

MECHANISM OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ AGONISTS IMPROVING INSULIN RESISTANCE IN GESTATIONAL DIABETES

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Abstract

This study examined how the Peroxisome Proliferator-Activated Receptor γ (PPAR γ) agonists rosiglitazone and GW1929 influence insulin resistance and cellular behavior in trophoblasts from gestational diabetes mellitus (GDM) with particular attention to the High Mobility Group Box 1 (HMGB1)/Nuclear Factor Kappa-B (NF- κ B) pathway. Primary trophoblast cells were assigned to four groups: untreated controls, insulin resistance (IR) induced with 10 μ mol/L insulin, and two IR groups additionally treated with 10 μ mol/L rosiglitazone or GW1929. Proliferation, apoptosis, and HMGB1/NF- κ B pathway activity were then compared across groups using standard statistical methods. Compared with controls, IR-treated cells showed lower glucose consumption and proliferation, higher apoptosis, and elevated HMGB1, NF- κ B p65 (mRNA and protein), along with increased TNF- α , IL-6, IL-17, TLR4, hs-CRP, and GSK-3 β ($p < 0.05$). Both rosiglitazone and GW1929 reversed these changes: glucose consumption and proliferation rose, apoptosis fell, and HMGB1, NF- κ B p65, and the inflammatory markers listed above all decreased relative to the IR group ($p < 0.05$). Together, these findings indicate that PPAR γ agonists can alleviate insulin resistance in GDM trophoblasts, support proliferation, and suppress apoptosis and inflammation, likely by dampening HMGB1/NF- κ B pathway activation.

Rezumat

Studiul a evaluat modul în care agonistii receptorului activat de proliferatorii peroxizomali gamma (*Peroxisome Proliferator-Activated Receptor γ* , PPAR γ), rosiglitazona și GW1929, influențează rezistența la insulină și comportamentul celular al trofoblastelor provenite din sarcini complicate de diabet zaharat gestațional (GDM), cu o atenție deosebită asupra căii de semnalizare High Mobility Group Box 1 (HMGB1)/Nuclear Factor Kappa-B (NF- κ B). Celulele trofoblastice primare au fost repartizate în patru grupuri: un grup martor netratat, un grup cu rezistență la insulină (IR) indusă prin administrarea a 10 μ mol/L insulină și două grupuri cu IR tratate suplimentar cu 10 μ mol/L rosiglitazonă, respectiv GW1929. Ulterior, proliferarea celulară, apoptoza și activitatea căii HMGB1/NF- κ B au fost comparate între grupuri. Comparativ cu grupul martor, celulele cu IR au prezentat un consum redus de glucoză și o capacitate proliferativă diminuată, o rată crescută a apoptozei și niveluri crescute de HMGB1 și NF- κ B p65 (ARNm și proteină), precum și concentrații crescute ale TNF- α , IL-6, IL-17, TLR4, hs-CRP și GSK-3 β ($p < 0,05$). Atât rosiglitazona, cât și GW1929 au crescut consumul de glucoză și proliferarea celulară, au redus apoptoza, iar expresia HMGB1, NF- κ B p65 și a markerilor inflamatori menționați anterior a fost semnificativ diminuată comparativ cu grupul IR ($p < 0,05$). În concluzie, aceste rezultate indică faptul că agonistii PPAR γ pot ameliora rezistența la insulină în trofoblastele asociate diabetului zaharat gestațional, pot stimula proliferarea celulară și pot inhiba apoptoza și inflamația, cel mai probabil prin suprimarea activării căii de semnalizare HMGB1/NF- κ B.

Keywords: gestational diabetes mellitus; PPAR γ agonists; HMGB1/NF- κ B p65 pathway; trophoblast cells; insulin resistance

Introduction

Insulin resistance (IR) and glucose metabolism disorders are considered pivotal physiological and pathological mechanisms underlying Gestational Diabetes Mellitus (GDM). The IR denotes an abnormal state where the biological effects of a certain amount of insulin production fail to meet expected levels. During pregnancy, hormones such as gestational hormones, prolactin and placental growth hormone all play roles in impairing insulin function (1, 2). Additionally, IR can trigger inflammatory responses, thereby impacting the normal progression of pregnancy (3). The HMGB1

engages in chronic inflammation by activating NF- κ B p65 through TLR (4).

The PPAR γ exhibits diverse biological functions, including regulation of metabolism, modulation of inflammatory responses, improvement of atherosclerosis and inhibition of tumorigenesis (5, 6). Moreover, PPAR γ can regulate the homeostasis of glucose metabolism, thus emerging as a critical target for treating type 2 diabetes mellitus. Li *et al.* (7) and Ni *et al.* (8) demonstrated that PPAR γ acts in regulating the maturation of human placental trophoblast cells, as well as modulating intracellular lipid accumulation and placental fatty acid transport, thereby

contributing to the maintenance of normal pregnancy processes. Consequently, abnormalities in PPAR γ function are closely associated with various pathological pregnancy outcomes. The PPAR γ agonists can enhance tissue sensitivity to insulin, decrease resistance in expression, improve IR, lower blood glucose (BG) levels and promote adipocyte differentiation while reducing lipid accumulation (9). Rosiglitazone, a PPAR γ agonist, belongs to the thiazolidinedione class of insulin sensitizers. Its mechanism is associated with the activation of the gamma subtype receptor of specific PPAR γ (10).

