

## Original Article

# PPAR $\gamma$ induces the paroxysm of endometriosis by regulating the transcription of MAT2A gene

Shun Zhang\*, Lingling Zhuang\*, Qian Liu, Xiaolin Yu, Qinghua Min, Minjie Chen, Qi Chen

*The First Affiliated Hospital of Nanchang University, No. 17 Yongwaizheng Street, Donghu District, Nanchang, Jiangxi, People's Republic of China. \*Co-first authors.*

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**Abstract:** Objective: To investigate the molecular mechanism of PPAR $\gamma$  impacting the paroxysm of endometriosis. Methods: Immunohistochemistry, qRT-PCR and Western Blot were used to determine the expression level of PPAR $\gamma$  and MAT2A in Eu, Ec and normal endometrial tissue (control). ESC and NSC were separately isolated. PPAR $\gamma$  was silenced in NSC and was up-regulated in ESC. Rosiglitazone (RSG) were used to incubate with ESC. Proliferation, apoptosis, invasion, and ultrastructure of cells were evaluated in vitro. The combination between PPAR $\gamma$  and the promoters of MAT2A was detected by dual-luciferase reporter assay. Results: MAT2A was up-regulated and PPAR $\gamma$  was down-regulated in Eu and Ec. The cell viability and the ability of migration and invasion declined greatly after up-regulating the expression of PPAR $\gamma$  or treating with RSG in ESC. Meanwhile, the expression level of MAT2A was significantly inhibited. Plenty of vacuoles and classical morphological changes of apoptotic cells were observed in the ESC with PPAR $\gamma$  over-expressed. The cell viability and the ability of migration and invasion of NSC with PPAR $\gamma$  silenced were promoted greatly. Meanwhile, the expression level of MAT2A was significantly up-regulated. Conclusion: The paroxysm and development of endometriosis were impacted by over-expressing PPAR $\gamma$  or introducing of RSG by inhibiting the transcription of MAT2A.

**Keywords:** PPAR $\gamma$ , MAT2A, endometriosis, paroxysm

## Introduction

Endometriosis is an estrogen-dependent gynaecological disorder that affects 6-10% of women of reproductive age group. It is characterized histologically by the presence of endometrial tissue at sites outside of the uterine cavity, primarily on the pelvic peritoneum and ovaries, resulting in severe pelvic pain, pain during intercourse, and infertility [1]. Up to now, the etiology and pathogenesis of endometriosis are not clear. There is growing evidence that eutopic endometrium from women with endometriosis has endogenous abnormalities, which permits endometrial tissue to attach, survive, invade, and results in the occurrence and development of endometriosis [2, 3].

As a dedifferentiated gene that regulate the cell growth, methionine adenosyl-transferase 2A (MAT2A) is an important target for mitogenic active cytokines, such as hepatocyte growth factor and leptin, to induce cell proliferation [4,

5]. High level of MAT2A was reported to induce cell proliferation, inhibit apoptosis and involved in the paroxysm and development of multiple types of malignant tumors, such as liver cancer, colon cancer, bladder cancer and prostate cancer [6-9].

Peroxisome proliferators activate receptors (PPAR $\gamma$ ) is a pleiotropic nuclear hormone receptor that can combine with particular DNA reaction components to compete with other transcription factors to influence particular gene with indirect, negative feedback and positive feedback regulations [10]. It is reported that PPAR $\gamma$  could exert important regulation function on cell cycle and apoptosis. And it is also a therapeutic target for the treatment of proliferative cardiovascular disease and cancer [11-13]. Rosiglitazone (RSG), an agonist of PPAR $\gamma$ , was reported to inhibit endometriosis [14] and improve the symptoms of endometriosis [15]. PPAR $\gamma$  is involved in multiple physiological and pathological process, including fat metabolism,

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glucose metabolism, cell proliferation and differentiation, tumorigenesis, inflammation and immune response. In current years, PPAR $\gamma$  is being focused in the field of gynecological disease by researchers. It is reported that PPAR $\gamma$  was expressed on endometrium and the activation of PPAR $\gamma$  may inhibit the growth of ectopic endometrium [16].

We got the hypothesis that PPAR $\gamma$  may change the biological character of endometrium, such as cell proliferation, apoptosis and invasion, by regulating the activation of transcriptional factor MAT2A. In present study, the pathogenesis of endometriosis was deeply claimed and new endometriosis diagnostic markers with higher specificity and sensitivity are investigated. The aim is to find more specified genetic target for future diagnosis and treatment of endometriosis.

### Materials and methods

#### *The collection of tissue samples*

The clinical samples were all collected from gynaecology and obstetrics department of the first affiliated hospital of Nanchang University. The samples of eutopic endometrium (Eu) and ectopic endometrium (Ec) were collected from 35 endometriosis patients after panhysterectomy. The samples of normal endometrium group (control) were collected from 35 women without endometriosis. The menstruation of all the patients are normal and there were no other malignant diseases, estrogen-dependent diseases, immune related diseases, surgical diseases, and inflammatory diseases on these patients. They have not received the treatments of gonadotropin-releasing hormone (GnRH) analogue, hormone or anti-inflammatory drugs within 6 months before the surgery. The endometrium was scraped immediately after the surgery, stored in the sterile medium containing 100 U/mL penicillin and streptomycin and transferred to the laboratory quickly for cell culture. The collection of human endometria got the authorization of all the patients and their family members. And the experiments were authorized by the ethical committee of the first affiliated hospital of Nanchang University.

#### *Immunohistochemistry*

The endometrium was separated out and put into the plate filled with pre-cool normal saline.

The tissues were embedded in paraffin, sectioned, and incubated with Caspase-3 and VEGF antibody (Bioss, 1:1000). After incubation at 4°C overnight and washed over by PBS buffer, the slides were incubated with goat anti-rabbit antibody at 37°C for 30 min. After washed over by PBS buffer, the slides were dyed with DAB agent for 5-10 min and re-dyed with hematoxylin for 3 min. The pictures were taken on the slides under inverted microscope (Olympus).

#### *The separation of primary cells from eutopic endometrium tissue and normal tissues in endometrium*

The endometrium tissues were washed over by PBS for 3 times to remove the bloodiness and cut into 1 mm<sup>3</sup> or smaller tissue blocks by eye scissors under sterile condition. 3 times volume of endometrium tissues buffer (0.1% type I collagenase and 0.25% trypsin containing EDTA) was added into the tissue. The mixture was transferred to the centrifuge tube after percussion for a while and digested for 20 min in the 37°C incubator. The supernatant was removed to stop the digestion and repeat the above operations for 3 times. DMEM/F12 medium containing 10% FBS was used to stop the digestion of the last collected tissues. The mixture was centrifuged at 1000 r/min for 5 min to remove the supernatant. The cells were re-suspended using DMEM/F12 medium containing 10% FBS and planted to the plate. The cells were incubated at 37°C in the incubator containing 5% CO<sub>2</sub>.

