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ORIGINAL RESEARCH

LncRNA HCP5 is Highly Expressed in Gestational Diabetes Mellitus to Suppress Insulin Secretion

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Purpose: LncRNA HCP5 has been reported to participate in high glucose-induced pathological processes, whereas its role in gestational diabetes mellitus (GDM) is unclear. This study aimed to explore the role of HCP5 in GDM.

Methods: This study enrolled a total of 220 pregnant women (gestational age = 1 month). A follow-up study was performed until delivery. The occurrence of GDM was checked every month during follow-up. Plasma samples were collected from all participants and expression of HCP5 was determined with RT-qPCR. The 220 patients were divided into high and low GDM groups, and GDM-free curves were plotted for both groups and compared. The ROC curve was plotted to explore the predictive value of plasma HCP5 on the day of admission for GDM. INS-1 cells were transfected with HCP5 expression vector or siRNA, and cell viability under high glucose was determined by the MTT assay. An ELISA was applied to determine insulin levels in the cell culture medium.

Results: During follow-up, the level of HCP5 was increased during pregnancy and the high HCP5 level group showed a significantly higher incidence of GDM. Plasma levels of HCP5 on the day of admission effectively separated GDM patients from healthy controls. HCP5 negatively regulated cell viability and insulin secretion under high glucose treatment.

Conclusion: HCP5 may act as a predictor for GDM, and it negatively regulated INS-1 cell viability and insulin secretion under high glucose conditions.

Keywords: HCP5, gestational diabetes mellitus, INS-1 cells, insulin

Introduction

Gestational diabetes mellitus (GDM) is a type of diabetes caused by glucose intolerance in pregnant women with no previous history of diabetes.[1,2] Differently from type 1 diabetes, which is caused by the insufficient production of insulin, the mechanism of GDM is unclear. Without proper treatment, GDM may cause a series of adverse events, such as early birth, stillbirth, and breathing difficulties.[3,4] GDM can also increase the long-term risk of cardiovascular diseases in mothers.[5] GDM can be treated with insulin injection or by maintaining a healthy diet.[6] However, insulin therapy or a healthy diet cannot fully prevent the offspring from developing metabolic disorders.[6] At present, early prediction and intervention are still important.

Previous studies have made numerous efforts to develop biomarkers for the early detection of GDM.[7–9] These biomarkers may include total cholesterol, triglycerides, low-density lipids, and serum uric acid.[7–9] Neural networks have also shown potential in the early detection of GDM.[8] However, these markers and methods are limited by unsatisfactory sensitivity and specificity.[7–9] The participation of molecular factors is a requirement for the development of GDM.[10] Some factors with important roles in GDM are likely biomarkers for this disease and its complications.[11] In many human clinical disorders, including GDM, lncRNAs indirectly affect protein synthesis, rather than directly coding proteins, to regulate disease development.[12] Therefore, lncRNAs are a goldmine for the development of biomarkers to detect GDM. However, the expression and function of most lncRNAs in GDM are unclear. LncRNA HCP5 has been reported to

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participate in high glucose-induced pathological processes,[13] suggesting its potential participation in GDM, but the role of LncRNA HCP5 is unclear. This study aimed to explore the role of HCP5 in GDM, with a focus on its clinical values.

Materials and Methods

Patients and Follow-Up

This study enrolled a total of 220 pregnant women (age = 26.7±3.1 years; gestational age = 1 month). All participants were enrolled at West China Second University Hospital between May 2019 and April 2020 after the Ethics Committee of this hospital had approved this study. The inclusion criteria were ≥18 years old, time from last menstrual period ≥1 month, and a pregnancy confirmed by β-hCG testing. Women who had diabetes before pregnancy were excluded.

A follow-up study was performed every month until delivery. Before 24 weeks, the criteria for GDM were the same as in non-pregnant women, with fasting blood glucose >7.1 mmol/L and glucose >11.1 mmol/L after a meal. At 24–28 weeks, GDM was diagnosed when at least two of three criteria were met: 1) fasting >95 mg/dL (5.1 mmol/L); 2) 1 hour >180 mg/dL (10 mmol/L); 3) 2 hours >155 mg/dL (8.5 mmol/L). All participants signed their informed consent. Plasma (fasting) samples obtained on the day of admission and every month during follow-up were stored in liquid nitrogen prior to use.

Cell Culture

Rat insulinoma INS-1 cells (Sigma-Aldrich), which are proven to be mycoplasma-free cells, were used in this study. RPMI 1640 Medium (Invitrogen) supplemented with FBS (10%), penicillin (1%), and streptomycin (1%) was applied to cultivate cells in an incubator, with humidity, CO_2, and temperature set to 95%, 5%, and 37°C, respectively. Cells were collected from passage 3 to 5 to be used in the subsequent assays.

