Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°2, 625-632
DOI: 10.24205/03276716.2020.4065

The role and mechanism of miR-210 in unexplained recurrent abortion

Benying Li[a*], Shifen Liu[b*], Yan Cao[a], Yaqi Zhang[c], Hai Xia[d], Teng Ma[e]

Abstract

Purpose: The cause of unexplained recurrent spontaneous abortion (URSA) is unknown. miR-210 regulates vascular endothelial cell proliferation and migration. However, whether it plays a role in URSA remains poorly understood. Our study intends to assess miR-210's role in URSA.

Materials and Method: Patients with recurrent abortion who underwent uterine surgery were selected as URSA group and normal early pregnancy patients who required termination of pregnancy were selected as a control group. The miR-210 level was detected by real time PCR. HTR-8/SVneo cells were assigned into NC group, miR-210 mimics group, and miR-210 inhibitor group, followed by analysis of cell proliferation by MTT assay, Caspase 3 activity, TNF-α and IL-6 secretion by ELISA, Bax/Bcl-2 expression by Real time PCR and PI3K/Akt signaling protein level by western blot.

Results: miR-210 was significantly lower in URSA group than control group (P <0.05). miR-210 mimics significantly up-regulated miR-210, promoted cell proliferation, decreased Caspase 3 activity and Bax level, upregulated Bcl-2, activated PI3K/Akt, and reduced TNF-α and IL-6 secretion (P <0.05). However, miR-210 inhibitor significantly down-regulated miR-210, inhibited cell proliferation, upregulated Caspase 3 activity and Bax, downregulated Bcl-2, inhibited PI3K/Akt, elevated TNF-α and IL-6 secretion (P <0.05).

Conclusions: miR-210 expression is recued in URSA. Targeting miR-210 can affect cell proliferation and apoptosis, and inhibit inflammatory factors secretion.

Keywords: Unexplained recurrent abortion; miR-210; trophoblast; PI3K / Akt signaling pathway.

Introduction

Recurrent spontaneous abortion (RSA) is a common disease in obstetrics, which often occurs in early pregnancy and in the same month of pregnancy [1, 2]. The causes of recurrent miscarriage are complicated, including genetics, endocrine disorders, reproductive system abnormalities, infectious diseases, blood group incompatibility, immune rejection and other factors [3, 4]. However, the etiology of a large proportion of patients with recurrent miscarriage is unknown and it is called as unexplain recurrent spontaneous abortion (URSA) [5]. With the changes in people's living environment, lifestyle and work stress, the incidence of URSA has increased recently, accounting for about 50% of recurrent miscarriages, increasing the difficulty of treatment and attracting the attention of obstetricians and gynecologists [6]. In several studies, the placenta has become a research focus. The formation of a functional placenta network is a key organ for maternal-fetal communication, which maintains nutrient supply and establishes maternal-fetal tolerance [7]. The main cells in the placental tissue are trophoblasts, which are the first to differentiate in the blastocysts. Trophoblasts are important cells for placenta formation, embryonic growth and development and successful completion of pregnancy [8, 9]. Therefore, trophoblast proliferation, differentiation, migration, and apoptosis are essential for the formation and function of the placenta. With the progress of pregnancy and the development of placenta, placental trophoblast proliferation, differentiation and apoptosis are

Running title: miR-210's role in abortion

[a] Department of Obstetrics, Yidu Central Hospital, Weifang Medical College, Weifang, Shandong, 262500, P.R. China
[b] Department of Pharmacy, Yidu Central Hospital, Weifang Medical College, Weifang, Shandong, 262500, P.R. China
[c] Department of Gynecology, Yidu Central Hospital, Weifang Medical College, Weifang, Shandong, 262500, P.R. China
[d] Department of Orthopedics, Yidu Central Hospital, Weifang Medical College, Weifang, Shandong, 262500, P.R. China
[e] Department of Breast surgery, Weifang people's Hospital, Weifang, Shandong, 262500, P.R. China
[*] These authors contributed equally to this work
Correspondence author: Dr. Teng Ma, No.151 Guangwen street, Kuiwen District, Weifang City, China. Tel: +86-0536-8192599; Fax: +86-0536-8192599; E-mail: kmvgv5569@163.com.

abnormal, the inflammatory environment is generated, the trophoblast is prone to cause repeated spontaneous abortion as well as fetal intrauterine growth restriction and other pregnancy diseases [10, 11].

