Original Research Article

COLEC12 expression participates in trophoblast insulin resistance, and reverses celecoxib-mediated inhibition of COX2-PGE2 axis in gestational diabetes

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Abstract

Purpose: To evaluate the contribution of collectin subfamily member 12 (COLEC12) in insulin resistance associated with gestational diabetes mellitus (GDM).

Methods: Insulin resistance was induced in human placental trophoblast (HTR-8/SVneo) cells in vitro by incubating them with 1 μM insulin for 48 h. Expression of COLEC12 was assessed using western blot and quantitative real time-polymerase chain reaction (qRT-PCR). Distribution of glucose transporters GLUT1 and GLUT4 was visualized using immunofluorescent staining. Furthermore, the level of reactive oxygen species (ROS) was evaluated by flow cytometry.

Results: Insulin-resistant HTR-8/SVneo (IR-HTR-8/SVneo) cells showed significantly elevated COLEC12 expression (p < 0.001). Knocking down of COLEC12 in these cells led to a significant reduction in GLUT1 expression, and increase in both glucose consumption and GLUT4 expression (p < 0.001). Furthermore, COLEC12 knockdown levels of ROS increased SOD expression in IR-HTR-8/SVneo, while knockdown of COLEC12 reduced p-insulin receptor substrate (IRS) (Ser307) protein expression, but increased p-IRS-1 (Tyr896), p-insulin receptor β (IRβ) (Tyr1361), and p-AKT levels. COLEC12 knockdown also decreased cyclooxygenase-2 (COX-2) expression, resulting in lower prostaglandin E2 (PGE2) levels in IR-HTR-8/SVneo. COLEC12 over-expression attenuated celecoxib-induced increase in glucose consumption, resulting in decreased COX2 and PGE2 levels.

Conclusion: COLEC12 in IR-HTR-8/SVneo cells has antioxidant effects that protect against insulin resistance. This protective effect is achieved through the down-regulation of COX-2/PGE2. These findings provide a potential strategy for the treatment of GDM.

Keywords: COLEC12, Insulin resistance, COX-2, PGE2, Oxidative stress, Gestational diabetes mellitus

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as the dysfunction of pancreatic β-cells and glucose intolerance, resulting in hyperglycemia that affects maternal health and fetal growth [1]. GDM affects approximately 15 % of pregnancies [2]. Therefore, effective strategies are needed for its prevention.

The placenta is the key organ for maternal and infant nutrition exchange, and is essential for healthy embryonic development during pregnancy [3]. Dysfunction of the placenta can result in the secretion of hormones that antagonize insulin, thus enhancing insulin resistance and exacerbating GDM development [4]. Therefore, placental function is an important therapeutic target for GDM. Inhibition of the activity, invasion, and migration of trophoblast leads to dysplasia of the placental tissues, resulting in GDM [5].

Collectin subfamily member 12(COLEC12) is a pattern recognition molecule, which recognizes fungi and bacteria and initiates complementary activation of the innate immune system [6]. COLEC12 is also involved in lipogenesis and lipolysis [7]. A genome-wide association study showed that COLEC12 contributed to the risk of metabolic syndromes [8]. Another genome-wide association study indicated an association between COLEC12 and diabetic retinopathy in patients with T2D [9].

The effects of COLEC12 on glucose consumption, insulin resistance, and oxidative stress in insulin-resistant HTR-8/SVneo were investigated. The related signaling pathway was also evaluated to identify potential targets for GDM therapy.

EXPERIMENTAL

Cell culture and treatment

HTR-8/SVneo (Procell, Wuhan, China) was incubated with 1-µM insulin (Sigma-Aldrich, St. Louis, MO, USA) for 48 h to induce insulin resistance. The IR-HTR-8/SVneo was treated with celecoxib (5 µM; Sigma-Aldrich) for an additional 24 h.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from both HTR-8/SVneo and IR-HTR-8/SVneo using a TRIzol kit (Life Technologies, Carlsbad, CA, USA). 2 µg of isolated RNA was reverse-transcribed into cDNAs, and COLEC12 mRNA was determined using a PreTaq II kit (Takara, Dalian, Liaoning, China), and the primers listed in Table 1.

Glucose consumption

IR-HTR-8/SVneo was transfected with siRNA targeting COLEC12 (siCOLEC12), pcDNA-COLEC12 (pc-COLEC12), or negative control siRNA (siNC, NC), obtained from Genepharma (Suzhou, China). After transfection, the cells were treated with 100 nM insulin for 30 min before collection of culture supernatants. The level of glucose consumption was measured using a glucose assay kit obtained from Nanjing Jiancheng Bioengineering Institute in Nanjing, China.

