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Original Article

miR-146a regulates inflammatory cytokines and reverses high-glucose- and high-insulin-induced insulin resistance in 3T3-L1 adipocytes by targeting *Traf6* through the NF-κB signaling pathway

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Abstract: Insulin resistance (IR) is a common feature of type 2 diabetes mellitus. Therefore, the molecular mechanism of IR in adipocytes is becoming a focal point of diabetes research. Emerging evidence suggests that miR-146a might be associated with diabetes and its related complications. However, the role of miR-146a in the development of IR remains unclear. In this study, we found that the expression of miR-146a was significantly inhibited in IR-3T3-L1 adipocytes compared with that in normal 3T3-L1 adipocytes, while that of *Traf6* was significantly promoted. The overexpression of miR-146a significantly inhibited the expression of *Traf6*, silenced the NF-κB signaling pathway, reduced the secretion of inflammatory cytokines, and increased glucose uptake in IR-3T3-L1 adipocytes. *Traf6* overexpression had no effect on the expression of miR-146a, but partially alleviated the effect of miR-146a in adipocytes transfected with miR-146a mimic. In conclusion, we demonstrated that miR-146a silenced the NF-κB signaling pathway, decreased the secretion of inflammatory cytokines, and improved glucose uptake in IR-3T3-L1 adipocytes by targeting *Traf6*. The findings provide information regarding a potential new therapeutic strategy to control IR in adipocytes.

Keywords: miR-146a, inflammatory cytokines, insulin resistance, adipocytes, *Traf6*, NF-κB

Introduction

Type 2 diabetes mellitus (T2DM), which leads to a high concentration of glucose in the blood, has harmful long-term effects on several body organs, especially the kidneys, eyes, heart, and nerves, and represents a major global public health problem [1]. Insulin resistance (IR) is a common feature of T2DM [2]. Recent research has suggested that dysfunctional adipocytes represent a key link between obesity and IR [3]. Therefore, the molecular mechanism of IR in adipocytes is becoming a focal point of research on T2DM. MicroRNAs (miRNAs or miRs) are a novel class of highly-conserved small non-coding RNAs that degrade their target mRNA sequences by binding to the mRNA's 3' untranslated region [4]. Evidence from recent studies has suggested that miRNAs regulate insulin secretion [5], sugar and lipid metabolism [6], and pancreatic β-cell development [7]. Thus, certain miRNAs might have applications in the prevention and treatment of T2DM and its related complications. It has been demonstrated that some miRNAs such as miR-21 and miR-320 regulate the development of IR [8, 9].

Several studies have provided evidence suggesting that miR-146a was associated with T2DM and its related complications by regulating the expression of interleukin-1 receptor-associated kinase-1 (*Irak1*) and tumor necrosis factor receptor associated factor 6 (*Traf6*), and reduced the expression of Il-6, TNF-α, and IL-1β [10-12]. However, the role of miR-146a in the development of IR remains unclear. In this study, we investigated the expression and effect of miR-146a in 3T3-L1 adipocytes with induced IR (IR-3T3-L1 adipocytes). The results showed that the expression of miR-146a was

significantly inhibited in IR-3T3-L1 adipocytes as compared with that in normal 3T3-L1 adipocytes. The overexpression of miR-146a significantly inhibited the activity of the NF-κB signaling pathway, reduced the secretion of inflammatory cytokines, and increased glucose uptake by inhibiting the expression of *Traf6*. In conclusion, we demonstrate that miR-146a improved glucose uptake in IR-3T3-L1 adipocytes by targeting *Traf6*. The findings provide information regarding a potential new therapeutic strategy to control IR.

Materials and methods

Cell culture and establishment of an IR model

3T3-L1 pre-adipocytes (ATCC, Manassas, VA, USA) were cultured and induced to differentiate into mature adipocytes, as described previously [13]. Briefly, 3T3-L1 pre-adipocytes were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco®, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco®) in a 5% CO_2 atmosphere at 37°C. For the induction of differentiation into mature adipocytes, 3T3-L1 pre-adipocytes were collected and cultured in differentiation media supplemented with 0.5 mmol/L 3-isobutyl-1-methylxantine, 10 mg/mL insulin, and 1 μmol/L dexamethasone (Sigma-Aldrich, St. Louis, MO, USA). At 2 days post-culture, the cells were cultured in DMEM media supplemented with 10% FBS and 10 mg/mL insulin, and replenished every other day with fresh media and insulin until day 9, when the cells were fully differentiated into adipocytes. To establish an IR model, 3T3-L1 adipocytes were cultured for 24 h at 37°C in DMEM supplemented with 10% FBS, 1 μmol/L insulin, and 0, 5, or 25 mmol/L glucose. As a control, 3T3-L1 adipocytes were preincubated for 24 h at 37°C in DMEM supplemented with 10% FBS, 1 nmol/L insulin, and 0, 5, or 25 mmol/L glucose.