MicroRNAs inhibit gene translation and reduce its stability to regulate gene expression, which in turn affects protein translation [12, 13]. miRNAs are important messengers and vectors for information transfer between cells and for regulating other cell biological functions [14]. miRNAs have been considered as powerful regulators of several cellular processes, such as cell differentiation, and apoptosis [15]. MiR-210 is abnormally expressed in various diseases such as cardiovascular diseases and tumors [16-18]. However, the relationship between miR-210 and URSA has not been reported. Our study intends to assess miR-210's role in URSA.

Materials and methods

Patients

Thirty patients with recurrent miscarriages (URSA group) who were admitted to our hospital's obstetrics and gynecology department and underwent hysterectomy from January 2018 to December 2018 were selected. Selection criteria: symptoms of vaginal bleeding and/or abdominal pain; B-mode ultrasonography without original cardiac tube pulsation; spontaneous abortions at 2 or more times; no abnormalities or diseases in anatomical structure, endocrinology, chromosomes, reproductive tract infection, immunity, etc. No medications previously. In the same period, normal early pregnancy patients requiring termination of pregnancy were selected as the control group. The selection criteria were: no threatened abortion symptoms; B-ultrasound examination showed normal intrauterine pregnancy and normal embryo development; no anatomy, endocrine, chromosomal, reproductive tract infection, immunity, etc. Abnormalities or diseases, no history of miscarriage. Patients in both groups underwent hysterectomy, and villous tissue was removed during the operation. 30 pregnant women in URSA group were singleton pregnancy, aged 22-47 years, mean age (32 ± 3.5) years, and pregnancy weeks 20-22 weeks and mean pregnancy week number (21 ± 1.5) weeks. The control group was consisted of 35 normal pregnant women during the same period, singleton pregnancy, age 20-42 years, mean age (31 ± 5.2) years, gestation weeks 20-26 weeks, and mean pregnancy weeks (22 ± 2.5) weeks. No significant differences were found regarding general clinical data such as age, gestational week, and body weight (P> 0.05) between the two groups. This study was performed by the medical ethics committee of our hospital. All participants signed the informed consent.

Main reagents and instruments

Placental trophoblast-derived cell line HTR-8 / SVneo was purchased from ATCC, USA. Trizol reagent, RNA extraction and reverse transcription (RT) kit, and real-time-PCR reagent were purchased from Invitrogen, USA. DMEM culture solution, fetal bovine serum (FBS), and green chain monoclonal antibody were from Hyclone, USA. Dimethyl sulfoxide, MTT powder was purchased from Gibco; rabbit anti-mouse PI3K / Akt and phosphorylated antibody, and HRP-labeled IgG was from Cell Signaling Company, USA. Caspase 3 active kit was from Shanghai Biyuntian Biotechnology Co., Ltd. miR-210 mimics and miR-210 inhibitor were designed and synthesized by Shanghai Gene Co., Ltd. The CO2 cell incubator was purchased from Thermo Corporation, USA.

Cell culture and grouping

HTR-8/SVneo cell lines were stored in liquid nitrogen, subcultured after resuscitation, and 3-8 passage logarithmic growth phase cells were selected for testing. Cells were cultured in 90% high glucose (25 mmol / L) complete medium with 10% fetal bovine serum (FBS) and separated into control group (NC group) (normal cells culture); miR-210 mimics group and miR-210 inhibitor group.

Cell transfection

The miR-210 mimics sequence was 5'-GUUGCAGAUCUAGGGUCA-3 '; 5'-GGAGUGUACACUUUA-3'. The miR-210 inhibitor sequence was 5'-GGAGUGUACUCUAGGGUCA-3 '; 5'-AGCUGUUGUACUUUA-3'. In a 6-well plate, when cell density reached a confluence of 70-80%, miR-210 mimics or miR-210 inhibitor liposomes were added to 200 μl of serum-free DMEM medium and mixed thoroughly. The mixed lipo2000 was mixed with the corresponding dilutions. Remove the serum from the cells, rinse gently with PBS, add 1.6ml serum-free medium, add each system, culture for 6h, replace the serum DMEM culture medium, and continue to culture for 48h for experimental research.

