An insight into the key genes and biological functions associated with insulin resistance in adipose tissue with microarray technology

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Abstract. In the present study, the key genes and biological functions associated with insulin resistance were investigated by comparing the gene expression profiles of adipose tissue obtained from insulin-sensitive and insulin-resistant patients. The gene expression data set GSE20950 was downloaded from the Gene Expression Omnibus, including 39 adipose tissue samples obtained from insulin-sensitive and insulin-resistant patients undergoing gastric bypass surgery. Adipose samples were divided into two groups (the insulin-sensitive and insulin-resistant groups) and the differentially expressed genes (DEGs) were screened out with packages of *R*. The interactions among DEGs were retrieved with Osprey and functional enrichment analysis was performed with the WebGestalt system. Information regarding the interaction network and enriched biological functions was combined to construct a functional interaction network. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was then conducted using the Database for Annotation, Visualization and Integrated Discovery. A total of 170 DEGs were detected in the insulin-sensitive group, 8 downregulated and 162 upregulated. Response to glucose stimulus was the most significantly over-represented functional term. The focal adhesion pathway was identified to be significant in the genes of the functional interaction network. The present study revealed key biological functions and DEGs in adipose tissues associated with insulin resistance, which may facilitate the development of novel therapies for insulin resistance and diabetes.

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Introduction

Diabetes is caused by an absolute or relative deficiency of insulin. It is one of the most prevalent of all chronic diseases, which is characterized by high blood sugar and multiple secondary complications. Obesity is a risk factor for insulin resistance, a precursor of type 2 diabetes, which involves a decreased response to insulin signaling in several peripheral tissues including adipose, liver and muscle (1,2). However, not all obese individuals are insulin-resistant (3).

Adipose tissue secretes numerous hormones and cytokines that function to regulate food intake and nutrient homeostasis, including insulin-like growth factor 2, leptin and resistin (4), and its role in the regulation of metabolism has been increasingly recognized in recent years (5). Previous studies have revealed a number of linkages between obesity and insulin resistance. Obesity-associated chronic inflammation in adipose tissue has a crucial role in the development of obesity-related insulin resistance (6-9). Adipocyte-derived cytokines are important in the pathogenesis of insulin resistance and type 2 diabetes (10). Hirosumi *et al* (11) indicated that the c-Jun amino-terminal kinase is a crucial mediator of obesity and insulin resistance. However, the molecular mechanisms underlying this effect remain elusive.

In the present study, the gene expression profiles of adipose tissue samples obtained from insulin-sensitive and insulin-resistant patients were compared with the aim of identifying differentially expressed genes (DEGs). Bioinformatic analysis, including interaction network analysis and functional enrichment analysis, were performed to disclose the key biological functions associated with insulin resistance. These findings may advance the understanding of insulin resistance and benefit the development of novel treatment strategies.

Materials and methods

Microarray data. Microarray data set GSE20950 (12) was downloaded from the Gene Expression Omnibus, including 39 subcutaneous and omental adipose samples, obtained from insulin-sensitive and insulin-resistant obese patients undergoing gastric bypass surgery. The platform was GPL570

[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA). The probe annotation files were also collected.

Raw data pre-treatment and differential analysis. All of the adipose samples were divided into two groups: The insulin-sensitive group and the insulin-resistant group. Using software *R*, CEL raw expression data were converted into gene expression values, which were then normalized by the median method (13,14). DEGs between the two groups were screened out with the limma package (15) and multiple testing corrections were applied on the P-value with the Benjamin-Hochberg method (16). Only the genes with a false discovery rate (FDR)<0.05 and |logFC(fold change)|>1 were selected as DEGs.

Interaction network and functional enrichment analysis. The interactions between DEGs were investigated with Osprey (17), which is designed to promote research into protein, protein interactions (PPIs) and protein complexes, and which integrates information from the Biomolecular Interaction Network Database (18) and the General Repository for Interaction Datasets (19), and contains >50,000 interactions.

Functional enrichment analysis was applied on the DEGs in the network using WebGestalt (20,21). P<0.05 was set as the threshold.

