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hsa_circ_0115355 promotes pancreatic β-cell function in patients with type 2 diabetes through the miR-145/SIRT1 axis

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a complex metabolic disease closely related to obesity, a growing global health problem. T2DM is characterized by decreased islet beta-cell mass and impaired insulin release from these cells, and this dysfunction is exacerbated by hyperglycemia (glucolipotoxicity). Circular RNAs (circRNAs) are abnormally expressed and play a regulatory role in T2DM.

Objective: This study aimed to evaluate the function and molecular mechanism of hsa_circ_0115355 in the progression of T2DM.

Methods: The regulatory effect of hsa_circ_0115355 on INS-1 cell function was assessed under glucolipotoxicity by MTT, flow cytometry analysis, and insulin secretion assay. Dual-luciferase experiments revealed a direct interaction of hsa_circ_0115355 with miR-145 and miR-145 with SIRT1. Furthermore, the regulatory role of the hsa_circ_0115355/miR-145/SIRT1 axis was verified by examining the function of INS-1.

Results: In this study, hsa_circ_0115355 was significantly underexpressed in both patients with T2DM and INS-1 cell lines. This study thus showed that hsa_circ_0115355 inhibits the occurrence and development of T2DM by regulating the expression of SIRT1 by adsorbing miR-145.

Conclusion: The underexpression hsa_circ_0115355 is also a potential novel diagnostic marker and therapeutic target for T2DM.

KEYWORDS
hsa_circ_0115355, INS-1, SIRT1, T2DM

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by decreased islet beta-cell mass leading to impaired insulin release. As an increasingly serious global health problem, the incidence of T2DM has continued to rise worldwide in recent years.[1] Patients with T2DM are at high risk for microvascular complications (including retinopathy, nephropathy, and neuropathy) and macrovascular complications (e.g., cardiovascular complications), which seriously affect the quality of life of patients.[2] A variety of pathophysiological disturbances, including environmental and genetic factors, contribute to pancreatic β-cell dysfunction in T2DM.[3] A hyperglycemic environment can lead to β-cell exhaustion, resulting in impaired insulin secretion and increased cellular apoptosis.[4,5] Nonetheless, the underlying molecular mechanisms for glucolipotoxicity-mediated pancreatic β-cell dysfunction remain yet to be completely understood.

MicroRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) have been reported to play important roles in T2DM.[6,7] Several miRNAs, such as miR-233, miR-107, and miR-103, are potentially involved in the occurrence and development of diabetes through lipid or glucose regulation, hepatic gluconeogenesis, insulin secretion, autophagy, and other molecular pathways.[8–10] In recent decades, lncRNAs such as ANRIL, MALAT1, Sox2OT, and MEG3 have all been shown to play important roles in diabetes pathology, particularly T2DM.[11–13] In addition, the role of circRNAs in the occurrence and development of T2DM has been recurrently revealed. Studies have shown that hsa_circ_CCNB1 and hsa_circ_0009024 can be used as potential biomarkers for T2DM[14]; Circ-Tulp4 activates the expression of cyclin D1 through soat1 expression and promotes cell cycle progression, thereby resisting and relieving INS-1 cell dysfunction under glycolipid toxicity.[15] These findings suggest that circRNAs may participate in the occurrence and development of T2DM through various mechanisms regulating INS-1 cell function.

Sirtuin1 (SIRT1), also known as the NAD-dependent deacetylase sirtuin-1, is encoded by a gene located on human chromosome 10q21.3.[16] SIRT1 is involved in a variety of pathways that regulate cellular functions, including those related to glycolipid metabolism, autophagy, inflammation, and circadian rhythm-related metabolism, as well as functions such as oxidative stress, apoptosis, and chromatin silencing. SIRT1 is involved in insulin regulation and thus plays a crucial role in the progression of diabetes.[17] High insulin resistance reduces SIRT1 expression, which, in turn, reduces insulin sensitivity. In patients with T2DM, SIRT1 is significantly downregulated and is associated with oxidative stress.[18] Inhibition of SIRT1 expression promotes inflammation in patients with T2DM.[19] Furthermore, aldehyde dehydrogenase 2 (ALDH2)/SIRT1 contributes to T2DM-induced retinopathy by inhibiting the oxidative stress pathway.[20] SIRT1 attenuates neuropathic pain in T2DM rats through epigenetic modification.[21] Several studies have also shown the role of SIRT1 in insulin secretion from INS-1 cells. Overexpression of SIRT1 in INS-1 cells enhances adenosine triphosphate (ATP) production by inhibiting uncoupling protein (UCP) 2, thereby mediating uncoupling metabolic regulation of glucose.[22] SIRT1 improves glucose tolerance by protecting INS-1 cells from various toxic stresses by inhibiting NF-κB signaling.[23] Thus, SIRT1 is an important molecule involved in the progression of T2DM and is also a potential target for therapy.

In this study, we found through microarray experiments that hsa_circ_0115355 is abnormally expressed in T2DM disease progression. Further validation in INS-1 cells, plasma from patients with T2DM, and normal individuals revealed a significantly low expression of hsa_circ_0115355 in cells and patients. We also found that hsa_circ_0115355 regulates the expression level of SIRT1 by competitively binding to miR-145, thereby mediating the functional regulation of INS-1 cell growth, apoptosis, and insulin secretion. Our study provides new insights into the pathogenesis and therapeutic targets of T2DM.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Pancreatic β-cell INS-1 cells were placed in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, and routinely cultured at 37 °C in a 5% CO_2 incubator. To establish a glucotoxicity model of INS-1 cells, these cells were cultured in DMEM with 25 mM glucose. The treated cells were named HG-INS-1.