2 Deoxyglucose transport assay

2-Deoxyglucose (2DG) transport was analyzed using a 2DG uptake measurement kit (Cosmo Bio, Tokyo, Japan), according to the manufacturer's instructions. Adipocytes in six-well plates were incubated in serum-free medium for 6 h and washed three times with Krebs-Ringer phosphate buffer (pH 7.4). Insulin was added to a final concentration of 1 μM and the cells were incubated at 37°C for 30 min at 37°C. 2DG was then added to a final concentration of 1 mM and the cells were incubated at 37°C for 20 min. The medium was then removed and the cells were gently washed three times with cooled phosphate-buffered saline containing 200 μM phloretin. Next, 3 mL 10 mM Tris-HCl buffer (pH 8.0) was added to each well and the adipocytes were disrupted using a microtip sonicator. The cell lysates were collected into fresh sterile plastic tubes and heat treatment was applied at 80°C for 15 min. The lysates were then centrifuged at 4°C and 15,000 × *g* for 20 min and each supernatant was transferred into a new tube. An aliquot of each supernatant was diluted ≥ 1:4 with 1 × sample diluent buffer and the optical density of each well at 420 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 3T3-L1 adipocytes, using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). For the quantification of miR-146a, total RNA was reverse transcribed to cDNA using an miRcute miRNA first-strand cDNA synthesis kit (TIANGEN Biotech, Beijing, China) and miR-146a expression was measured using an miRcute miRNA qPCR detection kit (SYBR® Green, TIANGEN). *U6* served as the internal reference. For the quantification of *Traf6*, total RNA was reverse transcribed into cDNA using a PrimeScript™ RT reagent kit with gDNA Eraser (Takara Bio, Ōtsu, Japan). qPCR was performed using Power SYBR® Green PCR master mix (Applied Biosystems, Foster City, CA, USA). β-actin was used as an internal control. qPCR was performed on a 7500 real-time PCR system (Applied Biosystems). Gene expression was measured in triplicate, quantified using the $2^{-\Delta\Delta CT}$ method, and normalized to an internal control. For miR-146a, the forward and reverse primers were 5'-GCGTGAGAACTGAATTCCA-3' and 5'-GTGCAGGGTCCGAGGT-3', respectively. For *U6*, the forward and reverse primers were 5'-CTCGCTTCGGCAGCACA-3' and 5'-AACGCTTCACGAATTTGCGT-3', respectively. For *Traf6*, the forward and reverse primers were 5'-TACTCATCAGAGAACAGATGC-3' and 5'-AAAGTACTGAATGTGCATGG-3', respectively. For βactin, the forward and reverse primers

were 5'-TGTCCACCTTCCAGCAGATGT-3' and 5'-AGCTCAGTAACAGTCCGCCTAGA-3', respectively.

miR-146a mimic construction, Traf6-pcDNA3.0 construction and transfection

A miR-146a mimic (mimic, 5'-UGAGAACUGAAUUCCAUGGGUU-3') and a negative control (NC, 5'-UUCUCCGAACGUGUCACGUTT-3') were synthesized by RiboBio (Guangzhou, Guangdong, China). The full coding sequence of *Traf6* (NM_145803) was synthesized by GENEWIZ (Suzhou, Jiangsu, China) and inserted into the pcDNA3.0 vector (*Traf6*-pcDNA3.0). For transient transfection, IR-3T3-L1 adipocytes were plated at 50% confluence and transfected with 200 nM miR-146a mimic or NC, using Lipofectamine® RNAiMAX transfection reagent (Invitrogen) according to the manufacturer's protocol. IR-3T3-L1 adipocytes were then transfected with miR-146a mimic and plated at 50% confluence and transfected with 200 nM *Traf6*-pcDNA3.0 or pcDNA3.0 using Lipofectamine® 3000 transfection reagent (Invitrogen). Cells were harvested 24 or 48 h after transfection for further analysis.

Western blotting

3T3-L1 adipocytes were washed twice with ice-cold phosphate-buffered saline and lysed in ice-cold radioimmunoprecipitation assay buffer containing 1 mmol/L phenylmethanesulfonyl fluoride and a cocktail of protease inhibitors (1:100 dilution; Beyotime, Nantong, China). Cell lysates were centrifuged at 4°C for 15 min at 13,000 × *g*. The protein content of each supernatant was quantified using a bicinchoninic acid protein assay kit (Beyotime). Samples containing 30 μg total protein were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Pall Corporation, Port Washington, NY, USA). The membranes were blocked at 37°C for 1 h in a buffer containing 0.1% Tween® 20 and 5% milk. For the detection of TRAF6, the membranes were incubated with antibodies against TRAF6 (1:1000; Abcam, Cambridge, UK) and then washed and incubated with a horseradish peroxidase-conjugated secondary antibody (1:10000 dilution) for 1 h at room temperature. Finally, the membranes were visualized by the enhanced chemiluminescence method and a densitometric analysis was performed using Image Pro-Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA). GAPDH served as a reference protein.

Inflammatory cytokine analysis

At 48 h post-treatment, the concentrations of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α secreted by the IR-3T3-L1 adipocytes were analyzed using a Bio-Plex Human cytokine group I 23-plex assay (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions.

Statistical analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used to perform all statistical analyses. Continuous variables are presented as the mean ± standard deviation. The significance of differences among multiple groups was analyzed by one-way analysis of variance (ANOVA) followed by a post-hoc least significant difference (*LSD*) test. The significance of differences between two groups was analyzed by the unpaired *t* test. *P* values <0.05 were considered statistically significant.

