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Down-regulated expressed protein HMGB3 inhibits proliferation and migration, promotes apoptosis in the placentas of fetal growth restriction

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ABSTRACT

Fetal growth restriction (FGR) is one of the major complications of pregnancy, which can lead to serious short-term and long-term diseases. High-mobility group box 3 (HMGB3) has been found to contribute to the development of many cancers. However, the role of HMGB3 in the pathogenesis of FGR is blank. Here, we measured the expression level of HMGB3 in the placenta tissues of six normal pregnancies and five FGR patients by western blotting and quantitative real-time polymerase chain reaction (qRT-PCR). CCK8 assay, transwell assay and flow cytometry were used to detect the functional effects of overexpression and silencing of HMGB3 on the HTR8/SVneo trophoblast cell line. The results showed that the protein levels of HMGB3 were significantly decreased in FGR placentas compared to normal controls, while mRNA levels of HMGB3 were not significantly altered. Furthermore, when overexpressed of protein HMGB3 of the trophoblast cells, the proliferation and migration abilities were significantly promoted, and the apoptosis abilities of these cells were statistically inhibited. Cell functional experiments showed the opposite results when the expression of HMGB3 was silent. And the expression of cell function-related genes PCNA, Ki67, Tp53, Bax, MMP-2 and E-cadherin was observed to show corresponding changes by qRT-PCR. The results of mass spectrometry showed that HMGB3 may directly or indirectly interact with 71 proteins. In summary, our results indicated that HMGB3 might be of very great significance to the pathogenesis of FGR and might play the role by leading the dysfunction of placental villous trophoblast cells and through the interaction with some other proteins.

1. Introduction

Fetal growth restriction (FGR) refers to a fetus who fails to achieve its genetically endowed growth potential, which is also known as intrauterine growth restriction (IUGR). FGR is a highly heterogeneous condition of global significance affecting up to 5–10% of pregnancies (Bamfo and Odibo, 2011; Mandruzzato et al., 2008; Miller et al., 2008). It remains a leading contributor to perinatal morbidity and mortality and serious adverse health outcomes in late life, such as stroke, type 2 diabetes, hypertension and coronary heart disease and so on (Bamfo and Odibo, 2011; Barker, 2006; Lackman et al., 2001). Its etiology is very complex and not fully understood. A large amount of causes for FGR have traditionally been subdivided into fetal, placental and maternal. However, impaired placental function may be the most important factor (Baschat and Hecher, 2004). Several elements have been identified to be associated with FGR, such as increased apoptosis in trophoblast, oxidative stress, deficient spiral arterial remodeling and abnormal angiogenesis (Ishihara et al., 2002; Levy et al., 2002; Maulik et al., 2006; Mert et al., 2012).

Fetal growth and development depend on intact placental function. It is the interface of nutrient and waste exchange between mother and fetus, which can regulate fetal growth and maternal physiology via the metabolism and production of a number of hormones and growth factors (Murphy et al., 2006). IGF-I and IGF-II have potent mitogenic

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activity which are expressed on distinct placental surfaces and induce somatic cell growth and proliferation (Hiden et al., 2009; Iniguez et al., 2010; Murphy et al., 2006). PlGF and VEGF are considered as major regulators of placental vascular development or angiogenesis (Regnault et al., 2003). There are many other important proteins to regulate the placental growth and development. However, dysregulated expression of some proteins could affect the function of placentas and play an important role in occurrence of FGR (Iniguez et al., 2010; Regnault et al., 2003; Street et al., 2006).

The High-mobility-group-box (HMGB) proteins have the unique DNA-binding domain, the HMG-box, which can bind strongly to DNA, affecting the structure of the double helix, bending the DNA, and play important roles in multiple processes in chromatin such as transcription, replication, recombination, DNA repair and genomic stability (Avgousti et al., 2016; Murugesapillai et al., 2017). Moreover, the HMGB proteins can also interact with other proteins including transcription regulators and histones (Avgousti et al., 2016; Murugesapillai et al., 2017). In our previous study, we constructed a large-scale comparative proteome profile of human placentas from normal and FGR pregnancies, which shows that HMGB3 was obviously down-regulated in the placenta of FGR (Miao et al., 2014). Recent studies have demonstrated that HMGB3 was high expressed in variety of tumors and affected tumor initiation and progression viaing promoting the tumor cells proliferation and migration and inhibiting tumor cells apoptosis (Gao et al., 2015; Li et al., 2015). And HMGB3 can regulate the balance between self-renewal and differentiation of hematopoietic stem cell by influencing the expression of other genes (Nemeth et al., 2006). In addition, animal experiments have indicated that HMGB3 has relationships with the differentiation of spermatogonia and the regulation of mesoderm formation and dorsoventral patterning (Bosseboeuf et al., 2014; Cao et al., 2012). Up to date, there is no research on HMGB3 and pregnancy-related diseases, and the pathological mechanism of HMGB3 in the placenta of FGR is unknown. Therefore, in our present study, we aimed to investigate the function and the potential mechanism of HMGB3 in the placentas of FGR and this may provide new theoretical basis and targets in the treatment of FGR.

