OncoTargets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

# Mi-Lnc70 Regulates the Progression of Murine Pancreatic β-Cell Line and Affects the Synthesis of Insulin and Glucagon

Wen Yuan, Dongxue Sun, Jing Wang, Yongli Yue, Xueling Li

State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock, College of Life Science, Inner Mongolia University, Hohhot, 010021, People's Republic of China

Correspondence: Yongli Yue; Xueling Li, State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock, College of Life Science, Inner Mongolia University, No. 24 Zhao Jun Road, Yu Quan Qu, Hohhot, Inner Mongolia, 010070, People's Republic of China, Tel/Fax +86 471 3679873; +86 471 3679807, Email yueyongli228@163.com; lixueling@imu.edu.cn



**Background:** Insulinoma, the most common type of pancreatic endocrine tumor, frequently induces hypoglycemia due to persistent hyperinsulinemia. Although Mi-Lnc70 expression progressively increases during pancreatic maturation in mice, the biological role of Mi-Lnc70 in pancreatic β cells remains elusive.

**Aim:** This study was designed to investigate the role of LncRNA-Mi-Lnc70 in the mouse pancreatic β-cell line MIN6.

**Methods:** We performed quantitative real-time PCR, cell counting kit-8 (CCK-8) assay, flow cytometry, transwell assay, wound healing assay, immunofluorescence staining, and Western blotting.

**Results:** The expression of Mi-Lnc70 was markedly elevated in mouse pancreatic β-cells (MIN6) compared to normal cells. Knockdown of Mi-Lnc70 markedly suppressed the proliferation, migration, and invasion capabilities of MIN6 cells but induced cell apoptosis and triggered G2/M phase cell cycle arrest. Moreover, Mi-Lnc70 knockdown influenced the expression profiles of pancreas-related lncRNAs and miRNAs and decreased the expression of islet-related genes and reduced the protein synthesis of INSULIN, GLUCAGON, and PDX1.

**Conclusion:** Mi-Lnc70 plays an important role in the proliferation, migration, and endocrine-related gene expression in pancreatic MIN6 cells, particularly in the synthesis of PDX1, INSULIN, and GLUCAGON.

**Keywords:** MIN6 cells, Mi-Lnc70, apoptosis, G2/M arrest, insulin, glucagon

## Introduction

Insulinoma is a type of pancreatic endocrine tumor that often causes hypoglycemia due to persistent hyperinsulinemia.[1] Accumulating evidence has shown that the expression of certain long non-coding RNAs (lncRNAs) in β-cells is regulated by extracellular glucose concentration-, indicating that lncRNAs may be involved in the regulation of insulin secretion.[2–6] The mapping of some pancreatic lncRNA genes has uncovered their association with human diabetes. Moreover, specific lncRNAs have been found to be dysregulated in pancreatic islets with type 2 diabetes (T2D), suggesting that lncRNAs may serve as potential regulatory factors in diabetes research.[2,7] These findings imply that lncRNAs may play a role in the synthesis or secretion of insulin in insulinoma.

Mutations or aberrant expression of protein-coding genes, as well as mutations and dysregulation of lncRNAs, play a significant role in the pathogenesis of cancer. Genome-wide association studies (GWAS) of tumor samples have identified a considerable number of lncRNAs associated with various types of cancer. LncRNAs may exhibit tumor suppressive or promoting functions, and their abnormal expression and mutations are closely related to tumorigenesis, metastasis, and tumor staging. They also have diagnostic and prognostic value.[8–11] For instance, a study revealed that lncRNA NUTF2P3-001 elevated K-Ras expression after hypoxia induction and facilitated the proliferation of Panc-1 and Bxpc-3 cell lines.[12] The long non-coding RNA (lncRNA) HOTTIP has been shown to enhance the expression of HOXA



Received: 18 March 2025
Accepted: 22 July 2025
Published: 3 September 2025

genes by binding to the WDR5/MLL1 complex. This interaction plays a crucial role in promoting the proliferation, survival, and migration of pancreatic cancer cells.[13]

Mi-Lnc70 is an islet-specific lncRNA located between the hepatic nuclear factor 1A (Hnf1a) and signal peptide peptidase-like 3 (Sppl3) genes on mouse chromosome 5, and it is a partial antisense transcript of Hnf1a. During pancreatic maturation in mice, the expression of Mi-Lnc70 gradually increases, although its expression level is not regulated by glucose.[9] HNF1A has been identified as a tumor suppressor in pancreatic cancer,[14–16] capable of inhibiting the proliferation and migration of pancreatic cancer cells.[16–18] Moreover, HNF1A is crucial for maintaining the function of human pancreatic α and β cells.[19,20] Mutations or deletions in HNF1A lead to pancreatic β cell dysfunction and are closely associated with the development of diabetes.[21–23] Given the significant role of HNF1A in pancreatic cell function and its proximity to Mi-Lnc70, this study aims to explore the biological function of Mi-Lnc70 in the mouse pancreatic β-cell line (MIN6), providing a foundation for elucidating the molecular mechanisms related to pancreatic tumors.

# Material and Methods

## Cell Line and Cell Culture

The murine pancreatic β-cell line MIN6 (RRID: CVCL_0431) was obtained from Xiubin Liang's laboratory in the Department of Pathophysiology at Nanjing Medical University and used for experimental studies. MIN6 cells were maintained in DMEM (Gibco) supplemented with 15% fetal bovine serum (FBS, Hyclone) and 50 μmol/L β-mercaptoethanol (Sigma) at 37°C in a humidified incubator with 5% $CO_2$. Prior to cell seeding, culture dishes were coated with gelatin (Sigma). Mouse embryonic fibroblast (MEF) cells were cultured in DMEM supplemented with 10% FBS. Cells were passaged at a 1:3 ratio using 0.05% trypsin (Gibco) and the medium was changed daily. All cell line usage and animal procedures were conducted in strict accordance with the guidelines established by the Inner Mongolia University Animal Care and Use Committee and were approved under license number IMU-MOUSE-2018-012.

## Cell Transfection

Cells were transfected with Mi-Lnc70-specific antisense locked nucleic acid (LNA) GapmeR using HiPerFect Transfection Reagent (QIAGEN), with nontargeting siRNA serving as a negative control. Twenty-four hours post-transfection, the culture medium was replaced with an appropriate volume of complete culture medium, and the cells were incubated for an additional 72 hours. Cell status was subsequently examined under a microscope. The sequences of the siRNAs targeting Mi-Lnc70 are detailed in Table S1.

## RNA Extraction and Real-Time PCR

Total RNA was extracted from cells using Trizol reagent (TransGene Biotech) according to the manufacturer's instructions. The extracted RNA was reverse transcribed into cDNA using the PrimeScript RT Reagent Kit with gDNA Eraser (RR047A, Takara). For miRNA reverse transcription, the TransScript® miRNA First-Strand cDNA Synthesis SuperMix (TransGene Biotech) was used. Quantitative real-time PCR was performed using TB GREEN™ Premix Ex Taq™ II (Takara) on a 7500 Real-Time PCR System (ABI Biosystems). The mouse U6 gene was used as the housekeeping gene for normalization. The relative expression levels of target genes were determined using the comparative CT method (ΔΔCT). Primer sequences used in the study are listed in Table S2.

