)	4.67 (3.1, 11.6)	.161
Stroke evaluation				
NIHSS on admission, points	5 (2, 12)	4 (2, 9)	12 (7, 18)	<.001
Infarct volumes, mm^3	8.3 (1.9, 31.6)	8.3 (1.6, 20.6)	30.7 (8.0, 214.9)	.039
ICH after 24 hours, n (%)	11 (11.7)	5 (8.3)	6 (28.6)	.030
Time to rt-PA treatment, min	141 (107, 187)	150 (110, 185)	140 (104, 208)	.931
Endovascular treatment, n (%)	20 (21.3)	9 (14.8)	9 (39.1)	.016
TOAST subtype, n (%)				.037
Large-artery atherosclerosis	32 (34.0)	22 (36.1)	7 (30.4)	
Cardioembolism	20 (21.3)	9 (14.8)	9 (39.1)	
Small-vessel occlusion	29 (30.9)	23 (37.7)	3 (13.0)	
Other determined/undetermined	13 (13.8)	7 (11.5)	4 (17.4)	
mRS on day 90, points	1 (0, 3)	1 (0, 1)	3 (3, 4)	-

Abbreviations: CRP, C-reactive protein; HbA1c, glycated hemoglobin; ICH, intracranial hemorrhage; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; rt-PA, recombinant tissue-type plasminogen activator; TOAST: Trial of Org 10172 in Acute Stroke Treatment.

Values are presented as the mean ± SD or median (interquartile range) for continuous variables and number (percentages) for categorical variables.

The *P* values reflect comparisons between the two groups stratified by mRS.

patients were large-artery atherosclerosis, 21.2% were cardioembolism, 30.9% were small-vessel occlusion, and 13.8% were other determined/undetermined etiologies.

miR-125b-5p and miR-206 Levels were Associated with Stroke Severity

The NIHSS score of patients was 5 (2, 12) on admission (in 94 patients), 2 (1, 7) at 24 hours (in 89 patients), and 1 (0, 5) on day 7 (in 88 patients) after thrombolysis. miR-125b-5p levels were correlated with NIHSS scores on admission ($R = .262$, $P = .014$; Table 2). miR-206 levels were correlated with NIHSS scores on day 7 after thrombolysis ($R = .341$, $P = .002$; Table 2). Besides, both miR-125b-5p and miR-206 levels were correlated with cerebral infarction volumes ($R = .292$, $P = .025$ and $R = .304$, $P = .030$; Table 2).

Additionally, miR-125b-5p and miR-206 levels were higher in patients with a moderate-to-severe stroke than in patients with a mild stroke (log_{10}-transformed, −2.71 ± 0.35 versus −3.02 ± .35, $P < .001$, and −2.95 ± .85 versus −3.42 ± .74, $P = .011$; Fig 2A and B; Supplementary Table S2).

Higher miR-125b-5p Levels were Found in Patients With an Unfavourable Outcome

At day 90 after stroke onset, 84 patients were followed up. The baseline characteristics of these subjects did not significantly differ from those of the 94 total patients (Supplementary Table S1). A total of 61 (72.6%) patients had a favorable outcome, and 23 (27.4%) patients had an unfavorable outcome. In the univariate analysis, patients with an unfavorable outcome were significantly older, had higher NIHSS scores on admission, included a greater

Table 2. *Correlation between different microRNAs and National Institutes of Health Stroke Scale (NIHSS) scores and stroke infarct volumes*

	NIHSS on admission		NIHSS at 24 h		NIHSS on day 7		Infarct volumes	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
miR-206	.161	.139	.189	.090	.341	**.002**	.304	**.030**
miR-125b-5p	.262	**.014**	.209	.058	.188	.091	.292	**.025**
miR-15a-3p	.187	.094	.174	.127	.143	.221	−.139	.294
miR-15a-5p	.060	.573	.158	.146	.071	.519	−.073	.562

Abbreviation: NIHSS, National Institutes of Health Stroke Scale.