2. Materials and methods

2.1. Patients and tissue samples

Placenta tissues were obtained from eleven pregnant women with no complication according to protocols approved by the Ethics Committee of The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital). All the mothers provided written informed consent before they had cesarean section delivery in The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University (Nanjing Maternity and Child Health Care Hospital). FGR refers to a fetus that has failed to achieve its genetically determined growth potential and was defined as EFW less than the 10th centile with respect to the reference value for current pregnancy age. For each placental sample, 3.0 g of tissue was dissected from the maternal side of the placentas (in the central part, exclusive of calcified area) and then frozen in liquid nitrogen prior to use.

2.2. Total RNA extracted and quantitative real-time PCR

A fraction of these samples was kept in TRIzol reagent (Invitrogen Technologies Co, USA, 5 mL/g), and total RNA was extracted, treated with RNase-free DNase, and quantified by spectrophotometer. Reverse transcription of cDNA was conducted using the reverse transcription kit (Takara, Tokyo, Japan). All the procedures were performed according to the manufacturer's instructions. qRT-PCR assays (Applied Biosystems) was used to quantify the expression level of mRNA in placentas and cells. qRT-PCR reactions were carried out according to the manufacturer's recommendation and performed triplicate with 384-well plates on ViiA7 real-time PCR System-Life Tech (Applied Biosystems, USA) with a standard absolute quantification thermal cycling program and using the SDS 2.4 software to determine the cycle threshold (Ct). After completion of the qRT-PCR amplification, the relative fold change was calculated based on the $2^{-\triangle\triangle Ct}$ method. GAPDH was used as internal control of mRNA for normalization.

Table 1
Clinical characteristics of study patients.

	Control(n = 6)	FGR(n = 5)	P-value
Maternal age(yerars)	28.17 ± 3.31	28.8 ± 2.5	0.736
Maternal BMI(Kg/m^2)	25.64 ± 1.98	27.08 ± 1.50	0.214
Gravidity	2.5 ± 0.84	2.8 ± 1.30	0.654
Gestational weeks	38.18 ± 2.26	36.12 ± 1.03	0.093
Birth weight(g)	3346.67 ± 663.19	2108.00 ± 150.57	**0.005

Data are presented as the means ± SD.
** P < 0.01.

2.3. Immunohistochemistry

Three pairs of placentas from FGR and healthy pregnant women were made into Parrffin-Embedded sections. The sections were deparaffinized and rehydrated with xylene, various concentrations of alcohol and distilled water. Sections were incubated in dark at room temperature for 25 min in 3% hydrogen peroxide solution after antigen retrieval using EDTA (pH 9.0) antigen retrieval solution (Servicebio, Wuhan, China) and then washed three times with PBS before being blocked with 3% Bovine Serum Albumin (Servicebio, Wuhan, China) for 30 min at room temperature. The HMGB3 antibody (1: 100, Abcam) was added to the section and incubated overnight. After washing three times in PBS, the sections were incubated with horseradish peroxidase (HRP) conjugated anti-rabbit antibodies for 1 h at room temperature. Immunoreactivity was visualized using a diaminobenzidine (DAB) kit (Servicebio, Wuhan, China). Finally, the sections were stained with hematoxylin 3 min and sealed with neutral gum.

2.4. Cell culture and cell transfection

The HTR8/SVneo trophoblast cell line was purchased from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China), which was derived from placental trophoblast. The HTR8/SVneo trophoblast cells were cultured in complete growth medium: RPMI1640 (WISET INC, Shanghai, China), supplemented with 10% fetal bovine serum (10%FBS) (Gibco, USA) under the condition of 37 °C and 5% CO2. Cell transfection was operated with Lipofectamine 2000 (Invitrogen, Carlsbad, USA) as manufacturer's protocol. For preparation, HTR8/SVneo trophoblast cells were seeded in six-well plates overnight until growing to 80 ~ 90%. Afterwards, 2.5 ug overexpression plasmid construction for HMGB3 (purchased from OriGene Technologies, Rockville, USA) and 5 ul Lipofectamine 2000 were separately diluted with Opti-MEM medium (Gibco, USA) without serum for 5 min and then were gently mixed befor incubating for 20 min at room temperature. The mixture was added into the HTR8/SVneo trophoblast cells in six-well plates which was washed twice with phosphate buffer saline (PBS) (WISET INC, Shanghai, China) and total culture system per well was 2 ml. After being cultivated at 37 °C with 5% CO2 for 6 h, cell culture medium of the six-well plates was replaced with RPMI1640 complete medium. Empty plasmid pcMV6-XL5 was used as a negative control. The negative control small interfering RNAs (siRNAs) and siRNAs of HMGB3 (si-HMGB3) were constructed by RiboBio Co., Ltd (Guangzhou, China). Cells were similarly transfected with the final concentration of 50 μM siRNAs as above.

