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Txnip inhibits porcine adipocyte differentiation through PPARγ and impairs the induction of glucose via ChREBP

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ABSTRACT - To explore the functions of *Txnip* and its mechanism in adipocyte differentiation, the preadipocytes were isolated from subcutaneous adipose tissue of three-day-old piglets and induced into adipogenic differentiation. The expression of *Txnip* and *ChREBP* was silenced, and the *Txnip* overexpression was achieved in the cells with transfection of the recombinant lentivirus strategies. *Txnip* silencing promoted the differentiation of porcine preadipocytes and *PPARγ* expression, and a *PPARγ* inhibitor reduced this facilitation. Instead, *Txnip* overexpression exerted a suppressive effect on the cell differentiation and *PPARγ* expression, and the PPARγ agonist offset this inhibition. High glucose stimulated the preadipocyte differentiation and expressions of *ChREBP* and *Txnip*. In contrast, *Txnip* expression was reduced by *ChREBP* silencing, suggesting glucose-regulated *Txnip* expression through the mediation of ChREBP. Moreover, the expressions of *ChREBP* and *Glut4* induced by high glucose and glucose uptake of the cells were reduced by *Txnip*-overexpression, but increased by *Txnip* silencing, while these changes of *Txnip* did not alter their expressions under low glucose. Collectively, Txnip could be an inhibitor of porcine preadipocyte differentiation, which attenuated the adipogenesis through the negative feedback regulation on *PPARγ*. Txnip impaired the induction of glucose on the preadipocyte differentiation through decreasing *Glut4* expression and glucose uptake and subsequent decrease of the expression and transcriptional activity of ChREBP.

Keywords: adipose tissue, lipid, pig

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1. Introduction

The body fat deposition in animals is characterized by the increment in adipogenesis at a cellular level. Adipogenesis is a comprehensive result of preadipocyte proliferating and differentiating into adipocytes. The adipocyte differentiation is regulated by a complex and coordinated gene expression program and many protein factors are involved in this process. Thioredoxin-interacting protein (Txnip), as the endogenous inhibitor, inhibits the reduction activity of thioredoxin (Trx) and then regulates many fundamental cellular processes, including differentiation (Hsiao et al., 2022), apoptosis (Schuster et al., 2018), and metabolism (Kim et al., 2018). Increasing evidence suggests Txnip plays a key physiological role in cell development (Chutkow and Lee, 2011; Park et al., 2018). However, it is unclear the function of *Txnip* and its mechanism in the adipogenesis of porcine adipocytes, are the primary cells for *de novo* lipogenesis.

The carbohydrate diet is the main energy source and substrate for *de novo* lipogenesis in pigs. It has been reported that glucose promoted *Txnip* expression in 3T3-L1 cells (Robinson et al., 2013) and cancer cells (Waldhart et al., 2017). A study on 3T3-L1 cells showed that ChREBP activation induces peroxisome proliferator-activated receptor γ (PPARγ) activity and promotes adipocyte differentiation by controlling the generation of PPARγ endogenous fatty acid ligand, which is regulated by *de novo* synthesis and desaturation of fatty acids (Witte et al., 2015). PPARγ is considered the main transcription factor regulating adipogenesis and plays a central role in adipocyte differentiation (Stachecka et al., 2019). The PPARγ activation invokes the expression of target genes related to adipogenesis, resulting in continuous fat accumulation (Mueller, 2014). Consequently, Txnip might participate in the induction of glucose on adipogenesis through ChREBP, which is interesting in regulating fat deposition in pigs.

In this report, we presented a mechanism whereby Txnip inhibited porcine adipocyte differentiation through the negative feedback regulation of PPARγ expression and transcriptional activity. Moreover, we confirmed that *Txnip* expression in the adipocyte was promoted by glucose. In turn, it hindered glucose uptake of the cells *via* down-regulating *Glut*4 expression, which reduced expression and activity of ChREBP and impaired the porcine adipocyte adipogenesis induced by glucose.

2. Material and Methods

2.1. Experimental animals

This research was carried out in Lanzhou, Gansu, China (Latitude 36°03' N and Longitude 103°40' E). Three-day-old male crossbred piglets (Duroc × Landrace × Large White) from different litters were used in this study. Research on animals was conducted according to the institutional committee on animal use (case no. 0307/2018).

2.2. Primary cell culture

Primary preadipocytes were prepared as previously described (Zhang et al., 2015) and maintained in the basal medium, DMEM/F12 medium (GIBCO/BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, ScienCell, Carlsbad, CA, USA), at 37 °C in humidified atmosphere with 5% CO_2. The cells grown to confluence were incubated to initiate differentiation with the adipogenic medium, containing 100 nmol L^{-1} insulin (Sigma-Aldrich), 1 μmol L^{-1} dexamethasone (Sigma-Aldrich), and 0.5 mmol L^{-1} IBMX (Sigma-Aldrich) in the basal medium, for three days. Then, a differentiation medium with 100 nmol L^{-1} insulin (Sigma-Aldrich) in the basal medium was used until sample collection. The medium was changed every other day.

