Am J Transl Res 2019;11(4):2339-2349
www.ajtr.org /ISSN:1943-8141/AJTR0092961

Original Article

Angiotensin II type i receptor agonistic autoantibodies induces apoptosis of cardiomyocytes by downregulating miR21 in preeclampsia: a mechanism study

Jin Wang[1*], Jiping Yue[1*], Qin Xia[2], Xiangying Jiao[1], Jianming Zhi[3]

[1]Key Laboratory of Cellular Physiology (Shanxi Medical University), Ministry of Education, and The Department of Physiology, Shanxi Medical University, Taiyuan, China; [2]Department of Pharmacy, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China; [3]Department of Anatomy and Physiology, School of Medicine, Shanghai Jiaotong University, Shanghai, China. [*]Equal contributors.

Received February 23, 2019; Accepted March 14, 2019; Epub April 15, 2019; Published April 30, 2019

Abstract: Angiotensin II type I receptor agonistic autoantibodies (AT_1-AA) in the plasma of preeclampsia patients can induce apoptosis of cardiomyocytes, and microRNA-21 (miR-21) can exert a protective effect on cardiomyocytes. But whether the pro-apoptotic effect of AT_1-AA is associated with miR-21 is unclear. The objective of the present study was to explore whether AT_1-AA induced cardiomyocyte apoptosis was related to its inhibitory of miR-21 expression. In vivo studies, the pregnant rats were divided into two groups: Sham group, Model group. The pathology, cell apoptosis, and relative protein expressions were evaluated by hematoxylin and eosin staining, and Western blot assay. The expression of microRNA was detected by gene microarray. In the cell experiment, the neonatal rat cardiomyocytes were divided into four groups: NC group, AT_1-AA group, and miR-21 group and AT_1-AA+miR-21 group. The cell apoptosis and relative proteins' expressions were measured by flow cytometry and Western blot assay. Results: Compared with the Sham group, miR-21 in the cardiac tissue of the model group was downregulated significantly; the expression of p-JNK, Bax and caspases-3 was increased, the expression of Bcl-2 was decreased, and the Bcl-2/Bax ratio became smaller. The expression of miR-21 in AT_1-AA treated cardiomyocytes was only 52% of the control group, with an apoptosis rate of 32.6%. In addition, the expression of pPTEN, pAKT and pFOXO3a in the model group was significantly higher than that in the NC group. The cardiomyocyte apoptosis rate in miR-21 overexpression group was only 23.7%, which was higher than that in the NC group, but significantly lower than that in AT_1-AA group. PTEN, AKT and FOXO3a phosporylation in miR-21 overexpression group was also lower than that in AT_1-AA group. AT_1-AA induced cardiomyocyte apoptosis by downregulating miR-21, and the PTEN/AKT/FOXO3a signal transduction pathway participated in this process. The result of the present study suggests that miR-21 may prove to be a new target for the diagnosis and treatment of preeclampsia and other cardiovascular diseases.

Keywords: Angiotensin II type I receptor agonistic autoantibodies (AT_1-AA), microRNA-21, apoptosis, cardiomyocyte, PTEN/AKT/FOXO3a signal

Introduction

The etiology and pathogenesis of preeclampsia remain elusive, but the role of angiotensin II type I receptor agonistic autoantibodies (AT_1-AA) in the development of preeclampsia has become increasingly prominent [1, 2]. Previous studies demonstrated that AT_1-AA existing in the plasma of preeclamptic patients could produce an agonist effect similar to Ang II by acting on 181-187 amino acids (AFHYESQ) on the second extracellular loop of AT_1 receptor (AT_1-R) [3]. Further study revealed that no desensitization occurred when AT_1-AA combined with AT_1-R and therefore they could act for a prolonged period of time and would produce more harm [3]. Animal studies demonstrated that intravenous (IV) injection of AT_1-AA to gestational-day 13 mice could induce hypertension, proteinuria, placental abnormality, and offspring of different sizes similar to the clinical presentation of eclampsia [1]. The mechanism of AT_1-AA inducing the development and progression of preeclampsia may be associated with its effect of

stimulating the tissue to produce soluble fms-Like Tyrosine-1, soluble Endoglin [4], tumor necrosis factor-α [5], and endothelin [6]. Levine *et al* [7] reported that the level of sFlt-1 began increasing 5-6 weeks before the onset of preeclampsia, and abnormal soluble Endoglin was detectable 2-3 months earlier than the presence of preeclamptic symptoms.

It was found in our previous study that low concentrations of AT_1-AA could amplify the response of Ang II to cardiac and vascular contractions by changing the spatial structure of AT_1-R and the intracellular level of Ca^{2+} [8]. This finding may explain why the concentration of Ang II in the plasma of preeclamptic patients was not high, but the vascular response to Ang II was increased. In addition, we successfully established a rat preeclampsia model by injecting AT_1-AA from the plasma of preeclampsia patients to the animal, and found that the susceptibility of the heart to ischemia was increased significantly in the modeled rats at four months postpartum [9].

microRNAs (miRNAs) are a group of endogenous non-coding single-stranded RNA molecules with about 22 nucleotides length. They participate in post-transcriptional regulation of gene expression in both animals and plants. Abnormal expression of miRNAs is associated with the development and progression of various diseases including preeclampsia [10], and therefore could be used as a new target for the diagnosis and treatment of diseases [11, 12]. The effect of miRNAs against cardiomyocyte apoptosis has aroused increasing attention. miR-21 cluster is recognized as playing an important role in regulating proliferation, apoptosis, cell cycle and other pivotal processes [13]. The expression of miR-21 was inhibited in ischemia/reperfusion injury (IRI)-induced cardiomyocyte apoptosis [14]. Choi e*t al* [15] found that the expression level of miR-21 was significantly reduced in the placental tissue of preeclampsia patients. However, the reason and pathogenic mechanism causing the reduced expression of miR-21 during the occurrence of preeclampsia remain unclear.