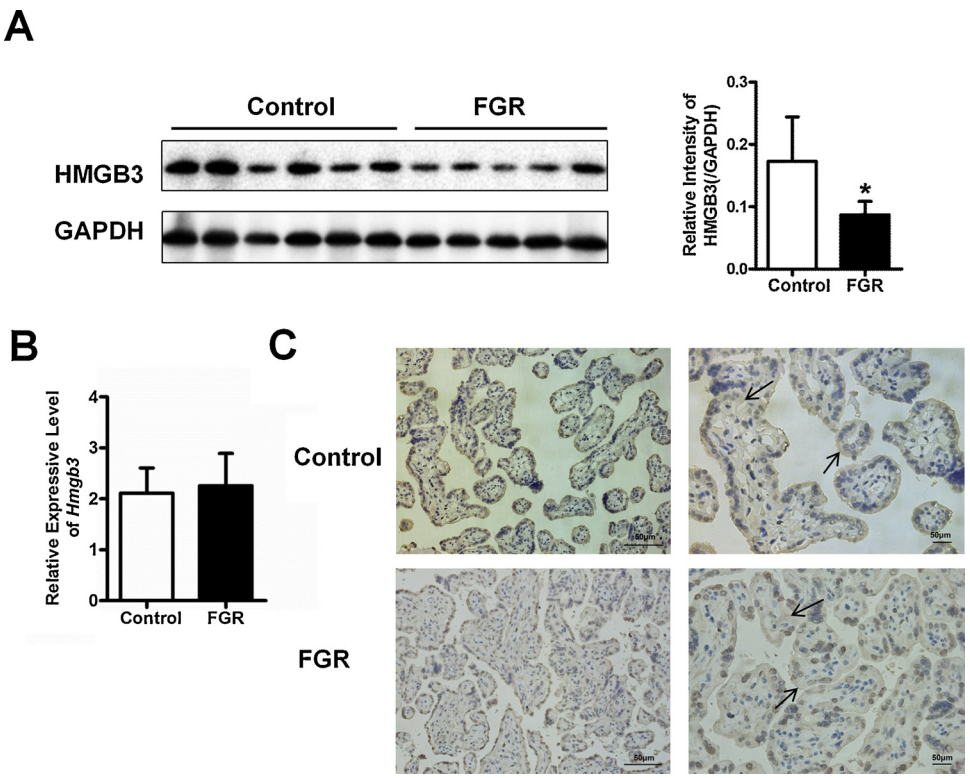

Fig. 1. HMGB3 expression in normal placenta tissue and FGR placenta. (A) Western blotting of HMGB3 protein expression in FGR placenta tissues (n = 5) compared with the normal pregnancy (n = 6). HMGB3 expression level was normalized to GAPDH. *P < 0.05. (B) qRT-PCR analysis of HMGB3 mRNA expression in FGR placenta tissues (n = 5). compared with the normal pregnancy (n = 6). The relative fold change was calculated based on the 2-△△Ct method. (C) Immunohistochemistry of HMGB3 in the placental tissues from normal and FGR pregnancies at 200× (left) and 400× (right) magnification.

2.5. Cell proliferation

The Cell Counting Kit-8 (CCK8) assay (Dojindo, Japan) was applied to test the cell proliferation as manufacturer's protocol. Briefly, the transfected cells were plated in 96-well plates at a density of 1×10^3 cells per well and cultured at 37 °C with 5% CO_2 for 0, 24, 48 and 72 h. 10 μl per well of CCK-8 was added to the cells and the 96-well plates were incubated for another 2 h. The absorbance was measured by a multifunctional microplate reader at 450 nm. All experiments were repeated three times independently.

2.6. Cell apoptosis

For cell apoptosis analysis, the HTR8/SVneo trophoblast cells were collected after being transfected for 48 h and stained with the Annexin V-FITC/Propidium Iodide Kit (BD Biosciences, Shanghai, China) followed the manufacturer's instructions. An apoptosis inducer kit (Beyotime, Shanghai, China) was used as a positive control. Data were analyzed with Kaluza Flow Cytometry Analysis Software (Beckman Coulter, Indianapolis, USA). All experiments were performed in triplicate independently.

2.7. Cell migration

Transwell assay was carried out to assess the migration capacity of the trophoblast cells. Prior to the experiment, 5×10^4 transfected HTR-8/SVneo cells were plated into each transwell(8 μm PET, Millipore, Switzerland). After incubation at 37 °C with 5% CO_2 for 48 h, these cells were fixed with 4% paraformaldehyde and stained with 1% crystal violet for 30 min respectively. A digital microscopy at 100× magnification was used to detect the number of migrated cells. 200 μL per well of 1% RIPA (Beyotime, Shanghai, China) was added to elute the migrated cells. The absorbances at 575 nm were measured by a multifunctional microplate reader. All experiments were performed in triplicate independently.

2.8. Western blotting

Western blot analysis of all samples which were collected from cells was performed as described previously(Miao et al., 2014). The membranes were blocked in Tris-Buffered Saline and 0.1% Tween 20 (TBST) containing 5% nonfat milk powder for 1 h, and incubated overnight with primary antibodies against HMGB3 (1:2500 dilution; Abcam Ab75782) and GAPDH (1:2000 dilution; ProteinTech Group, Chicago, USA). The membranes were then washed three times (20 min each) with TBST and incubated for 1 h with HRP-conjugated goat anti-rabbit IgG (1:2000 dilution; ProteinTech Group, Chicago, USA). Integrated density values were then calculated using FluorChem E system (ProteinSimple, California, USA). These values were then normalized to GAPDH level.

