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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

differentially expressed genes were selected according to the p value threshold ($p < 0.05$), followed by a secondary selection of >log2.

2.3. Gene ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes database (KEGG) pathway analysis

The GO categories were used to construct and facilitate the biologically meaningful annotation of genes. GO categories were examined using with the GO chart feature offered by the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Significant enrichment pathways were determined from these differentially expressed genes using the Kyoto Encyclopedia of Genes and Genomes database (KEGG), then the KEGG database was used to build the network of genes according to the relationship among the genes, proteins and compounds in the database.

2.4. Integrated analysis of DEGs and DMGs

The gene expression and methylation level were analyzed simultaneously in order to extract the genes overlapping between DEGs and DMGs. Correlations of greater than two were analyzed based on the level of mean methylation and the gene expression.

2.5. Validation of methylation of DMGs by bisulfate pyrosequencing and expression of DEGs by qRT-PCR

The methylation levels in the promoter region of DMGs were validated by bisulfate pyrosequencing. To find out whether the status of methylation in the gene promoter affected gene activation, an analysis of the mRNA expression with DMGs by quantitative PCR was performed. Microarray data were validated using SYBR-green-based quantitative real-time PCR for four genes (*HLA-DMB, HLA-DOA, MSLN* and *HSPA6*) from the VOAT samples.

3. Results

3.1. Illumina gene expression profile

The differentially expressed genes were screened out between the two groups. Compared with controls, 935 genes were commonly dysregulated in the GDM group, including 450 down-regulated DEGs and 485 up-regulated DEGs.

3.2. Differential expression analysis, gene ontology (GO) analysis, KEGG pathway analysis

The top five molecular functions (MF) were heparin binding, receptor binding, calcium ion binding, phosphatidylinositol-4,5-bisphosphate binding and natural killer cell lectin-like receptor binding. Cellular component (CC) of genes expressed differentially in the tissues of GDM were related to extracellular space, extracellular region, extracellular matrix, smooth muscle contractile fiber and membrane raft in the cellular component of GO terms. GO biological process (BP) terms, including antigen processing and presentation, extracellular matrix organization, positive regulation of cell-substrate adhesion, response to magnesium ion, and vascular smooth muscle contraction, were differentially expressed.

The top ten enriched pathways of the DEGs were renin-angiotensin system, graft-versus-host disease, allograft rejection, type I diabetes mellitus, viral myocarditis, antigen processing and presentation, staphylococcus aureus infection, autoimmune thyroid disease, asthma, bladder cancer.

3.3. DNA methylation microarray data

In the whole genome, 12 802 DMRs targeted 2866 genes, of which 5910 DMRs targeting 1298 genes showed significant hypomethylation in GDM relative to the controls, whereas 6892 DMRs targeting1568 genes showed hypermethylation. Additionally, there were 311 genes in the promoter region, of which 166 genes (53.4%) showed significant hypermethylation, whereas 145 genes (46.6%) showed the reciprocal pattern. Among them, the top three genes with CpG difference in the promoter region between the GDM and control group included 5′UTR in PSORS1C1, TSS1500 and TSS200 in DKFZp686A1627, and 1st Exon and TSS200 in PCDHB13.

Measurement of DNA methylation level along chromosomes showed that DMRs were mainly located in chromosome 6 (13.7%), chromosome 1 (9.5%) and chromosome 2 (7.9%) (detailed in Fig. 1). According to the CpG position in the genome, the DMR proportions of 1st Exo, 3′UTR, 5′UTR, Body, TSS1500 and TSS200 were 2.8%, 5.7%, 7.8%, 64.9%, 11.9% and 6.8%, respectively.

There were 14 miRNA genes with differentiated CpG sites in the promoter region between the two groups, including MIR-662, MIR-886, MIR-654, MIR-376, MIR-548, MIR-526, MIR-519, MIR-522, MIR-346, MIR-300, MIR-202, MIR-1228, MIR-133, and MIR-885.

