European Review for Medical and Pharmacological Sciences 2020; 24: 11761-11767

Correlation between serum microRNA-136 levels and RAAS biochemical markers in patients with essential hypertension

H.-T. CHU, L. LI, M. JIA, L.-L. DIAO, Z.-B. LI

[1]Department of Cardiovascular Medicine, Zaozhuang Municipal Hospital, Zaozhuang, Shandong, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the correlation between microRNA-136 levels and biochemical markers of renin-angiotensin-aldosterone system (RAAS) in patients with essential hypertension (EH).

PATIENTS AND METHODS: The subjects were divided into EH group (n=110) and healthy control group (n=110). MicroRNA-136 expression, angiotensin-converting enzyme (ACE) activity, and expression of renin (RA) and angiotensin II (Ang II), and aldosterone (ALD) in peripheral blood serum were examined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), equine glycylglycine glycine method, magnetic particle chemistry, and radioimmunoassay, respectively. In addition, the correlation between microRNA-136 and RAAS biochemical markers was estimated by Pearson linear regression. Meanwhile, ROC curve analysis was carried out to evaluate the potential of microRNA-136 for the diagnosis of EH. Follow-up data were recorded for assessing the influence of microRNA-136 on the prognosis in patients with EH.

RESULTS: It was found that microRNA-136 expression was remarkably elevated in peripheral blood serum of patients with EH, and the expression levels of biochemical markers of RASS, such as ACE, RA, Ang II, and ALD were also found higher than those in healthy controls. Meanwhile, a significant positive correlation was confirmed between microRNA-136 level and ACE activity, RA, Ang II, as well as ALD levels in patients with EH. In addition, the area under the ROC curve (AUC) was calculated as 0.8662, with a sensitivity of 82.73% and a specificity of 80.91%. After two-months medication intervention, patients with EH expressing a high level of microRNA-136 had better therapeutic efficacy than those with a low level.

CONCLUSIONS: In peripheral blood serum, microRNA-136 expression was dramatically negatively correlated with biochemical markers of RASS. High level of microRNA-136 predicts a good prognosis in patients with EH following medication. Therefore, microRNA-136 can be used as a potential biomarker for the diagnosis of EH.

Key Words:
Essential hypertension, MicroRNA-136, RAAS.

Introduction

Essential hypertension (EH), a syndrome with elevated blood pressure as the main clinical manifestation, is an important cause and risk factor for various cardiovascular and cerebrovascular diseases[1]. This syndrome can seriously affect the structure and function of the heart, brain, kidney, and other important organs, and eventually lead to the failure of these organs. Up to now, hypertension is still one of the main causes inducing cardiovascular death[2], but the pathogenesis of EH remains to be accurately determined.

MicroRNA (miRNA), first reported in 1993, is a class of endogenous non-coding RNA, able to be engaged in the transcription and expression of many genes[3,4]. MiRNAs exist in plasma, serum and other body fluids in a stable form, play an auxiliary role in disease diagnosis or evaluation of prognosis and recurrence condition and may be used as a new clinical marker. Therefore, the change of miRNA expression may be closely associated with the occurrence and progression of human diseases[5]. Hao et al[6] have demonstrated that miRNA can be involved in the occurrence, development, and outcome of hypertension. In addition, microRNA-136, located on chromosome 14, is reported to have the ability of suppressing the proliferation and invasion of colon cancer cells by targeting liver receptor homolog-1/Wnt signaling[7]. However, the correlation between miRNA-136 and essential hypertension has not been explored.

The activation of renin-angiotensin aldosterone system (RAAS) is a classic pathway for the development of hypertension and plays an important role in the pathogenesis of this disease.

Corresponding Author: Zaibo Li, BM; e-mail: jm681005@163.com

Currently, miRNAs are reported to be closely related to the overactivation of the RAAS system and can indirectly or directly affect blood pressure through a variety of pathways[8]. Renin, released and secreted by preglomerular cells, participates in regulating blood pressure and maintaining the dynamic balance of the environment in the body. Dysregulation of aldosterone, the final product of the renin-angiotensin system, can induce hypertension. Angiotensin-converting enzyme 2 (ACE2) generates angiotensin-7 peptide Ang (1-7) under the action of angiotensin metabolism, and thus acts as a negative regulator of the RAAS system. As a peptide, Apelin, the second catalytic compound of ACE2, has a protective effect on myocardial contractility and cardiovascular system. Apelin binds to its surface receptor APJ, a 7-transmembrane receptor with significant homology to the Ang II1 receptor (AT1R)[9,10]. In this study, the expression level of microRNA-136 in the serum of patients with EH was explored, and its correlation with the biochemical markers of RAAS was analyzed, so as to provide a new idea for the diagnosis and treatment of EH.