2.9. Immunoprecipitation and mass spectrometry

The transfected cell were divided into IgG group and myc group for immunoprecipitation (IP). 5 μl normal IgG antibody or Myc-tag antibody (1 μg/μl) and 5 μl pre-washed A/G beads were added to protein sample of each group for IP overnight. The above solution was washed 3 times with lysis buffer and centrifuged at 3000 rpm for 2 min. After being boiled with 70ul SDS loading buffer for 10 min, the protein solution was performed to verify the effect of IP by Western blotting. Then the strips of SDS-PAGE electrophoresis were excised into small pieces (~ 1 × 2 mm) and washed three times with 25 mM NH_4HCO_3 and 50% acetonitrile solution. After being dried completely under vacuum, the reduction reaction was performed at 56 °C for 1 h in an appropriate volume of 10 mM dithiotreitol and the alkylation was allowed to proceed in the dark for 45 min at room temperature in an appropriate volume of 55 mM iodoacetamide. Digestion was then carried out with 12.5 ng/μl of trypsin at 37 °C overnight. To extract the tryptic peptides from the gel pieces, an appropriate volume of 60% acetonitrile and 0.2% trifluoroacetate was added. Following 20 min of vortex and 5 min of sonication, the supernatant was taken and desalted with C18 ZipTip (Millipore). Half of the eluate was subjected to online electrospray

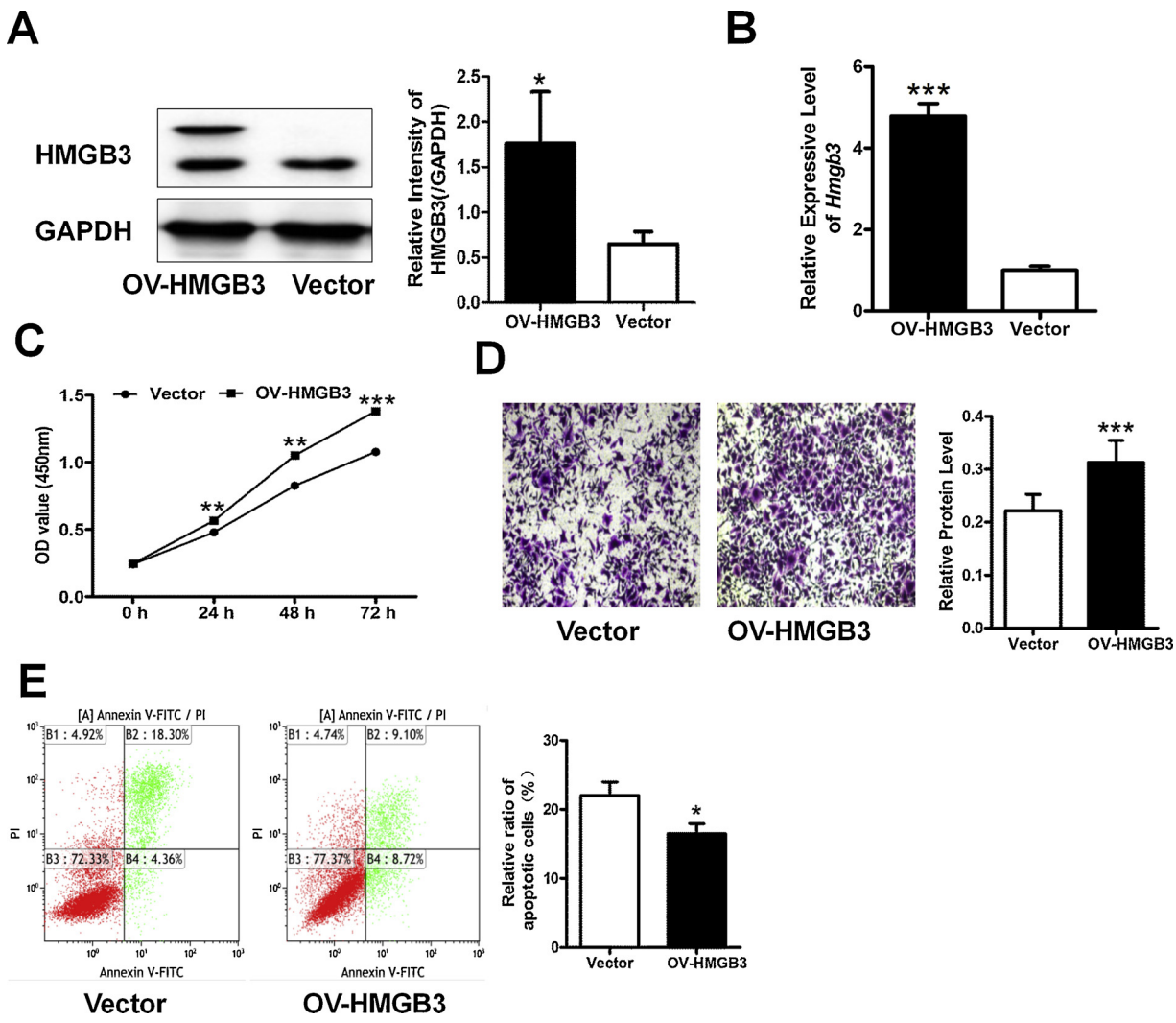

Fig. 2. Effects of Over-expression of HMGB3 on the Function of HTR-8/SVneo Trophoblast Cells. (A–B) HMGB3 expression of HTR-8/SVneo Trophoblast Cells after being transfected overexpression HMGB3 vsctor for 48 h by Western blotting and qRT-PCR analysis. (C) CCK8 assay applied to test the cell proliferation after being transfected for 0 h, 24 h, 48 h, 72 h. (D) Transwell assay applied to test the cell migration after being transfected for 48 h (magnification, 100×). (E) Flow Cytometry Analysis applied to evalue the cell apoptosis after being transfected for 48 h. * P < 0.05, ** P < 0.01, *** P < 0.001.

tandem mass spectrometry using the Nano ACQUITY UPLC system and set up to search the Uniprot-Crassostrea gigas database.

