PROTECTIVE EFFECT OF PGC-1A ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Guangqin Liu[#], Yifei Pei[#], Zaiping Jing, Liangxi Yuan, Jian Dong, Jian Zhou, Junmin Bao[*]
Department of Vascular Surgery, Changhai Hospital, The Navel Military Medical University, Shanghai, PR China
[#]*These authors contributed equally to this work as co-first author*

ABSTRACT

Objective: *PGC-1α achieves the protective effect of TNF-α-induced injury to human umbilical vein endothelial cells by inhibiting the Ca^{2+}/NFAT pathway.*

Methods: *Human umbilical vein endothelial cells were treated with 20, 40, and 80 ng/mL TNF-α to create three models with different degrees of cell injury. The human umbilical vein endothelial cells were cultured in vitro and transfected by interfering with the PGC-1α RNA lentivirus. The silencing of the PGC-1α gene was divided into two groups: the control group and the down-regulation of the PGC-1α gene group. The effects of different concentrations of TNF-α on apoptosis of human umbilical vein endothelial cells were determined by flow cytometry. The expression of intracellular genes was detected by real-time quantitative PCR, and the expression of intracellular protein was measured by western blot assay. The concentrations of mtROS and Ca^{2+} were measured.*

Results: *After human vein endothelial cells were treated for 24 h with different concentrations of TNF-α, the apoptosis rate gradually increased with the increase of TNF-α concentration, and the difference was statistically significant compared with the control group ($P<0.05$). The expression of PGC-1α mRNA in each group was significantly lower than that of the control group after human venous endothelial cells were treated for 24 h of with different concentrations of TNF-α ($P<0.05$). After treatment with different concentrations of TNF-α, the relative expression rate of PGC-1α protein in each group was remarkably lower than that in the control group ($P<0.05$). The expression of NFAT1 mRNA in the TNF-α 40 and 80 ng/mL treatment groups was significantly higher than that in the control group ($P<0.05$), and the expression of NFAT2 mRNA in different concentrations of the TNF-α treatment groups was markedly higher than that in the control group ($P<0.05$). The expression of NFAT1 protein in the TNF-α 40 and 80 ng/mL treatment groups was significantly higher than that in the control group ($P<0.05$), and the expression level of NFAT2 protein in different concentrations of TNF-α treatment group was clearly higher than that in the control group ($P<0.05$). The intracellular mROS and Ca^{2+} concentrations were significantly higher than that in the control group after the down-regulation of the PGC-1α gene expression ($P<0.05$). After down-regulating the expression of the PGC-1α gene, the levels of NFAT1, NFAT2, mRNA, and protein in the cells were significantly higher than those in the control group ($P<0.05$).*

Conclusion: *20, 40, and 80 ng/mL of TNF-α can induce different degrees of human umbilical vein endothelial cell injury. PGC-1α has a protective effect on this kind of cell injury, and its related mechanism may be to inhibit the Ca^{2+}/NFAT signalling pathway by reducing the intracellular mtROS concentration, and thus to protect damaged cells.*

Keywords: *PGC-1α, Ca^{2+}/NFAT pathway, TNF-α, human umbilical vein endothelial cell, injury, protective effect.*

DOI: 10.19193/0393-6384_2020_4_347

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Atherosclerosis, a common cardiovascular disease caused by many factors, is usually preceded by the accumulation of lipids and compound carbohydrates. Lipid metabolic disorders are the basis of its lesions, which are characterised by high specific chronic inflammation reflected by cell molecules. Vascular endothelial injury is an important manifestation of cardiovascular diseases, and such injury can lead to atherosclerosis by promoting the proliferation of macrophages and vascular smooth muscle cells. Inflammation and oxidative stress play a very important role in this process[(1)]. Inflammatory factors such as tumour necrosis factor-α (TNF-α) and lipopolysaccharide can cause vascular endothelial cell injury. TNF-α is rich in cells and plays an important role in maintaining the stability of the cardiovascular internal environment. It can promote the expression of adhesion molecules by acting on the