## Cell Counting Kit (CCK)-8 Assay

For the cell proliferation assay, $1\times10^4$ cells suspended in 100 μL of culture medium were seeded into each well of a 96-well plate. After cell attachment, 100 μL of CCK-8 reagent mixture (prepared by mixing 10 μL of CCK-8 reagent with 90 μL of DMEM) was added to each well. The plates were then incubated at 37°C in the dark for 2 hours. The absorbance at 450 nm was measured using a microplate reader (Molecular Devices). Data were analyzed using GraphPad Prism version 8.0.

## Cell Cycle Assay

Cells in the logarithmic growth phase were seeded into a 24-well plate at a density of $9\times10^5$ cells/mL. After 24 hours of culture, cells were transfected as described previously. At 72 hours post-transfection, MIN6 cells were collected, washed twice with cold DPBS, and immediately fixed in cold 75% ethanol at 4°C for a minimum of 4 hours. Following fixation, cells were washed once with DPBS and stained with a propidium iodide (PI) solution containing 50 μg/mL PI and 100 μg/mL RNase A. Cells were then incubated at 4°C for 30 minutes in the dark. Flow cytometric analysis was performed within 1 hour to determine the cell cycle distribution.

## Apoptosis Assay

Cells in the logarithmic growth phase were seeded into 24-well plates at a density of $9\times10^5$ cells/mL. After 24 hours of culture, cells were transfected with Mi-Lnc70-specific siRNA. At 72 hours post-transfection, cell pellets were collected. Apoptosis was assessed using the Annexin V-FITC Apoptosis Detection Kit (Solarbio) according to the manufacturer's instructions. Briefly, cells were resuspended in binding buffer, stained with Annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry within 1 hour to determine the apoptosis rate.

## Wound Healing Assay

MIN6 cells were seeded in 24-well plates at a density of $9\times10^5$ cells per well and cultured for 24 hours. A linear scratch was created in the cell monolayer using a 100 μL micropipette tip. Non-adherent cells were removed by washing with DPBS. Subsequently, cells were transfected with Mi-Lnc70-specific siRNA as described previously. Images of the scratch wounds were captured at 0 hours and 72 hours using a microscope. The wound-healing rate was calculated using the following formula: 100% × [(wound width at 0 h − wound width at 72 h) / wound width at 0 h].

## Transwell Migration and Invasion Assay

Transwell assays were conducted using a transwell chamber with a pore diameter of 8 μm (Corning). MIN6 cells, transfected with Mi-Lnc70-specific siRNA, were resuspended in serum-free medium and seeded into the upper chamber at a density of $5\times10^4$ cells in 100 μL. The lower chamber was filled with complete medium containing 10% fetal bovine serum as a chemoattractant. After 20–24 hours of incubation at 37°C in a 5% $CO_2$ incubator, cells that had migrated to the lower surface of the membrane were fixed with absolute ethanol and stained with DAPI (Beyotime) for 2–3 minutes at room temperature. Cells remaining on the upper surface were gently removed with a cotton swab. Images were captured using an inverted light microscope (Nikon). For the invasion assay, the procedure was identical to the migration assay, except that the upper chamber was coated with Matrigel to mimic the extracellular matrix.

## Cell Total Protein Extraction and Western-Blot

Cells were collected and lysed in lysis buffer (Thermo Fisher Scientific) supplemented with phenylmethylsulfonyl fluoride (Beyotime) on ice for 30 minutes. The lysates were then heated at 100 °C for 10 minutes, followed by centrifugation at 4°C for 10 minutes at 12000×g. The supernatant was collected, and protein concentration was determined using the BCA assay (Thermo Fisher Scientific). Total protein was separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% skim milk powder in TBST for 1 hour at room temperature, and then incubated with primary antibodies overnight at 4°C. After washing with TBST, membranes were incubated with secondary antibodies for 1 hour at room temperature. Target protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Thermo Fisher Scientific) and captured using an imaging analysis system (Bio-Rad). The primary and secondary antibodies used in this study are listed in Tables S3 and S4.

## Immunofluorescence

MIN6 cells were washed once with DPBS and fixed in 4% paraformaldehyde (PFA; Solarbio) for 30 minutes at room temperature. After fixation, cells were permeabilized with 0.5% Triton X-100 (Solarbio) for 30 minutes. Subsequently, cells were blocked for 2 hours at room temperature using a blocking solution containing 2% BSA, 2% goat serum, 2%

skim milk powder, and 0.15 M glycine in DPBS. The samples were then incubated with primary antibodies overnight at 4°C. Following three washes with DPBS, the samples were incubated with secondary antibodies for 1 hour at room temperature. After two additional washes with DPBS, the samples were stained with DAPI (0.5 mg/mL; Beyotime) for 3 minutes at room temperature. The samples were then washed twice with DPBS and mounted onto slides using antifading Mounting Medium (Solarbio). Fluorescent signals were visualized using a Nikon confocal laser-scanning microscope (Nikon, A1). The primary and secondary antibodies used in this study are listed in Tables S5 and S6.

## Statistical Analyses

Data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Statistical significance between two groups was determined using Student's *t*-test, whereas one-way analysis of variance (ANOVA) was employed to analyze data from more than three groups. Significance levels were defined as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

# Results

## Expression Analysis of Pancreatic Islet-Specific lncRNAs in MIN6 Cells

To elucidate the role of lncRNAs in insulinoma, we initially utilized real-time fluorescence quantitative PCR to measure the expression levels of several pancreatic islet-specific lncRNAs in the mouse pancreatic β-cell line MIN6. Our results revealed that Mi-Lnc70 exhibited a significantly higher expression level compared to other lncRNAs such as Mi-Lnc72, Mi-Lnc75, Mi-Lnc76, and Mi-Lnc80 (Figure 1A and B). This finding indicates that Mi-Lnc70 may exert a crucial regulatory function in mouse pancreatic β-cells. Therefore, we selected Mi-Lnc70 for further investigation.