2.10. Data statistical analysis

Data from at least three independent experiments were expressed as the mean ± standard deviation (SD). The differences between two groups were analyzed using the *Student's t-test.* The cell experiment data were analyzed by using *t* test. Significant difference was recognized as a *p* value less than 0.05. All analysis was performed with SPSS 23.0 software.

3. Results

3.1. Clinical characteristics of the study patients

Table 1 shows the clinical characteristics of the study patients include the females with the control group (n = 6) and FGR (n = 5). No complications were found in normal and FGR pregnancies. No significant differences were identified between the two groups with respect to maternal age, body mass index (BMI), gravidity and gestational weeks at delivery. The neonates of FGR had a significantly lower birth weight than those whose mothers were in the control group.

HMGB3 protein expression is downregulated in the placenta of FGR patients.

To investigate the role of HMBG3 in the development and progression of FGR, HMBG3 expression levels were detected by Western Blot and qRT-PCR. The results showed that HMGB3 protein expression was significantly decreased in the FGR placenta ($P < 0.05$) (Fig. 1A). However, no significant difference of HMGB3 expression was observed in mRNA level ($P > 0.05$) (Fig. 1B). And the level of HMGB3 protein expression was further confirmed by immunohistochemistry. As shown in Fig. 1C, the HMGB3 protein is mainly expressed in the cytoplasm of syncytiotrophoblasts and cytotrophoblasts and is lightly stained in placental tissue of FGR, indicating that the HMGB3 protein was downregulated in placental tissue of FGR compared with that of normal pregnancy.

3.2. Overexpression of HMGB3 promotes proliferation and migration and inhibits apoptosis of human trophoblast cells in vitro

To further investigate the potential role of HMGB3 in the pathogenesis of FGR, overexpression HMGB3 plasmid were transfected into the HTR-8/SVneo trophoblast and the empty plasmid were transfected as a control. First, after transfected for 48 h, the protein level of HMGB3 was confirmed to be overexpressed about 3-fold by Western Bloting