surface of vascular endothelial cells[2]. It can also bind to its receptor to form multi-molecular complexes that activate IkB kinase and induce phosphorylation of IkB-α. Free NF-kB can be transferred into the nucleus to promote the expression of various pro-inflammatory chemokines, resulting in the injury of human vascular endothelial cells[3]. Peroxisome proliferator-activated receptor (PPAR)-γ co-activating factor-1α (PGC-1α) is a transcriptional donor activating factor, and it is widely involved in such physiological processes as mitochondrial biosynthesis, hepatic glucose heterosis, and adaptive heat production[4]. In recent years, it has been shown that PGC-1α is involved in oxidative stress and angiogenesis, which can reduce the production of reactive oxygen species (ROS) and the expression of vascular wall adhesion molecules in the mitochondria and the cells induced by TNF-α. NFAT is a nuclear transcription factor that can promote the transcription of inflammatory genes. PGC-1α plays an inflammatory role in endothelial cells through the Ca^{2+}/NFAT pathway[6]. Therefore, the purpose of this study is to analyse the protective effect of PGC-1α on TNF-α-induced injury of human umbilical vein endothelial cells by inhibiting the Ca^{2+}/NFAT pathway.

Materials and methods

Experimental reagents and instruments

The human umbilical vein endothelial cell line was provided by Shanghai cell bank of Chinese Academy of Sciences. The TNF-α inducer was purchased from the R & D company (USA). Foetal calf serum was purchased from the Lanzhou Rongye Biotechnology Co., Ltd. Trizol was purchased from the Sigma company (USA). EP centrifuge tubes were purchased from the Axygen company (USA). The CCK-8 kit was purchased from Dojindo Chemistry, Japan. The BCA protein examination kit was purchased from Jiangsu Beyotime Biotechnology Co., Ltd. The anti-PGCα and anti-NFAT antibodies were purchased from the CST company.

The ultra-purification worktable was purchased from the AIRTECH company. The high-pressure steam steriliser was purchased from the American ZEALWAY company. The carbon dioxide incubator, low-temperature high-speed centrifuge, and automatic enzyme-reader were purchased from the Thermo Fisher company (USA). The inverted optical microscope was purchased from the Guangzhou Gangran Mechanical and Electrical Equipment Co., Ltd. The ultraviolet spectrophotometer and vertical electrophoresis were purchased from the Bio-Rad company (US). The real-time quantitative PCR instrument was purchased in ABI Prism7500.

Cell culture and grouping

The frozen human umbilical vein endothelial cell strain was placed in an incubator at 37 °C for melting. After centrifugation of the cell strain, the DMEN culture solution containing 10% foetal bovine serum was added, and the cells were cultured under the condition of 5% CO_2 at 37 °C. Cells at the logarithmic growth stage with good growth were taken for cell passage. Then trypsin digestion was carried out to prepare the cell suspension, which was stored at -80 °C for later use. The cultured cells were divided into the control group and treatment groups of 20, 40, and 80 ng/mL TNF-α.

The human umbilical vein endothelial cells were cultured in vitro and transfected by interfering with the PGC-1α RNA lentivirus. The silencing of PGC-1α gene was divided into two groups: the control group and the group for down-regulation of the PGC-1α gene.

Detection method

Apoptosis was detected by flow cytometry

The human umbilical vein endothelial cell strain with logarithmic growth was taken, and trypsin digestion was carried out to prepare the cell suspension. The cells were inoculated on a 6-well plate at a concentration of 1 × 105 ml, and the original medium was replaced with medium containing 20, 40, and 80 ng/mL TNF-α. After a series of operations, such as washing, digestion, and centrifugation, apoptosis was detected by flow cytometry.

