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Original Article

Prognostic value of serum miR-155 in intracerebral hemorrhage

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Abstract: Deregulation of microRNAs (miRNAs) has been demonstrated to be implicated in the pathogenesis of a number of human diseases including stroke. miR-155 is identified as a marker for pro-inflammation. However, its clinical significance in intracerebral hemorrhage (ICH) is poorly known. The aim of the current study was investigate the prognostic value of serum miR-155 in ICH. A total of 80 patients with ICH and 30 healthy controls were recruited in the study. Real-time PCR was used to compare the serum miR-155 level between ICH patients and healthy subjects. Then the clinical value of serum miR-155 was further determined. Our results showed that serum miR-155 was significantly elevated in patients with ICH. Serum miR-155 was able to predict 6 months mortality and 6 months unfavorable outcome with high accuracy. In addition, serum miR-155 was significantly associated with hematoma volume and National Institutes of Health Stroke Scale (NIHSS) score. Moreover, patients with higher serum miR-155 had significantly poorer 6-month mortality and 6-month unfavorable outcome. Taken together, serum miR-155 was upregulated in patients with ICH and it might have the potential to predict the prognosis for this deadly disease.

Keywords: Intracerebral hemorrhage, miR-155, serum, prognosis

Introduction

Intracerebral hemorrhage (ICH) is serious form of stroke leading to high morbidity and mortality [1]. It affects more than a million people around the world annually and accounts for 20-30% of all strokes [2]. ICH is a significant public health issue as no effective treatment is now available. Therefore, exploring biomarkers that associated with ICH contributes to early detection and risk stratification for this deadly disease, which is very important for improving the clinical outcome.

MicroRNAs (miRNAs) are a class of highly conserved, small non-coding RNAs with the length of 20-22 nucleotides [3]. They are important posttranscriptional regulators of gene expression either by inhibiting mRNA translation and/or promoting mRNA degradation [4]. miRNAs control nearly all the biological processes such as proliferation, differentiation, apoptosis and survival [5, 6]. Aberrant expression of miRNAs has been demonstrated to be implicated in the development of many neurological diseases including ICH [7, 8]. Zheng et al globally profiled the miRNAs between ICH patients with or without perihematomal edema (PHE). A number of differentially expressed miRNAs were identified and combining these differentially expressed miRNAs was able to discriminate the two groups with extremely high accuracy [9]. The expression level of serum miR-130a was significantly increased in patients with ICH. In addition, high serum miR-130a levels were positively correlated with severity of PHE and poor clinical outcome of ICH. Moreover, they also showed that miR-130a might exert its damage effects by reducing caveolin-1 expression in the rat model [10].

Inflammation is a risk factor for ICH progression and various studies have reported that miR-155 is a pro-inflammatory molecule [11, 12]. Therefore, we speculated that miR-155 might be involved in the pathogenesis of ICH. The aim

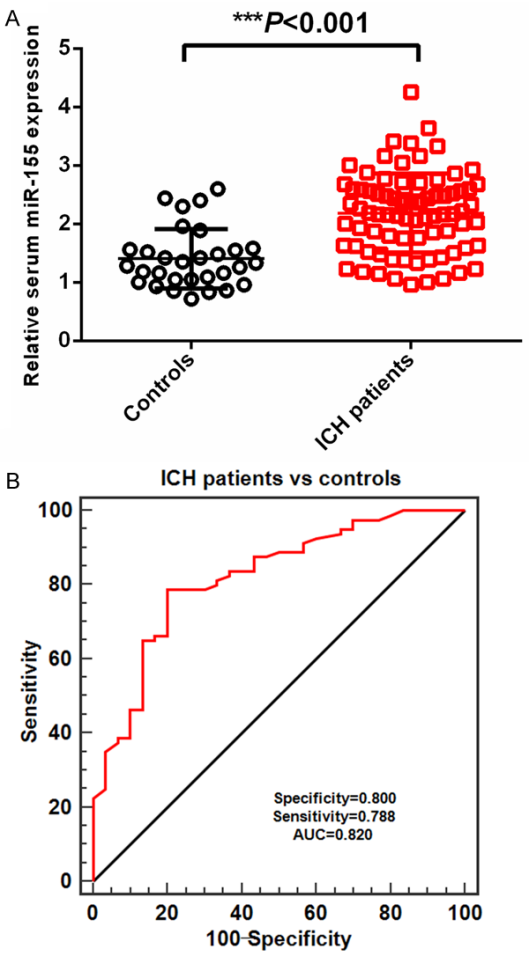

Figure 1. Serum miR-155 was upregulated in patients with ICH.

of the current study was to determine the clinical significance of serum miR-155 in patients with ICH.

