

Association of β -arrestin1 and p53-MDM2 signaling in the development of missed abortion

Ting Liu

Shandong University Qilu Hospital <https://orcid.org/0000-0003-4157-4812>

Yuyan Ma

Shandong University Qilu Hospital

Qihui Yin

Shandong University Qilu Hospital

Huanyu Zhou

Shandong University Qilu Hospital

Yan Fang (✉ fangyan7801@163.com)

Qilu Hospital of Shandong University

Research

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Abstract

Background

Missed abortion is a nonviable pregnancy before the 20th week of gestation with retained products of conception. The definite etiology and pathogenesis are not fully understood. β -arrestin1, the important dynamic multitask scaffold protein, it play an important regulatory role in interacting with G protein-coupled receptor (GPCR) to mediate its homologous desensitization and internalization. Recent studies have demonstrated that p53/Mdm2-mediated ubiquitination of the IGF-1R maybe closely related to G protein-coupled receptor kinases (GRK)/ β -arrestin1 system. Our previous studies have confirmed that the elevated expression of p53 and Mdm2 may be responsible for apoptosis during missed abortion. However, there was no information surrounding β -arrestin1 in missed abortion.

Methods

The mRNA levels of β -arrestin1 in villous samples of 31 missed abortion patients and 31 healthy controls were determined by real-time quantitative PCR. Immunohistochemistry was used to explore the expression and location of β -arrestin1, p53, Mdm2, VEGF, HIF-1 α in trophoblasts. We up-regulated and silenced the expression of β -arrestin1 by lentiviral transfection, transwell assays were performed to examine the influences of β -arrestin1 expression on cell invasion. Furthermore, we explored the expression of several important proteins which may be related to β -arrestin1.

Results

β -arrestin1 mRNA levels in the villous samples from women with missed abortion were found to be dramatically lower than in women who had normal pregnancies. The immunohistochemistry results showed that β -arrestin1 positive staining was significantly lower in missed abortion group than that in normal pregnancies group. Furthermore, the patients with missed abortion showed significantly higher levels of p53, Mdm2 and HIF-1 α , lower level of VEGF than healthy controls by immunohistochemistry. The protein expressions of p-ERK, p-AKT, p-p53 in HRT-8 cells were significantly downregulated by reducing β -arrestin1 expression, while the expression of p-NF- κ B, p-Mdm2 were enhanced. Overexpression of β -arrestin1 exhibited the adverse effect. The loss expression of β -arrestin1 expression was significantly related with decreased cell invasion ability.

Conclusion

Our data indicated that β -arrestin1 could play an important role in maintaining the maternal-fetal tolerance, the decreased β -arrestin1 expression in the villous samples may be related to the development of missed abortion.

Introduction

Missed abortion is a peculiar form of spontaneous abortion before 20 weeks' gestation, which is characterized by the unrecognized intrauterine death of the embryo or fetus without outside intervention[1]. At present, the prevalence of missed abortion appears to be a dramatically increasing trend, accounting for about 15% of clinically diagnosed pregnancies[2]. Plenty of etiologic factors, such as chromosomal and uterine abnormalities, immunological and endocrinological disorders, infections, hereditary thrombophilia and environmental factors, psychogenetic factors, have been demonstrated to be associated with the occurrence of missed abortion[3,4]. Although the definite etiology and pathogenesis are not fully understood, many studies have shown that cell apoptosis plays a key role in missed abortion. In normal pregnancy,

trophoblast cell apoptosis was observed to be an appropriate extent which facilitates the formation and development of villi and chorionic villi branches. However, excessive apoptosis of trophoblasts may cause villi dysplasia or cytotrophoblast cell degeneration, and the number of syncytiotrophoblasts was significantly decreased, eventually leading to the failure of pregnancy[5]. Identifying and characterizing key molecular markers involved in missed abortion are important for developing new therapeutic treatments.

β -arrestin1, the important dynamic multitask scaffold protein, plays an important regulatory role in interacting with G protein-coupled receptor (GPCR) to mediate its homologous desensitization and internalization[6]. The canonical function of β -arrestin1 involves binding to phosphorylated activated receptors which terminate G protein activation by preventing its access to the receptor cytoplasmic surface. β -arrestin1 also binds to multiple non-receptor partners, thereby regulating a variety of cell signaling pathways. It has been reported that β -arrestin1 mediates cell development and differentiation through signal transduction mechanisms and regulates the p53/Mdm2, MAPK, NF- κ B and PI3K/Akt signaling pathways[7,8,9]. Recent studies demonstrated that p53/Mdm2-mediated ubiquitination of the IGF-1R may be closely related with G protein-coupled receptor kinases (GRK)/ β -arrestin1 system. Our previous studies have confirmed that the elevated expression of p53 and Mdm2 may be responsible in the development of missed abortion[10,11,12]. However, there was no information surrounding the functional role of β -arrestin1 in missed abortion. Based on our former study, we speculated that β -arrestin1 may be an indispensable molecular factor participating in the process of missed abortion.