The gene expression in the cells was detected by real-time quantitative PCR

The human umbilical vein endothelial cell strain with logarithmic growth was taken and inoculated on 12-well plates. Then 1 ml of Trizol was added to each well and total RNA was extracted. The RNA reverse transcription was performed with reference to the PrimeScript kit specification of Takara company, the resulting cDNA was diluted 30 times with dd H_2O, and an internal reference was set. The PCR dilated gene fragment reaction was carried out using fluorescence quantitative PCR instrument, and the expression of genes in the relative quantitative samples was calculated. The expression of intracellular protein was detected by western blot assay. Human umbilical vein endothelial cells were treated

with 20, 40, and 80 ng/ml TNF-α for 24 h, and then the cell culture medium was discarded. After a series of operations such as washing, digestion, and centrifugation, 15 times diluted samples and standard products were added to a 96-well plate, and 200 μL BCA working liquid was added to each well according to the instructions of the BCA protein concentration kit. After incubation, the OD value at 540nm was measured, and the protein concentration was calculated.

Detection of mtROS concentration

The human umbilical vein endothelial cell strain with logarithmic growth was taken and inoculated on 12-well plates. The control group and the down-regulated PGC-1α gene group after cell adherence were replaced with the original medium with fresh DMEM medium. After 24 h, the cell culture medium was discarded and HBSS with 5 μmol / ml Mito SOX was added. After incubation, the cells were rinsed three times and photographed under a microscope, and the fluorescence intensity was analysed.

Detection of Ca^{2+} concentration

The human umbilical vein endothelial cell strain with logarithmic growth was taken and inoculated on 12-well plates. The control group and the down-regulated PGC-1α gene group after cell adherent were replaced with the original medium with fresh DMEM medium. After 24 h, the cells were rinsed and the Fluo-3AM working solution was added. After incubation, the cells were photographed under a microscope, and the fluorescence intensity was analysed.

Statistical method

The measurement data were represented by mean ± standard deviation ($\bar{x}\pm s$). A t-test was used to compare the groups, and single-factor variance analysis was used to compare multiple groups. $P<0.05$ indicates that the difference between groups has statistical significance. The data were analysed using the SPSS21.0 software package.

Results

The effect of different concentration of TNF- α on the apoptosis of human umbilical vein endothelial cells

Flow cytometry showed that after human vein endothelial cells were treated for 24 h with different concentrations of TNF-α, the apoptosis rate gradually increased with the increase of TNF-α concentration, and the difference was statistically significant compared with the control group ($P<0.05$). The results are shown in Figure 1.

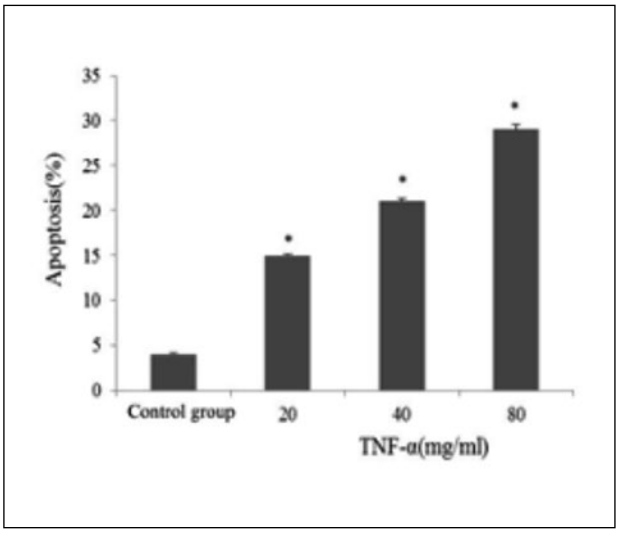

Figure 1: The effect of different concentration of TNF-α on the apoptosis of human umbilical vein endothelial cells.