Results

miR-146a expression was inhibited in IR-3T3-L1 adipocytes

To evaluate whether the IR-3T3-L1 adipocyte model was successfully established, insulin sensitivity of the cells was measured by the 2DG transport assay (**Figure 1A**). Under the same glucose concentration, addition of 1 μmol/L insulin significantly increased glucose transport of 3T3-L1 adipocytes compared with addition of 1 nmol/L insulin ($P<0.05$). In the presence of 1 μmol/L insulin, addition of 25 mmol/L glucose led to a significant decrease in the rate of glucose transport in 3T3-L1 adipocytes compared with the addition of 0 or 5 mmol/L glucose ($P<0.05$). The results indicated that high concentrations of glucose (25 mmol/L) and insulin (1 μmol/L) resulted in the development of IR in the 3T3-L1 adipocytes and suggested that the IR-3T3-L1 adipocyte model was successfully established. The expression of miR-146a showed a trend similar to that of glucose transport (**Figure 1B**). Under the same glucose concentration, addition of 1 μmol/L insulin led to a significant increase in

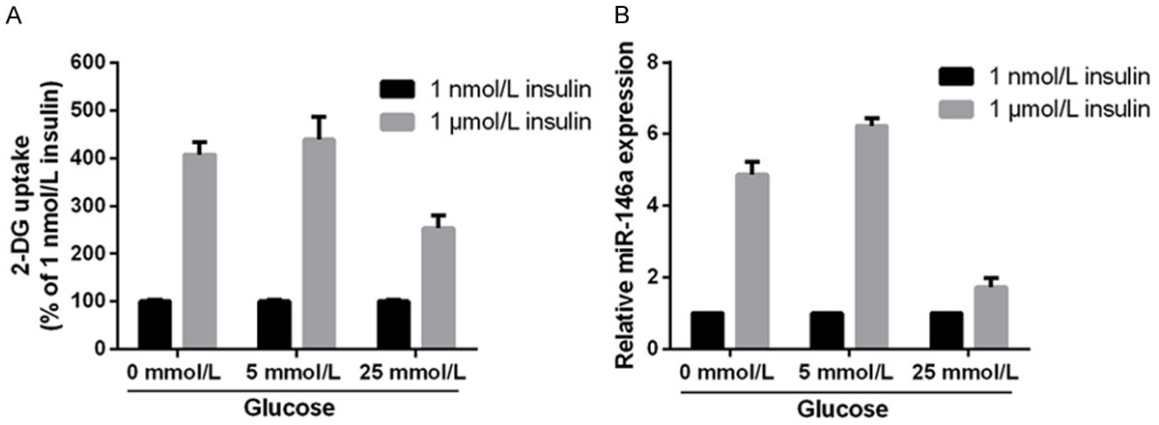

Figure 1. 2-Deoxyglucose (2DG) transport (A) and miR-146a expression (B) in 3T3-L1 adipocytes were analyzed using a 2DG uptake measurement kit and by quantitative real-time polymerase chain reaction (qRT-PCR), respectively.

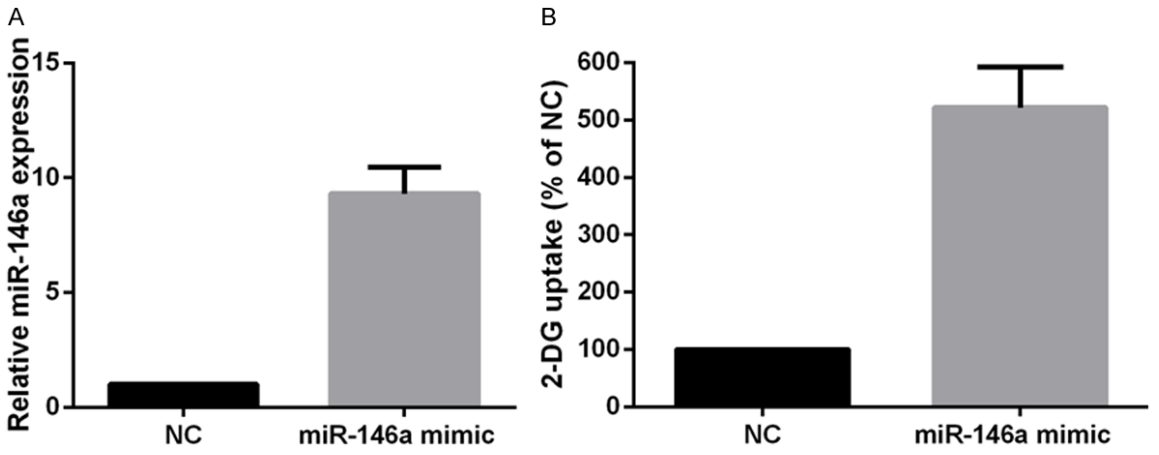

Figure 2. miR-146a expression (A) and 2DG transport (B) were analyzed by qRT-PCR and using 2-DG uptake measurement kit, respectively, at 48 h post-transfection in IR-3T3-L1 adipocytes that had been transfected with an miR-146a mimic.

