Original Article
Circular RNA DLGAP4 is down-regulated and negatively correlates with severity, inflammatory cytokine expression and pro-inflammatory gene miR-143 expression in acute ischemic stroke patients

Xiaoqi Zhu[1*], Ji Ding[1*], Beiyun Wang[2], Jie Wang[1], Min Xu[1]

[1]Department of Neurology, Tong Ren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]Department of Gerontology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China. *Equal contributors.

Abstract: This study aimed to investigate the expression of circular RNA DLGAP4 (circ-DLGAP4) and its correlation with severity, inflammation, inflammatory cytokine levels as well as microRNA-143 (miR-143) expression in acute ischemic stroke (AIS) patients. One hundred and seventy AIS patients and 170 non-AIS controls were enrolled in this study. PBMC and serum from all participants were collected. Circ-DLGAP4 and miR-143 expression in PBMC were detected by qPCR, and TNF-α, IL-1β, IL-6, IL-8, IL-17 as well as IL-22 expressions in serum were detected by ELISA. The information of CRP, ESR, and National Institutes of Health Stroke Scale (NIHSS) score of AIS patients was collected. PBMC circ-DLGAP4 was down-regulated in AIS patients compared with controls, and ROC curve analysis disclosed that PBMC circ-DLGAP expression had good value in predicting lower AIS risk with area under curve 0.816. Spearman's rank correlation test showed that PBMC circ-DLGAP4 expression negatively correlated with NIHSS score and CRP level in AIS patients. In addition, PBMC circ-DLGAP4 level also was negatively associated with serum expressions of TNF-α, IL-6, IL-8 as well as IL-22. Moreover, PBMC circ-DLGAP4 expression was negatively correlated with PBMC miR-143, and PBMC miR-143 was positively associated with NIHSS score, CRP, ESR, TNF-α, IL-1β, IL-6, IL-8, IL-17, as well as IL-22 levels. Circulating circ-DLGAP4 could serve as a novel biomarker for diagnosis and disease surveillance of AIS and is negatively correlated with inflammation and miR-143 expression in AIS patients.

Keywords: Acute ischemic stroke, circ-DLGAP4, severity, inflammation, miR-143

Introduction

Stroke, one of the leading causes of worldwide death and disability, mainly attacks people in low-income and middle-income countries [1, 2]. Acute ischemic stroke (AIS) is one of the most frequent types of strokes and the predominant cause of long-term disability, which subsequently brings a large burden to society every year [3]. As a critical medical emergency, AIS requires a timely evaluation and triage with the use of multiple assessment tools, including non-contrast cranial computed tomography (CT) scan, diffusion-weighted magnetic resonance image (MRI) and assessment scales, which are effective but also have their limitations, such as a restricted sensitivity to specific brain areas [4-7]. With respect to the management of AIS, intravenous recombinant tissue plasminogen activator (IV rtPA) within 4.5 hours after stroke onset remains to be the first choice in clinical practice, while due to lack of rapid identification of stroke symptoms prehospital, timely intervention of AIS patients is often hampered [8, 9].

Circular RNAs (circRNAs) are a novel class of non-coding RNAs with a structure of closed loop, being deficient in 5' and 3' polarity or a polyadenylated tail [10]. Recent studies elucidate that circRNAs have multiple functions, such as regulating translation and sponging microRNAs (miRNAs) [10-12]. Moreover, the roles of circRNAs in human diseases have been revealed by several studies, such as regulating biological process (for instance: stimulate the

neuron injury or promotes epithelial-mesenchymal transition), cells functions (for instance: modify cell proliferation, apoptosis, migration and invasion), and molecules signaling (for instance: regulate Wnt signaling and JAK-STAT signaling pathways) [13-16]. CircRNA DLGAP4, a novel circRNA that originates from the exons 8, 9 and 10 of gene DLGAP4, is a sponge for miR-143, and is recently found to reduce the neurological deficits, infarct areas, and blood-brain damage in mice models of stroke, and the gene DLGAP4 is reported to be related to functions of neurons and brain disorders [17, 18]. Therefore, we presumed that circ-DLGAP4 might have the potential to serve as a biomarker in AIS patients for diagnosis and disease surveillance.