Cell Transfection

Mycoplasma-free cells were transferred to a six-well plate, followed by cell culture to reach about 80% confluence. Then cells were transfected with LncRNA HCP5 vector (pcDNA3.1) or HCP5 siRNA using Lipofectamine 2000 (Invitrogen). Transfection experiments were repeated three times. Incubation with the transfection mixture (Lipofectamine 2000 +vector or siRNA) was performed for 6 h, and washing with fresh medium was performed three times. Cells were gathered at 48 h to perform the subsequent assays.

RNA Sample Preparations

Isolation of RNA from samples was carried out with the RNAstorm™ RNA Isolation Kit (Biotium). All steps were completed following instructions from Biotium. In brief, after cell lysis, the lysates were subjected to first step purification and gDNA removal using the column. After that, RNA binding columns were used for RNA binding, followed by adding DNase solution onto membranes to further digest gDNA. Digestion of gDNA was performed at room temperature for 20 min, followed by washing with washing buffers. After centrifugation for 5 min at 12,000 *g*, nuclease-free water was added to elute RNA. RNA samples were all stored in a tank containing liquid nitrogen prior to use.

Analysis of RNA Quality and RT-qPCR

RNA concentration was analyzed using a 2100 Bioanalyzer. Nuclease-free water was added into all RNA samples to make a final concentration of about 1500 ng/µL. The sample machine was also used to analyze the integrity of all RNA samples. The analysis revealed that a RIN value higher than 8.5 was reached in all cases. With about 1 µL RNA sample as the template, cDNA preparation was performed using the RT-IV system (Invitrogen). qPCRs were then performed with internal control 18S rRNA to quantify the expression levels of HCP5. Relative gene expression levels were determined by normalizing Ct values through the 2-delta delta Ct method. The primer sequence were as follows: 5′-TGAGAGCAGGACA GGAAAA-3′ (forward) and 5′-CCAACCAGACCCTAAGTGA-3′ (reverse); 18S ribosomal RNA (18S): 5′-CGCTCGCTCCTCTCCTACTT-3′ (forward) and 5′-CGGGTTGGTTTTGATCTGA TAA-3′ (reverse).

MTT Assay

Cells gathered at 48 h post-transfection were seeded onto a 96-well plate containing 10 mM D-glucose. Cell culture was performed under the aforementioned conditions for a further 48 h. After that, about 20 µL MTT (5 g/L, Sigma-Aldrich) was added to each well. Cells were incubated for 4 h, followed by the addition of 150 µL of DMSO. Finally, OD values were measured at 490 nM to reflect cell viability.

ELISA

At 48 h post-transfection, cell cultures were centrifuged and culture medium was collected. Insulin in the medium was detected using the Insulin Human ELISA Kit (Invitrogen). All steps were completed following the manufacturer's instructions.

Statistical Methods

Data comparisons and image preparation were performed using SPSS 17.0 software. Data comparisons were performed using two-tailed Student's *t*-test. The diagnostic value of plasma HCP5 on the day of admission for GDM was explored using the ROC curve, which was performed using potential GDM patients as true-positive cases and the remaining pregnant women as true-negative cases. The 220 pregnant women were divided into high and low HCP5 level groups (n=110). GDM-free curves were plotted using follow-up data and compared with the log rank test. A *p*-value of less than 0.05 was taken as statistically significant.

Results

Plasma Levels of HCP5 in GDM Patients and Control Women

The 220 pregnant women were followed up until delivery to monitor the occurrence of GDM. During follow-up, a total of 34 cases of GDM were diagnosed. The remaining 186 pregnant women in this study comprised the control group. Plasma levels of HCP5 at each time-point (during follow-up to the day on which GDM was diagnosed) were compared between GDM patients and controls. The results showed that HCP5 levels increased during pregnancy, and HCP5 levels were higher in GDM patients (n=34) than in the control group (n=186), even in early pregnancy (Figure 1). Therefore, increased plasma levels of HCP5 may participate in GDM.

Analysis of the Predictive Value of Plasma HCP5 on the Day of Admission for GDM

The predictive value of plasma HCP5 on the day of admission for GDM was assessed using the ROC curve, with potential GDM patients (n=34) as true-positive cases and the remaining pregnant women (n=186) as true-negative cases. Plasma levels of HCP5 on the day of admission effectively separated GDM patients from healthy controls (Figure 2). Therefore, plasma HCP5 may serve as an early predictive biomarker for GDM.

