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## Serum miR-126 and miR-146a levels in patients with acute cerebral infarction and their relationship with severity of the disease

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### ABSTRACT

**Objective:** To analyze the serum levels of miR-126, miR-146a and its relationship with infarction area, severity of disease and inflammatory reaction degree in patients with acute cerebral infarction (ACI).

**Methods:** A total of 75 cases with ACI treated in our hospital from April 2014 to October 2015 and 80 healthy cases were respectively selected as ACI group and control group for retrospective study. Patients' clinical data were collected, and the serum levels of miR-126, miR-146a, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 were detected.

**Results:** Serum contents of miR-126 ( $0.286 \pm 0.078$  vs.  $1.000 \pm 0.169$ ) and miR-146a ( $0.337 \pm 0.084$  vs.  $1.000 \pm 0.158$ ) in patients of ACI group were significantly lower than those of control group. Contents of IL-1 $\beta$  [ $(68.4 \pm 10.3)$  vs.  $(22.7 \pm 5.8)$  ng/L], TNF- $\alpha$  [ $(126.9 \pm 22.4)$  vs.  $(49.6 \pm 8.4)$  ng/L] and IL-6 [ $(89.3 \pm 14.7)$  vs.  $(34.8 \pm 5.9)$  ng/L] were obviously higher than those of control group. The bigger the infarction area was, the more severer the degree of nerve defect could be. The lower the serum levels of miR-126, miR-146a were, the higher the levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 could be. Levels of miR-126 and miR-146a were negative correlation with levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-6.

**Conclusions:** An abnormal decrease in serum levels of miR-126 and miR-146a in patients with ACI was closely related to the severity of disease. Through regulating the generation of inflammatory factors TNF- $\alpha$ , IL-1 $\beta$  and IL-6, miR-126 and miR-146a may get involved in the changes of cerebral infarction condition.

## 1. Introduction

Acute cerebral infarction (ACI) is a cerebrovascular disease which causes serious harm to human health, and its disability rate and fatality rate are relatively high<sup>[1–3]</sup>. Atherosclerosis is an important pathological basis for causing the occurrence and development of ACI. The formation and character change of atheromatous plaque are the core links of causing the

development and change of cerebral infarction condition<sup>[4–6]</sup>. At present, great progress has been made clinically in the diagnosis and treatment of cerebral infarction. With the development of thrombolytic therapy, interventional therapy, functional exercise during convalescence, neurotrophs, and other measures, prognosis in patients with cerebral infarction has been improved greatly. In spite of this, the pathogenesis of cerebral infarction, especially the pathogenesis of atherosclerosis is not clear yet. miRNA is a non-coding RNA with the length of 17–25 bp, and its function is to target and induce degradation or translation inhibition of mRNA, thereby causing gene silence at a post-transcriptional level. miRNA involves in various *in-vivo* post-transcriptional control of genetic expression, which has regulating effect on the mature of neurological function, formation of atheromatous plaque, immune and inflammatory response<sup>[7–9]</sup>. In the process of the occurrence and development of cerebral infarction and atherosclerosis, various miRNAs play an important role in regulation. Studies have shown that two

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miRNAs, miR-126 and miR-146a, can involve in the process of atherosclerosis by targeting and regulating inflammatory response, and their abnormal content relate to various cardiovascular and cerebrovascular diseases<sup>[10,11]</sup>. In the following study, we analyzed the serum levels of miR-126, miR-146a and their relationship with severity of disease in patients with ACI.

## 2. Materials and methods

### 2.1. Study objects

Using the method of retrospective study, 75 cases of patients with ACI treated in our hospital from April 2014 to October 2015 were selected as the ACI group. All patients met the diagnostic criteria of cerebral infarction defined by the Fourth National Academic Conference on Cerebral Vascular Disease. These patients were sent to the hospital after onset in 48 h, and did not receive thrombolytic and volume expansion therapies before collecting blood samples. Patients with merge malignant tumor, serious infection and autoimmune disease were excluded. Eighty healthy cases that match the data of ACI group patients were selected as control group. This study was approved by the Hospital Ethics Committees, and obtained the informed consent from subjects tested.

