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Serum MicroRNA-137 Serves as a Novel Biomarker for Cerebral Atherosclerosis Diagnosis and Cerebrovascular Event Prediction

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Abstract: MicroRNAs have been reported as biomarkers for various diseases, including cerebral atherosclerosis (AS). In this study, whether serum microRNA-137 (miR-137) could be used as a biomarker for diagnosing cerebral AS and predicting cerebrovascular event was investigated. Quantitative real-time PCR was used to measure the expression of miR-137 in serum. Logistic analysis was used to evaluate the risk factors for the occurrence of cerebral AS, and receiver operating characteristic curves were used to estimate the diagnostic value of miR-137 and other risk factors for AS occurrence. Furthermore, the prognostic value of miR-137 for patients with AS was estimated using Kaplan–Meier survival analysis and Cox regression analysis. The results indicated that serum miR-137 levels were decreased in patients with cerebral AS. The expression of miR-137 was negatively correlated with total cholesterol and low-density lipoprotein cholesterol levels in patients with cerebral AS. The levels of miR-137, total cholesterol, low-density lipoprotein cholesterol, and hypersensitivity C response protein may serve as risk factors for the occurrence of cerebral AS, and miR-137 had diagnostic value for AS screening. Cerebral AS patients with positive cerebrovascular events have low miR-137 expression. Patients with high miR-137 expression had a lower incidence of cerebrovascular adverse events (log-rank $P = 0.013$), and miR-137 was an independent prognostic marker for the prediction of cerebrovascular event occurrence in patients with cerebral AS. In conclusions, our findings indicate that serum miR-137 levels are decreased in patients with cerebral AS and may be a new biomarker for diagnosing cerebral AS and predicting cerebrovascular events.

Key Words: cerebral atherosclerosis, MiR-137, diagnosis, prognosis

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The authors report no conflicts of interest.

A signed written informed consent was obtained from each patient, and the experimental procedures were all in accordance with the guidelines of the Ethics Committee of Weifang People's Hospital. Written informed consent for publication was obtained from each participant. The data and materials are available from the corresponding author upon reasonable request.

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INTRODUCTION

Atherosclerosis (AS) is one of the main potential factors of cardiovascular disease (a disease with the highest mortality).[1] It seriously endangers human health, especially the health and intellectual activities of middle- and old-aged people. It is noted that cerebral AS may cause acute cerebral circulatory disorders and chronic cerebral ischemic symptoms, such as transient ischemic attack (TIA) and stroke. It has been known that the main cellular components of blood vessels are endothelial cells (ECs) and vascular smooth muscle cells (VSMCs).[2] Thus, cerebral AS, which has complex etiology and pathogenesis, is believed to be closely related to the inflammatory response caused by vascular endothelial injury and the dysfunction of vascular ECs and VSMCs.[3] In addition, the higher total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels are significantly related to the occurrence of cerebral AS.[4] The hypersensitivity C-response protein (hs-CRP) is a vital inflammatory factor related to the formation of AS.[5]

Small noncoding microRNAs (miRNAs) are single-stranded RNA molecules with a length of approximately 21–23 nucleotides. Generally speaking, miRNAs can induce mRNA degradation or inhibit mRNA translation.[6] It is well known that miRNAs are related to many important biological processes, including proliferation, invasion, and apoptosis.[7–9] MiRNAs seem to be potential biomarkers for various diseases, such as prostate cancer,[10] glioblastoma,[11] and liver cirrhosis.[12] In addition, miRNAs have been known to be associated with arteriosclerosis.[13] Moreover, miR-126-5p,[14] miR-126, and miR-143[15] have been shown to function as biomarkers for AS. A study on gene and miRNA profiling of human induced pluripotent stem cell–derived ECs showed that miR-137 was increased in human embryonic stem cell–derived ECs and human induced pluripotent stem cell–derived ECs during endothelial differentiation.[16] More importantly, studies have found that miR-137 can regulate the function of vascular ECs and VSMCs, and promote angiogenesis,[17,18] suggesting a vital role of miR-137 in vascular biology. The dysfunction of vascular ECs and VSMCs has been well known to be a key mechanism of the cerebral AS; consequently, miR-137 may be related to the pathogenesis of cerebral AS and stroke.