Based on our previous work, the present study was intended to explore whether cardiomyocyte apoptosis during preeclampsia was due to the suppression of miR-21 expression in an AT_1-AA induced preeclampsia rat model in vivo, and to investigate the involved mechanism of this suppression effect in cultured cardiomyocytes of lactating mice. The results will supply the evidence for the therapeutic strategy of preeclampsia.

Materials and methods

Animals

Healthy female Wistar rats aged 12 weeks and weighing 220-250 g and 3-day-old neonatal rats were purchased from the Experimental Animal Center of Shanghai Jiao Tong University School of Medicine (Shanghai, China). All animal procedures were performed according to the guidelines approved by the university.

Selection of preeclamptic patients and extraction of the antibody

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) criteria and upon approval from the ethics committee of the hospital, six normal pregnant women with negative AT_1-AA and six preeclampsia patients with the titer of AT_1-AA>1:640 were selected for the present study. All the subjects were primiparas and there was no significant difference in age between the two groups. Venous blood was drawn for the experiment. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the preeclampsia group were 148.2 ± 10.8 mmHg and 106.4 ± 8.4 mmHg respectively *vs.* 117.6 ± 6.8 mmHg and 76.8 ± 6.1 mmHg in the normal pregnancy group. Urine protein was 4235 ± 1870 mg/24 h in the preeclampsia group *vs.* 170 ± 84 mg/24 h in the normal pregnancy group. Plasma samples from each group were mixed to extract IgG by using Protein A-Sepharose CL-4B affinity chromatography. After antibody titer calibration and determination of the protein concentration, they were used for subsequent experiments.

Establishment of the preeclampsia rat model

The preeclampsia rat model was established as described previously [16]. Briefly, 12 pregnant rats were equally randomized to a sham group and a model group. There was no significant difference in age, weight and BP between the two groups. In the model group, 100 μl AT_1-AA PBS solution with the titer >1:160 was injected to the gestational-day 13 rats via the

tail vein, and a booster injection was given the following day. In the sham group, 100 μl IgG PBS from the normal pregnant women was injected into the gestational-day 13 rats via the tail vein. At day 19 of pregnancy, BP was measured by the tail-cuff method. The rats were placed in the metabolic cage to collect urine for protein analysis. At day 20 of pregnancy, the rats were anesthetized by ether inhalation anesthesia to remove the heart for subsequent experiments. At the same time, the fetus was removed by laparoscopy to measure weight and height.

Histological examination of the heart

After removing the left atrium, right atrium and right ventricular free wall, weight of the heart with the left ventricular (LV) free wall and interventricular septum retained was measured accurately to calculate the left ventricular mass index (LVMI) by using the following equation: LVMI = LV weight/body weight (mg/kg). A 2 mm^3 myocardial tissue was fixed in 0.25% glutaraldehyde and observed under the transmission electron microscope (TEM). A one-third LA was fixed in 10% formaldehyde, HE stained and placed under the optical microscope to observe the myocardial structure and calculate the cross-sectional area (CSA) of the cardiomyocytes. The remaining LA was stored at -70°C for subsequent RNA and protein extraction.

MicroRNA expression profile chip hybridization detection

100 mg of rat LV tissue was ground in liquid nitrogen. Total RNA was extracted by routine Trizol method, and the quality was detected by ultraviolet absorption and denaturing agarose gel electrophoresis. miRNA chip V3.0 hybridization was performed using miR crystal core · micro RNA microarray Kit (CapitalBio). Images were scanned using LuxS can10K/A Dual Channel Laser Scanner (Capi talBio). The chip image data were analyzed using LuxScan 3.0 image analysis software (CapitalBio). A 2-fold or less than 0.5-fold difference between the obtained values from the model and sham groups indicates a significant trend of upregulation or downregulation.

Cell apoptosis assay

Wistar lactating rats aged 1-3 days were provided by the Experimental Animal Center of Shanghai Jiao Tong University School of Medicine. According to the method described in the literature [17], the heart of the lactating rat was removed aseptically, sheared to pieces, and digested repeatedly with 0.1% trypsin and 0.1% collagenase. The cell suspension was filtered through a 200-mesh sieve, washed with DMEM, and cultured under 5% CO_2 and 37°C conditions. Cardiomyocytes were obtained by 60-min differential adherence and removal of fibroblasts.

Cells were evenly seeded to 12-well plates at a density of 5×10^5 cells/well. When cells grew to 80% confluence, the medium was replaced with serum-free DMEM/F12 and culture was continued for 16 h until cells were synchronized. Synchronized cells were divided into four groups: NC group, where the medium was added with IgG from the normal pregnant women (the protein concentration was equal to that of AT_1-AA group). AT_1-AA group, where the medium was added with AT_1-AA with a final titer of 1:160 and culture was continued for 48 h; miR-21 group, where cells were transfected with miR-21 mimic fragment; AT_1-AA+miR-21 group, where cells were first transfected with miR-21 mimic fragment and then the medium was added with AT_1-AA with a final titer of 1:160. Cells were harvested 48 h after culture for subsequent determinations.