Immunofluorescent staining

IR-HTR-8/SVneo with indicated transfections was seeded onto glass slides and then fixed with 10 % formalin for 10 min. The cells were then permeated with 0.1 % TritonX-100 and blocked in goat serum. Specific antibodies against GLUT1 or GLUT4 (obtained from Abcam in Cambridge, MA, USA) were incubated with cells overnight. The cells were washed with PBS and treated with Cy3-labeled secondary antibody. After incubation with DAPI obtained from Sigma-Aldrich, the cells were then photographed using a fluorescence microscope from Olympus in Tokyo, Japan.

Level of reactive oxygen species

IR-HTR-8/SVneo with indicated transfections were harvested and treated with the DCFH-DA solution from a ROS assay kit (Beyotime, Beijing, China) for 20 min. After treatment, the cells were analyzed using flow cytometry (ACEA in San Diego, CA, USA) to assess the fluorescence.

Assessment of superoxide dismutase (SOD) and prostaglandin E2 (PGE2)

To determine the SOD levels, the IR-HTR-8/SVneo was lysed in RIPA buffer obtained from Beyotime, and the proteins were isolated. The isolated proteins were then subjected to a Superoxide Dismutase Colorimetric Activity Kit. To assess PGE2 levels, the culture supernatants from the IR-HTR-8/SVneo were harvested and subjected to Prostaglandin E2 Human ELISA Kit, also obtained from Thermo Fisher Scientific, Suzhou, China.

Table 1: Primers for qRT-PCR

Gene	Forward	Reverse
COLEC12	5'-AATCCTTCGGTTACAAGCGGT-3'	5'-ACTGTGATTGTTAGCAAGGCAC-3'
GAPDH	5'-GAACGGGAAGCTCACTGG-3'	5'-GCCTGCTTCACCACCTTCT-3'

Western blot

Proteins were extracted from the cells using RIPA buffer (Beyotime), separated and then transferred onto nitrocellulose membranes. The membranes were then blocked and probed with specific antibodies: anti-COLEC12, anti-COX-2, anti-β-actin (1:2000), anti-p-IR (Tyr1361), anti-IRβ (1:3000), anti-p-IRS-1(Ser307), anti-p-IRS-1(Tyr896), anti-IRS-1 (1:4000), anti-p-AKT and anti-AKT (1:5000). The membranes were visualized using enhanced chemiluminescence (Sigma-Aldrich) after incubation with horseradish peroxidase-conjugated secondary antibody (1:5000). All antibodies used in this study were acquired from Abcam.

Statistical analysis

Data was collected in triplicate, and expressed as mean ± SEM. Statistical analysis was performed using SPSS software version 8.0, and significance of the differences was determined using either Student's t-test or one-way analysis of variance. A *p*-value of *< 0.05* was considered statistically significant.

RESULTS

COLEC12 was elevated in IR-HTR-8/SVneo

The COLEC12 mRNA expression was up-regulated in insulin-resistant HTR-8/SVneo (Figure 1 A). The increase in COLEC12 was accompanied by a corresponding increase in COLEC12 protein expression, as shown in Figure 1 B.
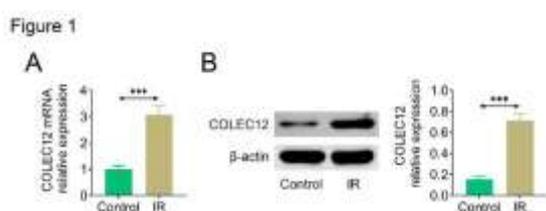

Figure 1: COLEC12 was elevated in IR-HTR-8/SVneo. COLEC12 mRNA (A) and protein (B) expression were up-regulated in IR-HTR-8/SVneo. ******$p < 0.01$, *******$p < 0.001$