Currently, the diagnosis of cerebral AS, however, is made by neuroimaging techniques, with the absence of circulating biomarkers. Therefore, studies regarding the

clinical significance of circulating miRNA expression in patients with cerebral AS are helpful for the early intervention of high-risk patients, and then decrease the occurrence of cerebrovascular adverse events. Thus, this study aimed to assess whether miR-137 could be used as a biomarker for cerebral AS diagnosis and prediction of cerebrovascular events.

MATERIAL AND METHODS

Patient Inclusion and Sample Collection

A total of 98 individuals admitted to the Weifang People's Hospital from January to December 2018 were enrolled and divided into 2 groups: cerebral AS group (n = 52) and control group (n = 46). In this study, the patient inclusion criteria were based on a previous study and slightly modified.[15] In brief, by magnetic resonance imaging or computed tomography examinations, none of the participants had a history of stroke. The examinations of cerebrovascular transcranial Doppler, magnetic resonance angiography, or computed tomography angiography were used to assess the AS and angiostenosis. Patients with cerebral AS and patients with vascular stenosis ≥50% were selected into the AS group. The subjects with no cerebral AS or with <50% degree of vascular stenosis were selected as the control group. The subjects with diagnosable diseases such as severe heart disease, recent myocardial infarction or angina pectoris, cardioembolism stroke of definite or uncertain etiology, severe infections or nephrosis or liver disease, thrombotic disease, and tumors were excluded. Blood samples of the 2 groups were collected via venipuncture, and then serum was obtained by centrifugation at 1500*g* at 4°C and stored at −80°C for further use.

Data Collection

The baseline characteristics of the participants, including age, sex, drinking, smoking, history of diabetes mellitus, and hypertension, were recorded at admission. Besides, we also recorded the laboratory parameters, including TC, triglyceride (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C), and hs-CRP. The excessive drinking was defined as drinking more than 25 g per day for adult males and drinking 15 g per day for adult females. People who smoke more than 20 cigarettes a day or who have been or are smoking for more than 6 months were defined as smokers. Fasting blood glucose ≥7.0 mM or diabetes diagnosed as requiring diet was defined as diabetes. Individuals with resting systolic blood pressure greater than or equal to 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg were defined as hypertension.

Follow-Up and End Points

The patients with cerebral AS were followed until the occurrence of cerebrovascular event or until October 2019 if there were no events. The end event was a composite outcome of TIA, stroke, and mortality. The diagnosis of TIA and stroke was based on the guideline of the American Heart Association/American Stroke Association.[19] The mortality was defined as cerebrovascular death. All protocols of this study were approved by the Ethics Committee of the Weifang People's Hospital, and informed consent was obtained from each participant.

RNA Extraction

Total RNA was extracted from the serum using TRIzol Reagent (Invitrogen, Carlsbad, CA). The purity and concentration of RNA were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Inc.). Then, the cDNA was synthesized from the obtained RNA by PrimeScript RT reagent kit (TaKaRa, Japan).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Serum miR-137 levels were measured using qRT-PCR, which was performed using the SYBR Green I Master Mix kit (Invitrogen, Carlsbad, CA) and 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) with following thermal cycling conditions: 95°C for 10 minutes, 95°C for 30 seconds, 60°C for 15 seconds, and 72°C for 15 seconds, for a total of 40 cycles. All procedures were performed based on the instructions of manufacturer. The expression of miR-137 was normalized to U6. Each sample was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: miR-137 forward, 5′-GCCGAGTTATTGCTTAAGAA-3′ and reverse, 5′-CTCAACTGGTGTCGTGGA-3'; U6 forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse, 5′-AACGCTTCACGAATTTGCGT-3'.

Statistical Analyses

All statistical analyses were performed using SPSS 21.0 software (SPSS, Inc, Chicago, IL) and GraphPad Prism 7.0 software (Inc, Chicago). All data were shown as mean ± SD. Student's *t* test or χ^2 test were used for comparison between groups. Correlations between indicators were analyzed by Pearson correlation analysis. Multivariate logistic analysis was used to evaluate the risk factors for AS occurrence. Receiver operating characteristic (ROC) curves were used to assess the diagnostic value of miR-137 and other risk factors for the occurrence of cerebral AS. Kaplan–Meier (KM) curves and log-rank test were used for survival analysis. Cox regression analysis was used to evaluate whether miR-137 was independently related to the occurrence of cerebrovascular adverse events of patients with AS. $P < 0.05$ indicated statistically significant.