Flow cytometry

Flow cytometry was performed according to the instructions of Annexin VFITC/PI apoptosiis assay kit (Biosea Biotechnology Co., Ltd., Beijing, China). After 48 h culture, the medium was discarded, and cells were digested by addition of pancreatin until they morphologically became round. Cells were then dispersed gently, added with DMEM to terminate digestion, and centrifuged at 1,000 r/min for 10 min. After discarding the supernatant, the remaining cells were washed with PBS twice and resuspended in binding buffer. The cell concentration was adjusted to 1×10^6/ml. The cell suspension was then added with fluorescence- labeled Annexin V and PI reagent and cultured away from light for 15 min. The apoptosis rate of each group was detected by flow cytometry within 1 h after culture. The same experiment was repeated three times.

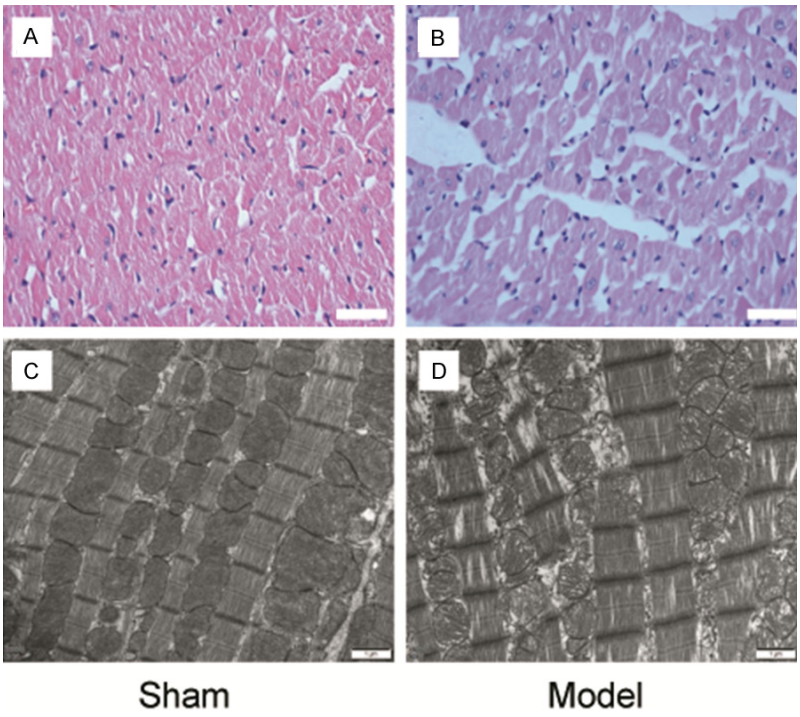

Figure 1. Morphological change of the cardiac tissue in pregnant rats was observed by HE staining and transmission electron microscopy in sham group (A, C) and model group (B, D). Original magnification (A, B) 400×; (C, D) 15000×.

Determination of the rat LV myocardial tissue and miR-21 expression in cultured cardiomyocytes by qRT-PCR

Total RNA was extracted from the cardiac tissue and cardiomycytes using the Trizol reagent according to the instructions. The miR-21 primer sequences are as follows: upstream 5'-CCTGCCTGAGCACCTCGTGC-3', downstream 5'-GACTGTGCGACTACCCCAA-3'; U6 primer upstream 5'-CTCGCTTCGGCAGCAC A-3', downstream 5'-AACGCTTCA CGAATTTGCG T-3'. The reaction conditions are as follows: denaturation 95°C 10 min; 95°C 15 s, 60°C 30 s, 40 cycles; extension 72°C 5 min. Using the RT-PCR reagent kit, RNA was reverse transcribed into cDNA and amplified to obtain 5 μL amplified product for subsequent 2% agar gel detection, photography and analysis.

Cell total protein extraction and Western blot assay

After 48-h treatment, the collected cells were added with RIPA lysis buffer and centrifuged to obtain the protein sample. Protein was quantitated by BCA method. 20 μg total protein was electrophoresed on SDS-PAGE. According to the size of the molecular mass Marker, appropriate protein was selected, transferred to the PVDF membrane, mounted with 5% skimmed milk for 2 h, added with the primary antibody, and cultured at 4°C overnight. After addition of horseradish peroxidase-labeled secondary antibody, cells were cultured at room temperature for 2 h, and developed according to the ECL chemiluminescence detection Kit. The result was photographed and analyzed with the BIO-RAD digital image analysis system. The relative expression level of the target protein was obtained by comparing the gray value of the target protein with that of β-actin.

Statistical analysis

All data are expressed as means ± SD. Differences between means were determined by the Student's unpaired t-test. The data were analyzed by using the SPSS13.0 software (SPSS Inc., Chicago, IL, USA). Probability values ≤0.05 was considered statistically significant.