3.4. Differential methylation analysis, Gene ontology (GO) analysis, KEGG pathway analysis

To find out which pathways and biological functions have been affected in the GDM, the biologically meaningful annotation of genes was constructed based on the GO analysis. In the study, after the GO categories detected by the analysis, a majority of differentially methylated genes were classified into the following functional categories, including antigen processing and presentation, cell growth and death, regulation of caspase activity/cell signal transduction, transcription, immune response. GO cellular component (CC) of genes hypomethylated differentially in the tissues of GDM were related to actin cytoskeleton, plasma membrane part, intrinsic to plasma membrane, while CC of genes hypermethylated differentially were related to extracellular matrix, intermediate filament cytoskeleton and intermediate filament.

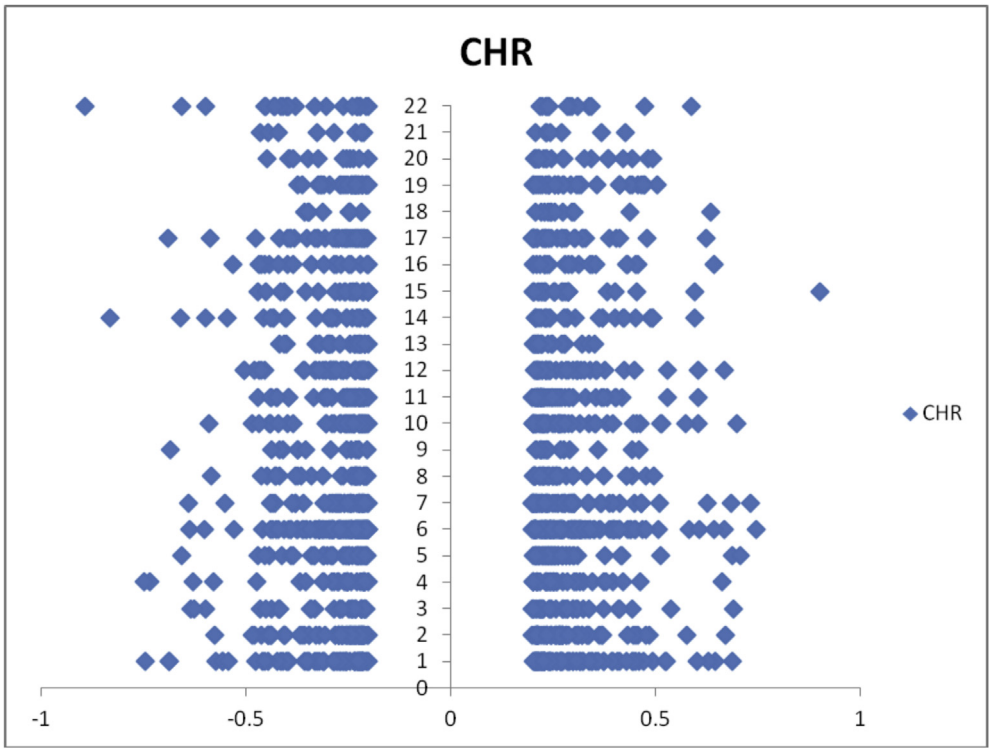

Fig. 1. Chromosome location distribution of DMRs in GDM. Global DNA methylation in human visceral omental adipose tissue plotted by gene region.

According to the results, antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, female pregnancy, pathogenesis and antigen processing and presentation were the main hypomethylated terms in the GO analysis. Regulation of caspase activity, positive regulation of multicellular organismal process and programmed cell death were the main hypermethylated terms (seen details in Fig. 2).

HLA-DRB1, HLA-DPB1 and HLA-DPA1 were mainly enriched in antigen processing of peptide, antigen processing and presentation, IL-6, SMAD3, ITGB2 and ANG were mainly enriched in regulation of cell death or apoptosis.

