Research Article

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Aberrant DNA methylation involved in obese women with systemic insulin resistance

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Abstract: Background: Epigenetics has been recognized as a significant regulator in many diseases. White adipose tissue (WAT) epigenetic dysregulation is associated with systemic insulin resistance (IR). The aim of this study was to survey the differential methylation of genes in obese women with systemic insulin resistance by DNA methylation microarray. Methods: The genome-wide methylation profile of systemic insulin resistant obese women was obtained from Gene Expression Omnibus database. After data preprocessing, differing methylation patterns between insulin resistant and sensitive obese women were identified by Student's t-test and methylation value differences. Network analysis was then performed to reveal co-regulated genes of differentially methylated genes. Functional analysis was also implemented to reveal the underlying biological processes related to systemic insulin resistance in obese women. Results: Relative to insulin sensitive obese women, we initially screened 10,874 differentially methylated CpGs, including 7402 hyper-methylated sites and 6073 hypo-methylated CpGs. Our analysis identified 4 significantly differentially methylated genes, including *SMYD3*, *UST*, *BCL11A*, and *BAI3*. Network and functional analyses found that these differentially methylated genes were mainly involved in chondroitin and dermatan sulfate biosynthetic processes. Conclusion: Based on our study, we propose several epigenetic biomarkers that may be related to obesity-associated insulin resistance. Our results provide new insights into the epigenetic regulation of disease etiology and also identify novel targets for insulin resistance treatment in obese women.

Keywords: obesity; insulin resistance; DNA methylation; bioinformatics

1 Introduction

Obesity, an emerging nutritional problem globally, has become the fifth leading risk for global deaths [1]. It is associated with low-grade inflammation resulting from chronic activation of the innate immune system, and can often lead to insulin resistance [2], a condition that is characterized by elevated circulating levels of insulin during fasting, despite normal or elevated glucose levels in the blood [3, 4]. The impaired ability of insulin to induce cellular responses is a pathophysiological mechanism that links obesity to metabolic disorders, such as type 2 diabetes and cardiovascular disease [5-7]. Recently, epigenetics has been recognized as a significant regulator in complex diseases. Both genetic and epigenetic factors have been implicated in the development of systemic insulin resistance. The association between insulin resistance and excess abdominal fat, particularly in intra-abdominal or visceral adipose tissue (VAT), is believed to be mediated by increased spontaneous hydrolysis of lipids (i.e. adipocyte lipolysis) [8, 9]. In addition, systemic IR is characterized by ectopic triacylglycerol accumulation in skeletal muscle and the liver [10-12].

As the best-studied epigenetic modification, DNA methylation is closely involved in several key biological processes [13]. Growing evidence has indicated that DNA methylation contributes to obesity associated insulin resistance [14-16]. Candidate gene methylation studies in animal models and humans have demonstrated methylation changes in the promoters of various genes that are implicated in obesity, appetite control and

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metabolism, insulin signaling, immunity, growth and circadian clock regulation [3, 17, 18]. Moreover, epigenome-wide association studies have been used to identify novel genes and pathways related to obesity and obesity-induced complications. Previous studies have identified several obesity-associated inflammatory genes, such as *UBASH3*, *TRIM3*, *HIF3A*, and *LY86* [19, 20]. Most recently, Fradin et al. [21] revealed 18 and 138 specific differentially methylated CpGs in moderately and severely obese children, respectively, relative to lean children. Global and site-specific differences in CpG methylation have been associated with obesity and insulin resistance [22-24]. However, to our knowledge, few studies have focused on the candidate methylated genes associated with systemic insulin resistance in obese women.

Hence, in this study, we attempted to identify differential DNA methylation of genes and the underlying biological processes involved in systemic insulin resistance in obese women based on the genome-wide methylation data. We anticipate that our work contribute to a better understanding of the underlying molecular mechanisms, and provide novel insights for the treatment of obesity-related systemic insulin resistance.

2 Materials and Methods

2.1 Genome-wide DNA methylation data

The global CpG methylation profile of omental adipose tissue in obese women with systemic insulin resistance was downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database of NCBI, with the accession number of GSE76394 [24]. In this dataset, there were a total of 79 samples available, including 39 visceral adipose tissue specimens from obese women with systemic insulin resistance (IR group) and 40 visceral adipose tissue samples from obese women without systemic insulin resistance (control group). DNA was assayed using the Illumina Human Methylation 450 Bead Chips (Illumina, San Diego, CA, USA). Detailed sample characteristics have been described in the previous study [24].

Before analysis of differential methylation sites, quantitative normalization and adjustment of DNA methylation data were performed using the Bioconductor Lumi [25] and BMIQ packages [26]. This dataset contained 485,577 probes (covering 21,231 genes). Since probes overlapping single nucleotide polymorphisms (SNPs) interfere with hybridization, probes containing SNPs with minimum allelic frequency < 0.05 were removed from the data, leaving 427,909 probes for subsequent analysis.