In the present study, we aimed to explore the possible modulatory effects of β -arrestin1 on missed abortion. We determined the expression status of β -arrestin1 in villous samples of missed abortion patients and normal controls at both mRNA and protein levels. Moreover, we detected the association between β -arrestin1 expression and several important molecules involved in apoptosis, angiogenesis and hypoxia. In addition, we performed a series of functional experiments and investigated the underlying molecular mechanism when β -arrestin1 was silenced or over-expressed in HTR-8 cells. The results demonstrated that β -arrestin1 expression was downregulated in missed abortion patients, altered expression of β -arrestin1 may be involved in some important signaling pathways to participate in the development of pregnancy loss in missed abortion.

Material And Method

Human subjects

The present study was approved by the Ethical Committee of Qilu Hospital, Shandong University, and there was no conflict of interest. This study involved 31 patients with first-trimester missed abortion and 31 healthy controls with normal induced abortion from January to December, 2019. Patients with chromosome abnormalities, autoimmune disorders, hormone treatment, clinical genital infections, recurrent pregnancy loss or any other systemic disease were excluded. Through ultrasound examination, abortion in the first trimester of pregnancy was defined as an intact gestational sac that lacks any fetal cardiac activity (6 weeks after the last menstrual period) or the maximum diameter of an intrauterine pregnancy exceeds 10 mm but there was no yolk sac, and empty fetal sac with confirmed gestational age not less than 6 weeks [13]. The 31 missed abortion patients included in this experiment confirmed 7–11 weeks' gestational age without successful pregnancy. The control group consisted of 31 women with unwanted pregnancies with healthy, viable intrauterine fetuses, they were also at 7–11 weeks' gestational age. All villous samples were washed with 0.9% NaCl immediately after collected by curettage or manual vacuum aspiration. Villous sample was frozen in a -80°C refrigerator for RNA extraction

Cell line and cell transfection

HTR-8 and Bewo cell lines were purchased from ATCC (via LGC Standards, Middlesex, UK) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA) in an atmosphere of 5% CO_2 at 37°C . To further explore the roles of β -arrestin1 in missed abortion, we transduced HTR-8 cells with the β -arrestin1 cDNA plasmid and small hairpin RNA (shRNA)

to change the original expression. All plasmids were designed by China National Pharmaceutical Group Co., Ltd. The lentiviral vectors GV492 and plko.1 were used to

improve the transduction efficiency. Followed by 5-7 days of selective culture with puromycin dihydrochloride ($2\ \mu\text{g}/\text{ml}$; Amresco, Solon, OH, USA), the stably silenced or over-expressing cell lines were established to perform the subsequent experiments.

Quantitative reverse-transcription polymerase chain reaction

Total RNA of HTR-8 cells or villous samples was extracted and cDNA was synthesized using Trizol reagent (Invitrogen). The concentration and purity of the isolated DNA was detected by NanoDrop ND-1000 spectrophotometer (Fisher Scientific, Madrid, Spain). Then, qPCR was performed using SYBR Green PCR Master Mix and the StepOne PlusTM Real-Time PCR System following the manufacturer's instructions. The relative expression of β -arrestin1 was determined with GAPDH as endogenous control. The primers used in this study were as follows: The primers used in this study are as follows: β -arrestin1-F: CGACAAAGGGACCCGAGTG; β -arrestin1-R: GCAGGTCAGCGTCACATAGA. GAPDH-F: AGAAGGCTGGGGCTCATTTG; GAPDH-R: AGGGGCCATCCACAGTCTTC.

Western Blot

Cells were lysed and the protein sample concentration was measured by a BCA protein assay kit (Thermo Scientific, MA, USA). Total proteins were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk and then incubated with primary antibodies specific to p53 (Abcam; diluted 1:1,000), Mdm2 (Abcam; diluted 1:500), β -arrestin1 (Abcam; diluted 500), p-ERK (Abcam; diluted 1:500), p-AKT (Abcam; diluted 1:1000) and P-NF-KB (Abcam; diluted 1:1,000). Followed by washing with TBST 4 times for 5 min, the membranes were then probed with the appropriate horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Then the immunoreactive protein bands were visualized with an ECL reagent (Wanleibio Co., Ltd.) by the ImageQuant LAS 4000 system (GE Healthcare Life Sciences, Logan, UT, USA). GAPDH served for the internal control.

Immunohistochemistry

30 paraffin-embedded missed abortion tissue samples and 31 paraffin-embedded normal pregnancies tissue samples were collected from the Qilu Hospital of Shandong University between April 2018 to May 2020. Immunohistochemical staining was performed on the villous tissue sections fixed in formalin and paraffin embedded. The sections were stored at 60°C overnight and were then deparaffinized and rehydrated through graded ethanol. After antigen microwave retrieval and endogenous peroxide quenching by 3% hydrogen peroxide, the sections were blocked by 10% goat serum for 15 min at 37 °C to suppress nonspecific background staining. Then, the sections were incubated with primary antibody against β -arrestin1 (1:100 dilution, Abcam, Cambridge, UK), p53 (1:100 dilution, Abcam, Cambridge, UK), Mdm2 (1:100 dilution, Abcam, Cambridge, UK), VEGF (1:50 dilution, Abcam, Cambridge, UK), HIF-1 α (1:100 dilution, Abcam, Cambridge, UK) overnight at 4 °C in a wet chamber. On the second day, the sections were incubated with biotinylated secondary antibody and labeled with diaminobenzamide (Gene Tech, Shanghai, China) to detect the immunoreactivity. PBS was used as negative controls. Brown-yellow granulation of cytoplasmic (β -arrestin1, VEGF, HIF-1 α) and nuclear (p53, Mdm2) staining was considered indicative of positive results. The immunohistochemical results were semi-quantitatively scored in accordance with the staining intensity and positive expression area by two independent pathologists who were blind to the patients' information based on previous study[14,15,16].