Rosiglitazone can increase the sensitivity of tissues such as skeletal muscle, liver and adipose tissue to insulin, enhance cellular glucose utilization and consequently lower BG levels while improving IR (11). Its primary characteristic lies in directly reducing IR, improving β -cell function and exerting long-term and sustained BG control. It is effective when utilized alone or in combination with other antidiabetic agents (12). However, in October 2017, Rosiglitazone was categorized as a Group 3 carcinogen. The GW1929 is a potent PPAR γ agonist belonging to the non-thiazolidinedione class of insulin sensitizers. It participates in regulating cell growth and gene expression and possesses anti-diabetic and neuroprotective effects (13). The GW1929 can reverse the excessive generation of reactive oxygen species in cisplatin-induced acute kidney injury cells, thereby mitigating renal tubular epithelial cell damage by inhibiting oxidative stress and cell apoptosis, ultimately exerting a renal protective effect (14). Moreover, in peri-menopausal rat models, GW1929 administration has been shown to reduce apoptosis in uterine and ovarian cells and improve uterine, ovarian and bone metabolism functions (15). However, the molecular mechanisms underlying the improvement of IR in GDM placental trophoblast cells by PPAR γ agonists, including GW1929, require further investigation.

To address this, this work isolated and cultured primary placental trophoblast cells from patients with GDM. The aim was to provide a reference for understanding the molecular mechanisms by which PPAR γ agonists improve IR in GDM.

Materials and Methods

Case information

Placental tissues from patients with GDM who underwent elective caesarean section in Taiyuan Maternal and Child Health Hospital from March 2023 to October 2025 were selected. A total of 153 patients met the diagnostic criteria for GDM, defined as fasting BG > 5.1 mmol/L 1 hr postprandial BG > 10.0 mmol/L or 2 hrs postprandial BG > 8.5 mmol/L during oral glucose tolerance test. Patients underwent caesarean section delivery, had singleton pregnancies and had no history of other diseases. Patients

with pregnancy complications such as preeclampsia, foetal intrauterine growth retardation, GDM and pre-term birth were excluded. This study was approved by the ethics committee of Taiyuan Maternal and Child Health Hospital and all participants provided informed consent before inclusion.

Isolation and cultivation of primary trophoblast cells

Within 30 min of placental delivery, approximately 50 g of fresh chorionic villi tissue from the placental trophoblast layer was obtained. The tissue was washed in pre-cooled physiological saline to remove impurities and blood. After thorough cutting, it was digested by shaking at 37°C and 120 rpm for 3 min. After adding 10 times the volume of pre-warmed foetal bovine serum (Gibco, USA) at 37°C, the supernatant was centrifuged at 1000 rpm for 10 min. Subsequently, the supernatant was discarded and the cell pellet was resuspended at 10 times the volume of pre-warmed Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) at 37°C. Cell digestion was repeated three times with the a procedure The suspension was collected and filtered through a 150 mesh sieve and the cell pellet was resuspended in a pre-warmed DMEM culture medium at 37°C. Cell separation was performed using a 5 - 70% Percoll density gradient separation solution, with the fraction between 35 - 50% Percoll density serving as the trophoblast cell fraction, with a density of approximately 1.048 - 1.062 g/mL. The cells were subjected to short tandem repeat (STR) analysis and Mycoplasma testing. The isolated trophoblast cells were resuspended in a pre-warmed DMEM culture medium at 37°C and their viability was confirmed (> 90%) using trypan blue staining.

Cell grouping and treatments

Primary trophoblast cells were cultured in DMEM (10% foetal bovine serum and 1% penicillin-streptomycin) and incubated at 37°C with 5% CO₂. Upon reaching 80% confluence, cells were dissociated using 0.25% trypsin and adjusted to 1×10^5 cells/mL. Subsequently, the cells were seeded into 12-well plates and maintained in routine culture for 24 hrs. Subsequently, cells were subjected to group treatments as follows: The Ctrl group consisted of primary trophoblast cells cultured under standard conditions. The IR group was treated with medium+10 mol/L insulin for 48 hrs. The IR+PPAR γ agonist Rosiglitazone (IR+Rsg) group was treated with medium+10 mol/L insulin for 24 hrs, followed by a change to medium+10 μ mol/L Rosiglitazone (Med Chem Express, USA) for continued culture. The IR+PPAR γ agonist GW1929 (IR+GW1929) group was treated with medium+10 mol/L insulin for 24 hrs, followed by a change to medium+10 μ mol/L GW1929 (Med Chem Express, USA) for continued culture.

Glucose consumption experiment

The glucose consumption assay was performed to assess the cellular IR status. The supernatant from each group of cells was centrifuged at 1000 rpm for

5 min and utilized for glucose content determination using a glucose assay kit (Shanghai Kehua Bio-Engineering Co., Ltd., China). Absorbance was measured at 505 nm utilizing a microplate reader (Varioskan LUX, Thermo Fisher Scientific, USA), with three replicate wells per group.