2.2 Differential methylation analysis

In this study, the raw methylation score for each probe was expressed as methylation β-values. The β-value is a quantitative methylation measure for CpGs, ranging from 0 (complete unmethylation) to 1 (full methylation). The β-values of individual CpGs from the two conditions were obtained, and the mean β-value difference of each CpG between two conditions was measured. CpG sites with absolute mean β-value differences < 0.05 were removed from this study. Moreover, a Student's t-test was employed to compare the β-values of CpGs between obese women with IR and those without IR. A threshold of $p < 0.01$ was used in this study. Statistically significant differences between groups were required to show a false discovery rate (FDR) <0.05 following multiple-test correction by the Benjamini–Hochberg method.

It is well known that methylation changes preferentially exist in genomic locations at intermediate methylation levels ($0.2 < \beta < 0.8$) [27]. To reduce non-variable CpGs, only CpGs with $0.2 <$ β-values < 0.8 were retained for subsequent analysis. In addition, a previous study indicated that many CpGs with β-value differences < 0.2 were difficult to reproduce [28]. Thus, only CpGs with mean β-value differences ≥ 0.2 were considered as significantly different in our study.

2.3 Network analysis

After obtaining differentially methylated genes in IR obese women, we performed a network analysis to explore co-regulated genes and reveal the biological functions of those proteins. In this study, the protein-protein interaction (PPI) data were obtained from the Search Tool for the Retrieval of Interacting Genes (STRING, http://string-db.org/) database. Each interaction in STRING is given a combined-score, reflecting the reliability of the interaction [29]. Here only the interactions with combined-score ≥ 0.4 were selected to construct the PPI sub-network, and the network was visualized by Cytoscape (v3.4.0, http://www.cytoscape.org/).

2.4 Functional analysis

Generally, co-regulated genes tend to perform similar biological functions. To analyze the underlying biological functions of genes involved in the differential methylation of CpGs, function analyses were implemented based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes database (KEGG), respectively, using the online tool Database for Annotation, Visualization and Integrated Discovery. The significance of biological functions was measured by Fisher's test, and p-values were adjusted by Benjamini & Hochberg false discovery rate. FDR < 0.01 was used here.

3 Results

3.1 Differential methylation analysis

This study focused on the differential methylation detected between insulin resistant and insulin sensitive obese women. This dataset contained 485,577 probes (covering 21,231 genes). Following methylation data preprocessing, the methylation data for 427,909 autosomal CpG sites in 79 individuals remained for differential methylation analysis. Initial analysis identified 10,874 differentially methylated CpGs (covering 5940 genes), including 7402 hyper-methylated sites and 3472 hypo-methylated CpGs, under the criteria of absolute mean β-value differences > 0.05 and p < 0.01. A distribution of differential methylation CpGs is illustrated in Figure 1.

Since methylation changes seldom fall within genomic regions at the extreme ends of methylation values (β-values < 0.2 or β-values > 0.8), only CpGs bearing 0.2 < β-values <0.8 were retained in our study, leaving 10,871 CpGs (covering 5938 genes) for subsequent analysis. Following the removal of CpGs with β-value differences < 0.2, only four CpGs were considered to show significantly differential methylation between insulin resistant and insulin sensitive obese women. These genes were *SMYD3*, *UST*, *BCL11A*, and *BAI3*. Figure 2 shows intuitively illustrated DNA methylation patterns of four differentially methylated genes.

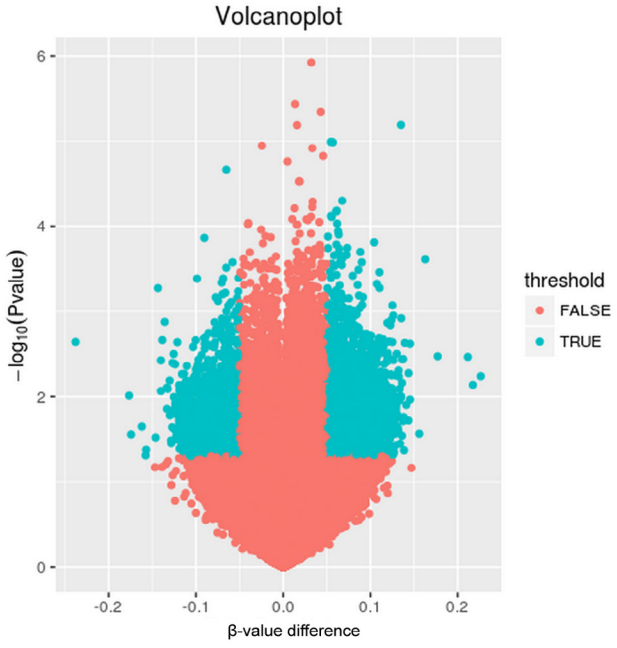

Figure 1. Volcano plot exhibits the distribution of differential methylation CpG sites in obese women with insulin resistance. The abscissa indicates the mean methylation differences between insulin resistant and insulin sensitive obese women, and the ordinate shows the log transformed p value. Only 4 CpG sites were considered to be significantly differentially methylated (blue). True, indicates the genes that are significantly up-regulated; False, indicates the genes that are significantly down-regulated.