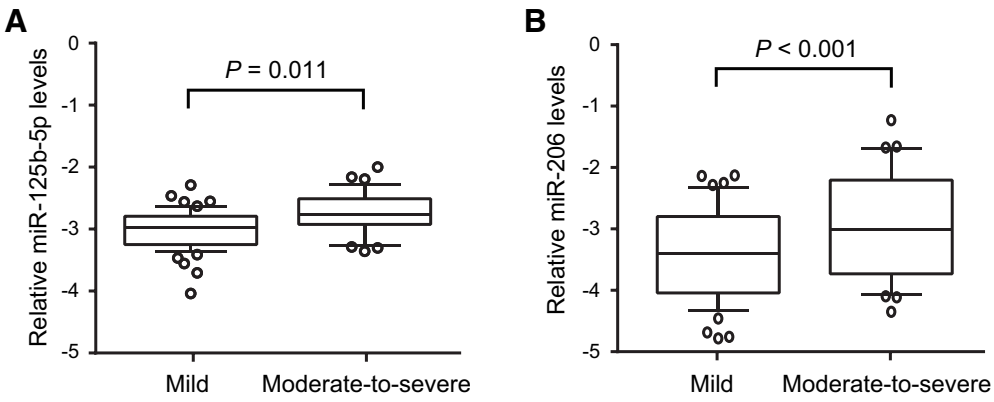

Figure 2. ***Comparison of plasma miR-206 (A) and miR-125b-5p (B) between patients stratified by National Institutes of Health Stroke Scale (NIHSS).*** *Box plots show the expression levels. Differences were compared by Student's* t*-test. Mild stroke was defined as an NIHSS score <8 on admission; Moderate-to-severe stroke was defined as an NIHSS score ≥8 on admission.*

percentage of history of stroke and smoking, and a greater likelihood of cardioembolism (Table 1). miR-125b-5p levels were significantly higher in the patients with an unfavorable outcome than in the patients with a favorable outcome (log_{10}-transformed, −2.67 ± .34 versus −2.97 ± .38, $P = .002$; Table 3 and Fig 3A). A subsequent multiple logistic regression analysis demonstrated that miR-125b-5p levels were independently predictive of an unfavorable outcome after adjusting for significant variables (adjusted odds ratios, 2.682, 95% CI 1.344-5.353, $P = .005$; Table 3). miR-206, miR-15a-3p, and miR-15a-5p levels did not differ between the patients grouped by outcome (Table 3).

miR-125b-5p Levels Showed Good Diagnostic Accuracy in Discriminating Patients With an Unfavorable Outcome

The ROC curve analysis showed that the AUC value of miR-125b-5p that discriminated the presence of an unfavorable outcome of stroke was 0.735 (95% CI 0.623-0.829, $P < .001$; Fig 3B), with a sensitivity of 86.36% and a specificity of 55.36%.

No Association was Found Between Different miRNAs and ICH

ICH complications were detected in 9 patients, no microRNA was independently associated with the

Table 3. *Relation of different microRNAs to functional outcome*

	mRS ≤2	mRS >2	*P* value	Adjusted OR (95% CI)*	Adjusted *P* value*
miR-206	−3.29 ± 0.76	−3.10 ± 0.93	.365	-	-
miR-15a-3p	−3.37 ± 0.61	−3.18 ± 0.66	.243	-	-
miR-15a-5p	−1.62 ± 0.56	−1.43 ± 0.62	.201	-	-
miR-125b-5p	−2.97 ± 0.38	−2.67 ± 0.34	.002	2.682 (1.344, 5.353)	.005

Abbreviations: CI, confidence interval; mRS, modified Rankin Scale; OR, odds ratio.
Log_{10}-transformed values were used.
*Adjusted by age, history of stroke, smoking, National Institutes of Health Stroke Scale on admission, and intracranial haemorrhage after 24 hours of endovascular treatment.

