ORIGINAL RESEARCH

miR-942-5p Regulates Proliferation, Invasion and EMT of Trophoblast Cells in Gestational Diabetes by Targeting the CEBPA

Qiaoxiang Chu, BM; Xiaorong Zhong, BM; Yuemei Lu, MM; Yunzhao Xu, MD

ABSTRACT

Objective • Gestational diabetes mellitus (GDM) is a prevalent metabolic disorder caused by abnormal glucose metabolism during pregnancy. Trophoblast dysfunction induced by hyperglycemia during pregnancy is the main factor leading to the development of GDM. In this study, we evaluated the expression of miR-942-5p in the placenta of patients with GDM and its regulation of trophoblast cell biological function.

Methods • HTR-8/SVneo trophoblast cells were incubated with glucose to establish *in vitro* models, and miR-942-5p mimics transfected cells were added. The expression levels of miR-942-5p, CCAAT-enhancer-binding protein alpha (CEBPA) and N-cadherin in tissues and cells were detected by reverse-transcription quantitative polymerase chain reaction (RT-qPCR), protein blotting and immunohistochemistry (IHC). MTT, flow cytometry and Transwell assays were used to determine changes in cell proliferation, apoptosis and invasion. Dual-luciferase reporter assay and Pearson analysis were used to confirm the association between miR-942-5p and CEBPA.

Results • miR-942-5p and N-cadherin were decreased in placental tissue and in human placental trophoblast cells (HTR-8/SVneo) exposed to high glucose (HG) conditions, while CEBPA was increased in placental tissue and HTR-8/SVneo exposed to HG conditions. Elevated levels of miR-942-5p suppressed apoptosis induced by HG and facilitated the proliferative and invasive capacities of HTR-8/SVneo. Mechanistically, we confirmed that miR-942-5p overexpression directly targeted CEBPA and suppressed CEBPA expression, while upregulating N-cadherin expression, which is involved in the EMT process of trophoblast cells and alleviated the dysfunction of trophoblast cells induced by HG in GDM.

Conclusion • Overexpression of miR-942-5p promotes proliferation, invasion and EMT of trophoblast cells by targeting and negatively regulating CEBPA. These findings offer novel understanding regarding the treatment of GDM. (*Altern Ther Health Med.* [E-pub ahead of print.])

Qiaoxiang Chu, BM, Obstetrics and Gynecology; Affiliated Hospital of Nantong University; Nantong, Jiangsu; China; Obstetrics and Gynecology; Hai'an People's Hospital; Nantong, Jiangsu; China. **Xiaorong Zhong, BM; Yuemei Lu, MM;** Obstetrics and Gynecology; Hai'an People's Hospital; Nantong, Jiangsu; China. **Yunzhao Xu, MD,** Obstetrics and Gynecology; Affiliated Hospital of Nantong University; Nantong, Jiangsu; China.

Corresponding author: Yunzhao Xu, MD
E-mail: xuyz@ntu.edu.cn

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to metabolic disorders caused by abnormal glucose metabolism during pregnancy, and is one of the common complications of pregnancy.[1] According to statistics, approximately 15.8% of pregnant women worldwide develop GDM.[2] As an important organ shared by the fetus and the mother during pregnancy, the formation and implantation of the placenta mainly depends on differentiation and invasive capabilities.[3] When GDM occurs, high blood sugar induces abnormal proliferation or apoptosis of placenta trophoblast cells, impairing placental development and causing adverse outcomes such as fetal growth restriction, stillbirth and neonatal respiratory distress syndrome.[4,5] Dysfunction of trophoblast cell function has become one of the important pathogenic factors in GDM, but how it causes dysfunction during the progression of GDM remains unexplained.

Epithelial-mesenchymal transition (EMT) has already been determined to be widely involved in various cellular pathophysiological processes, including embryonic development, tissue repair and cancer metastasis.[6,7] More and more studies have shown that EMT is pivotal in the process of placenta formation. He, et al.[8] found that interleukin (IL)-23 treatment at a high concentration reduced the expression of N-cadherin, inhibiting EMT of trophoblast cells, and

revealing a new target for improving recurrent spontaneous abortion. Davies, et al.[9] also demonstrated that accelerating the occurrence of EMT is an important therapeutic strategy for suppressing the onset of preeclampsia during pregnancy. N-cadherin, a key regulatory factor for trophoblast cell behavior, manipulates the transformation of syncytiotrophoblast cells from layered phenotypes and gives it the ability to proliferate and invade, thereby promoting placental development.[10] EMT, by modulating its proliferative and invasive potential, has been identified as an essential physiological process in GDM.[11,12]

MicroRNA (miRNA) is a non-coding RNA and that is typically 18 to 25 nucleotides in length. Through the modulation of target gene expression,[13] it influences the onset of diseases. MiR-942-5p is a newly discovered miRNA that participates in the progression of various diseases, including sepsis,[14] colorectal cancer[15] and melanoma,[16] by regulating cell proliferation, apoptosis, differentiation and metabolism. In the past decade or so, miRNA-942-5p pregnancy-related complications have also been reported. Zhang, et al.[17] found that, in the development of preeclampsia, miR-942-5p is reduced and its low expression inhibits the vitality of extravillous trophoblast (EVT) cells and human umbilical vein endothelial cell (HUVEC) angiogenesis. Ren, et al.[18] also showed that miR-942-5p is repressed in preeclampsia tissues, and overexpression of miR-942-5p enhances cell proliferation and suppresses cell apoptosis in HTR-8/SVneo. Dong, et al.[19] found that circFBXW7 could inhibit the EMT process of lung adenocarcinoma via sponge miR-942-5p. However, it still remains unclear whether or not miR-942-5p is related to GDM by regulating the biological behavior of placental trophoblast cells.