3.2 Network analysis

Based on the STRING PPI data, we explored co-regulated genes of the four identified differentially methylated

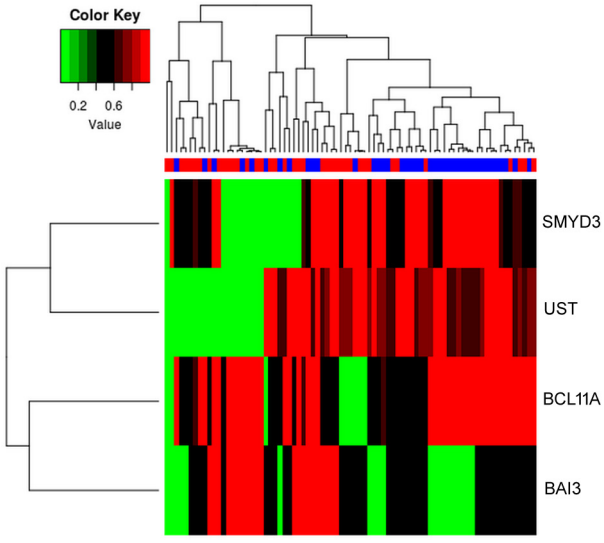

Figure 2. Hierarchical clustering illustrates DNA methylation patterns of the four differentially methylated genes in insulin resistant and insulin sensitive obese women. Each row is an individual CpG site and each column is a different sample. Color gradation from green to red denotes low to high DNA methylation, with color key ranging from 0 (no methylation; green) to 1 (complete methylation; red). The mean percent methylation value for each probe (red–blue scale) is the mean methylation value, after adjustment for covariates, for all samples.

genes. Under the criterion of edge combined-score ≥ 0.4, reliable interacting partners of the candidate genes were selected to construct the sub-networks using Cytoscape. Only the top 10 interactors were visualized in this study, as illustrated in Figure 3. Characteristics of the sub-networks related to the differentially methylated genes are shown in Table 1.

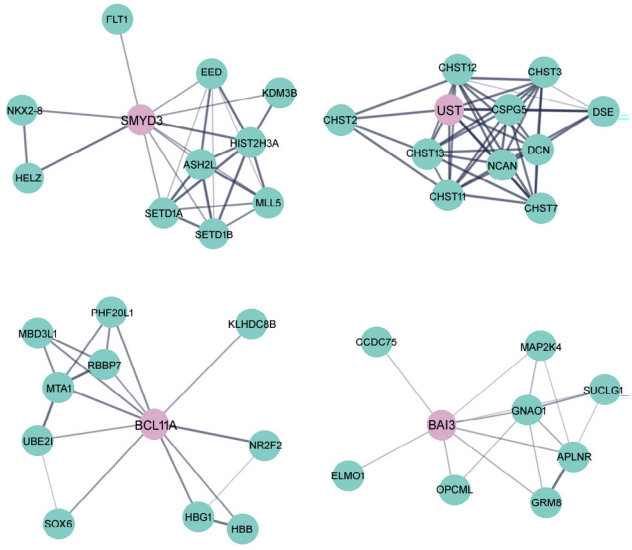

Figure 3. The protein-protein sub-networks of four differentially methylated genes and their co-regulated genes.

3.3 Functional analysis

After identifying four sub-networks, we further investigated their related functions. Our results showed that the differentially methylated genes and co-regulated genes were predominantly involved in two biological processes, namely, chondroitin sulfate ($p = 2.89\times10^{-13}$) and dermatan sulfate biosynthesis ($p = 3.45\times10^{-8}$) (Table 2). Interestingly, KEGG pathway analysis found that the differentially methylated genes were significantly enriched in the glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate pathway ($p = 9.60\times10^{-9}$).

4 Discussions

The frequency of obesity is growing quickly worldwide. Obesity is linked to many metabolic complications, including insulin resistance, hypertension, type 2 diabetes, nonalcoholic fatty liver disease, polycystic ovarian disease and several types of cancers [1, 6]. Epigenetics is known to play significant roles in complex diseases, and has attracted increasing attention from investigators, providing mechanisms where environmental factors can influence complex diseases. As reported, changes in DNA methylation may contribute to the metabolic improvements observed after bariatric surgery, and have an association with calorie restriction induced weight loss [4, 22, 23, 30].

