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TDAG51 Attenuates Impaired Lipid Metabolism and Insulin Resistance in Gestational Diabetes Mellitus Through SREBP-1/ ANGPTL8 Pathway

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Background: T-cell death-associated gene 51 (TDAG51) belongs to the transcription factor family and is involved in the energy homeostasis of the liver through the regulation of lipogenesis.

Aims: To evaluate the role of T-cell death-associated gene 51 in gestational diabetes mellitus.

Study Design: Experimental animal and human-sample study.

Methods: A total of 30 patients with GDM were enrolled in the study. TDAG51 expression in patients with gestational diabetes mellitus was assessed by Western blotting and quantitative reverse-transcription polymerase chain reaction. A high-fat and high-sugar diet was used to establish a gestational diabetes mellitus model. Mice with gestational diabetes were injected with lentivirus-mediated overexpression of TDAG51. Blood glucose was measured using a glucometer, and glucose and insulin tolerance tests were performed to detect insulin resistance. Liver and adipose tissues were subjected to hematoxylin-eosin staining. Cell apoptosis was detected by TUNEL staining. Human villous trophoblast cells (HTR-8/SVneo) were treated with a high-glucose medium to induce gestational diabetes mellitus.

Results: TDAG51 was downregulated in gestational diabetes mellitus and high glucose-induced HTR-8/SVneo. TDAG51 overexpression reduced the level of blood glucose, enhanced serum insulin, and attenuated glucose and insulin tolerance in gestational diabetes mellitus mice. TDAG51 overexpression also ameliorated impaired lipid metabolism and alleviated adipocyte hypertrophy and hepatic lipid droplets in gestational diabetes mellitus mice. The expressions of SREBP-1 and ANGPTL8 were upregulated in gestational diabetes mellitus and showed a negative correlation with TDAG51 in patients with gestational diabetes mellitus. TDAG51 increased the expressions of SREBP-1 and ANGPTL8 in gestational diabetes mellitus mice. TDAG51 overexpression reduced cell apoptosis and enhanced cell viability of high glucose-induced HTR-8/SVneo. Ectopic expression of ANGPTL8 attenuated the TDAG51-induced increase in cell viability and decrease in apoptosis in high glucose-induced HTR-8/SVneo.

Conclusion: TDAG51 alleviated impaired lipid metabolism and insulin resistance in gestational diabetes mellitus via downregulation of SREBP 1/ANGPTL8 pathway.

INTRODUCTION

Gestational diabetes mellitus (GDM) affects approximately 2-5% of normal pregnancies.[1] Dysfunction of pancreatic β-cells in patients with GDM induces insufficient production of insulin and leads to insulin resistance and disturbed metabolism, resulting in an increased risk of metabolic abnormalities, congenital anomalies, macrosomia, hydramnios, preeclampsia, and type 2 diabetes mellitus.[2] Therefore, lipid metabolism and glycometabolism alterations and insulin resistance are mainly implicated in the pathogenesis of GDM.[2] Moreover, the offspring of GDM women are susceptible to premature delivery, birth injury, macrosomia, respiratory distress syndrome, and high-glucose tolerance disorder.[3] Current strategies, including insulin treatment, diet, and lifestyle interventions, are widely used for GDM.[4] However, long-term usage of these strategies might affect the developing fetus.[4] Therefore, novel and effective strategies are beneficial for GDM treatment.

T-cell death-associated gene 51 (TDAG51) functions as a transcription factor in various cellular processes.[5] For example,

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TDAG51 promoted renal interstitial fibrosis and cell apoptosis in mice with chronic kidney disease.[6] TDAG51 exerts a protective effect against depression-like behavior and maternal care defects after parturition.[7] TDAG51 deletion regulated adipogenesis and induced insulin resistance, hepatic steatosis, and mature obesity.[8] However, TDAG51 overexpression reduced weight gain and improved insulin signaling.[9] TDAG51 was regarded as a potential target for obesity and insulin resistance.[7] TDAG51 was downregulated in the umbilical cord plasma of patients with GDM.[10] However, the role of TDAG51 in GDM remains unclear.