2.3. Construction and transfection of recombinant lentivirus expression vector

The coding region of porcine *Txnip* gene was generated by gene synthesis according to the Genebank (No. NM_001044614.2) and cloned into the lentivirus transfer vector pGLV5 (EF-1aF/GFP&Puro) by restriction endonuclease digestion with *Not*I and *Nsi*I to construct recombinant *Txnip* overexpression lentivirus LV5-*Txnip*. After transformed into competent *E. coli* DH5α cells and identified by restriction enzyme digestion and sequencing, the lentivirus LV5-*Txnip* was obtained by the homologous recombination between EF-1aF/GFP&Puro and packaging plasmid (pGag/Pol, pRev and pVSV-G) (GenePharma, Shanghai, China). The recombinant lentivirus was packaged and amplified in 293T cells. After purification, a virus titer was detected by the green fluorescent protein (GFP)-labeled method. An empty lentivirus vector (LV5-GFP) was used as the control. The primary porcine preadipocytes at 30 to 40% confluence were transfected with the lentivirus with polybrene (8 μg mL^{-1}) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

2.4. Lentivirus siRNA construction and transfection

The recombinant lentivirus harboring shRNA against porcine *Txnip* were designed by using a lentivirus system, and the sequences of the oligonucleotides used to create *Txnip*-siRNA were as follows: *Txnip*-shRNA-F is 5′-GATCCGGATCTAGTGGATGTCAATACttcaagagaGTATTGACATCCACTAGATCCTTTTTTG-3′ and *Txnip*–shRNA-R is 5′-AATTCAAAAAAGGATCTAGTGGATGTCAATACtctcttgaaGTATTGACATCCACTAGATCCG-3′. Negative siRNAs did not share sequence similarity with any reported *S. Scrofa* gene sequences and was used as control. Control-shRNA-F is 5′-GATCCGTTCTCCGAACGTGTCACGtttcaagagaACGTGACACGTTCGGAGAACTTTTTTG-3′ and control-shRNA-R is 5′-AATTCAAAAAAGTTCTCCGAACGTGTCACGttctcttgaaACGTGCACGTTCGGAGAACG-3′. The nucleotides were subcloned into the *BamH*I/*EcoR*I site of Lentiviral vector (pGLV3/H1/GFP+Puro). All the shRNA were confirmed by DNA sequencing. Porcine preadipocytes at density of 30-40% were transfected with *Txnip*-siRNA or control-siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

ChREBP-siRNA was constructed in our laboratory before, and the detailed information is described in the study by Zhang et al. (2015).

2.5. Cell treatments and determination of glucose content

The cultured preadipocytes transfected with *Txnip*-siRNA, *ChREBP*-siRNA, or LV5-*Txnip* grew to 70-80% confluence and were exposed to the adipogenic medium with 5 mmol L^{-1} glucose (0 d). The cells were harvested on 0-10 d after adipogenic inducing for the Oil Red O staining, real-time RT-PCR, and Western blotting assays.

To determine the role of *PPARγ* in the adipocyte differentiation regulated by *Txnip*, the *Txnip* silencing cells and the *Txnip* overexpression cells were treated separately with the PPARγ inhibitor GW9662 (15 μmol L^{-1}) and agonist rosiglitazone (1 μmol L^{-1}) for 48 h, and then the cellular lipid content was measured.

To investigate the effect of both *Txnip* silencing and overexpression on glucose uptake and utilization, the cells were cultured for 48 h under low glucose (5 mmol L^{-1}), and then cultured for 48 h in the medium containing 20 mmol L^{-1} glucose (Sigma-Aldrich). The medium was collected to determine glucose content by ELISA (Jonln, Shanghai, China). The relevant information of cell treatments sequence was described in detail in the corresponding legend of resulting graph.

2.6. Cellular lipid content measurement

At indicated times (see the legend of figures), the cellular lipid content analysis was performed according to a modified Oil Red O staining extraction from Ramírez-Zacarías et al. (1992). In brief, the cells cultured in 24-cell plates were rinsed twice with Ca^{2+} and Mg^{2+}-free PBS, and fixed in 10% neutralized formalin for 30 min. Cells were stained for 2 h by complete immersion in 0.2% Oil Red O (Sigma-Aldrich) prepared in 60% isopropanol solution followed by exhaustive rinse with water. Cell morphology was examined and photographed with a microscope. The stained culture dishes were subjected to dye extraction with isopropanol. The optical density (OD) of the solution was measured at 510 nm for quantification, using a UV-2102 PC ultraviolet spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China).

2.7. Real-time PCR

Total cellular RNA was extracted using Trizol reagent using standard techniques (Gibco/BRL, Grand Island, NY) and the cDNA obtained using a reverse transcription kit (Invitrogen). Real-time PCR was performed using a Superscript RT III enzyme kit (Invitrogen). SYBR Green was used as the detection reagent for quantification. The relative expression of each target gene was expressed using the

comparative threshold cycle ($2^{-\Delta\Delta CT}$) method and β-*actin* as an internal control. The specificity of the PCR amplification was always verified with melting curve analysis. The information of specific primers used in this study was listed in Table 1.

Table 1 - Primer for real-time PCR

Gene	Accession no.	Primer sequence (5′ to 3′)	Product length (bp)
Txnip	NM_001044614.2	S: AACAGGGGAGAATGAGATGGTG A: CTTGAGTTGGCTGGCTTGG	167
ChREBP	DQ372586.1	S: GCTCAACGCTGCCATCAA A: GTCCCGCATCTGGTCAAAG	88
PPARγ	NM_214379.1	S: AGGACTACCAAAGTGCCATCAAA A: GAGGCTTTATCCCCACAGACAC	142
Glut4	NM_001128433	S: AGTGGCTGGGAAGGAAGAAG A: TGAGGAACCGTCCAAGAATG	164
ACC1	NM_001114269.1	S: AAGGGCTGCCTCTAATG A: GATGTAAGCGCCGAACT	287
β-*actin*	AF054837	S: GATCGTGCGGGACATCAA A: AGGAAGGAGGGCTGGAAGAG	180

2.8. Western blotting

The cultured cells were scraped with protein lysis buffer (RIPA, Beyotime, Shanghai, China) supplemented by a protease inhibitor (Pierce, Rockford, IL, USA). Lysates were then quantitated, and twenty micrograms of protein were subjected to SDS-PAGE and transferred to PVDF membrane (Millipore, USA). Membranes were incubated with antibodies against PPARγ and β-actin (Santa Cruz Biotechnology).