Patients and Methods

Information Collection

110 patients with primary hypertension who were hospitalized in the hospital from May 2016 to December 2018 were collected, all in line with the diagnostic criteria for EH in the World Health Organization and the International Association of Hypertension. They were composed of 60 males and 50 females aged 44-78 years old, with an average of (59.6±7.2) years old. Patients with kidney disease, diabetes, or severe cardiovascular and cerebrovascular diseases were excluded. A total of 110 healthy people in the hospital were selected as the control group, consisting of 62 males and 48 females aged 48-75 years, with a mean of (58.8 ± 9.1) years old. There were no significant differences in age and gender between the two groups (p>0.05), which were comparable. The investigation was approved by the Ethics Committee of Zaozhuang Municipal Hospital, and an informed consent form was signed by each subject.

Blood Specimen Processing

About 4-5 mL of fasting venous blood was collected in the morning, inverted several times, let stand for 1 h at room temperature, and centrifuged at 1000 g for 15 min at 4°C. After that, the supernatant was collected in the enzyme-free Eppendorf (EP) tube and then stored at -80°C.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Detection

Total RNA in serum was extracted by TRIzol method (Invitrogen, Carlsbad, CA, USA) and then reversely transcribed into complementary deoxyribonucleic nucleic acid (cDNA). QRT-PCR was performed according to the protocol of TaqMan RNA Reverse Transcripts Kits (Thermo Fisher Scientific, Waltham, MA, USA). The primers were designed as follows: miR-136: 5'-ACUCCAUUUGUUUUGAUGAUGGA-3' (forward) and 5'-UCCAUCAUCAAAACAAAUGGAGU-3' (reverse), and U6: 5'-GCTTCGGCAGCACATATACTAAAAT-3' (forward) and 5'-CGCTTCACGAATTTGCGTGTCAT-3' (reverse).

Detection of ACE Activity

Equine glycylglycine can be hydrolyzed to hippuric acid and glycylglycine under the action of ACE. The hippuric acid was extracted with ethyl acetate, dissolved in a sodium chloride solution, and the amount of reduction of equine glycylglycine was measured at 228 nm. The serum ACE assay kit was purchased from Beyotime Biotech (Shanghai, China).

Serum Renin (RA), Angiotensin II (Ang II), Aldosterone (ALD) Detection

Automatic biochemical analyzer (Zhengzhou Antu Bioengineering Co., Ltd., model AutoLumo A2000, Zhengzhou, China) and intelligent immune counter (Beijing North Biotechnology Research Institute Co., Ltd., model FJ-2008PS, Beijing, China) were used. RA was examined by renin quantitative test kit using magnetic particle chemistry method, ALD was detected by ALD kit using radioimmunoassay, and Ang II was analyzed by radiation method.

Follow-Up

Patients recruited with EH orally took extended release nifedipine tablets, with 10 mg, bid, and they were followed up for 2 months. Therapeutic efficacy of medication was assessed as follows: Significant effect: diastolic blood pressure decline ≥ 10 mm Hg, and it dropped to normal or above 20 mm Hg. Valid: diastolic blood pressure decline < 10 mm Hg, but it dropped to normal or declined 10-19 mm Hg. Invalid: failed to achieve the above two standards. Total effective rate was the ratio

Table I. Baseline characteristics of healthy subjects and patients with EH.