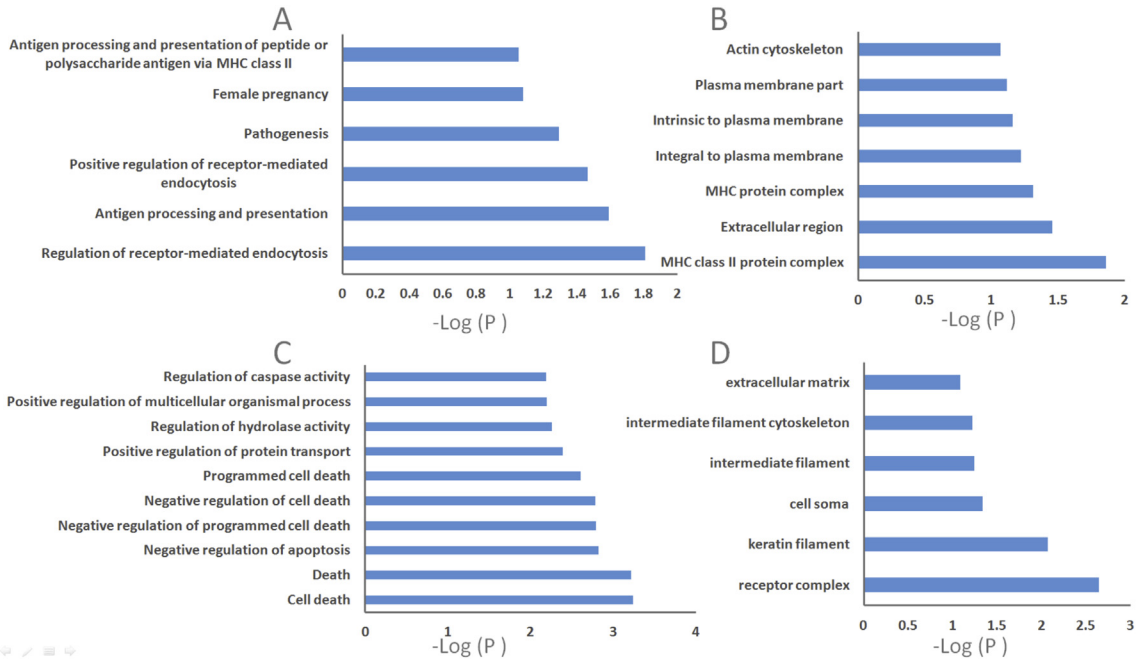

Fig. 2. A, C Genes showing differential methylation clustering by biological process. Gene Ontology analysis (A Hyper; C Hypo) B, D Genes showing differential methylation clustering by cellular component. Gene Ontology analysis (B.Hyper; D.Hypo).

