Biomedicine & Pharmacotherapy 94 (2017) 412–417

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Lentivirus-mediated miR-23a overexpression induces trophoblast cell apoptosis through inhibiting X-linked inhibitor of apoptosis

Lichun Li[a,1], Aiqin Hou[a,1], Xia Gao[a], Juan Zhang[a], Liping Zhang[a], Juan Wang[a], Hua Li[a,*], Yanbin Song[b,c,**]

[a] Department of Obstetrics, Affiliated Hospital, Yan'an University, Yan'an, Shaanxi 716000, China
[b] Department of Cardiology, Affiliated Hospital, Yan'an University, Yan'an, Shaanxi 716000, China
[c] The College of Life Science, Northwest University, Xi'an, Shaanxi 710069, China

ARTICLE INFO

Article history:
Received 27 March 2017
Received in revised form 14 July 2017
Accepted 19 July 2017

Keywords:
Preeclampsia
Trophoblast cell
Apoptosis
miR-23a
XIAP

ABSTRACT

Preeclampsia (PE) is a pregnancy-specific disorder representing a major cause of maternal and perinatal morbidity and mortality. MicroRNAs (miRNAs) have emerged as critical regulators in PE. However, the precise role of miRNAs in PE remains poorly understood. In this study, we aimed to investigate the potential role of miR-23a and the underlying mechanism in regulating trophoblast cell apoptosis. We found a significant increase of miR-23a expression in placental tissues from PE patients. Lentivirus-mediated miR-23a overexpression significantly induced apoptosis in trophoblast cells *in vitro*. X-linked inhibitor of apoptosis (XIAP) was identified as a target gene of miR-23a by bioinformatics analysis and dual-luciferase reporter assay. Overexpression of miR-23a significantly inhibited XIAP expression. Knockdown of XIAP also induced trophoblast cell apoptosis. Moreover, restoration of XIAP expression significantly abolished the miR-23 overexpression-induced trophoblast cell apoptosis. Taken together, our study demonstrates that miR-23a induces trophoblast cell apoptosis by inhibiting XIAP, which may contribute to PE. Our findings provide novel insights into understanding the pathogenesis of PE and suggest a potential therapeutic target in PE.

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disorder characterized by hypertension and proteinuria causing high maternal and perinatal morbidity and mortality [1]. Increased trophoblast cell apoptosis in the placenta has been suggested as a major cause of the pathogenesis of PE [2,3]. However, the underlying molecular mechanism involved in the trophoblast cell apoptosis during PE remains largely unknown.

MicroRNAs (miRNAs) are a subset of small, non-coding RNAs that negatively regulate gene expression in a post-transcriptional manner [4]. Briefly, miRNAs recognize the complementary sequences within the 3′-untranslated region (UTR) of target mRNA and bind the 3′-UTR to induce mRNA instability and degradation [4]. Therefore, they function in numerous biological processes, including cell apoptosis, differentiation and proliferation [5]. In recent years, miRNAs are emerging as important regulators in the development and progression of various diseases [6]. miRNAs have been found to be abundantly expressed in human placenta [7]. An increasing number of studies has reported that various miRNAs are differentially expressed in placenta tissues of PE patients [8,9]. miRNAs are involved in regulating trophoblast cell functions such as invasion and apoptosis [10,11]. Therefore, miRNAs have been proposed as potential biomarkers and therapeutic targets in PE [12,13]. It is essential to gain a better understanding of miRNAs in the pathogenesis of PE.

X-linked inhibitor of apoptosis (XIAP) is a member of the inhibitor of apoptosis protein family that potently inhibits cell apoptosis by directly interacting with and inhibiting caspase-3/7/9 [14,15]. XIAP is highly expressed in trophoblasts throughout placental development [16]. Inactivation of XIAP is correlated with trophoblast cell apoptosis in placenta [17]. XIAP protects

Abbreviations: PE, preeclampsia; miRNAs, microRNAs; XIAP, X-linked inhibitor of apoptosis; LV, lentiviral; NC, negative control; FBS, fetal bovine serum; RT-qPCR, Real-time quantitative polymerase chain reaction.

* Corresponding author at: Department of Obstetrics, Affiliated Hospital, Yan'an University, No. 43 North Street, Yan'an, Shaanxi 716000, China.
** Corresponding author at: Department of Cardiology, Affiliated Hospital, Yan'an University, No. 43 North Street, Yan'an, Shaanxi 716000, China.
E-mail addresses: huali_ya@163.com (H. Li), yanbin_songyb@163.com (Y. Song).

[1] These authors (Lichun Li and Aiqin Hou) have contributed equally to this study and share first authorship.

http://dx.doi.org/10.1016/j.biopha.2017.07.082
0753-3322/

trophoblast cells against Fas-induced apoptosis [17]. XIAP has been reported to be decreased in trophoblast cells during PE [18]. Therefore, XIAP plays an important role in the pathogenesis of PE serving as a potential therapeutic target for PE.

Several studies have highlighted the importance of miR-23a in regulating cell apoptosis [19,20]. Moreover, miR-23a has been found to be up-regulated in trophoblast cells treated with bacterial peptidoglycan [21]. However, the function of miR-23a in PE remains unknown. In this study, we aimed to investigate the potential role of miR-23a and the underlying mechanism in regulating trophoblast cell apoptosis. We found a significant increase of miR-23a expression in placental tissues from PE patients. Lentivirus-mediated miR-23a overexpression significantly induced apoptosis in trophoblast cells. XIAP was identified as a functional target gene of miR-23a. These results showed that miR-23a induced the apoptosis of trophoblast cells by inhibiting XIAP. Our study highlights the importance of miR-23a in trophoblast cell apoptosis and provides novel insights into understanding the pathogenesis of PE.