Results

Characteristics of pregnant and fetal rats

To observe the pathogenic effect of AT_1-AA during preeclampsia, we established a preeclampsia rat model by injecting AT_1-AA via the tail vein. At day 5 after AT_1-AA injection, SBP and DBP of the pregnant rats in the model group were significantly higher than those in the sham group (93.6 ± 6.5 mmHg and 143.7 ± 7.4 mmHg vs. 78.4 ± 6.2 mmHg and 110.3 ± 6.5 mmHg). In addition, urine protein in the model group was significantly higher than that in the sham group (10.13 ± 3.42 mg/24 h vs. 3.15 ± 0.82 mg/24 h). The weight and height of the offspring in the model group were significantly lower than those in the normal control group (4.37 ± 0.35 g and 3.55 ± 0.32 mm *vs.* 4.27 ± 0.52 g and 3.87 ± 0.41 mm). All these results

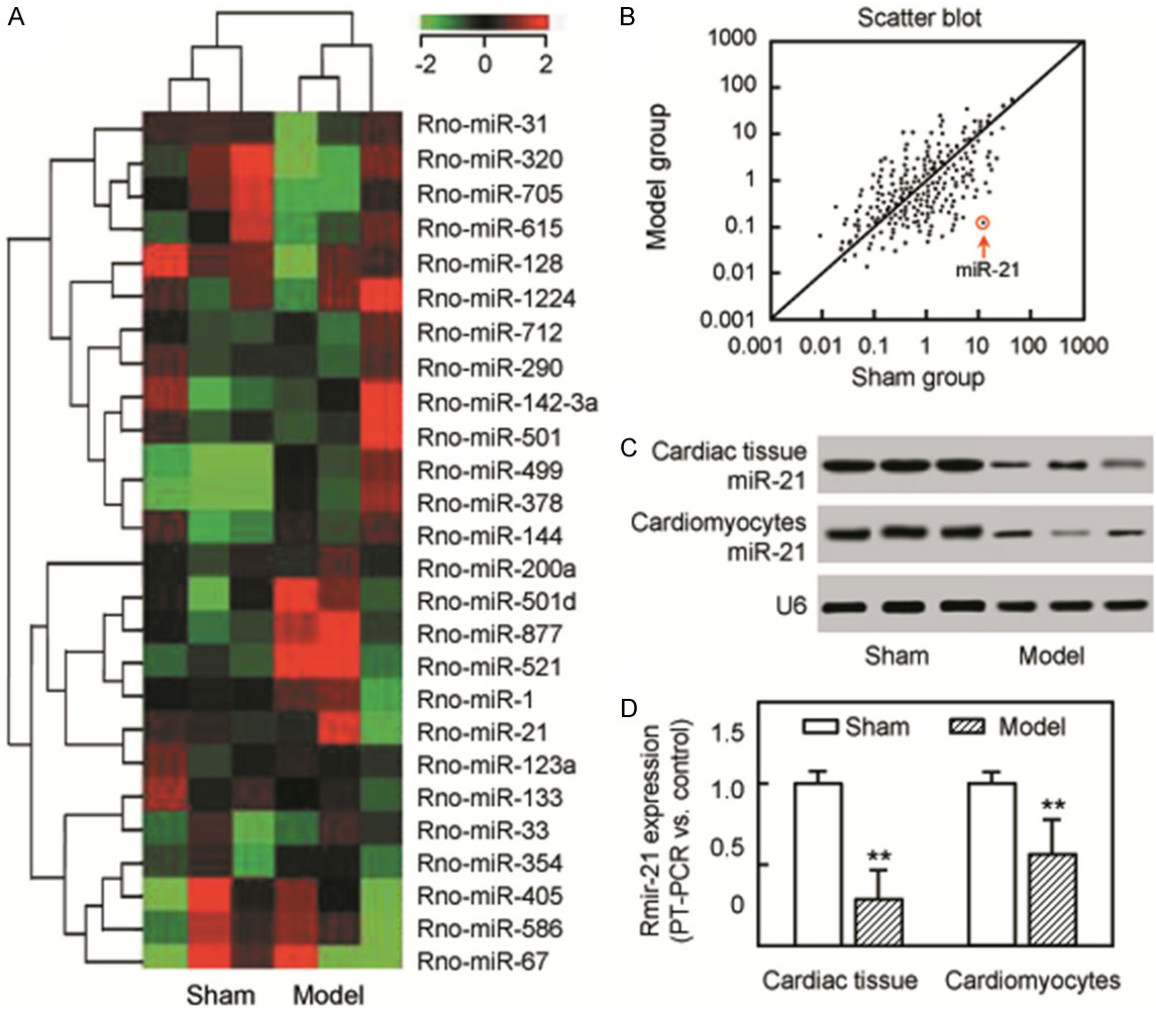

Figure 2. miRNA expression profiles and detection of miR-21 in the cardiac tissue and neonatal rat cardiomyocytes. A: Microarray chip analysis of miRNA expression in the cardiac tissue of control and model groups. Columns represent cell lines and rows show the relative expression level for individual miRNAs. The red and green colors indicate high of low expression, respectively. B: Scatter plot of signal intensity of gene chip. C, D: miR-21 level in the cardiac tissue and neonatal rat cardiomyocytes by Western Blot and qRT-PCR analysis.

indicate that the model was established successfully.