2.2 | Cell transfection

HG-INS-1 cells in the logarithmic growth phase were taken, trypsinized, and resuspended in DMEM without FBS to a density of 1×10^5 cells/ml. The cells were seeded in 6-well plates (2 ml/well) and routinely cultured for 24 h. According to the following groups, HG-INS-1 cells were transfected with Lipofectamine 2000 mixed with siRNA or vector, and the subsequent experiments were carried out after 48 h.

2.3 | qRT-PCR

Serum and cell total RNA were extracted by the TRIzol one-step method, and the RNA concentration and purity were detected on a NanoDrop. All RNAs were reverse transcribed into cDNA using a one-step reverse transcription kit and detected according to the instructions of the qPCR kit. For detect the expression of hsa_circ_0115355 and CSE1L by qRT-PCR, β-actin was used as the internal reference gene, and when the expression of miR-145 was detected by qRT-PCR, U6 was used as the internal reference gene.

2.4 | CCK-8 assay

HG-INS-1 cells in the logarithmic growth phase were seeded in 96-well plates, each well containing 1×10^4 cells, and placed in an incubator at 37°C, with 5% CO_2 for routine culture for 24 h. Then, 10 μl of CCK-8 solution was added to each well 1 h before the test and incubated in a 37 °C incubator for 3 h, after which, the optical density (D) value was detected at 450 nm using a microplate reader.

2.5 | Flow cytometry

HG-INS-1 cells were suspended in 500 μl flow cytometry binding buffer and then stained in the dark by adding 10 μl Annexin V/FITC and 5 μl propidium iodide for 30 min at room temperature. The apoptotic ratio of cells was detected on the machine (CytoFLEX S, Beckman Coulter, Inc., USA).

2.6 | ELISA

The INS-1 cells in the logarithmic growth phase were inoculated into 96-well culture plates, and the insulin levels secreted by INS-1 cells were determined following the detection steps provided in the insulin ELISA kit.

2.7 | Western blot

The total protein in cells was extracted with RIPA, and the protein concentration was detected by the BCA kit. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the target band was electrotransferred to Poly(vinylidene fluoride) membrane and was blocked with 5% nonfat milk powder at room temperature for 1 h. Antibody (Abcam, USA, ab8227) was incubated at room temperature for 2 h. Finally, ECL staining was performed, the images were acquired by a gel imager, and the gray value was analyzed by Image J.

2.8 | Bioinformatics analysis

We used the circBank database (http://www.circbank.cn) to predict the target binding relationship between hsa_circ_0115355 and miR-145, and the starBase database (https://starbase.sysu.edu.cn) to predict the potential of miR-145 and SIRT1 binding site.

2.9 | Dual-luciferase

We obtained constructs of hsa_circ_0115355 and SIRT1 pmir-GLO luciferase expression vectors, hsa_circ_0115355 and SIRT1 wild-type vectors (pmirGLO-hsa_circ_0115355-WT/pmirGLO-SIRT1-WT), hsa_circ_0115355 or SIRT1 mutant vectors (pmirGLO-hsa_circ_0115355-MUT/pmirGLO-SIRT1-MUT), hsa_circ_0115355 or SIRT1 wild-type vector, hsa_circ_0115355 or SIRT1 mutant vector, and miR-145 mimics, miR-NC (GenePharma, China). With each specified construct, Lipofectamine 2000 was mixed and then transfected into HEK 293T cells and 48h later, luciferase activity was detected with a dual fluorescein enzyme reporter gene detection kit.

2.10 | Statistical analysis

Experimental data were statistically analyzed with SPSS 20.0 statistical software. Graph Pad Prism 6 was used to draw pictures of experimental data. Then, a t test was used to compare the two groups, and the Pearson correlation coefficient test was used to analyze the correlation between genes. When $p < 0.05$, the difference was statistically significant.

3 | RESULTS

3.1 | hsa_circ_0115355 was significantly downexpressed in serum of patients with T2DM and high glucose-induced INS-1 cells

The qRT-PCR assay indicated significantly reduced expression of hsa_circ_0115355 in the serum of patients with T2DM compared with that in normal controls (Figure 1A). The expression of hsa_circ_0115355 in INS-1 cells cultured with high glucose was also significantly lower than that in normal cultured INS-1 cells (Figure 1B). Studies have shown that circRNAs can stably exist in tissues and cells and are not subject to exonuclease-mediated RNA degradation. We then treated the INS-1 cell line with RNase R, extracted total RNA, and found intact hsa_circ_0115355, indicating that it was resistant to RNase R; however, RNase R could degrade CSE1L mRNA (Figure 1C). Next, actinomycin D assays revealed a half-life of hsa_circ_0115355 transcripts exceeding 24h, indicating greater stability of this molecule than linear CSE1L mRNA transcripts in INS-1 cells (Figure 1D). Next, subcellular localization analysis revealed enriched hsa_circ_0115355 in the cytoplasm (Figure 1E).