Head MRI and diffusion-weighted imaging were carried out in patients of ACI group after admission, and the maximum diameter of infarct < 1.5 cm was considered as lacunar infarction; the maximum diameter of infarct 1.5–3.0 cm was considered as small infarct, and  $\geq 3.0$  cm was considered as large infarct. The National Institutes of Health Stroke Scale (NIHSS) was performed before treatment, NIHSS < 7 was slight type; 7–15 was moderate type, and > 15 was serious type.

### 2.2. Study methods

Reviewing the case data in patients of ACI group and the medical examination report in healthy group, gender, age, height, weight and blood pressure were collected. Peripheral blood in patients of ACI group was collected after admission. Peripheral blood of control group was collected during physical examination. Serum was took after the sodium citrate anticoagulation and kept at  $-80^{\circ}\text{C}$ . Proper amount of blood sample was used to extract total miRNA by using miRcute miRNA isolation kit (DP501). miRNA was reverse-transcribed into corresponding cDNA by using miRcute miRNA cDNA first-stand cDNA (KR201). Finally, contents of miR-126 and miR-146a were detected by using fluorescence PCR and ancillary SYBR Green (FP401). Besides, proper amount of blood sample was used to detect the content of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 by using ELIASA and ancillary ELIASA kit.

### 2.3. Statistical analysis

Using SPSS version 19.0 for entering and analyzing data, measurement data were expressed by mean  $\pm$  SD, and data between two groups were analyzed by *t*-test, and data among three groups were analyzed by ANOVA. Enumeration data were expressed by frequency and analyzed by *Chi*-square test. Correlations between two variables were tested by Pearson's correlation analysis. *P* < 0.05 was considered as statistical significance.

## 3. Results

### 3.1. Clinical data of two groups

Among 75 cases of ACI group, male was 45 and female was 30, with mean age of  $(54.2 \pm 7.2)$  years, body mass index (BMI)  $(22.1 \pm 3.9)$  kg/m<sup>2</sup>, systolic blood pressure (SBP)  $(135.9 \pm 16.9)$  mmHg, diastolic blood pressure (DBP)  $(84.5 \pm 10.1)$  mmHg. Among 80 healthy cases of control group, male was 50 and female was 30, with mean age of  $(52.8 \pm 8.4)$  years, BMI  $(22.8 \pm 4.4)$ , SBP  $(132.4 \pm 15.4)$  mmHg, DBP  $(83.9 \pm 11.4)$  mmHg. According to statistic analysis, there was no significant difference among gender, age, BMI, SBP, and DBP in patients with ACI (Table 1).

### 3.2. Comparison of serum index between two groups' objects

Contents of serum miR-126  $(0.286 \pm 0.078)$  vs.  $1.000 \pm 0.169$ , miR-146a  $(0.337 \pm 0.084)$  vs.  $1.000 \pm 0.158$  in patients of ACI group were significantly lower than those of control group, and contents of miR-126  $(0.286 \pm 0.078)$  vs.  $1.000 \pm 0.169$ , miR-146a  $(0.337 \pm 0.084)$  vs.  $1.000 \pm 0.158$  were obviously lower higher than those of control group. Contents of low density lipoprotein cholesterol, high density lipoprotein cholesterol, blood urea nitrogen and serum creatinine have no significant difference with control group (Table 2).

### 3.3. Serum index in patients of ACI group with different infarction area

The serum levels of miR-126  $(0.682 \pm 0.114)$  vs.  $0.392 \pm 0.063$  vs.  $0.167 \pm 0.032$ , miR-146a  $(0.705 \pm 0.121)$  vs.  $0.417 \pm 0.088$  vs.  $0.198 \pm 0.027$  and IL-1 $\beta$   $[(36.9 \pm 6.5)$  vs.  $(64.9 \pm 8.9)$  vs.  $(95.1 \pm 13.7)$  ng/L], TNF- $\alpha$   $[(70.5 \pm 11.4)$  vs.  $(104.2 \pm 13.2)$  vs.  $(160.3 \pm 26.9)$  ng/L], IL-6  $[(65.8 \pm 10.3)$  vs.  $(84.8 \pm 12.6)$  vs.  $(171.3 \pm 24.8)$  ng/L] in patients of ACI group with different infarction area have significant difference. The bigger infarction area was, the lower serum levels in miR-126 and miR-146a could be, and levels in IL-1 $\beta$ , TNF- $\alpha$  and IL-6 were higher (Table 3).