SREBP-1 is a transcription factor that regulates the synthesis of cholesterol and unsaturated fatty acids and is involved in innate immunity, cancer, insulin resistance, and metabolic syndrome.[11,12] Abnormal SREBP-1 expression was associated with the pathogenesis of diabetes.[13] SREBP-1 regulates the expression of genes involved in insulin signaling, such as PI3K, PI3KR3, and insulin receptor substrate 2.[14] The downregulation of SREBP-1 attenuated insulin resistance in mice fed a high-fat diet.[15] The inhibition of SREBP-1 contributed to the amelioration of gestational diabetes.[16] Since the loss of TDAG51 resulted in the upregulation of SREBP-1,[8] TDAG51 might be involved in insulin resistance and lipid metabolism in GDM through SREBP-1.

In this study, the effects of TDAG51 on insulin resistance and lipid metabolism of GDM mice were investigated. The underlying mechanism might provide a promising target for GDM.

MATERIALS AND METHODS

Patients' Samples

A total of 30 patients with GDM were recruited at Third Affiliated Hospital of Zunyi Medical University between 2017 and 2019, and 10 healthy pregnant women were enrolled as control. Participants, who provided written informed consent, were subjected to 75-g oral glucose tolerance test at 24-28 weeks of gestation. Patients with glucose levels 1 h post-load of 10.0 mmol/l, 2 h post-load of 8.5 mmol/l, or more than fasting 5.1 mmol/l were diagnosed with GDM. Patients with systemic infections, malignant tumors, abnormal liver and kidney function, and pregestational diabetes mellitus were excluded. The clinical characteristics of all the participants are shown in Table 1.

The study was approved by the Third Affiliated Hospital of Zunyi Medical University (approval no. 2016-021). Umbilical cord vein blood and placenta samples were collected from all the participants. A glucose analyzer (YSI, Yellow Springs, OH, USA) was used to detect the blood glucose level in each participant.

Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Umbilical cord vein blood and placenta samples were lyzed in Trizol (Sigma-Aldrich, St. Louis, MO, USA). Isolated RNAs were then reverse-transcribed and subjected to qRT-PCR analysis. The expressions of TDAG51 (forward, 5′-ATGCTGGAGAA CAGCGGCTGC-3′; reverse, 5′-GGTATGGCTGATTATGATC-3′), SREBP-1 (forward, 5′-ACTTCTGGAGGCATCGCAAGCA-3′; reverse, 5′-AGGTTCCAGAGGAGGCTACAAG-3′), and ANG PTL8 (forward, 5′-CTTCGGGCAAGCCTGTT-3′; reverse, 5′-GCTGTCCCGTAGCACCTTC-3′) were determined using the 2 -$^{\Delta\Delta}$CT method. GAPDH (forward, 5′-GGATTTGGTC GTATTGGG-3′; and reverse, 5′-GGAAGATGGTGATGGGATT-3′) was used as endogenous control.

Immunohistochemistry

Placenta samples were fixed, embedded, and then sliced. The sections were treated with 3% hydrogen peroxide and then blocked in 5% dry milk. Following incubation with primary antibody (anti-TDAG51; 1:50; Abcam, Cambridge, MA, USA), sections were incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:100; Nuoyang, Beijing, China). Sections were incubated with 3,3′-diaminobenzidine (Sigma-Aldrich) and measured under a microscope (Olympus, Tokyo, Japan).

Animal Models

A total of 40 male C57BL/6J and 80 female mice were fed with normal laboratory animal feed and kept in standard conditions. The experiment was approved by the Third Affiliated Hospital of Zunyi Medical University. The 80 female mice were divided into the control (n = 20) and GDM (n = 60) groups. The control group was fed with normal laboratory animal feed, and the GDM groups were fed with high-fat and high-sugar feed (65% common feed, 10% sugar, 10% yolk, and 15% lard). Eight weeks later, female and male mice (ratio of 2:1) were caged. Vaginal secretions or emboli were examined by microscopy next day, and mice were considered pregnant with an observation of the vaginal embolus or sperm. The gestational day was recorded as GD0. A total of 40 mice were successfully pregnant, with 10 female mice in the control group and 30 in the GDM group. The rest of the mice were abandoned.

TABLE 1. Clinical Characteristic of Participants and Pregnancy.