2.9. Statistical analysis

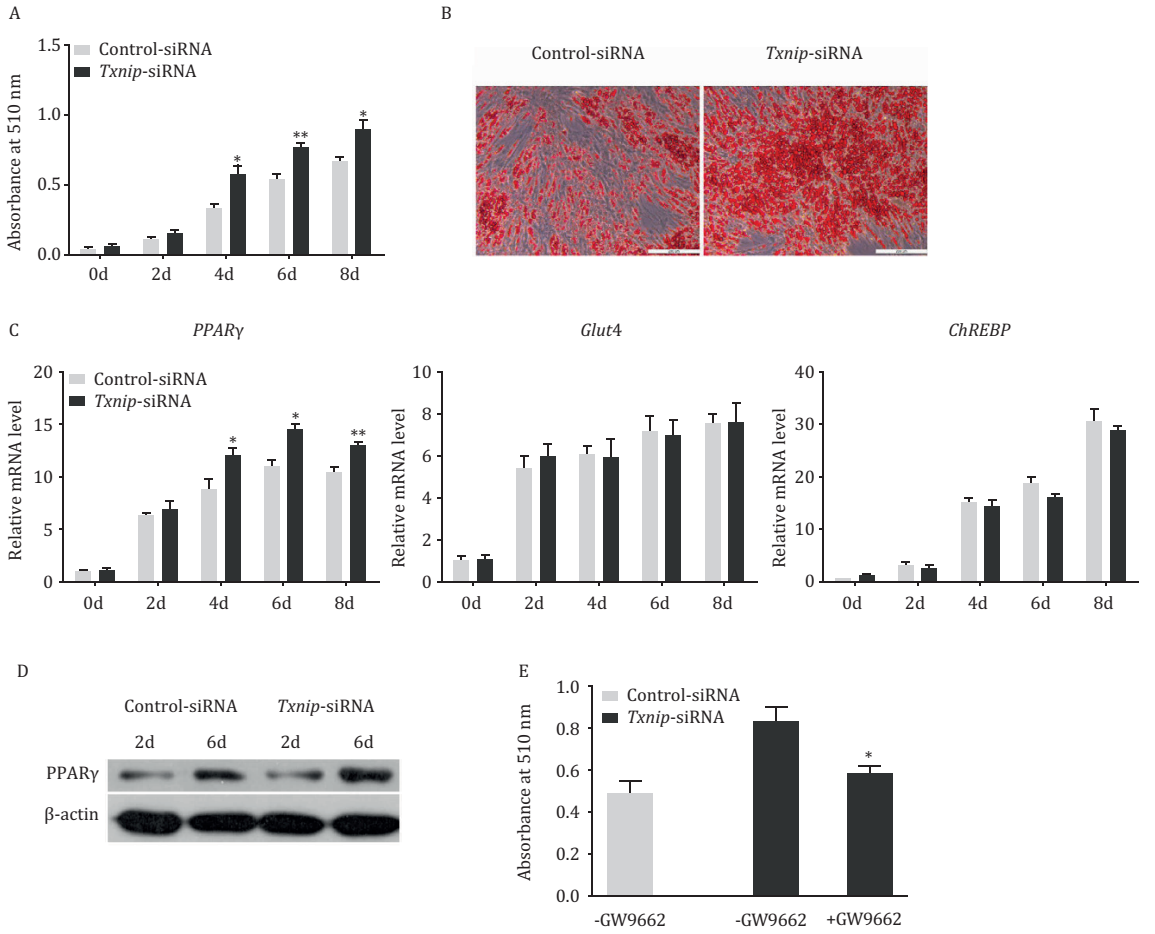
Data was presented as mean±SEM. Data were analyzed by ANOVA using SPSS version 17.0 software (SPSS science, Chicago, IL, USA). Duncan's multiple range tests was used for statistical comparisons. $P<0.05$ was regarded as statistically significant.

3. Results

3.1. *Txnip* expression silencing promoted porcine preadipocyte differentiation

To determine the function of Txnip in the differentiation of the porcine preadipocytes, lentivirus-mediated *Txnip* interference system was constructed. The silencing efficiency for *Txnip* expression in the preadipocytes transfected with *Txnip*-siRNA reached around 75%. The lipid content increased significantly with the adipogenic differentiation, and the content was higher in *Txnip* silencing cells than in control cells on days 4 to 8 ($P<0.05$) (Figure 1A). The increment effect of *Txnip* silencing on adipogenic differentiation on day 8 was also shown by the Oil Red O staining (Figure 1B). As expected, the lipid accumulation markedly increased by silencing of *Txnip* expression. Consistent with the lipid content in the cells, the mRNA expression of *PPARγ* increased significantly when *Txnip* expression was silenced. *Txnip* silencing did not affect the expressions of *ChREBP* and *Glut4* throughout the differentiation stage, although their expressions increased gradually with the preadipocyte differentiation (Figure 1C). In agreement with the mRNA expression, the expression level of PPARγ protein on day 6 increased significantly compared with that on day 2 in both control and *Txnip*-silencing cells and was significantly higher in *Txnip*-silencing cells than in control cells (Figure 1D).

Moreover, on day 4 after adipogenic inducing, the adipocytes transfected with *Txnip*-siRNA were exposed to 15 μmol L^{-1} of GW9662 (a PPARγ inhibitor) for 48 h, and the differentiation of the cells decreased markedly compared with that of untreated cells, which implied that the differentiation promoted by *Txnip* silencing was abated by the reduced PPARγ activity (Figure 1E).