#### *The determination of the concentration of RSG*

The endometrium cells were incubated with 0, 1  $\mu$ mol/L, 5  $\mu$ mol/L, 10  $\mu$ mol/L, 20  $\mu$ mol/L and 40  $\mu$ mol/L RSG for 48 h. The cell viability was detected by CCK8 assay.

#### *The detection of dual luciferin*

13 groups of plasmids were used to perform the study. 293T cells were planted in the 6-well plate and transfected with (1) PPAR $\gamma$  over-expressed plasmids (Thermo). (2) Vector (Thermo) with the sequence of wild PPRES1 (Genscript). (3) Vector with the sequence of mutant PPRES1. (4) Vector with the sequence of wild PPRES2 (Genscript). (5) Vector with the sequence of mutant PPRES2. (6) Vector with the sequence of wild PPRES3 (Genscript). (7)

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Vector with the sequence of mutant PPREs3. (8) Vector with the sequence of wild PPREs4 (Genscript). (9) Vector with the sequence of mutant PPREs4. (10) Vector with the sequence of wild PPREs5 (Genscript). (11) Vector with the sequence of mutant PPREs5. (12) Vector with the total length of sequence of wild PPREs (Genscript). (13) Vector with the total length of sequence of mutant PPREs. 25  $\mu$ L/well Opti-MEM (Thermo) was added into EP tubes. One tube was added with 0.75  $\mu$ L/well lipofectamine 3000 (Lip 3000, Thermo) and another tube was added with 1  $\mu$ L/well P3000 (Thermo). 1.2  $\mu$ g/well plasmids (pRL-SV40, Thermo) was added into the diluted Lip 3000 except for the control group. 293T cells were mixed with the diluted Lip 3000 according to above divided groups and placed under room temperature for 15 min. 50  $\mu$ L/well suspended cells were added into the wells of plate and collected 48 later.

### *Experimental group*

9 groups were divided in present study. (1) ESC; (2) ESC with PPAR $\gamma$  over-expressed (PPAR $\gamma$ ); (3) ESC with scrambled plasmids (Vector); (4) ESC incubated with 10  $\mu$ mol/L RSG (Solarbio R8470) (RSG); (5) ESC with PPAR $\gamma$  over-expressed incubated with 10  $\mu$ mol/L RSG (PPAR $\gamma$ +RSG); (6) ESC with scrambled plasmids incubated with 10  $\mu$ mol/L RSG (Vector+RSG); (7) NSC; (8) NSC with PPAR $\gamma$  silenced (sh PPAR $\gamma$ ); (9) NSC with scrambled plasmids (Vector). The endometrium cells were planted in the 6-well plate diluted to  $8 \times 10^4$  cells/well and incubated until all cells were adhere to the subface of wells and the density of cells reached 70%. 0.5 mL/well medium with no FBS were exchanged to incubate the endometrium cells. 2 sterile EP tubes were added with 125  $\mu$ L/tube Opti-MEM. One tube was added with 5  $\mu$ L Lip 3000 and another one was added with 2.5  $\mu$ g plasmids and 5  $\mu$ L P3000. The EP tubes were mixed individually and incubated under room temperature for 5 min. The solutions of the two tubes were mixed together and incubated under room temperature for 15 min. The mixture was divided into two equal duplicates and dropped into the wells accordingly. The cells were put back to the incubator and 0.5 mL medium containing 20% FBS was added into the wells 4 h post the transfection. 10

$\mu$ mol/L RSG was added into the wells 48 h post the transfection according to above groups and incubated for 48 h.

### *CCK8 assay for accessing proliferation of cells*

The cell density was settled as  $6 \times 10^3$  cells/well. 10  $\mu$ L of CCK-8 solution was added into each well of the plate using a repeating pipettor. The plate was incubated for 1-4 h and the absorbance at 450 nm was measured by a benchmark microplate reader (Bio-Rad, CA). Three independent assays were performed. The survival fraction was calculated according to the equation: inhibition rate =  $(OD_{\text{control}} - OD_{\text{drugs}}) / OD_{\text{control}}$ .

### *Cell migration assay*

Matrigel (BD) was coated uniformly on the surface of the transwell bottom after being diluted 1:2 with serum-free medium and cells were seeded in 24-well culture plates and grown to 90% confluence. Subsequently, the transwell chamber and medium was discarded after 24 h incubating and migrated cells were stained with 0.1% crystal violet for 10 min after being fixed with ethanol for 30 min. The picture was taken by inverted microscope (OLYMPUS). Three independent assays were performed.

### *Cell invasion assay*

Matrigel was coated uniformly on the surface of the transwell bottom after being diluted 1:3 with serum-free medium and cells were seeded in 24-well culture plates and grown to 90% confluence. The transwell chamber and medium were discarded after 24 h incubating and cells were stained with 0.1% crystal violet for 10 min after being fixed with ethanol for 30 min. The picture was taken by inverted microscope (OLYMPUS). Three independent assays were performed.

### *Transmission electron microscope*

Endometrium cells in logarithmic growth phase were seeded in 24-well plates at a density of  $1 \times 10^5$  cells/well, followed by treated with TM (50  $\mu$ g/mL) for 48 h. After washed with cold PBS for 3 times, cells were digested with trypsin and collected by centrifugation to remove the supernatant. Finally, cells were fixed with

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**Table 1.** The sequences of primers for PPAR $\gamma$ , MAT2A and GAPDH

Primer ID	Sequences	Length of the primer (bp)	Length of the product (bp)	Annealing temperature (°C)
PPAR $\gamma$ F	CCCAGGTTTGCTGAATGTG	18	197	57.8
PPAR $\gamma$ R	TGTCTGTCTCCGTCTTCTTGAT	20		
MAT2A F	TTGTGCTGCGAAATACCT	19	102	57
MAT2A R	CCCCAACCGCCATAAGT	17		
GAPDH F	CAATGACCCCTTCATTGACC	20	106	57.2
GAPDH R	GAGAAGCTTCCCCTTCTCAG	20		

3% glutaraldehyde and 1% citric acid, gradually dehydrated by acetone and embedded with dipropylene dicarboxylate. The solid was cut into ultrathin sections. Ultrastructural changes of endometrium cells were observed and photographed under transmission electron microscopy.

