



# Peroxisome proliferator-activated receptor gamma preserves intracellular homeostasis of insulin-resistant periodontal ligament stem cells

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**Background:** Diabetes and periodontitis develop and influence each other. The peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) agonist rosiglitazone (RSG) controls blood glucose and hence the systemic diseases associated with diabetes by increasing the sensitivity of tissues to insulin. However, whether and how RSG can treat diabetic periodontitis is poorly understood.

**Methods:** Insulin-resistant periodontal ligament stem cells (IR-PDLSCs) were induced by glucosamine (18 mM, 24 h) in the presence or absence of RSG or GW9662 (a PPAR $\gamma$  antagonist). The glucose uptake rate was tested to evaluate insulin sensitivity. A scratch test was carried out to measure cell proliferation and motility. We used 2,7-dichlorodihydrofluorescein diacetate (DFCH-DA) and JC-1 kits to detect oxidative stress (OS), and cytoskeleton staining and Calcein-AM/PI kits were used to determine cell viability. Interferon-gamma (IFN- $\gamma$ ) and interleukin-10 (IL-10) ELISA kits were used to evaluate inflammation levels. Finally, quantitative real-time polymerase chain reaction (qRT-PCR) and western blot (WB) analysis were used to assess the expression of osteogenic/odontogenic differentiation-related genes or proteins.

**Results:** Our results showed that RSG exhibited a protective effect on IR-PDLSCs, with increased insulin sensitivity and migration efficiency, an alleviation of glucosamine-induced OS, and a downregulated pro-inflammatory cytokine secretion through activation of PPAR $\gamma$  receptors. Moreover, RSG alleviated the suppressed odontogenic differentiation ability of IR-PDLSCs.

**Conclusions:** RSG preserves the biological functions of IR-PDLSCs in maintaining intracellular homeostasis by increasing insulin sensitivity, reducing OS, and suppressing inflammation.

**Keywords:** Diabetes; insulin resistance; periodontal ligament cell; PPAR $\gamma$

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## Introduction

As one of the most common metabolic diseases, diabetes will likely affect 500 million people worldwide by 2030 (1). Diabetes is the seventh leading cause of death (2), mainly resulting from diabetes mellitus complications, such as cardiovascular disease and diabetic nephropathy (3). More than 90% of patients have type 2 (T2) diabetes (2), characterized by tissue resistance to insulin (IR), decreased glucose consumption, increased glycogen synthesis, and subsequent hyperglycemia (4).

Diabetic periodontitis usually presents as serious damage to the periodontal tissue, which is often more severe than that of periodontitis patients without metabolic diseases. The main pathological features of diabetic periodontitis consist of large amounts of osteoclastic absorption, a ruptured and disordered arrangement of the collagen fibers, and significant infiltration of inflammatory cells to the damaged tissues. Furthermore, periodontal abscesses may develop for those with unsatisfactory blood glucose control, eventually leading to tooth loss (5). However, when there is good glycemic control, the severity of periodontitis can be significantly alleviated by apparent tissue regeneration (6), which demonstrates the importance of hyperglycemia in the development or aggravation of periodontitis. In fact, the interplay between periodontitis and diabetes may be bidirectional.

Insulin resistance and the inflammatory response are the two most important factors in the interaction between the two diseases (7). Periodontitis may aggravate diabetes as the recruited inflammatory cells and cytokines can enter into the systemic circulation to induce adipose insulin resistance (8); however, with periodontal therapy, diabetes can be effectively controlled with a decreased expression of serum TNF- $\alpha$  and C-reactive protein (CRP) by controlling local reactive oxygen species (ROS) levels and pro-inflammatory cytokine secretions (9), suggesting that glycemic control and anti-inflammatory therapy are equally important for tissue remodeling in diabetic periodontitis patients (10).

Periodontal ligament stem cells (PDLSCs) are the primary stem cells involved in periodontal regeneration and are derived from periodontal ligament. They have multidirectional potential to differentiate into osteoblasts, adipocytes, or other cells under proper induction to repair damaged tissues (11). However, in a high glucose environment, the activity of PDLSCs may be badly affected. Hyperglycemia can initiate and boost the inflammatory

reaction, resulting in an elevated level of inflammatory factors [such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6)] (7) and an obvious oxidative stress injury (12), making an unfavorable microenvironment to the PDLSC. Moreover, hyperglycemia can directly regulate the mitochondrial apoptosis pathway through modulating Bcl-2 and Bax expression (13,14), leading to PDLSC apoptosis and cell proliferation inhibition and thus, impairing the repair and regeneration process of periodontal tissue (15,16). Furthermore, excessive deposition of advanced glycosylated end products (AGEs) derived from hyperglycemia will greatly impair the metabolic pathway (17), breaking the balance of subgingival microbiome and inhibiting the activity of alkaline phosphatase and collagenase of PDLSC (18), which will exert an adverse effect on the differentiation and proliferation of cells. The impaired self-renewal, decreased cell mobility (19,20), suppressed osteogenesis potential and increased adipogenesis probability can cause progressive periodontal injury (20). Therefore, increasing insulin sensitivity for glycemic control to maintain intracellular homeostasis and promoting PDLSCs to function correctly is a feasible strategy for diabetic periodontal treatment.