3.2 | hsa_circ_0115355 induces proliferation and insulin secretion in HG-INS-1 cells and inhibits apoptosis

To explore the potential regulatory role of hsa_circ_0115355 in INS-1 cells, we transfected INS-1 cells with the pcd5 vector expressing hsa_circ_0115355 (or control) and transfected INS-1 cells with two siRNAs targeting hsa_circ_0115355 (or control sequence). Expression of pcd5-hsa_circ_0115355 significantly induced hsa_circ_0115355 overexpression in INS-1 cells (Figure 2A), whereas si-hsa_circ_0115355#1 and #2 effectively reduced the level of hsa_circ_0115355 in these cells (Figure 2B). Since si-hsa_circ_0115355#1 showed more robust knockdown efficiency than si-hsa_circ_0115355#2, si-hsa_circ_0115355#1 was used for further analysis and named si-hsa_circ_0115355. To examine the effect of hsa_circ_0115355 on the proliferation of HG-INS-1 cells, the CCK-8 proliferation assay showed that enhanced expression of hsa_circ_0115355 significantly increased the proliferation ability of HG-INS-1 cells at 96h after transfection, whereas knockdown of hsa_circ_0115355 effectively inhibited the proliferation of HG-INS-1 cells (Figure 2C). Compared with cells transfected with the control vector, the number of apoptotic HG-INS-1 cells was significantly reduced when transfected with pcd5-hsa_circ_0115355, indicating that hsa_circ_0115355 overexpression protects HG-INS-1 cells from apoptosis. Meanwhile, more apoptotic HG-INS-1 cells were observed in cells deficient in hsa_circ_0115355 expression (Figure 2D). The results of the ELISA kit showed that overexpression of hsa_circ_0115355 in HG-INS-1 cells promoted insulin secretion, whereas the knockdown of hsa_circ_0115355 inhibited
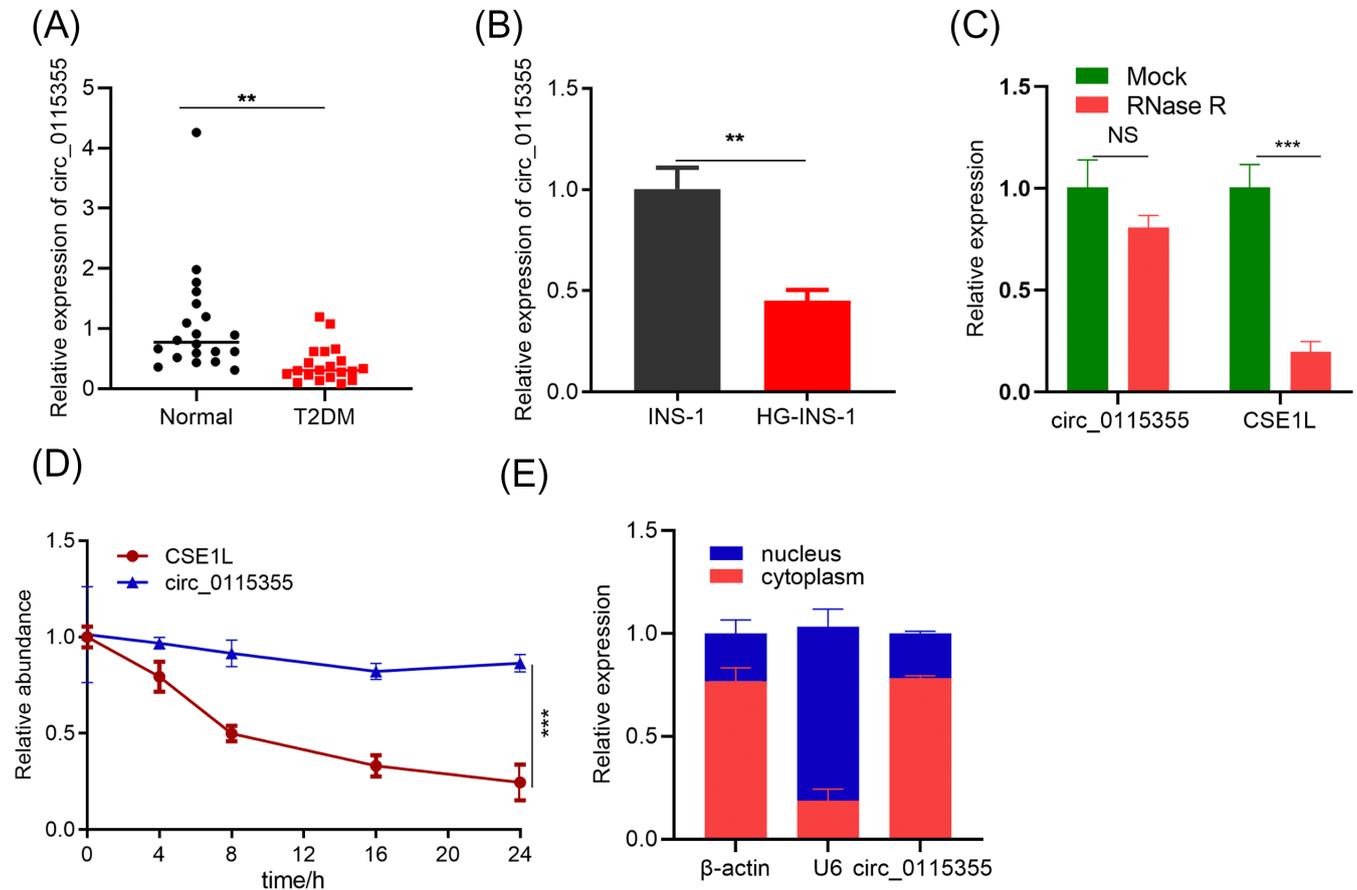
FIGURE 1 Overexpression of hsa_circ_0115355 in the serum of patients with T2DM and high glucose-induced INS-1 cells. qRT-PCR analysis of: (A). The expression of hsa_circ_0115355 in the serum of patients with T2DM, n = 20. (B). The expression of hsa_circ_0115355 in INS-1 and HG-INS-1 cells. (C) The expression of hsa_circ_0115355 and CSE1L mRNA expression in INS-1 cells with or without RNase R treatment. (D)The expression of hsa_circ_0115355 and CSE1L mRNA expression after actinomycin D treatment. (E) The ratio of hsa_circ_0115355 in the cytoplasm and nucleus of INS-1 cells