CCAAT-enhancer-binding protein alpha (CEBPA) is a member of the leucine zipper transcription factor family.[20] As an important transcriptional regulator, CEBPA is widely present in various body tissues and widely participates in cell proliferation, apoptosis, metabolism, differentiation, oncogene induction and tumor development.[21] Jiang, et al.[22] discovered that CEBPA can suppress the proliferation of mouse myeloid cells and simultaneously promote cell apoptosis. Huan, et al.[23] also found that CEBPA induces biological dysfunction in liver cancer cells by inhibiting EMT. Specifically, a 2009 report listed CEBPA as a target gene involved in the cellular function of GDM.[24] In this study, we hypothesized that CEBPA may participate in the progression of GDM by modulating the biological activities of trophoblast cells.

In summary, the aim of this study was to research the roles of miR-942-5p and CEBPA in GDM, their mutual relationship, investigate how the miR-942-5p/CEBPA axis works on trophoblast cell biological function and to establish a solid theoretical foundation for understanding the pathogenesis of GDM.

MATERIALS AND METHODS

Research Object and Specimen Collection

We selected 45 placental tissue specimens from caesarean section deliveries of pregnant women at Hai'an People's Hospital in China. Of these, 15 women were included in the GDM group; aged 23 to 35 years, average age 29.33 years; 30 normal pregnant women were included in the control group: aged 23 to 41 years, average age 30.22 years.

Table 1. Clinicopathological Factors in Healthy Pregnancies and Patients with Gestational Diabetes Mellitus

Characteristics	Healthy (n = 30)	GDM (n = 15)
Age (years)	30.22	29.33
BMI (kg/m^2)	21.68	24.10
Gestation (wks)	40	39
Fasting plasma glucose (mmol/l)	4.68	5.20
1-hour plasma glucose (mmol/l)	8.50	9.74
2-hour plasma glucose (mmol/l)	7.44	8.34
Fetal birth weight (grams)	3508.33	3503.33

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus

GDM group

Inclusion criteria for patients in the GDM group were hypertension and proteinuria occurring after 20 weeks of pregnancy; exclusion criteria were patients with diabetes and heart disease.

Normal group

The inclusion criteria for normal pregnant women were delivery after 38 weeks of pregnancy, normal blood pressure, negative urine protein and no pregnancy complications.

Prior to conducting this study, approval was obtained from the ethics committee of the hospital, and all participants signed consent forms (see Table 1).

Ethical Compliance

This study was approved by the ethics committee of the Affiliated Hospital of Nantong University. Signed written informed consent was obtained from all patients and/or guardians.

Cell culture and transfection

Human placental trophoblast cells HTR-8/SVneo and 293T were purchased from Pricella (Wuhan, China). Cells were resuscitated according to the manufacturer's instructions and then transferred to a complete culture medium and maintained in a humidified incubator at a temperature of 37°C with a 5% CO_2 atmosphere. When they reached the logarithmic phase of growth, they were divided into the *in vitro* model group and the control group with 25 mM and 5 mM glucose incubation, respectively.[25] Lipofectamine 8 was used to transfect HTR-8/SVneo in HG medium with miR-942-5p mimics and negative control (NC) mimics (Hunan Fenghui Biology, Changsha, China), and the transfected cells were used for subsequent experiments after 48 hours. All experiments were conducted independently 3 times.

Reverse-transcription quantitative polymerase chain reaction

The total RNA extracted from the tissues and cells of patients from each group was collected and subjected to

reverse transcription following the instructions provided by the TaKaRa kit. (TaKaRa, RR047A, Tokyo, Japan), and then RT-qPCR reaction was performed. The expression levels of miR-942-5p, CEBPA and N-cadherin were calculated using the 2-delta delta Ct ($2^{-\Delta\Delta CT}$) formula[26] (see Table 2).

Table 2. Quantitative Polymerase Chain Reaction Primers

Gene	Sequences (5'-3')	
miR-942-5p	F	CTTCTCTGTTTTGGCCATGTG
	R	CTCTACAGCTATATTGCCAGCCAC
U6	F	CGCTTCGGCAGCACATATAC
	R	AAATATGGAACGCTTCACGA
CEBPA	F	GAGTGGCGGCAGCGGC
	R	CAGTTCGCGGCTCAGCTGTT
N-cad	F	GACAATGCCCCTCAAGTGTT
	R	CCATTAAGCCGAGTGATGGT
GAPDH	F	GTGGTCTCCTCTGACTTCAA
	R	TCTCTTCCTCTTGTGCTCTTG

Western blotting

We collected embryonic tissues and HTR8-SVneo cells from the groups and extracted and measured protein concentration using the BCA assay kit (P0010; Beyotime, Shanghai, China). Proteins were diluted with protein buffer, separated via protein electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with skim milk for a period of 2 hours, the samples were incubated with primary antibodies for CEBPA (ab40761; Abcam, Cambridge, Massachusetts USA), N-cadherin (ab76011; Abcam), and β-actin (ab8227; Abcam) overnight at 4°C. After washing with tris buffered saline with tween (TBST), the samples were incubated with secondary antibodies for 2 hours at 4°C, and then enhanced chemiluminescence (ECL) substrate was added (180-5001; Tanon, Shanghai, China) for visualization. Gel images were captured with a gel imaging system, and β-actin was used as an internal reference.