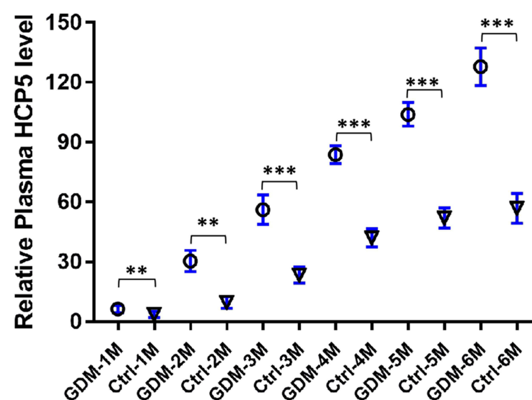

Figure 1 Plasma levels of HCP5 in GDM patients and control women. The 220 pregnant women were followed up until delivery to monitor the occurrence of GDM. During follow-up, a total of 34 cases of GDM were diagnosed. The remaining 186 pregnant women in this study were the control group. GDM-1M to 6M means HCP5 level at gestational age 1 month to 6 months in GDM patients. Ctrl-1M to 6M means HCP5 level at gestational age 1 month to 6 months in control patients. Data presented in this figure represent the average values of three technical replicates. **$p<0.01$, ***$p<0.001$.

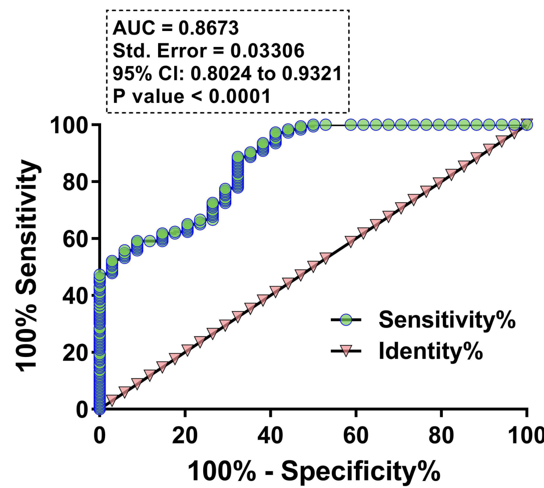

Figure 2 Analysis of the diagnostic value of plasma HCP5 on the day of admission for GDM. The diagnostic value of plasma HCP5 on the day of admission for GDM was assessed using the ROC curve, which was performed using potential GDM patients (n=34) as true-positive cases and the remaining pregnant women (n=186) as true-negative cases.

Association Between Plasma HCP5 and Occurrence of GDM

The 220 pregnant women were divided into high and low HCP5 level groups (n=110). GDM-free curves were plotted using follow-up data and compared using the log rank test. Curve analysis showed that, compared to the low HCP5 level group, the high HCP5 group experienced a significantly increased occurrence of GDM (Figure 3).

Role of HCP5 in the Viability of INS-1 and Insulin Secretion

INS-1 cells were transfected with HCP5 expression vector or siRNA, and transfections were confirmed at 48 h post-transfection using RT-qPCRs (Figure 4A and B) ($p<0.01$). The viability of INS-1 cells with HCP5 overexpression and siRNA silencing was analyzed by performing the MTT assay. HCP5 overexpression significantly decreased cell viability, while siRNA silencing increased cell viability (Figure 4C) ($p<0.01$). An ELISA was performed to determine levels of insulin in cell culture medium. HCP5 overexpression significantly decreased insulin secretion, while siRNA silencing increased insulin secretion (Figure 4D) ($p<0.01$). The level of insulin was negatively correlated with the level of HCP5 (Supplementary Figure 1).

Discussion

The expression pattern of HCP5 in GDM and its clinical values for this common clinical disorder have been explored in the present study. Moreover, the role of HCP5 in regulating insulin secretion was analyzed. We showed that HCP5 may

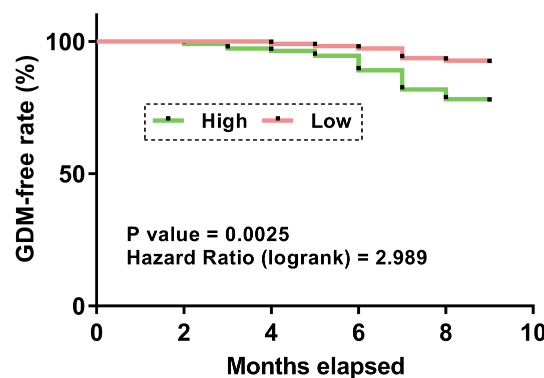

Figure 3 Association between plasma HCP5 and occurrence of GDM. The 220 pregnant women were divided into high and low HCP5 level groups (n=110). GDM-free curves were plotted using follow-up data and compared with the log rank test.