insulin secretion in these cells (Figure 2E). The above studies show that high glucose can induce functional impairment of INS-1 cells, whereas overexpression of hsa_circ_0115355 can promote the proliferation and insulin secretion and inhibit the apoptosis of INS-1 cells, and improve the functional impairment of HG-INS-1 cells.

3.3 | hsa_circ_0115355 is miR-145 sponge

hsa_circ_0115355 has been shown to play its role as a competing endogenous RNA. Bioinformatic analysis predicted a putative miR-145 binding site on hsa_circ_0115355 (Figure 3A). Knockdown of hsa_circ_0115355 could significantly promote the expression of miR-145, whereas overexpression of hsa_circ_0115355 significantly inhibited the expression of miR-145 (Figure 3B). Interestingly, knockdown or overexpression of miR-145 could significantly promote or inhibit, respectively, the expression of hsa_circ_0115355 (Figure 3C). Further dual-luciferase reporter analysis revealed a targeted binding relationship between hsa_circ_0115355 and miR-145 (Figure 3D).

Compared with normal INS-1 cells, the expression of miR-145 was significantly increased in HG-INS-1 cells (Figure 3E). In addition, high level of miR-145 was detected in the serum of patients with T2DM compared with the serum of healthy people (Figure 3F). Pearson correlation analysis showed that the expression of hsa_circ_0115355 in the serum of patients with T2DM was negatively correlated with the expression of miR-145 (Figure 3G). The above studies, therefore, showed that miR-145, as a target of hsa_circ_0115355, was negatively regulated by hsa_circ_0115355.

3.4 | The role of miR-145 in hsa_circ_0115355-mediated effects on INS-1 cell function

To further explore the biological functions of hsa_circ_0115355 in INS-1 cells through miR-145 regulation, we transfected HG-INS-1 cells with miR-NC+vector, miR-145 mimics+vector, and miR-145 mimics+hsa_circ_0115355, separately. Transfection of miR-145 mimics inhibited cell proliferation and insulin secretion, but this effect could be partially eliminated by overexpression of
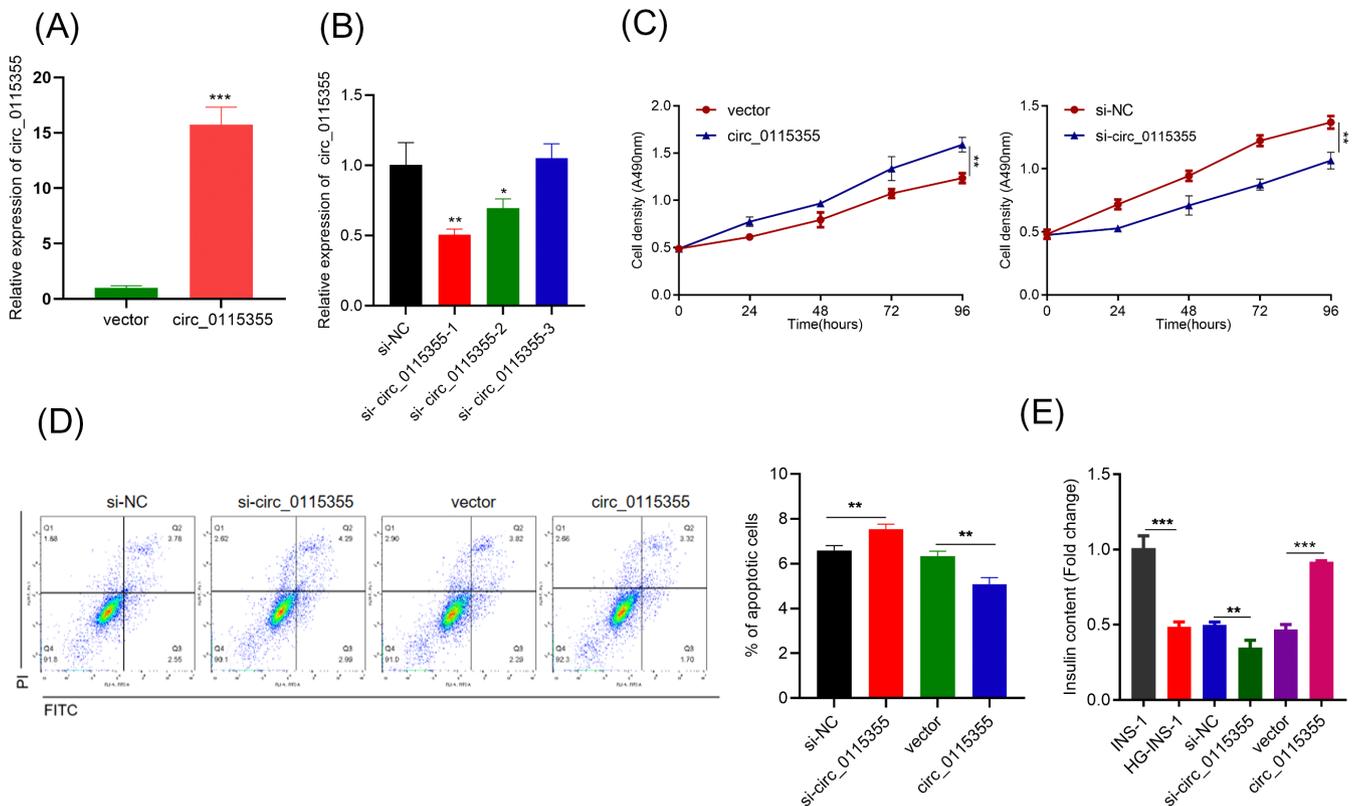
FIGURE 2 hsa_circ_0115355-induced proliferation and insulin secretion of HG-INS-1 cells and apoptotic inhibition. The effectiveness of hsa_circ_0115355 knockdown (A) and overexpression (B) was analyzed by qRT-PCR. (C) Determination of the proliferative capacity of HG-INS-1 cells by CCK8 assay. (D) Evaluation of the apoptotic capacity of HG-INS-1 cells by flow cytometry. (E) Evaluation of the insulin secretion capacity of INS-1 cells by ELISA