Porcine preadipocytes at the density of around 30~40% were transfected with Control-siRNA or *Txnip*-siRNA, and 48 h later, the cells were induced with adipogenic medium with 5 mmol/L glucose.
A: effect of *Txnip* silencing on the adipogenesis of porcine adipocytes; B: cells stained with Oil Red O on day 8 after adipogenic induction; C: mRNA expressions of adipogenic genes during differentiation by real-time PCR; D: PPARγ protein expressions on day 2 and 6 after adipogenic induction by western blotting; E: effect of PPARγ inhibitor on the adipogenesis on day 6 after adipogenic induction.
Data were presented as the means±SE, n = 9.
*(P<0.05) and **(P<0.01) refer to the significance between the different treatments on the same day.

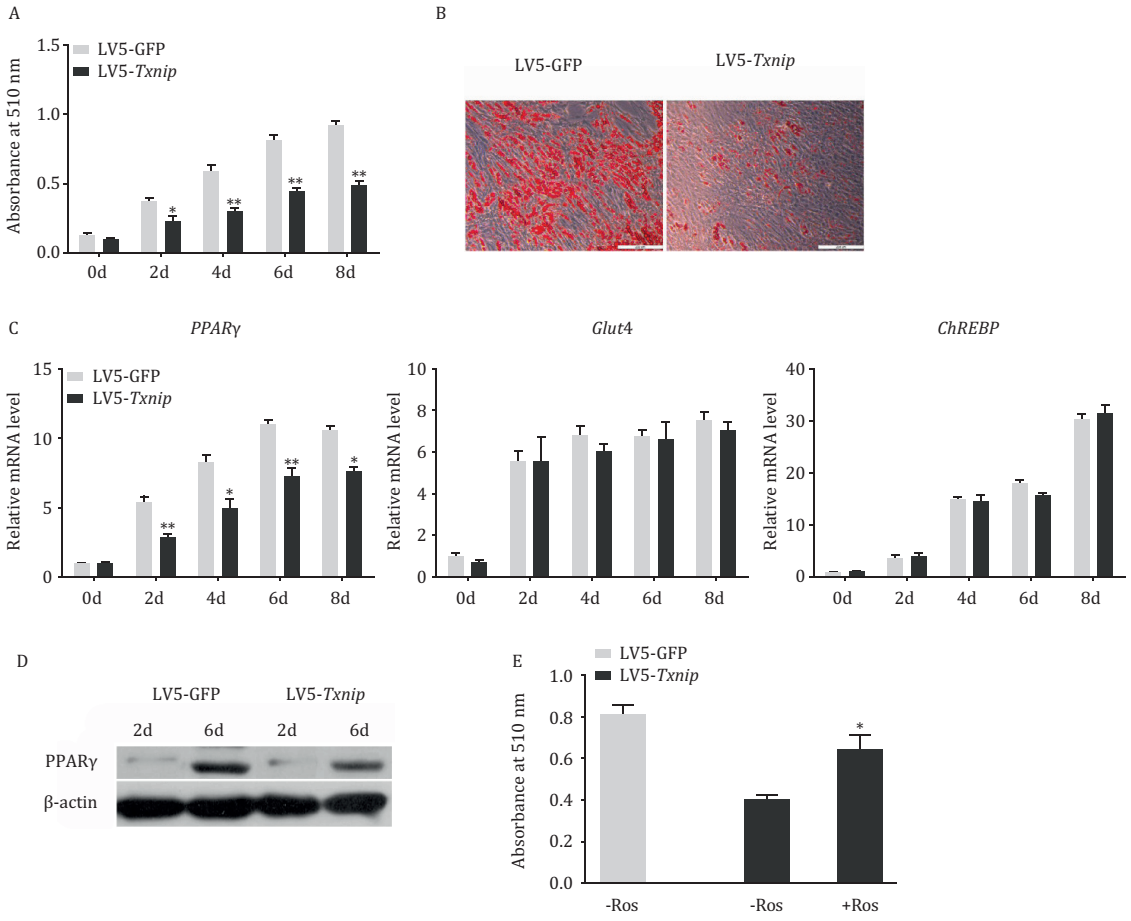
Figure 1 - *Txnip* silencing expression promoted porcine preadipocyte differentiation.

3.2. *Txnip* overexpression inhibited porcine preadipocyte differentiation

The lentivirus-mediated *Txnip* overexpression system was established further to investigate the role of Txnip in porcine preadipocyte differentiation. The preadipocytes were transfected with LV5-*Txnip*, and *Txnip* mRNA level was raised by about 80 folds compared with the cells transfected with LV5-GFP. *Txnip* overexpression inhibited porcine preadipocyte differentiation, which was verified by the decreased amount of lipid accumulation in the Oil Red O staining assay performed on 0-8 d after adipogenic inducing (Figures 2A and B). The mRNA expression of *PPARγ* was significantly lower in cells infected with LV5-*Txnip* than in cells infected with LV5-GFP on days 2 to 8 (P<0.05). In

accordance with this, the PPARγ protein expression level decreased significantly in the cells of *Txnip* overexpression (Figures 2C and D). *Txnip* overexpression had no effect on the expressions of *ChREBP* and *Glut4* throughout the differentiation (Figure 2C).

Txnip-overexpression cells were exposed to 1 μmol L^{-1} of rosiglitazone for 48 h, and the differentiation of the cells increased markedly compared with that of untreated cells, although it was still lower than that of the control cells (Figure 2E), which implied that the differentiation impaired by *Txnip* overexpression was relieved by the heightened PPARγ activity.



Porcine preadipocytes at the density of around 30~40% were infected with LV5-GFP or LV5-*Txnip*, and 48 h later, the cells were induced to differentiation in the adipogenic medium with 5 mmol/L glucose.
A: effect of *Txnip* overexpression on the adipogenesis of porcine adipocytes; B: cells stained with Oil Red O on day 8 after adipogenic induction; C: mRNA expressions of adipogenic genes during differentiation by real-time PCR; D: PPARγ protein expressions on day 2 and 6 after adipogenic induction by western blotting; E: effect of PPARγ agonist (Ros) on the adipogenesis on day 6 after adipogenic induction.
Data were presented as the means±SE, n = 9.
*(P<0.05) and **(P<0.01) refer to the significance between the different treatments on the same day.