RESULTS

Baseline Data of the Individuals in Two Groups

All participants were divided into AS groups (n = 52) and control group (n = 46). From the data in Table 1, no significant differences were found between the AS group and the control group in terms of age, sex, drinking, smoking, diabetes, hypertension, TG, and HDL-C. The significantly higher levels of TC, LDL-C, and hs-CRP were observed in the AS group than in the control group.

TABLE 1. Baseline Data of the Individuals in Two Groups

Variables	Control Group (n = 46)	AS Group (n = 52)	*P*
Age (yr)	62.000 ± 8.340	65.404 ± 11.793	0.106
Gender			
Female	20	20	0.614
Male	26	32	
Drinking			
No	33	32	0.286
Yes	13	20	
Smoking			
No	28	23	0.100
Yes	18	29	
Diabetes			
No	29	31	0.728
Yes	17	21	
Hypertension			
No	25	21	0.167
Yes	21	31	
TC (mmol/L)	4.200 ± 0.781	5.363 ± 1.202	<0.001
TG (mmol/L)	1.672 ± 0.752	1.802 ± 0.674	0.371
LDL-C (mmol/L)	2.878 ± 0.626	3.726 ± 0.713	<0.001
HDL-C (mmol/L)	1.219 ± 0.227	1.145 ± 0.188	0.081
hs-CRP (mg/L)	7.325 ± 11.637	14.319 ± 18.405	0.029

MiR-137 Expression was Decreased in Patients With AS

We detected the expression of miR-137 in AS and control groups by qRT-PCR and found the downregulation of miR-137 expression in patients with cerebral AS compared with the controls ($P < 0.001$, Fig. 1).

Correlation of miR-137 with Clinical Characteristics of Patients With AS

To explore whether miR-137 levels were associated with the development of cerebral AS disease, we performed the χ^2 test and Pearson correlation analysis. Table 2 showed that miR-137 expression was significantly negatively correlated with TC and LDL-C (all $P < 0.05$), suggesting that miR-137 may be associated with the development of cerebral AS.

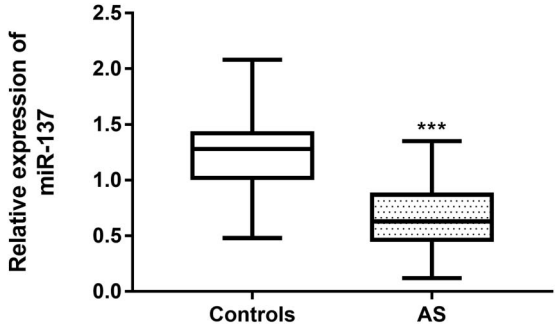

FIGURE 1. The expression of miR-137 in the control group (n = 46) and the cerebral AS group (n = 52). ***$P < 0.001$.

Diagnostic Performance of miR-137 for the Screening of AS

The miR-137 and all clinical characteristics were included in the logistic analysis to explore the risk factors for AS occurrence. The results showed that levels of miR-137, TC, LDL-C, and hs-CRP were significantly correlated with the occurrence of cerebral AS (Table 3). In addition, we further evaluated the diagnostic value of miR-137, TC, LDL-C, and hs-CRP for the screening of AS. As shown in Figure 2, the area under the curve (AUC) for miR-137 was 0.908 (with a specificity of 87%), and the AUCs for TC, LDL-C, and hs-CRP were 0.810 (with a specificity of 71.7%), 0.819 (with a specificity of 84.8%), and 0.624 (with a specificity of 32.6%), respectively. Therefore, miR-137 has a high diagnostic value for cerebral AS screening.