hsa_circ_0115355 (Figure 4A,B). In addition, the effect of miR-145 mimics on apoptosis of HG-INS-1 cells could be partially reversed by the elevated expression of hsa_circ_0115355 (Figure 4C).

3.5 | SIRT1 is a downstream target of miR-145

According to the theory of ceRNAs, circCSE1 and mRNA must share miR-145 to relieve mRNA repression by competitive binding with miR-145. Bioinformatics analysis was conducted using starBase and revealed that SIRT1 shares complementary binding sites with miR-145. For further validation, SIRT1 was selected because it plays an important role in islet beta-cell proliferation and regulation of insulin secretion (Figure 5A). Further experimental results showed that miR-145 mimic could significantly inhibit SIRT1 expression, whereas miR-145 inhibitor could promote its expression (Figure 5B). Knockdown of hsa_circ_0115355 significantly inhibited SIRT1 expression, whereas overexpression of hsa_circ_0115355 promoted the expression of SIRT1 (Figure 5C). The abovementioned mutual binding was confirmed by luciferase assay, which showed significantly inhibited relative luciferase activity of the SIRT1-WT+miR-145 mimic group compared to that of the SIRT1-MUT+miR-145 mimic group (Figure 5D). Thus, the relationship between SIRT1 and miR-145 was confirmed. Furthermore, the expression of SIRT1 was downregulated in HG-INS-1 cells compared

with normal INS-1 cells (Figure 5E). The above experimental data suggest that SIRT1 is a downstream target of miR-145.

3.6 | hsa_circ_0115355 regulates the in vitro function of INS-1 cells by regulating the miR-145/SIRT1 axis

To further explore the in vitro function of hsa_circ_0115355 in INS-1 cells by regulating the miR-145/SIRT1 axis, we transfected HG-INS-1 cells with si-NC+vector, si-SIRT1+vector, and si-SIRT1+hsa_circ_0115355, separately, and the transfection of si-SIRT1 indeed inhibited cell proliferation and insulin secretion, but overexpression of hsa_circ_0115355 could be partially eliminated (Figure 6A,B). In addition, the effect of si-SIRT1 on HG-INS-1 cell apoptosis could be partially reversed by the elevated expression of hsa_circ_0115355 (Figure 6C).