Changes of PGC-1α mRNA and protein levels in human umbilical vein endothelial cells treated with different concentrations of TNF-α

The results of real-time quantitative PCR detection showed that the expression of PGC-1α mRNA in each group was significantly lower than that of the control group after 24 h of treatment of human venous endothelial cells with 20, 40, and 80 ng/ml of TNF-α ($P<0.05$).

The western blot assay showed that, after treatment with different concentrations of TNF-α, the relative expression rate of PGC-1α protein in each group was remarkably lower than that in the control group ($P<0.05$). The decrease of PGC-1α mRNA and protein level depended on the stimulation concentration of TNF-α. The results are shown in Figure 2 and Table 1.

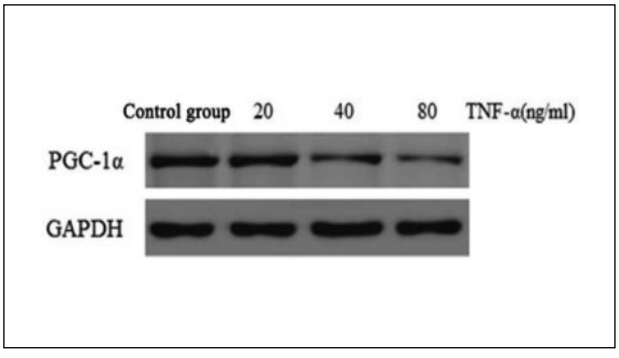

Figure 2: Changes of PGC-1α protein levels in human umbilical vein endothelial cells with the treatment of different concentrations of TNF-α.

Groups	Cases	PGC-1α mRNA	PGC-1α protein
Control group	5	1.00±0.01	2.15±0.35
TNF-α 20ng/ml	5	0.51±0.06*	1.57±0.05*
TNF-α 40ng/ml	5	0.08±0.04*#	0.78±0.07*#
TNF-α 80ng/ml	5	0.02±0.01*#△	0.51±0.11*#

Table 1: Changes of PGC-1α mRNA and protein levels ($\bar{x}\pm s$).

*Notes: Compared with the control group, *P<0.05; compared with TNF-α 20 ng/ml group, #P<0.05; Compared with TNF-α 40 ng/ml group, △P<0.05.*

Changes of NFAT1, NFAT2 mRNA, and protein levels in human umbilical vein endothelial cells with different concentrations of TNF-α

The real-time quantitative PCR detection showed that the expression of NFAT1 mRNA in the TNF-α 40 and 80 ng/mL treatment groups was significantly higher than that in the control group (P<0.05), and the expression level of NFAT2 mRNA in different concentrations of TNF-α treatment group was markedly higher than that in the control group (P<0.05). The western blot assay showed that the expression of NFAT1 protein in the TNF-α 40 and 80 ng/mL treatment groups was significantly higher than that in the control group (P<0.05), and the expression level of NFAT2 protein in different concentrations of TNF-α treatment group was clearly higher than that in the control group (P<0.05). The results are shown in Figure 3 and Table 2.

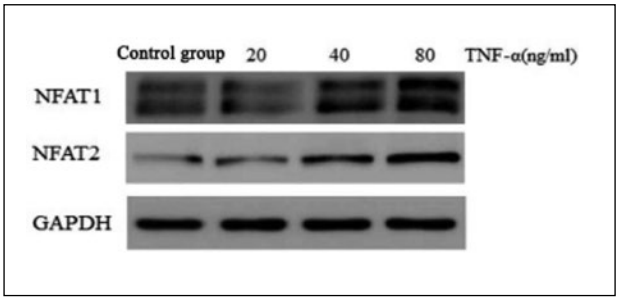

Figure 3: Changes of NFAT1 and NFAT2 protein levels.