Knockdown of COLEC12 promoted glucose consumption in IR-HTR-8/SVneo

IR-HTR-8/SVneo was transfected with siCOLEC12 or pc-COLEC12 to knockdown or overexpress its protein expression, respectively (Figure 2 A). Results showed that IR-HTR-8/SVneo had lower glucose consumption than control cells, which was reversed by COLEC12 knockdown and enhanced by COLEC12 overexpression (Figure 2 B). the over-expression of COLEC12 reduced glucose consumption, while the knockdown of COLEC12 increased glucose consumption in IR-HTR-8/SVneo (Figure 2 B). Furthermore, immunofluorescent staining showed that GLUT1 was reduced in IR-HTR-8/SVneo by the COLEC12 knockdown, and up-regulated in cells overexpressing COLEC12 (Figure 2 C). However, the over-expression of COLEC12 reduced GLUT4 distribution, while the COLEC12 knockdown enhanced GLUT4 distribution (Figure 2 C). The knockdown of COLEC12 promoted glucose consumption by decreasing GLUT1 and increasing GLUT4 expression in IR-HTR-8/SVneo.
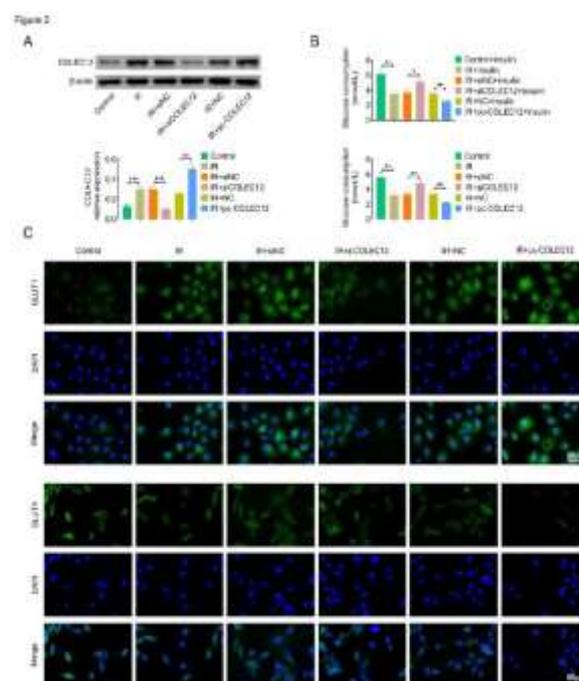

Figure 2: COLEC12 knockdown promoted glucose consumption in IR-HTR-8/SVneo. (A) IR-HTR-8/SVneo was transfected with siCOLEC12 or pc-COLEC12 to reduce or enhance COLEC12 protein expression, respectively; (B) Over-expression of COLEC12 increased glucose consumption, while COLEC12 knockdown showed opposite results in IR-HTR-8/SVneo; (C) Over-expression of COLEC12 reduced GLUT4 and enhanced GLUT4 distribution, while COLEC12 knockdown showed opposite results in IR-HTR-8/SVneo. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* control, IR+siNC, or IR+NC

COLEC12 knockdown reduced oxidative stress in IR-HTR-8/SVneo

The ROS was accumulated in IR-HTR-8/SVneo (Figure 3 A and B). The over-expression of COLEC12 enhanced the accumulation of ROS, whereas COLEC12 knockdown reduced ROS levels (Figure 3 A and B). The activity of SOD decreased in IR-HTR-8/SVneo (Figure 3 C). The

over-expression of COLEC12 further reduced SOD activity (Figure 3 C), while COLEC12 knockdown enhanced SOD activity (Figure 3 C).
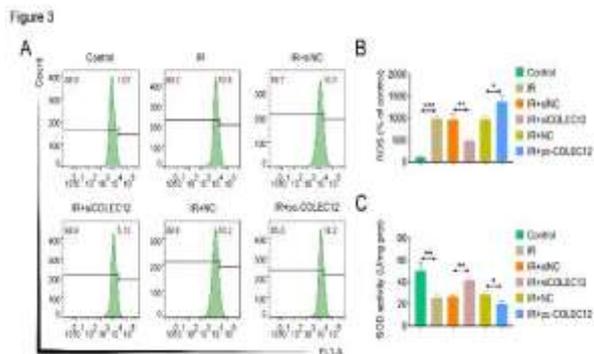

Figure 3: COLEC12 knockdown reduced oxidative stress in IR-HTR-8/SVneo. (A) Over-expression of COLEC12 enhanced ROS accumulation, while COLEC12 knockdown showed opposite results in IR-HTR-8/SVneo; (B) Relative ROS levels; (C) Over-expression of COLEC12 reduced SOD activity, while COLEC12 knockdown showed opposite results in IR-HTR-8/SVneo. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* control, IR+siNC, or IR+NC