Rosiglitazone (RSG) is one of several synthetic thiazolidinediones (TZDs) and plays an essential role in maintaining glucose homeostasis in T2 diabetes patients by increasing insulin sensitivity in skeletal muscle and adipose tissue. RSG treatment can increase glucose uptake by upregulating the mRNA expression of adiponectin (21) and decreasing hepatic glycogen production via activation of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) receptors (22). Moreover, RSG is crucial for maintaining intracellular homeostasis by preventing excessive ROS production. It suppresses mitochondrial conformation change and relieves mitochondrion swelling through its anti-inflammatory and antioxidant effects (23), which may play an important protective role in preventing brain or renal cellular damage (24). However, the effect of RSG or PPAR $\gamma$  on the treatment of diabetic periodontitis, especially on the intracellular homeostasis of IR-PDLSCs, remains to be elucidated. Therefore, we constructed a cellular insulin resistance model *in vitro*, similar to the pathogenesis of T2 diabetes, to study the biological response of PDLSCs to hyperglycemia and to explore the molecular mechanism of PPAR $\gamma$  during this process. We present the following article in accordance with the MDAR reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-2207/rc>).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of West China Stomatological Hospital of Sichuan University (permit No. WCHSIRB-D-2019-065). Informed consent was taken from all the participants' parents.

### *Cell isolation and culture*

Human PDLCs (hPDLCs), derived from the freshly extracted, intact premolars of healthy young people (aged 14–18), who needed orthodontic treatment, were isolated by the tissue block enzyme digestion method. The hPDLCs were cultured in complete medium [containing 10% fetal bovine serum (FBS), Hyclone, Logan, UT, USA; 1% 100 U/mL of penicillin, 100 µg/mL streptomycin and 90%  $\alpha$ -MEM medium] at 37 °C in a 5% CO<sub>2</sub> incubator. The medium was refreshed every other day, and the purified cells at passage two to passage five were chosen for later testing.

### *Cell viability*

To explore the effects of different concentrations of glucosamine (Beyotime, Shanghai, China), insulin, RSG (Merk, Darmstadt, Germany), or GW9662 (Merk) on cell viability, a cell count kit-8 (CCK-8; KeyGen Biotech, Nanjing, China) was used according to the manufacturer's instructions. Briefly, 5,000 hPDLCs were cultured by  $\alpha$ -MEM medium containing 2% FBS (low serum medium) for serum starvation for 24 hours; after that, the cells were separately treated with different concentrations of glucosamine, insulin, RSG, and GW9662 for 24 hours. Following incubation at 37 °C and 5% CO<sub>2</sub> for 1.5–2 hours with 10% CCK-8 reagent, the optical density (OD) value at 450 nm absorbance was recorded, and the cell viability was calculated for statistical analysis.

### *Insulin resistance model*

According to the above results, 18 mM glucosamine was applied to induce cell insulin resistance. The hPDLCs were seeded in 6-well plates at a density of  $5 \times 10^5$  and cultured in a low serum medium for 24 hours. Subsequently, they were divided into a control (C) group, a glucosamine (G) group, a glucosamine/rosiglitazone (GR) group, and a glucosamine/rosiglitazone/GW9662 (GRG) group. Groups

C and G were cultured in a low serum medium while groups GR and GRG were cultured with an extra 18 mM of glucosamine added to the low serum medium; 24 hours later, the medium in group G was replaced with 18 mM of glucosamine medium while the mediums in the GR and GRG groups were replaced with 10 µM of RSG medium and 10 µM RSG/10 µM GW9662 medium, respectively. Cell supernatants in the four groups were collected for glucose content testing and ELISA assay.

### *Glucose metabolism of IR-hPDLCs*

To analyze the cellular glucose metabolism, the hPDLCs in all groups were treated with 100 nM insulin for another 30 min. Then the cell supernatants were collected to test the glucose consumption values using the GOD-POD (Jiancheng Bioengineering Institute, Nanjing, China) method according to the manufacturer's instructions. Briefly, 10 µL of cell supernatant was incubated with 90 µL reaction buffer in a 96-well plate at 37 °C for 20 min; then, the OD value at 505 nm absorbance was recorded. After that, cells in each well were collected and counted to calculate the glucose content with the following formula: glucose content ( $\mu\text{mol}/10^4$  cell) =  $0.002 \times (\text{OD value in sample} - \text{OD value in blank}) / (\text{OD value in standard} - \text{OD value in blank})$ .

### *Biological characteristics of the IR-hPDLCs*

As described above, cells were divided into groups C, G, GR, and GRG.

### *Cell morphology*

To observe the effects of PPAR $\gamma$  receptors on cell morphology, the hPDLCs were inoculated on a sterilized cover glass. Then, cytoskeletal immunofluorescence (IF) staining was performed as previously described to determine the morphology of cells under different treatment conditions (23).

### *Cell migration*

The hPDLCs, seeded in 6-well plates at a density of  $5 \times 10^5$ , were separately treated with different stimulation. A pipette tip was used to scrape the monolayer on each plate, and the cells were then gently washed by PBS three times to remove the non-adherent cells. The cells were detected at 0, 12, 24, and 48 h under light microscopy (Nikon, Tokyo, Japan).