Histopathological change of the LV myocardium of the pregnant rats

To observe the effect of AT_1-AA on the cardiac structures during preeclampsia, we observed histological changes of the heart. It was found that LVMI and myocardial CSA of the model group were significantly larger than those of the sham group (2.87 ± 0.34 mmHg and 1085.4 ± 56.5 μm^2 *vs.* 2.14 ± 0.28 mmHg and 687.3 ± 49.6 μm^2). The histological result showed that in the sham group, the myocardium was evenly stained, the myocardial fibers were well arranged, and the markings were clear; the myocardial fibers were neatly arranged with bright and dark bands and Z line clearly seen, and the mean length of the sarcomeres was 1.63 ± 0.07 μm (**Figure 1A**, **1C**). In the model group, the myocytes were hypertrophic; cell density was decreased and the intercellular substance was increased, with large amounts of fibroblasts filled in; the cardiomyocytes were crinkled, the muscle fibers were broken and lyzed, the sarcomeres became unclear, the intercalated discs were broken, the nuclear membrane was unclear, and the nuclear chromatin was decreased; the number of mitochondria was decreased, the ridge and intima fusion partially or completely disappeared, while vacuoles we-

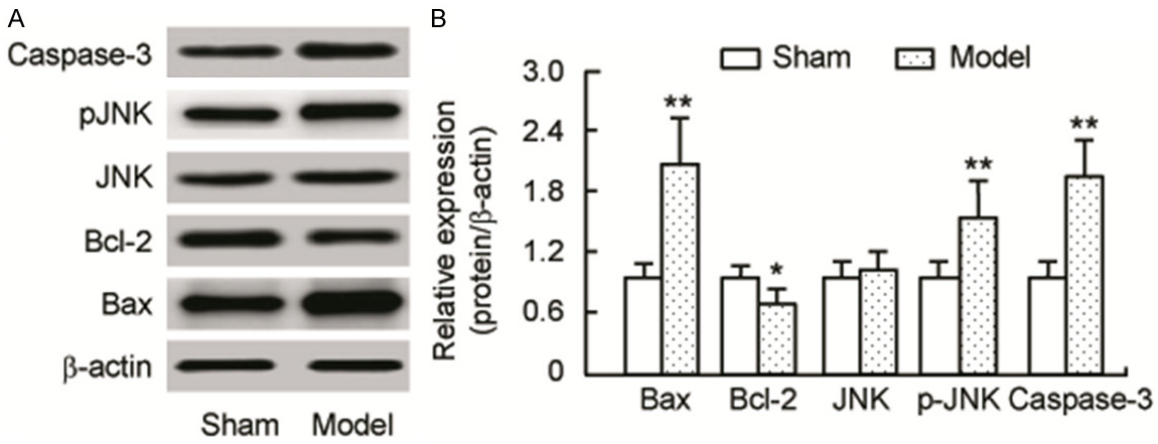

Figure 3. Western blot analysis of protein in the cardiac tissue involved in apoptosis. A: JNK, pJNK, Bcl-2, Bax, caspase-3 and β-Actin were used as internal control to normalize the results. B: Relative quantification of protein-expression levels. Results are expressed as means ± SE of triplicate experiments. *P<0.05 and **P<0.01 *vs.* control.

re present; the mean length of the sarcomeres was 1.85 ± 0.11 μm (**Figure 1B**, **1D**). The above results suggest that the adverse effect of AT_1-AA on the cardiac structures may be associated with its effect in inducing apoptosis of cardiomyocytes.

Expression of JNK, Bcl-2, Bax and caspase-3 protein in the cardiac tissue

To verify whether AT_1-AA could induce cardiomyocyte apoptosis in pregnant rats, we detected related proteins in the cardiac tissue of pregnant rats by Western blot, found that compared with the sham group, JNK protein was increases slight (not significantly, P<0.05), but p-JNK protein was increased. The p-JNK/JNK ratio in the model group was significantly higher than those in the control group (1.67 ± 0.45 vs. 1.07 ± 0.23, P>0.05). In addition, both the expression of Bcl-2 protein and the Bcl-2/Bax ratio were decreased significantly in the model group, while the expression of the downstream signaling molecule caspase-3 protein was increased. These results indicate that AT_1-AA activated caspase-3 by phosphorylating JNK protein, downregulating Bcl-2 and upregulating Bax, finally resulting in cell apoptosis (**Figure 3**).

Analysis of miR-21 expression

To clarify through which miRNA AT_1-AA exerted its adverse effect on the myocardium, we analyzed and compared the miRNA expression profiles in the heart tissue and screened out miRNAs that underwent changes. Compared with the sham group, there were 8 differentially expressed miRNAs in the cardiac tissue of the model group, including upregulation of miR-449a, miR-320, miR-301 and miR-133, and downregulation of miR-21, miR-1, miR-501 and miR-805, among which the expression of miR-21 decreased most obviously (**Figure 2A**, **2B**). Real-time PCR showed that the amount of miR-21 in the cardiac tissue of the model group was only 32.7% of the sham group (**Figure 2C**, **2D**).

Real-time PCR of the cultured myocardial miR-21 of the pregnant rats showed that the expression level of myocardial miR-21 in AT_1-AA treated group was only 56% of the sham group (**Figure 2C**, **2D**). These results suggest that the adverse effect of AT_1-AA on cardiomyocytes may be related to the suppression of miR-21 expression.