Prediction of target genes and dual luciferase assays

The TargetScan database was used to predict the binding site of miR-942-5p and CEBPA, and the fragment was then amplified. Subsequently, wild-type plasmid pmirGLO-WT-CEBPA and mutant plasmid pmirGLO-MUT-CEBPA were constructed separately. The 2 constructed plasmids, along with miR-942-5p mimics and NC mimics, were then transfected together into HEK-293T cells. After 48 hours, the luciferase activity of each group of cell lysates was determined.

MTT assays

Each well was seeded with 5×10^3 single cells in a 96-well plate, and after being cultured for 12, 24 and 48 hours, 20 μL of MTT (Beyotime, C0009S, Shanghai, China) at a concentration of 5 mg/mL was added to the sample and incubated for 4 hours. Then, 150 μL of dimethyl sulfoxide (DMSO) was added and the sample was shaken horizontally for 30 minutes, and the optical density (OD) of each well at 490 nm was measured with a microplate photometer (Multiskan; Thermo Fisher Scientific, Waltham, Massachusetts USA).

Flow cytometry

The instructions for the Annexin FITC/propidium iodide (PI) cell apoptosis detection kit (40302ES20; Yeasen, Shanghai, China) were strictly followed. Each group of HTR8-SVneo cells was mixed with an AnnexinV/FITC and binding buffer mixed solution at a dilution ratio of 1:40 and incubated for 30 minutes, then dissolved in a mixture of PI and binding buffer at a ratio of 1:40 and incubated in liquid for 15 minutes. Flow cytometry (Invitrogen, Carlsbad, California USA) was used to measure the apoptotic cell rate.

Invasion assays

Transwell chambers were coated with a layer of extracellular matrix gel (356234 BD; Biocoat, Franklin Lakes, New Jersey USA). The upper chamber was filled with serum-free RPMI 1640 medium containing a single-cell suspension, while the lower chamber was filled with medium containing 10% fetal bovine serum. After fixation with formaldehyde, the cells were stained with crystal violet (G1062; Solonbao, Beijing, China), and the transmembrane cells were observed and counted using an inverted microscope (Olympus CKX31; Tokyo, Japan).

Statistical analysis

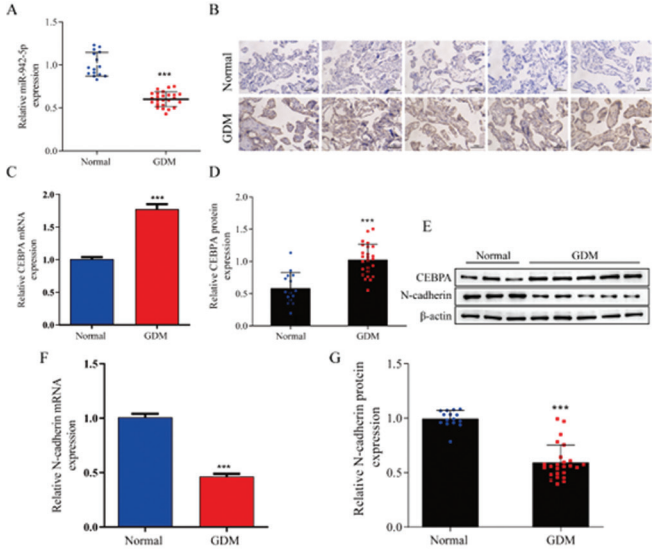
Statistical analysis was performed of the relevant data using GraphPad Prism 8 software (GraphPad, La Jolla, California USA). Statistical analysis was performed using t tests and one-way ANOVA to compare the differences between the 2 groups. A significance level of $P < .05$ was considered statistically significant.

RESULTS

miR-942-5p and N-cadherin Are Downregulated, While CEBPA is Upregulated in the Placental Tissue of Patients with GDM

The dysregulated expression of genes in the placenta, a key organ in the pathogenesis of GDM, is closely associated with the occurrence and development of GDM.[27] In this study, placental tissue from 30 normal pregnant women and 15 patients diagnosed with GDM were collected and analyzed. Compared with normal placental tissue, miR-942-5p expression was reduced in the placenta of patients with GDM (Figure 1A; $P < .001$). IHC staining results were shown in Figure 1B. Compared with normal placental tissue, a darker brown-yellow color was observed in the tissues of the GDM group, and there was more CEBPA-positive expression in the placental tissue of patients with GDM. In addition, RT-qPCR and protein blotting experiments indicated that CEBPA mRNA and expression of protein were notably increased in GDM placental tissue compared with normal placental tissue (Figure 1C-1E; $P < .001$). Furthermore, RT-qPCR and protein blotting experiments also indicated that N-cadherin mRNA and protein expression were manifestly decreased in GDM placental tissue compared with normal placental tissue (Figure 1E-1G; $P < .001$). These results emphasize that the low miR-942-5p and N-cadherin expression and high CEBPA expression in the placental tissue of patients with GDM may contribute to GDM progression.

Figure 1. miR-942-5p and N-cadherin are downregulated, while CEBPA is upregulated, in the placental tissue of patients with GDM. Placental tissue was collected and analyzed from 30 normal pregnant women and 15 patients with GDM. (**1A**) RT-qPCR was used to evaluate miR-942-5p in placental tissue; (**1B**) IHC staining was used to evaluate CEBPA protein in placental tissue; (**1C**) RT-qPCR was used to evaluate CEBPA mRNA in placental tissue; (**1D-1F**) Western blot detection of CEBPA and N-cadherin protein expression in placental tissue; (**1G**) RT-qPCR detection of N-cadherin mRNA expression in placental tissue. ***$P < .001$.