### Live-dead cell staining

The hPDLSCs inoculated on the sterilized cover glass were separately treated according to their different groups. After washing in PBS three times, the cells were sequentially incubated with propidium iodide (PI) and Calcein-AM in the dark at room temperature for 15 min to stain the dead and living cells, respectively, using a live-dead cell staining kit (KeyGen Biotech).

### Osteogenic/odontogenic differentiation of the IR-hPDLSCs

Cells were seeded in 6-well plates at a density of  $10^5$  and were divided into four groups as described above. After being cultured in the osteogenic induction medium (complete medium containing 5 mM L-glycerophosphate (Merk), 100 nM dexamethasone (Merk), and 50 mg/mL ascorbic acid (Merk) for 7 days, quantitative real-time polymerase chain reaction (qRT-PCR) and western blot (WB) assays were conducted.

### RT-PCR

The hPDLSCs were washed in PBS and then the cells were collected in a tube containing TRIzol lysate. After being stored at  $-80^\circ\text{C}$  for 1 week, the intracellular RNA was extracted as previously described (23). Real-time PCR was performed by denaturing at  $105^\circ\text{C}$  for 5 min followed by 40 cycles of PCR ( $95^\circ\text{C}$  for 30 s,  $60^\circ\text{C}$  for 30 s, and  $72^\circ\text{C}$  for 45 s). The relative expression levels were calculated using the  $2^{-\Delta\Delta\text{CT}}$  method and normalized to the internal control *GAPDH* gene. The specific primer information was as follows (5'-3'): *GAPDH* (forward: CTTTGGTATCGTGGAAGGACTC; reverse: GTAGAGGCAGGGATGATGTTCT); *COL-1* (forward: AACATGGAGACTGGTGAGACCT; reverse: CGCCATACTCGAACTGGAATC); *Runx2* (forward: CTTTACTTACACCCCGCCAGTC; reverse: AGAGATATGGAGTGCTGCTGGTC).

### WB assay

The WB assay was conducted as previously described (23) after the hPDLSCs were cultured in an osteogenic induction medium for 7 days. After being blocked by 5% nonfat dry milk (Bio-Rad Laboratories, Hercules, CA, USA), the membranes were separately incubated with a primary antibody (*GAPDH* (7E4) Mouse mAb, Zen, Chengdu, China; *DMP-1* Rabbit pAb, Biovision, San Francisco, CA, USA) in a 1:1,000 dilution at  $4^\circ\text{C}$  overnight. The band intensity of each protein was standardized to the corresponding *GAPDH* intensity by Image Lab software.

### ROS level of the IR-hPDLSCs

The oxidative stress (OS) level of the IR-hPDLSCs and the effect of PPAR $\gamma$  receptors on intracellular ROS were measured by JC-1 (Beyotime) and 2',7'-dichlorofluorescein diacetate (DCFH-DA, Merk) tests. Briefly, cells were washed three times in PBS and subsequently stained with JC-1 or 10  $\mu\text{M}$  DCFH-DA and kept in the dark at  $37^\circ\text{C}$  for 20 min. After the dyes were thoroughly washed away, the cells were observed by a confocal microscope for 30 min.

### Inflammation of the IR-hPDLSCs

Cell supernatants were collected for ELISA (DECO, Shanghai, China) detection of interleukin-10 (IL-10)/interferon-gamma (IFN- $\gamma$ ) concentrations following the manufacturer's instructions (23).