Real-time PCR

RNA was isolated and DNA reverse transcription synthesis was performed according to the kit instructions. The primers were synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Table 1). Real-time PCR reaction conditions: 55 °C 1 min,

92 °C 30 S, 58-60 °C 45 S, and 72 °C 35 S for 35 cycles. Data were collected using the PCR reactor software and GAPDH was used as a reference. Gene expression was analyzing using 2-ΔCt method.

MTT assay

After 24 hours of culture, 20 µl sterile MTT was added to the wells with 3 replicates at each time point for 4 hours followed by addition of 150 µl / well of DMSO for 10 min until the full dissolvent of purple crystals to detect the absorbance (A) value. Then, the proliferation rate of each group was calculated.

ELISA

TNF-α and IL-6 secretion in the supernatant of each group was assessed by ELISA in accordance with the kit instructions.

Caspase 3 activity test

The changes in Caspase3 activity in each group of cells were examined according to the kit instructions. Trypsin digested cells were centrifuged at 600g at 4 °C for 5min and cell lysate was lysed followed by addition of 2 mM Ac-DEVD-pNA to measure OD value change at 405nm to calculate Caspase3 activity changes.

Western blot

Extract HTR8 cell proteins from each group: add lysate, quantify the protein by Bradford method and store at -20 ℃ for Western blot experiments followed by separation on 10% SDS-PAGE gel separation and transferring protein to NC membrane which was then incubated with primary antibody diluted PI3K / Akt signal pathway monoclonal antibody at 4 ℃ overnight followed by incubation with 1: 2000 diluted sheep anti-rabbit secondary antibody and subsequent washing with PBST and development of color with chemiluminescence agent for 1 min.

Statistical processing

SPSS 16.0 software was utilized for analyzing data which were displayed as mean ± standard deviation (SD) and assessed by one-way ANOVA. P <0.05 indicates a difference.

Results

miR-210 level in the villi of URSA patients

miR-210 in villous tissue of URSA patients was significantly downregulated (P <0.05) (Figure 1).

Regulation of miR-210 expression

Transfection of miR-210 mimics significantly up-regulated its expression (P <0.05) which was significantly downregulated after miR-210 inhibitor transfection (P <0.05) (Figure 2).

miR-210's effect on cell proliferation

miR-210 mimics up-regulated its expression and significantly promoted cell proliferation (P <0.05) which was significantly inhibited by miR-210 inhibitor (P <0.05) (Figure 3).

miR-210's effect on Caspase 3 activity

Transfection of miR-210 mimics can up-regulate its expression and significantly inhibit Caspase 3 activity (P <0.05). miR-210 inhibitor down-regulated its expression and promoted Caspase 3 activity (P <0.05) (Figure 4).

miR-210's effect on cell apoptosis genes expression

Transfection of miR-210 mimics up-regulated its expression, and significantly downregulated Bax and upregulated Bcl-2 (P <0.05). Transfection of miR-210 inhibitor down-regulated its expression, significantly upregulated Bax and downregulated Bcl-2 (P <0.05) (Figure 5).

miR-210's effect on the secretion of inflammatory factors

miR-210 mimics up-regulated its expression and significantly decreased TNF-α and IL-6 (P <0.05). miR-210 inhibitor down-regulated its expression and significantly increased secretion of TNF-α and IL-6 (P <0.05) (Figure 6).

Regulation of miR-210 on PI3K / Akt signaling pathway

Transfection of miR-210 mimics up-regulated its expression and significantly activated PI3K/Akt phosphorylation (P <0.05). The miR-210 inhibitor group down-regulated miR-210 expression and inhibited PI3K/Akt activation (P <0.05) (Figure 7).

Discussion

In recent years, the incidence of recurrent miscarriage caused by various causes has increased year by year. The problem of early pregnancy tissue loss caused by maternal and embryonic causes has gradually been effectively resolved, but the cause of recurrent miscarriage in nearly half of the patients is still unknown. At which time a certain drug should be used to interfere with the occurrence of recurrent abortion or evaluate the therapeutic effect of a certain drug have become important tasks. The mechanism of URSA is complex and is related to trophoblasts proliferation and apoptosis

[19]. Trophoblast proliferation and villous angiogenesis play a vital role in normal pregnancy [20]. Studies have shown that during the early stages of pregnancy, the embryo is in a hypoxic environment, and that excessive apoptosis and insufficient angiogenesis in the villous tissue are closely related to the occurrence of URSA [21].