### Real-time RT-PCR

Total RNA of the cells was collected from the tissues using a RNA Extraction Kit (Takara) in terms of the instructions of the manufacture. RNA extracted was quantified with a NanoDrop spectrophotometer (NanoDrop Technologies). A specific RT primer was used to reverse-transcribe the complementary DNA. SYBR Premix Ex Taq<sup>TM</sup> (Takara) with an Applied Bio-Rad CFX96 Sequence Detection system (Applied Biosystems) was used in the real-time PCR procedure. The expression level of PPAR $\gamma$  and MAT2A was determined by the threshold cycle (Ct), and relative expression levels were calculated by the  $2^{-\Delta\Delta Ct}$  method after normalization with reference to the expression of U6 small nuclear RNA. The expression level of GAPDH in the tissue was taken as negative control. Three independent assays were performed. The information of the primers were shown in **Table 1**.

### Western blot assay

Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime) was used to isolate the proteins from the cells. Approximately 35  $\mu$ g of protein was separated on 12% SDS-polyacrylamide gel (SDS-PAGE). The gel was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore). The membrane was blocked with 5% nonfat dry milk in TBST (Tris buffered saline/0.1% Tween-20, pH 7.4) for 1 h at room temperature and incubated overnight with primary rabbit anti-human antibodies to PPAR $\gamma$  (Abcam, 1:1000) and MAT2A (Abcam, 1:1000).

A horseradish peroxidase-conjugated antibody against rabbit IgG (Abcam, 1:5000) was used as a secondary antibody. Blots were incubated with the ECL reagents (Beyotime, Jiangsu Province, China) and exposed to Tanon 5200-multi to detect protein expression. Three independent assays were performed.

### Statistical analysis

Results are presented as mean  $\pm$  SEM of at least three independent experiments. Each experiment was conducted in triplicate. Statistical significance among multiple groups was analyzed using Prism 7 software by one-way ANOVA followed by post-hoc Dunnett's test, while Student's t-test was applied for statistical analysis of two classes of data.  $P < 0.05$  was considered significant.

### Result

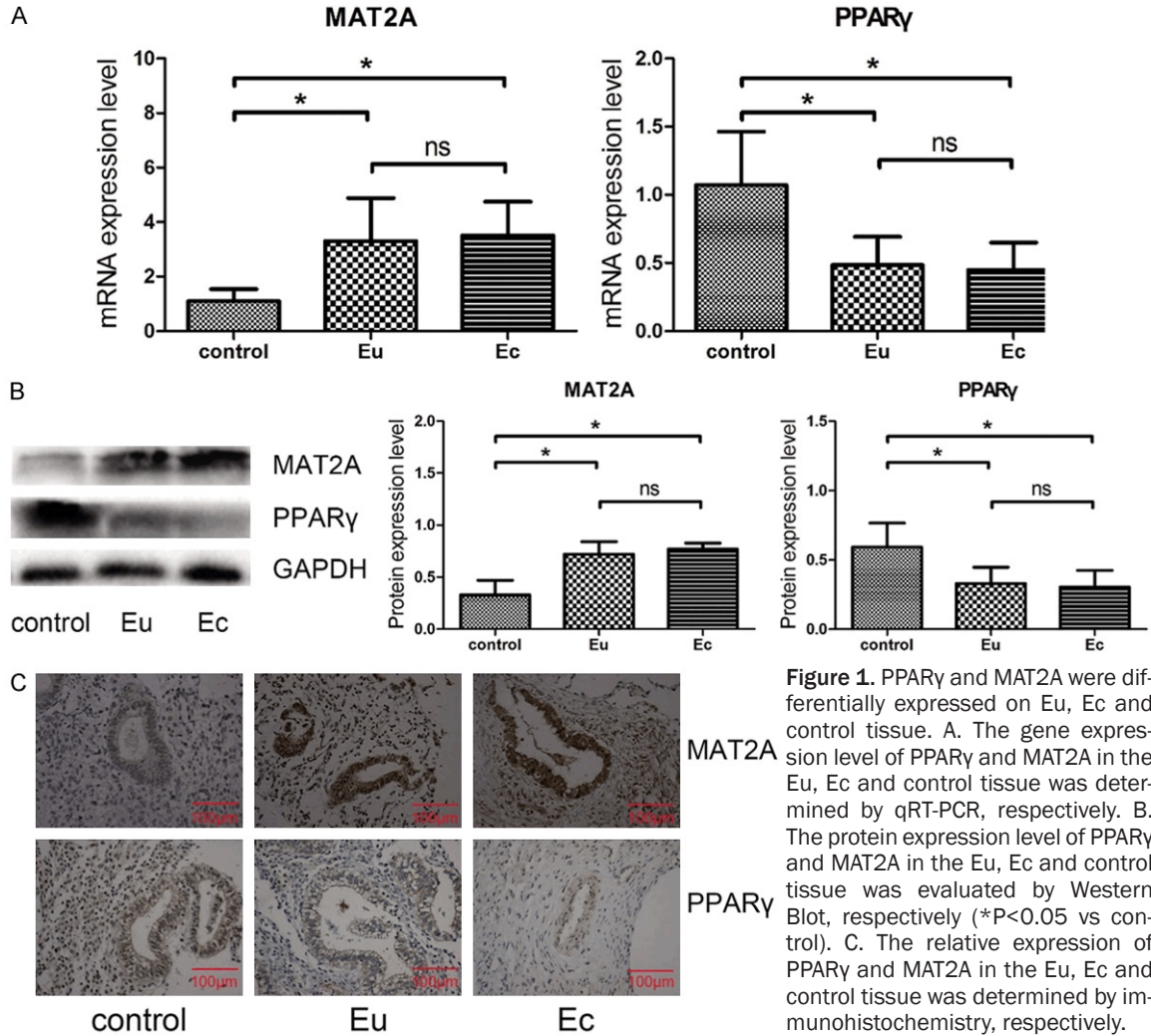
#### *The relative expression level of PPAR $\gamma$ and MAT2A in Eu, Ec and control tissue*

To evaluate the expressional difference of PPAR $\gamma$  and MAT2A in endometriosis, the clinical Eu, Ec and normal tissues were collected for detection. As shown in **Figure 1A** and **1B**, MAT2A was up-regulated and PPAR $\gamma$  was down-regulated in Eu and Ec, compared with control ( $*P < 0.05$  vs control). No significant difference on expression of MAT2A and PPAR $\gamma$  was observed between Eu and Ec. The expressional difference was verified by the results of immunohistochemistry, which were shown in **Figure 1C**.