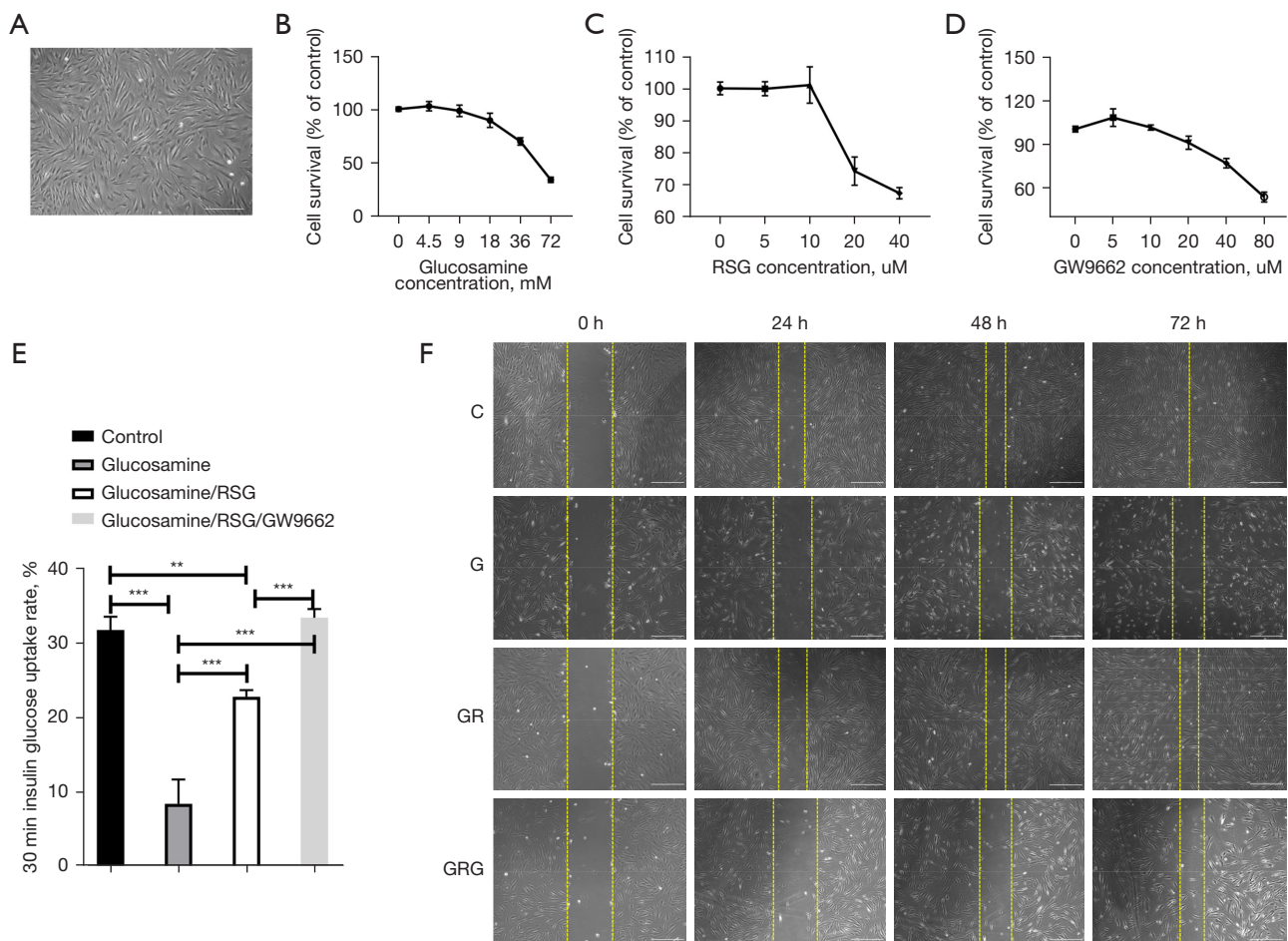
### Statistical analysis

All quantitative data were analyzed by SPSS 22.0 software, and all the data are presented as mean  $\pm$  SD. One-way analysis of variance (ANOVA) was used to analyze the differences among groups, while Student's *t*-test was used to compare the differences between two groups. A value of  $P < 0.05$  was considered statistically significant. All the experiments in this study were repeated at least three times.

## Results

### RSG increased the glucose uptake rate and migration of the glucosamine-induced PDLSCs

The isolated PDLSCs were well adhered to the plate with a long fusiform shape (Figure 1A). A CCK-8 test was conducted to explore the appropriate drug concentrations of glucosamine, RSG, and GW9662. The results showed that lower concentrations of the three chemical reagents did not affect cell viability, while more than 18 mM glucosamine, 10  $\mu\text{M}$  RSG, or 10  $\mu\text{M}$  GW9662 caused a decrease in PDLSCs' viability after culturing for 24 hours (Figure 1B-1D). Therefore, 18 mM glucosamine was applied to induce cellular insulin resistance, and 10  $\mu\text{M}$  RSG or 10  $\mu\text{M}$  GW9662 was used to study the role of PPAR $\gamma$  on the biological properties of the IR-PDLSCs. Results of the 30 min insulin glucose uptake rate showed that, compared to the glucosamine group, insulin glucose uptake increased from 8.48% to 22.84% with RSG treatment, indicating the efficiency of glucosamine in inducing insulin resistance