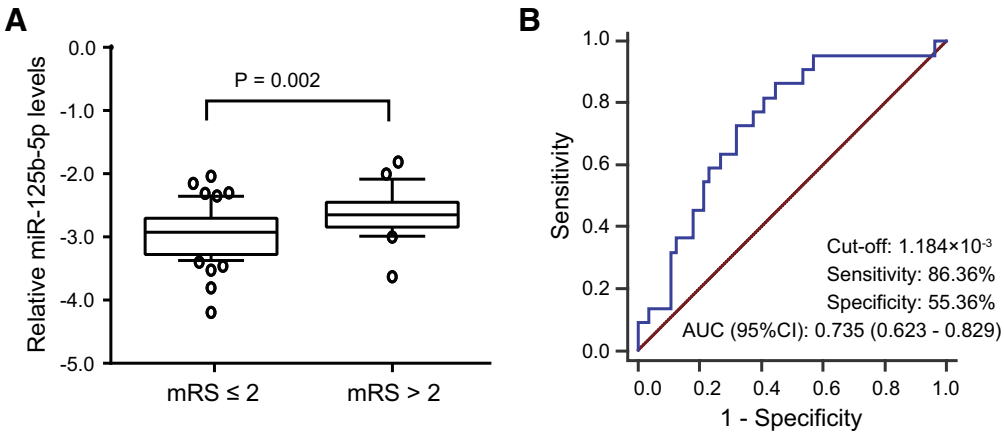

Figure 3. ***miR-125b-5p levels and stroke outcome.*** *(A) Comparison of the relative levels of plasma miR-125b-5p between patients stratified by mRS. Box plots show the expression levels. Differences were compared by Student's* t*-test. (B) Diagnostic accuracy of miR-125b-5p in discriminating patients with an unfavorable outcome. Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; mRS, modified Rankin Scale.*

occurrence of ICH (Supplementary Table S3). In addition, for only 2 patients who had symptomatic ICH, we did not analyse symptomatic ICH alone.

Discussion

The aberrant expression of miRNAs in patients with AIS and their clinical association with AIS have been reported in several studies.[22,26] Nevertheless, it has also been confirmed that a variety of miRNA expression is altered after thrombolysis treatment, whether in intracranial or extracranial.[27,28] Thus, whether these alternative miRNAs can also be used as independent predictors after recanalization has not been fully addressed. In addition, in view of the presence of cerebral I/R injury after recanalization, I/R injury-related miRNAs in circulation might be associated with AIS.

To the best of our knowledge, this study is the first to find that miR-125b-5p levels were significantly elevated in patients with an unfavorable outcome and showed good diagnostic accuracy in discriminating the presence of an unfavorable outcome after AIS. A previous study found that miR-125b-5p has potential clinical utility as a diagnostic marker for the occurrence of AIS, and the authors suggested platelet as a major source of elevated miR-125b-5p, which might be related to platelet aggregation or thrombus formation.[22] To some extent, our findings further agree with the above observation, as thrombolysis is the breakdown of blood clots by targeting fibrin.

In addition, we found that miR-125b-5p levels were correlated with NIHSS scores and infarct volumes, and miR-125b-5p levels were higher in patients with a moderate-to-severe stroke than with a mild stroke after thrombolysis, thus evidencing a relationship between miR-125b-5p and stroke severity. The possible role of miR-125b-5p in the central nervous system has not been completely elucidated. A recent study found that miR-125b-5p levels are increased in the middle cerebral artery occlusion model as well as the OGD model.[7] Overexpression of miR-125b-5p exacerbates the OGD-induced injury by reducing cystathionine β-synthase expression and subsequently hydrogen sulfide generation, which may be related to the antioxidant action as well as the antiapoptotic effect.[7] Additionally, miR-125b-5p may be involved in post-transcriptional regulation of the inflammatory and immune response, such as enhancing the activation of macrophage and its role in stimulating T cell activation,[29] attenuating monocyte chemoattractant protein-1 production in macrophages,[30] and regulating TNF-α expression in neonatal monocytes.[31] Overall, the specific functions of miR-125b-5p in the prognosis and severity after AIS in the present study remain at a speculative stage and require further investigation.

It should be noted that miR-125b-5p acts as an ischemic stress protector after acute myocardial infarction, opposing cardiomyocyte apoptosis by repressing pro-apoptotic bak1 (a mitochondrial protein) and klf13 (a zinc finger transcription factor).[8] The increased expression of miR-125b significantly decreases infarct size and prevents I/R injury in the myocardium.[9] These reports may indicate that the specific temporal and spatial expression patterns of miR-125b-5p have different biological functions.

We demonstrated that patients who had suffered a moderate-to-severe stroke had higher miR-206 levels than those who had a mild stroke. Although clinical studies of miR-206 in stroke are scarce, a previous basic study observed that miR-206 expression is increased and positively correlated with the infarct sizes in brain tissues after cerebral ischemia.[32] In addition, neuronal cells show increased cell survival and maintenance of neurites following OGD deprivation when miR-206 inhibitor is transfected.[32] Similarly, overexpression of miR-206 inhibits neuronal cell proliferation and viability, partly mediated by inhibition of orthodenticle homeobox 2, playing an

important role in neurodevelopment.[15] Furthermore, the inhibition of miR-206 has antiapoptotic and migration-promoting effects in mesenchymal stem cells during hypoxic preconditioning.[33]

On the other hand, numerous studies have confirmed that acute cerebral ischemia stimulates angiogenesis for the formation of new brain microvessels.[34] miR-206 was demonstrated to negatively regulate angiogenesis by inhibiting vascular endothelial growth factor and then controlling the strength of angiogenic signalling to the endothelium.[35] Additionally, knockdown of miR-206 in endothelial progenitor cells rescues their angiogenic and vasculogenic abilities, which is an important process in the repair of the vasculature.[36] Taken together, these data implicate role of the increased miR-206 in moderate-to-severe stroke rather than simple acute behavior, although the specific mechanisms still need to be clarified.