Variable	Control	Hypertension	χ^2/t	p
Age	58.8 ± 9.16	59.6± 7.22	0.719	0.473
Sex (male/female)	62/48	60/50	0.074	0.892
BMI (kg/m^2)	24.15 ± 3.28	24.73 ± 3.41	1.286	0.200
SBP (mm Hg)	124.21±17.35	141.73±20.09	6.922	<0.001
DBP (mm Hg)	81.32±8.07	92.11±9.19	9.253	<0.001
TC (mmol/L)	4.36±1.16	4.46±1.27	0.611	0.543
HDL-C (mmol/L)	1.48±0.64	1.34±0.43	1.904	0.058
LDL-C (mmol/L)	2.03±1.02	2.11±1.23	0.525	0.600
Glucose (mg/dL)	85.66±5.98	87.21±6.11	1.901	0.059
Smoking (n)	59	66	0.908	0.414

Note: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

of numbers of significant effect and valid cases to the total case number.

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM, Armonk, NY, USA). The measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± s). The comparison between groups was analyzed by *t*-test, and enumeration data comparison was achieved by the Chi-square test. Then, the correlation analysis was performed by Pearson correlation, and the receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of microRNA-136 for EH. The difference was statistically significant at $p<0.05$.

Results

Baseline Characteristics of Healthy Subjects and Patients with EH

Clinical data of recruited patients with EH and healthy subjects were collected. Significant differences in systolic and diastolic blood pressure were found between two groups ($p<0.05$). Nevertheless, no significant differences in age, sex, BMI, TC, HDL-C, and LDL-C were seen between EH patients and healthy controls ($p>0.05$, Table I), suggesting the comparable baseline characteristics between groups.

MicroRNA-136 Is Under-Expressed in Serum of Patients With EH

qPCR revealed that microRNA-136 expression in peripheral blood of patients with EH was remarkably higher than that of healthy controls (Figure 1), suggesting that microRNA-136 may be engaged in the development of hypertension.

Comparison of Serum ACE Activity and Levels of RA, Ang II and ALD in Peripheral Blood of Two Groups

It was found that serum ACE activity and RA, Ang II, and ALD levels in patients with EH were remarkably up-regulated compared with those in the healthy subjects (Table II), indicating that patients with EH contained higher RASS biomarkers in serum.

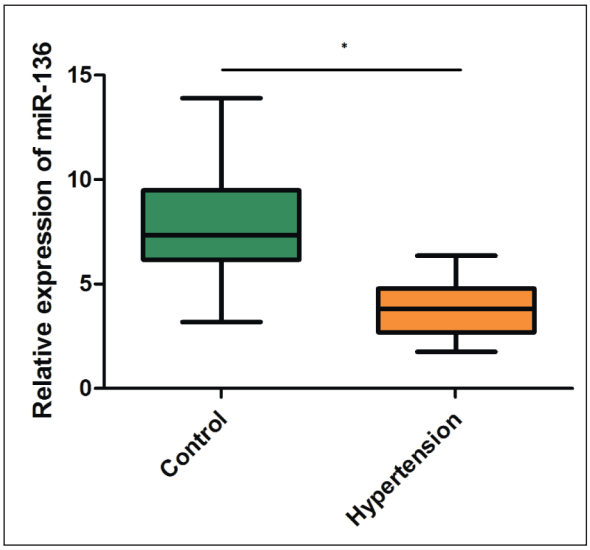

Figure 1. Low expression of microRNA-136 in the serum of patients with EH. Compared with the serum of healthy people, qRT-PCR shows that the expression level of microRNA-136 in the serum of patients with EH is remarkably reduced.

Table II. Comparison of serum ACE activity and levels of RA, Ang II, and ALD in peripheral blood.

Variable	Control	Hypertension	*t*	*p*
ACE activity (U/L)	105.37±25.13	171.20±31.39	17.171	<0.001
RA (μg/L)	2.92±0.43	3.13±0.51	3.302	0.001
Ang II (ng/L)	51.99±18.27	104.73±34.29	14.237	<0.001
ALD (ng/L)	30.15±19.07	51.32±18.10	8.445	<0.001

ACE: Angiotensin converting enzyme; RA: Renin; Ang II: Angiotensin II; ALD: Aldosterone.

Correlation Between Serum MicroRNA-136 in Peripheral Blood and RAAS Biochemical Markers in Patients With EH

To determine whether there exists a correlation between microRNA-136 and RAAS biochemical markers, Pearson correlation analysis was performed, and it was found that microRNA-136 expression was negatively correlated with ACE activity (r=-0.7874, p<0.001) (Figure 2A), RA (r=-0.3693, p<0.001) (Figure 2B), Ang II (r=-0.2743 p=0.0037) (Figure 2C), and ALD (r=-0.5378, p<0.001) (Figure 2D), which indicates that microRNA-136 can inhibit ACE activity and decrease the expression levels of RA, Ang II, and ALD.