Prognostic Value of miR-137 for the Prediction of Cerebrovascular Events

In this study, the occurrence of cerebrovascular adverse events in patients with cerebral AS was recorded, among which a total of 29 patients (TIA number: 21; stroke number: 5; mortality number: 3) had cerebrovascular events. As shown in Figure 3A, miR-137 expression was significantly decreased in patients with positive cerebrovascular adverse compared with that in patients with negative cerebrovascular adverse ($P < 0.001$). The median expression value of miR-137 was used as the cutoff value to classify the patients into high (n = 23) and low (n = 29) expression groups. The KM method was used to establish the curve of occurrence of cerebrovascular adverse events. The results of KM curve (Fig. 3B) suggested that the patients with low expression levels of miR-137 had a high possibility of occurrence of cerebrovascular adverse events. The results of Cox regression analyses (Table 4) showed that miR-137 was independently correlated with the occurrence of cerebrovascular adverse events. The above

TABLE 2. Correlation of miR-137 With Clinical Characteristics of Patients With Cerebral AS

	miR-137 Expression	
Categorical Variables	**χ^2**	***P***
Gender	0.439	0.508
Drinking	0.008	0.930
Smoking	0.216	0.642
Diabetes	3.502	0.061
Hypertension	0.027	0.870
	miR-137 Expression	
Metric Variables	**r value**	***P***
Age	−0.107	0.295
TC (mmol/L)	−0.443	0.011
TG (mmol/L)	−0.128	0.283
LDL-C (mmol/L)	−0.431	0.009
HDL-C (mmol/L)	−0.191	0.060
hs-CRP (mg/L)	−0.041	0.691

χ^2 test was used to analyze categorical variables; Pearson correlation analysis was used to assess metric variables.

TABLE 3. Multivariate Logistic Analysis for Patients With Cerebral AS

Variables	OR	95% CI	*P*
Age	1.123	0.089–1.246	0.089
Gender	0.586	0.052–3.852	0.365
Drinking	1.515	0.184–12.445	0.699
Smoking	2.357	0.302–18.387	0.413
Diabetes	2.546	0.363–17.849	0.347
Hypertension	1.103	0.101–3.137	0.321
TC (mmol/L)	1.714	1.019–3.526	0.032
TG (mmol/L)	3.334	0.546–7.362	0.192
LDL-C (mmol/L)	17.356	2.105–143.119	0.008
HDL-C (mmol/L)	0.213	0.003–14.255	0.471
hs-CRP (mg/L)	1.089	1.008–1.175	0.030
miR-137 expression	0.096	0.003–0.412	0.001

results indicated that miR-137 can function as an independent prognostic marker of cerebral AS to predict the occurrence of cerebrovascular adverse events.

DISCUSSION

As a chronic multifactorial vascular disease, AS is a critical cause of a variety of cardiovascular diseases.[20] However, the lack of antiatherosclerotic therapy remains a large challenge. The discovery of novel AS biomarkers can be used to screen cerebral AS and predict the occurrence of cerebrovascular events.

Numerous studies have shown that miRNAs play key roles in the occurrence and development of a variety of human diseases, such as cancer,[21] metabolic diseases,[22] and cardiovascular diseases.[23] In addition, miRNAs have been known to be related to the progression of arteriosclerosis,[13] such as miR-26a,[20] miR-33a, and miR-33b.[24] Moreover, miR-137 has been found to be associated with cerebrovascular diseases. For instance, Liu et al[17] found that miR-137 enhances the proliferation and angiogenesis of endothelial progenitor cells in mice with cerebral ischemic stroke. The study by Huang et al[25] suggested that miR-137 suppresses vasculogenesis in brain arteriovenous malformations. In this study, miR-137 expression was found to be decreased in patients with AS compared with controls. Besides, TC, LDL-C, and hs-CRP levels were significantly higher in patients with AS than in controls. Then, the research on the association of miR-137 expression with clinical characteristics of patients with AS showed that the expression level of miR-137 was significantly negatively correlated with the levels of TC and LDL-C. Therefore, we considered that miR-137 may be involved in the progression of cerebral AS.

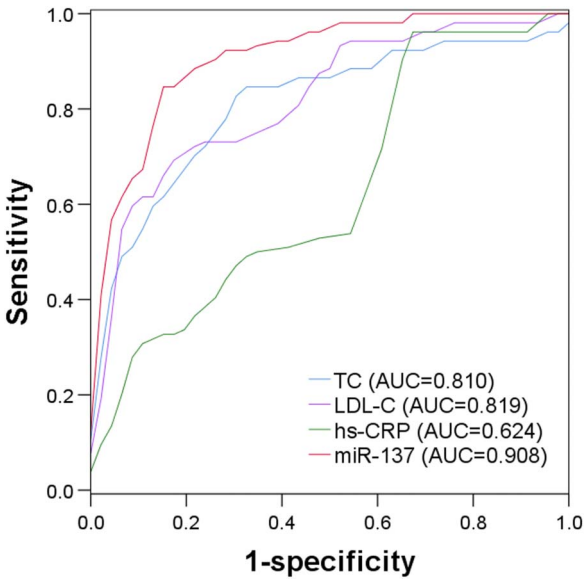

FIGURE 2. ROC curves for the screening of cerebral AS based on miR-137, TC, LDL-C, and hs-CRP levels. The AUC for miR-137 was 0.908, indicating that miR-137 has a high diagnostic value for cerebral AS screening. The number of patients was 52.