Our study failed to show a significant association between miR-15a-3p and miR-15a-5p and stroke in AIS patients with thrombolysis. Dysregulation of the miR-15a/16-1 cluster in plasma has been reported previously in stroke patients.[37] A recent study found that miR-15a levels are not only increased after stroke but also correlated with NIHSS scores in AIS patients, and patients with higher miR-15a levels had a better prognosis.[38] The different conclusions may be attributed to diverse clinical conditions, distinct sample collection times, and heterogeneity among different populations. In addition, alteplase treatment may change the expression of certain miRNAs.[27,28]

This study has several limitations. First, although our results were significant, our sample size was relatively small. Therefore, our preliminary results require further validation in larger studies. Second, although we included only blood samples collected at 24 hours after thrombolytic therapy to reduce the possibility of confounding by variation in time, we could not derive any information on the dynamic alteration. A longitudinal study including multiple time points covering the pre- and post-thrombolytic periods may better identify certain changes and determine the best time point. Finally, due to the focus of this study on clinical relevance, we did not completely elucidate the biological mechanisms. Cerebral ischemia can trigger biological reactions and alter pathological events in both intracranial and peripheral systems. It is unclear whether the altered levels of miRNAs are predominantly from the brain due to the disruption of the blood-brain barrier or from any other tissues. Further functional studies are needed to provide deeper insight into their sources and biological implications.

Conclusions

In conclusion, we provided preliminary evidence that the plasma levels of miR-125b-5p are elevated in patients with an unfavorable outcome and miR-125b-5p can be an independent prognostic marker in AIS patients receiving recanalization therapy. In addition, we showed that the levels of miR-125b-5p and miR-206 are associated with stroke severity. While the precise cellular sources and mechanisms underlying the observed elevations remain to be determined, we have expanded the knowledge and application of miRNAs in AIS patients with recanalization therapy.

Declarations of Interest

None.

Acknowledgements: The authors thank the nurses and students of the Neurology Department of our hospital who have substantially assisted with the study.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2019.02.026.

References

1. Donnan GA, Fisher M, Macleod M, et al. Stroke. Lancet 2008;371:1612-1623.
2. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317-1329.
3. Muruet W, Rudd A, Wolfe CDA, et al. Long-term survival after intravenous thrombolysis for ischemic stroke: a propensity score-matched cohort with up to 10-year follow-up. Stroke 2018;49:607-613.
4. Sun MS, Jin H, Sun X, et al. Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization Therapy. 2018;2018: 3804979.
5. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215-233.
6. Di Y, Lei Y, Yu F, et al. MicroRNAs expression and function in cerebral ischemia reperfusion injury. J Mol Neurosci 2014;53:242-250.
7. Shen Y, Shen Z, Guo L, et al. MiR-125b-5p is involved in oxygen and glucose deprivation injury in PC-12 cells via CBS/H2S pathway. Nitric Oxide 2018;78:11-21.
8. Bayoumi AS, Park KM, Wang Y, et al. A carvedilol-responsive microRNA, miR-125b-5p protects the heart from acute myocardial infarction by repressing pro-apoptotic bak1 and klf13 in cardiomyocytes. J Mol Cell Cardiol 2018;114:72-82.
9. Wang X, Ha T, Zou J, et al. MicroRNA-125b protects against myocardial ischaemia/reperfusion injury via targeting p53-mediated apoptotic signalling and TRAF6. Cardiovasc Res 2014;102:385-395.
10. Yin KJ, Deng Z, Hamblin M, et al. Peroxisome proliferator-activated receptor delta regulation of miR-15a in ischemia-induced cerebral vascular endothelial injury. J Neurosc 2010;30:6398-6408.
11. Yin KJ, Fan Y, Hamblin M, et al. KLF11 mediates PPAR-gamma cerebrovascular protection in ischaemic stroke. Brain 2013;136:1274-1287.