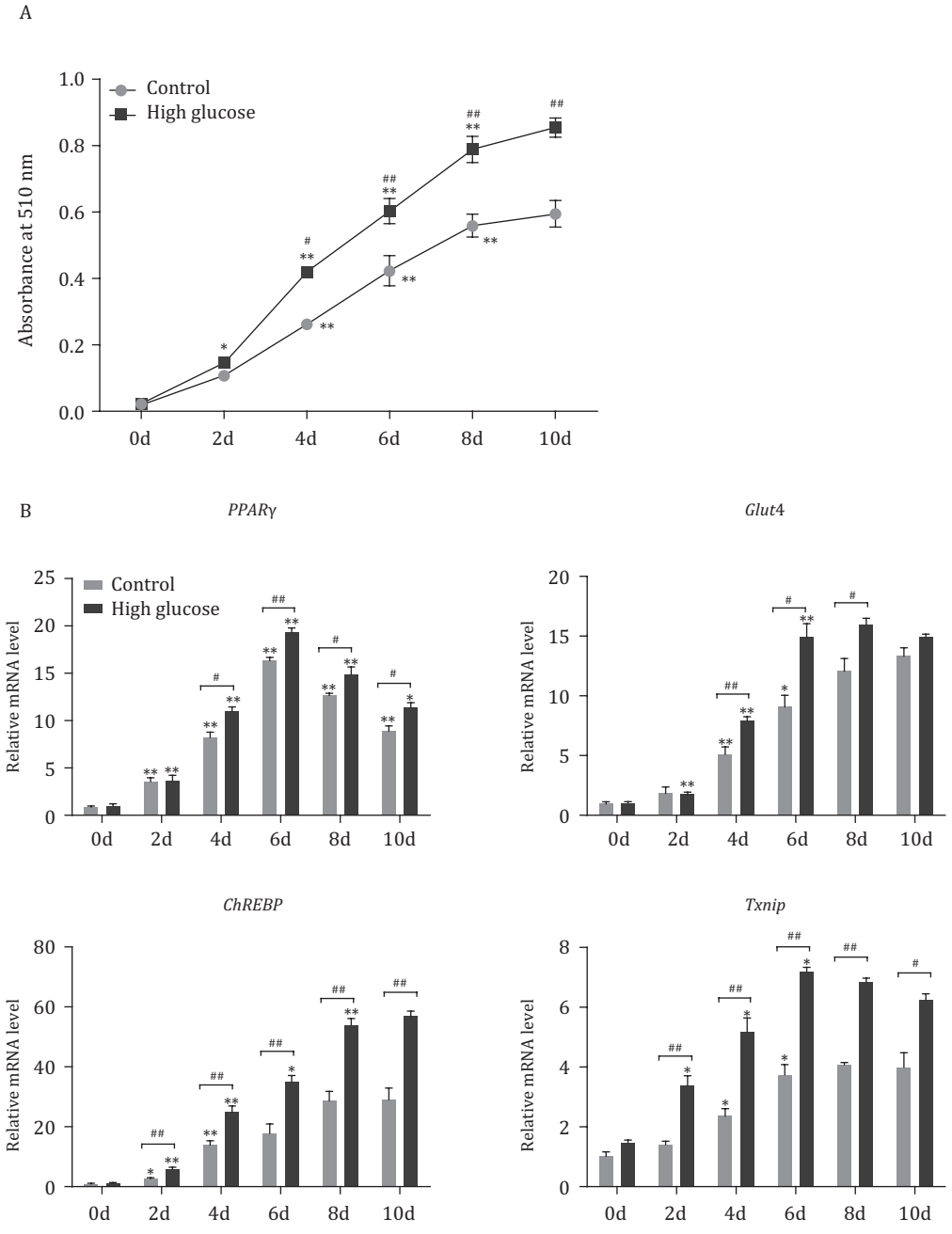
Figure 2 - *Txnip* overexpression inhibited porcine preadipocytes differentiation.

3.3. Differentiation and *Txnip* expression of porcine preadipocyte was promoted by glucose

Porcine preadipocytes growing to confluence were exposed to the adipogenic medium containing 5 mmol L^{-1} glucose (control) or 20 mmol L^{-1} glucose (high glucose), and the differentiation was determined (Figure 3A). The lipid content in both control and high-glucose-treated cells increased significantly at the onset of adipogenic induction, respectively (P<0.05). However, the lipid content

was higher in high-glucose-treated cells than in control cells from d 4 (P<0.05), which showed high glucose promoted the differentiation of porcine preadipocytes.

In addition, the expressions of *PPARγ*, *Glut4*, *ChREBP*, and *Txnip* increased with the prolonged culture time in both control and high-glucose-treated cells (P<0.01) (Figure 3B). The expressions of *PPARγ*, *Glut4*, and *Txnip* reached a maximum on day 6 after adipogenic induction (P<0.05), and the *PPARγ* expression decreased on days 8 and 10 compared with that on d 6 (P<0.05). *ChREBP* expression increased significantly from day 2 (P<0.05), and reached a maximum on day 8. Compared with low



A: effect of glucose on the differentiation of porcine adipocytes; B: effect of glucose on the expressions of adipogenic genes, β-*actin* as an internal control. The results were presented as means±SE, n = 9.
*(P<0.05) and **(P<0.01) refer to the significance compared with the value of the previous day.
#(P<0.05) and ##(P<0.01) refer to the significance between different treatments on the same day.