Thus, this study aimed to investigate the expression of circ-DLGAP4 and its correlation with severity, inflammation, inflammatory cytokine levels, as well as microRNA (miR)-143 expression in AIS patients.

Methods

Patients and controls

One hundred and seventy patients admitted with AIS at Tongren Hospital, Shanghai Jiao Tong University School of Medicine from January 2016 to September 2018 were consecutively enrolled in this case-control study. The inclusion criteria were as follows: (1) diagnosed as primary AIS confirmed by brain computed tomography angiography (CTA) scan or magnetic resonance angiography (MRA); (2) age above 18 years old; (3) admission within 24 hours of symptom onset. Patients were excluded if they had intracranial hemorrhage, severe infection or inflammatory diseases, complicated with hematologic malignancies or solid tumors, treated with immunosuppressive medicine currently, or were pregnant or lactating women. Besides, there were 170 patients with vascular risk factors (such as hypertension, hyperlipidemia, hyperuricemia) being recruited as controls during the same period, and all controls had no history of stroke, peripheral vascular disease, coronary ischemia, inflammatory diseases, autoimmune diseases or hematological malignancies. This study was approved by Institutional Review Board of Tongren Hospital, Shanghai Jiao Tong University School of Medicine. All patients and controls (or their guardians) provided written informed consents before enrollment.

Collection of patients' characteristics and blood samples

After the written informed consents were acquired, baseline characteristics of AIS patients and controls were recorded, which included age, gender, body mass index (BMI), smoke, hypertension, diabetes mellitus, hyperlipidemia, hyperuricemia and chronic kidney disease (CKD). As for the AIS patients, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also measured and recorded, and National Institutes of Health Stroke Scale (NIHSS) score was collected as well, which was evaluated by physician within 24 hours after admission and used to assess disease severity. AIS patients' blood samples were collected prior to any treatment (within 24 hours of symptom onset). Each sample was divided into two parts within 1 hour after collection, one was used for separation of peripheral blood mononuclear cell (PBMC) using the Ficoll density gradient centrifugation, and the other was centrifuged for the collection of serum. Then PBMC and serum were stored at -80°C for further detection. Controls' blood samples were collected after the acquisition of the written informed consents and used to separate PBMC, then stored at -80°C as well.

Detections of Circ-DLGAP4 and miR-143

Total RNA was extracted from PBMC using the TRIzol Reagent (Invitrogen, USA) for detections of circ-DLGAP4 and miR-143. Subsequently, for circ-DLGAP4 detection, RNase R (Epicentre, USA) was added for the digestion of linear RNA in total RNA. And for miR-143 detection, the total RNA was used for transcription. Afterward, the RNA was reversely transcribed into cDNA by PrimeScript™ RT reagent Kit (TAKARA, Japan), and PCR was conducted using QuantiNova SYBR Green PCR Kit (Qiagen, German). GAPDH was used as internal reference for circ-DLGAP4 detection, while U6 was used as internal reference for miR-143 detection. Lastly, the results were calculated using the $2^{-\Delta\Delta Ct}$ formula. Primers used in the study were as follows: Circ-DLGAP4: Forward: AAGTGAACAAGGGACGCTGAC; Reverse: ACTGCTCTGGACTGTGACTGA. miR-143: Forward: ACACTCCAGCTGGGGGGTGCAGTGCTGCATCTC; Reverse: TGTCGTGGAGTCGGCAATTC. GAPDH: Forward: TGACCACAGTCCATGCCATCAC; Reverse: GCCTGCTTCACCACCTTCTTGA. U6: Forward: CTCGCTTCGGCAGCACATATACTA; Reverse: ACGAATTTGCGTGTCATCCTTGC.