**Table 1**

Clinical data in two groups objects.

Group	Gender (male/female)	Age (year)	BMI (kg/m <sup>2</sup> )	SBP (mmHg)	DBP (mmHg)
ACI group ( <i>n</i> = 75)	45/30	54.2 $\pm$ 7.2	22.1 $\pm$ 3.9	135.9 $\pm$ 16.9	84.5 $\pm$ 10.1
Control group ( <i>n</i> = 80)	50/30	52.8 $\pm$ 8.4	22.8 $\pm$ 4.4	132.4 $\pm$ 15.4	83.9 $\pm$ 11.4
<i>P</i>	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

**Table 2**

Comparison of serum index between two groups' objects.

Group	miR-126/U6	miR-146a/U6	IL-1 $\beta$ (ng/L)	TNF- $\alpha$ (ng/L)	IL-6 (ng/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	BUN (mmol/L)	Scr ( $\mu$ mol/L)
ACI group ( <i>n</i> = 75)	0.286 $\pm$ 0.078	0.337 $\pm$ 0.084	68.4 $\pm$ 10.3	126.9 $\pm$ 22.4	89.3 $\pm$ 14.7	3.9 $\pm$ 0.6	1.7 $\pm$ 0.2	5.6 $\pm$ 0.55	68.1 $\pm$ 10.2
Control group ( <i>n</i> = 80)	1.000 $\pm$ 0.169	1.000 $\pm$ 0.158	22.7 $\pm$ 5.8	49.6 $\pm$ 8.4	34.8 $\pm$ 5.9	4.1 $\pm$ 0.5	1.6 $\pm$ 0.2	5.9 $\pm$ 0.9	66.3 $\pm$ 9.7
<i>P</i>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05

LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; Scr: Serum creatinine; BUN: Blood urea nitrogen.

**Table 3**

Serum index in patients of ACI group with different infarction area.

Different infarction area	miR-126/U6	miR-146a/U6	IL-1 $\beta$ (ng/L)	TNF- $\alpha$ (ng/L)	IL-6 (ng/L)
Lacunar infarct	0.682 $\pm$ 0.114	0.705 $\pm$ 0.121	36.9 $\pm$ 6.5	70.5 $\pm$ 11.4	65.8 $\pm$ 10.3
Small area infarcts	0.392 $\pm$ 0.063	0.417 $\pm$ 0.088	64.9 $\pm$ 8.9	104.2 $\pm$ 13.2	84.8 $\pm$ 12.6
Large area of infarcts	0.167 $\pm$ 0.032	0.198 $\pm$ 0.027	95.1 $\pm$ 13.7	160.3 $\pm$ 26.9	171.3 $\pm$ 24.8
<i>P</i>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

**Table 4**

Serum index in patients of ACI group with different severity.

Different severity	miR-126/U6	miR-146a/U6	IL-1 $\beta$ (ng/L)	TNF- $\alpha$ (ng/L)	IL-6 (ng/L)
Slight cerebral infarction	0.669 $\pm$ 0.104	0.712 $\pm$ 0.118	41.3 $\pm$ 7.1	73.1 $\pm$ 10.8	62.2 $\pm$ 9.8
Moderate cerebral infarction	0.415 $\pm$ 0.069	0.427 $\pm$ 0.091	62.2 $\pm$ 8.2	112.8 $\pm$ 12.8	82.1 $\pm$ 11.9
Serious cerebral infarction	0.154 $\pm$ 0.029	0.176 $\pm$ 0.024	92.5 $\pm$ 12.9	159.1 $\pm$ 22.5	168.5 $\pm$ 22.7
<i>P</i>	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

### 3.4. Serum index in patients of ACI group with different severity

Serum miR-126, miR-146a and levels of IL-1 $\beta$ , TNF- $\alpha$ , IL-6 in patients of ACI group with different severity have obviously difference. The bigger infarction area was, the lower levels in serum miR-126 (0.669  $\pm$  0.104 vs. 0.415  $\pm$  0.069 vs. 0.154  $\pm$  0.029) and miR-146a could be (0.712  $\pm$  0.118 vs. 0.427  $\pm$  0.091 vs. 0.176  $\pm$  0.024), and levels in IL-1 $\beta$  [(41.3  $\pm$  7.1) vs. (62.2  $\pm$  8.2) vs. (92.5  $\pm$  12.9) ng/L], TNF- $\alpha$  [(73.1  $\pm$  10.8) vs. (112.8  $\pm$  12.8) vs. (159.1  $\pm$  22.5) ng/L] and IL-6 were higher [(62.2  $\pm$  9.8) vs. (82.1  $\pm$  11.9) vs. (168.5  $\pm$  22.7) ng/L] (Table 4).