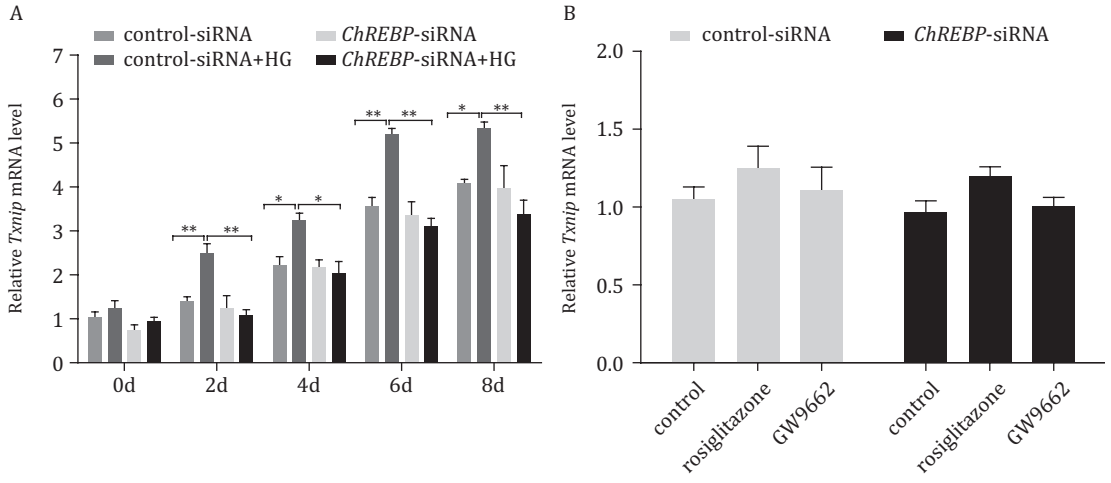
Figure 3 - Effect of glucose on adipogenesis.

glucose, high glucose treatment significantly improved the expressions of *PPARγ*, *ChREBP*, and *Txnip* from days 2 to 10, and *Glut*4 expression from days 4 to 8 after adipogenic induction (P<0.05). Thus, high glucose facilitated the expressions of *PPARγ*, *Glut*4, *ChREBP*, and *Txnip*.

3.4. *ChREBP* mediated the expression of *Txnip* induced by glucose

The previous studies showed that ChREBP is the main mediator of glucose-induced adipogenesis. To understand whether glucose regulates the expression of *Txnip* through *ChREBP*, preadipocytes transfected with *ChREBP*-siRNA were treated with high glucose, and then *Txnip* expression was detected (Figure 4). In low-glucose condition, there was no difference in the *Txnip* expression between control and *ChREBP* silencing cells (P>0.05). When cultured in high-glucose concentration, the *Txnip* expression in control-siRNA cells increased significantly on days 2 to 8 (P<0.05). In *ChREBP* silencing cells, however, *Txnip* expression was not affected by high-glucose treatment (P>0.05) and was markedly lower than in control-siRNA cells (P<0.01), indicating that *ChREBP* silencing decreased the *Txnip* expression induced by high glucose.

Furthermore, considering that high glucose stimulated *PPARγ* expression, we tested whether the *Txnip* mRNA expression induced by high glucose was affected by PPARγ activity. When the differentiating adipocytes transfected with control-siRNA were treated with rosiglitazone or with GW9662, the high glucose-induced *Txnip* expression was not altered. Similarly, the decreased *Txnip* expression by *ChREBP* silencing under high glucose was not changed by rosiglitazone or GW9662 (Figure 4B). These data suggested that transcription factor ChREBP, other than PPARγ, mediated *Txnip* expression induced by high glucose.



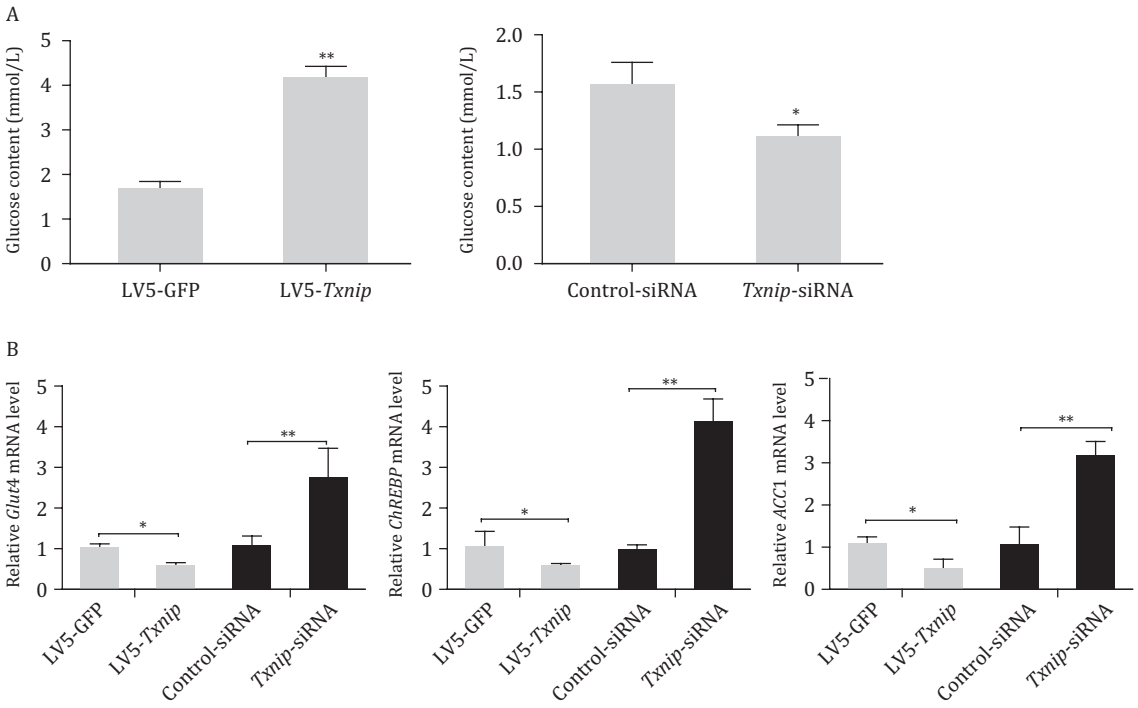
A: effect of glucose on *Txnip* expression in *ChREBP*-silencing cells; B: effect of rosiglitazone or GW9662 treatment for 48 h on *Txnip* expression in *ChREBP*-silencing cells on day 4 after adipogenic induction.
HG - 20 mmol/L of glucose.
The results were presented as means±SE, n = 9.
*(P<0.05) and **(P<0.01) refer to the significance difference between the treatments.