12. Sun M, Yamashita T, Shang J, et al. Time-dependent profiles of microRNA expression induced by ischemic preconditioning in the gerbil hippocampus. Cell Transplant 2015;24:367-376.
13. Yang X, Tang X, Sun P, et al. MicroRNA-15a/16-1 antagomir ameliorates ischemic brain injury in experimental stroke. Stroke 2017;48:1941-1947.
14. Hullinger TG, Montgomery RL, Seto AG, et al. Inhibition of miR-15 protects against cardiac ischemic injury. Circ Res 2012;110:71-81.
15. Wang R, Hu Y, Song G, et al. MiR-206 regulates neural cells proliferation and apoptosis via Otx2. Cell Physiol Biochem 2012;29:381-390.
16. Zhai C, Qian Q, Tang G, et al. MicroRNA-206 protects against myocardial ischaemia-reperfusion injury in rats by targeting Gadd45beta. Mol Cells 2017;40:916-924.
17. Jauch EC, Saver JL, Adams Jr. HP, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44:870-947.
18. Brott T, Adams Jr. HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864-870.
19. Adams Jr. HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.
20. He X, Bao Y, Shen Y, Wang E, et al. Longitudinal evaluation of serum periostin levels in patients after large-artery atherosclerotic stroke: a prospective observational study. Sci Rep 2018;8:11729.
21. Kvistad CE, Logallo N, Oygarden H, et al. Elevated admission blood pressure and stroke severity in acute ischemic stroke: the Bergen NORSTROKE Study. Cerebrovasc Dis 2013;36:351-354.
22. Tiedt S, Prestel M, Malik R, et al. RNA-Seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as potential biomarkers for acute ischemic stroke. Circ Res 2017;121:970-980.
23. Larrue V, von Kummer RR, Muller A, et al. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). Stroke 2001;32:438-441.
24. van Swieten JC, Koudstaal PJ, Visser M, et al. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604-607.
25. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008;3:1101-1108.
26. Scherrer N, Fays F, Mueller B, et al. MicroRNA 150-5p improves risk classification for mortality within 90 days after acute ischemic stroke. J Stroke 2017;19:323-332.
27. Zhang L, Chopp M, Liu X, et al. Combination therapy with VELCADE and tissue plasminogen activator is neuroprotective in aged rats after stroke and targets microRNA-146a and the toll-like receptor signaling pathway. Arterioscler Thromb Vasc Biol 2012;32:1856-1864.
28. Xiang W, Tian C, Lin J, et al. Plasma let-7i and miR-15a expression are associated with the effect of recombinant tissue plasminogen activator treatment in acute ischemic stroke patients. Thromb Res 2017;158:121-125.
29. Chaudhuri AA, So AY, Sinha N, et al. MicroRNA-125b potentiates macrophage activation. J Immunol 2011;187:5062-5068.
30. Lu JB, Yao XX, Xiu JC, et al. MicroRNA-125b-5p attenuates lipopolysaccharide-induced monocyte chemoattractant protein-1 production by targeting inhibiting LACTB in THP-1 macrophages. Arch Biochem Biophys 2016;590:64-71.
31. Huang HC, Yu HR, Huang LT, et al. miRNA-125b regulates TNF-alpha production in CD14+ neonatal monocytes via post-transcriptional regulation. J Leukocyte Biol 2012;92:171-182.
32. Liu FJ, Lim KY, Kaur P, et al. microRNAs involved in regulating spontaneous recovery in embolic stroke model. PLoS One 2013;8:e66393.
33. Zhang Y, Lei W, Yan W, et al. microRNA-206 is involved in survival of hypoxia preconditioned mesenchymal stem cells through targeting Pim-1 kinase. Stem Cell Res Ther 2016;7:61.
34. Greenberg DA, Jin K. From angiogenesis to neuropathology. Nature 2005;438:954-959.
35. Stahlhut C, Suarez Y, Lu J, et al. miR-1 and miR-206 regulate angiogenesis by modulating VegfA expression in zebrafish. Development 2012;139:4356-4364.
36. Tang Y, Zhang Y, Chen Y, et al. Role of the microRNA, miR-206, and its target PIK3C2alpha in endothelial progenitor cell function - potential link with coronary artery disease. FEBS J 2015;282:3758-3772.
37. Tan KS, Armugam A, Sepramaniam S, et al. Expression profile of MicroRNAs in young stroke patients. PLoS One 2009;4:e7689.
38. Lu WJ, Zeng LL, Wang Y, et al. Blood microRNA-15a correlates with IL-6, IGF-1 and acute cerebral ischemia. Curr Neurovasc Res 2018;15:63-71.