Cell invasion assay

The cell invasion experiment was performed using Corning Matrigel Invasion Chamber in 24-well plate (8- μ m pore size; Corning Life Sciences, Lowell, MA, USA). A total of 2.5×10^4 cells in 500 μ l of serum free medium were added to the top chamber. Growth medium (750 μ l) with 20% FBS was added into the lower chamber. After incubated for 48 hours at 37°C in a humidified incubator containing 5% CO₂, parts of cells were invaded to the lower surface of the upper chamber. Fixed the cells with 4% paraformaldehyde and stained with crystal violet. The cells on the upper surface of the upper chamber were gently removed by a cotton swab. Images of the stained cells were then captured under a microscope at $\times 100$ magnification and cells from at least five randomly selected fields were counted for each experiment.

Statistical analysis

SPSS (SPSS Inc, Chicago, IL, USA) 22.0 software was used to perform statistical analysis. Differences between groups were analyzed by the Student's t-test and one-way ANOVA based on three individual experiments. $P \leq 0.05$ was considered statistically significant.

Result

β -arrestin1 mRNA levels in villous samples of missed abortion and normal pregnancies

To explore the functional roles of β -arrestin1 in missed abortion, qRT-PCR analysis was performed to detect the mRNA expression levels of β -arrestin1 in the villous samples of missed abortion patients and healthy controls. The result showed that the expression of β -arrestin1 mRNA in villous samples was significantly decreased in patients with missed abortion compared with normal pregnancies ($P < 0.01$, Fig. 1A).

The Expression of β -arrestin1 in the villous samples of missed abortion patients and normal controls detected by immunohistochemistry

To further explore the expression sites and levels of β -arrestin1 proteins, we detected the β -arrestin1 expression in the 30 villous samples of missed abortion patients and 31 healthy controls by immunohistochemistry. The results showed that β -arrestin1 protein was expressed in both decidua and chorion of missed abortion and control groups, β -arrestin1 was primarily expressed in the cytoplasm of cytotrophoblasts and syncytiotrophoblasts, villous stromal cells (Fig. 1C). Results according to statistic analysis, there was significant differences between the two groups, β -arrestin1 positive staining was significantly lower in missed abortion group than that in normal pregnancies group ($P < 0.01$, Table 1).

p53, Mdm2, VEGF and HIF-1 α protein levels in villous samples of missed abortion and normal pregnancies

The immunoreactivity of p53, Mdm2, VEGF and HIF-1 α were also evaluated in both missed abortion group and normal pregnancy group. The p53 and Mdm2 proteins were mainly expressed in the nucleus of the villous trophoblast cells, syncytiotrophoblasts and extravillous cytotrophoblast cells. HIF-1 α protein was mainly expressed in the cytoplasm of the villous trophoblast cells, syncytiotrophoblasts, and extravillous cytotrophoblast cell columns, occasionally it can also be expressed in the nucleus of the villus syncytial trophoblast cells and extravillous cytotrophoblast cell columns. VEGF protein is mainly expressed in the cytoplasm of villus trophoblasts, syncytiotrophoblasts, extravillous cytotrophoblast column and interstitial cells of villi. The missed-abortion samples had significantly more p53, Mdm2 and HIF-1 α positive cells in the syncytiotrophoblast and extravillous trophoblastic cell columns, compared with normal pregnancies group ($P < 0.01$, $P < 0.01$, $P = 0.035$). The expression of VEGF in missed abortion was considerably lower than that of control group ($P = 0.026$, Fig. 2).

β-arrestin1 overexpression increases cell migration and invasion in HTR-8 cells

First of all, we examined β-arrestin1 expression in HTR-8, Bewo cells at protein expression levels respectively (Fig. 1B). And we found that β-arrestin1 was expressed at a relative lower level in HTR-8 cells. Therefore we established stable β-arrestin1 knockdown and overexpression cells using lentiviral-mediated gene delivery in HTR-8 cells. HTR-8 cells were transduced with the β-arrestin1 sh-RNA plasmid and β-arrestin1 cDNA plasmid. Compared with their vector and control groups, the efficiency of silencing or over-expression in these cell lines was at least 50% at the mRNA level (Fig. 3A). We carried out transwell assays to explore the effects of β-arrestin1 on HTR-8 cells mobility. Results revealed that overexpression of β-arrestin1 elevated the invasion potential of HTR-8 cells by 59% (P = 0.0338), and the invasion abilities of HTR-8 cells after siRNA β-arrestin1 treatment were remarkably reduced by 51% (P = 0.037, Fig. 3B).