MiRNAs have been considered as ideal candidate biomarkers for a variety of human diseases, including AS, mainly because of their specific expression patterns and high stability in blood samples.[26] For example, miR-326 expression could be used as a potential biomarker in patients with Behcet's disease.[27] Serum miR-21 and miR-221 were used as biomarkers for cerebrovascular diseases.[28] Besides, miR-30-5p might be a clinical biomarker for AS.[29] Thus, in this study, we also investigated the clinical significance of serum miR-137 in patients with cerebral AS.

After investigating the association between miR-137 and cerebral AS, the diagnostic and prognostic value of miR-137 in patients with cerebral AS was further studied. First of all, logistic analysis was performed to evaluate the risk factors for the occurrence of cerebral AS, and it is found that miR-137, TC, LDL-C, and hs-CRP levels were risk factors for AS occurrence. Further ROC analysis indicated that miR-137 had diagnostic value for cerebral AS screening. The specificity of AUC for miR-137 was 87.0%, and the specificities of AUCs for TC, LDL-C, and hs-CRP were 71.7%, 84.8%, and 32.6%, respectively. Thus, miR-137 has high specificity for AS screening. Then, patients with positive cerebrovascular adverse were found to have lower miR-137 expression than those with negative cerebrovascular events. The results of KM curves showed that AS patients with high miR-137 expression levels had a low possibility of developing cerebrovascular adverse events. Cox regression analyses indicated an independent correlation between miR-137 and the occurrence of cerebrovascular events. Increasing evidence suggests that miR-137 may function as a potential biomarker in some diseases. For instance, a study indicated that miR-137 has potential for diagnosis and prognosis in human neurological and neoplastic diseases.[30] Li et al[31] found that miR-137 may be a diagnostic biomarker for Parkinson's disease. MiR-137 might be a biomarker for diagnosing multiple sclerosis.[32] Lei et al[33] found that miR-137 had prognostic value in prolactinomas. Low miR-137 expression suggested a poor prognosis in patients with cutaneous melanoma.[34] Therefore, we consider that miR-137 may be a potential clinical tool for diagnosing cerebral AS and predicting the occurrence of cerebrovascular adverse events.

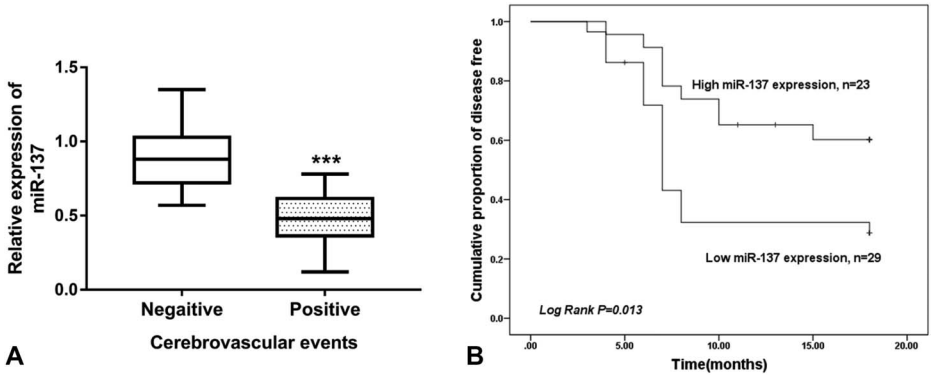

FIGURE 3. Prognostic value of miR-137 in predicting cerebrovascular events. A, Expression of miR-137 in cerebral AS patients with positive (n = 29) and negative (n = 23) cerebrovascular events. B, Kaplan–Meier survival curves of cerebral AS patients with high (n = 23) or low (n = 29) miR-137 expression levels (log-rank $P = 0.013$). (***$P < 0.001$ vs. cerebral AS patients with negative cerebrovascular events).