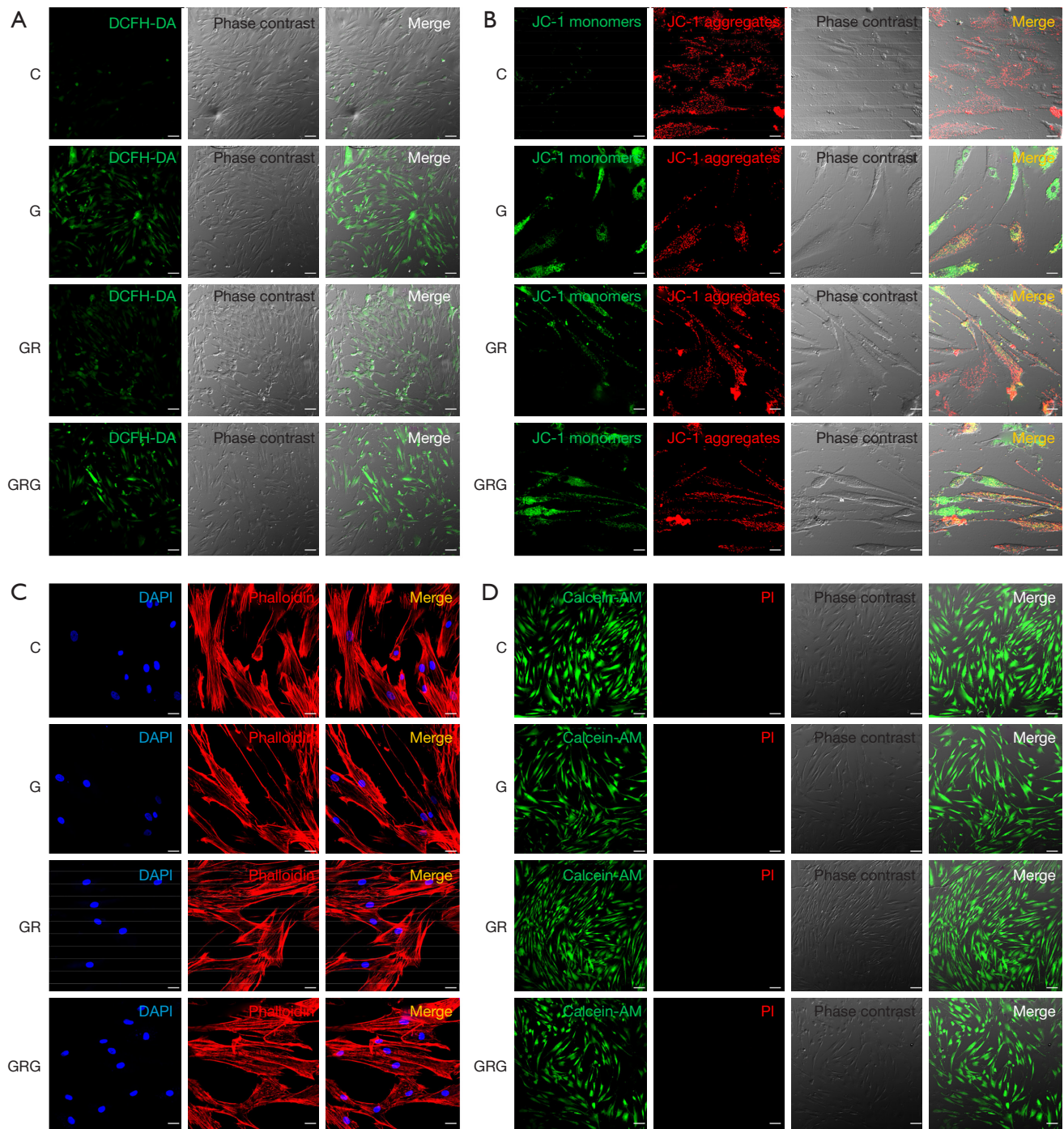


**Figure 1** RSG increases the glucose uptake rate and migration of the glucosamine-induced PDLSCs. (A) Second passage PDLSCs under the light microscope. Scale bar: 500  $\mu$ m. (B-D) Effects of different concentrations of glucosamine, RSG, and GW9662 on the viability of PDLSCs. (E) 30 min insulin glucose uptake rate of PDLSCs treated with or without 18 mM glucosamine, 10  $\mu$ M RSG, or 10  $\mu$ M RSG combined with 10  $\mu$ M GW9662. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . (F) Cellular migration of PDLSCs treated with or without 18 mM glucosamine, 10  $\mu$ M RSG, or 10  $\mu$ M RSG combined with 10  $\mu$ M GW9662 under a light microscope (BX43F, Olympus, Japan). Scale bar: 200  $\mu$ m. RSG, rosiglitazone; PDLSC, periodontal ligament stem cell; C, control group; G, glucosamine group; GR, glucosamine/rosiglitazone group; GRG, glucosamine/rosiglitazone/GW9662 group.

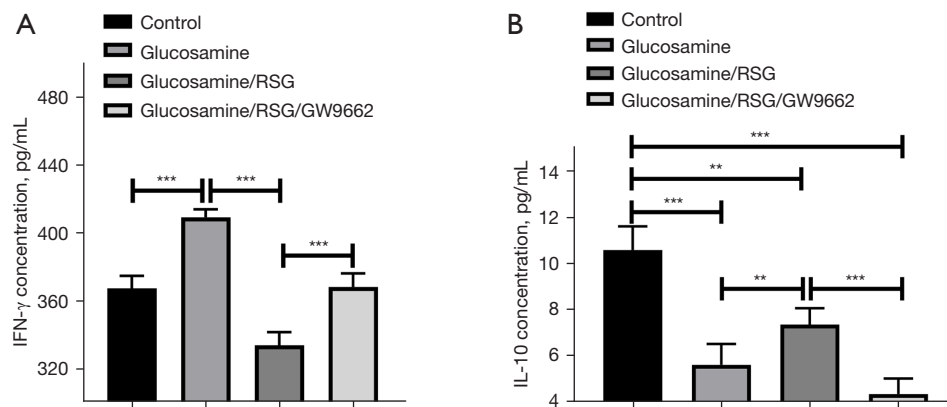
and RSG in treating the hyperglycemia caused by insulin resistance (Figure 1E). However, administration with GW9662 did not reverse the effect of RSG on IR-PDLSCs, suggesting that PPAR $\gamma$  might not be the only receptor for glucose control in PDLSCs (Figure 1E). The migration test demonstrated that RSG improved the impaired cellular migration ability of the IR-PDLSCs and that GW9662 partly reversed this effect (Figure 1F), indicating the protective role of PPAR $\gamma$  in preserving migration.