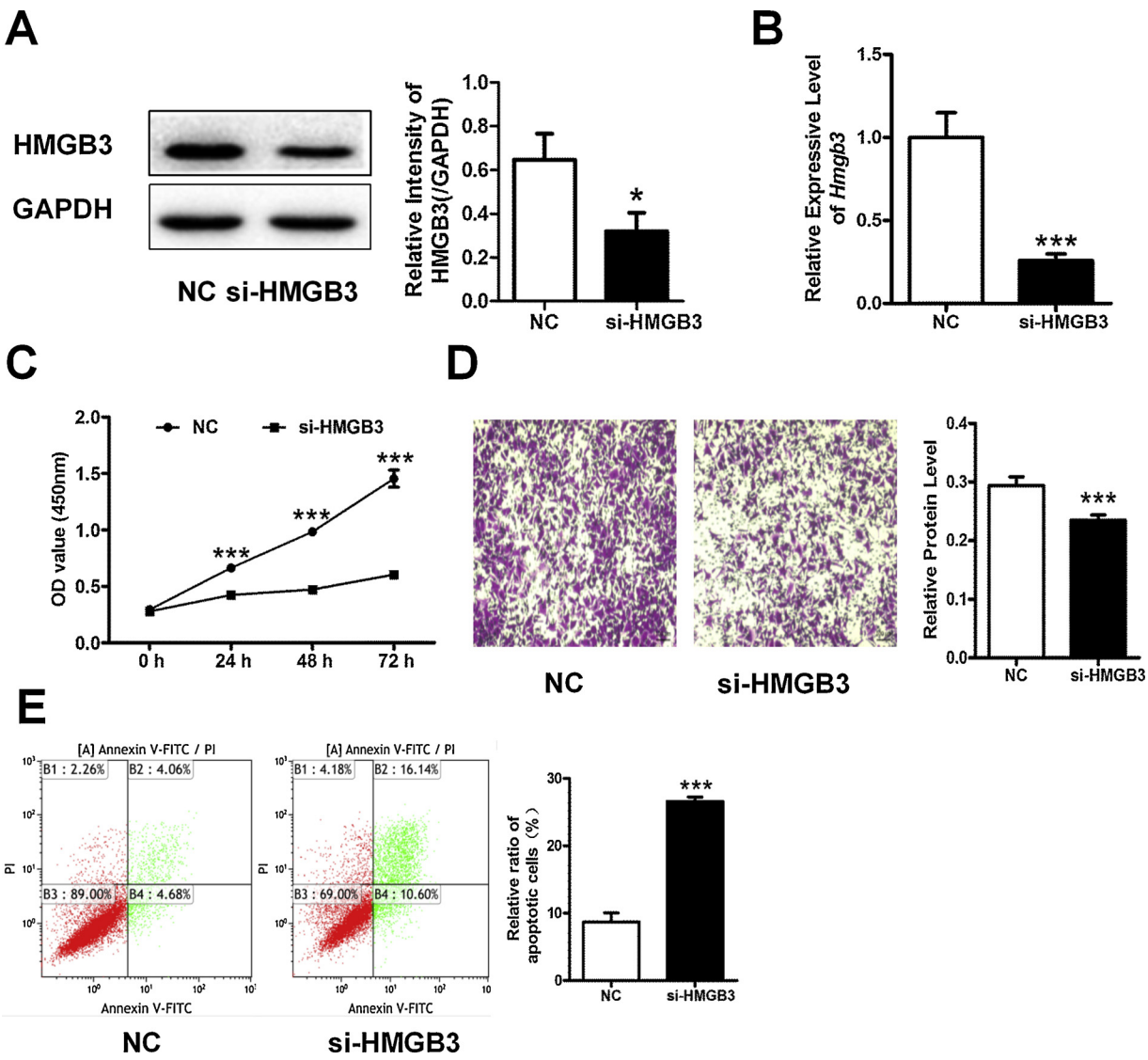

Fig. 3. Effect of down-regulated HMGB3 on the Function of HTR-8/SVneo Trophoblast Cells. (A–B) HMGB3 expression of HTR-8/SVneo Trophoblast Cells after being transfected small interfering HMGB3 for 48 h by Western blotting and qRT-PCR analysis. (C) CCK8 assay applied to test the cell proliferation after being transfected for 0 h, 24 h, 48 h, 72 h. (D) Transwell assay applied to test the cell migration after being transfected for 48 h (magnification, 100×). (E) Flow Cytometry Analysis applied to test the cell apoptosis after being transfected for 48 h. * P < 0.05, ** P < 0.01, *** P < 0.001.

(Fig. 2A) and 5-fold overexpression of HMGB3 mRNA was confirmed by qPCR (Fig. 2B). Then, CCK8 assay showed that the proliferation ability of HTR-8/SVneo cells was significantly enhanced at 24 h, 48 h and 72 h ($P < 0.05$) after HMGB3 overexpressed (Fig. 2C). Transwell assay showed that over-expression of HMGB3 also significantly promoted HTR-8/SVneo cell migration ($P < 0.05$) (Fig. 2D). Moreover, HTR-8/SVneo cells were transfected with plasmid and an apoptosis inducer kit. And then Flow cytometry analysis suggested that compared with the control group (22.02%), overexpression of HMGB3 significantly inhibited the apoptosis of trophoblastic cells (16.48%) ($P < 0.05$) (Fig. 2E).

3.3. Silencing of HMGB3 leads to less proliferation and migration and more apoptosis of human trophoblast cell line

To further confirm the function of HMGB3 in the pathogenesis of FGR, We reduced HMGB3 expression in HTR-8/SVneo trophoblasts cells using small interference RNA. As shown in Fig. 3A and B, the protein level of HMGB3 was decreased by 50% and the mRNA level was suppressed by 70–80% by transfection of small interference RNA. Similarly, the effects of HMGB3 on the function of HTR-8/SVneo cells were investigated by CCK8 assay, Transwell assay and flow cytometry. We found that the silencing of HMGB3 could significantly inhibit the proliferation of HTR-8/SVneo trophoblast cell in 24 h, 48 h, 72 h ($P < 0.05$) (Fig. 3C), while the migration of HTR-8/SVneo cells was also significantly inhibited($P < 0.05$) (Fig. 3D). In addition, compared with the control group, silencing of HMGB3 significantly promoted the apoptosis of HTR-8/SVneo trophoblasts (8.69% VS 26.59%, $P < 0.05$) (Fig. 3E).

3.4. HMGB3 may regulate the expression of several genes or interact with 71 proteins directly or indirectly

To further investigate how HMGB3 affected the trophoblast function, total RNA of HTR-8/SVneo trophoblast cell line was extracted after transfection for 48 h and the expression levels of PCNA, Ki67, Tp53, Bax, MMP-2 and E-cadherin were detected by qRT-PCR. The results showed that the expression of PCNA, Ki67 and MMP-2 was up-regulated after overexpression of HMGB3. Among them, the expression of Ki67 and MMP-2 was statistically up-regulated and the expression of