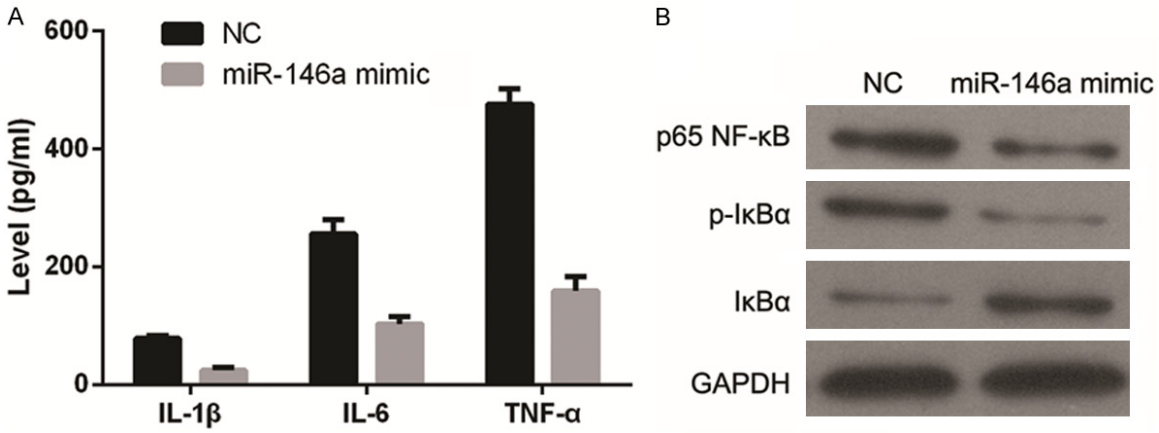

Figure 3. Effects of miR-146a on the NF-κB signaling pathway and the secretion of inflammatory cytokines in IR-3T3-L1 adipocytes. (A) miR-146a overexpression significantly reduced secretion of IL-1β, IL-6, and TNF-α in IR-3T3-L1 adipocytes. (B) miR-146a overexpression significantly inhibited expression of NF-κB p65 and p-IκBα, while it promoted expression of IκBα in IR-3T3-L1 adipocytes.

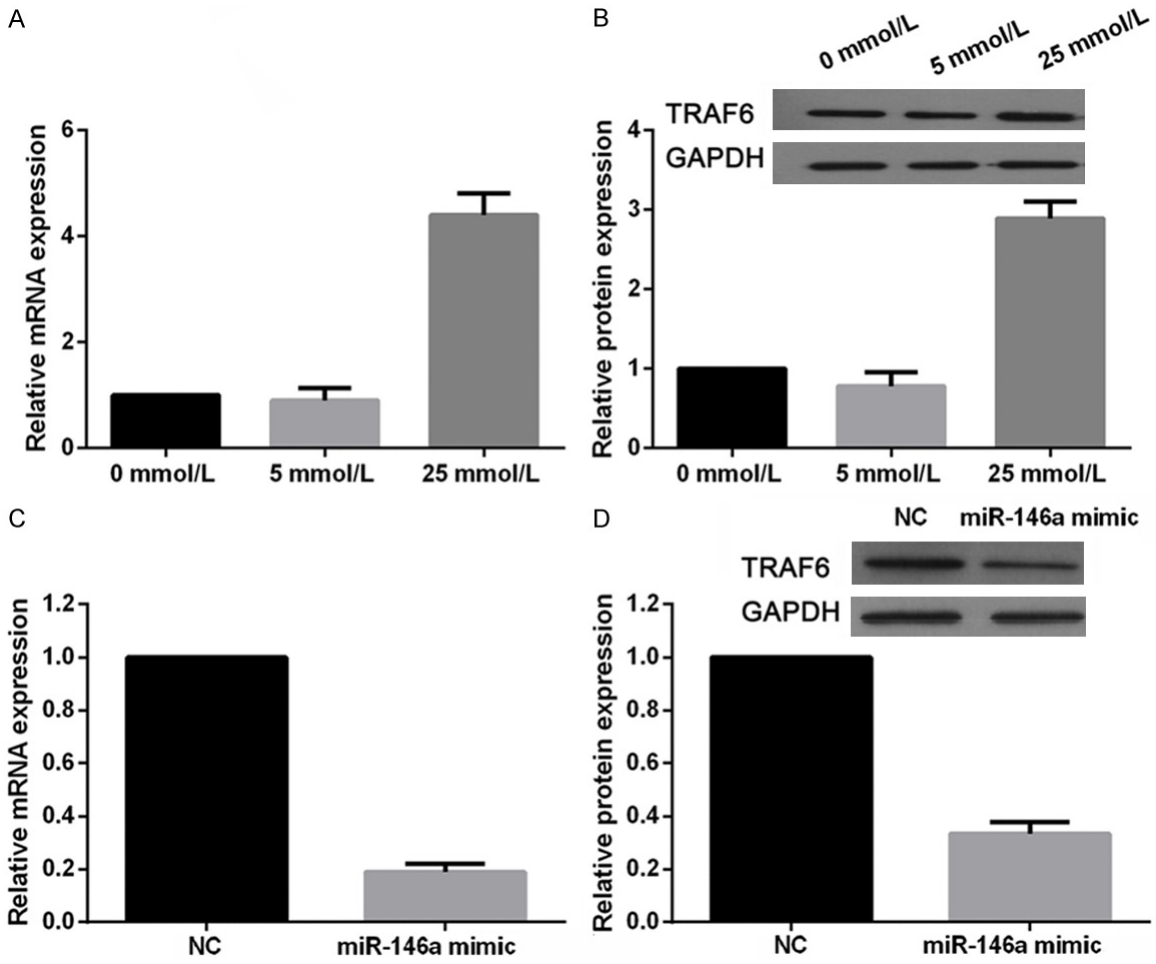

Figure 4. Expression of Traf6 was analyzed by qRT-PCR and Western blotting. (A and B) Expression of Traf6 was analyzed by qRT-PCR (A) and Western blotting (B) in IR-3T3-L1 adipocytes treated with 1 μmol/L insulin plus 0, 5, or 25 mmol/L glucose. (C and D) Expression of Traf6 was analyzed by qRT-PCR (C) and Western blotting (D) in miR-146a mimic-transfected IR-3T3-L1 adipocytes.

the expression of miR-146a as compared with the addition of 1 nmol/L insulin (P<0.05). In the presence of 1 μmol/L insulin, addition of 25 mmol/L glucose led to a significant decrease in the expression of miR-146a as compared with the addition of 0 or 5 mmol/L glucose (P<0.05). The results suggest that expression of miR-146a was inhibited in IR-3T3-L1 adipocytes as compared with that in 3T3-L1 adipocytes.