AT_1-AA induced apoptosis of cultured cardiomyocytes

To verify whether miR-21 downresulation participated in the effect of AT_1-AA in inducing apoptosis of cardiomyocytes, we transfected miR-21 mimic to cells to increase the expression level of miR-21, and detected apoptosis of the cultured cardiomycytes of pregnant rats by flow cytometry. It was found that the apoptosis rate in miR-21 group was insignificantly lower than that the NC group was (8.72 ± 1.09% *vs.* 9.67 ± 1.23%). The apoptosis rate in AT_1-AA group was 36.47 ± 5.42%, which was signifi-

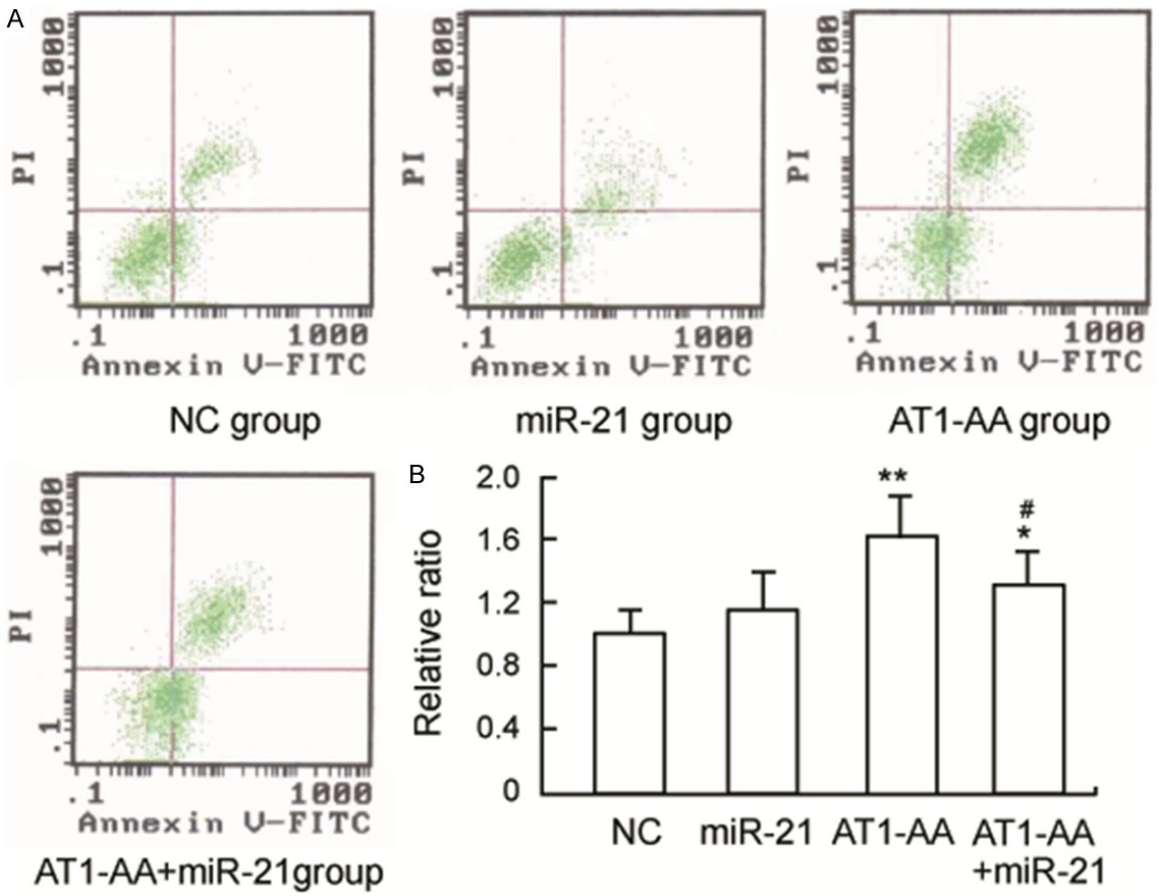

Figure 4. AT_1-AA induced neonatal rat cardiomyocyte apoptosis through downregulating miR-21 level. A: Primary cardiomyocytes were treated with 1:160 AT_1-AA for 48 h and then stained with annexin V-FITC and PI using annexin V-FITC apoptosis detection kit. Apoptosis was measured by flow cytometry. B: Quantitative assessment of the mean total cell death rate of three experiments (mean ± SD). FITC: fluorescein isothiocyanate; PI: propidium iodide. *P<0.01, **P<0.001 *vs.* NC group; #P<0.05, ##P<0.01 *vs.* AT_1-AA group.

cantly higher than that in the NC group (P<0.05). The apoptosis rate in AT_1-AA+miR-21 group was 18.65 ± 4.19%, which was significantly lower than that in AT_1-AA group (P<0.05). These results indicate that AT_1-AA could markedly increase the apoptosis rate of culture cardiomyocytes, probably by inhibiting the expression of miR-21 (**Figure 4**).

Expression of pPTEN, pAKT, pFOXO3a and FasL protein in cardiomyocytes of lactating rats

To further analyze the expression of downstream signaling molecules after miR-21 downregulation by AT_1-AA, we analyzed PTEN, Akt and FOXO1 48 h after AT_1-AA action by Western blot, and found that compared with the NC group, the expression of PTEN in miR-21 group was downregulated (P<0.05), but upregulated in AT_1-AA group (P<0.05). Compared with AT_1-AA group, the expression of PTEN was downregulated in AT_1-AA+mi-21 group (P<0.05).