#### *The determination of the concentration for RSG*

To screen the optimal concentration of RSG to be incubated with endothelial cells, the cell viability of endothelial cells was evaluated following incubated with 0, 1, 5, 10, 20 and 40

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**Figure 1.** PPAR $\gamma$  and MAT2A were differentially expressed on Eu, Ec and control tissue. A. The gene expression level of PPAR $\gamma$  and MAT2A in the Eu, Ec and control tissue was determined by qRT-PCR, respectively. B. The protein expression level of PPAR $\gamma$  and MAT2A in the Eu, Ec and control tissue was evaluated by Western Blot, respectively (\* $P < 0.05$  vs control). C. The relative expression of PPAR $\gamma$  and MAT2A in the Eu, Ec and control tissue was determined by immunohistochemistry, respectively.

$\mu\text{mol/L}$  RSG. As shown in [Figure S1](#), the cell viability of endometrium cells incubated with  $10 \mu\text{mol/L}$  RSG was significantly lower than control (\* $P < 0.05$  vs  $0 \mu\text{mol/L}$ ), with no obvious difference for other four concentrations. Therefore,  $10 \mu\text{mol/L}$  RSG was used for the subsequent experiments.

### The evaluation of the transfection of PPAR $\gamma$ over-expressed vector and PPAR $\gamma$ silenced vector

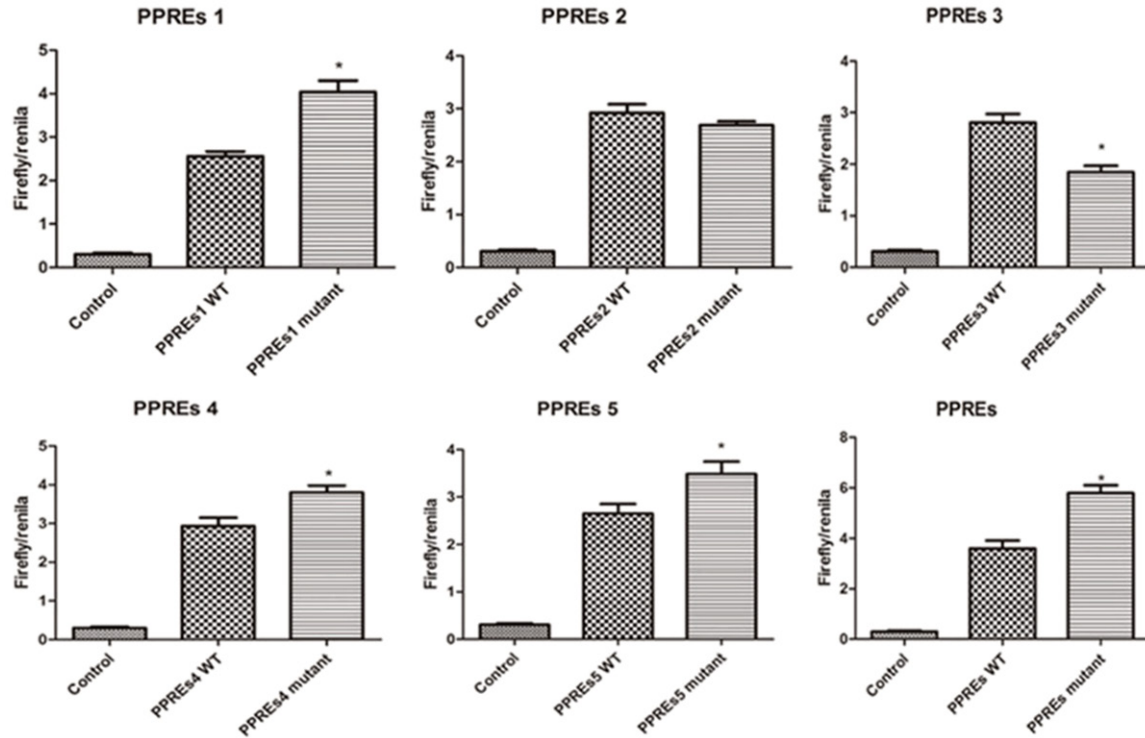
A PPAR $\gamma$  over-expressed vector and a PPAR $\gamma$  silenced vector were transfected into the endothelial cells to achieve a PPAR $\gamma$  over-expressed and a PPAR $\gamma$  knock-down endothelial cells, respectively. As shown in [Figure S2A](#), PPAR $\gamma$  was expressed as extremely higher level in the cells from PPAR $\gamma$  group than that from control group (\* $P < 0.05$  vs ESC), which indicated that the PPAR $\gamma$  over-expressed endometrium cell model was successfully established. As sh-

own in [Figure S2B](#), PPAR $\gamma$  in the cells from sh-PPAR $\gamma$  group was greatly down-regulated (\* $P < 0.05$  vs NSC), which indicated that the PPAR $\gamma$  silenced endometrium cell model was also successfully established.

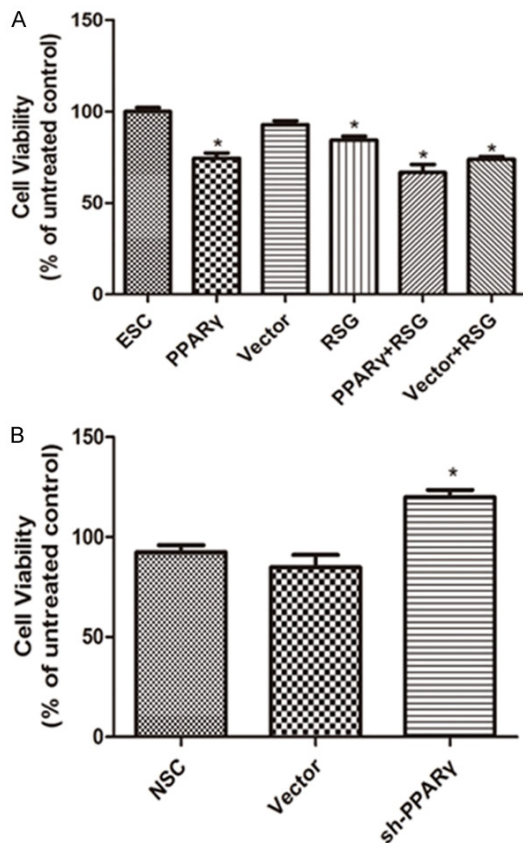
### The combination of PPAR $\gamma$ with the promoter of MAT2A detected by dual luciferin

To explore the interaction between PPAR $\gamma$  and the promoter of MAT2A, 6 sequences from the PPRES, including a total sequence and 5 partial sequences, were utilized to conduct the dual luciferin reporter assay. As shown in [Figure 2](#), the fluorescence intensity of PPRES, PPRES1, PPRES4 and PPRES5 mutant group increased greatly, compared with that of wild type group, respectively (\* $P < 0.05$  vs wild type). The results indicated that PPAR $\gamma$  may have the ability of combining PPRES total sequence, PPRES1, PPRES4 and PPRES5 sequence of the promoter of MAT2A.