Transfection of miR-146a mimic into IR-3T3-L1 adipocytes upregulated miR-146a expression and increased glucose uptake

To assess the biological function of miR-146a in IR-3T3-L1 adipocytes, the miR-146a mimic or NC was transiently transfected into IR-3T3-L1 adipocytes. miR-146a expression and glucose uptake were significantly increased in IR-3T3-L1 adipocytes transfected with the miR-146a mimic compared with in those cells transfected with the NC at 48 h post-transfection (P<0.05, **Figure 2A** and **2B**).

miR-146a overexpression inhibited the secretion of inflammatory cytokines by IR-3T3-L1 adipocytes

We assessed the effects of miR-146a on the activity of the NF-κB signaling pathway and the secretion of inflammatory cytokines. We found that miR-146a overexpression in IR-3T3-L1 adipocytes significantly reduced the secretion of IL-1β, IL-6, and TNF-α (**Figure 3A**), inhibited the expression of NF-κB p65 and p-IκBα, and promoted the expression of IκBα, silencing the NF-κB signaling pathway (**Figure 3B**).

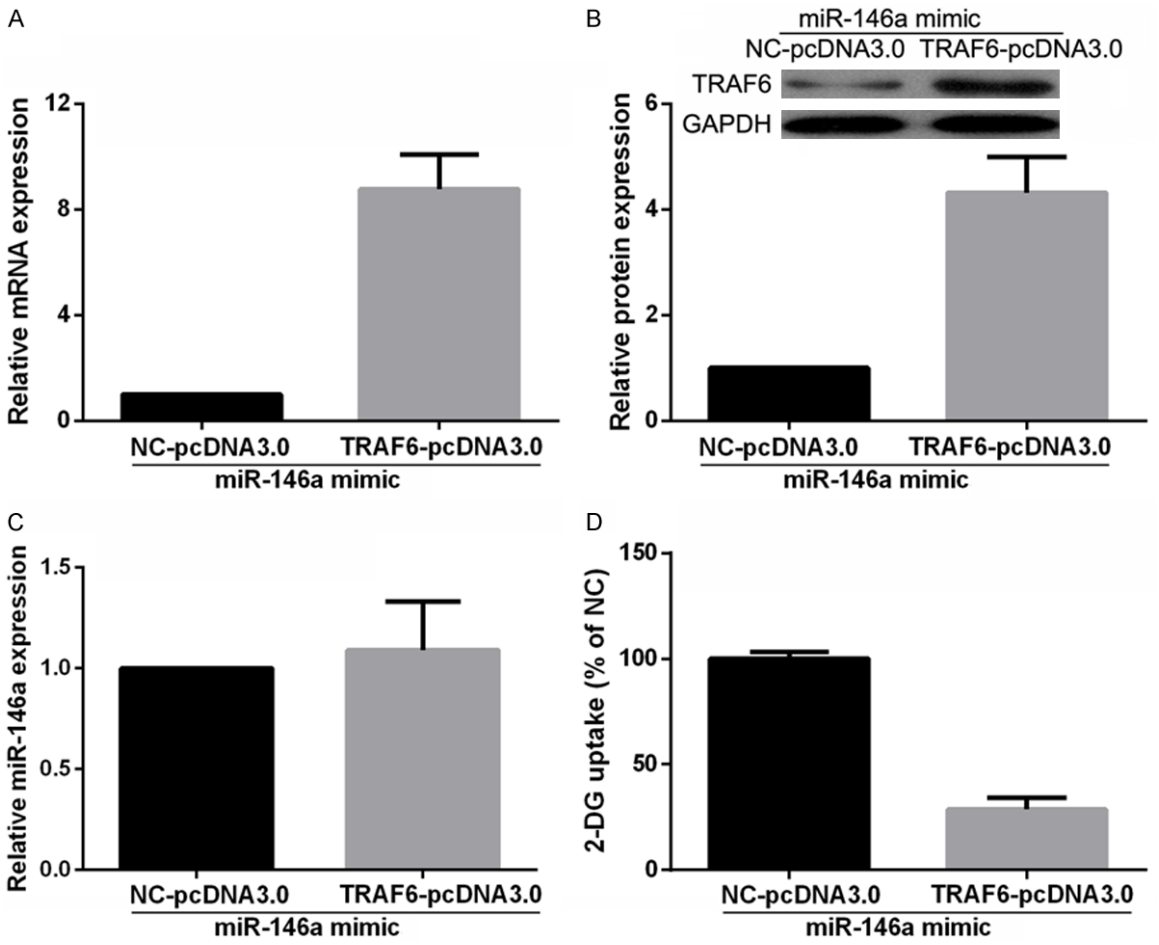

Figure 5. Effect of Traf6-pcDNA3.0 vector transfection on expression of Traf6 and miR-146a and glucose uptake. (A and B) Expression of Traf6 was analyzed by qRT-PCR (A) and Western blotting (B) in miR-146a mimic-transfected IR-3T3-L1 adipocytes after Traf6-pcDNA3.0 vector transfection. (C) Expression of miR-146a was analyzed by qRT-PCR in miR-146a mimic-transfected IR-3T3-L1 adipocytes after Traf6-pcDNA3.0 vector transfection. (D) 2-DG transport assay was analyzed using a 2-DG uptake measurement kit in miR-146a mimic-transfected IR-3T3-L1 adipocytes after Traf6-pcDNA3.0 vector transfection.