Characteristic	Control (n = 10)	GDM (n = 30)	*p* value
Age (Y)	28.30 ± 2.26[7.99]	29.07 ± 2.24[7.71]	0.3561
Gestation age (W)	39.10 ± 1.91[4.88]	39.53 ± 1.96[4.96]	0.5462
Pre-maternal BMI (kg/m^2)	20.18 ± 1.56[7.73]	22.26 ± 2.40[10.78]	0.0149*
Fasting blood (mmol/l)	4.15 ± 0.39[9.4]	5.89 ± 1.25[21.22]	<0.001***
1 hour blood glucose (mmol/l)	7.23 ± 0.30[4.15]	10.12 ± 0.34[3.36]	<0.001***
2 hour blood glucose (mmol/l)	6.28 ± 0.27[4.3]	8.42 ± 0.31[3.68]	<0.001***
Birth weight (g)	3,339 ± 291[8.72]	3633 ± 369[10.16]	0.0274*

GDM, gestational diabetes mellitus; body mass index (BMI), means weight (kg)/square of height (m^2); n, number; Y, year; W, week; Data are presented as mean ± SD, [] means Coefficient of Variance. Statistical analysis was determined by t-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

The lentivirus for TDAG51 overexpression (OE-TDAG51) was acquired from Genechem Co., Ltd. (Shanghai, China). Pregnant mice in the GDM group were divided into three groups: GDM (n = 10), GDM with vector (n = 10), and GDM with OE-TDAG51 (n = 10). The GDM with vector group and the GDM with OE-TDAG51 group were injected with lentivirus via the tail vein at a dose of $1x10^7$ U. Levels of blood glucose and serum insulin were detected at GD0, GD10, and GD20. Glucose and insulin tolerance tests were performed at GD15.

Measurement of Blood Glucose and Serum Insulin

Blood samples were obtained from mice in each group via tail venipuncture. A glucometer (Roche Diagnostics, Risch-Rotkreuz, Switzerland) was used to detect blood glucose levels. The serum level of insulin was detected using an ELISA kit (NJJCBIO Co., Ltd, Nanjing, China).

Measurement of Glucose and Insulin Tolerance

Mice were fasted for 6 h before intraperitoneally injected with 2.0 g/kg glucose. Blood glucose concentrations were analyzed by the glucometer at 0, 30, 60, 90, and 120 min. Mice were also fasted for 1 h before intraperitoneally injected with 1.0 mU/kg insulin for the insulin tolerance test. Blood glucose concentrations were also analyzed using a glucometer at 0, 30, 60, 90, and 120 min.

Measurement of Lipid Metabolism

On GD20, serum levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglyceride (TG) were measured using enzyme-linked immunoassay (ELISA) kits.

Hematoxylin-eosin Staining

On GD20, mice were sacrificed by cervical dislocation, and liver and adipose tissues were collected. Tissues were fixed, paraffin-embedded, and sliced. Sections were treated with hematoxylin and then subjected to eosin staining before observation under the microscope.

Cell Culture and Transfection

HTR-8/SVneo was grown in RPMI-1640 medium (BioTek company, Beijing, China) at 37 °C. Cells were treated with 25 nM glucose to induce a cell model of GDM. Cells were cultured in a medium with 5 nM glucose and set as a control group. Cells were transfected with pcDNA-TDAG51 or co-transfected with pcDNA-TDAG51 and pcDNA-SREBP-1 using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Viability Assay

HTR-8/SVneo were subjected to different transfections in 96-well plates and then cultured in a medium for another 24, 48, or 72 h. Cells were treated with CCK8 solution (Beyotime, Beijing, China) for 2 h, and absorbance at 450 nm was examined via a Microplate Autoreader (Thermo Fisher Scientific).

TUNEL Staining

On GD20, the placenta tissues of mice were fixed, paraffin-embedded, and sliced. Sections were then treated with 50 μg/ml proteinase K and incubated in a TdT enzyme working solution of the TUNEL kit (Nanjing KeyGen Biotechnology Co. Ltd., Nanjing, China). Sections were then treated with horseradish peroxidase working solution and incubated with 3,3′-diaminobenzidine. Sections were counter-stained by hematoxylin and measured under a microscope. HTR-8/SVneo were also fixed and then permeabilized with 0.2% Triton-X. Following incubation with the TdT enzyme working solution, cells were subjected to the same procedures to detect cell apoptosis.