Group	Cases	NFAT1 mRNA	NFAT2 mRNA	NFAT1 protein	NFAT2 protein
Control group	5	1.00±0.05	1.00±0.07	0.54±0.11	0.09±0.03
TNF-α 20 ng/ml	5	0.91±0.06	1.95±0.09*	0.51±0.03	0.18±0.03*
TNF-α 40 ng/ml	5	1.98±0.15*#	2.74±0.41*#	0.96±0.44*#	0.37±0.13*#
TNF-α 80 ng/ml	5	2.94±0.01*#△	3.75±0.16*#△	1.20±0.08*#△	0.65±0.05*#△

Table 2: Changes of NFAT1, NFAT2 mRNA and protein levels ($\bar{x}\pm s$).

*Notes: Compared with the control group, *P<0.05; compared with TNF-α 20 ng/ml group, #P<0.05; Compared with TNF-α 40 ng/ml group, △P<0.05.*

Effect of the down-regulation of PGC-1α on the concentration of mtROS and Ca^{2+} in human umbilical vein endothelial cells

The concentrations of intracellular mROS and Ca^{2+} were significantly higher than that in the control group after down-regulation of PGC-1α gene expression (P<0.05). The results are shown in Figure 4.

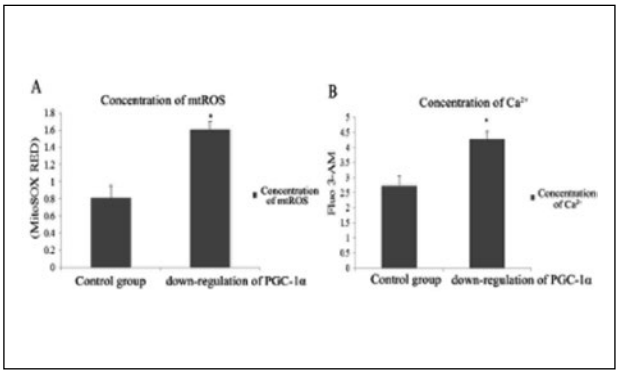

Figure 4: Effect of down-regulation of PGC-1α on the concentration of mtROS and Ca^{2+} in human umbilical vein endothelial cells.

*Notes: Compared with the control group, *P<0.05. A: mtROS concentration change; B: Ca^{2+} concentration change.*

Effect of down-regulation of PGC-1α on the expression of NFAT1, NFAT2, mRNA, and protein in human umbilical vein endothelial cells

After down-regulating the expression of PGC-1α gene, the levels of NFAT1, NFAT2 mRNA and protein in the cells were significantly higher than those in the control group (P<0.05). The results are shown in Figure 5 and Table 3.

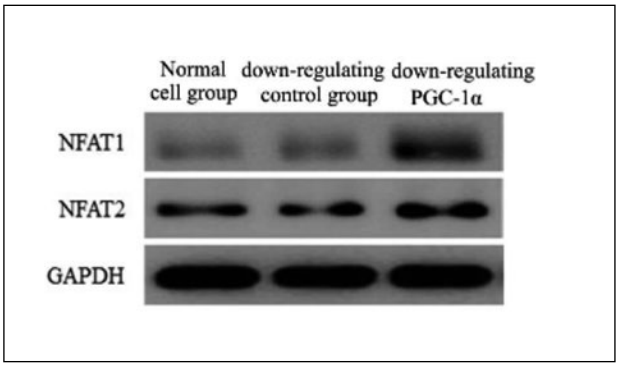

Figure 5: Changes of NFAT1 and NFAT2 protein levels.

Groups	Cases	NFAT1 mRNA	NFAT2 mRNA	NFAT1 protein	NFAT2 protein
Control group	5	1.00±0.04	1.00±0.06	0.57±0.02	0.62±0.03
Down-regulation of the PGC-1α gene group	5	2.34±0.02*	2.26±0.07*	0.96±0.09*	0.92±0.02*

Table 3: Changes of NFAT1, NFAT2 mRNAa and protein levels ($\bar{x}\pm s$).