Functional interaction network and pathway enrichment analysis. Based on the interaction network and functional enrichment analysis result, a functional interaction network was constructed.

Kyoto Encyclopedia of Genes and Genomes (KEGG) (22) pathway analysis was applied on the genes in the functional interaction network using the Database for Annotation, Visualization and Integrated Discovery. P<0.05 was set as the threshold.

Results

DEGs. The normalized gene expression profiles are revealed in Fig. 1. Differential analysis was performed between the insulin-sensitive and insulin-resistant groups. A total of 170 DEGs were identified in the insulin-sensitive group, of which 8 were downregulated and 162 were upregulated. This result suggested that numerous genes in the adipose tissue obtained from insulin-resistant patients were downregulated and thus contributed to insulin resistance.

Interaction correlation and relevant biological functions. Interactions among DEGs were retrieved using Osprey. In the present study, a total of 33 interactions were obtained (Table I).

Functional enrichment analysis was performed with WebGestalt for all of the gene interactions and eight functional terms were revealed (Table II). Response to glucose stimulus was the most significant functional term and three genes were included; neuronatin (NNAT), transforming growth factor beta receptor II (TGFBR2) and phosphatase and tensin homolog (PTEN).

Functional interaction network and significant pathways. The interaction network was integrated with the functional enrichment analysis result to construct the functional interaction network (Fig. 2).

Pathway enrichment analysis revealed that focal adhesion (hsa04510, P=0.03695) was over-represented in the genes (Fig. 3). Three DEGs were included in this pathway: Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β (PIK3CB), integrin α2 (ITGA2) and PTEN. Focal adhesions are specific types of large macromolecular assemblies through which mechanical force and regulatory signals are transmitted. They serve as the mechanical linkages to the extracellular cell matrix and as a biochemical signaling hub to concentrate and direct numerous signaling proteins at sites of integrin binding and clustering. As a result, focal adhesions

Table I. Interactions between the DEGs.

Interactor 1	Interactor 2
TXN	PTEN
RAD23B	ZFAND5
NOP16	GART
ITGA2	FLT4
PIK3CB	FLT4
MMP9	PIK3CB
ETS1	MMP9
PTEN	PIK3CB
ETS1	MCM8
PTEN	YES1
PTEN	IRS1
IRS1	LIPE
NDUFA7	ATP5O
ZFYVE16	PIK3CB
ITGA2	COL8A1
AZGP1	PIP
HEYL	MAML1
MMP9	PTEN
NOP16	POLR3E
FZD7	SFRP1
TGFBR2	ZFYVE16
ETS1	PURA
TXN	MMP9
MCM8	PTEN
TXN	DLST
TXN	ATP5O
MCM8	ANTXR2
AGPAT5	AGPAT9
IRS1	PIK3CB
DLST	ATP5O
NDUFA7	ENSG00000109390
MCM8	PURA
SMC3	BCLAF1

DEGs, differentially expressed genes; TGFBR2, transforming growth factor β receptor II; PTEN, phosphatase and tensin homolog; PIK3CB, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β; ITGA2, integrin α2.

Table II. Functional terms enriched in DEGs from the interaction network.

Term	Count	P-value	Genes
GO:0009749~response to glucose stimulus	3	0.000444	NNAT, TGFBR2, PTEN
GO:0006796~phosphate metabolic process	17	0.002208	SGK2, PIK3CB, FLT4, NDUFA7, PTPN4, PDK4, TGFBR2, WNK1, NDUFC1, PTEN, DUSP4, PDIK1L, DUSP16, BMP2K, ATP5O, YES1, LIPE
GO:0048008~platelet-derived growth factor receptor signaling pathway	3	0.008688	ZFAND5, TIPARP, PTEN
GO:0060021~palate development	3	0.023668	TGFBR2, TIPARP, PYGO2
GO:0046486~glycerolipid metabolic process	5	0.033067	PIK3CB, AGPAT9, CHPT1, PTEN, LIPE
GO:0051272~positive regulation of cell motion	4	0.036903	ETS1, MMP9, ITGA2, IRS1
GO:0010033~response to organic substance	11	0.042616	DUSP4, GATM, NNAT, TGFBR2, ITGA2, EDEM3, IRS1, PTEN, PGGT1B, TTC3, GART
GO:0007167~enzyme linked receptor protein signaling pathway	7	0.042853	ZFAND5, FLT4, ZFYVE16, TGFBR2, TIPARP, IRS1, PTEN

DEGs, differentially expressed genes. NNAT, neuronatin; TGFBR2, transforming growth factor β receptor II; PTEN, phosphatase and tensin homolog; PIK3CB, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β; ITGA2, integrin α2.