miR-146a regulate Traf6 expression in IR-3T3-L1 adipocytes

To demonstrate the association between *Traf6* and miR-146a in IR-3T3-L1 adipocytes, the expression of *Traf6* was detected in IR-3T3-L1 adipocytes treated with 1 μmol/L insulin plus 0, 5, or 25 mmol/L glucose. The results are shown in **Figure 4A** and **4B**. In the presence of 1 μmol/L insulin, the addition of 25 mmol/L glucose led to a significant increase in the expression of *Traf6* as compared with the addition of 0 or 5 mmol/L glucose ($P<0.05$). The results suggest that the expression of *Traf6* was promoted in IR-3T3-L1 adipocytes. Additionally, the cells transfected with the miR-146a mimic exhibited a significant reduction in their expression of TRAF6 (**Figure 4C** and **4D**). These data indicated that *Traf6* is a potential target of miR-146a in IR-3T3-L1 adipocytes.

Overexpression of Traf6 partially reversed the effect of miR-146a on glucose uptake in IR-3T3-L1 adipocytes transfected with a miR-146a mimic

To overexpress *Traf6*, the *Traf6*-pcDNA3.0 vector was constructed and transfected into IR-3T3-L1 adipocytes that had also been transfected with a miR-146a mimic. The cells were then harvested for qRT-PCR and Western blot analysis. The results showed that the transfection of the cells with the *Traf6*-pcDNA3.0 vector significantly promoted their expression of *Traf6*

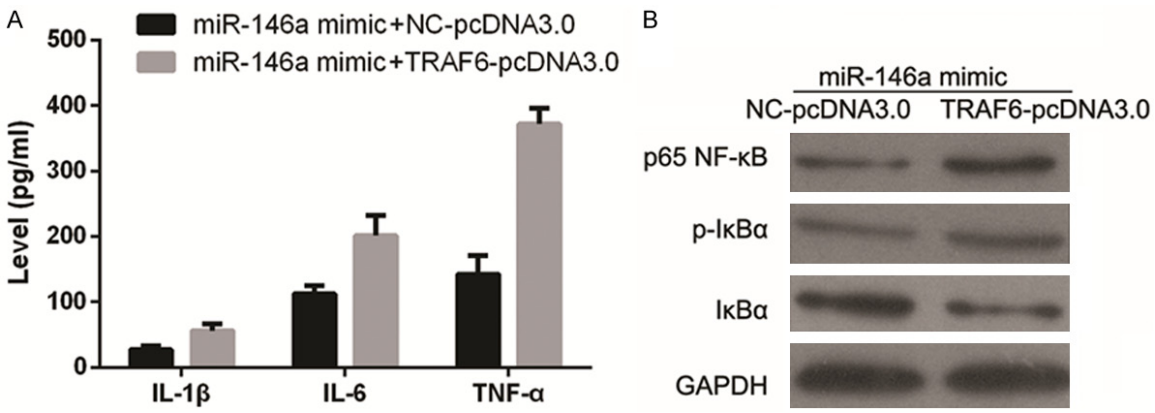

Figure 6. Effects of Traf6 on the NF-κB signaling pathway and secretion of inflammatory cytokines in IR-3T3-L1 adipocytes transfected with a miR-146a mimic. A: Traf6 overexpression significantly promoted secretion of IL-1β, IL-6, and TNF-α in miR-146a mimic-transfected IR-3T3-L1 adipocytes. B: Traf6 overexpression significantly inhibited expression of NF-κB p65 and p-IκBα, while it reduced expression of IκBα.

(**Figure 5A** and **5B**) but had no effect on their expression of miR-146a (**Figure 5C**) as compared with the transfection of NC-pcDNA3.0 into miR-146a mimic-transfected IR-3T3-L1 adipocytes. The results also showed that the transfection of the *Traf6*-pcDNA3.0 vector into miR-146a mimic-transfected IR-3T3-L1 adipocytes significantly inhibited glucose uptake as compared with the transfection of NC-pcDNA3.0 into the same cells (**Figure 5D**).

Overexpression of Traf6 partially reversed the effect of miR-146a on the NF-κB signaling pathway and inflammatory cytokines in IR-3T3-L1 adipocytes transfected with a miR-146a mimic

Finally, we assessed the effects of miR-146a and *Traf6* on the activity of the NF-κB signaling pathway and the secretion of inflammatory cytokines. We found that *Traf6* overexpression promoted the secretion of IL-1β, IL-6, and TNF-α, enhanced the expression of NF-κB p65 and p-IκBα, and inhibited the expression of IκBα, silencing the NF-κB signaling pathway in IR-3T3-L1 adipocytes transfected with a miR-146a mimic (**Figure 6A** and **6B**). Overexpression of Traf6 partially reversed the effect of miR-146a on NF-κB signaling pathway and inflammatory cytokines in IR-3T3-L1 adipocytes.