Western Blot

Tissues and cells were lysed to collect the supernatants. Protein samples were segregated and then transferred onto nitrocellulose membranes. Membranes were then incubated with primary antibodies: anti-TDAG51 and anti-GAPDH (1:2,000), anti-Bax (1:3,000), anti-cleaved caspase 3 and anti-cleaved PARP (1:4,000), and anti-SREBP-1 and anti-ANGPTL8 (1:5,000). The membranes were incubated with secondary antibodies (1:5,000) and then subjected to a chemiluminescence reagent kit (Beyotime). All antibodies were purchased from Abcam.

Statistical Analysis

All data were expressed as mean ± standard deviation and analyzed by Student's t-test or one-way analysis of variance. The Pearson correlation coefficient was used for the correlation analysis, and $p < 0.05$ was considered statistically significant.

RESULTS

TDAG51 was Reduced in GDM

To investigate the relationship between TDAG51 and GDM, the expression level of TDAG51 in GDM was evaluated. The expression of TDAG51 was downregulated in umbilical cord vein blood (Figure 1a and b) and placenta (Figure 1c and d) samples of the GDM group compared with that of the control group. Immunohistochemical analysis also confirmed the downregulation of TDAG51 in the placenta samples of the GDM group (Figure 1e). Moreover, a high expression of TDAG51 was significantly associated with fasting blood, 1-h blood glucose, 2-h blood glucose, and birth weight (Table 1), suggesting that TDAG51 might be involved in GDM.

TDAG51 Ameliorated Insulin Resistance in GDM

High-fat and high-sugar diets were used to establish a GDM model. TDAG51 was also decreased in the placenta samples of GDM mice (Figure 2a). To assess the effect of TDAG51 on GDM, GDM mice were injected with lentivirus-mediated overexpression of TDAG51 (Figure 2a). Blood glucose (Figure 2b) was increased, and serum insulin (Figure 2c) decreased in GDM mice. However, the overexpression of TDAG51 reduced blood glucose (Figure 2b) and enhanced serum insulin (Figure 2c) in GDM mice on GD10 and GD20. Moreover, data from the glucose tolerance test showed that the blood glucose level in GDM mice was higher than that in control mice after glucose injection (Figure 2d). The overexpression of TDAG51 reduced the blood glucose

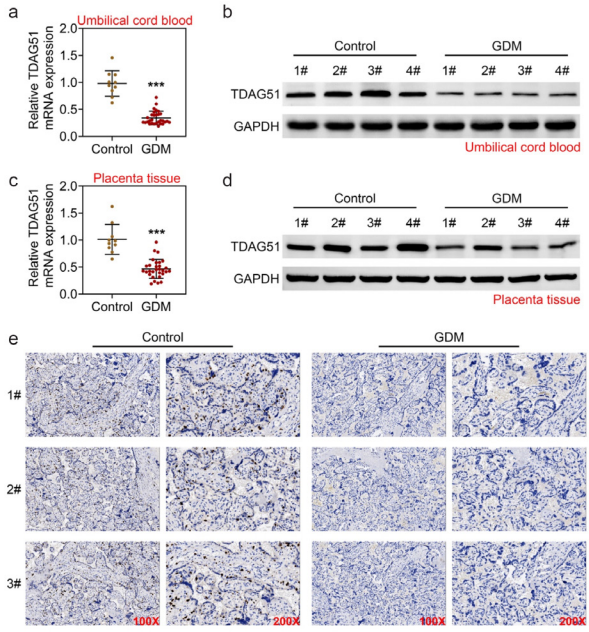

FIG. 1. TDAG51 was reduced in GDM
(a) mRNA expression of TDAG51 was downregulated in the umbilical cord vein blood of patients with GDM.
(b) Protein expression of TDAG51 was downregulated in the umbilical cord vein blood of patients with GDM.
(c) mRNA expression of TDAG51 was downregulated in the placenta of patients with GDM.
(d) Protein expression of TDAG51 was downregulated in the placenta of patients with GDM.
(e) Immunohistochemical analysis confirmed the downregulation of TDAG51 in the placenta samples of patients with GDM. *** vs. control, $p < 0.001$.