As highly conserved short RNAs, miRNAs are widely present in organisms and closely related to cell proliferation, apoptosis, tissue and organ development, etc., and involve in the pathophysiology of various diseases [22]. miR-210 is involved in multiple biological processes [23]. The research of cell functions such as invasion, differentiation, proliferation, and programmed apoptosis of trophoblasts is a hot spot in molecular cell research of URSA [24]. This study confirmed that compared to normal placental villous tissue, miR-210 expression was reduced in placental villous tissue in patients with URSA, implying that miR-210 involves in URSA, and may be related to hypoxia in the placenta of early pregnancy. Decreased miR-210 expression leads to insufficient angiogenesis of the placenta and causes nutritional supply failure [25].

Further analysis of in vitro cultured placental trophoblast cells confirmed that miR-210 inhibitor transfection significantly downregulates miR-210, inhibits trophoblast proliferation, promotes apoptosis and the production of inflammatory factors; whereas, miR-210 mimics elevate miR-210 level in trophoblast cells, increase the proliferation of trophoblasts, inhibit apoptosis and inflammation. The results suggest that miR-210 regulates trophoblasts proliferation and apoptosis. The PI3K/Akt signaling participates in the process of trophoblast invasion into the endometrium. It can be regulated by many factors and regulates various cell functions, such as cell growth and apoptosis, proliferation [26, 27]. Our study confirms that miR-210 downregulation in trophoblasts can inhibit the activation of the PI3K/Akt signaling pathway, thus inhibiting trophoblasts proliferation, promoting apoptosis, and inhibiting invasion process [28], which leads to insufficient invasion of trophoblasts into the endometrium, affecting insufficient placental function and causing miscarriage, while promoting the expression of miR-210 can activate PI3K/Akt signaling, promote trophoblasts proliferation and invasion and inhibit apoptosis, leading to amelioration of URSA. However, the exact mechanism by how miR-210 regulates PI3K/AKT signaling in trophoblasts is not investigated in our study, which is the main study limitation. We plan to investigate this in the future.

Acknowledgments

This work was supported by The Research project from Health Commission of Weifang. (NO. wfwsjk_2019_173).

Disclosure of conflict of interest

None.

Conclusion

MiR-210 expression is reduced in relapsed abortion of unknown cause. Targeting miR-210 expression in trophoblasts can affect cell proliferation by PI3K/Akt signaling, inhibit the secretion of inflammatory factors, and thus regulate the occurrence of recurrent abortions of unknown origin, suggesting that miR-210 might be involved in the pathogenesis of URSA and may be a novel therapeutic target for the treatment of URSA.

References

[1] Zhao X, Jiang Y, Ping Y, Guo H, He M and Feng X. Associations between tumor necrosis factor-alpha and interleukin-6 polymorphisms and unexplained recurrent spontaneous abortion risk: A meta-analysis. Medicine (Baltimore). 2019; 98(46): e17919.

[2] Dean DD, Agarwal S and Muthuswamy S. Defining the role of FMR1 gene in unexplained recurrent spontaneous abortion. J Assist Reprod Genet. 2019; 36(11): 2245-2250.

[3] Elias A, Hamoudi R, Schwartz N, Ron G and Elias M. Calibrated Automated Thrombogram During Pregnancy in Unexplained Recurrent Miscarriages: A Pilot Study. Isr Med Assoc J. 2019; 21(10): 681-685.

[4] Ou H and Yu Q. Efficacy of aspirin, prednisone, and multivitamin triple therapy in treating unexplained recurrent spontaneous abortion: A cohort study. Int J Gynaecol Obstet. 2020; 148(1): 21-26.

[5] Zhang Y, Li C, Fu S, Yang C, Song Y, Liu M, Wang Z, Liang P and Zhang J. NOD1 modulates decidual stromal cell function to maintain pregnancy in the early trimester. Cell Biochem Funct. 2019; 37(7): 464-473.

[6] Chen H, Cheng S, Liu C, Fu J and Huang W. Bioinformatics Analysis of Differentially Expressed Genes, Methylated Genes, and miRNAs in Unexplained Recurrent Spontaneous Abortion. J Comput Biol. 2019; 26(12): 1418-1426.