Materials and methods

Patients and samples

The study was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consents were obtained from participants or their family members. ICH was diagnosed based on computed tomography (CT) scans or magnetic resonance imaging (MRI) scans. Exclusion criteria included secondary brain hemorrhage due to other causes such as renal or hepatic insufficiency, anticoagulant treatment, malignancy, infectious diseases (within a month), surgery and major trauma. Those patients with severe primary diseases or missing of follow-up were also excluded from the study. A healthy control group was formed by 30 individuals who attended our hospital for physical examination.

Peripheral venous blood samples were drawn from patients with ICH on admission and from healthy controls at study entry. Serum was separated via centrifugation at 1,300 g for 10 min at 4°C within 1 h of collection. Then the samples were aliquoted and stored at -80°C until further analysis.

Real-time PCR

Total RNA was extracted from 400 μL of serum samples using a mirVana PARIS kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. The quantity of the extracted RNA was determined by Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Briefly, 5 μL of total RNA was reverse-transcribed to cDNA using TaqMan® MicroRNA Reverse Transcription Ki t (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using TaqMan miRNA probes (Applied Biosystems) on the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems). The relative expression fold change was calculated by using the $2^{-\Delta\Delta Ct}$ method. Each sample was examined in triplicate and RNU6B was used as the internal control for comparison.

Statistical analysis

All statistical analyses were performed using the SPSS statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism, version 5 (GraphPad, La Jolla, CA, USA). Kolmogorov-Smirnov test was performed to evaluate the normality of the distribution of data. Mann-Whitney U tests were employed to compare differences of serum miR-155 between two different groups as the data was not normally distributed. Chi-squared test was performed to test dichotomized or categorical independent variables. Receiver operating characteristics (ROC) curves were constructed and the area under the curve (AUC) was calculated to evaluate the predicted value of serum miR-155. Survival curves were estimated using the Kaplan-Meier method, and comparisons were made using the log rank test. A value of