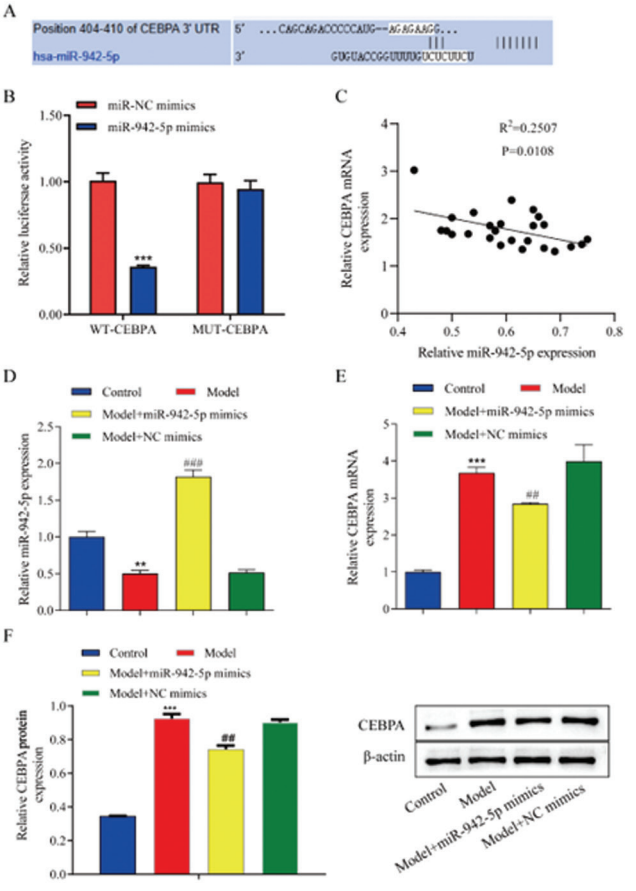
Abbreviations: CEBPA, CCAAT-enhancer-binding protein alpha; GDM, gestational diabetes mellitus; IHC, immunohistochemistry; RT-qPCR, reverse-transcription quantitative polymerase chain reaction.

CEBPA is a Direct Target of miR-942-5p

CEBPA, as an important regulatory factor in placental development, can serve as a downstream target gene for miRNA,[28] as it participates in the regulation of trophoblast cell's biological function.[29] In our study, the binding sites of miR-942-5p in 3'UTR of CEBPA were detected using TargetScan (Figure 2A). To verify the prediction results, WT-CEBPA or mut-CEBPA double luciferase report plasmid was used for luciferase Reporter gene determination (Figure 2B). miR-942-5p mimics reduced the luciferase activity of pmirGLO-WT-CEBPA ($P < .001$), but there was no remarkable change in pmirGLO-MUT-CEBPA. Meanwhile, A negative correlation was observed between the expression levels of miR-942-5p and CEBPA, as indicated by Pearson correlation analysis ($P = .0108$) (Figure 2C).

Therefore, we speculated that miR-942-5p negatively regulates CEBPA and affects the biological function of trophoblast cells. To test this hypothesis, we transfected miR-942-5p NC mimics into high glucose (HG)-induced HRT-8/SVneo. RT-qPCR was evaluated with the transfection efficiency of miR-942-5p mimics (Figure 2D). miR-942-5p mimics significantly promoted the expression of miR-942-5p in the model group ($P < .001$), while NC mimics showed no significant difference in the expression of miR-942-5p. Using

Figure 2. CEBPA is a direct target of miR-942-5p. We co-transfected miR-NC mimics and miR-942-5p mimics with pmirGLO-WT-CEBPA and pmirGLO-MUT-CEBPA into 293T cells, respectively; we transfected miR-942-5p mimics and NC mimics into HG-induced HRT-8/SVneo cells. (**2A**) TargetScan predicted the binding site between miR-942-5p and CEBPA; (**2B**) Detected the expression level of luciferase activity in 293T cells; (**2C**) Pearson correlation analysis was conducted to examine the correlation between miR-942-5p and CEBPA. (**2D**) RT-qPCR detection of miR-942-5p transfection efficiency; (**2E**) RT-qPCR detection of the impact of miR-942-5p mimics transfection on the expression of CEBPA mRNA in trophoblast cells; (**2F**) protein blotting detected the influence of miR-942-5p mimics transfection on the expression of CEBPA protein in trophoblast cells. The data is represented as mean ± SD (n=3); (***$P < .001$, **$P < .01$ vs Control Group, miR-NC mimics group; ###$P < .001$, ##$P < .01$, vs Model Group)



Abbreviations: CEBPA, CCAAT-enhancer-binding protein alpha; HG, high glucose; NC, negative control; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; SD, standard deviation.

