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Expression of miR-146a-5p in patients with intracranial aneurysms and its association with prognosis

H.-L. ZHANG[1], L. LI[2], C.-J. CHENG[1], X.-C. SUN[1]

[1]Department of Neurosurgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing, China
[2]School of Clinical Medicine, Gansu University of Chinese Medicine, Lanzhou, China

Abstract. – OBJECTIVE: The study aims to detect the association of miR-146a-5p with intracranial aneurysms (IAs).

PATIENTS AND METHODS: The expression of miR-146a-5p was compared from plasma samples between 72 patients with intracranial aneurysms (IAs) and 40 healthy volunteers by quantitative Real-time polymerase chain reaction (qRT-PCR). Statistical analysis was performed to analyze the relationship between miR-146a-5p expression and clinical data and overall survival (OS) time of IAs patients. Univariate and multivariate Cox proportional hazards have also been performed.

RESULTS: Notably, higher miR-146a-5p expression was found in plasma samples from 72 patients with intracranial aneurysms (IAs) compared with 40 healthy controls. Higher miR-146a-5p expression was significantly associated with rupture and Hunt-Hess level in IAs patients. Kaplan-Meier survival analysis verified that higher miR-146a-5p expression predicted a shorter overall survival (OS) compared with lower miR-146a-5p expression in IAs patients. Univariate and multivariate Cox proportional hazards demonstrated that higher miR-146a-5p expression, rupture, and Hunt-Hess were independent risk factors of OS in patients with intracranial aneurysms (IAs).

CONCLUSIONS: MiR-146a-5p expression may serve as a biomarker for predicting prognosis in patients with IAs.

Key Words:
Intracranial aneurysms, microRNAs, miR-146a-5p, Prognosis.

Introduction

Intracranial aneurysms (IAs) occur approximately to 2-3% of the population and exhibit genetic predisposition in selected patients[1,2]. Intracranial aneurysms (IAs) lead to spontaneous subarachnoid hemorrhage (SAH), ultimately disability and mortality[3]. Studies had indicated some risk factors associated with the formation of IAs, such as cigarette smoking, alcohol consumption, hypertension, and so on[4].

MicroRNAs (miRNAs) have been demonstrated to play key regulators in a series of biological processes[5]. The roles of different micro-RNAs (miRNAs) involved in the cardiovascular system have been widely recognized. Some of the miRNAs are significantly altered in IAs patients and function as biomarkers for IAs patients' prognosis[6]. miR-34b/c rs4938723CC and TP53 Arg72-Pro polymorphisms may be involved in the susceptibility to IA[7]. MiR-16 and miR-25 are independent risk factors for IAs occurrence[8]. MiR-92a and KLF2 have a negative correlation in intracranial aneurysm model, and miR-92a could directly target KLF2 in endothelial cells through a complementary sequence of 3'UTR region[9]. Patients with low miR-29a expression have longer disease-free survival (DFS) and overall survival (OS) in IAs patients[10]. However, the association of miR-146a-5p with intracranial aneurysms (IAs) was not reported so far.

In the study, we demonstrated that miR-146a-5p was notably higher expression in plasma samples from 72 patients with intracranial aneurysms (IAs) compared with healthy controls. Higher miR-146a-5p expression predicted a poor overall survival (OS) than patients with lower miR-146a-5p expression. Thus, our results indicated that miR-146a-5p expression may serve as a biomarker for predicting prognosis of IAs.

Patients and Methods

Patient Plasma Samples

72 intracranial aneurysms (IAs) patients were diagnosed using computed tomography (CT), computed tomography angiography (CTA) or digital subtraction angiography (DSA). The 40 con-

Corresponding Author: Xiaochuan Sun, MD; e-mail: sunxiaochuandn@163.com

trol plasma samples were from healthy volunteers without smoking history, high blood glucose and hypertension. Written informed consent was obtained from all patients or the first-degree relatives of the unconscious patients. The study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University.

Prognosis and Follow-up

Glasgow Prognosis Score (GOS) was used to assess the prognosis of IA; The GOS > 3 points was identified as good, while GOS ≤ 3 as poor. The overall follow-up time was three and a half years dated from discharge time of IAs patients after treatment to March 2017. Overall survival (OS) was calculated from the time when aneurismal neck clipping finished to the end of overall follow-up time.

Quantitative Real-time-PCR (QRT-PCR)

The total RNA from the plasma samples was isolated by using a QIAamp circulating nucleic acid kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The RNA was reversed transcription by Superscript First Strand cDNA synthesis system (Invitrogen, Carlsbad, CA, USA). SYBR® Prime Script™ RT-PCR Kit II (TaKaRa, Otsu, Shiga, Japan) was used to detect the mRNA expression. U6 was served as the internal control. The mRNA expression was analyzed using the $2^{-\Delta\Delta Ct}$ methods. Primers sequences for the study were as follow: β-actin forward sequence: 5'-CAGATCCCATCCACGCAGTT-3', β-actin reverse sequence: 5'-ATTGCACGTGTGGCAAGTTC-3'. MiR-146a-5p-forward sequence: 5'-GGCGATGAGAACTGAATTCCA-3'.