Real time fluorescence quantitative PCR detection of mRNA expression level

Cells from each group were lysed using Trizol to extract total RNA. After assessment of the concentration and purity of the extracted RNA, cDNA synthesis utilized the PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time) following instructions. The PCR reactions were performed utilizing TB Green® Premix Ex Taq™ II (Tli RNaseH Plus), Bulk kit. Reaction conditions comprised pre-denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec and extension at 72°C for 32 sec. The GAPDH served as the reference gene and the relative expression levels of HMGB1 and NF-κB p65 (encoded by RELA gene) were determined utilizing $2^{-\Delta\Delta CT}$ methodology. Primer sequences for PCR were employed as follows: HMGB1, forward 5'-GCTGACAAGGCTCGTTATGA-3', reverse 5'-CCTTTGATTTTGGGGCGGTA-3'; NF-κB p65(RELA), forward 5'-AACAGAGAGGATTTTCGTTTCCG-3', reverse 5'-TTTGACC TGAGGGTAA GACTTCT-3'; GAPDH, forward 5'-CTCACCAGGATGCACCAATGTT-3', reverse 5'-CGCGTTGCTCA CAATGTTTCAT-3'. Each experiment was performed in triplicate. All reagents were from Takara Biological Technology (Beijing) Co., Ltd., China.

Western blotting

Cells from each group were lysed using radioimmunoprecipitation assay (RIPA) buffer to extract total protein and its concentration was determined with bicinchoninic acid assay (Shanghai Beyotime Biotechnology Co., Ltd., China). Fifty micrograms of protein were loaded and separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by transfer onto polyvinylidene fluoride (PVDF) membranes and blocking with 5% skim milk powder for 2 hrs. Primary antibodies against HMGB1 (Catalog No. ab18256), NF-κB p65 (Catalog No. ab16502) and β-actin (Catalog No. ab8227) (diluted 1:2000; Abcam, UK) were applied and incubated overnight at 4°C. After membrane washing, secondary antibodies labelled with horseradish peroxidase (Catalog No. ab205718, diluted 1:5000; Abcam, UK) were applied and incubated at 25°C for 2 hrs. Membranes were developed using enhanced chemiluminescence reagents (Shanghai Beyotime Biotechnology Co., Ltd., China) to visualize protein bands with a gel imaging system. The β-actin served as internal control and Image J1.8.0.345 software (National Institutes of Health, America) determined relative grayscale values of target protein bands. Each experiment was conducted in triplicate.

Cell counting kit-8 (CCK-8) and EdU fluorescence staining for detecting cell proliferation activity

The cells utilized in this work were primary trophoblast cells in logarithmic growth phase, which were seeded into a 96-well plate at 5×10^3 cells per well and cultured for 48 hrs after grouping. Regarding instructions of CCK-8 assay kit (Beijing Solarbio Science and Technology Co., Ltd., China), CCK-8 reagent (10 μL) was applied and incubated for 2 hrs. Absorbance at 450 nm was then measured utilizing a microplate reader, with three replicate wells per group. Cells were seeded into a 24-well plate at 2×10^4 cells/well and cultured for 48 hrs after grouping. The original medium was discarded and cells were fixed with 4% paraformaldehyde at 25°C for 15 min. Then, 50 mmol/L EdU staining solution (Shanghai Beyotime Biotechnology Co., Ltd., China) was applied and cells were incubated at 37°C in the dark for 2 hrs. Afterward, 0.5 mL of Hoechst 33342 staining solution (Hyclone, USA) was applied and cells were incubated at 25°C in the dark for 15 min to visualize nuclear staining. Fluorescence intensity was determined utilizing ImageJ analysis software by randomly selecting five fields of view and calculating the EdU-positive expression rate. Each experiment was repeated thrice.

Flow cytometry and Hoechst 33342 fluorescence staining for detecting cell apoptosis

Cells were seeded into 6-well plates at 1×10^5 cells/well and cultured for 48 hrs after grouping. Following digestion with 0.25% trypsin, cells were rinsed with phosphate buffered saline (PBS). Cell apoptosis rate (AR) was assessed following the Annexin V-FITC/PI Cell Apoptosis Detection kit protocol (Shanghai Sigma-Aldrich, China). Cells were suspended in $1 \times$ binding buffer, to which 5 μL of Annexin V-FITC binding solution and 10 μL of PI binding solution were added per well. The mixture was gently mixed and left to incubate at 25°C in the dark for 15 min. Cell AR was promptly analysed utilizing a flow cytometer (CytoFLEX, Beckman Coulter, USA). Each experiment was replicated thrice.

Cells were seeded into 24-well plates at 2×10^4 cells/well and cultured for 48 hrs after grouping. After the original medium was discarded, cells were fixed with 4% paraformaldehyde overnight at 25°C. Subsequently, cells were permeabilized with 0.5% Triton X-100 for 15 min, washed and incubated with 0.5 mL of Hoechst 33342 staining solution at 25°C in the dark for 30 min. After mounting with neutral gum, fluorescence intensity was measured using ImageJ analysis software by randomly selecting five fields of view and calculating the Hoechst 33342-positive expression rate. Each experiment was repeated thrice.

ELISA (enzyme-linked immunosorbent assay) for detecting inflammatory factors

The cell culture supernatants from each group were centrifuged at 12,000 rpm for 10 min. The supernatants

were utilized for the detection of Tumor Necrosis Factor (TNF)- α , Interleukin (IL)-6, IL-17, Toll-Like Receptor 4 (TLR4), High-Sensitivity C-Reactive Protein (hs-CRP) and Glycogen Synthase Kinase-3 β (GSK-3 β) expression levels, regarding instructions of the assay kit: TNF- α ELISA Kit (KET0001; Shanghai Keyi Biotechnology Co., Ltd., China), IL-6 ELISA Kit (KET0006; Shanghai Keyi Biotechnology Co., Ltd., China), IL-17 ELISA Kit (KET017; Shanghai Keyi Biotechnology Co., Ltd., China), TLR4 ELISA Kit (KETL04; Shanghai Keyi Biotechnology Co., Ltd., China), hs-CRP ELISA Kit (KETCRP01; Shanghai Keyi Biotechnology Co., Ltd., China), GSK-3 β ELISA Kit (KETGSK3 β 01; Shanghai Keyi Biotechnology Co., Ltd., China). Each experiment was performed in triplicate.