Table 1. Characteristics of AIS patients and controls

Characteristics	AIS patients (N = 170)	Controls (N = 170)	P value
Age (years)	64.9 ± 12.5	63.9 ± 10.5	0.412
Gender (n/%)			0.381
Male	124 (72.9)	131 (77.1)	
Female	46 (27.1)	39 (22.9)	
BMI (kg/m²)	24.5 ± 2.8	24.2 ± 2.7	0.331
Smoke (n/%)	82 (48.2)	83 (48.8)	0.914
Hypertension (n/%)	157 (92.4)	149 (87.6)	0.148
Diabetes mellitus (n/%)	33 (19.4)	27 (15.9)	0.393
Hyperlipidemia (n/%)	73 (42.9)	71 (41.8)	0.826
Hyperuricemia (n/%)	55 (32.4)	55 (32.4)	1.000
CKD (n/%)	27 (15.9)	22 (12.9)	0.440
NIHSS score	7.9 ± 3.4	-	-
CRP (mg/L)	26.0 (20.1-34.7)	-	-
ESR (mm/H)	30.1 (22.9-40.3)	-	-
TNF-α (pg/mL)	55.5 (40.3-86.7)	-	-
IL-1β (pg/mL)	5.1 (3.5-7.4)	-	-
IL-6 (pg/mL)	40.1 (33.1-49.0)	-	-
IL-8 (pg/mL)	53.9 (40.1-75.5)	-	-
IL-17 (pg/mL)	86.3 (56.0-113.9)	-	-
IL-22 (pg/mL)	68.1 (50.7-93.8)	-	-

Data are presented as mean ± standard deviation, count (%) or median (25th-75th quantiles). Comparison was determined by t test or Chi-square test. P value < 0.05 was considered significant. AIS, acute ischemic stroke; BMI, body mass index; CKD, chronic kidney disease; National Institutes of Health Stroke Scale (NIHSS) score; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TNF-α, tumor necrosis factor α; IL, interleukin.

Measurements of inflammatory cytokines

For AIS patients, the levels of inflammatory cytokines including tumor necrosis factor α (TNF-α), interleukin (IL) 1β (IL-1β), IL-6, IL-8, IL-17 and IL-22 in serum were measured by use of Enzyme-Linked Immunosorbent Assay (ELISA) (Abcam, Massachusetts, USA) kits according to the manufacturer's instructions.

Statistical analysis

SPSS 21.0 statistical software (SPSS Inc, Chicago, USA) and GraphPad Prism 7.00 software (GraphPad Software, La Jolla, USA) were used for statistical analysis and figures making. Count data were expressed as count (percentage); continuous data were described as mean ± standard deviation if normally distributed, and as median (25th-75th quantiles) if not normally distributed. Comparison of count data was determined by Chi-square test; comparison of continuous data was determined by t test or Wilcoxon rank sum test. Correlation

analysis was analyzed by Spearman's rank correlation test. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were used to assess the value of Circ-DLGAP4 level in distinguishing AIS from the control. Reported statistical significance levels were all two-sided, and P value < 0.05 was considered significant.