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**Figure 2.** The correlation between PPAR $\gamma$  and the promoter of MAT2A was evaluated by the dual luciferin reporter assay (\* $P < 0.05$  vs wild type).



**Figure 3.** The proliferation ability of endometrium cells from each group. A. The cell viability of endometrium cells in ESC, PPAR $\gamma$ , vector, RSG, PPAR $\gamma$ +RSG and vector+RSG group was determined by CCK8 assay, respectively (\* $P < 0.05$  vs ESC). B. The cell viability of endometrium cells in NSC, vector and sh-PPAR $\gamma$  group was detected by CCK8 assay, respectively (\* $P < 0.05$  vs NSC).

*The proliferation ability of endometrium cells from each group*

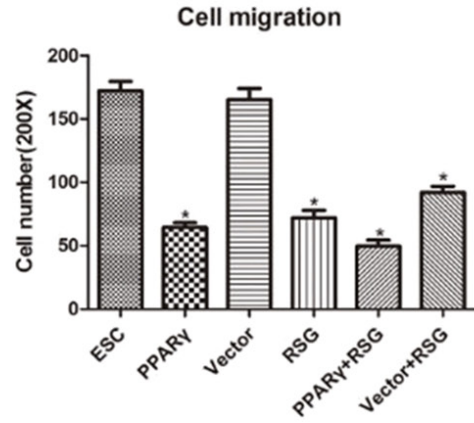
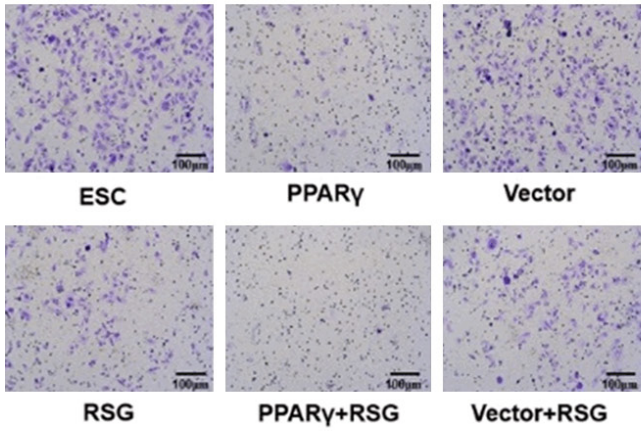
**Figure 3A** showed that the cell viability of endometrium cells from PPAR $\gamma$ , RSG, PPAR $\gamma$ +RSG and vector+RSG group was significantly lower than that from ESC group, respectively (\* $P < 0.05$  vs ESC). As shown in **Figure 3B**, the cell viability of endometrium cells from sh-PPAR $\gamma$  group was increased greatly, compared with that from NSC group (\* $P < 0.05$  vs NSC). These results indicated that PPAR $\gamma$  may inhibit the proliferation of endometrium cells.

*The migration and invasion ability of endometrium cells from each group*

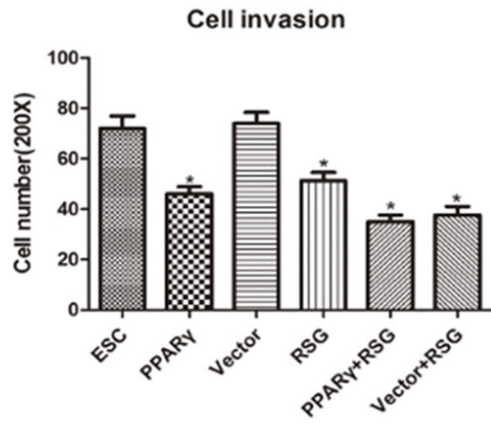
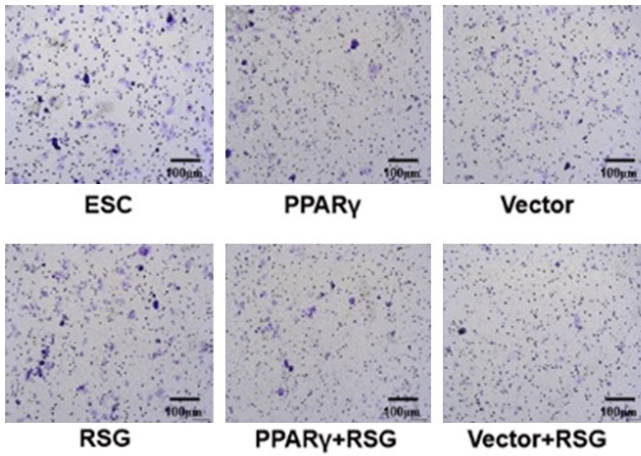
As shown in **Figure 4A** and **4B**, the numbers of cells that migrate or invade over the transwell

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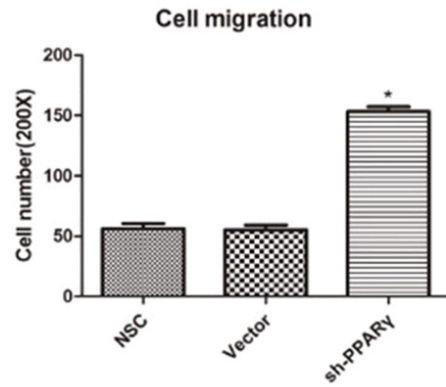
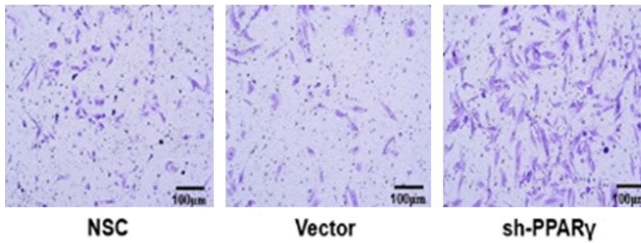
A