Discussion

IR is defined as a reduction in the responsiveness of cells or tissues to insulin. IR mainly occurs in adipose tissue, liver, and skeletal muscle, and the cells comprising those tissues abundantly express insulin receptors on their outer membranes. IR mainly manifests as a decrease in glucose uptake by muscle and adipose tissue alongside a reduction in glucose output by the liver. In the present study, we investigated the role of miR-146a in the development of IR in 3T3-L1 adipocytes. This study showed that miR-146a expression was downregulated and *Traf6* expression was upregulated in IR-3T3-L1 adipocytes as compared with that in 3T3-L1 adipocytes. Additionally, overexpression of miR-146a significantly promoted glucose uptake in IR-3T3-L1 adipocytes, while overexpression of *Traf6* had no effect on the expression of miR-146a but significantly inhibited glucose uptake in miR-146a mimic-transfected IR-3T3-L1 adipocytes. These results showed that *Traf6* overexpression partially alleviated the effect of miR-146a on glucose uptake, suggesting that miR-146a promoted glucose uptake by regulating *Traf6*. Previous studies have reported that miR-146a can regulate disease progression by targeting *Traf6* [14, 15], which is concordant with the results of the present study.

Inflammatory factors such as C-reactive protein, TNF-α, and IL-6 have been associated with the development of T2DM [16-18]. Inflammatory factors play important roles in the diagnosis, prevention, and treatment of T2DM [19, 20] and the concept of regulating such factors has provided new ideas for the development of new drugs for treating T2DM. Previous studies have

found that decreased expression of miR-146a promotes the expression of inflammatory factors, contributing to disease progression in chronic obstructive pulmonary disease [21] and lupus nephritis [22]. In this study, we found that miR-146a overexpression significantly reduced the secretion of IL-1β, IL-6, and TNF-α by IR-3T3-L1 adipocytes, while upregulation of *Traf6* expression partially promoted their secretion in miR-146a mimic-transfected IR-3T3-L1 adipocytes. These results showed that *Traf6* overexpression partially alleviated the inhibitory effect of miR-146a on the secretion of IL-1β, IL-6, and TNF-α, suggesting that miR-146a inhibited their secretion by regulating *Traf6*. The results are similar to those of a previous study, which showed that miR-146a inhibited the production of IL-1β, IL-6, and TNF-α by regulating *TRAF6* in lupus nephritis [22].

NF-κB is involved in inflammation, apoptosis, and the immune response [23, 24]. Silencing of NF-κB has been reported to protect adipocytes from inflammation and IR-induced injury, and it would be helpful to understand the regulatory mechanisms of NF-κB signaling in IR-3T3-L1 adipocytes to elucidate the pathogenesis of insulin tolerance and provide potential new therapeutic targets [25, 26]. In this study, we found that miR-146a overexpression significantly inhibited expression of NF-κB p65 and p-IκBα, while it promoted expression of IκBα in IR-3T3-L1 adipocytes. Experimental overexpression of *Traf6* partially promoted expression of NF-κB p65 and p-IκBα, while it inhibited expression of IκBα in miR-146a mimic-transfected IR-3T3-L1 adipocytes. These results showed that *Traf6* overexpression partially alleviated the inhibitory effect of miR-146a on the NF-κB signaling pathway, suggesting that miR-146a silenced the NF-κB signaling pathway by regulating *Traf6*, which corresponds with the results of previous studies in ischemia/reperfusion injury [27] and osteoarthritis-derived chondrocytes [28].

In conclusion, this study demonstrates that miR-146a overexpression can silence the NF-κB signaling pathway, inhibit secretion of inflammatory factors, and promote glucose uptake by targeting *Traf6*. Based on the results of the present study, miR-146a could represent a potential new therapeutic target for controlling IR.

Disclosure of conflict of interest

None.

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References

[1] Naqshbandi M, Harris SB, Esler JG and Antwi-Nsiah F. Global complication rates of type 2 diabetes in indigenous peoples: a comprehensive review. Diabetes Res Clin Pract 2008; 82: 1-17.

[2] Chakraborty C. Biochemical and molecular basis of insulin resistance. Curr Protein Pept Sci 2006; 7: 113-121.

[3] Guilherme A, Virbasius JV, Puri V and Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008; 9: 367-377.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P and Stoffel M. A pancreatic islet-specific microRNA regulates insulin secretion. Nature 2004; 432: 226-230.

[6] Teleman AA, Maitra S and Cohen SM. Drosophila lacking microRNA miR-278 are defective in energy homeostasis. Genes Dev 2006; 20: 417-422.

[7] Latreille M, Hausser J, Stutzer I, Zhang Q, Hastoy B, Gargani S, Kerr-Conte J, Pattou F, Zavolan M, Esguerra JL, Eliasson L, Rulicke T, Rorsman P and Stoffel M. MicroRNA-7a regulates pancreatic beta cell function. J Clin Invest 2014; 124: 2722-2735.

[8] Ling HY, Hu B, Hu XB, Zhong J, Feng SD, Qin L, Liu G, Wen GB and Liao DF. MiRNA-21 reverses high glucose and high insulin induced insulin resistance in 3T3-L1 adipocytes through targeting phosphatase and tensin homologue. Exp Clin Endocrinol Diabetes 2012; 120: 553-559.

[9] Ling HY, Ou HS, Feng SD, Zhang XY, Tuo QH, Chen LX, Zhu BY, Gao ZP, Tang CK, Yin WD, Zhang L and Liao DF. CHANGES IN microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. Clin Exp Pharmacol Physiol 2009; 36: 1440-1681.