[7] Eskandarian M and Moazzeni SM. Uterine Dendritic Cells Modulation by Mesenchymal Stem Cells Provides A Protective Microenvironment at The Feto-Maternal

Interface: Improved Pregnancy Outcome in Abortion-Prone Mice. Cell J. 2019; 21(3): 274-280.
[8] Yang W, Wu Z, Yu M, Peng X, Lu W, Feng W and Kang X. Characteristics of midluteal phase uterine artery hemodynamics in patients with recurrent pregnancy loss. J Obstet Gynaecol Res. 2019; 45(7): 1230-1235.
[9] Yuan M, Huang L, Leung WT, Wang M, Meng Y, Huang Z, Pan X, Zhou J, Li C, Sima Y, Wang L, Zhang Y and Ying C. Sperm DNA fragmentation valued by SCSA and its correlation with conventional sperm parameters in male partner of recurrent spontaneous abortion couple. Biosci Trends. 2019; 13(2): 152-159.
[10] Ye Y, Kuhn C, Kosters M, Arnold GJ, Ishikawa-Ankerhold H, Schulz C, Rogenhofer N, Thaler CJ, Mahner S, Frohlich T, Jeschke U and von Schonfeldt V. Anti-alpha-enolase antibody is a novel autoimmune biomarker for unexplained recurrent miscarriages. Ebiomedicine. 2019; 41(610-622.
[11] Zhao X, Li Q, Yu F, Lin L, Yin W, Li J and Feng X. Gene polymorphism associated with endothelial nitric oxide synthase (4VNTR, G894T, C786T) and unexplained recurrent spontaneous abortion risk: A meta-analysis. Medicine (Baltimore). 2019; 98(4): e14175.
[12] Bar I, Theate I, Haussy S, Beniuga G, Carrasco J, Canon JL, Delree P and Merhi A. MiR-210 Is Overexpressed in Tumor-infiltrating Plasma Cells in Triple-negative Breast Cancer. Journal of Histochemistry & Cytochemistry. 2019;
[13] Feng H, Wu R, Zhang S, Kong Y, Liu Z, Wu H, Wang H, Su Y, Zhao M and Lu Q. Topical administration of nanocarrier miRNA-210 antisense ameliorates imiquimod-induced psoriasis-like dermatitis in mice. J Dermatol. 2019;
[14] Petrozza V, Costantini M, Tito C, Giammusso LM, Sorrentino V, Cacciotti J, Porta N, Iaiza A, Pastore AL, Di Carlo A, Simone G, Carbone A, Gallucci M and Fazi F. Emerging role of secreted miR-210-3p as potential biomarker for clear cell Renal Cell Carcinoma metastasis. Cancer Biomark. 2019;
[15] Kapral M, Wawszczyk J and Weglarz L. Regulation of MicroRNA-155 and Its Related Genes Expression by Inositol Hexaphosphate in Colon Cancer Cells. Molecules. 2019; 24(22):
[16] Jana A, Narula P, Chugh A and Kulshreshtha R. Efficient delivery of anti-miR-210 using Tachyplesin, a cell penetrating peptide, for glioblastoma treatment. International Journal of Pharmaceutics. 2019; 572(
[17] Ikert H and Craig PM. Chronic exposure to venlafaxine and increased water temperature reversibly alters microRNA in zebrafish gonads (Danio rerio). Comp Biochem Physiol Part D Genomics Proteomics. 2019; 33(100634.
[18] Yang ZY, Zhao N, Cui J, Wu HS, Xiong JX and Peng T. Exosomes derived from cancer stem cells of gemcitabine-resistant pancreatic cancer cells enhance drug resistance by delivering miR-210. Cellular Oncology. 2019;
[19] Qiao XR, Wang L, Liu M, Tian Y and Chen T. MiR-210-3p attenuates lipid accumulation and inflammation in atherosclerosis by repressing IGF2. Biosci Biotechnol Biochem. 2020; 84(2): 321-329.
[20] Logiodice F, Lombardelli L, Kullolli O, Haller H, Maggi E, Rukavina D and Piccinni MP. Decidual Interleukin-22-Producing CD4+T Cells (Th17/Th0/IL-22+and Th17/Th2/IL-22+, Th2/IL-22+, Th0/IL-22+), Which Also Produce IL-4, Are Involved in the Success of Pregnancy. International Journal of Molecular Sciences. 2019; 20(2):
[21] Xu YJ, Ban YJ, Ran LM, Yu YR, Zhai SS, Sun ZZ, Zhang JZ, Zhang M, Hong T, Liu R, Ren LD and Hu LL. Relationship between unexplained recurrent pregnancy loss and 5,10-methylenetetrahydrofolate reductase) polymorphisms. Fertility and Sterility. 2019; 111(3): 597-603.
[22] Li D, Li J, Jia B, Wang Y, Zhang J and Liu G. Genome-wide identification of microRNAs in decidual natural killer cells from patients with unexplained recurrent spontaneous abortion. Am J Reprod Immunol. 2018; 80(5): e13052.
[23] Pasculli B, Barbano R, Rendina M, Fontana A, Copetti M, Mazza T, Valori VM, Morritti M, Maiello E, Graziano P, Murgo R, Fazio VM, Esteller M and Parrella P. Hsa-miR-210-3p expression in breast cancer and its putative association with worse outcome in patients treated with Docetaxel. Scientific Reports. 2019; 9(
[24] Wang H, Zhao Y, Luo R, Bian X, Wang Y, Shao X, Li YX, Liu M and Wang YL. A positive feedback self-regulatory loop between miR-210 and HIF-1alpha mediated by CPEB2 is involved in trophoblast syncytiolization: implication of trophoblast malfunction in preeclampsia. Biol Reprod. 2019;
[25] Bavil FM, Karimi-Sales E, Alihemmati A and Alipour MR. Effect of ghrelin on hypoxia-related cardiac angiogenesis: involvement of miR-210 signalling pathway. Archives of Physiology and Biochemistry. 2019;