*Notes: Compared with the control group, *P<0.05.*

Discussion

Atherosclerosis is a kind of chronic injury inflammatory reaction. Its pathogenesis is not clear, but it is closely related to obesity, metabolic syndrome, and other diseases. Lipids deposited under endarterium are characteristic of its pathological changes. This study shows that the destruction of vascular endothelial cells, the proliferation of vascular smooth muscle cells, infection, and abnormal lipid metabolism are closely related to the formation of atherosclerotic plaques[7]. Vascular endothelial cells can not only maintain the integrity of blood vessels, but also the origin of oxidative injury. Oxidative stress is involved in the process of endothelial cell injury, and inflammation is involved in the process of atherosclerosis[8]. TNF-α is a pro-inflammatory factor secreted by activated mononuclear cells. It can promote apoptosis of endothelial cells by mediating oxidative stress, and it may also mediate the inflammatory response and induce an inflammatory response of the vascular smooth muscle cells[9]. This study used an injury model of human umbilical vein endothelial cells induced by different concentrations of TNF-α. Flow cytometry showed that the apoptosis rate increased gradually with the increase of TNF-α concentration. This suggests that TNF-α can induce injury of human umbilical vein cells, and it is speculated that TNF-α may be one mechanism of cell injury. In 1998, PGC-1α was found in the brown adipose tissue of mice by foreign scholars. Human PGC-1α gene is on chromosome 4p15.1, contains 2394 bases, and comprises 13 exons. It has obvious tissue expression specificity, and it is highly expressed in skeletal muscle, the heart, the kidney, and other tissues rich in mitochondria[10]. Recent studies have found that PGC1α is the central regulatory factor of mitochondrial function and biosynthesis, which can activate the NBF-1 target gene through transcription and participate in the regulation and transcription of the mitochondrial respiratory gene. In addition, PGC-1α has diverse biological effects on potential integrated transcription factors, and it plays a key role in the biosynthesis and oxidative stress of the mitochondria[11]. Activation of T-cell nuclear factor NFAT affects cell damage and apoptosis. It mainly exists in the cytoplasm of cells. After activation, it can promote the expression of inflammation-related genes and induce inflammation, in which the expression of NFAT1 and NFAT2 is most obvious in the endothelial cells[12]. Studies have found that NFAT can induce apoptosis and inflammation of vascular smooth muscle cells by inhibiting insulin[13]. The results showed that the levels of PGC-1α gene and protein were significantly down-regulated and NFAT1, NFAT2 gene, and protein were remarkably up-regulated in the injury of human umbilical vein cells induced by TNF-α. This suggests that PGC-1α and NFAT activation may be involved in TNF-α-induced injury of human umbilical vein cells.

Oxidative stress is an unbalanced state between oxidant and antioxidant. ROS plays a key role in oxidative stress, which can cause local inflammation by activating BF-kB and other nuclear transcription factors to release a variety of factors[14].

Oxidative stress can also cause Ca^{2+} influx. Related studies have shown that the migration of endothelial cells is related to the M4 transient receptor potential channel induced by oxidative stress-regulated Ca^{2+}[15]. This study showed that the intracellular mtROS, Ca^{2+} concentration, NFAT1, NFAT2 mRNA, and protein levels were significantly higher than those in the control group after down-regulating the PGC-1α gene expression. This suggests that PGC-1α can reduce the generation of mtROS and decrease the Ca^{2+} concentration, inhibit the expression level of NFAT1 and NFAT2, and play a protective role in damaged cells.

In conclusion, 20, 40, and 80 ng/ml TNF-α can lead to different degrees of human umbilical vein endothelial cell damage, and PGC-1α has a protective effect on this kind of cell injury. The related mechanism may be to reduce the level of the intracellular mtROS concentration and further inhibit the Ca^{2+}/NFAT signalling pathway, to protect the damaged cells.