The effect of β-arrestin1 on the expression of p-ERK, p-AKT, p-NF-κB, p-p53, p-Mdm2

To uncover the possible mechanism of β-arrestin1 in missed abortion, we tested the expression levels of several key molecules in p53 signaling pathway and some apoptosis-related proteins by western blot. The p-ERK, p-AKT and p-p53 were downregulated in HTR-8 cells when β-arrestin1 was silenced. Moreover, p-NF-κB and p-Mdm2 were upregulated in these cells compared with the control cells. Whereas, overexpression of β-arrestin1 enhanced the expression levels of p-ERK, p-AKT and p-p53, while p-NF-κB, p-Mdm2 were downregulated. Our results suggested that β-arrestin1 overexpression may have some relationship with p-ERK, p-AKT, p-p53, p-NF-κB and p-Mdm2 in vitro (Fig. 4).

Discussion

Missed abortion is considered to be the most common complications of pregnancy. From the immunological point, pregnancy is somewhat analogous to organ transplantation. The fetus possesses the antigens of both maternal and paternal, it is semi-allogeneic. A normal immune system of pregnant woman can not only protect mother and fetus from foreign pathogens, but also tolerate semi-allogeneous[17]. An appropriate immune status, embryo quality and the physiological status of decidua are indispensable for a successful pregnancy. According to the previous study, normal pregnancy has a certain degree of trophoblast cell apoptosis, which is conducive to the formation and development of villi and chorionic villi branches[18]. However, excessive apoptosis of trophoblasts may lead to abnormal villi development or cytotrophoblast degeneration, and even lead to pregnancy failure[19, 20, 21]. In addition, a study by Chen reported that adequate angiogenesis in the villi plays an important role in maintaining early pregnancy[22]. Therefore, apoptosis and angiogenesis may be closely related to the occurrence of missed abortion.

Arrestins were originally discovered to be the main protein component of the retinal photoreceptor region, and also called "retinal S antigen" because of their involvement in uveitis. There are four members of the arrestin family: β-arrestin1, β-arrestin2, x-arrestin, and s-arrestin[23]. The classical functions of β-arrestin1 and β-arrestin2 were initially identified to mediate desensitization, sequestration, and recycling of G protein-coupled receptors (GPCRs), whereas s-arrestin and x-arrestin can only be found in the vision

system[24]. Further investigations established that β -arrestin system is a major hub that controls almost the entire GPCR signal network. There is increasing evidence suggests that β -arrestin1 acts as modulator in a number of intracellular signaling pathways, such as extracellular regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK3), apoptosis-signal-regulating kinase 1(ASK1), insulin-like growth factor I (IGF-1), phosphoinositide 3-kinase (PI3K-Akt), and wingless-type MMTV integration site family members 5A and 3A (Wnt5a and Wnt3A)[25–29], which play an important role in the regulation of various cellular functions in both normal and malignant cells.

In addition, recent studies have shown that the downstream of IGF-1R has an attractive β -arrestin1 antagonistic effect on p53 activation. β -arrestin1 moves to the nucleus and acts as an adaptor protein to promote the binding and degradation of p53 through E3-ubiquitin ligase Mdm2, thereby accumulating DNA damage[30]. However, when Mdm2 recognizes the target p53 and binds to the HIF-1 α subunit, it mediates the degradation of HIF-1 α , reduces the expression of VEGF, inhibits angiogenesis, stops the cell cycle and increases apoptosis and eventually leads to missed abortion[31]. Our previous studies have confirmed that p53 and Mdm2 genes play a vital role in the development of villus tissue during early pregnancy, and ultimately affect the outcome of pregnancy[32]. However, there was no study have been made regarding the role of β -arrestin1 in early pregnancy. Based on the former studies, we speculated that β -arrestin1 may be closely related with missed abortion.

In the present study, we collected the villous samples of missed abortion and healthy controls. At first, we detected the expression of β -arrestin1 by qRT-PCR. We found that the expression of β -arrestin1 was significantly downregulated in missed abortion group. To further investigate the expression sites and protein levels of β -arrestin1, we measured the expression of β -arrestin1 of missed abortion and induced abortion groups by immunohistochemistry. We found that β -arrestin1 positive staining mainly expressed in the nuclei of villous cytotrophoblast, syncytiotrophoblast and extravillous trophoblastic cell column. Consistent with the qRT-PCR results, the expression of β -arrestin1 in missed abortion group was significantly lower than normal controls. The results evidently proved the correlation between the low expression of β -arrestin1 and undesirable pregnancy outcome.

We also detected the expressions of p53, Mdm2 immunoreactivity in both the missed abortion groups and normal pregnancy, and we found that there was significant differences between the two groups. P53 is a vital apoptosis-regulating gene which is precisely controlled to keep the balance between cell death and proliferation. Mdm2 is the main negative regulator of p53, it ubiquitinates p53 and promotes its proteasome degradation. In turn, p53 stimulates Mdm2 transcription[33]. Therefore, Mdm2 and p53 form a regulatory feedback loop which strongly affected by cellular stress. Our former studies have confirmed that Mdm2 and p53 may be a genetic risk factor for missed abortion[11]. This fact strongly supports the critical role of p53 and Mdm2 in the regulation of embryogenesis. In the present study, the expressions of p53 and Mdm2 protein in villous samples of missed-abortion patients are significantly higher than in those from normal pregnancies. The result suggests that the lack of β -arrestin1 in villous samples may lead to increased apoptosis, and then subsequently activates P53-mediated apoptosis.