4 | DISCUSSION

In T2DM, glucolipotoxicity and dysfunction in β-cells lead to insulin deficiency as a key factor in disease progression.[24,25] T2DM characterizes abnormal apoptosis and senescence of β-cells. For example,
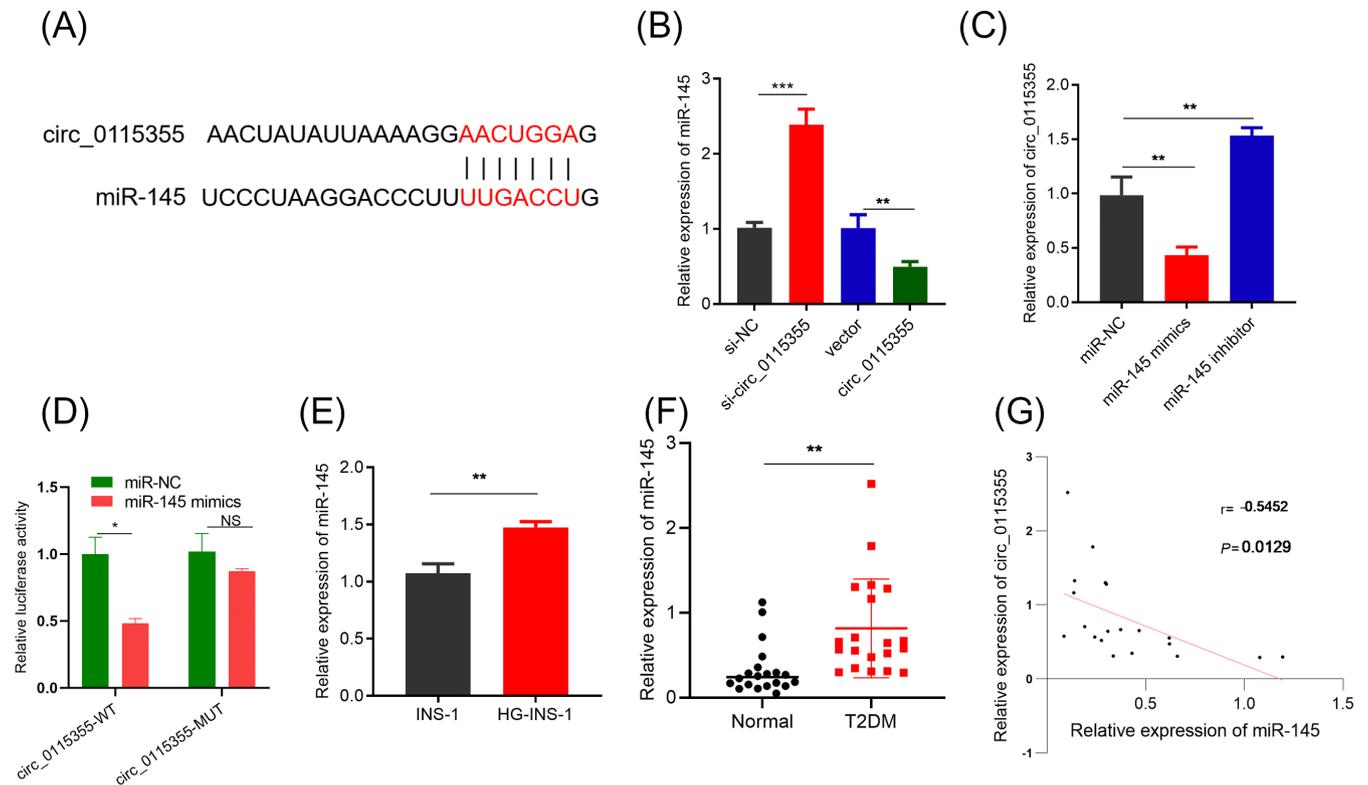
FIGURE 3 hsa_circ_0115355 is a miR-145 sponge. (A) Prediction of the binding sites of hsa_circ_0115355 and miR-145 from the database. (B) Analysis of the expression of miR-145 by qRT-PCR. (C) Analysis of the expression of hsa_circ_0115355 by qRT-PCR. (D) Analysis of the binding between hsa_circ_0115355 and miR-145 by luciferase activity assay. (E) miR-145 expression in INS-1 and HG-INS-1 cells. (F) Detection of miR-145 expression in serum of patients with T2DM by qRT-PCR. (G) Pearson analysis of the correlation between hsa_circ_0115355 and miR-145 expression in serum of patients with T2DM
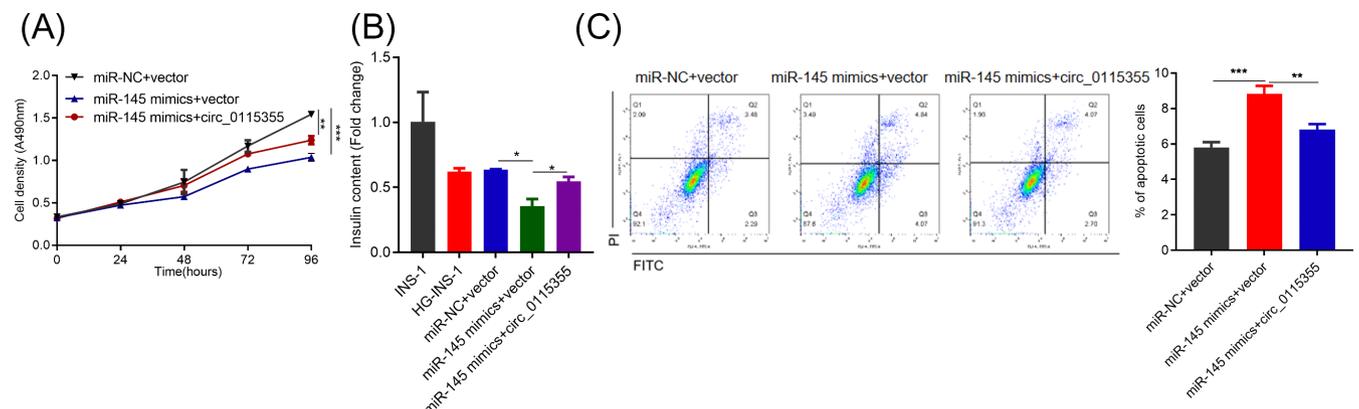
FIGURE 4 MiR-145 is involved in hsa_circ_0115355-mediated effects on INS-1 cell function. (A) Assessment of the proliferative capacity of HG-INS-1 cells by CCK8 assay. (B) Evaluation of the insulin secretion capacity of INS-1 cells by ELISA. (C) Evaluation of the apoptotic capacity of HG-INS-1 cells by flow cytometry

some free fatty acids may trigger endoplasmic reticulum stress to impair β-cell function and survival; in T2DM, there are more β-galactosidase-positive cells and high expression of p16 INK4A, C-C Motif Chemokine Ligand 4, and interleukin-6 accelerate cellular senescence and dysfunction.[26] Long-term exposure to high glycolipid conditions also results in decreased beta-cell proliferation and increased apoptosis. A persistent imbalance between excessive β-cell apoptosis and restricted proliferation results in loss of β-cell mass

and decreased insulin secretion.[27] Therefore, for T2DM control, stable regulation of glucose metabolism by β-cells under controlled glycolipid toxicity is critical.