RT-qPCR and Western blot analysis (Figures 2E-2F), miR-942-5p mimics obviously restrained the expression of CEBPA in HRT-8/SVneo in the model group ($P < .01$), while NC mimics showed no significant difference in CEBPA expression. These results indicate that miR-942-5p may directly target the negative regulation of CEBPA expression and have an impact on the biological function of trophoblast cells.

miR-942-5p Activates the EMT Process in Trophoblast Cells

The EMT process of trophoblast cells plays a crucial part in embryonic development, and the epithelial marker N-cadherin is a key regulatory factor for the proliferative and invasive capacities of trophoblast cells.[9] Analysis of RT-qPCR and protein blotting are shown in Figures 3A-3B**.** MiR-942-5p mimics obviously promoted the expression of N-cadherin in the model group cells ($P < .05$), while NC mimics showed no obvious change in the expression of N-cadherin. These findings demonstrate that miR-942-5p promotes the EMT process in trophoblast cells by promoting the expression of N-cadherin.

miR-942-5p Suppresses Trophoblast Cell Apoptosis While Enhancing Proliferation and Invasion in Trophoblast Cells

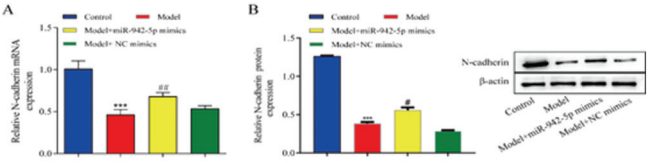
Next, we studied the effect of miR-942-5p on the apoptosis, proliferation and invasion ability of trophoblast cells. First, by detecting cell apoptosis with flow cytometry (Figure 4A), we found that miR-942-5p mimics significantly inhibited HG-induced apoptosis of HRT-8/SVneo ($P < .001$), while NC mimics showed no notable difference in the effect of apoptosis on HRT-8/SVneo. Subsequently, we used MTT and Transwell to measure cell proliferation and invasion, respectively; the results are shown in Figures 4B-4C. MiR-942-5p mimics significantly enhanced the HG-induced proliferative and invasive capacities of HRT-8/SVneo ($P < .05$), while NC mimics had no notable effect. These findings emphasize that miR-942-5p can inhibit trophoblast cell apoptosis and promote trophoblast cell proliferation and invasion.

DISCUSSION

During pregnancy, the hyperglycemia-induced abnormal proliferation and invasion of the trophoblast layer plays a crucial role in GDM. Therefore, studying the mechanisms of trophoblast cell proliferation and invasion and finding new effective molecular targets are of great importance in treating GDM. Increasing evidence suggests that miRNA expression is abnormal in placental tissue from patients with GDM, providing a basis for studying GDM occurrence.[30,31]

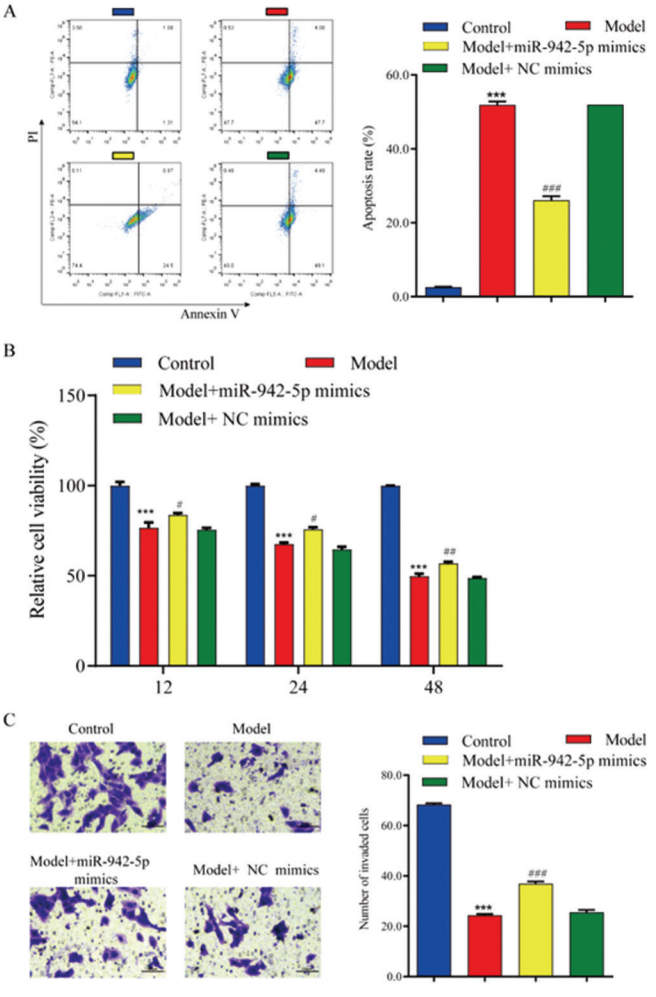
MiRNA is a regulatory, non-coding small RNA. It can bind to the 3'UTR of specific mRNA, causing rapid degradation and low gene expression.[32] The placenta is a pivotal organ in GDM, and the ectopic expression of miRNA is closely associated with the onset of GDM. Specifically, miRNA is involved in GDM as it regulates the biological function of trophoblast cells. Studies have reported that overexpressed miR-1323 in GDM negatively regulates TP53INP1, inducing dysfunction of trophoblast cells.[33] Downregulated miRNA-29b in GDM regulates trophoblast migration and invasion through an HIF3A-dependent mechanism and changes placental development.[34] miR-942-5p, as a newly discovered miRNA, is involved in many diseases due to its regulation of cell biological function.[35,36] miR-942-5p has been proven to serve as a regulator in cell invasion and migration. By targeting DKK3, it promotes proliferation and invasion of human melanoma cells,[16] and

Figure 3. MiR-942-5p activated the EMT process in trophoblast cells. (**3A**) RT-qPCR was used to detect the expression of N-cadherin mRNA in placental tissue; (**3B**) Protein blotting was used to detect the expression level of N-cadherin protein. The data is represented as mean ± SD (n=3); (***$P < .001$, vs Control Group, ##$P < .01$, #$P < .05$, vs Model Group).



Abbreviations: EMT, epithelial to mesenchymal transition; SD, standard deviation.