Statistical analysis

Data were denoted as Mean $\pm$ Standard Deviation. Employing SPSS 26.0 (IBM, USA), a Student's *t*-test was adopted for comparisons between groups, while one-way analysis of variance followed by a LSD-*t* test was applied for comparisons among groups. The $p < 0.05$ meant statistically significant.

Results and Discussion

Impact of PPAR γ agonists on IR in cultured cells under IR conditions

Glucose consumption demonstrated the impact of PPAR γ agonists on IR in trophoblast cells of GDM patients under IR conditions (Figure 1). It was found that the glucose consumption of cells in IR group was greatly inferior to Ctrl group ($p < 0.05$), while that in IR+Rsg group and IR+GW1929 group was markedly superior to IR group ($p < 0.05$).

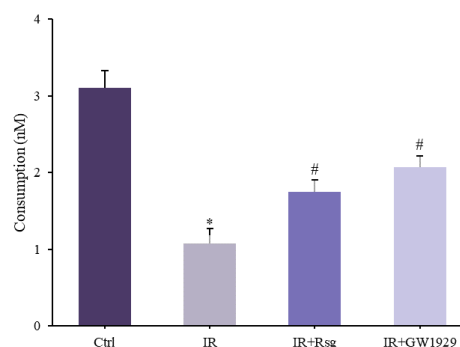


Figure 1.

Comparison of glucose consumption among different groups of cultured cells

* $p < 0.05$ vs. Ctrl group and # $p < 0.05$ vs. IR group

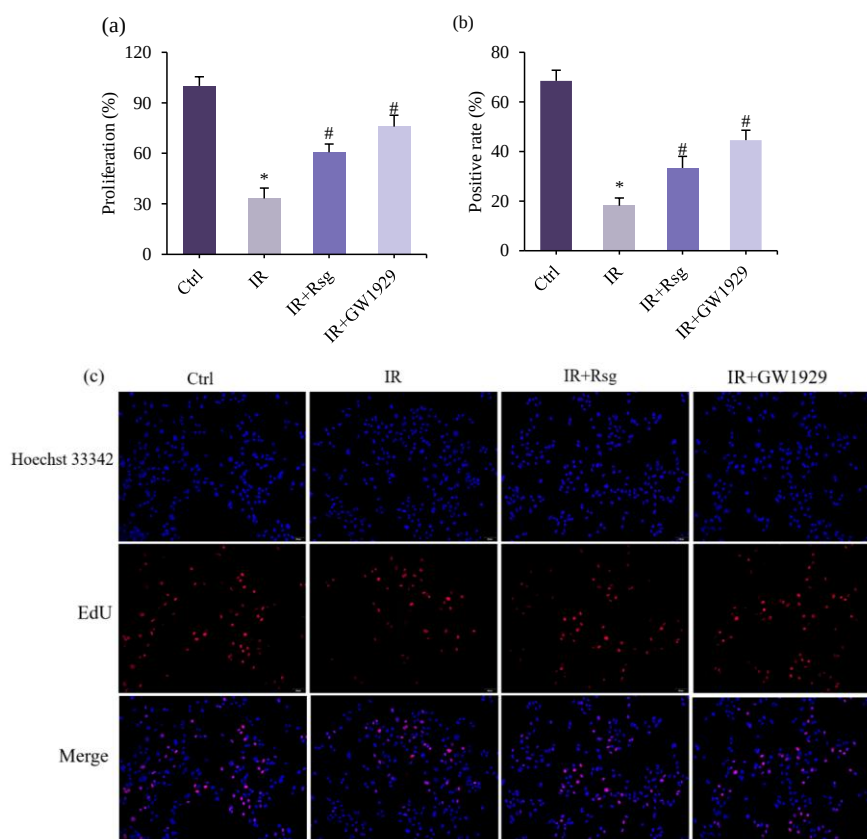


Figure 2.

Comparison of PRs among different groups of cultured cells; (a) Cell PR measured by the CCK-8 assay; (b) Cell EdU-positive staining rate; (c) Fluorescent staining observation of cells ($\times 400$)

* $p < 0.05$ vs. Ctrl group and # $p < 0.05$ vs. IR group

Effects of PPAR γ agonists on cell proliferation in cultured cells under IR conditions

Influence of PPAR γ agonists on proliferation rate (PR) of trophoblast cells from GDM patients under IR conditions was explored (Figure 2). The cell PR and EdU positivity rate in the IR group were significantly lower than those in the Ctrl group ($p < 0.05$) (Figure 2a). In contrast, the IR+Rsg and IR+GE1929 groups exhibited significantly higher cell PRs and EdU positivity rates compared to the IR group ($p < 0.05$) (Figure 2b). The Hoechst 33342 staining labels live

cell nuclei with blue fluorescence, while EdU staining marks cell nuclei with red fluorescence (Figure 2c).

Effects of PPAR γ agonists on apoptosis of cultured cells under IR conditions

The effects of PPAR γ agonists on the AR of trophoblast cells from GDM patients under IR conditions were illustrated in Figure 3. The AR of cells in IR group was markedly superior to Ctrl group ($p < 0.05$), while the AR of cells in IR+Rsg group and IR+GW 1929 group was greatly inferior to IR group ($p < 0.05$).