To further understand the relationship between β -arrestin1 and p53, Mdm2 in missed abortion, we silenced and up-regulated the expression of β -arrestin1 by lentiviral transfection. Through cell experiments, we found that knockdown of β -arrestin1 by siRNA transfection significantly suppressed p53 expression, stimulated Mdm2 activation. Furthermore, overexpression of β -arrestin1 exhibited the adverse effect. These data suggested that β -arrestin1 was associated with cell apoptosis may be dependent on p53/Mdm2 interaction.

In the early stage of embryonic development, angiogenesis is the most basic condition for embryo implantation and endometrial development. Angiogenesis requires the interaction of a variety of factors, including various hormones, growth factors, and soluble extracellular matrix molecules. VEGF is the most important regulator of angiogenesis[34]. The formation of placental trophoblast vessels is strictly regulated by oxygen concentration [35]. HIF-1 α can regulate local oxygen pressure. HIF-1 α -mediated transcriptional activation contributes to the increase of VEGF gene expression during hypoxia, but high levels of HIF-1 α may inhibit the secretion of VEGF[36]. Excessive and prolonged hypoxia can induce apoptosis, the mechanism of HIF-1 α inducing apoptosis is that p53 protein can bind to HIF-1 α through oxygen degradation domain (ODD) during hypoxia, enhance the stability of p53. To study the correlation between VEGF and HIF-1 α expression and missed abortion, we further detected the expression of VEGF and HIF-1 α by immunohistochemistry. We found that HIF-1 α expression in the missed abortion villous samples to be significantly higher than in control samples, but there was significantly lower VEGF in the samples from missed abortions. In this way, these findings corroborate those of previous reports showing that β -arrestin1 deficiency may form a negative effect in neovascularization.

Previous studies have revealed that β -arrestin1 binds to mGluR1 to activate ERK and mediate the effect of TNFR1 on the NF-KB activity, and β -arrestin1 interacts with GSK3 β to regulate the activity of p-Akt [37]. We therefore assessed ERK, Akt and NF-K expression and phosphorylation status in cells at differential expression level of β -arrestin1 in the HTR-8 cells. We found that down-regulation of β -arrestin1 cause high levels of p-NF-KB expression and a remarkable decrease in the level of p-ERK/p-AKT. Overexpression of β -arrestin1 showed the adverse effect. The results demonstrated that β -arrestin1 overexpression stimulates ERK, AKT signaling, but has a suppression effect on NF-KB signaling pathway.

In the first trimester of pregnancy, the placenta-derived villous cytotrophoblast cells proliferate or differentiate through two different ways to form the external layer of the multinucleated syncytiotrophoblast or the extremely invasive extravillous trophoblasts (EVTs)[38, 39]. Acquiring invasiveness is essential for EVT to constitute a connection between the uterine wall and placenta, which ensure enough blood flow to the growing fetus. Insufficient EVT invasion of the spiral artery wall was identified to be closely related with many early pregnancy complications, such as missed abortion, intrauterine growth restriction or pre-eclampsia [40]. However, the molecular mechanisms of EVT differentiation and invasion are still poorly understood. Recently, β -arrestin1 has been reported to act as regulators of EMT and be involved in cell invasion, migration and tumor metastasis. Zeng et al found that β -arrestin1 facilitate the cell migration and invasion of prostate cancer cells by interacting with β -catenin [37]. In this study, we explored the role of β -arrestin1 in HTR-8 cell invasion, the present data

indicated that overexpression of β -arrestin1 promote cell invasion, silencing of β -arrestin1 significantly inhibited the invasive ability. The results suggested that β -arrestin1 may serve a promotive role in cell invasion.

In conclusion, for the first time, we confirmed that there may be a significantly association between missed abortion and reduced expression of β -arrestin1. The β -arrestin1 depletion leads to increased apoptosis and decreased invasion ability. Furthermore, β -arrestin1 was found to be involved in the regulation of the Akt, NF- κ B, ERK pathway. Exploring the exact mechanism will hopefully identify more new and effective strategies to diagnose and treat the patients with missed abortion.

Abbreviations

IHC: Immunohistochemistry; qRT-PCR: Quantitative real-time PCR; EVT: extremely invasive extravillous trophoblasts

Declarations

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Availability of supporting data

Please contact with author for data requests.