### ***RSG alleviated glucosamine-induced OS without harming the cytoskeleton or survival of the PDLSCs by activating PPAR $\gamma$ receptors***

The results of DCFH-DA staining indicated that ROS levels in the IR-PDLSCs were significantly upregulated with an enhanced fluorescence intensity compared to PDLSCs, indicating that glucosamine induced obvious intracellular OS injury (Figure 2A). However, the glucosamine-induced



**Figure 2** RSG alleviates glucosamine-induced OS without harm to the cytoskeleton and survival of the PDLSCs by activating PPAR $\gamma$  receptors. (A) The ROS level measured by DCFH-DA on a fluorescence microscope. Scale bar: 200  $\mu$ m. (B) The mitochondrial membrane potential detected by JC-1 staining. Scale bar: 100  $\mu$ m. (C) Cytoskeleton stained by Phalloidin. Scale bar: 100  $\mu$ m. (D) Living and dead cells detected by Calcein-AM/PI staining. Scale bar: 200  $\mu$ m. RSG, rosiglitazone; OS, oxidative stress; PDLSC, periodontal ligament stem cell; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; DCFH-DA, 2',7'-dichlorofluorescein diacetate; C, control; G, glucosamine; GR, glucosamine/rosiglitazone; GRG, glucosamine/rosiglitazone/GW9662.



**Figure 3** RSG modulates the glucosamine-induced inflammation via PPAR $\gamma$  receptors. (A) The IFN- $\gamma$  concentration detected by ELISA assay. \*\*\*, P<0.001. (B) The IL-10 concentration detected by ELISA assay. \*\*, P<0.01; \*\*\*, P<0.001. RSG, rosiglitazone; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; IFN- $\gamma$ , interferon-gamma; ELISA, enzyme linked immunosorbent assay; IL-10, interleukin-10.

PDLSCs demonstrated a downregulated ROS expression when treated with RSG. Furthermore, this effect was partly reversed by GW9662, indicating the importance of RSG PPAR $\gamma$  in regulating the intracellular ROS level (Figure 2A). JC-1 staining showed a green fluorescence in the glucosamine-induced PDLSCs, which might result from insufficient capacity to form J-aggregates with a relatively low mitochondrial membrane potential (Figure 2B). The mitochondrial depolarization process directly disrupted intracellular homeostasis with an upregulated ROS level. Consistent with the results obtained in Figure 2A, RSG treatment also alleviated OS injury, with a relatively high mitochondrial membrane potential with red fluorescence. Similarly, GW9662 treatment blocked the antioxidation effect of RSG, confirming the importance of RSG PPAR $\gamma$  in regulating OS. To further study the effects of PPAR $\gamma$  on the morphology and cytotoxicity of the glucosamine-induced PDLSCs, cytoskeleton and Calcein-AM/PI staining were performed, and the results found no side effects of RSG or GW9662 on cells (Figure 2C,2D). These results demonstrated the efficiency of RSG in controlling OS via PPAR $\gamma$  receptors.

#### RSG modulated glucosamine-induced inflammation via PPAR $\gamma$ receptors

Our results showed that the concentration of inflammatory cytokine IFN- $\gamma$  was significantly upregulated in the group of IR-PDLSCs with 409.8 pg/mL (P<0.001), while RSG treatment decreased it to a normal level of 334.3 pg/mL (P<0.001) (Figure 3A). Furthermore, the anti-

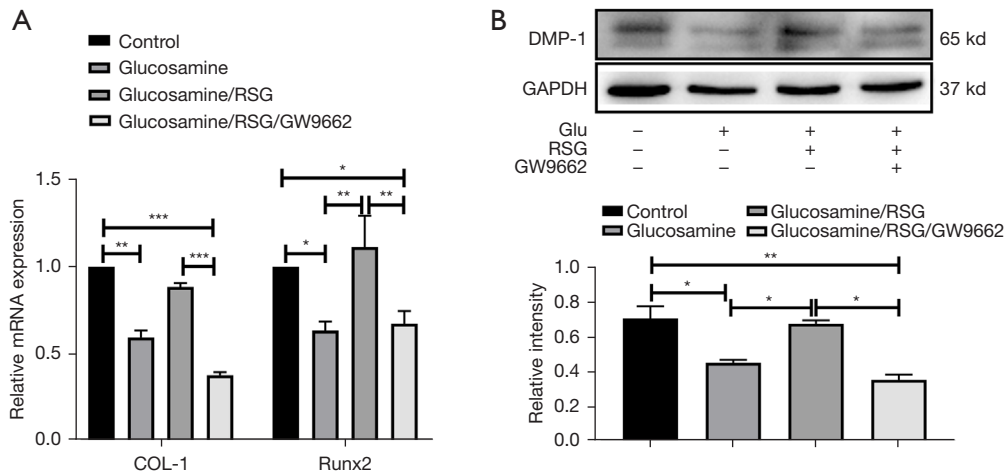
inflammatory factor IL-10 expression in the RSG group was increased compared to the glucosamine group (7.4 vs. 5.6 pg/mL, P<0.01, respectively) (Figure 3B). Additionally, the anti-inflammatory effect of RSG on glucosamine-induced PDLSCs was partly reversed by GW9662, indicating the importance of the RSG PPAR $\gamma$  receptors in modulating inflammatory reactions.