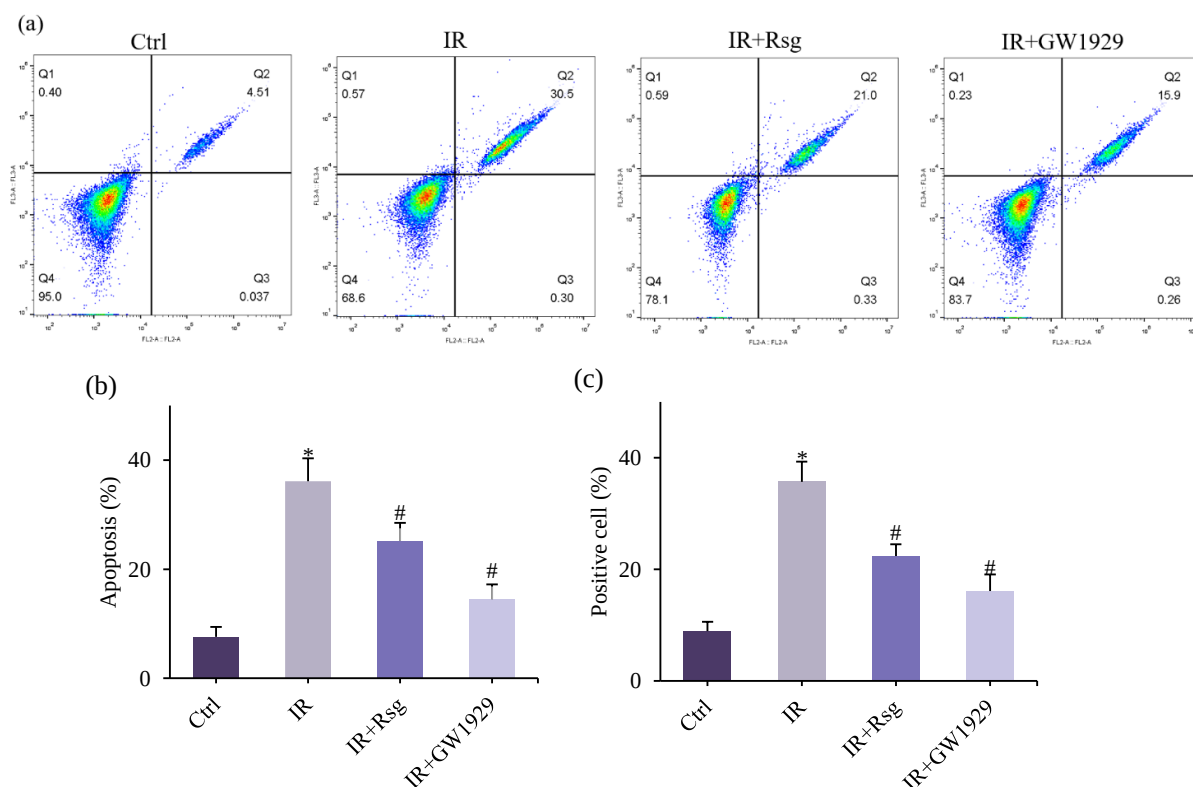


Figure 3.

Comparison of AR among cultured cells in each group; (a) Flow cytometry analysis of cell apoptosis;

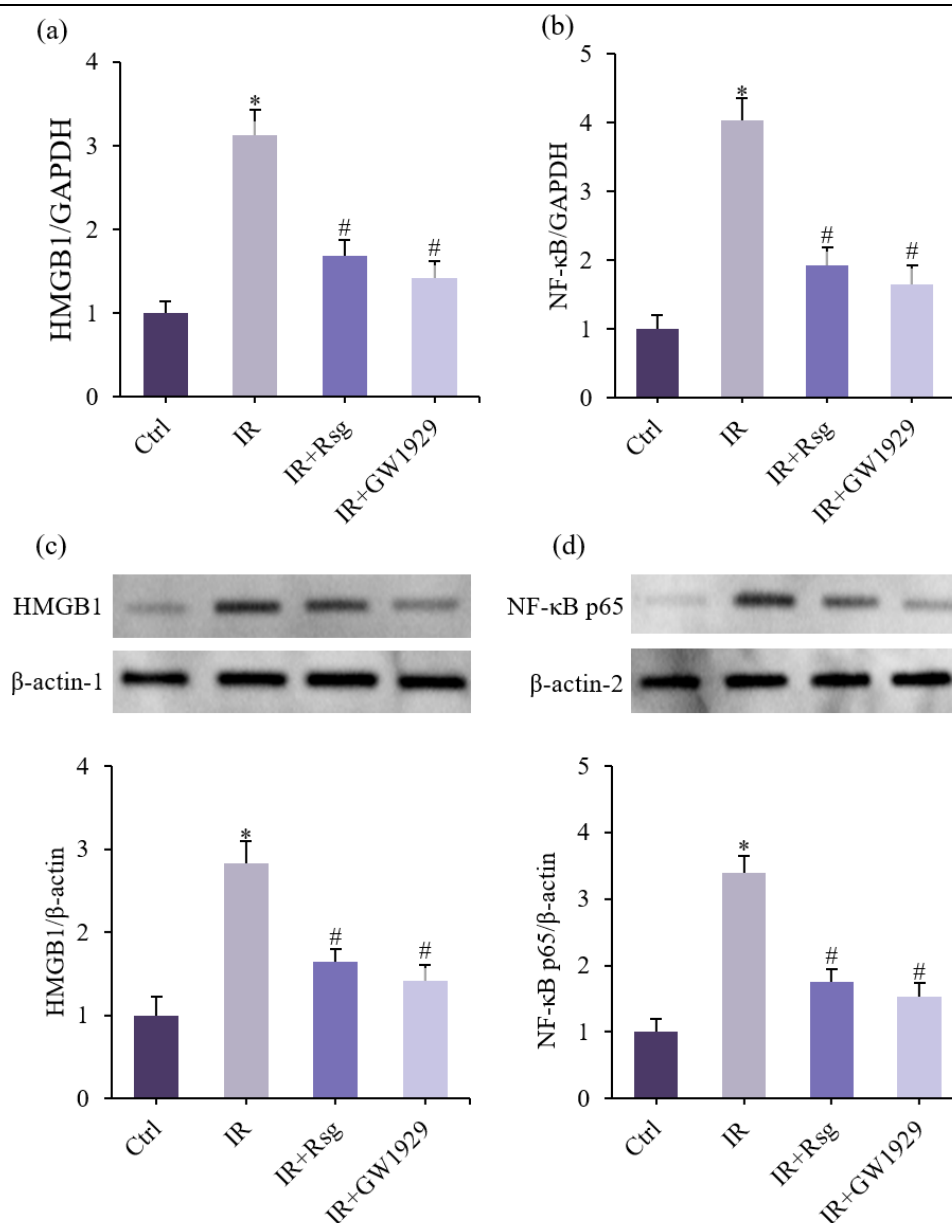
(b) AR of cells; (c) Rate of apoptotic-positive cells