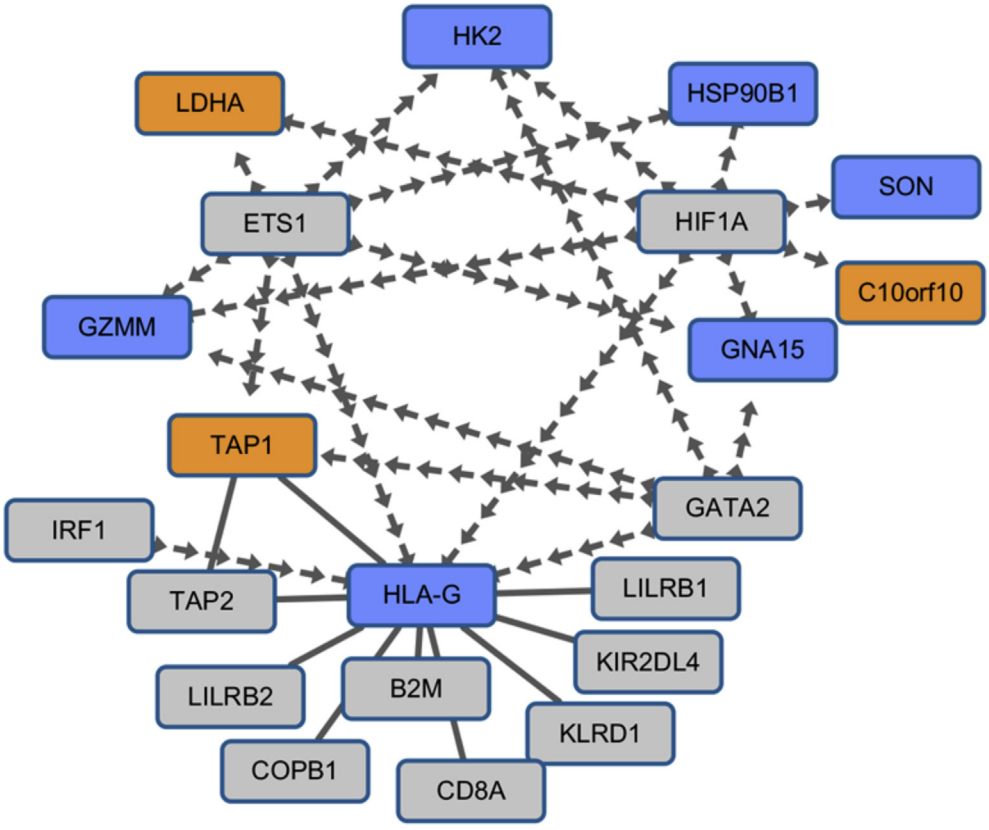

Fig. 3. Network of genes with significant differences in methylation ($p < 0.05$). The blue icons indicate hypomethylation, and orange icons indicate hypermethylation.

KEGG pathway analyses were applied to determine the roles played by the differentially methylated genes in the biological pathways. KEGG pathway analysis has shown that most of the differentially methylated genes were related with graft-versus-host disease, type I diabetes mellitus, antigen processing and presentation and allograft rejection(seen details in Table 1).

3.5. Integrated analysis of DEGs and DMGs

The level of gene expression and the mean methylation difference for each gene were analyzed to construct the correlations between gene expression and methylation status. The overlapping 7 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* show the hypermethylation and the up-regulated expression, while *HSPA6* shows the hypomethylation and down-regulated expression. A typical negative correlation between gene expression and DNA methylation level were only found in *MSLN* with significantly hypermethylated in CpG island and downregulated transcription. There is no gene was found to be significantly hypomethylated in CpG islands and unregulated transcription.

Table 1
Functional pathways the differentially methylated genes are involved in OVAT with GDM.

Term	KEGG Pathway	Genes	Counts
hsa05332	Graft-versus-host disease	*IL6, HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-G*	5
hsa04940	Type I diabetes mellitus	*HLA-DRB1, HLA-DPA1, HLA-DPB1, HSPD1, HLA-G*	5
hsa04612	Antigen processing and presentation	*HLA-DRB1, TAP1, HSPA6, HLA-DPA1, HLA-DPB1, HLA-G*	6
hsa05330	Allograft rejection	*HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-G*	4
hsa05416	Viral myocarditis	*HLA-DRB1, ITGB2, HLA-DPA1, HLA-DPB1, HLA-G*	5
hsa04672	Intestinal immune network for IgA production	*IL6, HLA-DRB1, HLA-DPA1, HLA-DPB1*	4
hsa05320	Autoimmune thyroid disease	*HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-G*	4
hsa05310	Asthma	*HLA-DRB1, HLA-DPA1, HLA-DPB1*	3
hsa04740	Olfactory transduction	*OR2AG1, OR2A5, OR52M1, OR52B4, OR4S2, OR8A1, OR4C6, OR10K1, OR6N2*	9
hsa04514	Cell adhesion molecules (CAMs)	*HLA-DRB1, ITGB2, HLA-DPA1, HLA-DPB1, HLA-G*	5

3.6. Validation of methylation of DMGs by bisulfite-pyrosequencing and expression of DEGs by qRT-PCR

The methylation status and expression of *HLA-DMB, HLA-DOA, MSLN* and *HSPA6* were selected to examine for their potential functions related to the molecular mechanisms of GDM, which have not been reported previously. The examined CpG positions located in or near genomic regions are consistent with specific microarray probes.

HLA-DMB, HLA-DOA, MSLN and *HSPA6* showed significantly different methylation level. The four candidate genes showed significantly different expression level, except for *HLA-DOA*. Among them, only one gene named *MSLN* showed a strong negative correlation between gene expression changes and DNA methylation level (seen details in Fig. 4).