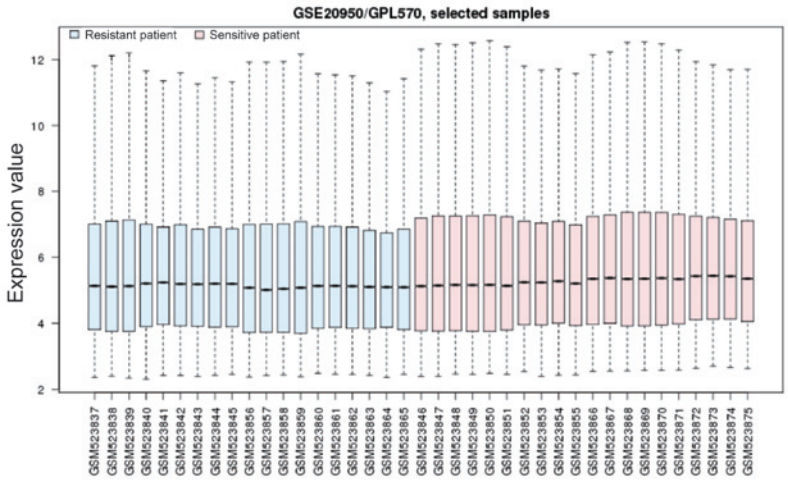

Figure 1. Box plot for normalized gene expression data. Adipose tissue samples from insulin-resistant patients are in blue and those from insulin-sensitive patients are in pink. The medians (black lines) are almost at the same level, indicating a good performance of normalization.

may lead to insulin-resistance and therefore require further investigation.

Discussion

Adipose tissues have an important role in the development of insulin resistance and thereby contribute to the incidence of type 2 diabetes. However, not all obese individuals exhibit insulin resistance. Therefore, understanding the underlying mechanisms involved is important, as it may benefit the development of novel therapeutic strategies for diabetes treatment. In the present study, the transcriptome of adipose tissues obtained from insulin-sensitive and insulin-resistant patients were compared with the aim of identifying the key DEGs and the associated biological functions. A total of 170 DEGs were obtained, the majority of which were upregulated in the insulin-sensitive group. The interactions among DEGs were retrieved and the functional enrichment analysis on these DEGs revealed eight significant functional terms, the majority of which were associated with metabolism and signaling pathways. In the KEGG pathway analysis, focal adhesion was identified to be significant.

The results revealed that response to glucose stimulus was the most significant biological function. This was in accordance with the role of adipose tissue in insulin resistance. Three DEGs were included in this term: NNAT, TGFBR2 and PTEN. TGFBR2 has previously been associated with obesity (23). It has been proposed that upregulation of TGFBR2 induced by high extracellular glucose, may contribute to distal tubular hypertrophy in diabetic nephropathy (24). A study by Yang *et al* (25) demonstrated that TGF-β signaling in hepatocytes participates in steatohepatitis through the regulation of cell death and lipid metabolism. PTEN is a key negative regulator of insulin-stimulated glucose uptake *in vitro* and *in vivo* (26). Lo *et al* (27) identified that an increase in PTEN gene expression appears to be associated with the development

