ORIGINAL RESEARCH

MALAT1 exacerbates immune dysregulation in gestational diabetes by modulating the miR-576-5p/HNRNPU axis: Implications for inflammatory pathogenesis

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Abstract

Background

Gestational diabetes mellitus (GDM) is characterized by systemic immune dysregulation and chronic inflammation that contribute to insulin resistance and β-cell dysfunction. The long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been implicated in several inflammatory disorders; however, its role in GDM-related immune and metabolic disturbances remains unclear.

Methods

A mouse model of GDM and insulin-resistant trophoblast cells were used to evaluate MALAT1 expression and its effects on inflammatory cytokines and β-cell apoptosis. Gene expression was analyzed by qRT-PCR and Western blotting, while histological and biochemical assays assessed pancreatic morphology, serum glucose, and inflammatory cytokine levels. Functional and rescue experiments were performed to explore the molecular interaction between MALAT1, miR-576-5p, and HNRNPU.

Results

MALAT1 expression was markedly elevated in GDM mice and insulin-resistant trophoblasts, correlating with increased levels of TNF-α, IL-1β, and IL-6, as well as enhanced β-cell apoptosis. Silencing MALAT1 alleviated hyperglycemia, preserved pancreatic islet structure, and reduced inflammatory mediator secretion. Mechanistically, MALAT1 acted as a competing endogenous RNA by sponging miR-576-5p to upregulate HNRNPU, thereby promoting NF-κB activation. HNRNPU overexpression reversed the anti-inflammatory and anti-apoptotic effects of MALAT1 knockdown.

Conclusion

MALAT1 promotes immune-metabolic dysfunction in GDM through the miR-576-5p/HNRNPU/NF-κB axis. Targeting this pathway may offer a novel therapeutic strategy for reducing inflammation-associated complications in GDM.

Keywords: Gestational diabetes mellitus, MALAT1, HNRNPU, miR-576-5p, immune dysregulation, pro-inflammatory cytokines

Introduction

Gestational diabetes mellitus (GDM) is widely acknowledged to be a common complication encountered in the time of pregnancy, and is marked by glucose intolerance, a condition that either manifests for the first time or is initially detected during the gestational period[1]. This condition has a significant global impact, affecting roughly 15% of pregnant women worldwide. GDM not merely a temporary concern during pregnancy; rather, it carries substantial risks for both the mother and the developing fetus. Moreover, women who suffer from GDM have an elevated risk of developing type 2 diabetes mellitus (T2DM) along with cardiovascular diseases in the postpartum period[2]. Currently, the management of GDM primarily revolves around dietary adjustments and pharmacological interventions, including the use of metformin and insulin[3]. Research indicates that GDM patients often exhibit varying degrees of insulin resistance (IR) or insufficient insulin secretion[4]. IR denotes a diminished responsiveness of target organs to insulin[5]. On a molecular level, IR manifests as a reduced ability of insulin, whether naturally circulating or externally administered, to lower blood glucose levels[6]. While some degree of IR is a normal physiological adaptation during pregnancy, facilitating the transfer of glucose from the mother to the fetus and supporting normal fetal growth and development, severe IR can lead to maternal hyperglycemia[7]. Consequently, delving into the pathogenesis of IR assumes paramount importance in the quest for effective GDM treatments.

Long non-coding RNAs (lncRNAs) constitute a unique subset of functional RNA molecules. They are distinguished by their extended length, typically exceeding 200 nucleotides, and notably lack the ability to encode proteins[8]. These RNA entities exert regulatory influence over protein-coding genes through a variety of sophisticated mechanisms[9]. For instance, they can form intricate complexes with their target genes, thereby modulating gene activity[10]. Additionally, lncRNAs are capable of interacting with microRNAs (miRNAs), either promoting their degradation or impeding their translation and post-transcriptional modification processes[11]. LncRNAs have a pervasive role in both the physiological and pathological aspects that occur within the body. They are crucial for sustaining cellular homeostasis, ensuring that cells function optimally under normal conditions. Additionally, lncRNAs are instrumental in the body's response to disease states, helping to regulate and potentially mitigate the effects of various pathologies[12]. Numerous studies have manifested that aberrant lncRNA expression profiles are closely related to the pathogenesis of complex diseases, including cancer, cardiovascular disease, neurological disease and diabetes[13]. The expanding corpus of knowledge on lncRNAs not only enhances our comprehension of the mechanisms underlying diseases but also paves the way for the creation of tailored therapeutic approaches and the identification of diagnostic biomarkers[14].

LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) belongs to one of the most extensively investigated and evolutionarily conserved lncRNAs and is located on the short arm of human chromosome 11q13.1[15]. A substantial body of research has established its significant involvement in a myriad of pathological processes, spanning diabetes and an array of malignant neoplasms[16,17]. Notably, it has been documented that MALAT1 expression is higher among GDM patients than pregnant women without GDM[18]. Besides, Abdulle and his colleague suggested that MALAT1 expression showed upregulation in different diabetic-related diseases, GDM included[19]. However, the specific function and related mechanism of MALAT1 in regulating IR in GDM still warrant further clarification.

Consequently, the current research aimed to explore the role and molecular intricacies of MALAT1 in governing IR during GDM. Based on our findings, it is plausible to hypothesize that fluctuations in MALAT1 expression levels could potentially offer valuable insights into developing novel therapeutic methods for GDM.