Authors' contributions

Ting Liu: conceptualization and validation

Yuyan Ma: methodology and analysis

Qihui Yin: writing—original draft preparation

Huanyu Zhou: design and interpretation

Yan Fang: project administration and funding acquisition

Ethics approval and consent to participate

The present study was approved by the Ethical Committee of Qilu Hospital, Shandong University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

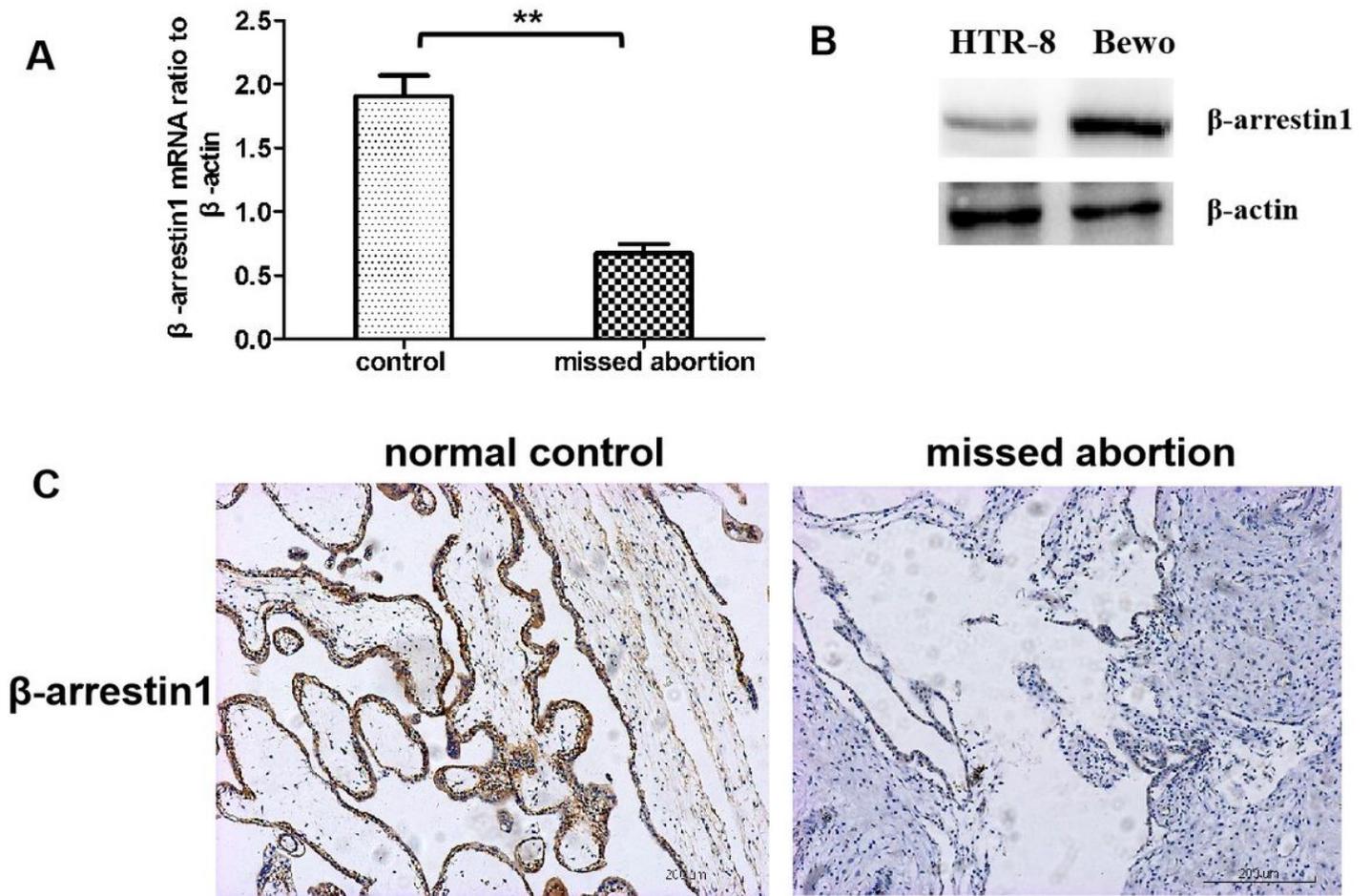


Figure 1

β -arrestin1 protein and mRNA levels in villous samples. (A) PCR analysis was performed to assess the relative mRNA levels of β -arrestin1 in missed-abortion and control villous samples; $**P < .01$. β -actin served as a loading control. (B) The expression of β -arrestin1 in HTR-8 and Bewo cells. (C) The expression of β -arrestin1 protein in the villous samples of 30 missed abortion patients and 31 healthy controls detected by immunohistochemical analysis.