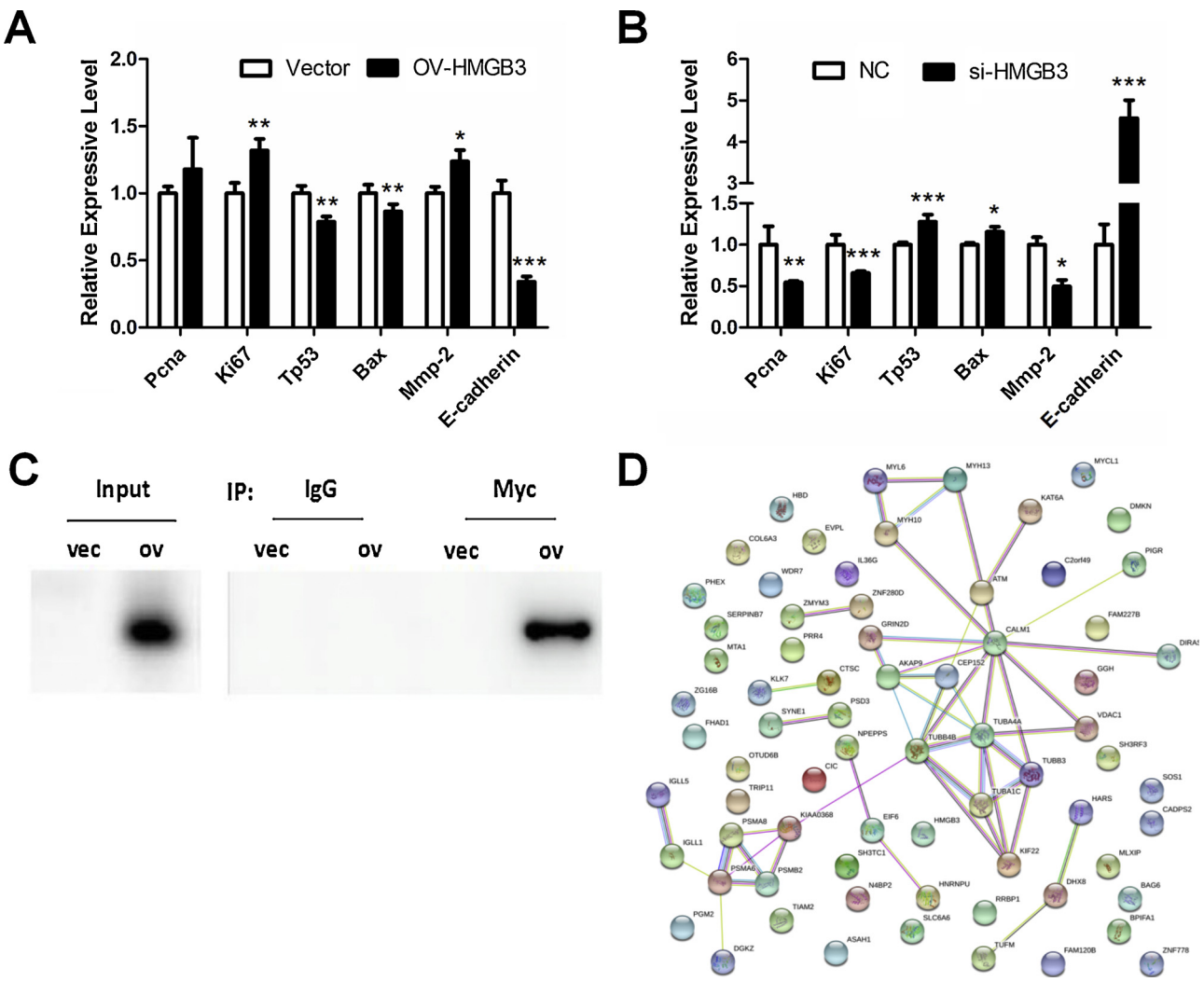

Fig. 4. Mechanism of HMGB3 regulating cell function. (A) The expression of Pcna, Ki67, Tp53, Bax, MMP-2 and E-cadherin mRNA expression after being transfected overexpression HMGB3 vsctor for 48 h by qRT-PCR. (B) The expression of Pcna, Ki67, Tp53, Bax, MMP-2 and E-cadherin mRNA expression after being transfected small interfering HMGB3 for 48 h by qRT-PCR. (C) Western blotting results showed that the protein sample containing HMGB3 was successfully extracted by immunoprecipitation. (D) Protein interaction network map of Mass Spectrometry results for proteins that may bind to HMGB3 using the STRING Database. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2
The sequence of primers used for qRT-PCR.

Gene name	Forward primer	Reverse primer
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
HMGB3	CCCAGAGGTCCCTGTCAATTT	CGATCATAGCGCACTTTATCTGC
PCNA	ACACTAAGGGCCGAAGATAACG	ACAGCATCTCCAATATGGCTGA
Ki67	ACGCCTGGTTACTATCAAAAGG	CAGACCCATTTACTTGTGTTGGA
Tp53	GGCCATCTACAAGCAGTCA	TTCTGTCATCCAAATACTCCA
Bax	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT

Tp53, Bax and E-cadherin was statistically down-regulated at the same time ($P < 0.05$) (Fig. 4A). While after silencing of HMGB3 expression, lower levels of PCNA, Ki67 and MMP-2 expression and higher levels of Tp53, Bax and E-cadherin expression was observed ($P < 0.05$) (Fig. 4B). Table 2 shows the sequences of all primers for these genes. Western blotting showed that a protein sample containing HMGB3 was successfully extracted by IP (Fig. 4C). Mass spectrometry analysis of the electrophoresis strip showed that 252 proteins in myc group and 243 proteins in non-specific IgG group were identified in the IP samples of the control empty vector group. While in the IP samples of over-expressed HMGB3 group, 222 proteins from myc group and 191 proteins from IgG group were identified respectively. By removing non-specific binding or repeating proteins from the control group, we found that HMGB3 may interact directly or indirectly with 71 proteins. The direct or indirect functional correlation of these proteins was investigated using the STRING database (http://string-db.org/) to obtain an interaction network map of these proteins (Fig. 4D).