Figure 4 - *ChREBP* silencing decreased the *Txnip* expression in differentiating porcine adipocytes induced by high glucose.

3.5. Txnip inhibited glucose uptake in the porcine adipocytes via Glut4

To determine the effect of Txnip on glucose uptake and utilization, the differentiating adipocytes were treated with 20 mmol L^{-1} of glucose for 48 h. Then the expressions of *Glut*4, *ChREBP*, and *ACC*1 in the cells and the glucose content in the medium surrounding the cells were detected. Glucose content in the surrounding medium was higher in *Txnip*-overexpression cells than in the cells transfected

with LV5-GFP (P<0.01), indicating glucose uptake was decreased by *Txnip* overexpression (Figure 5A). Instead, *Txnip* silencing decreased the glucose content in the surrounding medium (P<0.05), which showed glucose uptake of the cells was promoted (Figure 5A). The expressions of *Glut*4, *ChREBP*, and *ACC*1 induced by high glucose were significantly decreased in *Txnip*-overexpression cells compared with LV5-GFP cells (P<0.05), and were increased in *Txnip*-siRNA cells compared with control-siRNA cells (Figure 5B), although the expressions of *Glut*4 and *ChREBP* were not affected by both *Txnip* overexpression and silencing under low glucose condition (P<0.01) (Figures 1C and 2C). These data suggested that *Txnip* overexpression could reduce glucose uptake of the cells through downregulation of *Glut*4 expression, thereby decreasing the expression and transcriptional activity of ChREBP, while *Txnip* silencing exerted the opposite effects.



The adipocytes on day 4 after adipogenic induction were cultured in the medium containing 20 mmol/L glucose for 48 h.
A: the medium surrounding the cells was collected to measure glucose content by ELISA; B: expressions of *Glut*4, *ChREBP*, and *ACC*1 mRNA detected by real-time PCR.
β-*actin* as an internal control.
Data are presented as means±SE, n = 9.
*P<0.05, **P<0.01.

Figure 5 - Effect of *Txnip* overexpression or silencing on the glucose uptake (A) and relative mRNA expressions (B) of differentiating adipocytes.

4. Discussion

The thioredoxin system, which is composed of NADPH, thioredoxin reductase (TrxR), and thioredoxin (Trx), is a key antioxidant system in defense against oxidative stress through its disulfide reductase activity regulating protein dithiol/disulfide balance (Dagdeviren et al., 2023). Thioredoxin-interacting protein (Txnip) directly binds to Trx and inhibits its reducing activity through disulfide exchange, regulating cellular redox status and linking between redox regulation and the pathogenesis of diseases (Yoshihara et al., 2014). Here, we showed that the differentiation of porcine preadipocytes was significantly reduced by *Txnip* overexpression and stimulated by *Txnip* silencing, which suggested Txnip was an inhibitor of adipocyte differentiation. Other studies also supported this view. Chutkow

and Lee (2011) reported that Txnip inhibited the differentiation of 3T3-L1 preadipocytes and endogenous Txnip protein was rapidly degraded at the onset of adipogenesis, and loss of Txnip increased adipogenesis in culture and adiposity *in vivo*. Txnip influenced adipocyte development *in vivo*, and played an important role in glucose homeostasis and lipid metabolism (Chutkow and Lee, 2011; Kim et al., 2007).

PPARγ is a member of the nuclear receptor superfamily of ligand-dependent transcription factors and is highly expressed in adipose tissue of humans and animals, and its expression is markedly induced during adipogenesis (Lee et al., 2019). Several studies have established that PPARγ is a molecular switch for adipocyte development both *in vitro* and *in vivo* (Aprile et al., 2018; Mueller, 2014). PPARγ was induced during the adipogenic differentiation of 3T3-L1 cells, a prerequisite for adipocyte differentiation (Poulsen et al., 2012). In this study, *PPAR*γ expression in porcine preadipocytes increased rapidly at the early stage of differentiation (2 d), peaked at the middle stage (6 d), and decreased significantly at the late stage (8-10 d), which was consistent with the results observed in 3T3-L1 preadipocytes (Ji et al., 2015). Furthermore, it was observed that *Txnip* overexpression resulted in a remarkable downregulation of *PPAR*γ expression in transcript and protein levels, and conversely, its expression markedly increased after the silencing of *Txnip* expression. In addition, when the *Txnip* overexpression cells were treated with rosiglitazone to activate PPARγ, the inhibition of *Txnip* overexpression on adipocyte differentiation was partially restored. However, when *Txnip* silencing cells were treated with GW9662 to inhibit PPARγ activity, the enhanced cell differentiation was abated. It was suggested that Txnip inhibited the differentiation of porcine preadipocytes by reducing the expression and transcriptional activity of PPARγ. *Txnip* promoter contained multiple PPARγ binding sites, and *Txnip* expression was negatively regulated by PPARγ (Qi et al., 2009). *Txnip* expression was highly upregulated when PPARγ was depleted from anaplastic thyroid cancer cells (Morrison et al., 2014). Chutkow et al. (2010) also demonstrated that Txnip was a negative regulator of *PPAR*γ expression, and in turn, PPARγ activation suppressed *Txnip* expression. Therefore, Txnip could inhibit the differentiation of porcine preadipocytes through negative feedback to regulate *PPAR*γ expression.