3.7. Differential methylation analysis, Gene ontology (GO) analysis, KEGG pathway analysis

To find out which pathways and biological functions have been affected in the GDM, the biologically meaningful annotation of genes was constructed based on the GO analysis. In the study, after the GO categories detected by the analysis, a majority of differentially methylated genes were classified into the following functional categories, including antigen processing and presentation, cell growth and death, regulation of caspase activity/cell signal transduction, transcription, immune response. GO cellular component (CC) of genes hypomethylated differentially in the tissues of GDM were related to actin cytoskeleton, plasma membrane part, intrinsic to plasma membrane, while CC of genes hypermethylated differentially were related to extracellular matrix, intermediate filament cytoskeleton and intermediate filament.

According to the results, antigen processing and presentation of peptide or polysaccharide antigen via MHC class ll, female pregnancy, pathogenesis and antigen processing and presentation were the main hypomethylated terms in the GO analysis. Regulation of caspase activity, positive regulation of multicellular organismal process and programmed cell death were the main hypermethylated terms (seen details in Fig. 2).

HLA-DRB1, HLA-DPB1 and *HLA-DPA1* were mainly enriched in antigen processing of peptide, antigen processing and presentation, *IL-6, SMAD3, ITGB2* and ANG were mainly enriched in regulation of cell death or apoptosis.

KEGG pathway analyses were applied to determine the roles played by the differentially methylated genes in the biological pathways. KEGG pathway analysis has shown that most of the differentially methylated genes were related with graft-versus-host disease, type I diabetes mellitus, antigen processing and presentation and allograft rejection (seen details in Fig. 2).

3.8. Integrated analysis of DEGs and DMGs

The level of gene expression and the mean methylation difference for each gene were analyzed to construct the correlations between gene expression and methylation status. The overlapping 7 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* show the hypermethylation and the up-regulated expression, while *HSPA6* shows the hypomethylation and down-regulated expression. A typical negative correlation between gene expression and DNA methylation level were only found in *MSLN* with significantly hypermethylated in CpG island and downregulated