Results

Clinical characteristics of sepsis patients and controls

No difference of characteristics between AIS patients and controls was found (**Table 1**), and the mean age of AIS patients and controls were 64.9 ± 12.5 year and 63.9 ± 10.5 years, respectively (P = 0.412). There were 124 (72.9%) males and 46 (27.1%) females among AIS patients, and 131 (77.1%) males as well as 39 (22.9%) females in the controls (P = 0.381). In addition, the mean values of BMI were 24.5 ± 2.8 kg/m² and 24.2 ± 2.7 kg/m² in AIS patients and controls, respectively (P = 0.331). The number of patients who had hypertension in AIS patients and controls were 157 (92.4%) and 149 (87.6%), respectively (P = 0.148), and there were respectively 33 (19.4%) and 27 (15.9%) patients who had diabetes mellitus in AIS patients and controls (P = 0.393). Additionally, the numbers of patients with hyperlipidemia were 73 (42.9%) and 71 (41.8%) (P = 0.826), and the numbers of patients who had hyperuricemia were 55 (32.4%) and 55 (32.4%) in AIS patients and controls, respectively (P = 1.000). 27 (15.9%) AIS patients and 22 (12.9%) controls hasd complication of chronic kidney disease (CKD) (P = 0.440). Additionally, in AIS patients, the mean NIHSS score was 7.9 ± 3.4, median CRP, ESR, TNF-α, IL-1β, IL-6, IL-8 and IL-17 levels were 26.0 (20.1-34.7) mg/L, 30.1 (22.9-40.3) mm/H, 55.5 (40.3-86.7) pg/mL, 5.1 (3.5-7.4) pg/mL, 40.1 (33.1-49.0) pg/mL, 53.9 (40.1-75.5) pg/mL, 86.3 (56.0-113.9) pg/mL and 68.1 (50.7-93.8) pg/mL, respectively. The other clinical characteristics of AIS patients and controls are listed in **Table 1**.
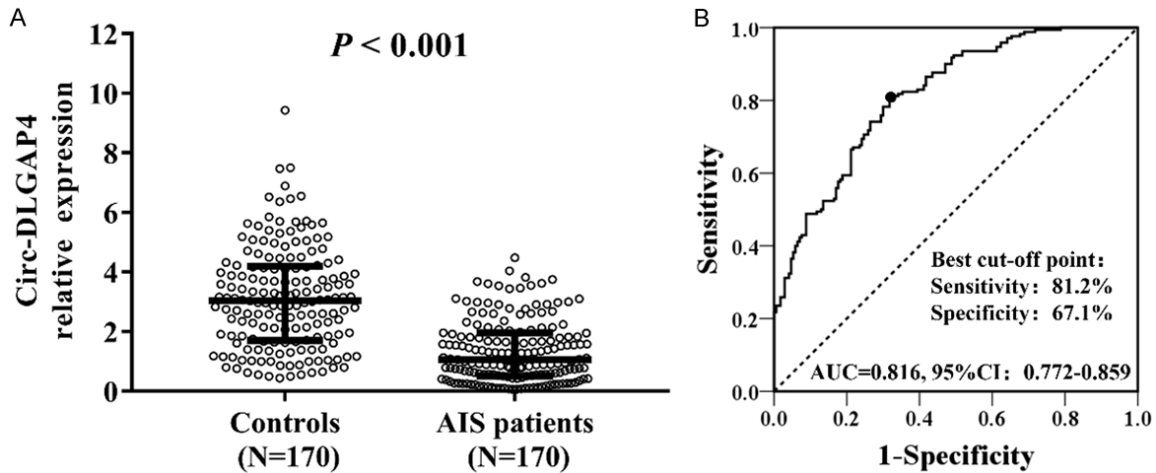
Figure 1. PBMC circ-DLGAP4 expression in predicting AIS risk. PBMC circ-DLGAP4 was down-regulated in AIS patients compared with controls (A) and had a good value in predicting AIS risk (B). Comparison between two groups was determined by Wilcoxon rank sum test. ROC curve was performed to evaluate the value of circ-DLGAP4 for predicting AIS risk. P < 0.05 was considered significant. AIS, acute ischemic stroke; ROC, receiver operating characteristic.
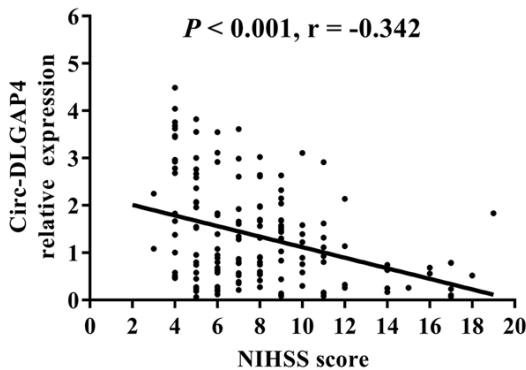

Figure 2. Correlation of PBMC circ-DLGAP4 with AIS severity. PBMC circ-DLGAP4 expression was negatively associated with NIHSS score. Spearman test was used to assess the correlation of circ-DLGAP expression with NIHSS score. P < 0.05 was considered significant. AIS, acute ischemic stroke; NIHSS, National Institutes of Health Stroke Scale.

Predictive value of PBMC circ-DLGAP4 for AIS risk

The PBMC circ-DLGAP4 was down-regulated in AIS patients compared with controls (P < 0.001) (**Figure 1A**). The AUC of PBMC circ-DLGAP4 for differentiating AIS patients from controls was 0.816 (95% CI: 0.772-0.859), and the sensitivity as well as specificity were 81.2% and 67.1% at the best cut-off point (**Figure 1B**), suggesting that PBMC circ-DLGAP4 had good value for predicting AIS risk.

Association of PBMC circ-DLGAP4 with NIHSS score

PBMC circ-DLGAP4 expression was negatively correlated with NIHSS score in AIS patients (P < 0.001, r = -0.324), which indicated that PBMC circ-DLGAP4 expression was reversely correlated with disease severity of AIS (**Figure 2**).