Figure 4. miR-942-5p inhibits trophoblast cell apoptosis and enhances proliferation and invasion in trophoblast cells. (**4A**) The effect of flow cytometry on trophoblast cell apoptosis; (**4B**) MTT analysis trophoblast cell proliferation at 12h, 24h, and 48h; (**4C**) Transwell detected trophoblast cell invasion ability. The data is represented as mean ± SD (n=3)' ***$P < .001$; vs Control Group, ###$P < .001$, ##$P < .01$, #$P < .05$, vs Model Group).



Abbreviations: SD, standard deviation.

by promoting proliferation, migration and invasion of cervical cancer cells, leads to exacerbation of cervical cancer.[37] In this study, we found that the expression of miR-942-5p was low in the placenta of patients with GDM. In addition, we transfected miR-942-5p mimics into HG-induced trophoblast cells and found that overexpression of miR-942-5p enhanced the proliferative and invasive capacities of trophoblast cells, alleviating the progression of GDM.

Transcription factor CEBPA regulates terminal differentiation and cell proliferation in many tissues. Increasing evidence suggests that CEBPA is an important regulatory factor in trophoblasts, affecting placental formation and fetal development by regulating trophoblast cell biology.[38,39] In this study, we found high expression of CEBPA in the placenta of patients with GDM. In addition, CEBPA can be regulated as a downstream target gene by miRNA.[40,41] We speculated that CEBPA may be regulated as a downstream target gene of miR-942-5p. According to bioinformatics analysis and dual-luciferase reporter assay, we confirmed the binding relationship between miR-942-5p and CEBPA. Specifically, miR-942-5p reduced the expression of CEBPA by combining with the 3'UTR of CEBPA, and we confirmed via Pearson analysis that miR-942-5p regulates CEBPA expression negatively in GDM. Given the importance of miR-942-5p and CEBPA in GDM pathogenesis, we explored their roles in regulating trophoblast cell function and attempted to determine their effects on GDM pathogenesis. Our results showed that overexpression of miR-942-5p leads to a decrease in the expression of CEBPA in HRT-8/SVneo cells, thereby enhancing cell proliferation and invasion.

Epithelial to mesenchymal transition (EMT) is a significant biological process involving intricate molecular events and signaling pathways that occur in epithelial cells, epigenetic regulation and other mechanisms to transform into cells with mesenchymal phenotypes.[42] Characterized by the loss of epithelial phenotypes and the acquisition of mesenchymal phenotypes, it plays a key part in regulating trophoblast cell proliferation and invasion in GDM.[11] Increasing evidence suggests that the epithelial marker N-cadherin is the key regulatory factor for trophoblast cell proliferation and invasion. Its dysregulation-induced trophoblast cell dysfunction is closely associated with the development and occurrence of pregnancy-related complications.[43,44] In this study, we consistently found low expression of N-cadherin in the placental tissue of patients with GDM. In particular, the occurrence of EMT is also controlled by miRNA regulatory factors, which maintain the necessary epithelial homeostasis by inhibiting critical mesenchymal markers (including N-cadherin), thus preventing EMT-mediated malignant tumors such as pancreatic and colorectal cancer.[45-47] In our study, we found that miR-942-5p mimics promoted the EMT process of HRT-8/SVneo via upregulation of N-cadherin expression induced by HG, enhancing the proliferation and invasion ability of trophoblast cells.

In conclusion, the overexpression of miR-942-5p has been found to be associated with promotion of the EMT process in trophoblast cells via its negative regulation of CEBPA, enhancing the proliferative and invasive capacities of trophoblast cells and alleviating CDM progress. of x. In this study, the effects of miR-942-5p and CEBPA on GDM were examined at both the tissue and cellular levels, fully demonstrating the role of overexpressed miR-942-5p in preventing and treating GDM.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Affiliated Hospital of Nantong University.

CONFLICT OF INTEREST

None.

FUNDING

None.