[26] Dai Y, Shen JL, Zheng XY, Lin TY and Yu HT. Integrated analysis of hypoxia-induced miR-210 signature as a potential prognostic biomarker of hepatocellular carcinoma: a study based on The Cancer Genome Atlas. Journal of Zhejiang University-Science B. 2019; 20(11): 928-932.
[27] Huang ZY, Du GZ, Huang XM, Han L, Han XM, Xu B, Zhang Y, Yu MM, Qin YF, Xia YK, Wang XR and
[28] Lu CC. The enhancer RNA lnc-SLC4A1-1 epigenetically regulates unexplained recurrent pregnancy loss (URPL) by activating CXCL8 and NF-kB pathway. Ebiomedicine. 2018; 38(162-170.
[29] Yang Y, Cheng L, Deng X, Yu H and Chao L. Expression of GRIM-19 in unexplained recurrent spontaneous abortion and possible pathogenesis. Mol Hum Reprod. 2018; 24(7): 366-374.

Table and Figure legends

Table 1. Primer sequences.

Gene	Forward 5'-3'	Reverse 5'-3'
GAPDH	AGTAGTCACCTGTTGCTGG	TAATACGGAGACCTGTCTGGT
miR-210	ACTTGTTCGACATTCCTGG	TCTAAGTAGCGAGCACCA
Bax	CGTTGGATGTTCCGACCT	AGTTCAGCGATCAGCGCGTA
Bcl-2	ACCATTGATGTGTTTGCAG	ATTCCGACCGCGTCGACAGC

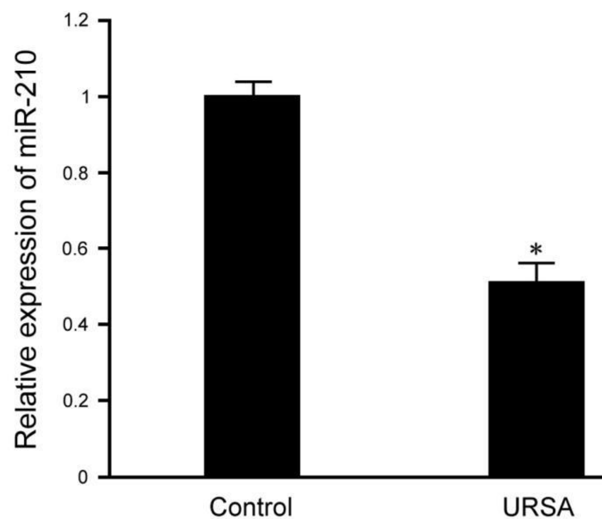

Figure 1. **MiR-210 expression in the villi of URSA patients. Compared with the control group, * P <0.05.**