Association of PBMC circ-DLGAP4 with inflammatory markers and cytokines

In AIS patients, the PBMC circ-DLGAP4 level was negatively associated with CRP (P = 0.007, r = -0.208), and serum TNF-α (P = 0.015, r = -0.187), IL-6 (P <0.001, r = -0.333), IL-8 (P = 0.024, r = -0.173) and IL-22 (P = 0.003, r = -0.229) (**Table 2**). However, the PBMC circ-DLGAP4 expression was not associated with ESR (P = 0.761, r = -0.023), IL-1β (P = 0.437, r = -0.060) or IL-17 (P = 0.720, r = -0.028) serum levels. These results suggested that PBMC circ-DLGAP4 negatively associated with inflammation level in AIS.

Association of PBMC circ-DLGAP4 with PBMC miR-143

Since miR-143 has been reported to be a target of circ-DLGAP4 in stroke models, we further evaluated the correlation of PBMC circ-DLGAP4 with PBMC miR-143 [17], and found that PBMC circ-DLGAP4 was negatively correlated with

Table 2. Correlation of Circ-DLGAP4 and inflammation markers and cytokines

Markers and cytokines	Circ-DLGAP4	
	P value	Correlation coefficient (r)
CRP	0.007	-0.208
ESR	0.761	-0.023
TNF-α	0.015	-0.187
IL-1β	0.437	-0.060
IL-6	< 0.001	-0.333
IL-8	0.024	-0.173
IL-17	0.720	-0.028
IL-22	0.003	-0.229

Correlation analysis was determined by Spearman's rank correlation test. P value < 0.05 was considered significant. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TNF-α, tumor necrosis factor α; IL, interleukin.

Table 3. Correlation of miR-143 and NIHSS score, inflammation markers, and cytokines

Markers and cytokines	miR-143	
	P value	Correlation coefficient (r)
NIHSS score	0.008	0.203
CRP	< 0.001	0.379
ESR	< 0.001	0.307
TNF-α	< 0.001	0.361
IL-1β	0.001	0.256
IL-6	0.003	0.230
IL-8	< 0.001	0.275
IL-17	0.002	0.233
IL-22	< 0.001	0.309

Correlation analysis was determined by Spearman's rank correlation test. P value < 0.05 was considered significant. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TNF-α, tumor necrosis factor α; IL, interleukin.
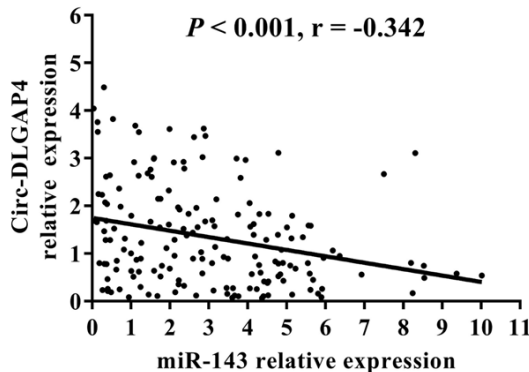

Figure 3. Correlation of PBMC circ-DLGAP4 expression with miR-143 PBMC expression. Circ-DLGAP4 PBMC expression was negatively associated with miR-143 expression in PBMC. Spearman test was used to assess the correlation of circ-DLGAP expression with miR-143 expression in PBMC. P < 0.05 was considered significant.

PBMC miR-143 expression in AIS patients (P < 0.001, r = -0.342) (**Figure 3**).

Association of miR-143 with AIS severity and inflammation

In addition, we further analyzed the association of PBMC miR-143 with NIHSS score, inflammation and inflammatory cytokines, which disclosed that the PBMC miR-143 expression was positively correlated with NIHSS score (P = 0.008, r = 0.203), CRP (P < 0.001, r = 0.379) level, ESR (P < 0.001, r = 0.307) level, and serum expressions of TNF-α (P < 0.001, r = 0.361), IL-1β (P = 0.001, r = 0.256), IL-6 (P =

0.003, r = 0.230), IL-8 (P < 0.001, r = 0.275), IL-17 (P = 0.002, r = 0.233) and IL-22 (P < 0.001, r = 0.309) (**Table 3**).