## 4. Discussion

Atherosclerosis is an important pathological basis causing ACI. The change of plaque and the rupture of fibrous cap will activate coagulation process, causing thrombogenesis, so as to result in nerve function deficit by various pathophysiology<sup>[12–15]</sup>. At present, the character change in arterial plaque and the follow-up molecular mechanism of nerve function deficit have not been completely clarified. miRNA is an endogenous non-coding single-stranded RNA with the length of 21–25 bp, involving with *in-vivo* regulation in various genetic expression and multiple biological processes. Previous studies indicated that the change of miRNAs content is related to atherosclerosis, plaque characteristic change and rupture, nerve function deficit, inflammation and imbalance of immunoreactions, in which abnormal contents of two miRNAs, miR-126 and miR-146a are related to various cardiovascular and cerebrovascular diseases<sup>[16–20]</sup>. We analyzed the above two miRNAs contents in serum, and the analysis indicated that an abnormal decrease in serum miR-

126 and miR-146a of patients with ACI is related to infarction area and neurologic impairment degree.

Inflammatory response is an important pathological change throughout various pathological processes of ACI. And links such as atherosclerosis, formation of plaque, character changes, formation of thrombus and neuron damage are involved in the activation of inflammatory reaction, aggregation of inflammatory cell and the synthesis and release of inflammatory factors<sup>[21–23]</sup>. IL-1 $\beta$  is an immunomodulatory factor with extensive biological activity, which can regulate the synthesis, secretion and release of various inflammatory mediators, making synergistic effect in inflammatory response<sup>[24]</sup>. TNF- $\alpha$  is an initial factor of inflammatory response in the process of cerebral infarction and ischemia reperfusion, which can directly cause brain tissue damage, induce cascade amplification of inflammatory response in local tissue and aggravate inflammatory response<sup>[22]</sup>. IL-6 has various biological functions, which can recruit inflammatory cell in local tissue, activate expression of inflammatory factor, as well as simultaneously cause edema of brain tissue and aggravate neurologic deficit<sup>[25]</sup>. We analyzed the content of inflammatory factor in serum, and results have shown that contents of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 in patients with ACI elevating abnormally were related to infarction area and neurologic impairment degree.

NF- $\kappa$ B is an important transcription factor regulating inflammatory response *in vivo*, which can initiate expression of various inflammatory factors after activating<sup>[26,27]</sup>. In addition, when inflammatory response of body was activated, interleukin-1 receptor-associated kinase 1 and tumor necrosis factor receptor associated factor-6 were activated and were able to promote the generation of various inflammatory factors<sup>[28,29]</sup>. Previous studies have indicated that miR-126 and miR-146a can targeting regulate NF- $\kappa$ B, IRAK-1 and TRAF-6 and

influence the generation of inflammatory factors<sup>[30–32]</sup>. In order to further prove if miR-126 and miR-146a involve in the occurrence of cerebral infarction and the development of disease through targeting and regulating the generation of inflammatory factors TNF- $\alpha$ , IL-1 $\beta$  and IL-6. Pearson's correlation analysis was used to verify the relationship of serum levels of miR-126 and miR-146a with TNF- $\alpha$ , IL-1 $\beta$  and IL-6. Results showed that levels of miR-126 and miR-146a were negatively correlated with levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-6, which suggested that miR-126 and miR-146a might involve in regulating the expressions of TNF- $\alpha$ , IL-1 $\beta$  and IL-6, and it can not only directly target toward the above 3'UTR of inflammatory factor mRNA to inhibit the expression of gene, but also indirectly regulate the generation of the above inflammatory factor by targeting TRAF-6, IRAK-1 and NF- $\kappa$ B. Specific molecular pathways that miR-126 and miR-146a regulate the generation of TNF- $\alpha$ , IL-1 $\beta$  and IL-6, were also needed to verify by *in-vitro* study.