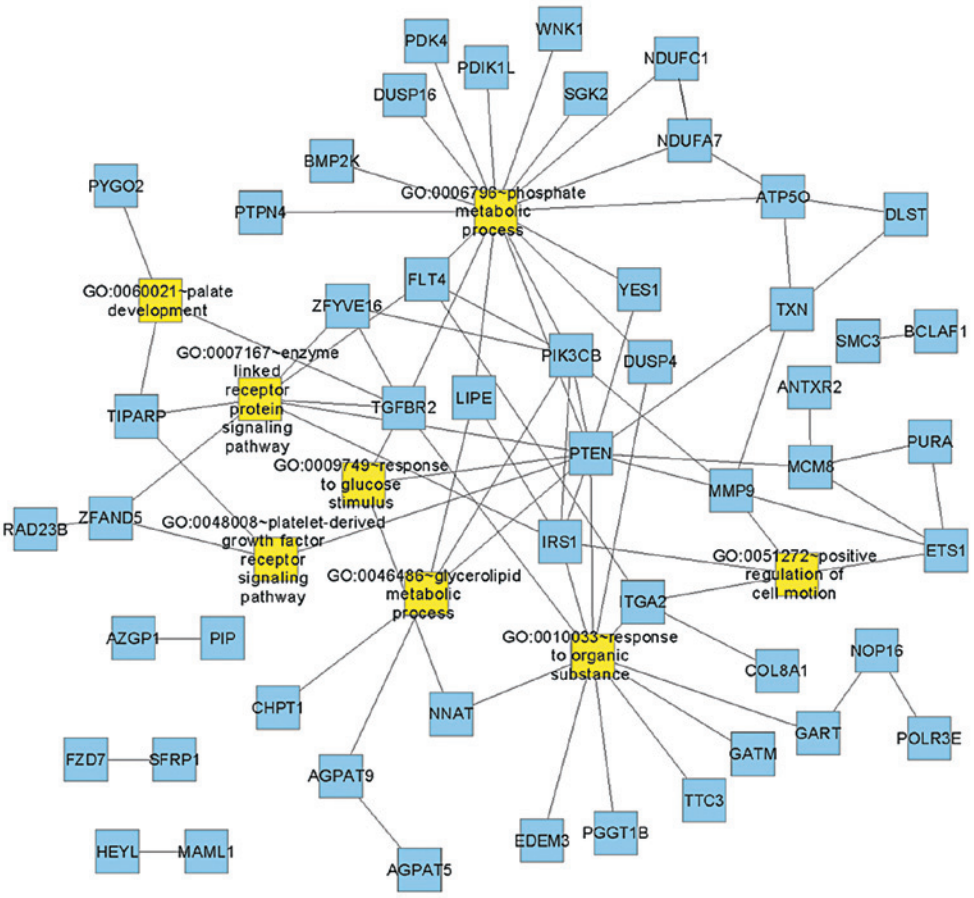

Figure 2. Functional interaction network. Blue rectangles represent differentially expressed genes and the yellow rectangles represent the over-represented functional terms. TGFBR2, transforming growth factor β receptor II; PTEN, phosphatase and tensin homolog; PIK3CB, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β; ITGA2, integrin α2.