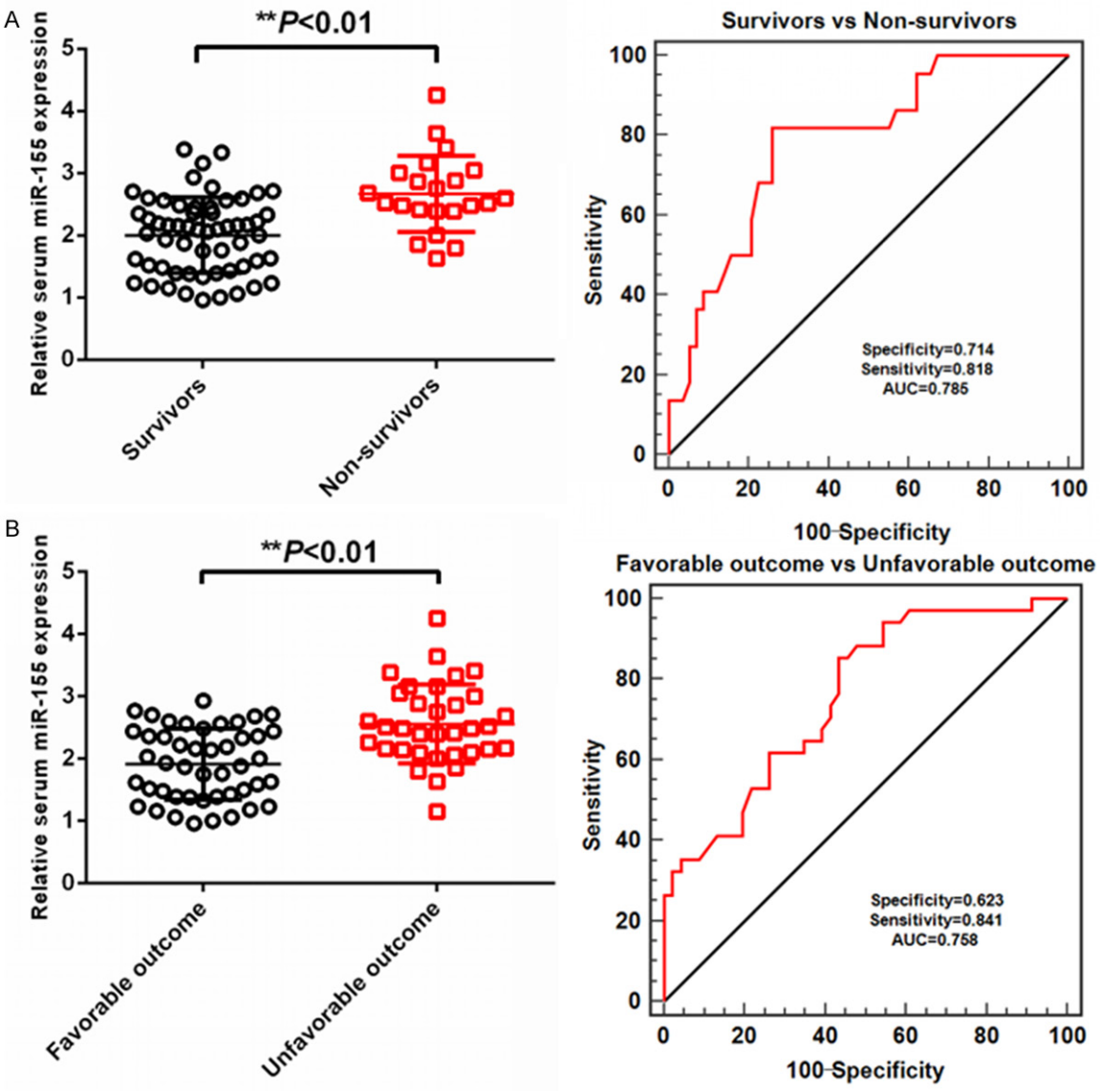

Figure 2. The predicted value of serum miR-155 for 6 months mortality and 6 months unfavorable outcome.

$P<0.05$ was considered as statistically significant.

Results

Serum miR-155 was upregulated in ICH patients

We compared the expression levels of miR-155 in the serum samples derived from 80 ICH patients and 30 healthy controls by real-time PCR. Our results showed that serum miR-155 levels were significantly higher in ICH patients compared to healthy volunteers (***$P<0.001$) (**Figure 1A**). ROC curve was constructed to evaluate the prediction value of serum miR-155. The results revealed that serum miR-155 was able to discriminate the ICH patients from healthy controls with good performance (AUC = 0.820, specificity = 0.800 sensitivity = 0.788) (**Figure 1B**).

The predictive value of serum miR-155 for 6 month mortality and unfavorable outcome

We then evaluated the miR-155 levels between 6 month survivors and non-survivors. Our data demonstrated the expression level of serum miR-155 was significantly elevated in 6 month non-survivors compared to non-survivors

Table 1. The correlation between serum miR-155 levels and clinicopathological parameters of ICH

Paremeters	Serum miR-155 expression		*P*
	Low (n = 37)	High (n = 43)	
Age (year)	64.5+7.3	65.2+8.6	0.065
Gender			0.204
Female	19	16	
Male	18	27	
Hypertension			0.955
No	5	6	
Yes	32	37	
Diabetes mellitus			0.184
No	21	18	
Yes	16	25	
Neurological deterioration			0.179
No	32	32	
Yes	5	11	
Intraventricular hemorrhage			0.111
No	30	28	
Yes	7	15	
Blood platelet count (10^9/L)	162.5 ± 48.9	167 ± 53.2	0.073
Hematoma volume (mL)	21.4 ± 5.7	35.8 ± 8.3	<0.001
NIHSS score	7.3 ± 2.2	14.3 ± 3.6	<0.001

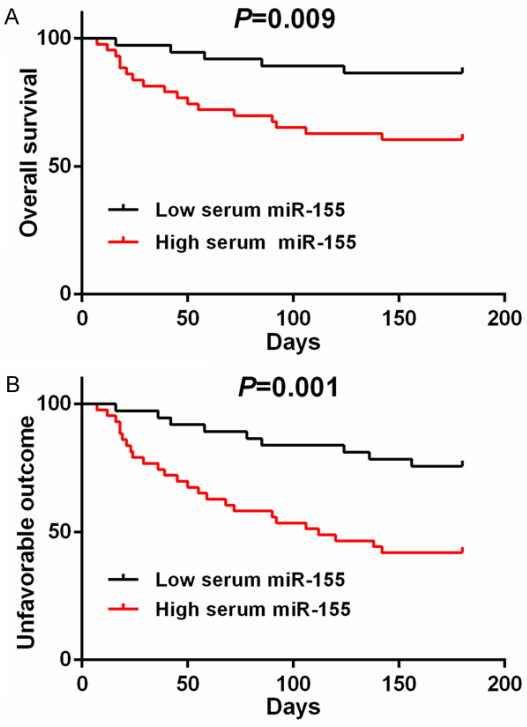

Figure 3. The association between serum miR-155 levels and 6 months mortality and unfavorable outcome.

