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Research paper

Gene expression profiles reveal effect of a high-fat diet on the development of white and brown adipose tissues

Hyeng-Soo Kim [a], Zae Young Ryoo [a], Sang Un Choi [b], Sanggyu Lee [a,*]

[a] School of Life Science, BK21 plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 702-701, Republic of Korea
[b] Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea

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ABSTRACT

Because of the recent discovery of brown adipose tissues tissue in adult humans, brown adipose tissues have garnered additional attention. Many studies have attempted to transform the precursor cells within the white adipocyte cultures to Brite (brown-in-white) cells by using genomic modification or pharmacological activation in order to determine the therapeutic effect of obesity. However, genome-scale analysis of the genetic factors governing the development of white and brown adipose tissues remains incomplete. In order to identify the key genes that regulate the development of white and brown adipose tissues in mice, a transcriptome analysis was performed on the adipose tissues. Network analysis of differentially expressed genes indicated that *Trim30* and *Ucp3* play pivotal roles in energy balance and glucose homeostasis. In addition, it was discovered that identical biological processes and pathways in the white and brown adipose tissues might be regulated by different genes. *Trim30* and *Ucp3* might be used as genetic markers to precisely represent the stage of obesity during the early and late stages of adipose tissue development, respectively. These results may provide a stepping-stone for future obesity-related studies.

1. Introduction

Obesity is a metabolic disorder associated with the diet and level of physical activity of an affected individual, as well as other social and cultural factors. The most critical factor affecting obesity is the expression (quantity) and size of adipose tissue in the body. Adipose tissue plays a role in lipid storage, adipokine secretion, thermogenesis, and glucose homeostasis, thereby regulating energy balance (Rosen and Spiegelman, 2006). Adipose tissue can be divided into the classic brown, brite (brown-in-white), and white adipocytes (Petrovic et al., 2010). Brown adipose tissue (BAT) has a different origin and function compared to the brite and white adipose tissues (WAT). WAT is derived from various cells, such as mesenchymal stem cells, vascular endothelial cells, and neural crest stem cells, and plays a role in the storage of excess energy and mobilization of free fatty acids (FFA) when required. On the other hand, BAT originates from the myotomal precursors and vascular endothelial cells, and expresses an abundance of mitochondria. BAT also expresses multilocular small lipid droplets, which are expended during non-shivering thermogenesis in response to cold stimulation, assisting in maintaining a homeostatic body temperature (Timmons et al., 2007).

For many years, BAT was known to exist only in rodents and newborn babies. However, recent studies have shown that BATs are also expressed in the cervical, supraclavicular, and paraspinal regions of adult humans. Therefore, extra attention has been focused on BAT, because of its thermogenic capacity and potential for reducing obesity (Celi, 2009; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Sacks and Symonds, 2013). The pharmacological activation of BAT, by agents such as the beta-3 adrenergic receptor (ADRB3) agonist, induces weight loss and improves insulin-sensitization in rodents (Arch, 2002). In addition, the use of a peroxisome proliferator-activated receptor γ (PPARγ) agonist, such as thiazolidinedione (TZD), not only improves insulin resistance and increases mitochondrial biogenesis for fatty acid oxidation, but also promotes the browning of white adipose tissue via the stabilization of the PRDM16 protein and SirT1-dependent deacetylation of PPARγ in rodents (Spiegelman, 1998; Wilson-Fritch et al., 2004; Ohno et al., 2012; Qiang et al., 2012). Brite cells are transformed from white adipocytes or develop from precursor cells within white adipocyte cultures by genomic modification, for example through the ectopic expression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1a) and the forkhead box 14 C2 (FOXC2) (Cederberg et al., 2001; Tiraby et al., 2003). In addition, previous research has shown that the over-expression of cyclooxygenase (COX-2) in WATs led to

Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; HFD, high-fat diet; ND, normal diet; PPI, protein–protein interaction; GSEA, gene set enrichment analysis; DEG, differentially expressed gene; FDR, false discovery rate.

* Corresponding author at: School of Life Science and Biotechnology, Kyungpook National University, Daegu, Republic of Korea.
E-mail address: slee@knu.ac.kr (S. Lee).

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increased prostaglandin synthesis and de novo BAT recruitment to the stromal-vascular cell fraction of the murine adipose tissue, resulting in increased energy expenditure and protection against obesity (Vegiopoulos et al., 2010). The discovery of such regulatory factors has important implications for the development of potential obesity treatment strategies.

In addition, the body fat content, including the fat-free mass and total body fat, reaches a peak in people between 40 and 70 years of age in the US, depending on the ethnicity and gender (Chumlea et al., 2002). Aging reduces the sensitivity to sympathetic tone, and induces changes in the endocrine control of BAT formation, weakening the regeneration in brown adipogenic progenitor cells. In addition, mitochondrial biogenesis and bioenergetics are also reduced in these cells via the decline in the expression of uncoupling protein (UCP)-1 in the inner mitochondrial membrane (Graja and Schulz, 2014).

