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Original Article

Analysis of whole genome-wide methylation and gene expression profiles in visceral omental adipose tissue of pregnancies with gestational diabetes mellitus

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Abstract

Background: DNA methylation is the most extensively studied epigenetic modification which had been suspected to be involved in the progress of gestational diabetes mellitus (GDM) . It is vital to investigate the expression profile and methylation profile in the GDM adipose tissue samples to learn more about the relationship between the two profiles.
Methods: Illumina Human Methylation 450 k DNA Analysis Beadchip and whole Human Gene Expression Array were selected to screen for methylation and gene expression in the omental visceral adipose tissue of pregnant women. Validation of methylation of DMGs was conducted by bisulfate pyrosequencing and expression of DEGs by q RT-PCR.
Results: Global gene methylation profiling and whole genome expression profiling were conducted in visceral omental adipose tissue (VOAT) between GDM and normal pregnancies. Compared with controls, 935 genes were commonly dysregulated in the GDM group, including 450 down-regulated DEGs and 485 up-regulated DEGs. The Seven overlapping genes between DEGs and DMGs were extracted, including *C10orf10*, *FSTL1, GSTT1, HLA-DPB1, HLA-DRB5, HSPA6* and *MSLN*. Among them, *C10orf10*, *FSTL1, GSTT1, HLA-DPB1, HLA-DRB5* showed hypermethylation and up-regulated expression, while *HSPA6* show hypomethylation and down-regulated expression. Typical negative correlation between gene expression and DNA methylation level was only found in MSLN with significant hypermethylation in the CpG island and downregulated transcription. No gene was found to be significantly hypomethylated in the CpG islands and unregulated transcription.
Conclusion: We found that antigen processing and presentation pathway and immune-related genes were closely associated with gestational diabetes mellitus in the visceral omental adipose tissue of Chinese pregnant women, based on the integration analysis of expression and methylation profiles. These results may be valuable for the prognostic biomarkers and future therapeutic targets.

Keywords: Adipose tissue; Genome; Methylation; Omental; Profile

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Gestational diabetes mellitus (GDM) is a kind of metabolic disorder with abnormal glucose tolerance during firstly recognized in the pregnancy, which clinically diagnosed is in the second trimester of gestation. GDM is associated with poor short-term and long-term health outcomes both for mothers and their offspring. The prevalence has been increasing globally worldwide, varying from 3% to 14%.[1] In Tianjin, China, the prevalence of GDM increased from 6.9% in 2008, to 8.8% in 2009, and 9.9% in 2010.[2] In Kunming and Beijing, China, the prevalences of IGT and GDM are 12.4, 13.3% and 3.1%, 14.7%, respectively.[3]

A number of risk factors have been reported to be associated with GDM, including ethnicity, obesity, first-degree family history of diabetes, maternal age and parity, but the mechanisms through which these factors act on the pathway of GDM remain unclear. Studies support that the epigenetic alterations of the fetus of a GDM mother could be the main mechanism of the transgenerational transmission of GDM and type II diabetes, which suggests that epigenetics plays an important role in the process of GDM.[4–6]

The most studied epigenetic marker is DNA methylation, which plays a major role in the regulation of gene expression. Generally, DNA hypermethylation is correlated with suppression of gene expression, while hypomethylation with overexpression of genes. Altered DNA methylation in cytosine–phosphate–guanine (CpG) regions may result in altered gene expression. Several genes, including *ADIPOR*, *leptin* and lipoprotein lipase showed evidence for association between the promoter methylation and GDM.[7–9] Attention has been focused on DNA methylation and gene expression in diseases.[10]

Subcutaneous and visceral are the two main types of white adipose tissue in humans. These various adipose tissues have distinct biochemical properties and functions influencing metabolic risk. A recent study reported genome-wide promoter methylation and transcriptome analysis of subcutaneous and omental adipose in obese vs lean individuals.[11] The studies of epigenetic modifications in the placenta and cord blood from GDM have also been reported.[12–14] However, the study of whole-genome methylation screening and molecular genetic differences in the visceral omental adipose tissue with GDM is still limited.

Given lots of evidence for the involvement of epigenetics in GDM, together with the role that adipose tissue plays in diseases progression, we hypothesized that the aberrant expression of genes attributed by epigenetic alteration could be involved in the pathway of the insulin resistance or metabolic disturbance. The integrative analysis of expression and methylation profiles is crucial for identification of promising target genes and therapies.

In this study, the integration of genome-wide analysis of DNA methylation profiles and whole genome expression profiles in VOAT from pregnancies was performed in order to investigate the relationship of the methylation and expression profile in GDM adipose tissues. The methylation alterations and expression of four loci (*HLA-DMB, HLA-DOA, MSLN* and *HSPA6*) were validated for their potential functions in GDM.

2. Methods

VOAT samples were obtained from patients (N = 50) in two groups during C-section: (1) GDM(N = 26); (2) control group (N = 24). Three samples each for whole-genome expression and methylation profiles were selected from the two groups, individually. Women with GDM were enrolled in this study, diagnosed by 75 g oral glucose tolerance test (OGTT) during the 24th to 28th week of gestation at the patient out clinic of the Obstetrics Department in the 1st Affiliated Hospital of Kunming Medical College. Informed consent was obtained from all investigated subjects. Approval for the use of the samples was given by the Ethics Committee of Kunming Medical College. The diagnosis of GDM was made by OGTT 75 g, according to the World Health Organization criteria. Exclusion criteria for participation include multiple gestations, infection, pregnancy with complications, congenital or chromosomal abnormalities of the fetus, a family history of diabetes, and pregnancy with alcohol or drug abuse.

2.1. Visceral omental adipose tissue collection

1 cm × 1 cm × 1 cm visceral omental adipose tissues were obtained after C-section, immediately frozen in liquid nitrogen and stored in an ultrafreezer at −80 °C. DNA extraction from OVAT samples was carried out using the QIAamp DNA Mini Kit (Qiagen, Germany) following manufacture 's protocol.

2.2. Genome-wide DNA methylation microarray and expression microarray

The Illumina Human Methylation 450 k DNA analysis Beadchip platform was used to assess the genome-wide DNA methylation in six samples. All tests were duplicated twice. The six arrays passed standard quality control metrics using methylumi packages. The Beadchip manufacturing process includes hybridization-based quality controls of each array feature, allowing consistent production of high-quality, reproducible arrays. A laser scans Beadchips at two wavelengths simultaneously and creates an image file. The Illumina Genome Studio Methylation module are used for extracting genome-wide DNA methylation data from data files collected from Hiscan Reader. P-values were adjusted for multiple testing according to the false discovery rate (FDR) procedure of Benjamini-Hochberg. The DMRs were selected based on the probes that exhibited different methylation status between GDM cases and controls. 5% FDR were selected as significant DMRs.

The Whole-Human Gene Expression Array (Affymetrix Gene Chip® PrimeView™, *Homo sapiens*, Affymetrix, CA, USA) was selected to screen for gene expression in the visceral omental adipose tissue of pregnant women. The data were normalized, and Log2 ratio data were converted into P value scores using the Kolmogorov–Smirnov test. The

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