Compared with the NC group, the expression of pPTEN, pAKT and pFOXO3a in AT_1-AA group was upregulated significantly (P<0.05). Compared with AT_1-AA group, the expression of pPTEN, pAKT and pFOXO3a in miR-21 overexpression group was downregulated significantly (P<0.05). All the above results suggest that AT_1-AA induced cardiomyocyte apoptosis probably via the miR-21-PTEN/AKT/FOXO3a signaling pathway (**Figure 5**).

Discussion

Preeclampsia is a disease seriously endangering both the mother and the fetus. The role of AT_1-AA in the pathogenesis of preeclampsia has

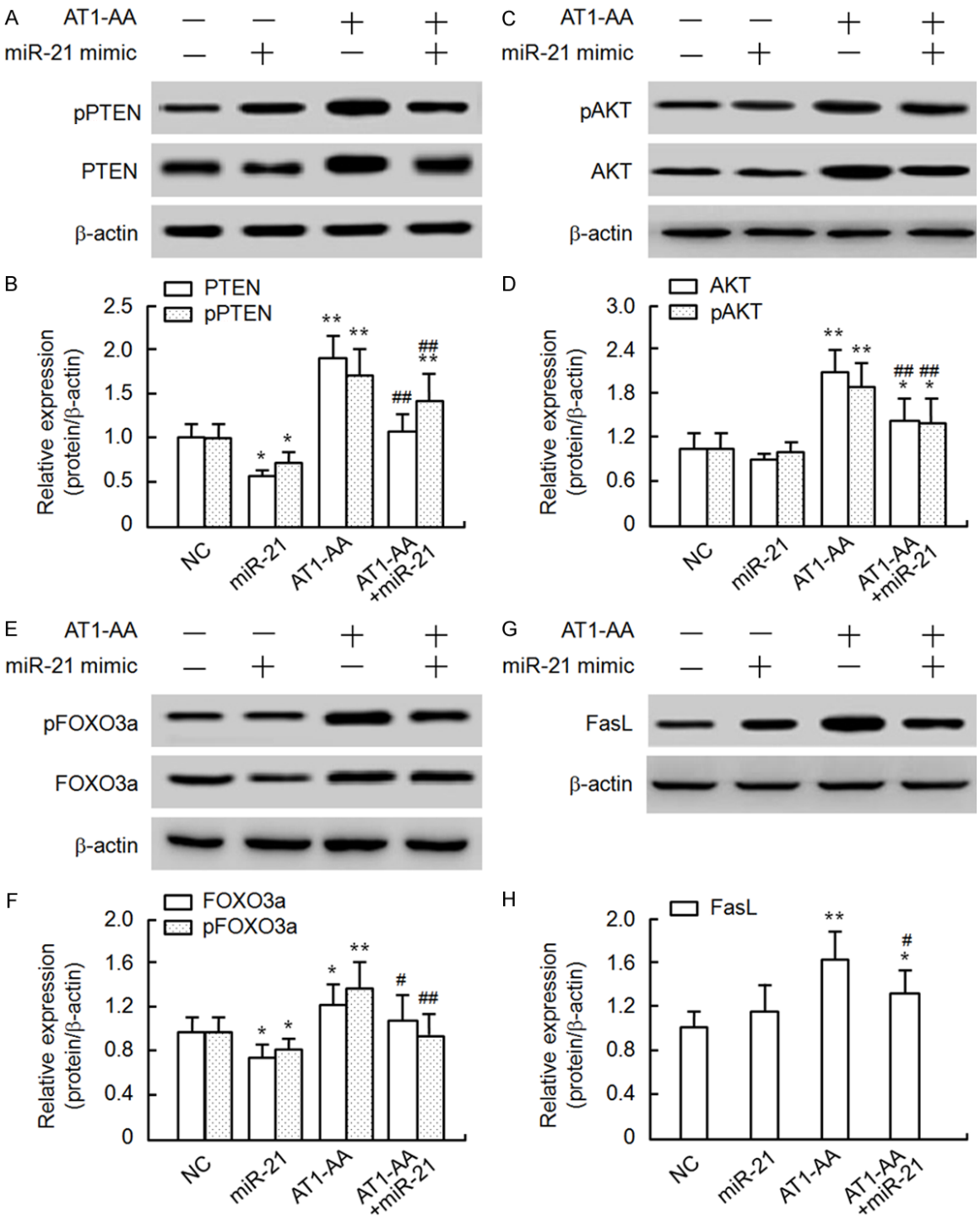

Figure 5. The cardiomyocyte apoptosis-inducing effect of AT_1-AA was mediated through the miR-21-PTEN-AKT signal transduction pathway. A, C, E and G: Western blot analysis of the expression and phosphorylation of PTEN, Akt, and FOXO3a in cultured neonatal rat cardiomyocytes treated AT_1-AA and/or miR-21 mimic. B, D, F and H: Quantification of PTEN, p-PTEN, Akt, p-Akt, FOXO3a, and p-FOXO3a expression in different treatment groups. Data were obtained by densitometry and normalized using β-actin as loading control. Values are expressed in relative optical density and represented as mean ± SD. For each column, *n*=4-6, *P<0.01, **P<0.001 *vs*. NC group; #P<0.05, ##P<0.01 *vs*. AT_1-AA group.

aroused increasing attention. But as the exact action mechanism of AT_1-AA is unclear, it is impossible to take effective preventive measures. The target of the pathological effect of

AT_1-AA is the AT_1 receptor (AT_1-R) on the cell membrane. Many in vitro experiments have demonstrated that Losartan and other AT_1-R inhibitors could effectively antagonize the pathological effect of AT_1-AA [16, 17]. But as AT_1-R participates in the fetal development, it is impossible to use AT_1-R antagonists as a treatment for preeclampsia patients with the presence of AT_1-AA in their plasma. It is therefore necessary to find the downstream target of AT_1-AA action so as to provide a new way of thinking and a new therapeutic target for the treatment of preeclamsia and other cardiovascular diseases.