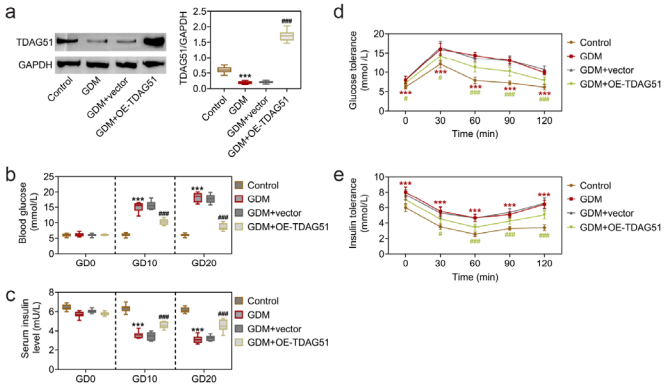

FIG. 2. TDAG51 ameliorated insulin resistance in GDM
(a) The protein expression of TDAG51 was downregulated in the placenta of GDM mice, and the injection with lentivirus-mediated overexpression of TDAG51 enhanced the expression of TDAG51.
(b) The overexpression of TDAG51 reduced the blood glucose in GDM mice on GD10 and GD20.
(c) The overexpression of TDAG51 enhanced serum insulin in GDM mice on GD10 and GD20.
(d) The overexpression of TDAG51 reduced the blood glucose levels in GDM mice after glucose injection.
(e) The overexpression of TDAG51 reduced the serum insulin levels in GDM mice after insulin injection. *** vs. control, $p < 0.001$. #, ### vs. GDM, $p < 0.05$, $p < 0.001$.

levels in GDM mice (Figure 2d). The insulin tolerance test also demonstrated that the overexpression of TDAG51 reduced serum insulin levels in GDM mice (Figure 2e), indicating that TDAG51 attenuated insulin resistance in GDM mice.

TDAG51 Ameliorated Impaired Lipid Metabolism in GDM

The HDL level was downregulated, and the TG, TC, and LDL levels were upregulated in GDM mice (Figure 3a). The overexpression of TDAG51 enhanced HDL and reduced TG, TC, and LDL levels in GDM mice (Figure 3a). Histological analysis showed that lipid droplets were accumulated in the liver (Figure 3b) and adipose (Figure 3c) tissues of GDM mice. However, the overexpression of TDAG51 alleviated hepatic lipid droplets (Figure 3b) and adipocyte hypertrophy (Figure 3c), revealing that TDAG51 attenuated impaired lipid metabolism in GDM mice.

TDAG51 Ameliorated Placenta Apoptosis in GDM

Apoptosis of placenta cells of samples from GDM mice was promoted compared with the control mice (Figure 4a). However, the overexpression of TDAG51 reduced cell apoptosis (Figure 4a). TDAG51 also decreased Bax, cleaved caspase 3, and cleaved PARP in the placenta samples of GDM mice (Figure 4b).

TDAG51 Downregulated SREBP-1 and ANGPTL8 Expressions in GDM

Protein expressions of SREBP-1 and ANGPTL8 were increased in the placenta samples of GDM mice (Figure 5a). The overexpression of TDAG51 decreased SREBP-1 and ANGPTL8 expressions in GDM mice (Figure 5a). SREBP-1 and ANGPTL8 levels were also upregulated in the placenta samples of the GDM group (Figure 5b) and showed a negative correlation with TDAG51 (Figure 5c).