COLEC12 knockdown activated insulin signaling pathways

Insulin treatment induced the up-regulation of the p-IRβ (Tyr1361), p-IRS-1 (Tyr896), and p-AKT expression, and reduced p-IRS-1 (Ser307) in HTR-8/SVneo. However, these protein expressions were reversed in IR-HTR-8/SVneo. Over-expression of COLEC12 further enhanced the expression of p-IRS-1 (Ser307) and decreased the expressions of p-IRβ (Tyr1361), p-IRS-1 (Tyr896), and p-AKT (Figure 4). Furthermore, COLEC12 knockdown showed opposite results.
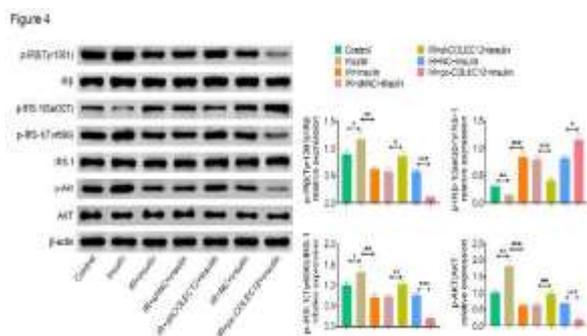

Figure 4: COLEC12 knockdown activated insulin signaling in IR-HTR-8/SVneo. Over-expression of COLEC12 enhanced expression of p-IRS-1 (Ser307) and reduced p-IRβ (Tyr1361), p-IRS-1 (Tyr896), and p-AKT expressions in IR-HTR-8/SVneo. COLEC12 knockdown showed the opposite results. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* control, IR+siNC, or IR+NC

COLEC12 knockdown reduced COX-2/PGE2 expression and reversed celecoxib-induced inhibition of COX-2/PGE2 signaling

COX-2 protein expression was up-regulated in IR-HTR-8/SVneo (Figure 5 A). The over-expression of COLEC12 enhanced the expression of COX-2 (Figure 5 A), while COLEC12 knockdown reduced COX-2 (Figure 5 A). Furthermore, PGE2 levels were also elevated in IR-HTR-8/SVneo (Figure 5 B). The over-expression of COLEC12 enhanced PGE2 levels (Figure 5 B), while COLEC12 knockdown reduced PGE2 levels (Figure 5 B), indicating that COLEC12 knockdown suppressed COX-2/PGE2 signaling. Celecoxib decreased COX-2 (Figure 5 C) and PGE2 (Figure 5 D) levels in IR-HTR-8/SVneo, but simultaneously increased glucose consumption (Figure 5 E). However, over-expression of COLEC12 inhibited the celecoxib-induced increase in glucose consumption (Figure 5 E), decrease in COX2 (Figure 5 C) and PGE2 (Figure 5 D) expression.
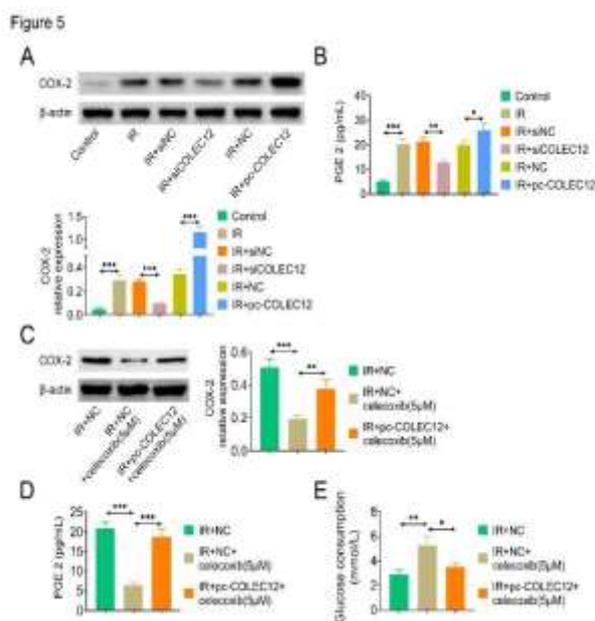

Figure 5: COLEC12 knockdown decreased COX-2/PGE2. (A) Over-expression of COLEC12 increased COX-2 expression, while COLEC12 knockdown decreased COX-2 expression in IR-HTR-8/SVneo. Moreover, PGE2 levels were also elevated; (B) Over-expression of COLEC12 increased PGE2 expression levels, while COLEC12 knockdown decreased PGE2 expression levels. COLEC12 attenuated celecoxib-induced decrease in COX2; (C) COLEC12 attenuated celecoxib-induced decrease in COX-2 expression; (D) COLEC12 attenuated celecoxib-induced decrease in PGE 2 expression; (E) COLEC12 attenuated celecoxib-induced increase in glucose consumption. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* control, IR+siNC, or IR+NC