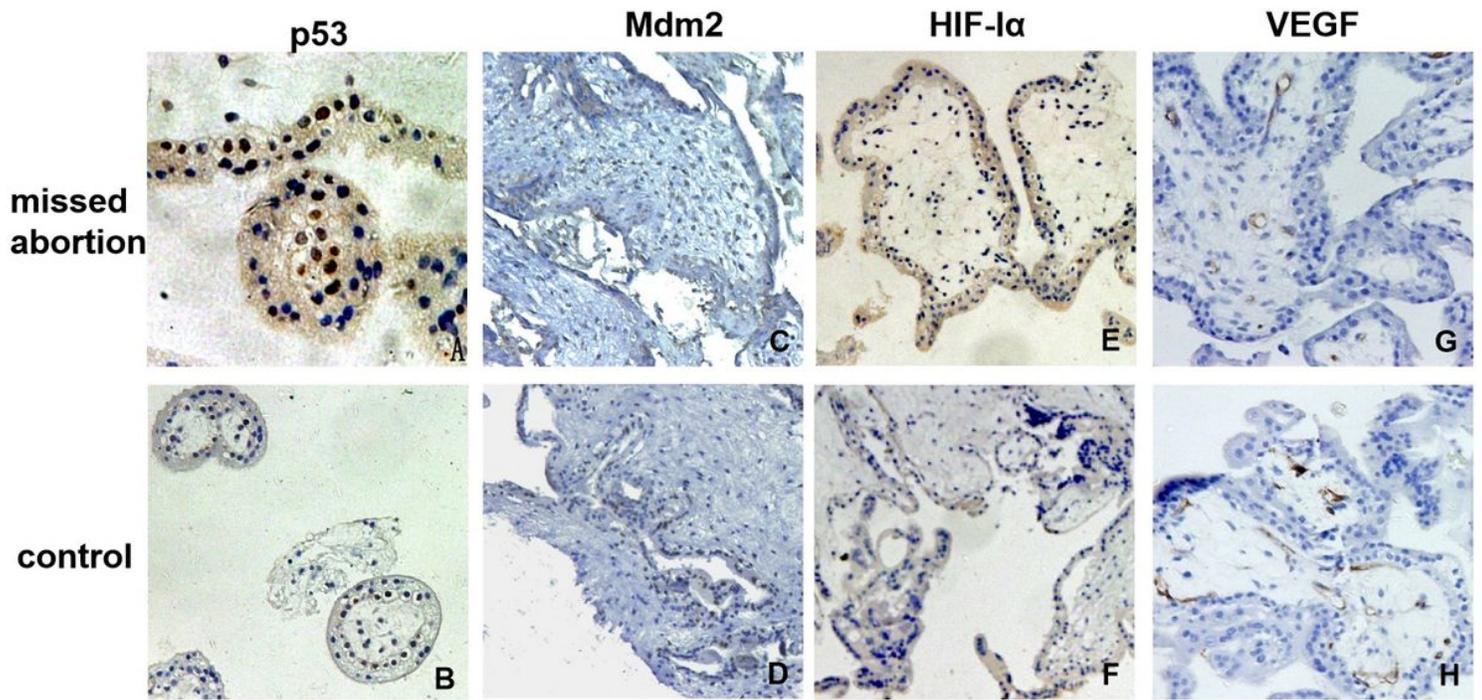


Figure 2

Immunohistochemical analysis was performed to determine the locations of p53, Mdm2, HIF-1 α , and VEGF in the trophoblasts. The missed-abortion samples had significantly more p53, Mdm2 and HIF-1 α positive cells in the syncytiotrophoblast and extravillous trophoblastic cell columns, compared with normal pregnancies group. The expression of VEGF in missed abortion was considerably lower than that of control group. (A, B, G, H original magnification, $\times 200$. C, D, E, F magnification, $\times 400$).