Evaluation of the Value of MicroRNA-136 in the Diagnosis of EH

ROC curve analysis showed that the area under the ROC curve was AUC=0.8662, p<0.001, 95% CI=0.8175-0.9148. When the cut-off value was 4.33, the sensitivity was 82.7% and the specificity was 80.91% (Figure 3). This result suggests that microRNA-136 in serum can be indeed used as a potential biomarker for the diagnosis of EH.

The Influence of MicroRNA-136 on the Therapeutic Efficacy in EH

Patients recruited with EH were classified into two groups (n=55 in each group) based on the median serum level of microRNA-136 in them. These patients were followed up for 2 months. According to the recorded follow-up data, therapeutic efficacy was evaluated. As data showed, after two-month medication, the total effective rate was 90.91% (50/55) in high microRNA-136 expression group and 63.64% (35/55) in low microRNA-136 expression group (p<0.05, Table III). It is concluded that highly expressed microRNA-136 contributes to EH treatment.

Discussion

Hypertension is a cardiovascular syndrome affected by multiple factors including heredity and environment, and its incidence is closely correlated with abnormal activity of renin-angiotensin-aldosterone system (RAAS)[11,12]. Angiotensin converting enzyme 2 (ACE2) is the key enzyme of RAAS system, which can inhibit the inflammatory response by regulating the production of angiotensin (Ang) II/Ang (1-7) to improve the cardiovascular remodeling phenomenon induced by hypertension[13]. In recent years, a variety of microRNAs (miRNAs) has been identified one after another[14,15], which can play a vital part in the occurrence and development of hypertension *via* regulating different pathophysiological processes such as cell proliferation, differentiation, metabolism, and apoptosis[16-18].

The expressions of some miRNAs in the peripheral blood of patients with EH, such as miRNA-126, miRNA-143, miRNA-145 and miRNA-133, are down-regulated, while some other miRNAs, such as miRNA-1, miRNA-296-5p, and miRNA-let-7e are conversely up-regulated. In addition, some miRNAs are found negatively correlated with blood pressure, such as miRNA-21, miRNA-143 and miRNA-145, while the opposite result is observed in miRNA-9, miRNA-126, and miRNA-133 expression[14-16,19]. Moreover, in spontaneous hypertensive rats (SHR), dysregulation of miRNA-155, miRNA-130a, and miRNA-208 are discovered, and their expression levels are negatively correlated with blood pressure[20].

Therefore, miRNA expression may be involved in the incidence of hypertension to some extent, suggesting that miRNA may serve as a potential target for the treatment of hypertension. MicroRNA-136 is one of the many dysregulated