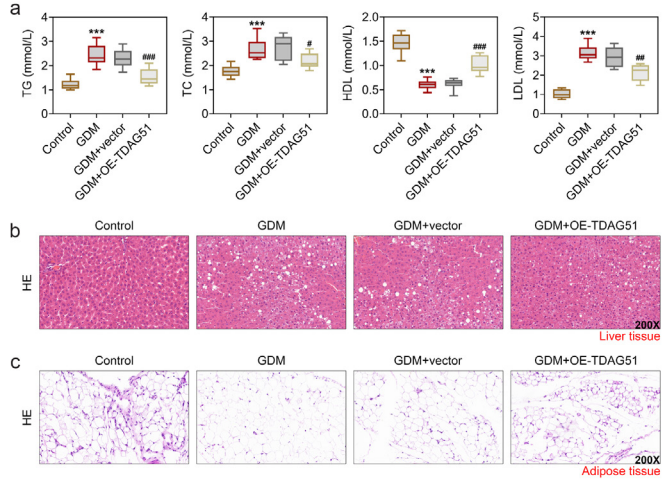

FIG. 3. TDAG51 ameliorated impaired lipid metabolism in GDM
(a) The overexpression of TDAG51 enhanced HDL and reduced TG, TC, and LDL levels in GDM mice.
(b) The histological analysis showed that lipid droplets accumulated in the liver of GDM mice, whereas TDAG51 overexpression alleviated hepatic lipid droplets.
(c) Lipid droplets accumulated in the adipose of GDM mice, whereas the overexpression of TDAG51 alleviated adipocyte hypertrophy. *** vs. control, $p < 0.001$. #, ##, ### vs. GDM, $p < 0.05$, $p < 0.01$, $p < 0.001$.

TDAG51 Increased the Cell Viability of High Glucose-Induced HTR-8/SVneo

High glucose was used to induce GDM in HTR-8/SVneo. TDAG51 was downregulated, whereas SREBP-1 and ANGPTL8 were upregulated in high glucose-induced HTR-8/SVneo (Figure 6A). The overexpression of TDAG51 reduced the expressions of SREBP-1 and ANGPTL8 (Figure 6a). Cell viability was decreased by high-glucose treatment, whereas the overexpression of TDAG51 increased the viability of high glucose-induced HTR-8/SVneo (Figure 6b). TDAG51 also suppressed cell apoptosis (Figure 6c and d).

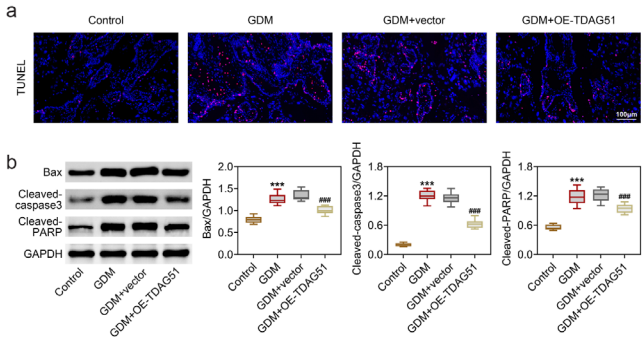

FIG. 4. TDAG51 ameliorated placenta apoptosis in GDM
(a) The overexpression of TDAG51 reduced cell apoptosis of the placenta in GDM mice.
(b) The overexpression of TDAG51 decreased the protein expressions of Bax, cleaved caspase 3, and cleaved PARP in the placenta samples of GDM mice. *** vs. control, $p < 0.001$. ### vs. GDM, $p < 0.001$.

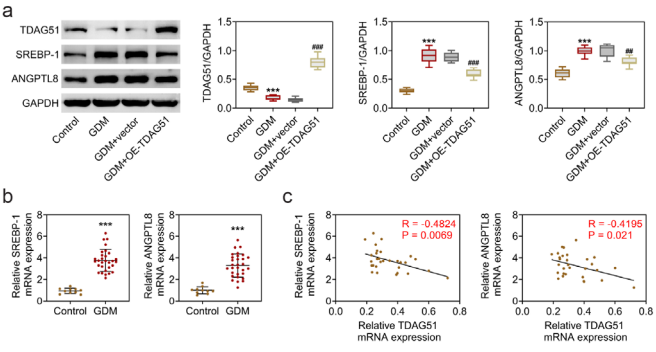

FIG. 5. TDAG51 downregulated SREBP-1 and ANGPTL8 expressions in GDM
(a) The protein expressions of SREBP-1 and ANGPTL8 were increased in the placenta samples of GDM mice, and the overexpression of TDAG51 decreased the expressions of SREBP-1 and ANGPTL8 in GDM mice.
(b) The expressions of SREBP-1 and ANGPTL8 were upregulated in the placenta samples of patients with GDM.
(c) SREBP-1 and ANGPTL8 showed a negative correlation with TDAG51 in patients with GDM. *** vs. control, $p < 0.001$. ### vs. GDM, $p < 0.001$.