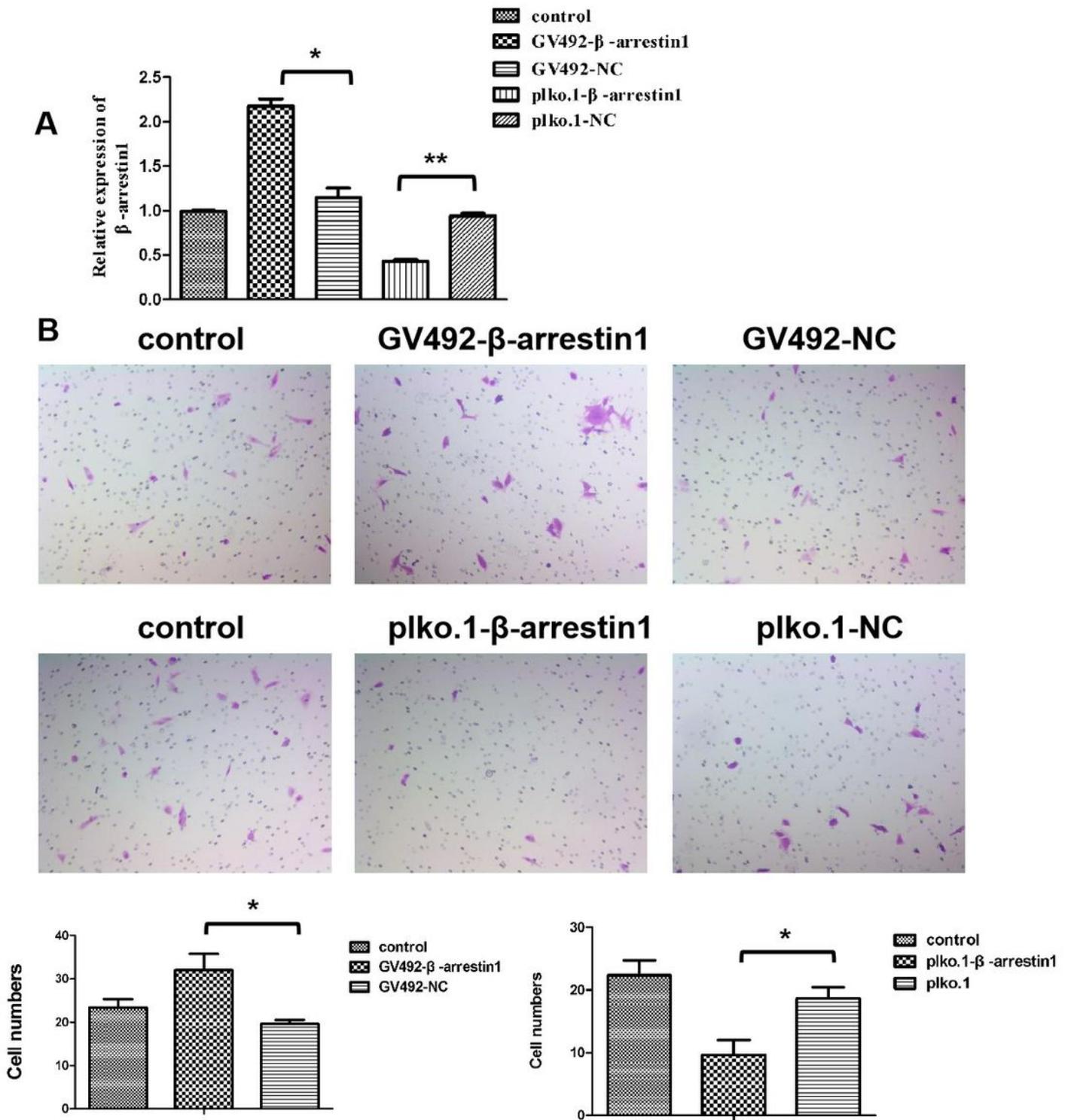


Figure 3

The effect of β -arrestin1 expression on cell migration and invasion of HTR-8 cells A. The efficiency of silencing or over-expression in these cell lines was determined at the mRNA level by PCR. B. Overexpression of β -arrestin1 elevated the invasion potential of HTR-8 cells, while the invasion abilities of HTR-8 cells after siRNA β -arrestin1 treatment were remarkably reduced.

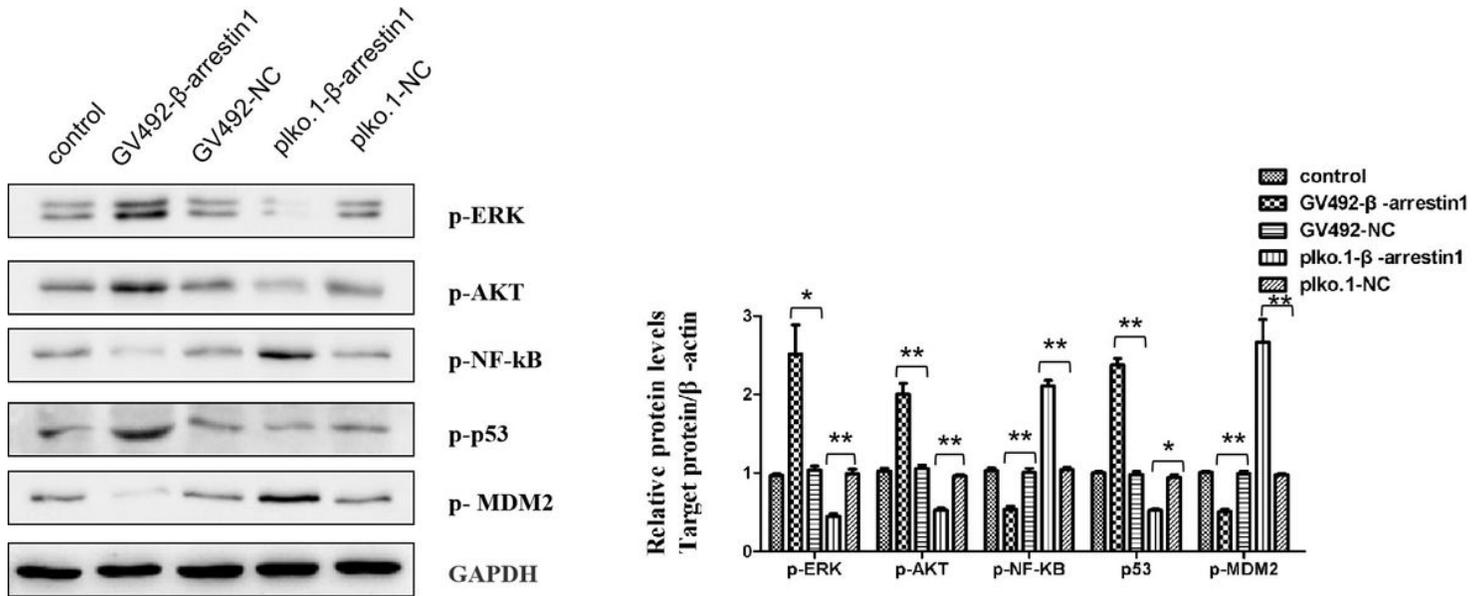


Figure 4

The effect of β -arrestin1 on the expression of p-ERK, p-AKT, p-NF-KB, p-p53, p-Mdm2. The p-ERK, p-AKT and p-p53 were downregulated in HRT-8 cells when β -arrestin1 was silenced. Moreover, p-NF-KB and p-Mdm2 were upregulated in these cells compared with the control cells. Whereas, overexpression of β -arrestin1 enhanced the expression level of p-ERK, p-AKT and p-p53, while p-NF-KB, p-Mdm2 was downregulated.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)