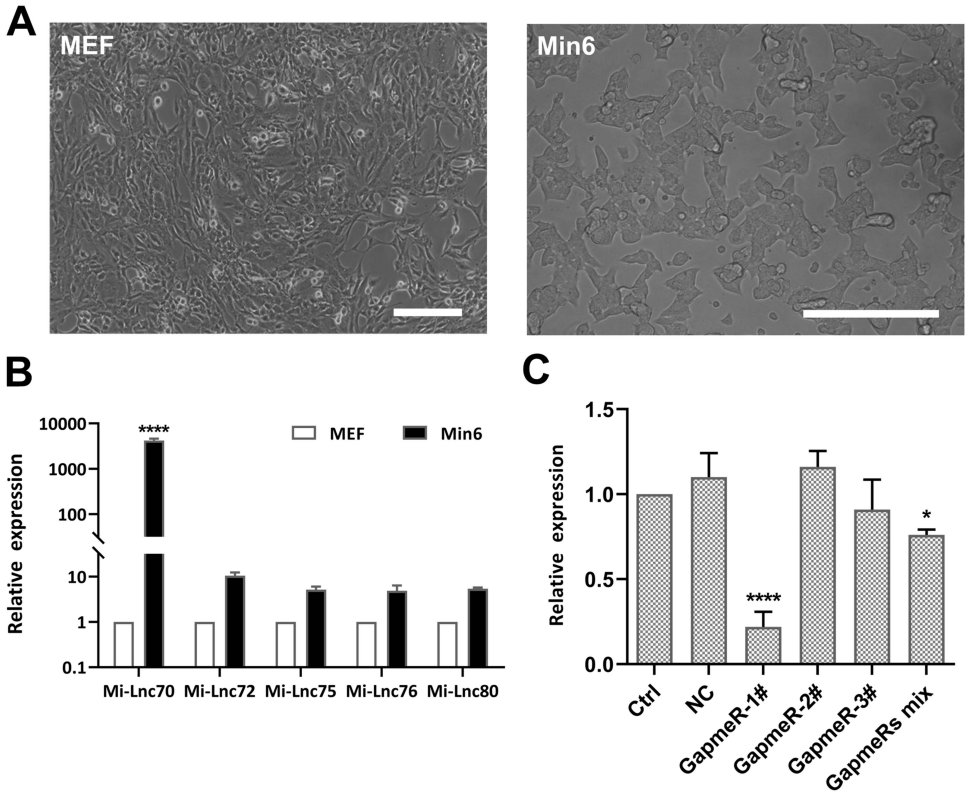



**Figure 1** The expression of pancreatic islet-specific lncRNAs was analyzed in MIN6 cells. (**A**) Morphological in MEF and MIN6 cells during culture. Scale bars: 100 μm. (**B**) The relative expression of pancreatic islet-specific Mi-LncRNAs in the MIN6 cells. ****$p < 0.0001$. (**C**) The relative expression of Mi-Lnc70 in the MIN6 cells under different transfection conditions is presented below. GapmeR-1#/2#/3# are different siRNAs designed for Mi-Lnc70, and GapmeRs mix is a mixture of GapmeR-1#, GapmeR-2# and Gapme-R3#. *$p < 0.05$, ****$p < 0.0001$.

To explore the role of Mi-Lnc70 in pancreatic β-cells, we employed GapmeR antisense oligonucleotides to specifically knockdown Mi-Lnc70 (si-Lnc70) and used a nonsense oligonucleotide as a negative control (NC). Post-transfection, the expression level of Mi-Lnc70 in the si-Lnc70 group was significantly reduced compared to both the NC group and the blank control group (Figure 1C).

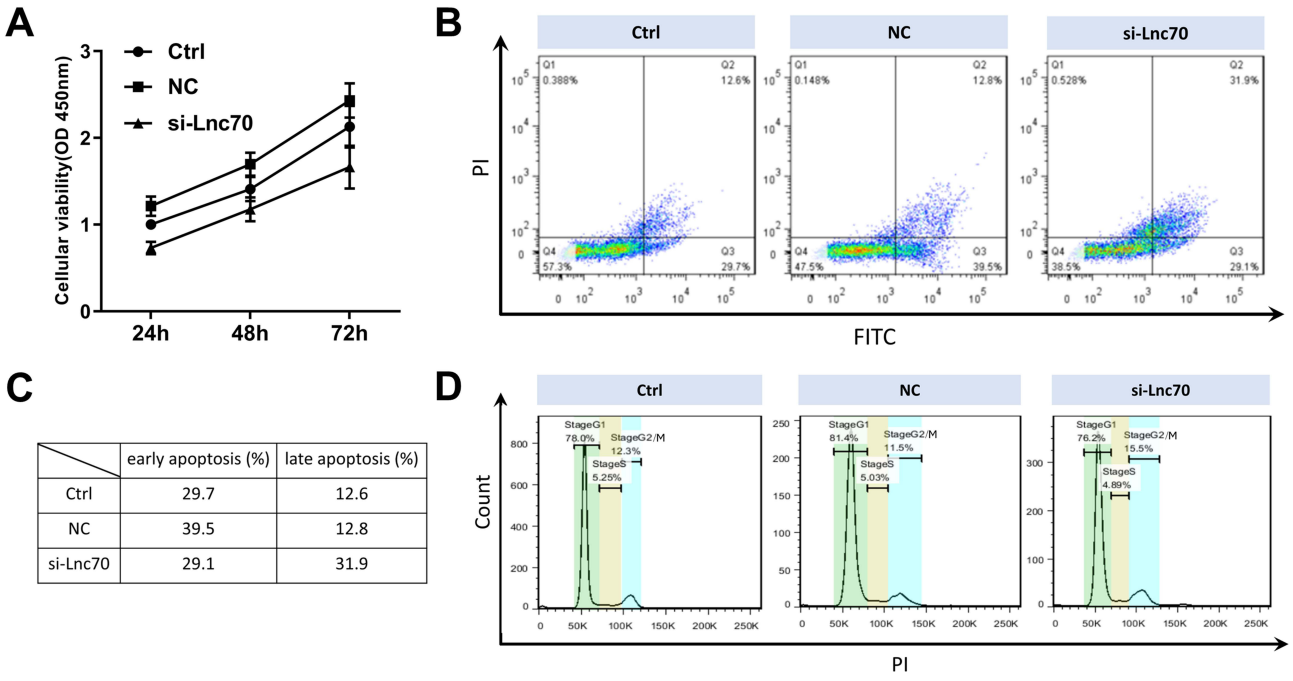
## Inhibition of MIN6 Cell Proliferation by Knocking Down Mi-Lnc70

To elucidate the role of Mi-Lnc70 in mouse pancreatic β cells, we first investigated the effect of Mi-Lnc70 knockdown on the proliferation of MIN6 cells in vitro. The CCK-8 assay was used to assess the impact of Mi-Lnc70 on cell proliferation. We constructed a Mi-Lnc70 knockdown group in the MIN6 cell line and found that the proliferation ability of the si-Mi-Lnc70 group was significantly lower than that of the negative control (NC) group and the blank control group (Figure 2A).

Apoptosis detection in the MIN6 cell line after transfection revealed that the proportion of late apoptotic cells (Q2: 31.9%) increased, while the proportion of live cells decreased following Mi-Lnc70 knockdown (Figure 2B and C). Furthermore, flow cytometry analysis of the cell cycle demonstrated an increased proportion of cells in the G2/M phase after Mi-Lnc70 knockdown, indicating G2/M phase arrest (Figure 2D). Collectively, these results indicate that down-regulation of Mi-Lnc70 induces G2/M phase arrest in MIN6 cells, thereby reducing their proliferation ability and promoting apoptosis.

## Knockdown of Mi-Lnc70 Reduces the Migration and Invasion Abilities of MIN6 Cells

To further investigate the function of Mi-Lnc70 in mouse pancreatic β cells, we examined the effects of Mi-Lnc70 knockdown on the migration, invasion, and wound-healing abilities of MIN6 cells in vitro. The Transwell assay was employed to assess the migration and invasion capabilities of MIN6 cells. As depicted in Figure 3A and B, the migration capacity of the si-Mi-Lnc70 group exhibited a substantial decline. Specifically, it was diminished by approximately 39.7% relative to the blank control group and by approximately 42.3% in comparison with the negative control (NC) group. Consistent with the migration results, the invasive capacity of the MIN6 cell line within the si-Mi-Lnc70 group




| | early apoptosis (%) | late apoptosis (%) |
|---|---|---|
| Ctrl | 29.7 | 12.6 |
| NC | 39.5 | 12.8 |
| si-Lnc70 | 29.1 | 31.9 |

**Figure 2** The Mi-Lnc70 construct was found to promote the proliferation of MIN6 cells. (**A**) The activity of MIN6 cells transfected with NC and si-Lnc70. (**B**) The detection of apoptosis in MIN6 cells transfected with NC and si-Lnc70. (**C**) The ratio of early apoptotic cells to late apoptotic cells in (**B**). (**D**) The cell cycle of MIN6 cells transfected with NC and si-Lnc70 was also analyzed.