In order to determine the differences between BAT and WAT, previous study groups have analyzed the transcriptome of both tissues at specific time points following a high-fat diet, and investigated the pathways and functions of the differentially expressed genes. Whole-genome transcriptional analysis must be conducted at multiple time points since the analysis at single time points is not sufficient for the elucidation of the developmental status and differences between BAT and WAT. In order to identify the key genes regulating WAT and BAT development, we performed a serial genome-wide transcriptome analysis of WAT and BAT extracted from mice provided with a normal, as well as high-fat, diet for 24 weeks. Our results might provide new insights into the physiological basis of obesity.

2. Materials and methods

2.1. Animals and tissue collection

The animals were fed with normal (ND) and high-fat diet (HFD) in order to evaluate the changes in gene expression levels in the adipose tissues of C57BL/6J mice, a diet-induced obesity-prone animal model (Table S1). Eighteen mice from each of the ND and HFD groups were anesthetized with ether at 2, 4, 8, 20, and 24 weeks after a 12 h fasting period. Epididymal adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were removed and stored in liquid nitrogen for RNA preparation. All experiments with animals were conducted using protocols approved by the Ethics Committee for animal studies of the Kyungpook National University.

2.2. RNA extraction and microarray

The WAT and BAT extracted from the 18 ND and HFD mice at each time point were homogenized using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The total RNA was isolated according to the manufacturer's protocols. The purity and integrity of the RNA samples were assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Total RNA from the ND and HFD groups were pooled within three sample sets, as described previously (Do et al., 2010). The pooled RNA was stored at −80 °C until further experimentation. Total RNA from six sample sets for each time point was amplified and purified using the Ambion Illumina RNA amplification kit (Ambion, Foster City, CA, USA) as per the manufacturer's protocols, in order to yield biotinylated cRNA. Labeled cRNA samples were hybridized to a mouse-6 expression bead array (Illumina MouseWG-6 v2 Expression BeadChip; Illumina, San Diego, CA, USA) according to the manufacturer's protocols. The raw data of the spot fluorescent intensities was extracted using the Illumina Beadstudio software.

2.3. Computational analyses

The quality of the expression data was assessed and the raw data were quantile normalized using a bead array (Figs. S1, S2) (Dunning et al., 2007). The genes expressed differentially between the mice provided with HFD and ND were selected for each time point using the linear models for microarray data (Limma) (Smyth, 2004) based on the False Discovery Rate (FDR), applying the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). The functional annotation tool DAVID (http://david.abcc.ncifcrf.gov/) was used for gene ontology (GO) mining and pathway analysis (Huang et al., 2008). The Michigan molecular interactions (MiMI) Cytoscape plugin was utilized to determine genome-based protein–protein interactions (Jayapandian et al., 2006). The hub objects analyzer (Hubba) was utilized to define the hub, all key genes, and the significant network computing base at the bottleneck nodes, based on centrality statistics (Lin et al., 2008). The distribution of the curated gene sets was compared between the mice provided with HFD and ND by gene set enrichment analysis (GSEA software v2.0) using the default parameters (Subramanian et al., 2005). The curated gene sets obtained from the molecular signature database (MSigDB v4.0) were utilized for all GSEA analyses. The epididymal white adipose tissue microarray data was deposited and exploited in the gene expression omnibus (GEO) database (GEO accession number: GSE39549). The complete expression data for brown adipose tissues was also submitted to the GEO database (GEO accession number: GSE64718).

2.4. Statistical analysis

The selection of genes expressed differentially between mice fed with ND and HFD was analyzed using the Limma package in R statistical language; FDR < 0.1 was considered to be statistically significant. The chromosome distribution, biological processes of gene ontology, and the pathways resulting from the differentially expressed genes were analyzed using the DAVID platform; a modified Fisher exact p-value < 0.01 was considered statically significant.

3. Results

3.1. Gene expression profiles of white and brown adipose tissues

A microarray analysis was performed on serial adipose tissue samples collected over 24 weeks in order to identify the changes in gene expression in WAT and BAT of mice, in response to a high-fat diet. The gene expression levels were compared between the high-fat and normal diet groups every week, resulting in the identification of differentially expressed genes (DEGs) with statistical significance (FDR < 0.1) (Table 1, Table S2). The number of DEGs in WAT was approximately half of the total transcripts analyzed at 2 weeks; a drastic decrease was observed in the number of DEGs within 4 and 8 weeks after initiation. There was a gradual increase in the number of DEGs at 20 and 24 weeks. These results indicated that the WAT responded in terms of changes in the gene expression levels early on after commencing the high-fat diet. However, BAT displayed a different trend. The number of DEGs in BAT showed a small but steady increase 2, 4, 8, and 20 weeks after initiation of the diet. However, this number increased drastically at 24 weeks. In addition, the DEGs were presented in only a few chromosomes of the adipose tissues, such as chromosomes 11 and 19 in WAT and chromosome 11 in BAT (Table S3). In particular, the genes located on chromosome 11 were expressed differentially in the WAT and

Table 1
The distribution of differentially expressed genes in the white and brown adipose tissues of mice fed with a high-fat diet, compared to that observed in mice fed with a normal diet.

		Week				
		2	4	8	20	24
Epididymal fat	Up	6983	228	377	1851	2655
	Down	9716	132	181	1867	2456
Interscapular brown fat	Up	197	162	348	542	1845
	Down	178	130	446	449	1579