Discussion

In this study, we discovered that: (1) PBMC circ-DLGAP4 was decreased in AIS patients compared with controls and had a good value for predicting the risk of AIS; (2) PBMC circ-DLGAP4 expression negatively correlated with AIS severity; (3) PBMC circ-DLGAP4 expression negatively associated with CRP, and serum TNF-α, IL-6, IL-8 and IL-22 levels in AIS patients; (4) PBMC circ-DLGAP4 expression was negatively correlated with miR-143 expression in AIS patients.

A previous study elucidates that plasma circ-DLGAP4 expression is decreased in AIS patients compared with the non-stroke controls, and is also reduced in cerebral artery occlusion stroke mouse models compared with non-stroke mouse models; in addition, the further experiments in this study reveal that up-regulating circ-DLGAP4 reduces the neurological deficit, infarct areas and blood-brain damage in brain tissue of stroke mouse models, and circ-DLGAP4 sponges miR-143 which subsequently leads to the reduction of homologous to the E6-AP C-terminal domain E3 ubiquitin protein ligase 1 (HECTD1) expression [17]. As miR-143 is a sponge of apoptosis protein Bcl-2 [19], and circ-DLGAP4 is abundantly expressed in myocytes and is a sponge of miR-143, a study presumes that circ-DLGAP4 might be able to

repress cardiomyocytes apoptosis through sponging miR-143 [20]. To our best knowledge, there is still no study that identifies circ-DLGAP4 as a biomarker for diagnosis or progression of AIS, therefore, our study was the first study that evaluated circ-DLGAP4 expression in AIS patients and assessed its correlation with risk and severity of AIS, which showed that PBMC circ-DLGAP4 was down-regulated in AIS patients and had a good value in predicting AIS risk, which might derive from that: (1) circ-DLGAP4 might take part in reducing pathologic damage in AIS patients through down-regulating HECTD1 by sponging miR-143, which then mediates the expressions of tight junction protein (TJP) and mesenchymal cell markers and subsequently reduces EMT in brain tissue [17, 19, 20]; (2) it was possible that circ-DLGAP4 reduced pathologic features of AIS through mediating gene DLGAP4, the dysregulation of which was reported to be related to early-onset cerebellar ataxia [21].

Inflammation is crucial in AIS etiology and is claimed to be associated with reperfusion injury, and there have been studies illuminating that circRNAs play critical roles in inflammation; for example, an animal experiment shows that there are 191 differentially expressed circRNAs in the cortex in mouse models of traumatic brain injury, and the enrichment analysis reveals that these miRNAs are mainly related to inflammation [22]. Another *in vitro* experiment illustrates that in nucleus pulposus (NP) cells obtained from NP tissue of patients after spinal surgery, up-regulated circ_4099 increases the expression of Collagen II and Aggrecan while suppresses the pro-inflammatory factors expressions including IL-1β, TNF-α and prostaglandin E2 (PGE2) [23]. As for circ-DLGAP4, only limited data reveals that it is a sponge for miR-143 in stroke models [17, 20], and the latter is proven to be a pro-inflammatory gene [24, 25], thus, we assessed the correlation of PBMC circ-DLGAP4 with laboratory indexes reflecting inflammation and inflammatory cytokines, and the data showed that PBMC circ-DLGAP4 was negatively correlated with CRP, TNF-α, IL-6, IL-8 and IL-22 levels. Those data indicated that circ-DLGAP4 was inversely correlated with inflammation level in AIS patients, which could be explained by that that circ-DLGAP4 decreased inflammation in AIS through sponging pro-inflammatory microRNAs, such as miR-143, which is a pro-inflammatory miRNA [17].

CircRNAs are known as miRNAs sponges in various diseases. For instance, a previous study reveals that TNF-α induces the expression of circ-4099, which subsequently mediates extracellular matrix (ECM) composition by sponging miR-616-5p and reduces miR-616-5p suppression of Sox9 in patients with intervertebral disc degeneration [23]. However, the studies reporting circRNAs as miRNA sponges in stroke and cardiac-cerebrovascular diseases are very few. A previous study reveals that circ fibroblast growth factor receptor 2 (FGFR2) enhances cell proliferation of myoblast differentiation via sponging miR-133a-5p and miR-29b-1-5p [26]. Another study elucidates that circ-SATB2 advocates vascular smooth muscle cell proliferation through regulating miR-939 [27]. These studies indicate that circRNA might play a role in cardiac-cerebral vascular diseases; however, as for AIS, there is only one study elucidating that circ-DLGAP4 acts as a miR-143 sponge in stroke mouse models [17]. In this study, we discovered that PBMC circ-DLGAP4 was negatively correlated with miR-143, and our further anlayses showed that PBMC miR-143 was positively associated with disease severity and inflammation of AIS, which indicated that circ-DLGAP4 might play a predominent role in etiology by sponging miR-143 in AIS.