Knowing that miRNAs play important roles in cell differentiation, proliferation, apoptosis, DNA methylation and tumorigenesis, their expression profiles may provide important information for research on the pathophysiological status [18]. Many studies have demonstrated that dysregulation of miRNAs is associated with the pathogenesis of preeclampsia [15, 19]. As placental miRNAs can be released to mother's blood circulation [20], detection of miRNAs in the plasma of pregnant women would be of great significance for the diagnosis and prediction of pregnancy-associated diseases. Choi *et al* [15] conducted a microarray analysis on miRNA expression profiles in the placental tissue of pregnant women with severe preeclamsia and normal pregnant women, and found that the expression of miR-21 was reduced significantly. In the present study, we established a preeclampsia model in pregnant rats by injection AT_1-AA IgG extracted from the plasma of preeclampsia patients into gestational-day 13 rats via the tail vein. miRNA chip test of the cardiac tissue showed that miR-21 was downregulated significantly in the model group. RT-PCR showed that the amount of miR-21 expression in the model group was about one-third of the amount in the sham group. In addition, AT_1-AA could also reduce the expression of miR-21 in cultured cardiomyocytes. Using related analysis software, we screened most abnormally expressed target genes of miR-21 and analyzed their functions, finding that miR-21 cell cycle and proliferation were associated with apoptosis and PTEN. Detection of apoptosis-associated proteins in the myocardial tissue of the model group showed that phosphorylated JNK protein was increased, the apoptosis-inhibiting gene Bcl-2 was decreased, the apoptosis-promoting gene Bax was increased, the Bcl-2/Bax ratio was decreased, and the expression of Bcl-2/Bax downstream caspase-3 was also increased significantly. Our in vitro experiment also demonstrated that AT_1-AA was able to downregulate the expression of miR-21 and further lead to apoptosis of cardiomyotes of the lactating rats. Overexpression of miR-21 in cardiomyocytes could inhibit AT_1-AA induced cardiomyocyte apoptosis significantly.

Current studies have discovered that phosphatase and tensin homologue deleted on chromosome 10 (PTEN) gene is one of the four target genes of miR-21 in mediating the cardiovascular effect, and also the only tumor suppressor gene with protein esterase and phosphatase activities. It plays important roles in regulating fetal development, cell growth, differentiation, apoptosis and migration [21]. Studies in recent years showed that PTEN overexpression could increase cell apoptosis, and the PTEN/AKT/FOXO3a pathway was an important pathway of regulating cell apoptosis [22]. Sayed *et al* [23] found that PTEN was the miR-21 target gene on hypoxia-induced apoptosis of cardiomyocytes. Roy *et al* [24] reported that PTEN was the miR-21 target gene on cardiac fibroblasts of IR-induced cardiac remodeling. Our experiment demonstrated that AT_1-AA was able to promote phosphorylation of PTEN, AKT and FOXO3a, thus increasing the expression of pPTEN, pAKT, pFOXO3a and FasL protein. Our study revealed that the effect of miR-21 on AT_1-AA induced cardiomyocyte apoptosis may need activation of the PTEN/AKT/FOXO3a signal transduction pathway. This finding may provide a new clue and way of thinking for the research of the mechanism of miR-21 against AT_1-AA induced cardiomyocyte apoptosis.

In summary, both our in vitro and in vivo experiments demonstrated that AT_1-AA could promote cardiomyocyte apoptosis by inhibit the expression of miR-21; miR-21 overexpression could significantly attenuate the effect of AT_1-AA in inducing cardiomyocyte apoptosis; and the miR-21 downstream PTEN/AKT/FOXO3a signal transduction pathway participated in this process. Whether miR-21 and the PTEN/AKT/FOXO3a signal transduction pathway could be used as a target point to antagonize the effect of AT_1-AA in inducing cardiomyocyte apoptosis needs further investigation.

Acknowledgements

This work was supported by the grants from the National Natural Science Foundation of China (No. 81670232).

Disclosure of conflict of interest

None.

Abbreviations

miR-21, microRNA-21; AT_1-AA, Angiotensin II type I receptor agonistic autoantibodies; AT1 R, AT1 receptor; IV, intravenous; miRNA, microRNAs; SBP, systolic blood pressure; DBP, diastolic blood pressure; LV, left ventricular; LVMI; left ventricular mass index; TEM, transmission electron microscope; CSA, cross-sectional area; PTEN, phosphatase and tensin homologue deleted on chromosome 10.

Address correspondence to: Xiangying Jiao, Key Laboratory of Cellular Physiology (Shanxi Medical University), Ministry of Education, and The Department of Physiology, Shanxi Medical University, Taiyuan, China. Tel: +86-351-4135766; E-mail: jiaoxyty@163.com; Jianming Zhi, Department of Anatomy and Physiology, School of Medicine, Shanghai Jiaotong University, Shanghai, China. Tel: +86-21-6384-6590 Ext. 776446; E-mail: zhjm169@gmail.com

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