In conclusion, an abnormal decrease in serum levels of miR-126 and miR-146a in patients with ACI is closely related to the severity of disease. miR-126 and miR-146a might involve in the change of severity of cerebral infarction through regulating inflammatory factors TNF- $\alpha$ , IL-1 $\beta$  and IL-6.

### Conflict of interest statement

The authors report no conflict of interest.

### References

- [1] Fahed R, Redjem H, Blanc R, Labreuche J, Robert T, Ciccio G, et al. Endovascular management of acute ischemic strokes with tandem occlusions. *Cerebrovasc Dis* 2016; **41**(5–6): 298-305.
- [2] Liu D, Sun W, Scalzo F, Xiong Y, Zhang X, Qiu Z, et al. Early magnetic resonance imaging predicts early neurological deterioration in acute middle cerebral artery minor stroke. *J Stroke Cerebrovasc Dis* 2016; **25**(2): 469-74.
- [3] Nolte CH, Audebert HJ. Management of acute ischemic stroke. *Dtsch Med Wochenschr* 2015; **140**(21): 1583-6.
- [4] Kim YB, Park KY, Chung PW, Kim JM, Moon HS, Youn YC. Brachial-ankle pulse wave velocity is associated with both acute and chronic cerebral small vessel disease. *Atherosclerosis* 2016; **245**: 54-9.
- [5] Ojha R, Huang D, An H, Liu R, Du C, Shen N, et al. Distribution of ischemic infarction and stenosis of intra- and extracranial arteries in young Chinese patients with ischemic stroke. *BMC Cardiovasc Disord* 2015; **15**(1): 158.
- [6] Psychogios K, Stathopoulos P, Takis K, Vemmu A, Manios E, Spegos K, et al. The pathophysiological mechanism is an independent predictor of long-term outcome in stroke patients with large vessel atherosclerosis. *J Stroke Cerebrovasc Dis* 2015; **24**(11): 2580-7.
- [7] Li SC, Khan M, Caplin M, Meyer T, Öberg K, Giandomenico V. Somatostatin analogs treated small intestinal neuroendocrine tumor patients circulating microRNAs. *PLoS One* 2015; **10**(5): e0125553.
- [8] Baglioni M, Russo F, Geraci F, Rizzo M, Rainaldi G, Pellegrini M. A new method for discovering disease-specific miRNA-target regulatory networks. *PLoS One* 2015; **10**(4): e0122473.
- [9] Xie F, Jones DC, Wang Q, Sun R, Zhang B. Small RNA sequencing identifies miRNA roles in ovule and fibre development. *Plant Biotechnol J* 2015; **13**(3): 355-69.
- [10] Wei Y, Nazari-Jahantigh M, Neth P, Weber C, Schober A. MicroRNA-126, -145, and -155: a therapeutic triad in atherosclerosis? *Arterioscler Thromb Vasc Biol* 2013; **33**(3): 449-54.
- [11] Bao MH, Xiao Y, Zhang QS, Luo HQ, Luo J, Zhao J, et al. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. *Int J Mol Sci* 2015; **16**(7): 14305-17.
- [12] Yang H, Zhu Y, Geng Z, Li C, Zhou L, Liu QI. Clinical value of black-blood high-resolution magnetic resonance imaging for intracranial atherosclerotic plaques. *Exp Ther Med* 2015; **10**(1): 231-6.
- [13] Kim BJ, Yoon Y, Lee DH, Kang DW, Kwon SU, Kim JS. The shape of middle cerebral artery and plaque location: high-resolution MRI finding. *Int J Stroke* 2015; **10**(6): 856-60.
- [14] Li S, Sun X, Bai YM, Qin HM, Wu XM, Zhang X, et al. Infarction of the corpus callosum: a retrospective clinical investigation. *PLoS One* 2015; **10**(3): e0120409.
- [15] Kim JM, Jung KH, Chu K, Lee ST, Ban J, Moon J, et al. Atherosclerosis-related circulating microRNAs as a predictor of stroke recurrence. *Transl Stroke Res* 2015; **6**(3): 191-7.
- [16] Fuentes E, Palomo I, Alarcón M. Platelet miRNAs and cardiovascular diseases. *Life Sci* 2015; **133**: 29-44.