https://doi.org/10.2147/OTT.S523599

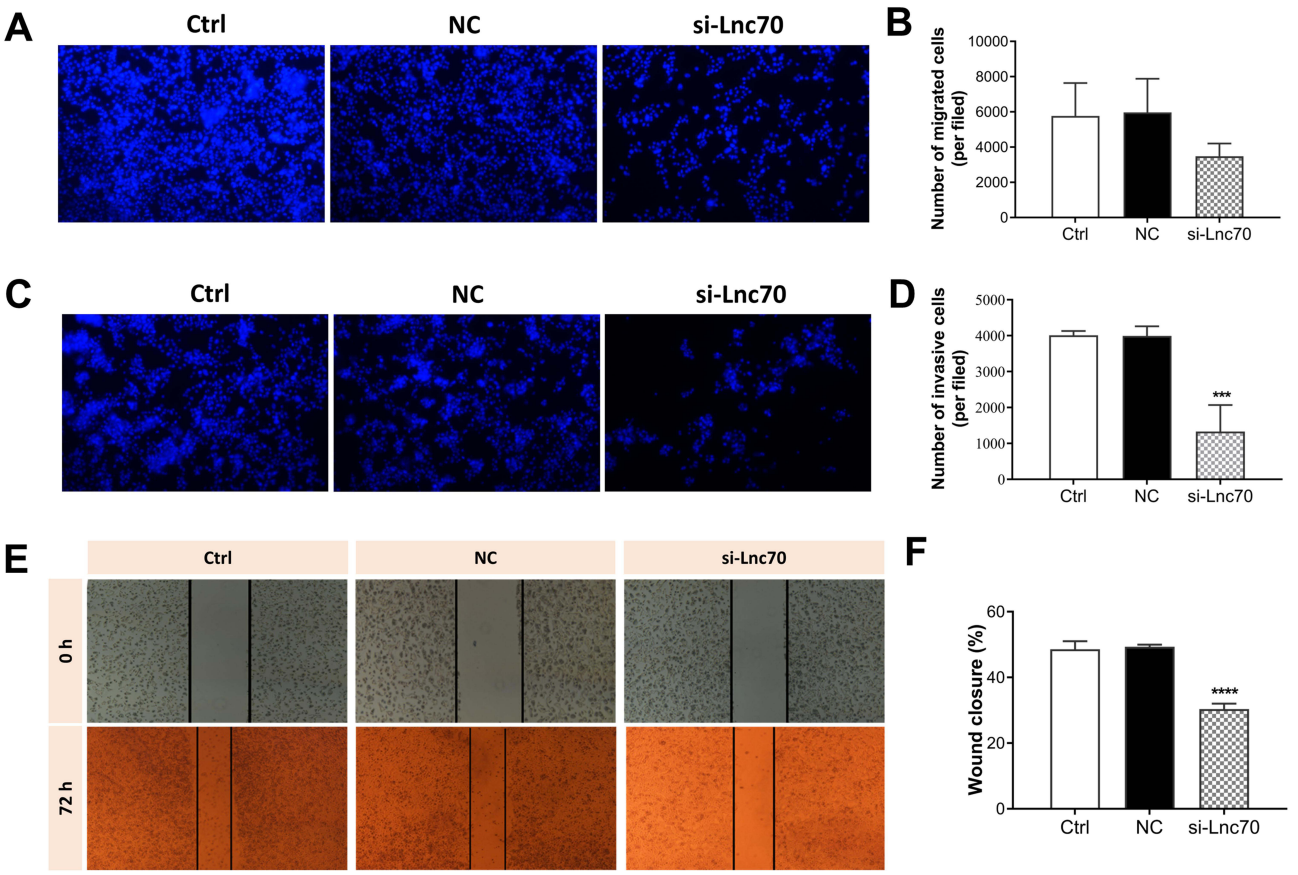



**Figure 3** Mi-Lnc70 enhanced the migration and invasion ability of MIN6 cells. (**A** and **B**) Transwell assays were employed to assess the migratory capacity of MIN6 cells transfected with NC and si-Lnc70. (**C** and **D**) Transwell invasion assays demonstrated the invasion capacity of MIN6 cells transfected with NC and si-Lnc70. ***$p < 0.001$. (**E** and **F**) Wound healing assay of MIN6 cells transfected with NC and si-Lnc70. ****$p < 0.0001$.

was likewise markedly attenuated. Quantitatively, it was decreased by approximately 66.8% when juxtaposed with the blank control group and by approximately 66.7% relative to the negative control (NC) group, as illustrated in Figure 3C and D. Additionally, wound-healing assays revealed that Mi-Lnc70 knockdown led to a decreased wound-healing capacity in MIN6 cells (Figure 3E and F). Collectively, these findings suggest that downregulation of Mi-Lnc70 results in reduced migration, invasion, and wound-healing abilities in MIN6 cells.

## Knockdown of Mi-Lnc70 Reduces the Expression of Pancreas-Related LncRNAs and MiRNAs in MIN6 Cells

To elucidate the underlying mechanisms, we examined the impact of Mi-Lnc70 knockdown on the expression of pancreas-related lncRNAs and miRNAs in MIN6 cells. Comparative analysis of the relative expression levels of these lncRNAs and miRNAs before and after transfection revealed significant changes in the expression profiles of multiple lncRNAs and miRNAs in MIN6 cells following Mi-Lnc70 knockdown. Among the lncRNAs, the expression levels of Mi-Lnc77, Mi-Lnc87, Mi-Lnc78, and Mi-Lnc85 were upregulated, while Mi-Lnc12 and Mi-Lnc67 showed no significant changes. In contrast, the expression levels of Mi-Lnc71, Mi-Lnc72, Mi-Lnc75, Mi-Lnc76, and Mi-Lnc80 were significantly downregulated (Figure 4A–C).

Furthermore, the knockdown of Mi-Lnc70 in MIN6 cells also affected the expression of pancreas-related miRNAs. While the expression levels of miR-195-3p, miR-15a-5p, and miR-30d-3p remained comparable to those in untransfected cells, the expression levels of miR-23b-3p, miR-124-1-3p, miR-124-1-5p, miR-16-1-5p, miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p were significantly decreased, with the most pronounced reductions observed for miR-7, miR-195-5p, and miR-15b-3p (Figure 4D and E). These findings suggest that Mi-Lnc70 may regulate the expression of genes involved