TABLE 4. Prognostic Value of miR-137 for the Prediction of Cerebrovascular Events

Variables	HR	95% CI	*P*
Age	1.022	0.982–1.064	0.286
Gender	0.419	0.146–1.201	0.106
Drinking	2.666	0.937–7.586	0.066
Smoking	1.731	0.686–4.369	0.245
Diabetes	3.146	0.094–6.545	0.125
Hypertension	1.644	0.633–4.268	0.307
TC (mmol/L)	1.160	0.818–1.645	0.404
TG (mmol/L)	1.015	0.492–2.095	0.667
LDL-C (mmol/L)	2.167	0.964–4.978	0.068
HDL-C (mmol/L)	0.338	0.017–6.858	0.480
hs-CRP (mg/L)	1.864	0.800–3.933	0.105
miR-137 expression	0.211	0.059–0.754	0.017

CONCLUSIONS

In conclusion, our study indicates that miR-137 expression is significantly decreased in patients with cerebral AS and may be a risk factor for disease onset. The aberrant expression of miR-137 in cerebral AS may be a candidate biomarker for disease screening and cerebrovascular event prediction. The clinical value of miR-137 is needed to be confirmed using a larger study population in further studies, the comparison of miR-137 with other miRNAs (such as diagnostic value and specificity) that have been studied in the context of cerebrovascular disease and AS should be performed in the future study. The diagnosis and treatment of cerebral AS may be improved with further understanding of the role of miR-137 in the future.

REFERENCES

1. Loppnow H, Werdan K, Buerke M. Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. *Innate Immun.* 2008;14:63–87.
2. Patsch C, Challet-Meylan L, Thoma EC, et al. Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. *Nat Cel Biol.* 2015;17:994–1003.
3. Son DJ, Jung YY, Seo YS, et al. Interleukin-32alpha inhibits endothelial inflammation, vascular smooth muscle cell activation, and atherosclerosis by upregulating Timp3 and reck through suppressing microRNA-205 biogenesis. *Theranostics.* 2017;7:2186–2203.
4. Gong DM, Zhang YL, Chen DY, et al. Endothelial GPR124 exaggerates the pathogenesis of atherosclerosis by activating inflammation. Cellular physiology and biochemistry. *Int J Exp Cell Physiol Biochem Pharmacol.* 2018;45:547–557.
5. Teng L, Meng R. Long non-coding RNA MALAT1 promotes acute cerebral infarction through miRNAs-mediated hs-CRP regulation. *J Mol Neurosci.* 2019;69:494–504.
6. Cui R. Linking miR-138 and USP10-P53 signaling. *Oncotarget.* 2019; 10:6541–6542.
7. He L, Zhu W, Chen Q, et al. Ovarian cancer cell-secreted exosomal miR-205 promotes metastasis by inducing angiogenesis. *Theranostics.* 2019; 9:8206–8220.
8. Suzuki R, Amatya VJ, Kushitani K, et al. miR-182 and miR-183 promote cell proliferation and invasion by targeting FOXO1 in mesothelioma. *Front Oncol.* 2018;8:446.
9. Zhang M, Zhang Q, Hu Y, et al. miR-181a increases FoxO1 acetylation and promotes granulosa cell apoptosis via SIRT1 downregulation. *Cel Death Dis.* 2017;8:e3088.
10. Mohammadi Torbati P, Asadi F, Fard-Esfahani P. Circulating miR-20a and miR-26a as biomarkers in prostate cancer. *Asian Pac J Cancer Prev.* 2019;20:1453–1456.
11. ParvizHamidi M, Haddad G, Ostadrahimi S, et al. Circulating miR-26a and miR-21 as biomarkers for glioblastoma multiform. *Biotechnol Appl Biochem.* 2019;66:261–265.
12. Ghaempoor M, Arefian E, Mohebbi SR, et al. Circulating miR-625 as an emerging biomarker for liver cirrhosis. *Clin Lab.* 2019;65.
13. Lu Y, Thavarajah T, Gu W, et al. Impact of miRNA in atherosclerosis. *Arteriosclerosis, Thromb Vasc Biol.* 2018;38:e159–e70.
14. Schober A, Nazari-Jahantigh M, Wei Y, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat Med.* 2014;20:368–376.
15. Gao J, Yang S, Wang K, et al. Plasma miR-126 and miR-143 as potential novel biomarkers for cerebral atherosclerosis. *J Stroke Cerebrovasc Dis.* 2019;28:38–43.
16. Wang L, Su W, Du W, et al. Gene and MicroRNA profiling of human induced pluripotent stem cell-derived endothelial cells. *Stem Cel Rev Rep.* 2015;11:219–227.
17. Liu XL, Wang G, Song W, et al. microRNA-137 promotes endothelial progenitor cell proliferation and angiogenesis in cerebral ischemic stroke mice by targeting NR4A2 through the Notch pathway. *J Cell Physiol.* 2018;233:5255–5266.
18. Pan J, Li K, Huang W, et al. MiR-137 inhibited cell proliferation and migration of vascular smooth muscle cells via targeting IGFBP-5 and modulating the mTOR/STAT3 signaling. *PloS One.* 2017;12:e0186245.
19. Powers WJ, Derdeyn CP, Biller J, et al. 2015 American heart association/American stroke association focused update of the 2013 guidelines for the early management of patients with acute ischemic stroke regarding endovascular treatment: a guideline for healthcare professionals from the American heart association/American stroke association. *Stroke.* 2015; 46:3020–3035.
20. Feng M, Xu D, Wang L. miR-26a inhibits atherosclerosis progression by targeting TRPC3. *Cel Biosci.* 2018;8:4.