* $p < 0.05$ vs. Ctrl group and # $p < 0.05$ vs. IR group

Impact of PPAR γ agonists on HMGB1/NF- κ B pathway in cultured cells under IR conditions

The impact of PPAR γ agonists on the mRNA expression levels of HMGB1/NF- κ B pathway-related genes in cultured cells was assessed from patients with GDM under IR conditions (Figure 4a and Figure 4b). The relative expression levels of HMGB1 and NF- κ B p65 (RELA) mRNA in IR group were markedly superior to those in Ctrl group ($p < 0.05$), while in the IR+Rsg and IR+GE1929 groups, the relative expression levels of HMGB1 and NF- κ B mRNA were substantially inferior to IR group ($p < 0.05$).

The impact of PPAR γ agonists on the protein expression levels of HMGB1/NF- κ B pathway-related proteins in cultured cells from patients with GDM under IR conditions was illustrated in Figure 4c and Figure 4d. It was found that the relative expression levels of HMGB1 and NF- κ B p65 proteins in IR group were markedly superior to those in Ctrl group ($p < 0.05$). However, in the IR+Rsg and IR+GE1929 groups, the relative expression levels of HMGB1 and NF- κ B p65 proteins were notably lower than IR group ($p < 0.05$).

**Figure 4.**

Comparison of HMGB1 and NF-κB expression levels in cultured cells among different groups;

(a) HMGB1 mRNA expression levels; (b) NF-κB mRNA expression levels;

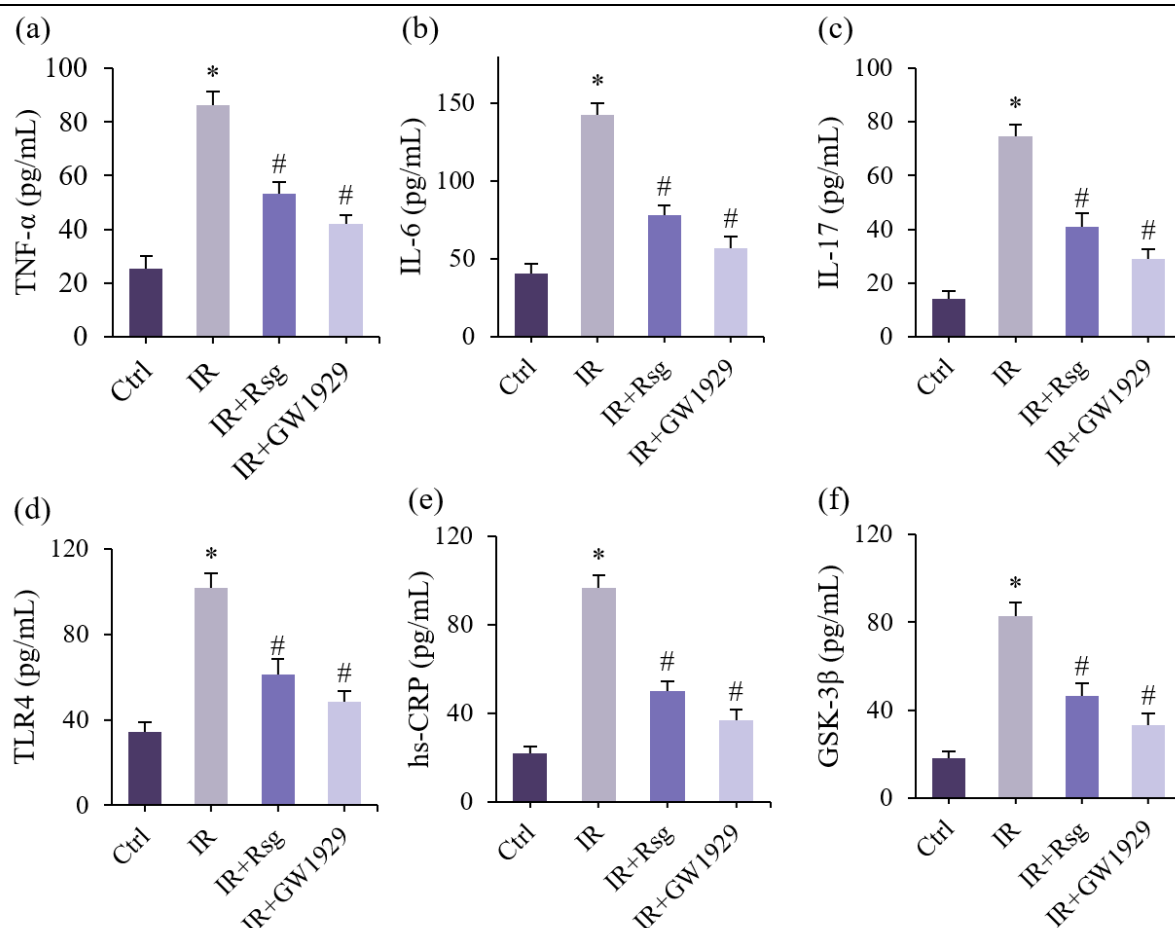
(c) HMGB1 protein expression levels; (d) NF-κB p65 protein expression levels