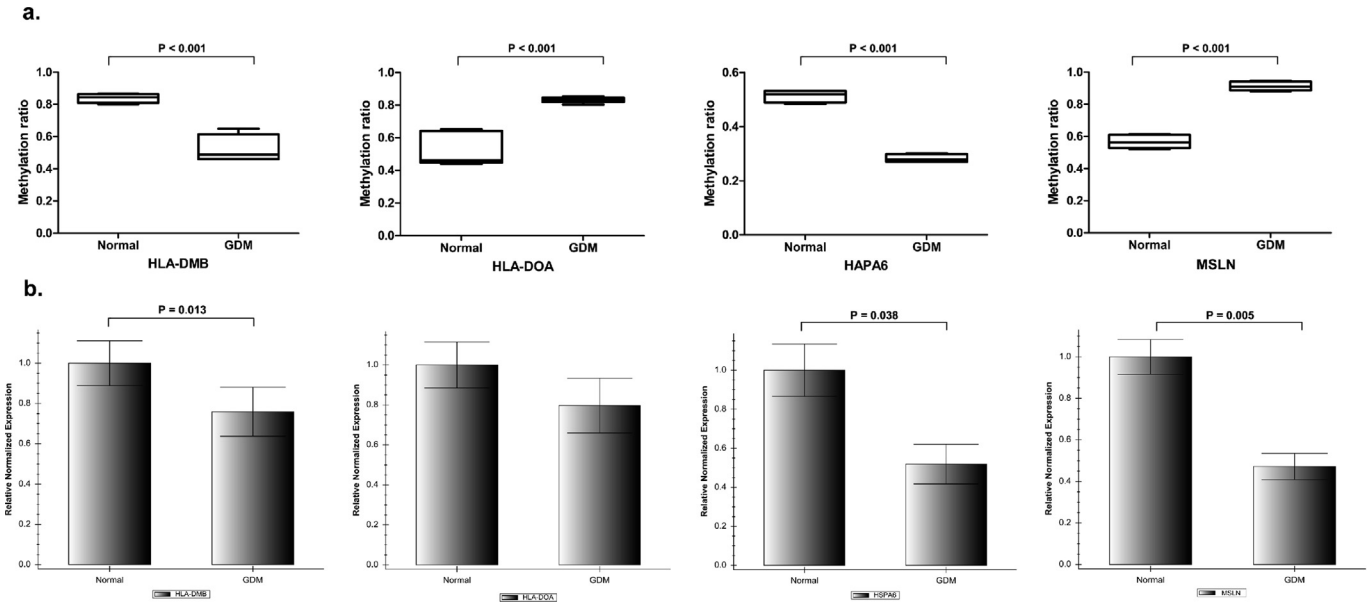

Fig. 4. The validation of methylation profiles and expression of 4 candidate genes (*HLA-DMB, HLA-DOA, MSLN* and *HSPA6*) a. Methylation level *HLA-DMB, HLA-DOA, MSLN* and *HSPA6* in GDM and control VOAT samples, including GDM (N = 26), control group (N = 24). b. Expression level of *HLA-DMB, HLA-DOA, MSLN* and *HSPA6* in GDM and control VOAT samples, including GDM (N = 26), control group (N = 24).

transcription. There is no gene was found to be significantly hypomethylated in CpG islands and unregulated transcription.

3.9. Validation of methylation of DMGs by bisulfite-pyrosequencing and expression of DEGs by qRT-PCR

The methylation statuses and expressions of *HLA-DMB, HLA-DOA, MSLN* and *HSPA6* were selected to examine for their potential functions related to the molecular mechanisms of GDM, which had not been reported previously. The examined CpG positions located in or near genomic regions were consistent with specific microarray probes.

HLA-DMB, HLA-DOA, MSLN and *HSPA6* showed significantly different methylation levels. The four candidate genes showed significantly different expression levels, except for *HLA-DOA*. One gene, *MSLN*, showed a strong negative correlation between gene expression changes and DNA methylation level (see details in Fig. 4).

4. Discussion

In this study, the expression profiles and methylation profiles of six samples were investigated to learn more about the relationship between the two profiles. A number of regions were differentially methylated and expressed between GDM VOAT and healthy controls. As far as we know, this is the first study to integrate the analysis of expression and methylation profile in VOAT with GDM in Chinese pregnant women. This study provides a solid basis for future research focused on the role DNA methylation plays in potential dysfunction in GDM, as well as role DNA methylation plays in GDM. However, there are still several limitations in the study.

Firstly, we only selected VOAT samples to investigate the analysis of expression and methylation profile, not other parts of adipose tissue, because VOAT is more strongly associated with increased risk of GDM. The various adipose tissues with distinct structural and biochemical properties function to influence metabolic risk, as for the different development origins of these tissues. Subcutaneous and visceral are two main types of white adipose tissue in humans, depositing throughout the body. Macartney-Coxson et al. reported that majority of these CpG sites were less methylated in subcutaneous than in omental adipose tissue. We did not perform inter-adipose tissue comparisons. Since the profiles are adipose tissue deposit specific, the difference in profiles between the SAT and VOAT should be elucidated in the future.

Secondly, the seven overlapping genes between DEGs and DMGs were extracted by integrating analysis of methylation and gene expression data, not by establishing the Epigenetic Module based on Differential Networks (EMDN) algorithms simultaneously analyzing DNA methylation and gene expression data. The reasons follow: First of all, the design of algorithms requires large-scale sample-matched methylation and gene expression profiles. Secondly, it has become evident that methylation has a complex relationship with gene expression. The epigenetic module is required for exploring and quantifying the complex correlation based on more studies. An effective algorithms model is needed to integrate the two profiles in the future.

Thirdly, the variability in global methylation in adipose tissue might be related to fat distribution and alterations in glucose metabolism or homeostasis. We did not observe the association between the profiles and related phenotypes much further, such as measures of fat distribution (waist measurement, WHR) and glucose homeostasis (HbA1c).