Statistical Analysis

Statistical analyses were performed using the SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Student's *t*-test was used to compare the expression of miR-146a-5p in IAs patients and healthy controls. The χ^2-test was used to examine the association between the expression of miR-146a-5p and clinicopathological factors. Univariate and multivariate Cox proportional hazards analyzed the relative risk factors for intracranial aneurysms (IAs). $p<0.05$ were considered statistically significant.

Results

Expression of miR-146a-5p is Upregulated in Plasma Samples from IAs Patients

In the study, we performed the qRT-PCR to assess the relative expression levels of miR-146a-5p from plasma samples of 72 intracranial aneurysms (IAs) patients and 40 healthy volunteers. As listed in Figure 1A, the results showed that miR-146-5p expression levels were notably upregulated in 72 (49/72) intracranial aneurysms (IAs) patients, compared with healthy controls (IAs patients *vs.* control health volunteers; 1.65 ± 0.41 *vs.* 1.05 ± 0.042).

Relative expression of miR-146a-5p Associated with Clinicopathological Factors

According to the mean expression level of miR-146a-5p in IAs patients, we divided IAs patients into two groups: higher miR-146a-5p expression group (miR-146a-5p expression > mean expression rate) and lower miR-146a-5p expression group (miR-146a-5p expression < mean expression rate). The relative clinicopathologic factors of IAs patients was collected and showed in Table I, we assessed the relationship between miR-146a-5p expression and the clinicopathologic factors of IAs patients. The χ^2-test results showed that higher miR-146a-5p expression was positively associated with rupture (p=0.003) and advanced Hunt-Hess level (p=0.015) in IAs patients (Table I).

Association of miR-146a-5p Expression with GOS and OS of IAs Patients

To further explore the clinical significance of miR-146a-5p expression in IAs patients, we assessed the association of miR-146a-5p expression with OS of IAs patients. Glasgow Prognosis Score (GOS) was used to assess the prognosis of IAs; The GOS > 3 points was identified as good, while GOS≤3 as poor. Univariate and multivariate Cox proportional hazards demonstrated that higher miR-146a-5p expression (HR: 2.188, 95% CI: 1.133-2.588, $p<0.05$), rupture (HR: 2.088, 95% CI: 1.399-2.899, $p<0.05$) and Hunt-Hess level (HR: 2.235, 95% CI: 1.428-3.766, $p<0.05$) were risk factors of prognosis for intracranial aneurysms (IAs) (Table II). Moreover, we observed that higher miR-146a-5p expression group showed a poor outcome compared with lower miR-146a-5p expression group in IAs patients (Figure 1B). Thus, miR-146a-5p expression may serve as a prognostic marker for IAs patients.

Discussion

MicroRNAs could target gene expressions through decreasing transcription or post-transcriptional inducing mRNA decay[11]. Previous stu-