* $p < 0.05$ vs. Ctrl group and # $p < 0.05$ vs. IR group

Effects of PPAR γ agonists on inflammatory response in IR-conditioned cultured cells

The impact of PPAR γ agonists on the levels of inflammatory factors in cultured cells of GDM patients under IR conditions was assessed using the ELISA method (Figure 5). It was observed that TNF- α (Figure 5a), IL-6 (Figure 5b), IL-17 (Figure 5c), TLR4 (Figure 5d), hs-CRP (Figure 5e) and GSK-3 β (Figure 5f) in IR group cells were markedly superior to those in Ctrl group ($p < 0.05$). Conversely, TNF- α , IL-6, IL-17, TLR4, hs-CRP and GSK-3 β in IR+Rsg and IR+GE1929 group cells were substantially lower than IR group ($p < 0.05$).

The results of this study revealed that treatment with PPAR γ agonists increased glucose consumption, enhanced cell proliferation and reduced apoptosis. Additionally, mRNA and protein expression levels of HMGB1 and NF-κB were decreased and the levels of inflammatory factors (TNF- α , IL-6, IL-17, TLR4, hs-CRP and GSK-3 β) were significantly reduced. These findings indicate that PPAR γ agonists effectively improved IR, promoted cell proliferation and inhibited apoptosis and inflammatory responses, primarily by downregulating the activity of the HMGB1/NF-κB pathway.

**Figure 5.**

Comparison of expression levels of inflammatory factors in cultured cells among different groups;

(a) TNF-α; (b) IL-6; (c) IL-17; (d) TLR4; (e) hs-CRP and (f) GSK-3β

* $p < 0.05$ vs. Ctrl group and # $p < 0.05$ vs. IR group

Trophoblast cells, crucial components of the placenta, are located at the outermost layer of the maternal-foetal barrier. During placental development, these trophoblast cells generate and secrete significant amounts of cytokines and hormones, which regulate placental formation and maintain their normal biological functions (16, 17). However, in the context of gestational diabetes, a hyperglycaemic environment can markedly disrupt the physiological functions of trophoblast cells. Additionally, high glucose levels can stimulate inflammatory responses in the placental trophoblast layer, leading to dysregulated secretion of inflammatory factors and pathological IR in patients with gestational diabetes (18). Glucose uptake, serving as the primary energy source for cell survival, is maintained by normal insulin secretion and functions to stabilize BG levels. The relationship between glucose homeostasis defects and IR is confirmed by the impaired glucose consumption capacity observed in insulin-resistant cell models (19). An insulin-resistant trophoblast cell model was established and a drastic reduction in glucose consumption was observed in this model through glucose consumption experiments, indicating the successful

fabrication of the insulin-resistant model. Many studies applied high-concentration insulin treatment to establish IR models and assessed the extent of IR by evaluating glucose consumption capability through glucose consumption assays (20, 21). Subsequently, the cell model was treated with the PPAR γ agonists Rosiglitazone and GW1929, resulting in a significant increase in glucose consumption after treatment. This suggests that the IR state of the cell model can be improved by PPAR γ agonists, thereby enhancing its glucose consumption capacity. Rosiglitazone, belonging to the thiazolidinedione class of hypoglycaemic agents, can activate PPAR γ within target cells and increase the sensitivity of these cells to insulin, thus improving glucose utilization by target cells (22, 23). Similarly, GW1929 can also activate PPAR γ and exert its biological effects.