4. Discussion

Trophoblast is the main cell type of extra-embryonic tissue and the early stage of placental development occurs in a relatively hypoxic

environment, which is beneficial for the proliferation of cytotrophoblast cells (Red-Horse et al., 2004). In addition, apoptosis is thought to be a normal part of villus trophoblast renewal and syncytiotrophoblasts formation (Scifres and Nelson, 2009). Many histopathological changes are observed in the placenta of FGR, including villus morphological changes, villus infarction and maternal vascular changes (Mifsud and Sebire, 2014). It suggested that placental dysfunction may be one of the important causes of FGR. Therefore, we first investigated the expression of HMGB3 in the FGR placenta. Compared with normal placenta, the protein HMGB3 expression is down-regulated in FGR placenta. Notably, our experiments show that HMGB3 can stimulate HTR-8/SVneo trophoblast proliferation and migration, while inhibiting cell apoptosis of trophoblasts. The impact is consistent with previous researches about functions of HMGB3 on tumor cell (Li et al., 2015). These results suggest that the abnormal low expression of HMGB3 protein in the placenta may promote the development of FGR by causing trophoblasts dysfunction.

The damage and repair of placenta and the dysplasia of villus are the characteristics of placenta in FGR due to excessive apoptosis of the syncytiotrophoblasts (Redline, 2008). Increased apoptosis and less cell proliferation was observed in the IUGR placenta, which may be associated with higher expression of pro-apoptosis genes p53, p21, and Bax in the trophoblast (Heazell et al., 2011). The staining intensity of PCNA, Ki67 and cyclin D3 was observed to be significantly reduced in all regions of the IUGR placental sample by immunohistochemical staining (Unek et al., 2014). These studies are consistent with our above results. Silencing of HMGB3 may decrease the expression of proliferation-related genes PCNA and KI67 and increase the expression of apoptosis-related genes Bax and TP53. In contrast, overexpression of HMGB3 may cause opposite changes of these genes expression. In addition, the invasion of trophoblast cells into endometrium is a critical step in the embryo implantation process, including migration and invasion of trophoblast cells (Liu et al., 2016). Extracellular matrix metalloproteinase MMP family can disrupt the extracellular matrix and promotes cell migration and invasion. E-cadherin is a Ca2 + -dependent cell surface protein present at the cell-adhesion junction, which is considered to be an inhibitor of trophoblast and cancer cell migration and invasion (Liu et al., 2016). In our experiments, overexpression of HMGB3 could promote the migration of trophoblast cells. At the same time, higher expression of MMP-2 and lower expression of E-cadherin were detected. These results further suggest that HMGB3 may have an effect on trophoblast function and may function by regulating the expression levels of these genes.

Our study also found that HMGB3 may interact directly or indirectly with 71 proteins whose functions involve immune response, oxidative stress, cell cycle regulation and catabolism. Among these 71 proteins, many proteins may play a role in the pathogenesis of FGR. In the placenta of IUGR induced by intraperitoneal injection of dexamethasone (0.4 mg/kg), lower level of metastasis tumor antigen (MTA) -1 in the nucleus of the basal area was detected, while higher level of MTA1 and lower level of MTA2 and MTA3 was observed in the cytoplasm of placental labyrinth area, accompanied by more cell death and changes in the expression of PCNA and TP53 (Alqaryyan et al., 2017). The MTA family is a family of co-regulatory proteins involved in cell cycle regulation, including MTA1, MTA2 and MTA3, which encode six different protein isoforms that are involved in regulating cell differentiation, hormonal action, cell proliferation and epithelial-mesenchymal transition (Alqaryyan et al., 2017). They also can regulate transcriptional activity through histone deacetylation and chromatin remodeling to affect embryonic development, homeostasis and tumorigenesis (Alqaryyan et al., 2017). In normal development of the placenta, the expression of MTA1 and estrogen receptors was increased along with pregnancy advance, suggesting that protein MTA1 plays an important role in regulating estrogen action and placental growth (Alqaryyan et al., 2017). In addition, HMGB3 may also interact with voltage-dependent anion channel-1 (VDAC1). Hypoxia induced an increase in the expression of VDAC1 and heme oxygenase-1 and C-terminally truncated heme oxygenase-1 was significantly decreased in FGR placenta (Oh et al., 2016). VDAC1 and heme oxygenase-1 appear to be co-localized in the basement membrane of syncytiotrophoblastic cells (Oh et al., 2016), suggesting that changes of VDAC1 expression induced by hypoxia may play a role in the pathogenesis of FGR. What's more, VDAC1 is involved in a variety of cellular biological processes. It may control cytochrome release by interacting with anti-apoptotic proteins, regulate the transport of mitochondrial calcium and metabolites, exert NADH-ferricyanide reductase activity in the plasma membrane and mediate the activity of plasma membrane electron transport (Oh et al., 2016). VDAC1 on the plasma membrane may also be a receptor for the angiogenesis regulator Kringle5 on endothelial cells and participate in the regulation of the angiogenesis process (Oh et al., 2016).

In conclusion, HMGB3 protein expression is abnormally down-regulated in the placenta of FGR patients. Overexpression and knock-down experiments show that HMGB3 can regulate the proliferation, migration and apoptosis of trophoblast cells, further leading to the disorder of placental function. Our results also suggest that the above regulation may through regulating the expression of genes involved in trophoblast function or directly or indirectly interacting with various proteins. Altogether, our findings provide more data for understanding the mechanism of HMGB3 in the FGR.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2018.11.007.

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