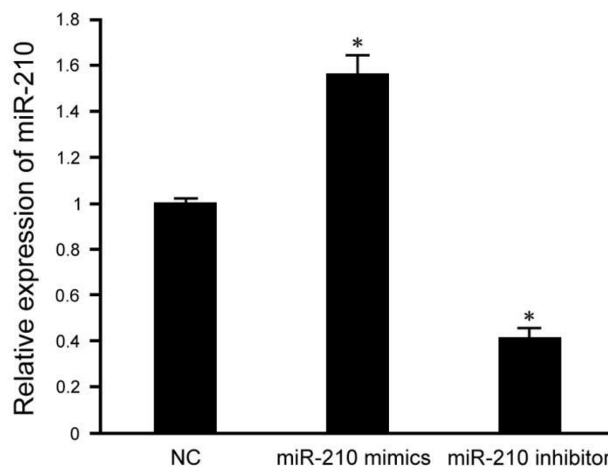

Figure 2. **Regulation of miR-210 expression in HTR-8 / SVneo cells. Compared with NC group, * P <0.05.**

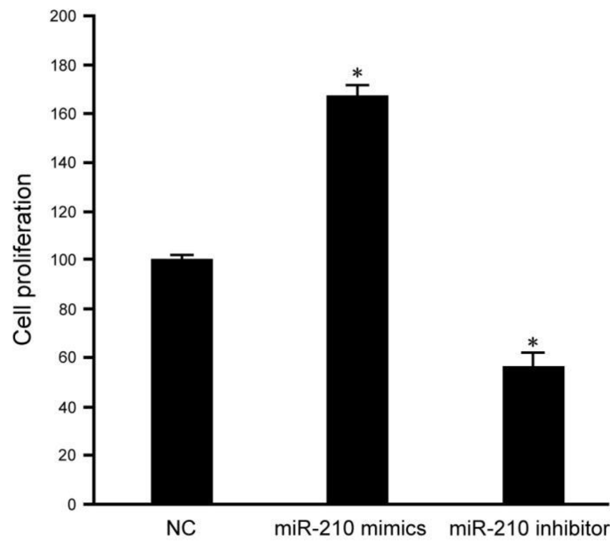

Figure 3. **Effect of regulating miR-210 on the proliferation of trophoblast cells HTR-8 / SVneo cells. Compared with NC group, * P <0.05.**

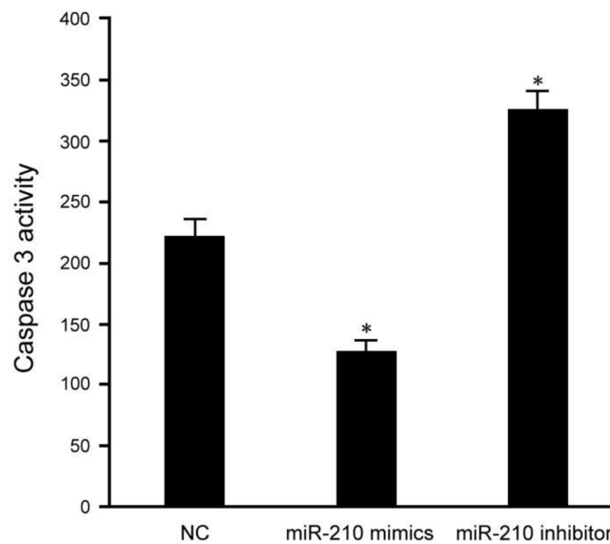

Figure 4. **Effect of regulation of miR-210 on the activity of Caspase 3 in trophoblast cells HTR-8 / SVneo cells. Compared with NC group, * P <0.05.**