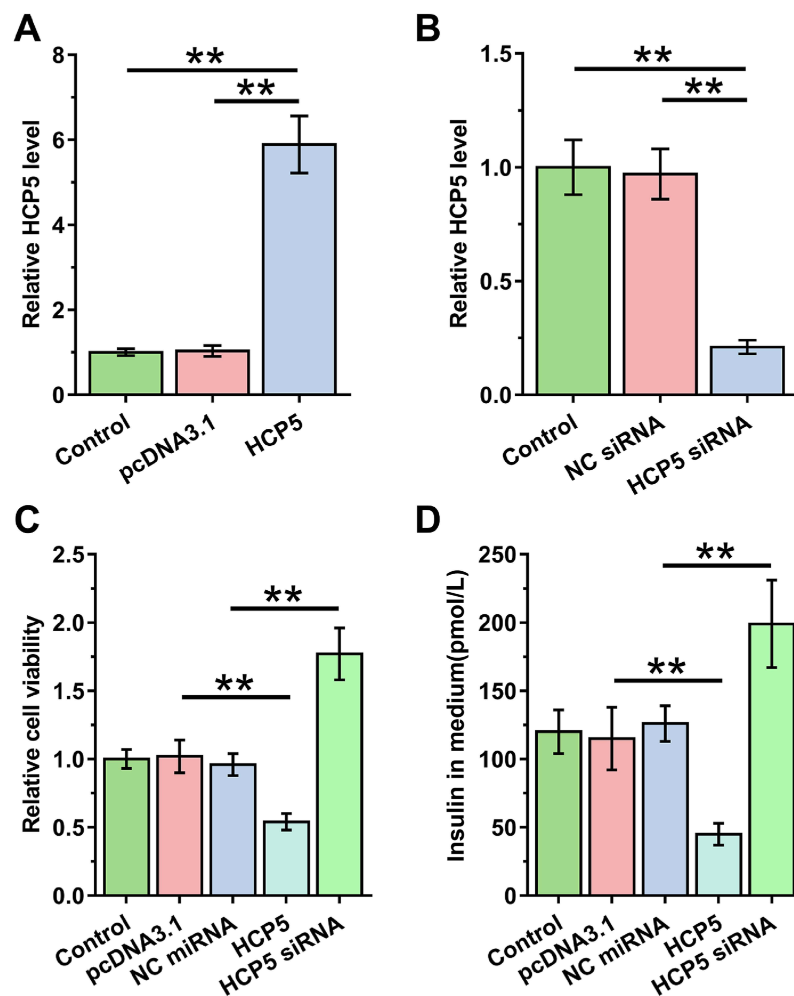

Figure 4 Role of HCP5 in the viability of INS-1 and insulin secretion. INS-1 cells were transfected with HCP5 expression vector or siRNA, and transfections were confirmed at 48 h post-transfection using RT-qPCRs (**A** and **B**). The viability of INS-1 cells with HCP5 overexpression and siRNA silencing was analyzed by performing the MTT assay (**C**). ELISA was performed to determine levels of insulin in cell culture medium (**D**). ***p*<0.01.

serve as a potential predictive biomarker for GDM. HCP5 may negatively regulate insulin secretion to promote GDM progression.

The functionality of HCP5 has been well studied in cancer biology.[14,15] For instance, HCP5 is overexpressed in cervical cancer and suppresses miR-15a to upregulate MACC1, thereby promoting tumor progression.[14] HCP5 is also highly expressed in bladder cancer and increases the movement of cancer cells by sponging miR-29b-3p.[15] In a recent study, *HCP5 was reported to interact with the axis of miR-93-5p/HMGA2, and its knockdown suppresses the proliferation, inflammation, and fibrosis of human glomerular mesangial cells induced by high glucose conditions*.[16] Therefore, HCP5 may participate in diabetes. The present study is the first to report the upregulation of HCP5 in GDM.

Although GDM is not caused by the reduced production of insulin, the application of exogenous insulin through direct injection improves the condition of both mothers and offspring.[17] Therefore, increasing the insulin level is helpful for the recovery from GDM. The present study showed that HCP5 could negatively regulate the viability of insulinoma INS-1 cells and the secretion of insulin from these cells. Therefore, HCP5 may promote GDM progression by suppressing insulin secretion.

Our study showed that plasma levels of HCP5 at the gestational age of 1 month were sensitive enough to separate potential GDM patients from controls. Moreover, high plasma HCP5 levels in pregnant women were closely associated with a high incidence of GDM during pregnancy. Therefore, measuring the plasma levels of HCP5 in the early stage of

pregnancy may assist in the identification of individuals with a high risk of GDM, thereby preventing the development of GDM by the early application of interventional approaches.

In conclusion, increased expression of HCP5 may contribute to the development of GDM by reducing insulin secretion. Moreover, HCP5 may serve as a potential predictive biomarker for GDM.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available owing to the research design, but are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Research Ethics Committee of West China Second University Hospital and was in line with the Declaration of Helsinki. Written informed consent was provided by all patients and controls.

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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.

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Disclosure

All the authors have declared that there was no potential conflict of interest in the research.

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