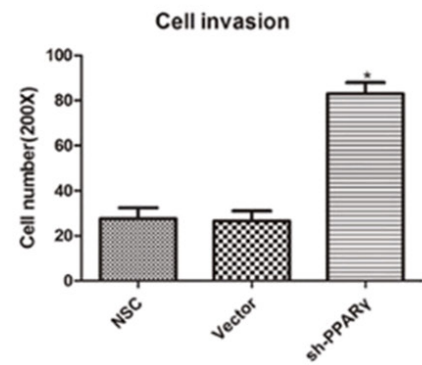
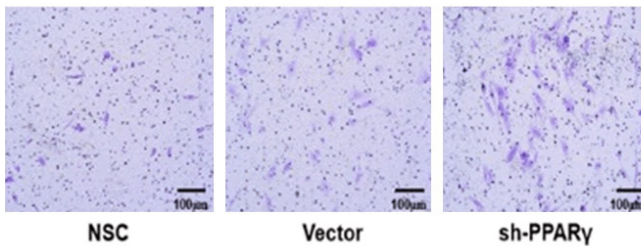
B



C



D



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**Figure 4.** The migration and invasion ability of endometrium cells from each group. A. Cell migration assay was performed on endometrium cells from ESC, PPAR $\gamma$ , vector, RSG, PPAR $\gamma$ +RSG and vector+RSG group (\*P<0.05 vs ESC). B. Cell invasion assay was performed on endometrium cells from ESC, PPAR $\gamma$ , vector, RSG, PPAR $\gamma$ +RSG and vector+RSG group (\*P<0.05 vs ESC). C. Cell migration assay was performed on endometrium cells from NSC, vector and sh-PPAR $\gamma$  group (\*P<0.05 vs NSC). D. Cell invasion assay was performed on endometrium cells from NSC, vector and sh-PPAR $\gamma$  group (\*P<0.05 vs NSC).

declined greatly from PPAR $\gamma$ , RSG, PPAR $\gamma$ +RSG and vector+RSG group, compared with that from ESC group (\*P<0.05 vs ESC). **Figure 4C** and **4D** showed that the numbers of cells that migrate or invade over the transwell from sh-PPAR $\gamma$  group were significantly higher than that from NSC group (\*P<0.05 vs NSC). The results implied that PPAR $\gamma$  may inhibit migration and invasion ability of endometrium cells.

### *The cellular structure of endometrium cells in each group*

TEM was used to detect the cellular microstructure of endothelial cells. As shown in **Figure 5A** and **5B**, the cellular structure of endometrium cells from NSC, vector, and sh-PPAR $\gamma$  group was relatively integrated. The cytomembrane, structure of endoplasmic reticulum and mitochondria of endometrium cells from PPAR $\gamma$  and RSG group was integrated, with chromatin agglutination and a small number of vacuoles in the cytoplasm. Large amounts of vacuoles occur in the cytoplasm of endometrium cells from PPAR $\gamma$ +RSG group, with typical morphological changes of apoptotic cells.

### *The expression level of PPAR $\gamma$ and MAT2A in endometrium cells from each group*

**Figure 6A** showed the mRNA and protein expression levels of PPAR $\gamma$  and MAT2A in the endometrium cells. In sh-PPAR $\gamma$  group, the expression of PPAR $\gamma$  declined and the expression of MAT2A was increased greatly, compared with NSC group (\*P<0.05 vs NSC). As shown in **Figure 6B**, the expression level of MAT2A in the endometrium cells from PPAR $\gamma$ , RSG, PPAR $\gamma$ +RSG and Vector+RSG group was significantly lower than that from ESC group (\*P<0.05 vs ESC). On the contrary, PPAR $\gamma$  in the endometrium cells from above group was greatly up-regulated (\*P<0.05 vs ESC).

## Discussion

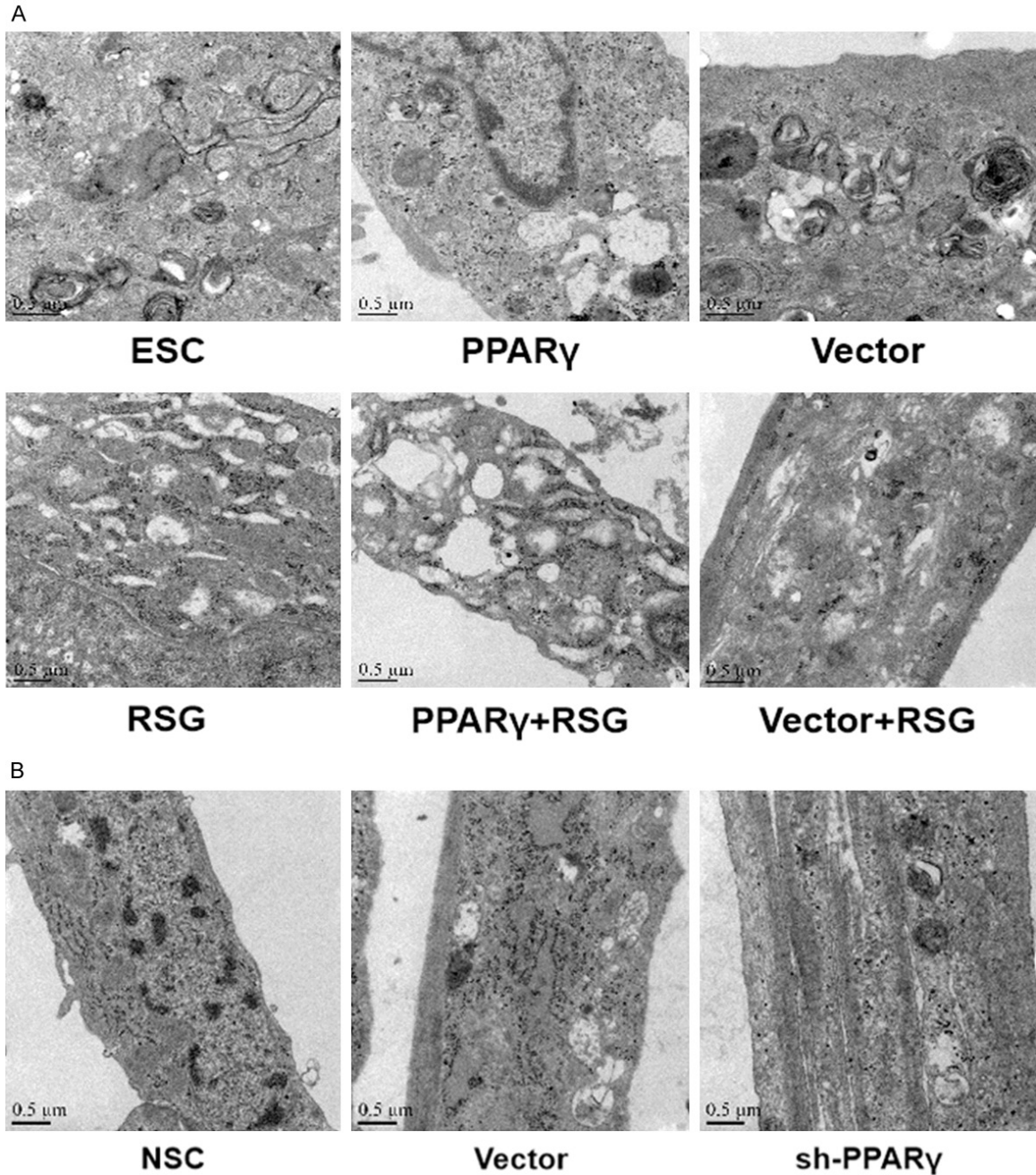
Although endometriosis is a nonmalignant disease, it has the same biological characteris-