REFERENCES

1. Fiskin G, Sahin N; Nonpharmacological Management of Gestational Diabetes Mellitus: Diaphragmatic Breathing Exercise. *Altern Ther Health M*. 2021;27:90-96.
2. Yuen L, Saeedi P, Riaz M, et al. Projections of the prevalence of hyperglycaemia in pregnancy in 2019 and beyond: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pr*. 2019;157:107841. doi:10.1016/j.diabres.2019.107841
3. Zhang C, Wang L, Chen J, Song F, Guo Y. Differential Expression of miR-136 in Gestational Diabetes Mellitus Mediates the High-Glucose-Induced Trophoblast Cell Injury through Targeting E2F1. *Int J Genomics*. 2020;2020:3645371. doi:10.1155/2020/3645371
4. Ke W, Chen Y, Zheng L, Zhang Y, Wu Y, Li L. miR-134-5p promotes inflammation and apoptosis of trophoblast cells via regulating FOXP2 transcription in gestational diabetes mellitus. *Bioengineered*. 2022;13(1):319-330. doi:10.1080/21655979.2021.2001219
5. Peng HY, Li MQ, Li HP. High glucose suppresses the viability and proliferation of HTR8/SVneo cells through regulation of the miR137/PRKAA1/IL6 axis. *Int J Mol Med*. 2018;42(2):799-810. doi:10.3892/ijmm.2018.3686
6. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428. doi:10.1172/JCI39104
7. Bakir B, Chiarella AM, Pitarresi JR, Rustgi AK. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol*. 2020;30(10):764-776. doi:10.1016/j.tcb.2020.07.003
8. He S, Ning Y, Ma F, Liu D, Jiang S, Deng S. IL-23 Inhibits Trophoblast Proliferation, Migration, and EMT via Activating p38 MAPK Signaling Pathway to Promote Recurrent Spontaneous Abortion. *J Microbiol Biotechnol*. 2022;32(6):792-799. doi:10.4014/jmb.2112.12056
9. E Davies J, Pollheimer J, Yong HE, et al. Epithelial-mesenchymal transition during extravillous trophoblast differentiation. *Cell Adh Migr*. 2016;10(3):310-321. doi:10.1080/19336918.2016.1170258
10. Kokkinos MI, Murthi P, Wafai R, Thompson EW, Newgreen DF. Cadherins in the human placenta--epithelial-mesenchymal transition (EMT) and placental development. *Placenta*. 2010;31(9):747-755. doi:10.1016/j.placenta.2010.06.017
11. Lai R, Ji L, Zhang X, et al. Stanniocalcin2 inhibits the epithelial-mesenchymal transition and invasion of trophoblasts via activation of autophagy under high-glucose conditions. *Mol Cell Endocrinol*. 2022;547:111598. doi:10.1016/j.mce.2022.111598
12. Liu X, Wang J, Fu X, et al. Thin endometrium is associated with the risk of hypertensive disorders of pregnancy in fresh IVF/ICSI embryo transfer cycles: a retrospective cohort study of 9,266 singleton births. *Reprod Biol Endocrinol*. 2021;19(1):55. doi:10.1186/s12958-021-00738-9
13. Zhu W, Shen Y, Liu J, et al. Epigenetic alternations of microRNAs and DNA methylation contribute to gestational diabetes mellitus. *J Cell Mol Med*. 2020;24(23):13899-13912. doi:10.1111/jcmm.15984
14. Lu Q, Zhang D, Liu H, Xu H. miR-942-5p prevents sepsis-induced acute lung injury via targeting TRIM37. *Int J Exp Pathol*. 2021;102(4-5):192-199. doi:10.1111/iep.12413
15. Zhou L, Chen Q, Wu J, et al. miR-942-5p Inhibits Proliferation, Metastasis, and Epithelial-Mesenchymal Transition in Colorectal Cancer by Targeting CCBE1. *BioMed Res Int*. 2021;2021:9951405. doi:10.1155/2021/9951405
16. Zhang W, Mao K, Liu S, Xu Y, Ren J. miR-942-5p promotes the proliferation and invasion of human melanoma cells by targeting DKK3. *J Recept Signal Transduct Res*. 2021;41(2):180-187. doi:10.1080/10799893.2020.1804280
17. Zhang Y, Huang G, Zhang Y, et al. MiR-942 decreased before 20 weeks gestation in women with preeclampsia and was associated with the pathophysiology of preeclampsia in vitro. *Clin Exp Hypertens*. 2017;39(2):108-113. doi:10.1080/10641963.2016.1210619
18. Ren J, Cai J. circ_0014736 induces GPR4 to regulate the biological behaviors of human placental trophoblast cells through miR-942-5p in preeclampsia. *Open Med (Wars)*. 2023;18(1):20230645. doi:10.1515/med-2023-0645
19. Dong Y, Qiu T, Xuan Y, et al. circFBXW7 attenuates malignant progression in lung adenocarcinoma by sponging miR-942-5p. *Transl Lung Cancer Res*. 2021;10(3):1457-1473. doi:10.21037/tlcr-21-230
20. Zahnow CA. CCAAT/enhancer-binding protein beta: its role in breast cancer and associations with receptor tyrosine kinases. *Expert Rev Mol Med*. 2009;11:e12. doi:10.1017/S1462399409001033
21. Renfro Z, White BE, Stephens KE. CCAAT enhancer binding protein gamma (C/EBP-γ): an understudied transcription factor. *Adv Biol Regul*. 2022;84:100861. doi:10.1016/j.jbior.2022.100861
22. Jiang J, Gao Q, Gong Y, et al. MiR-486 promotes proliferation and suppresses apoptosis in myeloid cells by targeting Cebpa in vitro. *Cancer Med*. 2018;7(9):4627-4638. doi:10.1002/cam4.1694
23. Huan H, Wen X, Chen X, et al. C/EBPα Short-Activating RNA Suppresses Metastasis of Hepatocellular Carcinoma through Inhibiting EGFR/β-Catenin Signaling Mediated EMT. *PLoS One*. 2016;11(4):e0153117. doi:10.1371/journal.pone.0153117
24. Enquobahrie DA, Williams MA, Qiu C, Meller M, Sorensen TK. Global placental gene expression in gestational diabetes mellitus. *Am J Obstet Gynecol*. 2009;200(2):206.e1-206.e13. doi:10.1016/j.ajog.2008.08.022
25. Zhang L, Zeng M, Tang F, Chen J, Cao D, Tang ZN. Circ-PNPT1 contributes to gestational diabetes mellitus (GDM) by regulating the function of trophoblast cells through miR-889-3p/PAK1 axis. *Diabetol Metab Syndr*. 2021;13(1):58. doi:10.1186/s13098-021-00678-9
26. Xu Y, Wang W, Chen J, et al. High neuropilin and tolloid-like 1 expression associated with metastasis and poor survival in epithelial ovarian cancer via regulation of actin cytoskeleton. *J Cell Mol Med*. 2020;24(16):9114-9124. doi:10.1111/jcmm.15547

27. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015;36(7):709-715. doi:10.1016/j.placenta.2015.04.006
28. Liu A, Pan W, Zhuang S, Tang Y, Zhang H. Cancer cell-derived exosomal miR-425-3p induces white adipocyte atrophy. *Adipocyte*. 2022;11(1):487-500. doi:10.1080/21623945.2022.2108558
29. Zhu G, Fei T, Li Z, Yan X, Chen YG. Activin Regulates Self-renewal and Differentiation of Trophoblast Stem Cells by Down-regulating the X Chromosome Gene Bcor. *J Biol Chem*. 2015;290(36):22019-22029. doi:10.1074/jbc.M115.674127
30. Chen X, Huang J, Peng Y, Han Y, Wang X, Tu C. The role of circRNA polyribonucleotide nucleoside transferase 1 on Gestational Diabetes Mellitus. *Cell Mol Biol (Noisy-le-grand)*. 2022;68(6):148-154. doi:10.14715/cmb/2022.68.6.24
31. Iljas JD, Guanzon D, Elfeky O, Rice GE, Salomon C. Review: bio-compartmentalization of microRNAs in exosomes during gestational diabetes mellitus. *Placenta*. 2017;54:76-82. doi:10.1016/j.placenta.2016.12.002
32. Rykova E, Ershov N, Damarov I, Merkulova T. SNPs in 3'UTR miRNA Target Sequences Associated with Individual Drug Susceptibility. *Int J Mol Sci*. 2022;23(22):13725. doi:10.3390/ijms232213725
33. Liu L, Zhang J, Liu Y. MicroRNA-1323 serves as a biomarker in gestational diabetes mellitus and aggravates high glucose-induced inhibition of trophoblast cell viability by suppressing TP53INP1. *Exp Ther Med*. 2021;21(3):230. doi:10.3892/etm.2021.9661
34. Sun DG, Tian S, Zhang L, et al. The *miRNA-29b* Is Downregulated in Placenta During Gestational Diabetes Mellitus and May Alter Placenta Development by Regulating Trophoblast Migration and Invasion Through a *HIF3A*-Dependent Mechanism. *Front Endocrinol (Lausanne)*. 2020;11:169. doi:10.3389/fendo.2020.00169
35. Wang J, Jiang C, Li N, et al. The circEPSTI1/mir-942-5p/LTBP2 axis regulates the progression of OSCC in the background of OSF via EMT and the PI3K/Akt/mTOR pathway. *Cell Death Dis*. 2020;11(8):682. doi:10.1038/s41419-020-02851-w
36. Luo N, Gao HM, Wang YQ, Li HJ, Li Y. MiR-942-5p alleviates septic acute kidney injury by targeting FOXO3. *Eur Rev Med Pharmacol Sci*. 2020;24(11):6237-6244. doi:10.26355/eurrev_202006_21521
37. Yuan DD, Jia CD, Yan MY, Wang J. Circular RNA hsa_circ_0000730 restrains cell proliferation, migration, and invasion in cervical cancer through miR-942-5p/PTEN axis. *Kaohsiung J Med Sci*. 2021;37(11):964-972. doi:10.1002/kjm2.12443
38. Bégay V, Smink J, Leutz A. Essential requirement of CCAAT/enhancer binding proteins in embryogenesis. *Mol Cell Biol*. 2004;24(22):9744-9751. doi:10.1128/MCB.24.22.9744-9751.2004
39. Ding DC, Chu TY, Liu HW. Dedifferentiation of human uterine polyp stem cells into embryo-like cells during inducing pluripotency. *Int J Biol Sci*. 2018;14(11):1586-1598. doi:10.7150/ijbs.23401
40. Wang C, Ren R, Hu H, et al. MiR-182 is up-regulated and targeting Cebpa in hepatocellular carcinoma. *Chin J Cancer Res*. 2014;26(1):17-29. doi:10.3978/j.issn.1000-9604.2014.01.01
41. Liang WC, Wang Y, Liang PP, et al. MiR-25 suppresses 3T3-L1 adipogenesis by directly targeting KLF4 and C/EBPα. *J Cell Biochem*. 2015;116(11):2658-2666. doi:10.1002/jcb.25214
42. Savagner P. Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity. *Curr Top Dev Biol*. 2015;112:273-300. doi:10.1016/bs.ctdb.2014.11.021
43. Li M, Zhang H, Kong Q. Long non-coding RNA IGF2-AS promotes trophoblast cell proliferation, migration, and invasion by regulating miR-520g/N-cadherin axis. *J Obstet Gynaecol Res*. 2021;47(9):3047-3059. doi:10.1111/jog.14886
44. Wu D, Shi L, Hong L, Chen X, Cen H. MiR-135a-5p promotes the migration and invasion of trophoblast cells in preeclampsia by targeting β-TrCP. *Placenta*. 2020;99:63-69. doi:10.1016/j.placenta.2020.07.028
45. Wong CH, Lou UK, Fung FK, et al. CircRTN4 promotes pancreatic cancer progression through a novel CircRNA-miRNA-lncRNA pathway and stabilizing epithelial-mesenchymal transition protein. *Mol Cancer*. 2022;21(1):10. doi:10.1186/s12943-021-01481-w
46. Jaca A, Govender P, Locketz M, Naidoo R. The role of miRNA-21 and epithelial mesenchymal transition (EMT) process in colorectal cancer. *J Clin Pathol*. 2017;70(4):331-356. doi:10.1136/jclinpath-2016-204031
47. Wang W, Wu Q, Wang Z, et al. Development of a Prognostic Model for Ovarian Cancer Patients Based on Novel Immune Microenvironment Related Genes. *Front Oncol*. 2021;11:647273. doi:10.3389/fonc.2021.647273