In this study, we investigated the DNA methylation patterns of insulin resistance in obese women. Our work was undertaken with the purpose of identifying unique methylation biomarkers associated with obesity-related insulin resistance using a genome wide analysis.

Our initial analysis revealed extensive changes to the DNA methylation profiles between insulin resistance and insulin sensitive obese women, including 10,874 differentially methylated CpGs (covering 5940 genes). It is well established that CpG islands can be found in promoter regions [31]. Approximately 60% of all human genes, particularly ubiquitously expressed housekeeping genes, are transcribed from CGI promoters, making these loci critical functional elements in the human genome. Multiple lines of evidence suggest that transcriptional

Table 1. The characteristics of the sub-networks related to differentially methylated genes.

Sub-network	Origin	Gene number	Edge number	Mean degree	Clustering coefficient
A	SMYD3	11	27	4.91	0.922
B	UST	11	46	8.36	0.908
C	BCL11A	11	19	3.45	0.815
D	BAI3	9	16	3.56	0.835

Table 2. The biological processes of the differentially methylated genes and the co-regulated gene mainly participated in

GO Term	Genes	FDR
GO:0030206~chondroitin sulfate biosynthetic process	CHST7, CHST12, CHST11, CHST3, CHST13, CSPG5, DCN, NCAN, DSE	0.000000000000289
GO:0030208~dermatan sulfate biosynthetic process	CHST12, UST, CSPG5, DCN, NCAN, DSE	0.0000000345

activity is required for protecting CGI promoters from DNA methylation. For instance, a strong active promoter is required to maintain the unmethylated paternal allele of the imprinted Airn CGI in murine embryonic stem cells (ESCs) [32]. Recent human methylome data show that the level of protection against DNA methylation at promoter regions is directly correlated to transcriptional output [33]. Taken together, these findings imply that transcription initiation at CGI promoters is crucial for resisting DNA methylation.

Further analysis identified four significantly differentially methylated genes, including *SMYD3*, *UST*, *BCL11A*, and *BAI3*. Functional analysis found that the differentially methylated genes are mainly involved in glycosaminoglycan and chondroitin sulfate/dermatan sulfate biosynthetic processes.

SMYD3, a member of SET and MYND-domain family, is a histone methyltransferase that catalyzes methylation of various histone amino-acid residues, and its expression is altered in various human diseases [34-37]. A previous study indicated that SMYD3 was an obesity-related gene and associated with body mass index (BMI) in pig and human [38]. Rossi et al. [39] also found that SMYD3 was significantly hypermethylated in obese women and diet-induced obese mice. UST (uronyl-2-sulfotransferase) catalyzes the transfer of a sulfate group to dermatan or chondroitin sulfate. Dermatan sulfate is one of a major glycosaminoglycans abundant in eukaryotes and its complex template-independent biosynthesis involves at least 21 different enzymes [40, 41]. Glycosaminoglycans are large linear polysaccharides constructed of repeating disaccharide units with chondroprotective effects [42]. Dermatan and chondroitin sulfates form the polysaccharide part of chondroitin sulfate proteoglycans, which are an abundant subtype of proteoglycans in the extracellular matrix of the nervous system [43]. Obesity is a chronic inflammatory process, characterized by activation of the immune system and endothelium [44,45]. Interestingly, dermatan sulfate as an anticoagulant and antithrombotic glycosaminoglycan, also has anti-inflammatory activity [46]. Chondroitin sulfate has been shown to play a role in the activation of pro-inflammatory pathways in obesity in obese animal studies [47]. In chondroitin sulfate-treated obese mice, a reduction of the number of macrophages in arteries and serum inflammatory cytokines pointed to a possible role for chondroitin sulfate in interfering with the inflammatory response of endothelium and monocytes in obesity [48-50]. Previous studies have also indicated that chondroitin sulfate treatment could alleviate hyperglycemia and improve glucose metabolism in insulin resistant mice [51, 52].

In conclusion, this work revealed extensive DNA methylation changes and identified four significantly differentially methylated genes in obesity-related insulin resistance. Chondroitin and dermatan sulfate biosynthetic processes were attributed to being the underlying biological processes influencing the pathogenesis of obesity-related insulin resistance. Our study proposes several epigenetic marks related to obesity- associated insulin resistance, and provides new insights into epigenetic regulation in disease etiology and new targets for disease treatment.

Ethical approval: The conducted research is not related to either human or animals use.

Conflict of Interest: Authors state no conflict of interest.

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