tics as tumor, such as easy to relapse and migrate. And no matter for tumor or endometriosis, migration or invasion was a complicated progress regulated by multiple factors, with cell proliferation, angiogenesis, cell adhesion, apoptosis, cell migration and other biological progress involved. Abnormality tends to occur on the biomolecules within the ectopic endometrium from endometriosis patient, including the activation of oncogene [17, 18], the excessive secretion of estrogen, the excessive expression of cytokines, prostaglandins, metalloproteinases and angiogenic factors [19]. In present study, endometrium cells were isolated from the endometriosis patients in our department. The agonists of PPAR $\gamma$  were reported to be potential inhibitors of cell proliferation [20] and could induce apoptosis [21]. They also could exert anti-angiogenesis effect by reducing the expression level of VEGF [22]. It is reported that the formulation of ectopic endometrium vascular was inhibited and the ectopic lesions was narrowed when RSG, an agonist of PPAR $\gamma$ , was used to treat endometriosis patient [23-25]. In present study, optimal concentration of RSG was picked out based on the impact of RSG on cell proliferation.

It is reported that the mRNA and protein expression level of MAT2A in the endometrium from endometriosis patients was more than 3 times that from control [26], which indicated that the abnormal expression of MAT2A was closely related with the paroxysm of endometriosis. Prachi [27] reported that the expression level of MAT2A increased greatly when T cells was activated or deteriorated, which would result in the activation of cell proliferation and induce apoptosis [28]. It is reported that in T cells, the activity of methionine adenosyltransferase (MAT) II was inhibited and the expression of apoptosis related factor ligands (FasL) was induced by silencing the expression of MAT2A, which would lead to the up-regulation of caspase-3 and induce apoptosis [29]. Komal Ramani [30] reported that cis-acting element (PPREs) was involved in the promoter of MAT2A in the hematopoietic



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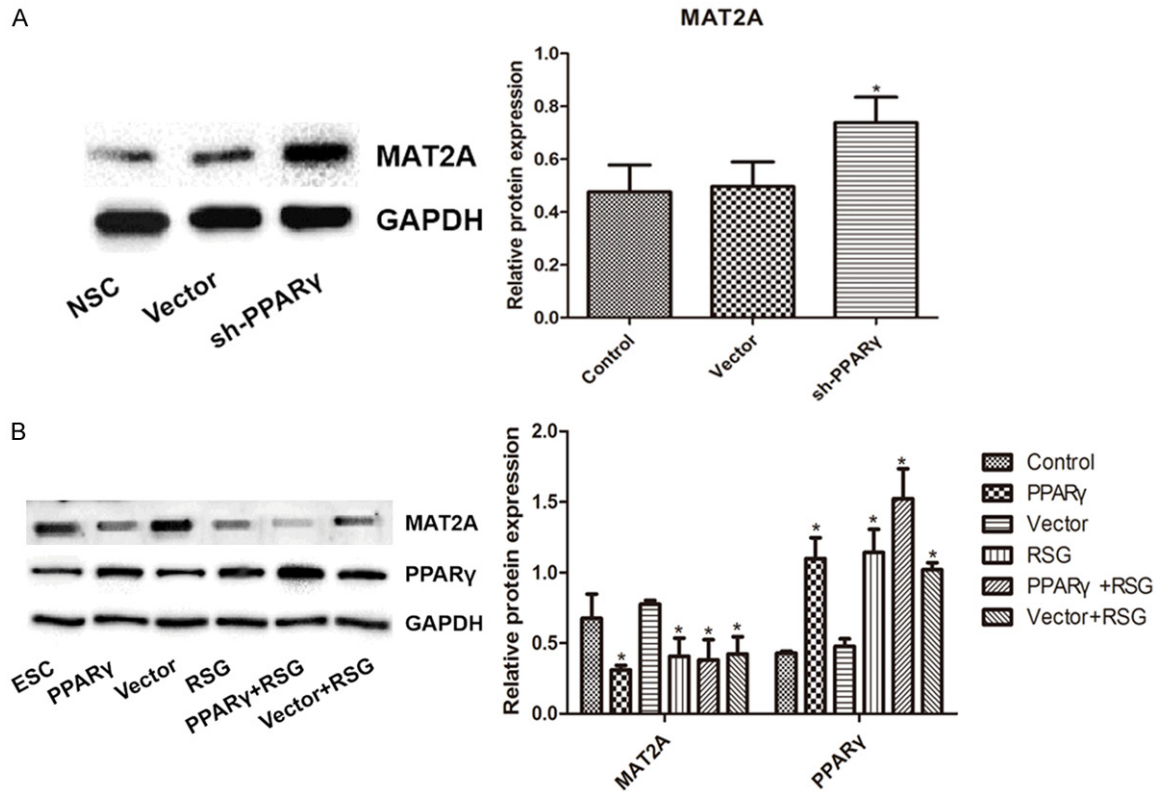


**Figure 5.** The cellular structure of endometrium cells in each group detected by transmission electron microscope. A. The cellular structure of endometrium cells from ESC, PPAR $\gamma$ , vector, RSG, PPAR $\gamma$ +RSG and vector+RSG group. B. The cellular structure of endometrium cells from NSC, vector and sh-PPAR $\gamma$  group.

stem cells (HSCs) of rats and could interact with the trans-acting factor of PPAR $\gamma$  to regulate the inactive or active of cells. RSG was reported to inhibit the expression of MAT2A and the activity of MAT2A's promoter, in which way, the activity of MAT2A and the combination with its promoter was inhibited. Also RSG could induce the combination of PPAR $\gamma$  with

MAT2A PPREs to prevent its transcriptional activity [31]. Present study indicated that the promoters of MAT2A (PPREs1, PPREs4 and PPREs5), could combine with PPAR $\gamma$ . In the PPAR $\gamma$  over-expressed or RSG incubated endometrium cells, PPAR $\gamma$  was up-regulated and MAT2A was down-regulated greatly. These results indicated that there were negative corre-

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**Figure 6.** The expression level of PPAR $\gamma$  and MAT2A in endometrium cells from each group. A. The expression level of PPAR $\gamma$  and MAT2A was detected by qRT-PCR and Western Blot in the endometrium cells from ESC, PPAR $\gamma$ , vector, RSG, PPAR $\gamma$ +RSG and vector+RSG group, respectively (\* $P < 0.05$  vs ESC). B. The expression level of PPAR $\gamma$  and MAT2A was detected by qRT-PCR and Western Blot in the endometrium cells from NSC, vector and sh-PPAR $\gamma$  group, respectively (\* $P < 0.05$  vs NSC).

lation between the expression level of PPAR $\gamma$  and MAT2A.