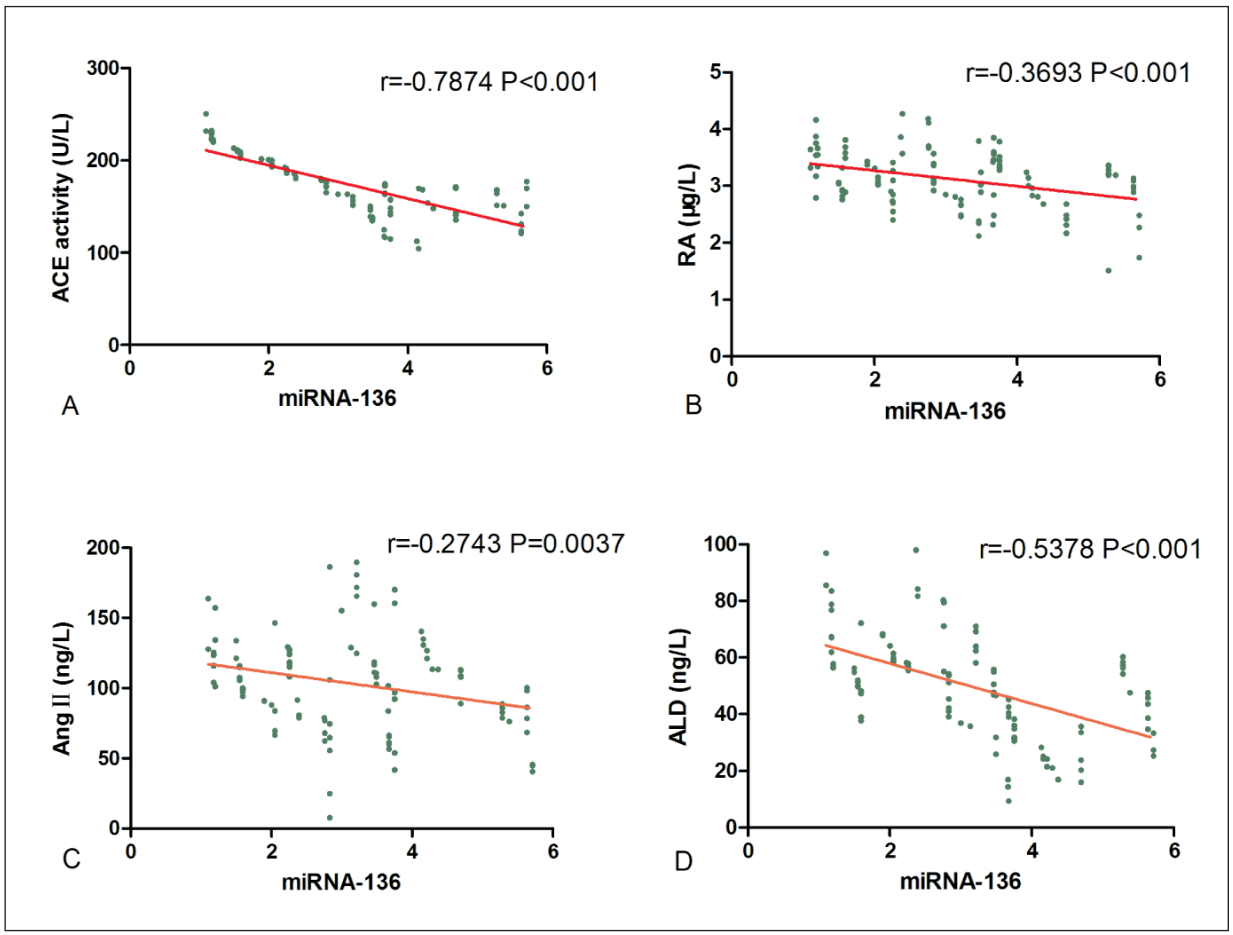

Figure 2. Correlation between serum microRNA-136 levels in peripheral blood and RAAS biochemical markers in patients with EH. **A**, Correlation between microRNA-136 expression and peripheral blood ACE activity. **B**, Correlation between microRNA-136 expression and peripheral blood RA. **C**, Correlation between microRNA-136 expression and peripheral blood Ang IIs. **D**, Correlation between microRNA-136 expression and peripheral blood ALD.

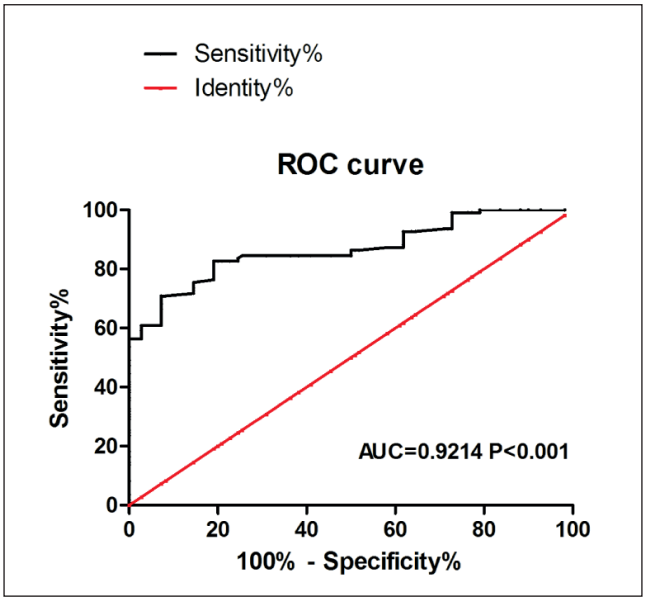

Figure 3. ROC curve evaluates the value of microRNA-136 in the diagnosis of EH. The area under the ROC curve (AUC) is 0.8662, $p<0.001$. When the cut-off value is 4.330, the sensitivity is 82.73% and the specificity is 80.91%.