To our best knowledge, this was the first study revealing the diagnostic value of PBMC circ-DLGAP4 for AIS, and its correlation with inflammation and miR-143 expression in AIS patients, which provided novel insight on the value of circRNAs in AIS in clinical practice. However, there were still several limitations in this study: (1) the AIS patients who were enrolled in our study were with mean NIHSS score of 7.9 ± 3.4, indicating that the majority of our patients were in mild (NIHSS score less than 6) or moderate stages (NIHSS score between 6 and 13) of AIS; therefore, the value of circ-DLGAP4 in patients with severe AIS might not be well evaluated in our study [28]; (2) There might be selection bias in this study, which resulted from that our study was conducted in a single center and the patients as well as controls mainly come from Eastern China.

Taken together, circulating circ-DLGAP4 could serve as a novel biomarker for diagnosis and disease surveillance of AIS and is negatively correlated with inflammation and miR-143 expression in AIS patients.

Disclosure of conflict of interest

None.

Address correspondence to: Min Xu, Department of Neurology, Tong Ren Hospital, School of Medicine, Shanghai Jiao Tong University, 1111 Xianxia Road, Shanghai 200336, China. Tel: +86-21-52039999; Fax: +86-21-52039999; E-mail: musong971597@163.com

References

[1] Hankey GJ. Stroke. Lancet 2017; 389: 641-654.

[2] Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C; Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010) and the GBD Stroke Experts Group. Global and regional burden of stroke during 1990-2010: findings from the global burden of disease study 2010. Lancet 2014; 383: 245-254.

[3] Writing Group Members, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart disease and stroke statistics-2016 update: a report from the american heart association. Circulation 2016; 133: e38-360.

[4] Hankey GJ and Blacker DJ. Is it a stroke? BMJ 2015; 350: h56.

[5] Whiteley WN, Wardlaw JM, Dennis MS and Sandercock PA. Clinical scores for the identification of stroke and transient ischaemic attack in the emergency department: a cross-sectional study. J Neurol Neurosurg Psychiatry 2011; 82: 1006-1010.

[6] Brazzelli M, Chappell FM, Miranda H, Shuler K, Dennis M, Sandercock PA, Muir K and Wardlaw JM. Diffusion-weighted imaging and diagnosis of transient ischemic attack. Ann Neurol 2014; 75: 67-76.

[7] Brunser AM, Hoppe A, Illanes S, Diaz V, Munoz P, Carcamo D, Olavarria V, Valenzuela M and Lavados P. Accuracy of diffusion-weighted imaging in the diagnosis of stroke in patients with suspected cerebral infarct. Stroke 2013; 44: 1169-1171.

[8] Asif KS, Lazzaro MA and Zaidat O. Identifying delays to mechanical thrombectomy for acute stroke: onset to door and door to clot times. J Neurointerv Surg 2014; 6: 505-510.

[9] Faiz KW, Sundseth A, Thommessen B and Ronning OM. Prehospital delay in acute stroke and TIA. Emerg Med J 2013; 30: 669-674.

[10] Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF and Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 2013; 19: 141-157.

[11] Jeck WR and Sharpless NE. Detecting and characterizing circular RNAs. Nat Biotechnol 2014; 32: 453-461.

[12] Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H and Shan G. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol 2015; 22: 256-264.

[13] Li P, Chen H, Chen S, Mo X, Li T, Xiao B, Yu R and Guo J. Circular RNA 0000096 affects cell growth and migration in gastric cancer. Br J Cancer 2017; 116: 626-633.

[14] Guo JN, Li J, Zhu CL, Feng WT, Shao JX, Wan L, Huang MD and He JD. Comprehensive profile of differentially expressed circular RNAs reveals that hsa_circ_0000069 is upregulated and promotes cell proliferation, migration, and invasion in colorectal cancer. Onco Targets Ther 2016; 9: 7451-7458.