[10] Wang G, Gu Y, Xu N, Zhang M and Yang T. Decreased expression of miR-150, miR146a and

miR424 in type 1 diabetic patients: association with ongoing islet autoimmunity. Biochem Biophys Res Commun 2017; [Epub ahead of print].

[11] Chen S, Feng B, Thomas AA and Chakrabarti S. miR-146a regulates glucose induced upregulation of inflammatory cytokines extracellular matrix proteins in the retina and kidney in diabetes. PLoS One 2017; 12: e0173918.

[12] Lo WY, Peng CT and Wang HJ. MicroRNA-146a-5p mediates high glucose-induced endothelial inflammation via targeting interleukin-1 receptor-associated kinase 1 expression. Front Physiol 2017; 8: 551.

[13] Ling HY, Wen GB, Feng SD, Tuo QH, Ou HS, Yao CH, Zhu BY, Gao ZP, Zhang L and Liao DF. MicroRNA-375 promotes 3T3-L1 adipocyte differentiation through modulation of extracellular signal-regulated kinase signalling. Clin Exp Pharmacol Physiol 2011; 38: 239-246.

[14] Hung PS, Liu CJ, Chou CS, Kao SY, Yang CC, Chang KW, Chiu TH and Lin SC. miR-146a enhances the oncogenicity of oral carcinoma by concomitant targeting of the IRAK1, TRAF6 and NUMB genes. PLoS One 2013; 8: e79926.

[15] Gao M, Wang X, Zhang X, Ha T, Ma H, Liu L, Kalbfleisch JH, Gao X, Kao RL, Williams DL and Li C. Attenuation of cardiac dysfunction in polymicrobial sepsis by MicroRNA-146a is mediated via targeting of IRAK1 and TRAF6 expression. J Immunol 2015; 195: 672-682.

[16] Ghiasi R, Ghadiri Soufi F, Mohaddes G, Alihemmati A, Somi MH, Ebrahimi H, Mirzaie Bavil F and Alipour MR. Influance of regular swimming on serum levels of CRP, IL-6, TNF-alpha in high-fat diet-induced type 2 diabetic rats. Gen Physiol Biophys 2016; 35: 469-476.

[17] Preciado-Puga MC, Malacara JM, Fajardo-Araujo ME, Wrobel K, Kornhauser-Araujo C and Garay-Sevilla ME. Markers of the progression of complications in patients with type 2 diabetes: a one-year longitudinal study. Exp Clin Endocrinol Diabetes 2014; 122: 484-490.

[18] Laake JP, Stahl D, Amiel SA, Petrak F, Sherwood RA, Pickup JC and Ismail K. The association between depressive symptoms and systemic inflammation in people with type 2 diabetes: findings from the South London Diabetes Study. Diabetes Care 2014; 37: 2186-2192.

[19] Hivert MF, Sun Q, Shrader P, Mantzoros CS, Meigs JB and Hu FB. Circulating IL-18 and the risk of type 2 diabetes in women. Diabetologia 2009; 52: 2101-2108.

[20] Hu FB, Meigs JB, Li TY, Rifai N and Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004; 53: 693-700.

[21] Osei ET, Florez-Sampedro L, Tasena H, Faiz A, Noordhoek JA, Timens W, Postma DS, Hackett TL, Heijink IH and Brandsma CA. miR-146a-5p plays an essential role in the aberrant epithelial-fibroblast cross-talk in COPD. Eur Respir J 2017; 49.

[22] Zheng CZ, Shu YB, Luo YL and Luo J. The role of miR-146a in modulating TRAF6-induced inflammation during lupus nephritis. Eur Rev Med Pharmacol Sci 2017; 21: 1041-1048.

[23] Yates LL and Gorecki DC. The nuclear factor-kappaB (NF-kappaB): from a versatile transcription factor to a ubiquitous therapeutic target. Acta Biochim Pol 2006; 53: 651-662.

[24] Tokunaga F and Iwai K. Linear ubiquitination: a novel NF-kappaB regulatory mechanism for inflammatory and immune responses by the LUBAC ubiquitin ligase complex. Endocr J 2012; 59: 641-652.

[25] Priyanka A, Shyni GL, Anupama N, Raj PS, Anusree SS and Raghu KG. Development of insulin resistance through sprouting of inflammatory markers during hypoxia in 3T3-L1 adipocytes and amelioration with curcumin. Eur J Pharmacol 2017; 812: 73-81.

[26] Priyanka A, Sindhu G, Shyni GL, Preetha Rani MR, Nisha VM and Raghu KG. Bilobalide abates inflammation, insulin resistance and secretion of angiogenic factors induced by hypoxia in 3T3-L1 adipocytes by controlling NF-kappaB and JNK activation. Int Immunopharmacol 2017; 42: 209-217.

[27] He X, Zheng Y, Liu S, Shi S, Liu Y, He Y, Zhang C and Zhou X. MiR-146a protects small intestine against ischemia/reperfusion injury by down-regulating TLR4/TRAF6/NF-kappaB pathway. J Cell Physiol 2018; 233: 2476-2488.

[28] Zhong JH, Li J, Liu CF, Liu N, Bian RX, Zhao SM, Yan SY and Zhang YB. Effects of microRNA-146a on the proliferation and apoptosis of human osteoarthritis chondrocytes by targeting TRAF6 through the NF-kappaB signalling pathway. Biosci Rep 2017; 37.