- [17] dos Santos AM, Metzinger L, Haddad O, M'baya-Moutoula E, Taïbi F, Charnaux N, et al. miR-126 is involved in vascular remodeling under laminar shear stress. *Biomed Res Int* 2015; **2015**: 497280.
- [18] Dong S, Xiong W, Yuan J, Li J, Liu J, Xu X. MiRNA-146a regulates the maturation and differentiation of vascular smooth muscle cells by targeting NF- $\kappa$ B expression. *Mol Med Rep* 2013; **8**(2): 407-12.
- [19] Chen T, Li Z, Jing T, Zhu W, Ge J, Zheng X, et al. MicroRNA-146a regulates the maturation process and pro-inflammatory cytokine secretion by targeting CD40L in oxLDL-stimulated dendritic cells. *FEBS Lett* 2011; **585**(3): 567-73.
- [20] Li Z, Wang S, Zhao W, Sun Z, Yan H, Zhu J. Oxidized low-density lipoprotein upregulates microRNA-146a via JNK and NF- $\kappa$ B signaling. *Mol Med Rep* 2016; **13**(2): 1709-16.
- [21] Ye L, Cai R, Yang M, Qian J, Hong Z. Reduction of the systemic inflammatory induced by acute cerebral infarction through ultra-early thrombolytic therapy. *Exp Ther Med* 2015; **10**(4): 1493-8.
- [22] Yukami T, Yagita Y, Sugiyama Y, Oyama N, Watanabe A, Sasaki T, et al. Chronic elevation of tumor necrosis factor- $\alpha$  mediates the impairment of leptomeningeal arteriogenesis in db/db mice. *Stroke* 2015; **46**(6): 1657-63.
- [23] Albertsson AM, Bi D, Duan L, Zhang X, Leavenworth JW, Qiao L, et al. The immune response after hypoxia-ischemia in a mouse model of preterm brain injury. *J Neuroinflammation* 2014; **11**: 153.
- [24] Yang B, Zhao H, Bin X, Wang YB, Zhang J, Cao YK, et al. Influence of interleukin-1 beta gene polymorphisms on the risk of myocardial infarction and ischemic stroke at young age *in vivo* and *in vitro*. *Int J Clin Exp Pathol* 2015; **8**(11): 13806-13.
- [25] Kumar P, Yadav AK, Kumar A, Sagar R, Pandit AK, Prasad K. Association between interleukin-6 (G174C and G572C) promoter gene polymorphisms and risk of ischaemic stroke: a meta-analysis. *Ann Neurosci* 2015; **22**(2): 61-9.
- [26] Seibold K, Ehrenschrwender M. p62 regulates CD40-mediated NF $\kappa$ B activation in macrophages through interaction with TRAF6. *Biochem Biophys Res Commun* 2015; **464**(1): 330-5.
- [27] Calvayrac O, Rodríguez-Calvo R, Martí-Pamies I, Alonso J, Ferrán B, Aguiló S, et al. NOR-1 modulates the inflammatory response of vascular smooth muscle cells by preventing NF $\kappa$ B activation. *J Mol Cell Cardiol* 2015; **80**: 34-44.
- [28] Weinberg EO, Genco CA. Directing TRAF-ic: cell-specific TRAF6 signaling in chronic inflammation and atherosclerosis. *Circulation* 2012; **126**(14): 1678-80.
- [29] Guo L, Chen CH, Zhang LL, Cao XJ, Ma QL, Deng P, et al. IRAK1 mediates TLR4-induced ABCA1 downregulation and lipid accumulation in VSMCs. *Cell Death Dis* 2015; **6**: e1949.
- [30] Park H, Huang X, Lu C, Cairo MS, Zhou X. MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. *J Biol Chem* 2015; **290**(5): 2831-41.
- [31] Liu R, Liu C, Chen D, Yang WH, Liu X, Liu CG, et al. FOXP3 controls an miR-146/NF- $\kappa$ B negative feedback loop that inhibits apoptosis in breast cancer cells. *Cancer Res* 2015; **75**(8): 1703-13.
- [32] Feng X, Tan W, Cheng S, Wang H, Ye S, Yu C, et al. Upregulation of microRNA-126 in hepatic stellate cells may affect pathogenesis of liver fibrosis through the NF- $\kappa$ B pathway. *DNA Cell Biol* 2015; **34**(7): 470-80.