Table III. The influence of microRNA-136 on the therapeutic efficacy in EH.

Groups	Effect after one month			Effect after two months		
	Significant effect	Valid	Invalid	Significant effect	Valid	Invalid
High level of microRNA-136 (n=55)	28	9	18	34	16	5
Low level of microRNA-136 (n=55)	21	13	21	22	13	20
χ^2	1.803	0.909	0.358	5.238	0.421	11.647
p	0.25	0.475	0.69	0.035	0.666	0.001

miRNAs in tumor tissues[21]. Jeong et al[22] reported that microRNA-136 can enhance paclitaxel's inhibitory effect on ovarian cancer cell proliferation by inhibiting the activity of cancer stem cells by targeting Notch3. In this study, microRNA-136 expression was found decreased in serum of patients with EH.

RAAS system is able to interact with miRNA[17,23]. ACE, the main product of Ang II, can promote cardiovascular proliferation, inflammatory reaction, and oxidative stress reaction, and thus participate in the process of hypertension mediated cardiovascular remodeling by targeting angiotensin type 1 (AT1) receptor. Meanwhile, the ACE2/Ang (1-7)-Mas receptor axis exerts anti-inflammatory response as well as anti-oxidation and anti-angiogenic remodeling effects by counteracting the above-mentioned effects of ACE/Ang II signaling[8,24]. Chen et al[24] considered that miRNAs act by activation of the RAAS system *via* the ACE2-Ang II-AT1R pathway. Kemp et al[25] found that microRNA-483-3p can directly act on specific parts of RAAS, and that microRNA-483-3p can regulate the balance and homeostasis of ACE, ACE2 and AT2R levels, suggesting that miRNAs may function as regulators of the RASS system, which reveals a complex relationship between miRNA and Ang II sensitivity in the RAS system. In this study, Pearson regression analysis manifested that miRNA-136 level in peripheral blood serum of patients with EH has significant negative association with RAAS biochemical markers. However, the relevant mechanism needs further research and exploration. In addition, through ROC curve analysis, it was found that microRNA-136 had the potential to be a new diagnostic molecular marker for hypertension.

Conclusions

These data indicate that serum microRNA-136 expression is remarkably reduced in patients with EH, while the expression of biochemical markers of RAAS system, such as ACE activity and RA, Ang II, ALD, are increased. It can be concluded that microRNA-136 exerts an inhibitory effect on RAAS system activity and may play a pivotal role in the progression of primary hypertension.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Huston JH, Maron BA, French J, Huang S, Thayer T, Farber-Eger EH, Wells QS, Choudhary G, Hemnes AR, Brittain EL. Association of mild echocardiographic pulmonary hypertension with mortality and right ventricular function. JAMA Cardiol 2019 Sep 18. doi: 10.1001/jamacardio.2019.3345. [Epub ahead of print].
2) Krakoff LR. Worldwide hypertension: the growing threat and the potential opportunities. Ann Glob Health 2016; 82: 225-226.
3) Collins RL, Brand H, Redin CE, Hanscom C, Antolik C, Stone MR, Glessner JT, Mason T, Pregno G, Dorrani N, Mandrile G, Giachino D, Perrin D, Walsh C, Cipicchio M, Costello M, Stortchevoi A, An JY, Currall BB, Seabra CM, Ragavendran A, Margolin L, Martinez-Agosto JA, Lucente D, Levy B, Sanders SJ, Wapner RJ, Quintero-Rivera F, Kloosterman W, Talkowski ME. Defining the diverse spectrum of inversions, complex structural variation, and chromothripsis in the morbid human genome. Genome Biol 2017; 18: 36.
4) Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.
5) Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203-222.
6) Hao Y, Wu BG, Shi J, Chen YL, Sun ZQ, Zheng LQ, Zhang XG, Geng N, Li TJ, Li H, Sun YX. Association of tag SNPs of GPx-3 with essential hypertension

in rural Han Chinese in Fuxin, Liaoning, China. Chin Med J (Engl) 2011; 124: 2113-2116.