In recent years, with the rapid development of high-throughput sequencing technology and bioinformatics technology, more circRNAs have been screened and verified for their expression and function. However, functions of a large number of circRNA still have not been functionally annotated. In one study, more than
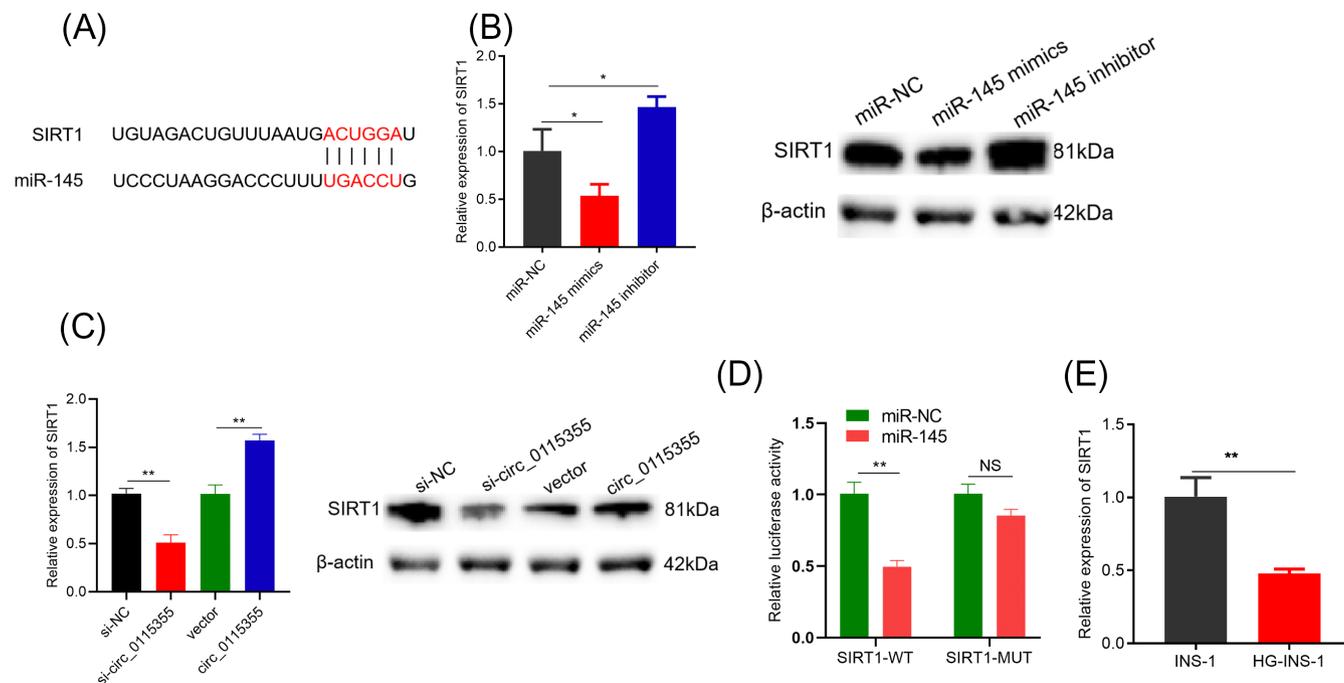
FIGURE 5 SIRT1 is a downstream target of miR-145. (A) Bioinformatics analysis shows that SIRT1 binds to miR-145. (B and C) The expression of SIRT1 was detected by qRT-PCR and western blot. (D) Analysis of the binding between SIRT1 and miR-145 by luciferase activity assay. (E) Analysis of SIRT1 expression by qRT-PCR
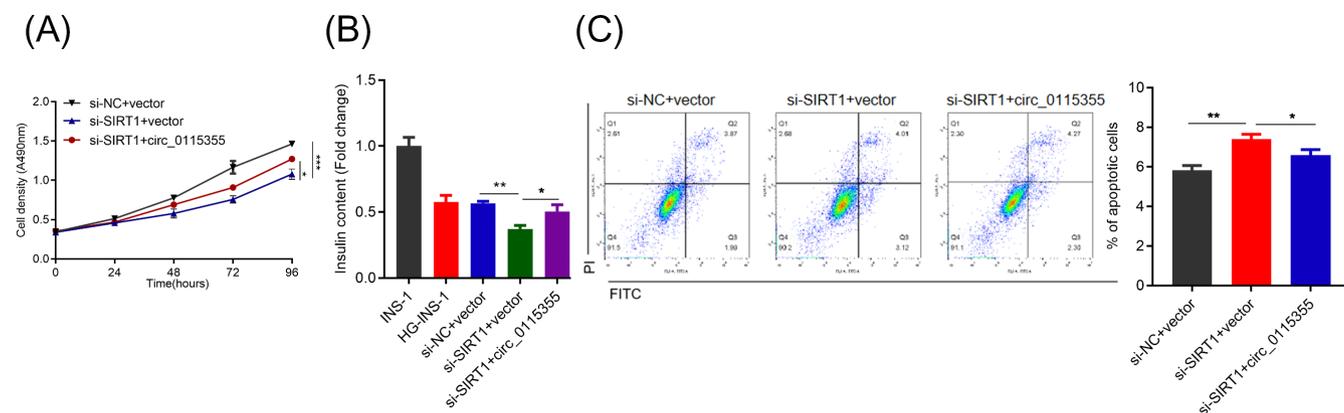
FIGURE 6 hsa_circ_0115355 regulates the in vitro function of INS-1 cells by regulating the miR-145/SIRT1 axis. (A) Assessment of the proliferative capacity of HG-INS-1 cells by CCK8 assay. (B) Evaluation of the insulin secretion capacity of INS-1 cells by ELISA. (C) Evaluation of the apoptotic capacity of HG-INS-1 cells by flow cytometry