(*P*<0.01). In addition, ROC analysis showed that serum miR-155 had good discriminatory capacity for predicting 6 month mortality (AUC = 0.785, specificity = 0.714, sensitivity = 0.818) (**Figure 2A**).

As regards to 6 month unfavorable outcome, the expression level of serum miR-155 was also higher in patients suffering from unfavorable outcome compared with those without unfavorable outcome (*P*<0.01). Serum miR-155 could differentiate the patients with 6 month unfavorable outcome from patients with 6 month favorable with relative good performance (AUC = 0.758, specificity = 0.623, sensitivity = 0.841) (**Figure 2B**).

The association between serum miR-155 and clinicopathological parameters of ICH

The median value of serum miR-155 was used to divide the ICH patients into high and low serum miR-155 group. Our analysis showed that high serum miR-155 level was positively associated with hematoma volume (*P*<0.001) and NIHSS score (*P*<0.001), indicating that serum miR-155 levels might be closely related with the progression of ICH. However, it was not correlated with age, gender, hypertension, diabetes, neurological deterioration, Intraventricular hemorrhages and blood platelet count (**Table 1**).

The prognosis value of serum miR-155 in ICH

We then investigated the association between serum miR-155 levels and prognosis of ICH. Compared with patients with low serum miR-155 level, patients with high miR-155 level had significantly shorter 6-month overall survival time (*P* = 0.009) (**Figure 3A**). Similarly, patients in the low serum miR-155 group also suffered significantly worse 6 month unfavorable outcome in comparison with those in the high serum miR-155 group (*P* = 0.001) (**Figure 3B**).

Discussion

Numerous studies have showed that circulating miRNAs expression changed dynamically fol-

lowing stroke, indicating that these molecules are key regulators in the initiation and development of stroke [13, 14]. In addition, miRNAs are highly stable in the circulation system, which enables them to be promising biomarkers for disease diagnosis and prognosis [15]. For instance, Wang et al compared the differentially expressed miRNAs in the clinical samples between ICH patients and healthy volunteers. They found that the expression level of miR-21-5p was significantly downregulated in the specimens (blood/haematoma) from patients compared to those blood samples from healthy controls [16].

In this study, we demonstrated that serum miR-155 was remarkably increased in ICH patients. In addition, it had high accuracy in predicting 6 month mortality and 6 month unfavorable outcome. Serum miR-155 was strongly correlated with hematoma volume and NIHSS score. Moreover, the 6 month overall survival and unfavorable outcome was worse in patients with high serum miR-155 levels, suggesting that miR-155 plays an important role in the progression of ICH and serum miR-155 might have promising clinical value for predicting the prognosis of ICH.

miR-155 is multifunctional molecule which has been shown to be involved in a variety of biological processes including, but not limited to, haematopoiesis, inflammation and immunity. Aberrant expression of miR-155 is closely associated with many diseases such as cardiovascular diseases, cancer and virus infection [12, 17]. One the one hand, miR-155 upregulation can be induced by different kinds of substance such as bacterial lipopolysaccharide (LPS) and inflammatory mediators (TNF-α, IFN-β). On the other hand, miR-155 might act as a pro-inflammatory molecule which is able to further exacerbate the inflammation process. For example, miR-155 was demonstrated to activate the production of proinflammation mediators IL-6 and TNF-α [18]. Therefore, miR-155 might play a key role in the amplification mechanisms of inflammation. Increasing evidence has shown that inflammatory responses are implicated in the pathophysiological processes of brain injury following ICH [19]. We speculate that the inflammatory mediators were significantly elevated in patients suffering ICH, which can increase the expression of miR-155. The upregulated miR-155 then pass the blood brain barrier and enter the circulation system, leading to our findings that miR-155 was significantly upregulated in ICH patients. In addition, miR-155 aggravates the inflammation process which might cause secondary injuries to brain. Therefore, we can observe the phenomenon that miR-155 upregulation was correlated with poor clinical outcome of ICH patients. However, one limitation of current study was the relative small sample. Further studies with larger samples should carry out the validation of the clinical value of serum miR-155 in ICH. The concrete molecular mechanisms responsible for the role of miR-155 in ICH also require for further investigations.

In conclusion, our results proved that elevated serum miR-155 was significantly associated with adverse clinical events in patients with ICH. The predictive value of serum miR-155 has great potential to monitor the prognosis of ICH.

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Disclosure of conflict of interest

None.

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