#### RSG preserved the osteogenic/odontogenic differentiation ability of the IR-PDLSCs by activating PPAR $\gamma$ receptors

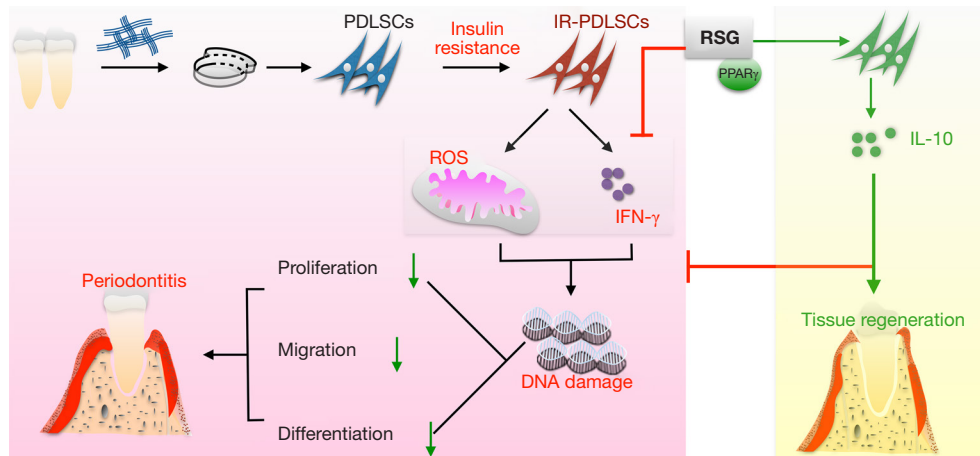
The RT-PCR results showed that glucosamine treatment significantly impaired the expression of the osteogenic differentiation-related genes collagen I (COL-1) and runt-related transcription factor 2 (Runx2) (P<0.01), while RSG treatment partly blocked this side effect with an increased Runx2 expression (P<0.01) (Figure 4A). Consistent with this result, WB results also showed that RSG treatment increased the expression of the odontogenic differentiation-related protein dentin matrix protein 1 (DMP-1) in the IR-PDLSCs (P<0.01) (Figure 4B). All these effects were partly reversed by GW9662, indicating that PPAR $\gamma$  might play a significant role in preserving the multiple differentiation potentials of glucosamine-induced PDLSCs (Figure 5).

#### Discussion

Similar to other stem cells, some biological behaviors are shared by PDLSCs (25,26). Consistent with our results, a previous study demonstrated a decreased self-renewal rate and migration efficiency and a suppressed



**Figure 4** RSG preserves the osteogenic/odontogenic differentiation ability of the glucosamine-induced PDLSCs by activating PPAR $\gamma$  receptors. (A) The relative mRNA expression of COL-1 and Runx 2 at 7 days. (B) The western blot analysis of the odontogenic differentiation-related protein DMP-1 at 7 days. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001. RSG, rosiglitazone; PDLSC, periodontal ligament stem cell; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; COL-1, collagen I; Runx2, runt-related transcription factor 2; DMP-1, dentin matrix protein 1.



**Figure 5** PPAR $\gamma$  activation preserves the biological functions of insulin-resistant periodontal ligament stem cells by maintaining intracellular homeostasis. PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; IR-PDLSC, insulin-resistant periodontal ligament stem cell; RSG, rosiglitazone; ROS, reactive oxygen species; IFN- $\gamma$ , interferon-gamma; IL, interleukin.

expression of osteogenic differentiation-related genes of the mesenchymal progenitor cells (MPCs) with high glucose culture (27). Meanwhile, some studies also found that high glucose concentrations damaged the biological function of PDLSCs by suppressing cell proliferation and osteogenic differentiation ability via OS injury, demonstrating the side effects of high glucose on cells (20,28,29). Unlike the previous study, we constructed an insulin-resistant model by applying glucosamine, which made PDLSCs insensitive to

insulin stimulation and subsequently decreased their glucose uptake rate. Compared with direct high glucose stimulation (20,29), the pathological model in this study was more similar to the pathogenesis of T2 diabetes. Thus, our study verifies the vital role of glucose homeostasis in maintaining cell function.