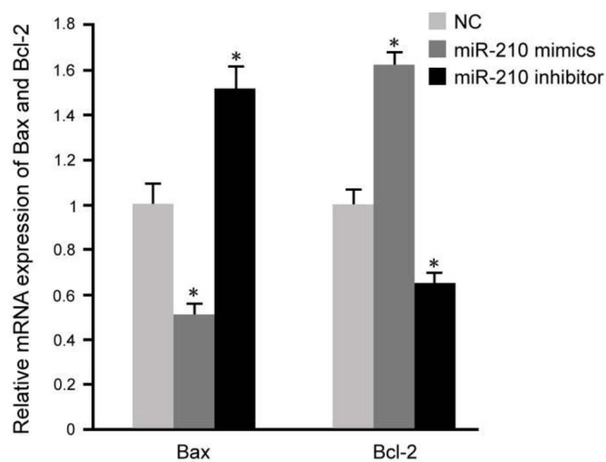

Figure 5. **Effect of regulating miR-210 on apoptotic genes of trophoblast cells HTR-8 / SVneo cells. Compared with NC group, * P <0.05.**

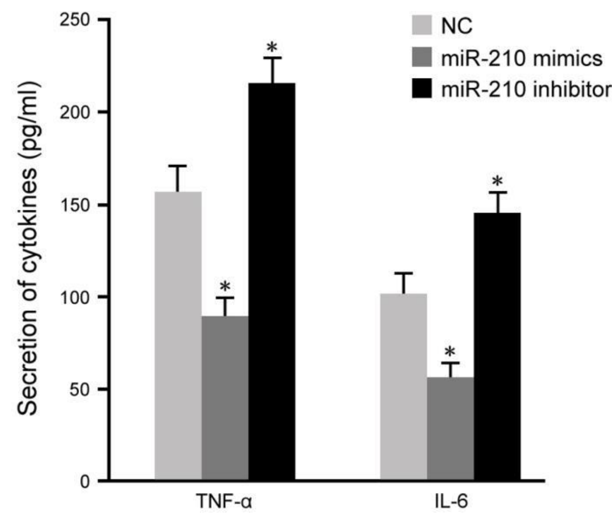

Figure 6. **Effect of regulating miR-210 on the secretion of inflammatory factors in trophoblast cells HTR-8 / SVneo cells. Compared with NC group, * P <0.05.**

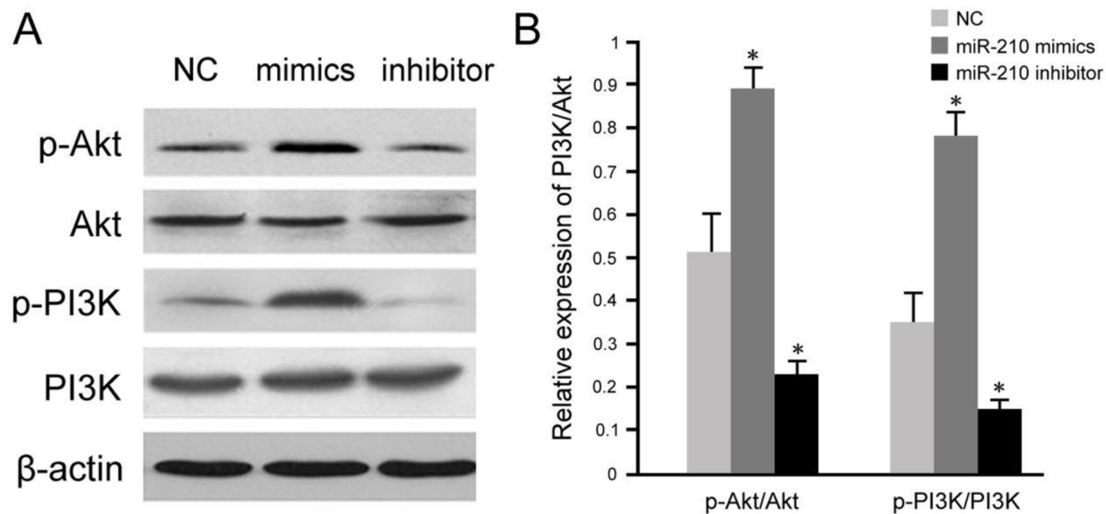

Figure 7. **Effect of regulating miR-210 on the PI3K / Akt signaling pathway in trophoblast cells HTR-8 / SVneo cells. A Western blot analysis of the effects of miR-210 on the PI3K / Akt signaling pathway of trophoblast HTR-8 / SVneo cells; B regulation of the effect of miR-210 on the PI3K / Akt signaling pathway of trophoblast HTR-8 / SVneo cells Statistical analysis, compared with NC group, * P <0.05.**