TDAG51 Ameliorated High Glucose-Induced Cell Injury in HTR-8/SVneo

The overexpression of SREBP-1 attenuated the TDAG51-induced reduced ANGPTL8 expression in high glucose-induced HTR-8/SVneo (Figure 7a). SREBP-1 also weakened the TDAG51-induced increase in cell viability (Figure 7b) and decrease in apoptosis (Figure 7c) in high glucose-induced HTR-8/SVneo.

DISCUSSION

A previous study showed that TDAG51 was decreased during adipogenesis, and TDAG51 was negatively associated with fatty liver in mice with hepatic steatosis.[8] Therefore, TDAG51 was regarded as a potential target for obesity and insulin resistance.[8] This study found that TDAG51 ameliorated insulin resistance and attenuated the impaired lipid metabolism in GDM mice.

In line with a previous study that TDAG51 was downregulated in umbilical cord plasma of patients with GDM,[10] our study also confirmed the decreased TDAG51 expression in umbilical cord vein blood and placenta samples of patients with GDM. GDM refers to abnormal glycometabolism during pregnancy, and a high-fat and high-sugar diet decreased serum insulin and increased blood glucose level, leading to insulin resistance.[17] Therefore, high-fat and high-sugar diets were used for animal GDM models.[18] Blood glucose was upregulated, whereas serum insulin was downregulated in GDM mice. Moreover, the glucose and insulin tolerance

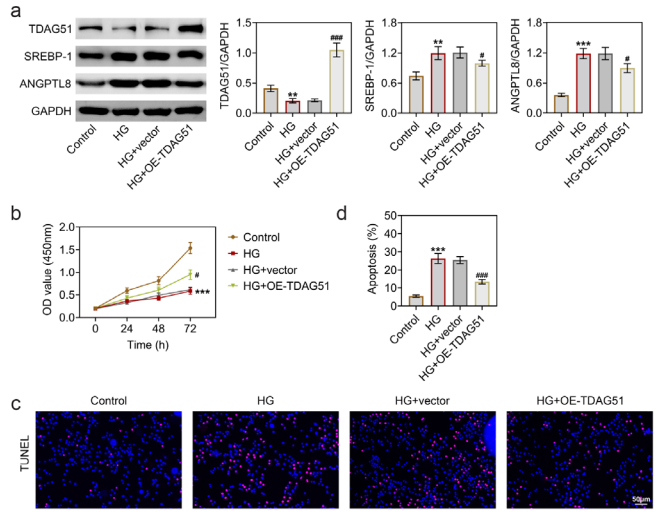

FIG. 6. TDAG51 increased the cell viability of high glucose-induced HTR-8/SVneo
(a) TDAG51 was downregulated, and SREBP-1 and ANGPTL8 were upregulated in high glucose-induced HTR-8/SVneo cells, whereas the overexpression of TDAG51 reduced the expressions of SREBP-1 and ANGPTL8.
(b) The overexpression of TDAG51 increased the cell viability of high glucose-induced HTR-8/SVneo.
(c) The overexpression of TDAG51 suppressed cell apoptosis of high glucose-induced HTR-8/SVneo.
(d) Relative cell apoptosis of high glucose-induced HTR-8/SVneo cells. **, *** vs. control, $p < 0.01$, $p < 0.001$. #, ### vs. HG (high glucose), $p < 0.05$, $p < 0.001$.

tests confirmed insulin resistance in GDM mice. TDAG51 was decreased in the placenta samples of GDM mice. TDAG51 loss induces a decrease in glucose disposal, increases plasma-free fatty acids, and stimulates insulin resistance.[8] Our results showed that the overexpression of TDAG51 decreased blood glucose levels and increased serum insulin levels to ameliorate insulin resistance in GDM mice.