In the study, the methylation microarray results revealed that the average level of genome-wide DNA methylation was slightly, but significantly, higher in GDM compared to control (GDM: 0.5469 vs control: 0.5251, $p < 0.05$). The level of genome-wide expression was significantly higher in GDM (GDM: 5.7703 vs control: 5.633, $p < 0.05$). We mapped these DMRs to their chromosomal locations, and a bias was observed toward chromosomes 6, 1 and 2. It had been observed that, in placenta with GDM, there was a bias towards chromosomes 1, 2 and 11.[14,15] Several genomic regions showed significantly higher global DNA methylation levels in VOAT with GDM compared with control, including 1st Exo, 3′UTR, 5′UTR, Body, TSS1500 and TSS200. Among them, body gene region had the highest level of DMRs. Varley et al. described the relationship between DNA methylation and gene expression, indicating that promoter methylation is associated with gene silencing and gene body methylation is associated with gene expression [Deaton et al., 2011; Aran et al., 2011; Maunakea et al., 2010]. Further investigation is needed to understand how and why. We are hopeful that further integrated analysis will shed light on the causes and consequences of this complex methylation pattern in GDM.

The mRNA expression was regulated by epigenetic modifications.[16,17] Therefore, we want to test whether those genes with differential mRNA expression exhibit differential DNA methylation in VOAT with GDM. Based on the integration analysis, we found 7 individual genes with differential mRNA expression and change with DNA methylation, including *C10orf10, FSTL1, GSTT1, HLA-DPB1, HLA-DRB5, HSPA6* and *MSLN*. Among these 7 genes, only *MSLN* had decreased mRNA expression together with significantly hypermethylated in CpG island based on the integration of expression and methylation profiles. Women with GDM are characterized by disturbances in insulin secretion and inflammation. It had been reported that MSLN has shown potential as serum biomarkers in cancers, including pancreatic cancer.[18] Circulating MSLN was prognostic for survival in both gastric and ovarian cancers.[19] Early studies suggested that the pathogenesis of GDM is a defect of pancreatic islet beta cell functions. The correlation between MSLN methylation status and GDM deserves further research.

Previously, our group observed that GDM, compared to normal placenta, displayed a higher level of inflammatory factors such as IL-1 and IL-6. In the study, 8% of the up-regulated genes were related to the immune response, including chemokines and chemokine receptors, MHC molecules, interleukins, complement receptors and lymphocyte receptors. In the study, the genes with difference in the GO analysis were observed in immune-related genes or pathways, in both methylation profile and expression profile suggests that

antigen process and presentation may be involved in the pathogenesis of GDM. Y. H Zhao et al. showed that two significantly different GDM-associated pathways in blood and placenta, supporting function distributions is in terms of immune-related genes. Our findings highlight a significant role of the MHC region in the presentation of GDM, with a general down-regulation of HLA genes among GDM-exposed VOAT adipose tissues.

An interaction network of genes with significant methylation alteration was built based on the KEGG database. In the network, *HK2, HSP90B1, SON, GNA15, GZMM, HLA-G, TAP1, LDHA* and *C10ORF10* were in the core of the network (see details in Fig. 3). In particular, the HLA genes, including *HLA-DRB1, HLA-DPB1, HLA-DPA1* and *HLA-G*, were significantly methylated. HLA-G was involved in inducing immune tolerance via interaction with inhibitory receptors present on natural killer cells, T and B lymphocytes, and antigen-presenting cells.[20,21] HLA-G was considered to be a mediator of induction of feto-maternal tolerance during pregnancy,[22] which had been reported to affect the cytotoxicity of natural killer and $CD8^+$T cells, $CD4^+$T lymphocyte functions and dendritic cell maturation in several autoimmune diseases, viral infections, cancer diseases and transplantation.[23−26] HLA-G was also postulated to protect the islet cells of the pancreatic tissue in the pathogenesis of GDM.[27] Y.H. Zhao et al. reported that immune-related genes may play important roles in the genomic expression profiles of blood and placenta, and also supported that the significant changes in *HLA-G* expression in both blood and placenta.

In conclusion, we found that antigen processing and presentation pathway and immune-related genes were closely associated with GDM in VOAT of Chinese pregnant women, based on the integration analysis of expression and methylation profiles. These results may be valuable for the identification of prognostic biomarkers and future therapeutic targets.

Acknowledgments

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