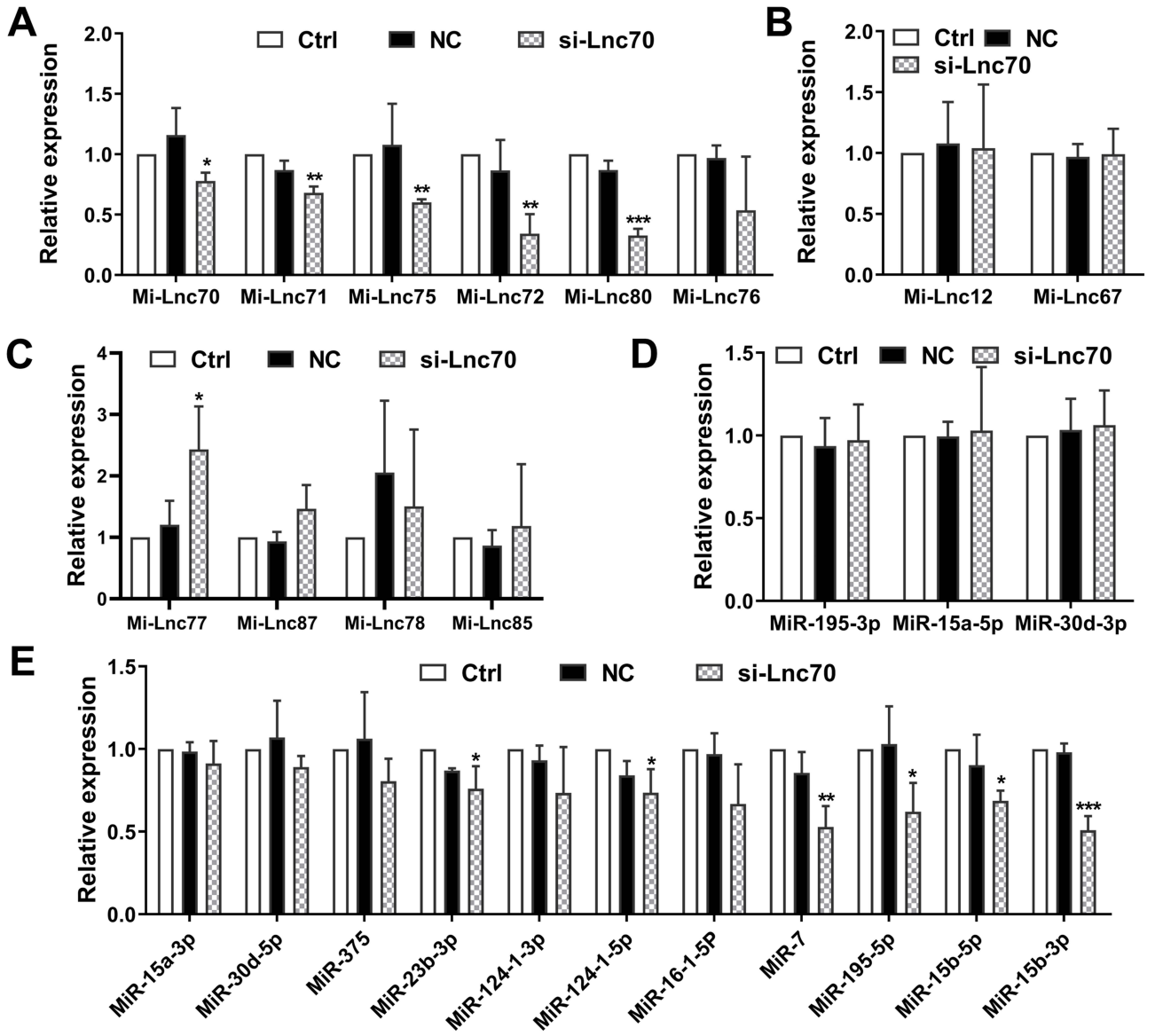



**Figure 4** Knockdown of Mi-Lnc70 had effects on the expression of pancreas-related lncRNAs and miRNAs in MIN6 cells. (**A**) The lncRNAs with reduced expression level are presented. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**B**) The lncRNAs with unchanged expression level are presented. (**C**) The lncRNAs with increased expression level are presented. *$p < 0.05$. (**D**) The miRNAs with unchanged expression level are presented. (**E**) The miRNAs with reduced expression level are presented. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

in the proliferation, apoptosis, migration, and invasion abilities of MIN6 cells by modulating the expression of specific miRNAs, particularly miR-7, miR-195-5p, and miR-15b-3p.

## Knockdown of Mi-Lnc70 Reduces the Synthesis of Secretion-Related Proteins in MIN6 Cells

To investigate the effect of Mi-Lnc70 knockdown on insulin synthesis in MIN6 cells, we quantified the relative expression levels of insulin and related factors post-transfection. Figure 5A showed that the knockdown of Mi-Lnc70 resulted in a significant downregulation of the expression levels of insulin and its associated factors, which encompass *Insulin1, Insulin2, Somatostatin, Pax4, MafA, Isl-1, NeuroD1, Pax6, Pdx1, Nkx6.1*, and *Glucagon*. Immunofluorescence staining revealed diminished expression of PDX1, C-peptide, insulin, glucagon, and somatostatin in MIN6 cells following Mi-Lnc70 knockdown. Notably, a significant reduction in somatostatin expression was observed in MIN6