21. Zhu L, Deng H, Hu J, et al. The promising role of miR-296 in human cancer. *Pathol Res Pract.* 2018;214:1915–1922.
22. Deiuliis JA. MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. *Int J Obes.* 2016;40:88–101.
23. Zhao Y, Ponnusamy M, Zhang L, et al. The role of miR-214 in cardiovascular diseases. *Eur J Pharmacol.* 2017;816:138–145.
24. Price NL, Rotllan N, Canfran-Duque A, et al. Genetic dissection of the impact of miR-33a and miR-33b during the progression of atherosclerosis. *Cel Rep.* 2017;21:1317–1330.
25. Huang J, Song J, Qu M, et al. MicroRNA-137 and microRNA-195* inhibit vasculogenesis in brain arteriovenous malformations. *Ann Neurol.* 2017;82:371–384.
26. Liu G, Liu W, Guo J. Clinical significance of miR-181a in patients with neonatal sepsis and its regulatory role in the lipopolysaccharide-induced inflammatory response. *Exp Ther Med.* 2020;19:1977–1983.
27. Jadideslam G, Ansarin K, Sakhinia E, et al. Expression levels of miR-21, miR-146b and miR-326 as potential biomarkers in Behcet's disease. *Biomarkers Med.* 2019;13:1339–1348.
28. Tsai PC, Liao YC, Wang YS, et al. Serum microRNA-21 and microRNA-221 as potential biomarkers for cerebrovascular disease. *J Vasc Res.* 2013;50:346–354.
29. Zhou Z, Chen Y, Zhang D, et al. MicroRNA-30-3p suppresses inflammatory factor-induced endothelial cell injury by targeting TCF21. *Mediators Inflamm.* 2019;2019:1342190.
30. Mahmoudi E, Cairns MJ. MiR-137: an important player in neural development and neoplastic transformation. *Mol Psychiatry.* 2017;22:44–55.
31. Li N, Pan X, Zhang J, et al. Plasma levels of miR-137 and miR-124 are associated with Parkinson's disease but not with Parkinson's disease with depression. *Neurol Sci.* 2017;38:761–767.
32. Ehya F, Abdul Tehrani H, Garshasbi M, et al. Identification of miR-24 and miR-137 as novel candidate multiple sclerosis miRNA biomarkers using multi-staged data analysis protocol. *Mol Biol Res Commun.* 2017;6:127–140.
33. Lei C, Jing G, Jichao W, et al. MiR-137's tumor suppression on prolactinomas by targeting MITF and modulating wnt signaling pathway. *J Clin Endocrinol Metab.* 2019;104:6391–6402.
34. Li N. Low expression of mir-137 predicts poor prognosis in cutaneous melanoma patients. *Med Sci Monitor.* 2016;22:140–144.