Material and methods

Ethics statement

The care and handling of mice throughout this study adhered strictly to the guidelines and protocols sanctioned by the Institutional Animal Care and Use Committee of our hospital. Every possible measure was taken to ensure that the experimental mice experienced minimal unnecessary distress or suffering.

Construction of GDM mouse model

Cyagen (Suzhou) Biotechnology Co., Ltd., Suzhou, China provided seven-week-old female (weighing 16-17 g) as well as male C57BL/6J mice (weighing 17-18 g). The mice were kept in an environment with precisely regulated conditions. They were housed at a temperature that stayed within the range of 20-25□ , while the relative humidity was consistently maintained between 45%-55%. Additionally, they were exposed to a 12-hour light/dark cycle to mimic natural day-night patterns. After a one-week acclimation period, mice were randomly assigned to experimental or control groups.

The control group consisted exclusively of age-matched pregnant female mice housed under identical conditions as the experimental group. Male mice were not included in the control group. To confirm pregnancy, female mice were cohabited with males at a 2:1 ratio overnight. Pregnancy was verified via vaginal smear for sperm detection, with the day of confirmation designated as gestation day 0 (GD0).

On GD6, the pregnant mice received an intraperitoneal injection of streptozocin (40 mg/kg, Sigma, USA), following a previously established protocol (20). Blood samples were gathered from the tail vein of each mouse. The blood glucose levels were measured utilizing the glucose oxidase-peroxidase method. Mice exhibiting blood glucose levels ≥ 11.1 mmol/L within 48 hours of the injection, and maintaining this level for three consecutive days, were deemed to have successfully developed the experimental model.

Animal grouping

A total of 24 mice that had been successfully modeled were randomly and evenly separated into 4 groups, with every group comprising six mice. The groups were as follows: the control group; the GDM group, which consisted of GDM-induced mice; the GDM+sh-NC group, where GDM mice received an injection of sh-NC; and the GDM+sh-MALAT1 group, in which GDM mice were injected with sh-MALAT1. Both the sh-NC and sh-MALAT1 were prepared at a concentration of 20 μg and dissolved in 2.5 mL of normal saline prior to administration. The solutions were then injected into the tail veins of the respective mice. As a control, an additional six normal pregnant mice were included in the study and designated to be the normal group. Mice in both normal and GDM groups were administered an equal volume of normal saline via tail-vein injection. On the 18th day of gestation (GD18), the mice were anesthetized and euthanized. The pancreatic tissues were then carefully harvested and utilized for the relevant experimental detections.

Detection of glucose and insulin

The fasting blood glucose (FBG) levels were determined using the glucose oxidase-peroxidase method, with the assay kit sourced from Sigma, USA. To measure the fasting serum insulin (FINS) levels, enzyme-linked immunosorbent assay (ELISA) kits tailored for mouse insulin, produced by Beyotime, China, were utilized. Using the measured FBG and FINS values, the homeostatic model assessment-insulin resistance index (HOMA-IR) was computed following the formula (FBG × FINS)/22.5.

Hematoxylin-eosin (HE) staining

Following fixation of the pancreatic tissues using 4% paraformaldehyde overnight, the tissue was thoroughly rinsed with running water for 4 hours to remove any residual fixative. Subsequently, the tissues were dehydrated utilizing a train of ethanol solutions with increasing concentrations. This was followed by a 30-minute treatment with xylene to further prepare the tissue for embedding. The dehydrated tissue was then embedded in paraffin wax. Using a microtome, the paraffin-embedded tissue was cut into 5 μm-thick sections. These sections were subjected to deparaffinization by immersion in xylene for 15 minutes, followed by a train of ethanol washes. Next, the sections were then stained with hematoxylin (obtained from Solarbio, China) for 5 minutes to highlight the nuclei. After a brief rinse, the sections were counterstained with eosin (sourced from Sangon, China) for 3 minutes. Finally, the stained sections were dehydrated once more using a graded ethanol series and xylene.

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Table 5. Comparison of the mean scores the pregnant women obtained from the PIKQ and UIAS in terms of some variables (n= 255)

	PIKQ		UIAS	
Variables	Mean±SD	p-value	Mean±SD	p-value
Age				
<30	8.12±2.79	0.73	42.34±3.33	0.96
≥30	8.01±2.45		42.32±3.66	
Education Status				
Primary school	6.86±2.84	0.00[b]	41.37±3.15	0.00[b]
High school	8.16±2.67		42.31±3.48	
University and above	8.90±2.10		43.05±3.57	
Gravida				
Primigravida	8.14±2.78	0.47	43.18±3.26	0.94
Multigravida	8.03±2.55		41.80±3.33	
Parity				
Nulliparous	8.01±2.74	0.24	43.19±3.21	0.00[b]
Primiparous	8.38±2.51		41.97±3.39	
Multiparous	7.62±2.66		41.21±3.80	
UI during pregnancy (n=130)				
Yes	8.24±2.27	0.305	42.16±3.13	0.414
No	7.90±2.98		42.52±3.81	
Pregnant women presenting to healthcare services with a complaint of UI (n=130)				
Yes	8.20±2.38	0.882	42.88±3.94	0.059
No	8.26±2.22		41.79±2.57	