Clinically, it can be challenging to treat diabetic periodontitis patients, as they often exhibited extremely severe alveolar bone resorption and rapid attachment



loss (10). Diabetes and periodontitis may be a reciprocal causation and form a vicious circle. Diabetes has been considered as an underlying factor of periodontitis and specifically, abnormal response of the immune system and the changes of lipid metabolism pathway (17) can be ascribed as the major reasons for the susceptibility to periodontitis of diabetes patients. Generally, there are lots of pro-inflammatory factors released from the hyperglycemia-induced dysfunctional immune cells, which can decrease the defense function and easily lead to periodontal tissue damage (12); with the further development of diabetes, high level of free fatty acid (FFA) derived from lipid metabolism disorder will result in skeletal muscle and adipose tissue insulin resistance (30), decreasing glucose uptake and muscle glycogen synthesis resulting in hyperglycemia and subsequent excessive deposition of AGEs (12,17). AGEs are considered strong simulators for activating inflammatory signaling pathways to release various pro-inflammatory cytokines and ROS (31), ultimately affecting the biological functions of PDLSCs and resulting in progressive damage and the delayed repair of periodontal tissue. Therefore, when diabetes is effectively controlled (12), the excessive deposited AGEs could be subsequently eliminated with reduced IL-6 and TNF- $\alpha$  expression, which would be favorable for inhibiting progressive alveolar bone loss and beneficial for tissue regeneration.

As an effective insulin sensitizer, RSG might play an essential role in blood glucose concentration control in T2 diabetes (22) by increasing the hepatic glucose uptake rate while decreasing the hepatic glucose synthesis efficiency (32), as well as increasing the mRNA expression of adiponectin by activating the adiponectin promoter in the PPAR $\gamma$  pathway (33). PPAR $\gamma$  activation can alleviate inflammatory response through regulating the biological behavior of monocytes and macrophages (33) in the innate immune system. In inflammatory bowel disease or LPS-induced acute inflammation, PPAR $\gamma$  activation exhibits considerable anti-inflammatory activity to slows down the pathological process (34) by blocking nuclear factor kappa-B (NF- $\kappa$ B) and protein kinase B (Akt) pathways in a dose-dependent manner and furthermore, this anti-inflammatory effect can be reversed by PPAR $\gamma$  antagonist GW9662 (35). The effective role of PPAR $\gamma$  activation in inflammation control was further verified in the rat periodontitis model, where RSG could effectively inhibited LPS-induced acute and chronic inflammation and the subsequent receptor activator of nuclear factor kappa-B ligand (RANKL) mediated bone resorption (36). Based

on the benefits of PPAR $\gamma$  receptor on microenvironments regulation and blood glucose control, RSG might be effective in periodontium regeneration of diabetes patients. Therefore, RSG or PPAR $\gamma$  receptors might play an important role in improving the biological functions of IR-PDLSCs. However, in our study, despite the upregulated insulin sensitivity of the IR-PDLSCs with an improved glucose uptake rate when RSG was administrated, the PPAR $\gamma$  antagonist GW9662 did not reverse the positive effect of RSG on glycemic control, suggesting that PPAR $\gamma$  is not the only RSG receptor that maintains the glucose homeostasis of PDLSCs. Although the specific mechanisms of RSG PPAR $\gamma$  in controlling blood glucose in PDLSCs are still controversial, the good glycogen regulation of RSG suggests it is a valid choice for diabetic periodontal treatment.

In addition to the important role RSG plays in modulating glycometabolism, the regulatory role of RSG in the local environment has also been verified (24), which is of vital importance to cell functioning (28). A previous study found that RSG significantly reduced mitochondrial swelling and removed the depolarization of mitochondrial membrane potential in high glucose cultured brain cells, resulting in a noticeable reduction of intracellular OS levels (37). Protecting cells from high glucose-induced oxygenation confirms RSG's beneficial effect in controlling cellular OS. Similar to this result, we also showed a reduction in mitochondrial membrane potential with a relatively low OS level in IR-PDLSCs after RSG treatment; Meanwhile, the expression of proinflammatory factor-like IFN- $\gamma$  induced by insulin resistance was significantly downregulated while anti-inflammatory factor-like IL-10 was significantly upregulated by RSG, confirming the antioxidant and anti-inflammatory effects of RSG on IR-PDLSCs.

Intracellular microenvironment homeostasis plays a decisive role in regulating the differentiation of cells and thus determines final tissue regeneration results (28). It has been found that controlling the OS response to hyperglycemia by administrating the antioxidant erythropoietin (EPO) significantly improves PDLSCs' biological functioning (28), including their self-renewal ability, migration, and osteogenic differentiation. Similar to this result, we also found an improvement in the biological behavior of IR-PDLSCs. They showed an enhanced osteogenic/odontogenic differentiation ability when intracellular homeostasis was maintained by RSG administration, resulting in reduced OS injury and

inflammatory reactions. As evidenced by the reversal effect of GW9662 on the positive effects of RSG on cells, PPAR $\gamma$  receptors may play an essential role in regulating the IR-PDLSC microenvironment.

In line with our results, a previous study reported that the osteogenic differentiation ability of bone marrow mesenchymal stem cells (BMSCs) was significantly increased after glucose metabolism disturbance was corrected, resulting in elevated bone repair efficiency (38). Therefore, RSG should be recommended as a routine treatment for diabetic periodontitis because of its beneficial effects on cells sensitive to insulin. RSG enhances glycemic control to improve intracellular homeostasis, reducing oxidative damage and inflammation and increasing the osteogenic differentiation ability of IR-PDLSCs.

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### Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-2207/rc>

*Data Sharing Statement:* Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-2207/dss>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-2207/coif>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of West China Stomatological Hospital of Sichuan University (permit No. WCHSIRB-D-2019-065). Informed consent was taken from all the participants' parents.

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