The activation of PPAR $\gamma$  was involved in multiple physiological and pathological process. Current researches indicated that the activation of PPAR $\gamma$  could exert anti-gynecologic malignant tumor activity, which may be related to its apoptotic inducing ability [32]. The activation of PPAR $\gamma$  was also reported to be effective in the protection of nervous system [33], which was related to its activity of anti-inflammatory and antioxidant stress response [34, 35]. In present study, PPAR $\gamma$  was highly expressed in endometrium cells by transfecting the vector over-expressed with PPAR $\gamma$ . It is reported that in multiple types of tumor cells, after PPAR $\gamma$  was activated, the proliferation of tumor cells was inhibited, which resulted in the differentiation and apoptosis of tumor cells [36-39]. Present study indicated that the proliferation ability of endometrium cells declined greatly after over-expressed with PPAR $\gamma$  or

incubated with RSG, along with the decrease of migration and invasion ability. In addition, classical morphological changes of apoptotic cells were observed. On the contrary, the ability of proliferation, migration and invasion was improved by silencing the expression of PPAR $\gamma$  in normal endometrium cells. These results indicated that the expression of PPAR $\gamma$  has great impact on the paroxysm of endometriosis.

Taken together, over-expressed with PPAR $\gamma$  or incubated with RSG in ESC could decline the ability of proliferation, migration, and invasion of cells by decreasing the transcriptional activity of MAT2A gene. In addition, the in vitro characteristic of ESC could be achieved by silencing the expression of PPAR $\gamma$  in NSC.

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Qi Chen, The First Affiliated Hospital of Nanchang University, No. 17 Yongwaizheng Street, Donghu District, Nanchang, Jiangxi, People's Republic of China. E-mail: chenqi\_112cq@126.com

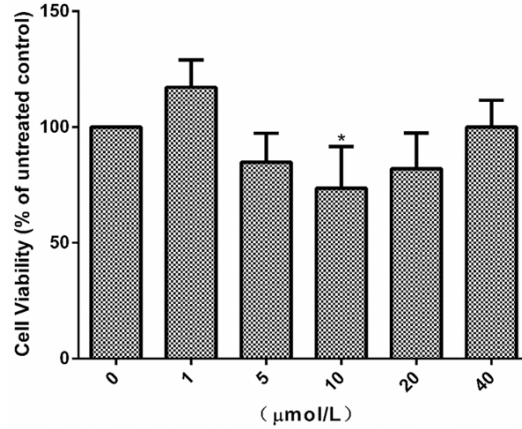
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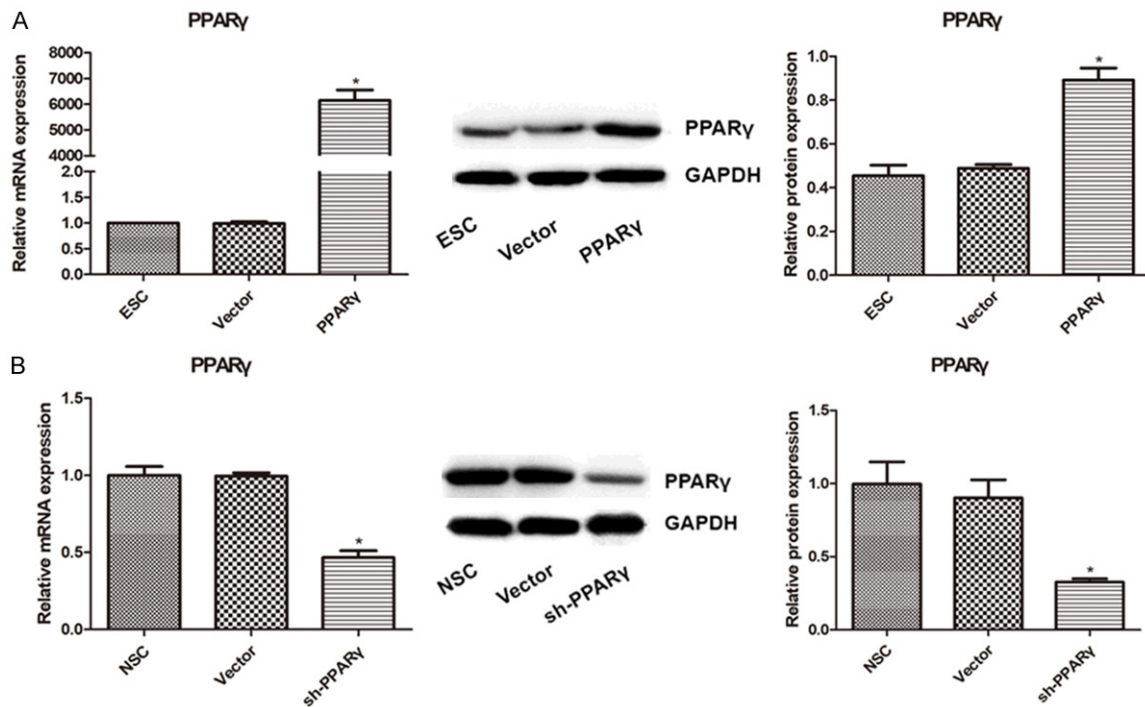
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**Figure S1.** The viability of cells was detected by CCK8 assay after incubating with different concentrations of RSG (\* $P < 0.05$  vs control).



**Figure S2.** The evaluation of the transfection of PPAR $\gamma$  over-expressed vector and PPAR $\gamma$  silenced vector. A. The relative mRNA expression level and protein expression level of PPAR $\gamma$  in the cells from ESC, vector and PPAR $\gamma$  group were detected by qRT-PCR and Western Blot, respectively (\* $P < 0.05$  vs ESC). B. The relative mRNA expression level and protein expression level of PPAR $\gamma$  in the cells from NSC, vector and sh-PPAR $\gamma$  group were detected by qRT-PCR and Western Blot, respectively (\* $P < 0.05$  vs NSC).