References

1) Zhu ML, Wang G, Wang H, Guo YM, Song P, et al. Amorphous nano-selenium quantum dots improve endothelial dysfunction in rats and prevent atherosclerosis in mice through Na+/H+ exchanger 1 inhibition. Vascul Pharmacol 2019; 115: 26-32.

2) Wang C, Yu X, Yan Y, Yang W, Zhang S, et al. Tumor necrosis factor-α: a key contributor to intervertebral disc degeneration. Acta Biochim Biophys Sin (Shanghai) 2017; 49: 1-13.

3) Tang J, Wu ZY, Dai RJ, Ma J, Gong GZ. Hepatitis B virus-persistent infection and innate immunity defect: Cell-related or virus-related? World J Clin Cases 2018; 6: 233-241.

4) Jacoupy M, Hamon-Keromen E, Ordureau A, Erpapazoglou Z, Coge F, et al. The PINK1 kinase-driven ubiquitin ligase Parkin promotes mitochondrial protein import through the presequence pathway in living cells. Sci Rep 2019; 9: 11829.
5) Atala A. Re: The Metabolic Co-Regulator PGC1α Suppresses Prostate Cancer Metastasis. J Urol 2017; 198: 1212-1214.
6) Sharabi K, Lin H, Tavares CDJ, Dominy JE, Camporez JP, et al. Selective Chemical Inhibition of PGC-1α Gluconeogenic Activity Ameliorates Type 2 Diabetes. Cell 2017; 169: 148-160.
7) Barbato E, Toth GG, Johnson NP, Pijls NH, Fearon WF, et al. A Prospective Natural History Study of Coronary Atherosclerosis Using Fractional Flow Reserve. J Am Coll Cardiol 2016; 68: 2247-2255.
8) Chagwedera DN, Ang QY, Bisanz JE, Leong YA, Ganeshan K, et al. Nutrient Sensing in CD11c Cells Alters the Gut Microbiota to Regulate Food Intake and Body Mass. Cell Metab 2019; 30: 364-373.
9) Mafu TS, September AV, Shamley D. The potential role of angiogenesis in the development of shoulder pain, shoulder dysfunction, and lymphedema after breast cancer treatment. Cancer Manag Res 2018; 10: 81-90.
10) Xing F, Luan Y, Cai J, Wu S, Mai J, et al. The Anti-Warburg Effect Elicited by the cAMP-PGC1α Pathway Drives Differentiation of Glioblastoma Cells into Astrocytes. Cell Rep 2017; 23: 468-481.
11) Zhang X, Ji R, Sun H, Peng J, Ma X, et al. Scutellarin ameliorates nonalcoholic fatty liver disease through the PPARγ/PGC-1α-Nrf2 pathway. Free Radic Res 2018; 52: 198-211.
12) Li H, Gao S, Ye J, Feng X, Cai Y, et al. COX-2 is involved in ET-1-induced hypertrophy of neonatal rat cardiomyocytes: role of NFATc3. Mol Cell Endocrinol 2014; 382: 998-1006.
13) Zhang Y, Storey KB. Regulation of gene expression by NFAT transcription factors in hibernating ground squirrels is dependent on the cellular environment. Cell Stress Chaperones 2016; 21: 883-894.
14) Pottosin I, Zepedajazo I. Powering the plasma membrane Ca2+-ROS self-amplifying loop. J Exp Bot 2018; 69: 3317-3320.
15) Cobley JN, Noble A, Jimenez-Fernandez E, Valdivia Moya MT, Guille M, et al. Catalyst-free Click PEGylation reveals substantial mitochondrial ATP synthase sub-unit alpha oxidation before and after fertilisation. Redox Biol 2019; 26: 101258.

Acknowledgement:
General Program of NSFC: Clinical Basic Research on Plasma MiRNA as Biomarker of Acute Aortic Dissection, (No. 81370441).

Corresponding Author:
Junmin Bao
Email: uwr81t@163.com
(China)