[15] Wang K, Gan TY, Li N, Liu CY, Zhou LY, Gao JN, Chen C, Yan KW, Ponnusamy M, Zhang YH and Li PF. Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. Cell Death Differ 2017; 24: 1111-1120.

[16] Lin SP, Ye S, Long Y, Fan Y, Mao HF, Chen MT and Ma QJ. Circular RNA expression alterations are involved in OGD/R-induced neuron injury. Biochem Biophys Res Commun 2016; 471: 52-56.

[17] Bai Y, Zhang Y, Han B, Yang L, Chen X, Huang R, Wu F, Chao J, Liu P, Hu G, Zhang JH and Yao H. Circular RNA DLGAP4 ameliorates ischemic stroke outcomes by targeting miR-143 to regulate endothelial-mesenchymal transition associated with blood-brain barrier integrity. J Neurosci 2018; 38: 32-50.

[18] Rasmussen AH, Rasmussen HB and Silahtaroglu A. The DLGAP family: neuronal expression, function and role in brain disorders. Mol Brain 2017; 10: 43.

[19] Liu M, Jia J, Wang X, Liu Y, Wang C and Fan R. Long non-coding RNA HOTAIR promotes cervical cancer progression through regulating BCL2 via targeting miR-143-3p. Cancer Biol Ther 2018; 19: 391-399.

[20] Wang S, Chen J, Yu W and Deng F. Circular RNA DLGAP4 ameliorates cardiomyocyte apoptosis through regulating BCL2 via targeting miR-143 in myocardial ischemia-reperfusion injury. Int J Cardiol 2018; 279: 147.

[21] Minocherhomji S, Hansen C, Kim HG, Mang Y, Bak M, Guldberg P, Papadopoulos N, Eiberg H, Doh GD, Mollgard K, Hertz JM, Nielsen JE, Ropers HH, Tumer Z, Tommerup N, Kalscheuer VM and Silahtaroglu A. Epigenetic remodelling and dysregulation of DLGAP4 is linked with early-onset cerebellar ataxia. Hum Mol Genet 2014; 23: 6163-6176.

[22] Jiang YJ, Cao SQ, Gao LB, Wang YY, Zhou B, Hu X, Pu Y, Li ZL, Wang Q, Xiao X, Zhao L, Wang S, Liang WB and Zhang L. Circular ribonucleic acid expression profile in mouse cortex after traumatic brain injury. J Neurotrauma 2018; 36: 1018-1028.

[23] Wang H, He P, Pan H, Long J, Wang J, Li Z, Liu H, Jiang W and Zheng Z. Circular RNA circ-4099 is induced by TNF-alpha and regulates ECM synthesis by blocking miR-616-5p inhibition of Sox9 in intervertebral disc degeneration. Exp Mol Med 2018; 50: 27.

[24] Yang Z, Wang J, Pan Z and Zhang Y. miR-143-3p regulates cell proliferation and apoptosis by targeting IGF1R and IGFBP5 and regulating the Ras/p38 MAPK signaling pathway in rheumatoid arthritis. Exp Ther Med 2018; 15: 3781-3790.

[25] Yu B, Zhao Y, Zhang H, Xie D, Nie W and Shi K. Inhibition of microRNA-143-3p attenuates myocardial hypertrophy by inhibiting inflammatory response. Cell Biol Int 2018; 42: 1584-1593.

[26] Chen X, Ouyang H, Wang Z, Chen B and Nie Q. A novel circular RNA generated by FGFR2 gene promotes myoblast proliferation and differentiation by sponging miR-133a-5p and miR-29b-1-5p. Cells 2018; 7.

[27] Mao YY, Wang JQ, Guo XX, Bi Y and Wang CX. Circ-SATB2 upregulates STIM1 expression and regulates vascular smooth muscle cell proliferation and differentiation through miR-939. Biochem Biophys Res Commun 2018; 505: 119-125.

[28] Schlegel D, Kolb SJ, Luciano JM, Tovar JM, Cucchiara BL, Liebeskind DS and Kasner SE. Utility of the NIH Stroke Scale as a predictor of hospital disposition. Stroke 2003; 34: 134-137.