Lipid metabolic disorder is a critical regulator of GDM onset.[17] Moreover, patients with GDM showed lower levels of LDL and higher levels of TG, TC, and LDL than women with normal pregnancies.[19] Our study also identified dysregulated lipid metabolism in GDM mice. Furthermore, lipid droplets were accumulated in the liver and adipose tissues of GDM mice. TDAG51 deficiency enhanced lipid accumulation in adipocytes and promoted adiposity with enlarged adipocytes.[8] Our results indicated that the overexpression of TDAG51 enhanced HDL and reduced TG, TC, LDL, and lipid accumulation in GDM mice. SREBP-1 is essential for the synthesis of cholesterol and unsaturated fatty acids, and TDAG51 loss promoted the upregulation of SREBP-1.[8] SREBP-1 was upregulated in GDM, and the overexpression of TDAG51 reduced SREBP-1 expression in GDM mice. Considering that the downregulation of SREBP-1 attenuated insulin resistance in mice fed a high-fat diet,[15] TDAG51 may ameliorate insulin resistance and impaired lipid metabolism in GDM mice through the inhibition of SREBP-1.

ANGPTL8 is expressed in the liver and adipose tissues.[20] ANGPTL8 is involved in lipid and glucose metabolism and pathogenesis of metabolic diseases.[20] The knockdown of ANGPTL8 reduced glucose uptake and ameliorated insulin resistance in HTR-8/SVneo.[21] Moreover, ANGPTL8 was elevated in the plasma of women with GDM,[22] and a high ANGPTL8 expression was considered a predictor of GDM.[23] Our results showed that ANGPTL8 was elevated in GDM and negatively correlated with TDAG51 in patients with GDM. The silencing of SREBP-1 decreased ANGPTL8 expression in HepG2 cells.[24] In this study, the overexpression of TDAG51 reduced ANGPTL8 expression in GDM mice. TDAG51 enhanced cell viability but reduced apoptosis of high glucose-induced HTR-8/SVneo. Furthermore, TDAG51 attenuated the TDAG51-induced increase in cell viability and decrease in apoptosis of high glucose-induced HTR-8/SVneo, revealing that TDAG51 might alleviate GDM through the downregulation of SREBP-1/ANGPTL8.

Collectively, TDAG51 ameliorated insulin resistance and impaired lipid metabolism in GDM mice. TDAG51 reduced SREBP-1 and ANGPTL8 expressions and TDAG51 reduced high glucose-induced cell injury through the downregulation of SREBP1 and ANGPTL8. Therefore, TDAG51/SREBP-1/ANGPTL8 might be a novel target for GDM. However, whether TDAG51 can bind to the promoter of SREBP-1 and regulates the expression should be assessed in future research.

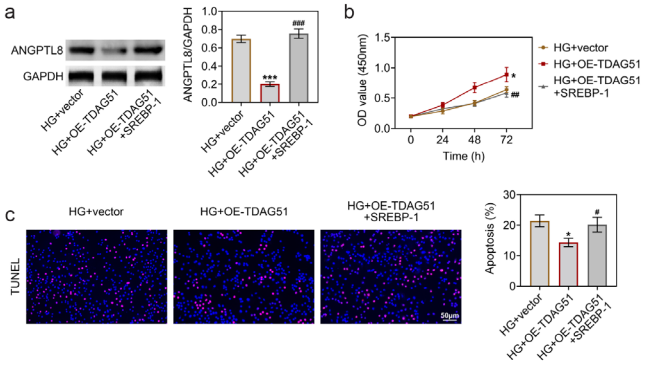

FIG. 7. TDAG51 ameliorated high glucose-induced cell injury in HTR-8/SVneo
(a) The overexpression of SREBP-1 attenuated the TDAG51-induced decrease in ANGPTL8 expression in high glucose-induced HTR-8/SVneo.
(b) The overexpression of SREBP-1 weakened the TDAG51-induced increase in cell viability in high glucose-induced HTR-8/SVneo.
(c) The overexpression of SREBP-1 weakened the TDAG51-induced decrease in apoptosis in high glucose-induced HTR-8/SVneo. *, *** vs. control, $p < 0.05$, $p < 0.001$. #, ## vs. HG, $p < 0.05$, $p < 0.01$.

Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the standards upheld by the Ethics Committee of Third Affiliated Hospital of Zunyi Medical University and with those of the 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects (approval no: 2016-021).

Informed Consent: Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- X.W., B.X.; Design- X.W., B.X.; Data Collection or Processing- X.W., B.X.; Analysis or Interpretation- X.W., B.X.; Literature Search- X.W., B.X.; Writing- X.W., B.X.

Conflict of Interest: No conflict of interest was declared by the authors.

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