DISCUSSION

A previous study using a genome-wide association approach revealed an association between COLEC12 and diabetic retinopathy in patients with T2D [15]. It was also reported to increase the risk of metabolic syndrome [14]. The current study investigated the role of COLEC12 in GDM, and observed that it was potentially linked to oxidative stress and insulin resistance, through its regulation of the COX-2/PGE2 axis. Proliferation, differentiation, and migration of trophoblast are crucial for proper placental function, as any dysfunction of the placenta can lead to the development of GDM [4]. Elevated levels of insulin negatively impacted the properties of trophoblast [10], resulting in dysplasia of placental tissues, leading to GDM [11]. Therefore, IR-HTR-8/SVneo is used as a cell model for GDM [11]. In this particular study, it was found that treatment with insulin led to a decrease in glucose consumption, suppressed insulin signaling, and induced insulin resistance in the cells.

COLEC12 was elevated in IR-HTR-8/SVneo. Further functional assays demonstrated that COLEC12 knockdown increased glucose consumption. Additionally, it was found that excess glycogen accumulated in the placenta of mice with GDM [11], and GLUT1 is important for glucose uptake in the placenta and increases in the placenta of GDM women [12], while GLUT4, an insulin-responsive glucose transporter, decreases in the placenta of GDM women [13]. GLUT1 distribution was up-regulated, while GLUT4 was down-regulated in IR-HTR-8/SVneo, and the knockdown of COLEC12 reduced the distribution of GLUT1 and enhanced GLUT4 expression. Insulin signaling is essential for glucose uptake, during which insulin binds to the receptor, induces internalization and phosphorylation of IRβ, and promotes the phosphorylation of IRS-1 and AKT, resulting in the regulation of GLUT1 and GLUT4 [14]. Defects in insulin signaling occur in GDM [13], and the p-IRS-1 level is enhanced, while p-IRβ, p-IRS-1, and p-AKT expression levels are reduced in IR-HTR-8/SVneo [11]. Knockdown of COLEC12 decreased p-IRS-1 (Ser307) and increased p-IRβ (Tyr1361), p-IRS-1, and p-AKT expression, thus contributing to glucose consumption in GDM.

Patients with GDM are diagnosed with chronic hyperglycemia during pregnancy. Chronic insulin resistance induces dysfunction of pancreatic β-cells and results in hyperglycemia, thus contributing to the pathogenesis of GDM [15]. ROS accumulation inhibits glucose uptake through the regulation of IRS-1 and GLUT4, thus contributing to GDM-associated insulin resistance [16]. COLEC12 knockdown enhanced the SOD activity and reduced ROS levels in IR-HTR-8/SVneo cells, thus exerting antioxidant effects against GDM-associated insulin resistance. The secretion of pro-inflammatory cytokines induces ROS accumulation, promotes oxidative stress, and participates in the pathogenesis of GDM [15]. COLEC12 knockdown may induce anti-inflammatory effects in IR-HTR-8/SVneo cells to protect against GDM. COX-2 catalyzes the synthesis of anti- and pro-inflammatory prostaglandins, such as PGE2, and regulates the inflammation and insulin resistance in T2D [17]. COX-2-mediated oxidative stress is associated with T2D [18]. The inhibition of COX-2 promotes palmitate-induced insulin resistance [17]. PGE2, in a COLEC12-dependent manner, mediates Th17 responses and dendritic cell conditioning in *H. pylori*-infected gastric stromal cells, whereas the knockdown of COLEC12 inhibits the expression of PGE2 [19]. Results in the present study showed that COX-2 and PGE2 expressions were up-regulated in IR-HTR-8/SVneo cells, whereas, the knockdown of COLEC12 reduced the expressions of COX-2 and PGE2. Furthermore, COLEC12 attenuated celecoxib-induced glucose consumption and the enhancement in COX2 and PGE2 expression levels.

CONCLUSION

The findings of this study suggest that COLEC12 is involved in oxidative stress and glucose consumption in IR-HTR-8/SVneo, and its knockdown reduces GLUT1 distribution, but enhances glucose consumption and GLUT4 expression by activating insulin signaling. Furthermore, COLEC12 knockdown exerts antioxidant effects, which may be attributed to its down-regulation of COX-2/PGE2. Therefore, targeting COLEC12 is a potential strategy for achieving GDM therapy.

DECLARATIONS

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Beibei Jin and Lin Yu designed the study and performed the experiments; Mi Hu and Jie Chen supervised data collection, analyzed and interpreted the data; Beibei Jin and Lin Yu prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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