Despite the traditional view of insulin as a pro-proliferative and anti-apoptotic factor, exposure of trophoblast cells to insulin results in DNA damage, increased apoptosis and decreased cell survival (24). This study found that the cell PR and EdU positivity rate in the IR group were significantly lower compared to the Ctrl group, while the apoptosis rate was

higher. In contrast, the IR+Rsg and IR+GE1929 groups exhibited higher cell PRs and EdU positivity rates, along with lower apoptosis rates compared to the IR group. These results suggest that under IR conditions, trophoblast cell proliferation activity is reduced and apoptosis is increased in patients with gestational diabetes. However, treatment with Rsg and GE1929 restores trophoblast cell proliferation and reduces apoptosis. This indicates that the PPAR γ agonists Rosiglitazone and GW1929 not only enhance cell proliferation but also significantly decrease apoptosis rates. The PPAR γ agonists may alleviate IR-associated apoptotic stress by modulating intracellular signalling pathways, thereby protecting cell survival. In insulin-resistant states, the proliferative activity of trophoblast cells from GDM patients is reduced, accompanied by an increase in AR (25). Trophoblast cells in GDM patients exhibit abnormal apoptosis, affecting placental tissue growth and leading to foetal overgrowth, emphasizing the critical role of trophoblast cell biology and its regulatory mechanisms in maintaining placental homeostasis. Wójcik *et al.* (26) found that the expression level of PPAR γ in blood leukocytes of GDM patients is markedly superior to that in normal parturient and its expression level is correlated with BG concentration (26). However, Gao *et al.* (27) discovered that the expression level of PPAR γ in adipose and placental tissues of GDM patients is dramatically inferior to that in healthy parturient and it is negatively correlated with glucose levels. The precise mechanisms underlying the role of PPAR γ in regulating GDM remain to be further elucidated. However, overall, given its involvement in glucose homeostasis and adipose tissue development and function, PPAR γ may serve as a key regulator in GDM progression. Current study findings demonstrate that treatment with PPAR γ agonists, Rosiglitazone and GW1929, leads to increased proliferative activity and reduced apoptosis in trophoblast cells under insulin-resistant conditions. This suggests that PPAR γ agonists may exert therapeutic effects on GDM by improving IR and modulating trophoblast cell behaviour.

The HMGB1 is widely expressed in eukaryotic cells and upon stimulation and aberrant expression, it can bind to receptors such as TLR4 and exert biological effects such as inducing inflammation and promoting angiogenesis (28, 29). The HMGB1 can stimulate insulin secretion and is thus an important factor influencing the occurrence of IR. It was found that under insulin-resistant conditions, there was a marked increase in HMGB1 mRNA and protein expression levels in trophoblast cells from GDM patients. This finding is in line with the results reported by Santangelo *et al.* (30), who noted elevated HMGB1 expression level in fetal membrane tissue of GDM patients. Similarly, Giacobbe *et al.* (31) observed increased serum HMGB1 levels in GDM patients, which were

positively correlated with BG levels during the 75 g oral glucose tolerance test. The HMGB1 can interact with the receptor of advanced glycation end products and induce NF- κ B transcription, thereby cooperatively inducing the deactivation of insulin receptor substrates 1 and 2 with inflammatory factors, ultimately leading to the occurrence of IR (32). The NF- κ B is a crucial class of inflammatory-related transcription factors participating in regulating the transmission of various inflammation-related genes and signaling pathways. The NF- κ B complex (*via* its p65 subunit) can induce the differentiation of macrophages and lymphocytes through cytokine stimulation, ultimately regulating inflammatory responses (33). Additionally, NF- κ B is involved in regulating the release of inflammatory factors (34). Under insulin-resistant conditions, there was a prominent increase in NF- κ B p65 (RELA) mRNA and protein expression levels in trophoblast cells from GDM patients. This finding was in line with those reported by Liu *et al.* (35), who found that NF- κ B/NLRP3 was activated in GDM mice, leading to increased expression levels. The HMGB1 and NF- κ B are both markers of chronic inflammation and their levels are associated with GDM and IR. Furthermore, this study found that treatment with the PPAR γ agonists Rosiglitazone and GW1929 improved the abnormal expression levels of HMGB1 and NF- κ B in trophoblast cells under insulin-resistant conditions, while also inhibiting inflammatory factors TNF- α , IL-6, IL-17, TLR4, hs-CRP and GSK-3 β . Ye *et al.* (36) demonstrated that Rosiglitazone can ameliorate lipid metabolism abnormalities in Polycystic Ovary Syndrome (PCOS) rats and reduce ovarian tissue oxidative stress, while also suppressing NF- κ B expression. Hwang *et al.* (37) further showed that the use of Rosiglitazone, a ligand-activated PPAR γ agonist, notably inhibits HMGB1 release in lipopolysaccharide-induced macrophages, thereby improving cellular inflammatory responses. The PPAR γ agonists Rosiglitazone and GW1929 can suppress HMGB1 and NF- κ B expression in trophoblast cells under insulin-resistant conditions, leading to the inhibition of IF release and consequently improving the inflammatory status of GDM. However, thiazolidinediones pose serious side effects, leading to restrictions on their use. Some researchers argued that Rosiglitazone may be beneficial in reducing the risk of cardiovascular diseases (38,39). Currently, there is no evidence from animal experiments to confirm the teratogenic effects of Rosiglitazone. Therefore, the risk of using thiazolidinediones during pregnancy has not been conclusively determined and further research is needed in the future.

Conclusions

The PPAR γ agonists can ameliorate IR in trophoblast cells of GDM patients, promote cell proliferation,

inhibit apoptosis and improve the inflammatory state. This effect may be associated with suppression of HMGB1/NF- κ B p65 pathway activation. However, the mechanisms underlying the effects of PPAR γ agonists on the pathological states of inflammation and oxidative stress in trophoblast cells after treating GDM remain to be further investigated.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article. The other data generated in the present study may be requested from the corresponding author.

Ethics approval and consent to participate

All experimental procedures were approved by the Committee of Taiyuan Maternal and Child Health Hospital. Informed consent was obtained from all patients included in the study.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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