5000 circRNAs were detected using RNA sequencing, of which 346 were abnormally expressed in diabetic mice.[15] In this study, we analyzed the function and regulatory role of aberrantly expressed hsa_circ_0115355 in T2DM.

Our experimental results showed significant underexpression of hsa_circ_0115355 in patients with T2DM, and the results were confirmed in the INS-1 cell model under high glucose conditions. Analysis of INS-1 growth, apoptosis, insulin secretion, and other functional experiments revealed that overexpressed hsa_circ_0115355 could promote the growth of INS-1 cells under glucolipotoxicity, inhibit cell apoptosis, and increase insulin secretion. Conversely, the knockdown of hsa_circ_0115355 in INS-1 also confirmed this experimental result. We also found, through database analyses and dual-luciferase

experiments, that there are binding sites for hsa_circ_0115355 and miR-145. The regulatory role of miR-145 in metabolic diseases such as diabetes and atherosclerosis has often been reported. For example, miR-145 partially inhibits cell proliferation and induces apoptosis by targeting OPG and KLF5, thereby improving macrophage infiltration, weight loss, and improving glucose metabolism.[28] MiR-145 micelles regulate vascular smooth muscle cell phenotype by regulating attenuates atherosclerosis.[29] Further, exosomal miR-145 delivered from mesenchymal stem cells to human umbilical vein endothelial cells downregulates junctional adhesion molecule-A expression to inhibit atherosclerosis.[30] The expression level of miR-145-5p is significantly downregulated in T2DM, which may be a potential diagnostic biomarker for T2DM.[31] These studies have further demonstrated that

miR-145 may have an important regulatory role in the progression of diabetes and also suggest that hsa_circ_0115355 may regulate the progression of diabetes through miR-145.

Based on our experiments and analyses, we further examined the mechanism by which hsa_circ_0115355 participates in diabetes regulation via miR-145. Bioinformatics analysis revealed that SIRT1 is a downstream target of miR-145. SIRT1 is a NAD+-dependent deacetylase; it has been identified as a molecule essential for caloric restriction-related antiaging strategies. Past studies have shown an important link between dysfunction of SIRT1 activation and the pathogenesis of aging and obesity-related diseases such as diabetes, cardiovascular disease, and neurodegenerative diseases.[32] Accordingly, our study showed that miR-145 and SIRT1 are negatively regulated, and hsa_circ_0115355 and SIRT1 are positively regulated. Meanwhile, SIRT1 indeed mediated INS-1 cell growth, apoptosis, and insulin secretion. Therefore, we confirmed that SIRT1 is a downstream target of the hsa_circ_0115355/miR-145 axis, and hsa_circ_0115355 promotes the expression of SIRT1 by adsorbing miR-145.

Although we could elucidate the mechanism of hsa_circ_0115355-mediated enhanced expression of SIRT1 through the adsorption of miR-145 and thus its role in the progression of diabetes, some aspects still need to be improved. First, we did not analyze the expression of miR-145 and SIRT1 in patients with T2DM. At the same time, the clinical bed case data can be analyzed to assess the correlation between the expression level of hsa_circ_0115355 and the diagnosis, prognosis, and other factors of T2DM. Second, we validated the role of the hsa_circ_0115355/miR-145/SIRT1 axis through cellular functional experiments, but these need to be corroborated through animal experiments. Therefore, by constructing an animal model, we can better verify the regulatory role of the hsa_circ_0115355/miR-145/SIRT1 axis. Finally, SIRT1 is considered a potential therapeutic target for metabolic diseases such as diabetes and atherosclerosis; hence, the therapeutic effects of SIRT1 and its regulatory molecule hsa_circ_0115355 can be better verified through animal models.

In conclusion, our study showed that hsa_circ_0115355 competitively binds to miR-145 to abolish the inhibitory effect of miR-145 on SIRT1, thereby promoting INS-1 cell growth, inhibiting apoptosis, and increasing insulin secretion. Our findings offer new insights into the pathogenesis of T2DM and provide a new theoretical basis for developing agents for the treatment of T2DM.

AUTHOR CONTRIBUTIONS

Ying Dai and Jianhua Wang designed research. Ying Dai and Xudan Ma analyzed data. Ying Dai, Xudan Ma, Jiangnan Zhang, Shuting Yu, and Yuchao Zhu performed research. Ying Dai and Jianhua Wang wrote the article. Ying Dai, Yuchao Zhu, and Jianhua Wang contributed to discussion and the proofreading of the article. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

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