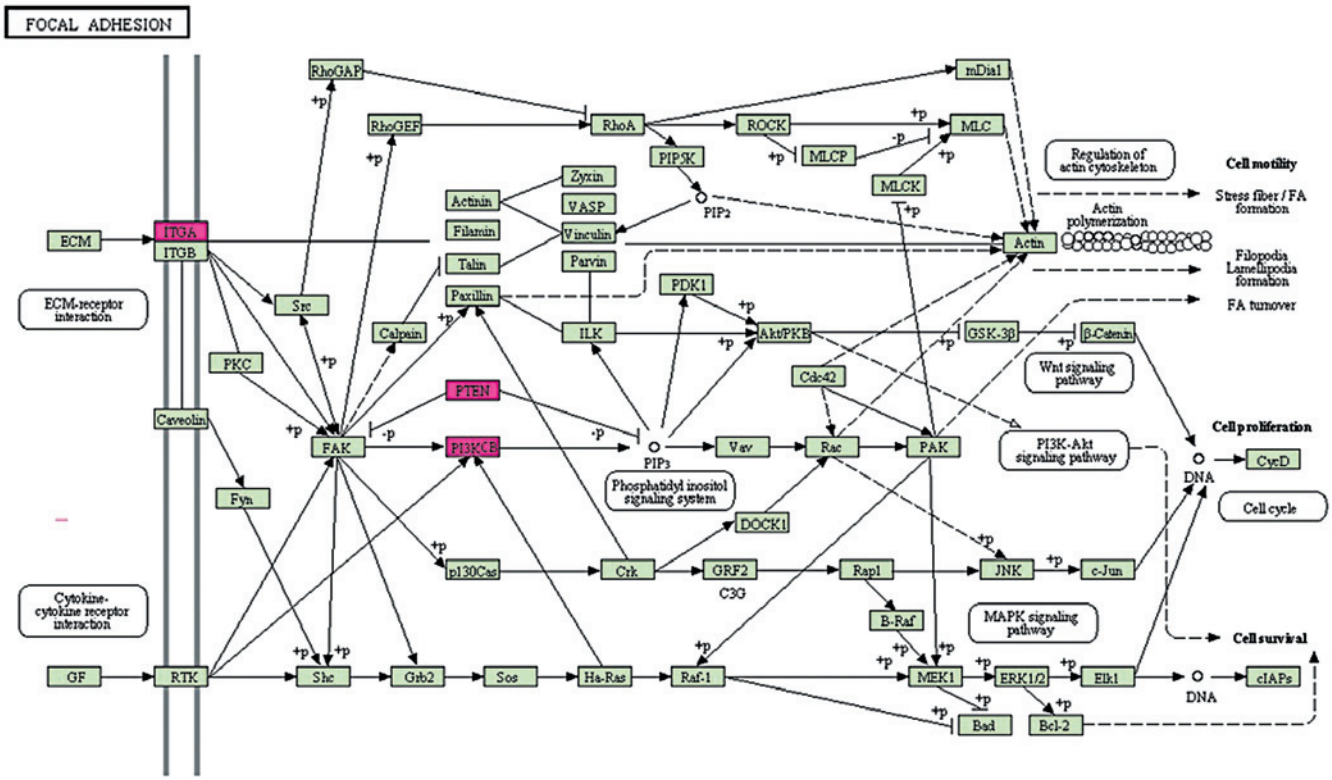

Figure 3. Focal adhesion pathway. Differentially expressed genes are shown in red and the other genes are represented in green. ECM, extracellular matrix; PTEN, phosphatase and tensin homolog; PIK3CB, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit β; ITGA2, integrin α2.

of insulin resistance. Butler *et al* (28) found that inhibition of PTEN expression reversed hyperglycemia in diabetic mice. Nakashima *et al* (29) reported that PTEN negatively regulates insulin signaling in 3T3-L1 adipocytes. Tang *et al* (30) further indicated that PTEN suppresses insulin signaling through the phosphatidylinositol 3-kinase/Akt pathway in 3T3-L1 adipocytes.

The focal adhesion pathway is closely associated with the insulin signaling pathway. Cell adhesion and focal adhesion kinases regulate insulin receptor substrate-1 expression (31). Baron *et al* (32) identified that p125Fak focal adhesion kinase is a substrate for the insulin and insulin-like growth factor-I tyrosine kinase receptors. Bisht *et al* (33) reported that focal adhesion kinase regulates insulin resistance in skeletal muscle. Three DEGs were identified in this pathway, including PIK3CB, ITGA2 and PTEN. PIK3CB is an isoform of the catalytic subunit of phosphoinositide 3-kinase (PI3K), which is important in the signaling pathways involving receptors on the outer membrane of eukaryotic cells. Le Stunff *et al* (34) identified a variant GATA-binding site in the PIK3CB promoter is a *Cis*-acting expression quantitative trait locus for this gene and attenuates insulin resistance in obese children, confirming the involvement of this gene in insulin resistance. In another study by Clément *et al* (35) it was suggested that the *cis*-regulatory rs361072 promoter variant of PIK3CB is associated with insulin resistance.

In conclusion, the present study described the molecular signatures of adipose tissues obtained from insulin-resistant and insulin-sensitive obese patients. Through comparative analysis, a number of DEGs and relevant biological functions were revealed. These findings provided a theoretical basis and direction for novel strategies for the therapeutic management of diabetes.

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