https://doi.org/10.2147/OTT.S523599

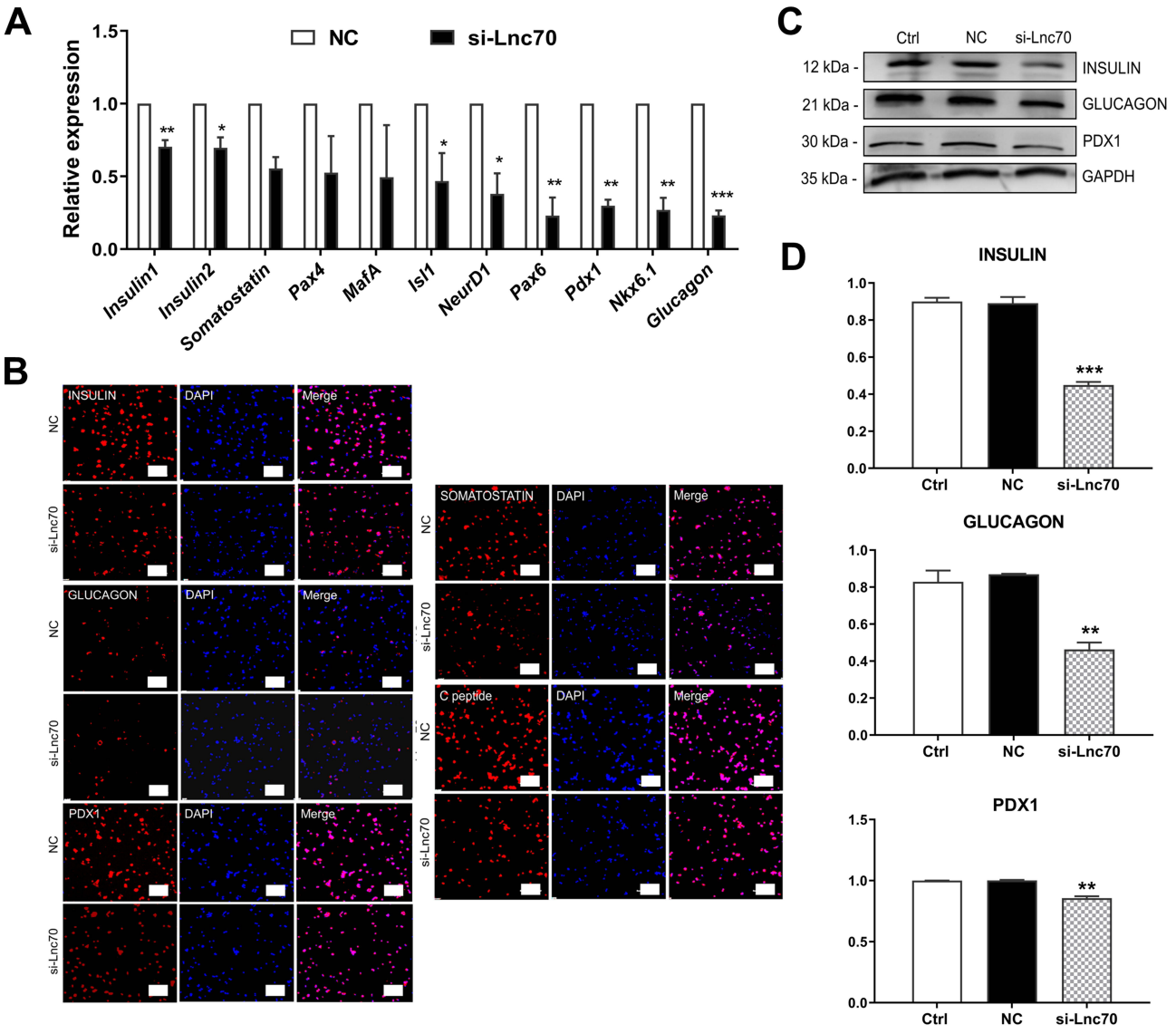



**Figure 5** Mi-Lnc70 affects the synthesis of islet-related secreted proteins in MIN6 cells. (**A**) Relative expression levels of insulin and related genes in MIN6 cells transfected with NC and si-Lnc70. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (**B**) Immunofluorescence staining of insulin and related genes in MIN6 cells transfected with NC and si-Lnc70 (n=3). Nuclei were stained with DAPI. Scale bars, 100 μm. (**C** and **D**) Expression of INSULIN, GLUCAGON and PDX1 proteins in MIN6 cells transfected with NC and si-Lnc70. **$p < 0.01$, ***$p < 0.001$.

cells transfected with si-Lnc70 (Figure 5B). Western blot analysis further revealed that the protein expression levels of insulin, glucagon, and PDX1 in Mi-Lnc70 knockdown cell lines were significantly downregulated compared with those in negative control cells and non-transfected cells, as shown in Figure 5C and D. Collectively, these findings indicate that the knockdown of Mi-Lnc70 inhibits the synthesis of PDX1, INSULIN, and GLUCAGON in MIN6 cells (Figure 6).

## Discussion

The most significant biological characteristics of tumor cells are their capacities for migration and invasion, which are closely related to the degree of differentiation of the tumor cells. Poorly differentiated tumor cells typically exhibit enhanced proliferative capacity. Previous studies have demonstrated that Malat1 can regulate the proliferation, migration, and invasion of cancer cells by modulating the expression of multiple downstream genes, including cell cycle regulatory genes such as *p21, p27*, and *B-Myb*. A reduction in Malat1 expression can result in the arrest of cancer cells in the G2/M phase of the cell cycle.[24] In this study, we observed that the downregulation of Mi-Lnc70 in MIN6 cells led to significant

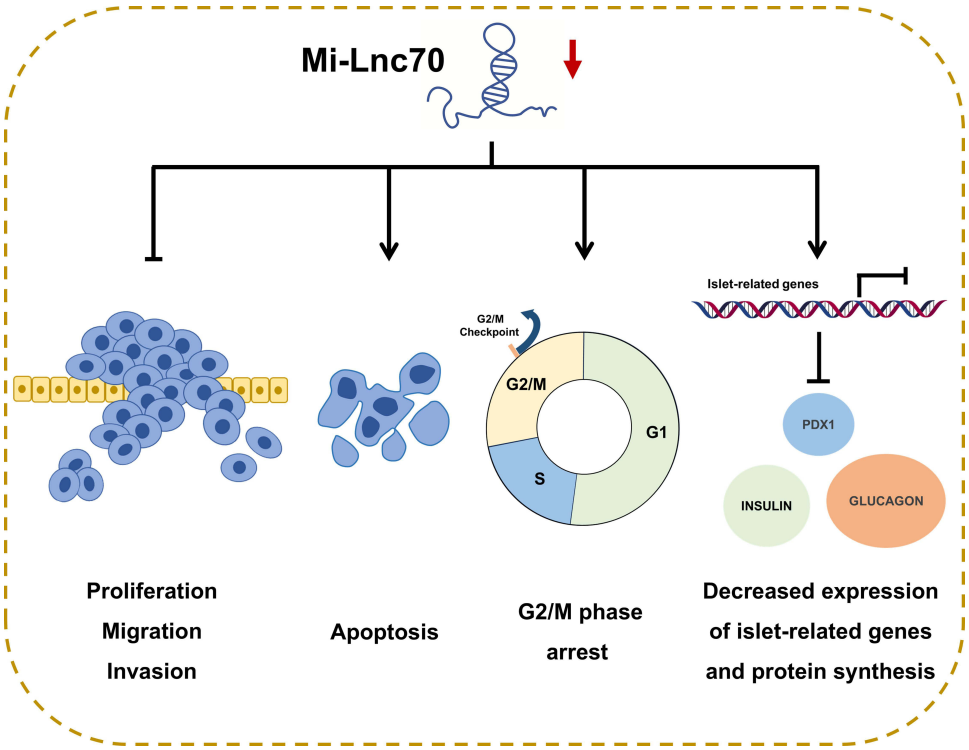



**Figure 6** Mi-Lnc70 Promotes Pancreatic β-Cell Progression and Affects the Synthesis of PDX1, Insulin, and Glucagon. Mi-Lnc70 was found to be significantly increased in MIN6 cells. Knockdown of Mi-Lnc70 significantly inhibited the proliferation, migration, and invasion of MIN6 cells while promoting cell apoptosis and G2/M phase arrest of the cell cycle. Additionally, Mi-Lnc70 knockdown reduced the expression of pancreas-related lncRNAs and miRNAs in MIN6 cells, as well as the synthesis of secretion-related proteins, including PDX1, INSULIN, and GLUCAGON.

inhibition of migration and invasion abilities, decreased wound-healing capacity, increased cell apoptosis, and cell cycle arrest in the G2/M phase. These findings suggest that Mi-Lnc70 plays a crucial role in regulating the biological behavior of MIN6 cells, potentially through mechanisms similar to those involving Malat1.

The miRNAs are pivotal regulators of gene expression, influencing multiple cellular pathways and the development and progression of cancer.[25] Long non-coding RNAs (lncRNAs) can act as competing endogenous RNAs (ceRNAs) or natural miRNA sponges, significantly impacting gene expression through post-transcriptional regulation. This regulatory mechanism involves competition for binding to shared miRNAs, leading to mutual regulation. Data from human pancreatic ductal adenocarcinoma (PDAC) tissues and adjacent normal tissues have shown a significant inverse correlation between miR-216a and Malat1. Overexpression of miR-216a or knockout of Malat1 has been found to affect the cell cycle of pancreatic cancer cells, with miR-216a overexpression inducing effects similar to those of si-Malat1, including G2/M phase arrest.[26] Additionally, lncRNA HNF1A-AS1 can interact with miRNAs and play a role in various cancers. HNF1A-AS1 can competitively bind to miRNAs (such as miR-149-5p, miR-30b-3p, miR-124) to relieve the inhibitory effects of these miRNAs on target genes, thereby promoting the expression of the target genes.[27–29] Conversely, HNF1A-AS1 can also synergize with miRNAs (such as miR-32-5p, miR-30b-5p, miR-363) to relieve the inhibition of downstream target genes, promoting the expression of common downstream target genes.[30–32] In this study, we observed a significant positive correlation between certain pancreatic development-related miRNAs and Mi-Lnc70, including miR-23b-3p, miR-124-1-5p, miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p. We hypothesize that miR-23b-3p, miR-124-1-5p, miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p may bind to Mi-Lnc70 and regulate each other. However, Mi-Lnc70 may function as a ceRNA or may cooperate with miRNAs to regulate downstream genes. Therefore, the specific mechanisms by which Mi-Lnc70 interacts with miR-23b-3p, miR-124-1-5p, miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p in MIN6 cells remain to be elucidated. Future studies should focus on deciphering these interactions to better understand the regulatory roles of Mi-Lnc70 in pancreatic β-cell function and tumorigenesis.