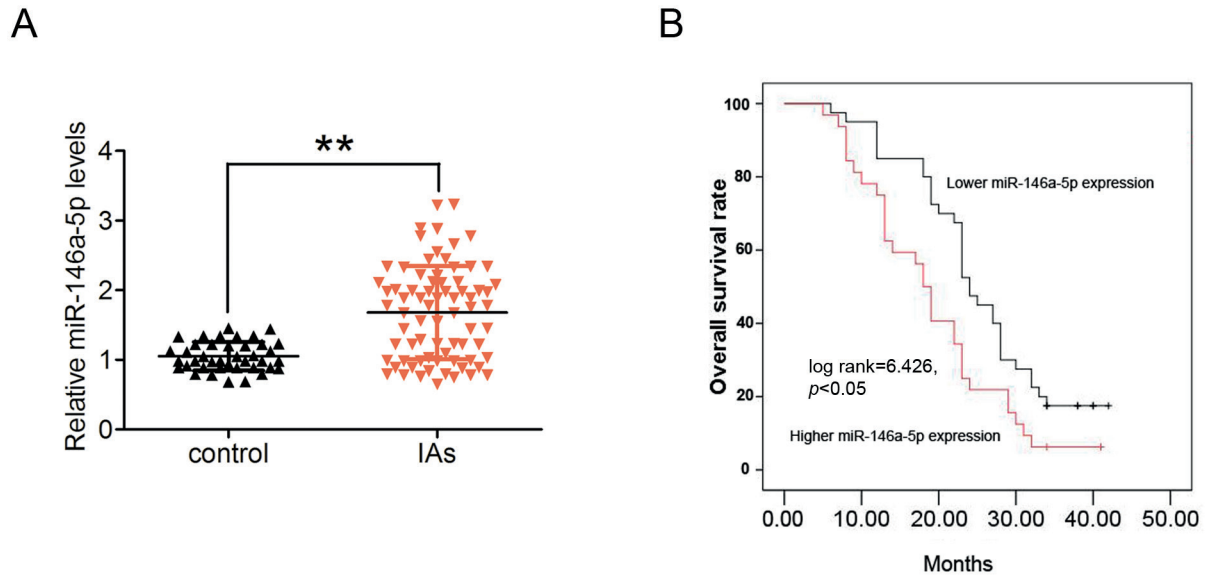

Figure 1. Expression of miR-146a-5p is upregulate in plasma samples from IAs patients. ***A,*** QRT-PCR was performed to assess the relative expression levels of miR-146a-5p from plasma samples of 72 intracranial aneurysms (IA) patients compared to 40 healthy controls, $^{**}p<0.01$. ***B,*** Patients who had a higher miR-146a-5p expression had a poor overall survival time compared with lower higher miR-146a-5p expression in plasma samples (n=72, log rank test).

Table I. Association between MiR-146a-5p expression and clinical factors in patients with intracranial aneurysms (IAs).

Prognostic factors	Number of patients	MiR-146a-5p		*p*-value
		Low (n=40)	High (n=32)	
Age				0.131
≤60	48	24	26	
>60	24	16	8	
Gender				0.081
Female	39	18	21	
Male	33	22	11	
Aneurysm size (cm)				0.556
<10	52	30	22	
>10	20	10	10	
History of hypertension				0.185
Present	32	15	17	
No	40	25	15	
History of smoking				0.624
Present	27	14	13	
No	45	26	19	
Blood glucose (mmol/L)				0.273
>5	18	8	10	
<5	54	32	22	
Rupture				0.003*
Unruptured	27	21	6	
Ruptured	45	19	26	
Hunt-Hess level				0.015*
Level I-III	51	33	18	
Level IV-V	21	7	14	
Aneurysm location				0.598
Anterior circulation aneurysm	34	20	14	
Posterior circulation aneurysm	38	20	18	

*$p < 0.05$.

Table II. Univariate and multivariate Cox proportional hazards analysis of miR-146a-5p expression and prognosis of IAs patients.

	Univariate Cox analysis		Multivariate Cox analysis	
Factor	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Age	0.983 (0.443-1.566)	0.556		
Gender	1.226 (0.668-1.922)	0.448		
Aneurysm size (cm)	1.136 (0.889-1.566)	0.406		
History of hypertension	1.124 (0.615-1.884)	0.398		
History of smoking	0.899 (0.445-1.566)	0.589		
Blood glucose (mmol/L)	0.566 (0.331-1.122)	0.698		
Rupture	2.244 (1.566-3.144)	0.001*	2.088 (1.399-2.899)	0.001*
Hunt-Hess level	2.668 (1.677-4.008)	0.001*	2.235 (1.428-3.766)	0.001*
Aneurysm location	1.089 (0.542-1.889)	0.455		
miR-146a-5p	2.334 (1.322-2.743)	0.001*	2.188 (1.133-2.588)	0.001*

*$p < 0.05$.

dies have demonstrated that miR-146a-5p plays important roles in some human diseases, such as miR-146a-5p acts as a negative regulator of TGF-β signaling in skeletal muscle after acute contusion[12]. MicroRNA-146a promotes cell migration and invasion in human colorectal cancer via carboxypeptidase M/src-FAK pathway[13]. MiR-146a-5p mediates epithelial-mesenchymal transition of oesophageal squamous cell carcinoma via targeting Notch2[14]. However, the association between miR-146a-5p and intracranial aneurysms (IAs) remains larger unknown. We demonstrated that miR-146a-5p was a notably higher expression in plasma samples from patients with intracranial aneurysms (IAs) compared with 40 healthy controls. The χ^2-test was used to examine the association between the expression of miR-146a-5p and clinical factors of IAs patients. We demonstrated that higher miR-146a-5p expression was significantly associated with rupture and Hunt-Hess levels in IAs patients.

In the previous study, some risk-factors related to IA are identified as hypertension, cigarette smoking and excessive alcohol intake, and so on[4,15]. Recent evidence also showed that some microRNAs were involved in IAs progression. By genome-wide miRNA screening analysis, Liu et al[16] found that more than 100 miRNAs were abnormally expressed in human IAs tissues. Wang et al[10] reported that miR-29a expression, tumor aneurysm, rupture, and Hunt-Hess were risk factors to the prognosis of IAs. Further, we demonstrated that higher miR-146a-5p expression predicted a poor overall survival (OS) than patients with lower miR-146a-5p expression in IAs patients. Univariate and multivariate Cox proportional hazards demonstrated that higher miR-146a-5p expression, rupture, and Hunt-Hess were risk factors for intracranial aneurysms (IAs).

Conclusions

We identified that miR-146a-5p expression was upregulated in IAs and associated with a poor overall survival (OS). Moreover, miR-146a-5p functions as an independent risk factor of OS for intracranial aneurysms (IAs) patients. These results indicated that miR-146a-5p may be a potential biomarker in the development of intracranial aneurysms.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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