Glucose, the main substrate for lipogenesis in mammals that utilize carbohydrates as the major energy source, induced adipocyte differentiation and *de novo* lipogenesis. ChREBP is a glucose-responsive transcription factor that plays a critical role in converting excess carbohydrates to triglycerides through *de novo* lipogenesis (Herman et al., 2012; Uyeda and Repa, 2006). ChREBP has been established to be a key transcription factor in adipogenesis induced by glucose in hepatocytes, 3T3-L1 cells, and rat adipocytes (Burgess et al., 2008; Herman et al., 2012; Iizuka et al., 2012). Glucose stimulated *ChREBP* expression and activated its transcriptional activity through the intermediate glucose-6-phosphate, and thus regulated the expressions of target genes, including *Txnip* and *ACC*1 (Jeong et al., 2011; McFerrin and Atchley, 2012). In the present study, the adipogenesis was significantly stimulated, and the expressions of *ChREBP* and *Txnip* were upregulated in porcine adipocytes when treated with high glucose. In accordance with the result, *ChREBP* and *Txnip* expressions in 3T3-L1 and human adipocytes were elevated by high glucose concentrations (Peshdary et al., 2019; Witte et al., 2015). However, *Txnip* expression in *ChREBP*-silencing cells was not affected by high glucose. Although PPARγ activation suppressed *Txnip* expression (Chutkow et al., 2010), *Txnip* expressions in both control cells and *ChREBP*-silencing cells under high glucose were not altered by PPARγ inhibitor or agonist. There are two ChoREs on the promoter of *Txnip* gene (Yu and Luo, 2009), and the glucose-stimulated *Txnip* expression was mediated by *ChREBP* (Cha-Molstad et al., 2009). Thereupon, it was indicated that glucose promoted the *Txnip* expression through ChREBP in porcine adipocytes; that is, ChREBP, rather than PPARγ, is a mediator of glucose-induced *Txnip* expression.

Txnip has been described as a major regulator of glucose metabolism (Gunes et al., 2015; Parikh et al., 2007). However, how Txnip regulated the adipocyte differentiation induced by glucose was unclear. Therefore, we investigated the effect of Txnip on expressions of *ChREBP*, *Glut*4, and *ACC*1 in differentiating adipocytes, as well as on glucose uptake. Glut4 is generally regarded as the main glucose transporter in adipocytes, skeletal muscle cells, and other cells with high glucose metabolism, and the high concentration of glucose enhances glucose uptake and metabolism by inducing *Glut*4 expression

(Ehebauer et al., 2020; Jung and Bu, 2020; Kwon et al., 2020). Aguiari et al. (2008) found that high glucose significantly improved the expression of *Glut*4 in primary rat adipose-derived stem cells. The expressions of *ChREBP* and *Glut*4 induced by high glucose were reduced by *Txnip* overexpression and further increased by *Txnip* silencing, while their basal expression under the low glucose culture was not altered by these changes of *Txnip*. Meanwhile, the uptake of cells to glucose in the surrounding medium was reduced by *Txnip* overexpression and raised by *Txnip* silencing. As a physiologic regulator of peripheral glucose uptake, *Txnip* overexpression repressed cellular glucose uptake, while *Txnip* silencing increased glucose uptake in adipocytes and skeletal muscle (Parikh et al., 2007; Wu et al., 2013). Similarly, *Txnip* negatively regulated glucose uptake in cells, and excessive glucose entering the cell induced Txnip protein to limit glucose uptake (Waldhart et al., 2017). A glucose-sensing module (GSM), composed of a low-glucose inhibitory domain (LID) and a glucose-response activation conserved element (GRACE), in ChREBP structure mediated glucose responsiveness of ChREBP. The transactivation activity of GRACE was inhibited by LID under low glucose concentration, and this inhibition was reversed by high glucose in an orientation-sensitive manner (Li et al., 2006). Accordingly, downregulation of *Glut*4 expression resulted in a decreased glucose uptake in the adipocytes, which reduced the expression and transactivation activity of ChREBP, thus decreasing the expressions of its target genes related to adipogenesis, such as *ACC*1. These results showed that Txnip impaired the induction of glucose on the porcine preadipocyte differentiation through decreasing *Glut*4 expression and glucose uptake and subsequent decrease of the expression and transcriptional activity of ChREBP.

5. Conclusions

Our results suggested that Txnip could be an inhibitor of porcine preadipocyte differentiation, which attenuated adipogenesis of the cells through a negative feedback regulation on PPARγ. Glucose induced the *Txnip* expression by the mediation of ChREBP. However, Txnip hindered glucose uptake of the cells via decreased *Glut*4 expression, resulting in a decreased expression and activity of ChREBP, which impaired the porcine adipocyte adipogenesis induced by glucose. The present study provides a reference for investigating the adipogenesis regulated by glucose.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.X. Lu. Funding acquisition: J.X. Lu and G.H. Zhang. Investigation: X.N. Dou, F. Wei, J. Zhang, C.C. Tian, L. Fu and S.S. Jiang. Methodology: X.N. Dou, F. Wei, J. Zhang and C.C. Tian. Project administration: X.N. Dou and F. Wei. Resources: L. Fu and S.S. Jiang. Supervision: G.H. Zhang. Writing – original draft: X.N. Dou and F. Wei. Writing – review & editing: J.X. Lu and G.H. Zhang.

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