https://doi.org/10.2147/OTT.S523599

Previous studies have demonstrated that a fraction of lncRNAs are primary functional targets of islet-specific transcription factors and key cis-regulatory determinants of transcriptional programs in islet cells. Additionally, a considerable proportion of lncRNAs exhibit lineage-specific expression patterns. MafA and Pdx1 play pivotal roles in maintaining the development and maturation of pancreatic β cells. By binding to the insulin promoter region, they regulate related transcription factors, thereby influencing the synthesis and secretion of insulin.[33,34] For instance, lncRNA MALAT1 can inhibit the expression of PDX1 and insulin secretion by reducing H3 histone acetylation of the PDX1 promoter, thereby inducing β-cell dysfunction.[35] Downregulation of lncRNA HOTTIP regulates insulin secretion and the cell cycle in islet β cells via the inhibition of the MEK/ERK pathway.[36] Moreover, lncRNA PLUTO can regulate the transcription of PDX1 in mature pancreatic β cells.[37,38] Our results showed that downregulation of Mi-Lnc70 led to decreased expression of miRNAs such as miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p, as well as decreased expression of downstream factors such as *PDX1, MafA*, and *Nkx6.1*, ultimately resulting in reduced insulin synthesis. Therefore, we speculate that Mi-Lnc70 may bind to miRNAs such as miR-7, miR-195-5p, miR-15b-5p, and miR-15b-3p, acting as a ceRNA or coordinately regulating the expression of downstream factors such as *PDX1* and *MafA* in MIN6 cells. This regulatory mechanism may ultimately lead to a reduction in insulin synthesis. Future studies should focus on elucidating the specific interactions between Mi-Lnc70 and these miRNAs, as well as their downstream targets, to better understand the regulatory roles of Mi-Lnc70 in pancreatic β-cell function and insulinoma pathogenesis.

In summary, our study demonstrates that the downregulation of Mi-Lnc70 significantly inhibits the migration, invasion, and wound-healing capacities of MIN6 cells while simultaneously promoting cell apoptosis. Additionally, the expression of pancreas-related genes, as well as multiple lncRNAs and miRNAs, is concomitantly reduced. These findings highlight the crucial regulatory role of Mi-Lnc70 in MIN6 cells. However, it is important to acknowledge that in vitro models have inherent limitations. Therefore, the precise mechanisms by which Mi-Lnc70 regulates the mouse pancreatic β-cell lineage require further validation through in vivo studies. Future research should focus on elucidating the detailed molecular pathways and interactions involving Mi-Lnc70 in pancreatic β-cell function and tumorigenesis, ultimately providing a more comprehensive understanding of its role in insulinoma.

## Conclusion

Mi-Lnc70 plays an important role in the proliferation, migration, and endocrine-related gene expression in pancreatic MIN6 cells, particularly in the synthesis of PDX1, INSULIN, and GLUCAGON.

## Abbreviations

lncRNAs, long non-coding RNAs; CCK-8, cell counting kit-8; miRNAs, micro RNAs; PDX1, pancreatic and duodenal homeobox 1; EMT, epithelial-mesenchymal transition; PI3K/ AKT, phosphoinositide 3-kinase/protein kinase B; K-Ras, kirsten rat sarcoma virus; APC, adenomatous polyposis coli; ERK, extra-cellular signal regulated kinase; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; T2D, type 2 diabetes; NUTF2P3-001, nuclear transport factor 2 pseudogene 3-001; MALAT1, metastasis associated lung adenocarcinoma transcript 1; HOTTIP, HOXA distal transcript antisense RNA; HOXA, homebox A10; WDR5/MLL1, WD repeat-containing protein 5/mixed lineage leukemia 1; Sppl3, signal peptide peptidase-like 3; Hnf1a, hepatic nuclear factor 1A; B-Myb, Myb-related protein B; PDAC, pancreatic ductal adenocarcinoma; PAX4, paired box 4; PAX6, paired box 6; Nkx6.1, NK6 Homeobox 1; Isl-1, Islet-1; NeuroD1, neurogenic differentiation 1; MafA, MAF BZIP transcription factor A.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Data Sharing Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

## Consent for Publication

All authors have provided their consent for publication.

## Acknowledgments

We thank Dr. Xiubin Liang of the Department of Pathophysiology of Nanjing Medical University for supplying the MIN6 cells. This paper has been uploaded to China National Knowledge Infrastructure (CNKI) as a thesis: https://www.cnki.net/.

## Funding

This work was financially supported by the National Natural Science Foundation of China (81471001).

## Disclosure

The authors declared that there is no competing interest in this work.