7) Yuan Q, Cao G, Li J, Zhang Y, Yang W. MicroRNA-136 inhibits colon cancer cell proliferation and invasion through targeting liver receptor homolog-1/Wnt signaling. Gene 2017; 628: 48-55.

8) Nossent AY, Hansen JL, Doggen C, Quax PH, Sheikh SP, Rosendaal FR. SNPs in microRNA binding sites in 3'-UTRs of RAAS genes influence arterial blood pressure and risk of myocardial infarction. Am J Hypertens 2011; 24: 999-1006.

9) Zhang Z, Chen L, Zhong J, Gao P, Oudit GY. ACE2/Ang-(1-7) signaling and vascular remodeling. Sci China Life Sci 2014; 57: 802-808.

10) Yu XH, Tang ZB, Liu LJ, Qian H, Tang SL, Zhang DW, Tian GP, Tang CK. Apelin and its receptor APJ in cardiovascular diseases. Clin Chim Acta 2014; 428: 1-8.

11) Weir MR, Bushinsky DA, Benton WW, Woods SD, Mayo MR, Arthur SP, Pitt B, Bakris GL. Effect of Patiromer on hyperkalemia recurrence in older chronic kidney disease patients taking RAAS inhibitors. Am J Med 2018; 131: 555-564.

12) Gant CM, Laverman GD, Vogt L, Slagman M, Heerspink H, Waanders F, Hemmelder MH, Navis G. Renoprotective RAAS inhibition does not affect the association between worse renal function and higher plasma aldosterone levels. BMC Nephrol 2017; 18: 370.

13) Pacurari M, Kafoury R, Tchounwou PB, Ndebele K. The renin-angiotensin-aldosterone system in vascular inflammation and remodeling. Int J Inflam 2014; 2014: 689360.

14) Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, Ma X, Lau WB, Rong R, Yu X, Wang B, Li Y, Xiao C, Zhang M, Wang S, Yu L, Chen AF, Yang X, Cai J. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. Circulation 2011; 124: 175-184.

15) Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. MicroRNA-9 and microRNA-126 expression levels in patients with essential hypertension: potential markers of target-organ damage. J Am Soc Hypertens 2014; 8: 368-375.

16) Batkai S, Thum T. MicroRNAs in hypertension: mechanisms and therapeutic targets. Curr Hypertens Rep 2012; 14: 79-87.

17) Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. J Clin Invest 2013; 123: 11-18.

18) Bronze-da-Rocha E. MicroRNAs expression profiles in cardiovascular diseases. Biomed Res Int 2014; 2014: 985408.

19) Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. Differential expression of vascular smooth muscle-modulating microRNAs in human peripheral blood mononuclear cells: novel targets in essential hypertension. J Hum Hypertens 2014; 28: 510-516.

20) Wu WH, Hu CP, Chen XP, Zhang WF, Li XW, Xiong XM, Li YJ. MicroRNA-130a mediates proliferation of vascular smooth muscle cells in hypertension. Am J Hypertens 2011; 24: 1087-1093.

21) Liu H, Xie X, Yang X, Li Y, Wang Y, Xu D. Enhanced inflammatory damage by microRNA-136 targeting Klotho expression in HK-2 cells by modulating JAK/STAT pathway. Pharmazie 2017; 72: 265-271.

22) Jeong JY, Kang H, Kim TH, Kim G, Heo JH, Kwon AY, Kim S, Jung SG, An HJ. MicroRNA-136 inhibits cancer stem cell activity and enhances the anti-tumor effect of paclitaxel against chemoresistant ovarian cancer cells by targeting Notch3. Cancer Lett 2017; 386: 168-178.

23) Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L, Braun T. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest 2009; 119: 2634-2647.

24) Chen LJ, Xu R, Yu HM, Chang Q, Zhong JC. The ACE2/apelin signaling, microRNAs, and hypertension. Int J Hypertens 2015; 2015: 896861.

25) Kemp JR, Unal H, Desnoyer R, Yue H, Bhatnagar A, Karnik SS. Angiotensin II-regulated microRNA 483-3p directly targets multiple components of the renin-angiotensin system. J Mol Cell Cardiol 2014; 75: 25-39.