2. Materials and methods

2.1. Clinical sample collection

Human placental tissues were collected from Affiliated Hospital, Yan'an University under a protocol approved by the Institutional Human Experiment and Ethic Committee of Affiliated Hospital, Yan'an University. Placental tissues were collected following delivery at term by cesarean section. The tissues were washed with sterile phosphate buffered saline (PBS), snap-frozen in liquid nitrogen and then stored at −80 °C for RNA extraction. PE was defined as either severe hypertension or severe proteinuria in accordance with the International Society for the Study of Hypertension in Pregnancy. Normal pregnancy without pre-eclampsia or any other complications was used as a control.

2.2. Cell culture

The human first trimester trophoblastic cell line 3A was purchased from the American Type Tissue Culture Collection (ATCC, Manassas, VA, USA) and grown in RPMI1640 (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS), 10 mM HEPES, 0.1 mM minimum essential medium non-essential amino acids, 1 mM sodium pyruvate and 1% penicillin-streptomycin solution (Gibco). Also, 293T cells were purchased from ATCC and grown in DMEM (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin solution (Gibco). Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2.

2.3. Cell treatments

The lentiviral vector LV-miR-23a and lentiviral negative control (LV-NC) vector were purchased from GenePharma (Shanghai, China). Cells were infected with LV-miR-23a or LV-NC by polybrene (Sigma, St. Louis, MO, USA) following the manufacturer's protocols. Briefly, cells were plated into a 12-well plate and cultured for 24 h. The medium were then replaced by complete medium containing 20 μl lentiviral particle stock and polybrene (5 μg/ml). After culture for 48 h, cells were harvested for analysis. XIAP siRNA and NC siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and transfected into cells according to the manufacturer's instructions. Briefly, cells were seeded into a 6-well tissue culture plate at a density of 2×10^5 cells/well and cultured for 24 h. A total of 1 μg siRNA were mixed with 6 μl Transfection Reagent (Santa Cruz Biotechnology) and 200 μl siRNA Transfection Medium (Santa Cruz Biotechnology) and incubated for 45 min at room temperature. Cells were incubated with the mixture for 6 h. Thereafter, the normal growth medium were added and cultured for 48 h before being collected for analysis. XIAP cDNA without 3′-UTR was inserted into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). The vector was then transfected by using Lipofectamine2000 (Invitrogen).

2.4. Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using Trizol (Invitrogen) and reverse-transcribed into cDNA using the miScript Reverse Transcription Kit (QIAGEN, Dusseldorf, Germany) and M-MLV reverse transcriptase (TAKARA, Dalian, China). RT-qPCR was performed with SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) and appropriate primers. The primer sequences were as follows: miR-23a, forward: 5′-AGCGGATCACATTGCCAGGG-3′ and reverse: 5′-GTGCAGGGTCCGAGGT-3′; U6, forward: 5′-CTCGCTTCGGCAGCACA-3′ and reverse: 5′-AACGCTTCACGAATTTGCGT-3′; XIAP, forward: 5′-ACCGTGCGGTGCTTTAGTT-3′ and reverse: 5′-TGCGTGGCACTATTTT-CAAGATA-3′; and GAPDH, forward: 5′-CCAGAACATCATCCCTGCCT-3′ and reverse: 5′-CCTGCTTCACCACCTTCTTG-3′. U6 and GAPDH were used as the internal control. The relative gene expression was analyzed by the $2^{-D\Delta Ct}$ method.

2.5. MTT assay

Cells were plated in 96-well plates at a density of 1×10^4 cells/well and cultured overnight. Cells were treated with 20 μl of MTT (Sigma; 5 mg/ml) and incubated for 4 h. The formazan crystals were dissolved by adding 200 μl of dimethyl sulfoxide (DMSO; Sigma). The absorbance at 490 nm was detected by a microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

2.6. Apoptosis assay

Cell apoptosis was measured by flow cytometry using Annexin V-FITC/PI (Beyotime Biotechnology, Haimen, China). Cells were digested with trypsin (2.5 g/l) and washed with PBS. Cells were resuspended in 500 μl of binding buffer and double-stained with PI and FITC-conjugated Annexin V for 30 min at 4 °C according to the manufacturer's instructions. The samples were detected by a BD FACSCanto Flow Cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed by BD FACS Diva software.

2.7. Caspase-3 activity assay

A total of 2×10^6 cells were lyse by 100 μl lysis buffer (Beyotime Biotechnology) followed by centrifugation at 16,000g for 15 min at 4 °C. Then, 10 μl protein samples, 10 μl Ac-DEVD-pNA (2 mM; Beyotime Biotechnology) and 80 μl reaction buffer were mixed and incubated for 1–4 h at 37 °C. Once color changed obviously, absorbance of the solution at 405 nm was measured using a spectrophotometer (Bio-Tek Instruments).

2.8. Luciferase reporter assay

The predicted 3′-UTR of XIAP mRNA (positions 957–1157) containing the seed sequence of the miR-23a binding site was cloned into the pmirGLO dual-luciferase vector (Promega, Madison, WI, USA) downstream of the luciferase reporter gene. Mutations were introduced into the 3′-UTR of XIAP (CAAUGUGA to GAUUCUCA) using a QuikChange Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, CA, USA). The 293T cells were transfected with pmirGLO dual-luciferase vector and infected with LV-miR-23a. After culture for 48 h, the luciferase activity was examined using the Dual-Luciferase Assay System (Promega).