## References

1. Fu J, Zheng H, Cui Q, et al. Nfe2l1-silenced insulinoma cells acquire aggressiveness and chemoresistance. *Endocrine-Related Cancer*. 2018;25(3):185–200. doi:10.1530/ERC-17-0458
2. Moran I, Akerman I, van de Bunt M, et al. Human beta cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. *Cell Metab*. 2012;16(4):435–448. doi:10.1016/j.cmet.2012.08.010
3. Yang W, Lyu Y, Xiang R, Yang J. Long noncoding RNAs in the pathogenesis of insulin resistance. *Int J Mol Sci*. 2022;23(24):16054. doi:10.3390/ijms232416054
4. Sathishkumar C, Prabu P, Mohan V, Balasubramanyam M. Linking a role of lncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes. *Human Genomics*. 2018;12(1):41. doi:10.1186/s40246-018-0173-3
5. Formichi C, Nigi L, Grieco GE, et al. Non-coding RNAs: novel players in insulin resistance and related diseases. *Int J Mol Sci*. 2021;22(14):7716. doi:10.3390/ijms22147716
6. Zhong G, Yang Q, Wang Y, Liang Y, Wang X, Zhao D. Long noncoding RNA X-inactive specific transcript (lncRNA XIST) inhibits hepatic insulin resistance by competitively binding microRNA-182-5p. *Immunity, Inflammation and Disease*. 2023;11(11):e969. doi:10.1002/iid3.969
7. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet*. 2014;15(1):7–21. doi:10.1038/nrg3606
8. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. *Cancer Res*. 2017;77(15):3965–3981. doi:10.1158/0008-5472.CAN-16-2634
9. Bartonicek N, Maag JL, Dinger ME. Long noncoding RNAs in cancer: mechanisms of action and technological advancements. *Mol Cancer*. 2016;15(1):43. doi:10.1186/s12943-016-0530-6
10. Kornfeld JW, Bruning JC. Regulation of metabolism by long, non-coding RNAs. *Front Genet*. 2014;5:57. doi:10.3389/fgene.2014.00057
11. Vitiello M, Tuccoli A, Poliseno L. Long non-coding RNAs in cancer: implications for personalized therapy. *Cell Oncology*. 2015;38(1):17–28. doi:10.1007/s13402-014-0180-x
12. Bermont F, Hermant A, Benninga R, et al. Targeting mitochondrial calcium uptake with the natural flavonol kaempferol, to promote metabolism/secretion coupling in pancreatic beta-cells. *Nutrients*. 2020;12(2):538. doi:10.3390/nu12020538
13. Cheng Y, Jutooru I, Chadalapaka G, Corton JC, Safe S. The long non-coding RNA HOTTIP enhances pancreatic cancer cell proliferation, survival and migration. *Oncotarget*. 2015;6(13):10840–10852. doi:10.18632/oncotarget.3450
14. Hoskins JW, Jia J, Flandez M, et al. Transcriptome analysis of pancreatic cancer reveals a tumor suppressor function for HNF1A. *Carcinogenesis*. 2014;35(12):2670–2678. doi:10.1093/carcin/bgu193
15. Kalisz M, Bernardo E, Beucher A, et al. HNF1A recruits KDM6A to activate differentiated acinar cell programs that suppress pancreatic cancer. *THE EMBO Journal*. 2020;39(9):e102808. doi:10.15252/embj.2019102808
16. Luo Z, Li Y, Wang H, et al. Hepatocyte nuclear factor 1A (HNF1A) as a possible tumor suppressor in pancreatic cancer. *PLoS One*. 2015;10(3):e0121082. doi:10.1371/journal.pone.0121082
17. Yu Y, Liang S, Zhou Y, Li S, Li Y, Liao W. HNF1A/CASC2 regulates pancreatic cancer cell proliferation through PTEN/Akt signaling. *Journal of Cellular Biochemistry*. 2019;120(3):2816–2827. doi:10.1002/jcb.26395
18. Gong Y, Dai HS, Shu JJ, Liu W, Bie P, Zhang LD. LNC00673 suppresses proliferation and metastasis of pancreatic cancer via target miR-504/HNF1A. *Journal of Cancer*. 2020;11(4):940–948. doi:10.7150/jca.32855
19. Qian MF, Bevacqua RJ, Coykendall VM, et al. HNF1α maintains pancreatic α and β cell functions in primary human islets. *JCI Insight*. 2023;8(24). doi:10.1172/jci.insight.170884.
20. Nhj N, Ghosh S, Bok CM, et al. HNF4A and HNF1A exhibit tissue specific target gene regulation in pancreatic beta cells and hepatocytes. *Nature Communications*. 2024;15(1):4288. doi:10.1038/s41467-024-48647-w

21. Miyachi Y, Miyazawa T, Ogawa Y. HNF1A mutations and beta cell dysfunction in diabetes. *Int J Mol Sci*. 2022;23(6):3222. doi:10.3390/ijms23063222
22. Chen Y, Jia J, Zhao Q, et al. Novel loss-of-function variant in HNF1a induces β-cell dysfunction through endoplasmic reticulum stress. *Int J Mol Sci*. 2022;23(21).
23. Nocera D, Menniti M, Belviso S, et al. Functional characterization of p.Pro409His variant in HNF1A, a hypomorphic mutation involved in pancreatic β-cell dysfunction. *Acta diabetologica*. 2019;56(8):883–888. doi:10.1007/s00592-019-01298-6
24. Zhang Y, Tang X, Shi M, Wen C, Shen B. MiR-216a decreases MALAT1 expression, induces G2/M arrest and apoptosis in pancreatic cancer cells. *Biochem Biophys Res Commun*. 2017;483(2):816–822. doi:10.1016/j.bbrc.2016.12.167
25. Hussen BM, Hidayat HJ, Salihi A, Sabir DK, Taheri M, Ghafouri-Fard S. MicroRNA: a signature for cancer progression. *Biomed Pharmacother*. 2021;138:111528. doi:10.1016/j.biopha.2021.111528
26. Kato M, Wang L, Putta S, et al. Post-transcriptional up-regulation of Tsc-22 by Ybx1, a target of miR-216a, mediates TGF-beta-induced collagen expression in kidney cells. *J Biol Chem*. 2010;285(44):34004–34015. doi:10.1074/jbc.M110.165027
27. Liu L, Chen Y, Li Q, Duan P. lncRNA HNF1A-AS1 modulates non-small cell lung cancer progression by targeting miR-149-5p/Cdk6. *Journal of Cellular Biochemistry*. 2019;120(11):18736–18750. doi:10.1002/jcb.29186
28. Liu HT, Ma RR, Lv BB, et al. LncRNA-HNF1A-AS1 functions as a competing endogenous RNA to activate PI3K/AKT signalling pathway by sponging miR-30b-3p in gastric cancer. *British Journal of Cancer*. 2020;122(12):1825–1836. doi:10.1038/s41416-020-0836-4
29. Guo X, Zhang Y, Liu L, Yang W, Zhang Q. HNF1A-AS1 regulates cell migration, invasion and glycolysis via modulating miR-124/MYO6 in colorectal cancer cells. *OncoTargets and Therapy*. 2020;13:1507–1518. doi:10.2147/OTT.S231249
30. Wu J, Li R, Li L, et al. MYC-activated lncRNA HNF1A-AS1 overexpression facilitates glioma progression via cooperating with miR-32-5p/SOX4 axis. *Cancer Medicine*. 2020;9(17):6387–6398. doi:10.1002/cam4.3186
31. Ma C, Wang H, Zong G, et al. EGR1 modulated LncRNA HNF1A-AS1 drives glioblastoma progression via miR-22-3p/ENO1 axis. *Cell Death Discovery*. 2021;7(1):350. doi:10.1038/s41420-021-00734-3
32. Bi Y, Mao Y, Su Z, Du J, Ye L, Xu F. Long noncoding RNA HNF1A-AS1 regulates proliferation and apoptosis of glioma through activation of the JNK signaling pathway via miR-363-3p/MAP2K4. *Journal of Cellular Physiology*. 2021;236(2):1068–1082. doi:10.1002/jcp.29916
33. Cesana M, Cacchiarelli D, Legnini I, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell*. 2011;147(2):358–369. doi:10.1016/j.cell.2011.09.028
34. Ndlovu MN, Van Lint C, Van Wesemael K, et al. Hyperactivated NF-kappaB and AP-1 transcription factors promote highly accessible chromatin and constitutive transcription across the interleukin-6 gene promoter in metastatic breast cancer cells. *Mol Cell Biol*. 2009;29(20):5488–5504. doi:10.1128/MCB.01657-08
35. Ding H, Wang F, Shi X, et al. LncRNA MALAT1 induces the dysfunction of β cells via reducing the histone acetylation of the PDX-1 promoter in type 1 diabetes. *Experimental and Molecular Pathology*. 2020;114:104432. doi:10.1016/j.yexmp.2020.104432
36. Xu X, Tian J, Li QY. Downregulation of HOTTIP regulates insulin secretion and cell cycle in islet β cells via inhibiting MEK/ERK pathway. *European Review for Medical and Pharmacological Sciences*. 2018;22(15):4962–4968. doi:10.26355/eurrev_201808_15636
37. Streicher SA, Lim U, Park SL, et al. Genome-wide association study of pancreatic fat: the multiethnic cohort adiposity phenotype study. *PLoS One*. 2021;16(7):e0249615. doi:10.1371/journal.pone.0249615
38. Akerman I, Tu Z, Beucher A, et al. Human pancreatic β cell lncRNAs control cell-specific regulatory networks. *Cell Metab*. 2017;25(2):400–411. doi:10.1016/j.cmet.2016.11.016