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Comparison of gene expression profiles between white and brown adipose tissues of rat by microarray analysis

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Abstract

To characterize the energy metabolism in brown adipose tissue (BAT), the differences in gene expression profiles between BAT and white adipose tissue (WAT) were analyzed using a high-density cDNA microarray. RNAs isolated from two adipose tissues were hybridized to an Agilent rat cDNA Microarray that contained about 14,500 cDNA probe sets. The expression levels of 499 cDNA/ESTs were found to be at least 5-fold higher or lower in BAT than in WAT. Consistent with our previous findings, high expression levels of genes encoding uncoupling protein 1, muscle-type carnitine palmitoyltransferase and some other proteins involved in energy metabolism in BAT were found. Most of the genes encoding mitochondrial proteins, such as subunits of ATP synthase, cytochrome *c* oxidase, and NADH dehydrogenase, were highly expressed, reflecting possible differences in the cellular content of mitochondria between BAT and WAT. However, the expression levels of several genes encoding mitochondrial protein, such as liver mitochondrial aldehyde dehydrogenase and dicarboxylate carrier, were remarkably lower in BAT. These results may give important clues to understand the unique energy metabolism in BAT.

Keywords: Brown adipose tissue; White adipose tissue; Energy metabolism; Microarray analysis

1. Introduction

It is well known that white (WAT) and brown (BAT) adipose tissues are present in mammals and represent counter actors in energy metabolism. The physiological role of WAT is to accumulate excess energy as fat, and that of BAT is its expenditure as heat. The unique function of BAT is the result of the presence of uncoupling protein 1 (UCP1) in mitochondria. In most tissues, the oxidation of fuel substrates results in ATP production through oxidative phosphorylation driven by the electrochemical gradient of H^+ ($\Delta\mu H^+$) across the mitochondrial inner membrane. However, in BAT, UCP1 dissipates $\Delta\mu H^+$ by acting as a H^+-conductor, thus increasing the capacity of oxidation of respiratory substrates without ATP synthesis (for reviews, see Refs. [1–4]).

It is generally thought that UCP1 is essential for efficient energy expenditure in BAT, but other mechanisms must be operative for effective thermoregulation. Based on this hypothesis, we attempted to identify other genes for which their expression levels are specifically regulated in BAT. In our previous studies, we showed that mRNA of muscle-type carnitine palmitoyltransferase (M-CPTI) may be expressed dominantly in BAT [5] and found an increase

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Abbreviations: ACC, acetyl-CoA carboxylase; BAT, brown adipose tissue; CC, carnitine carrier; Cy3 and Cy5, cyanine 3 and cyanine 5; DIC, dicarboxylate carrier; EST, expressed sequence tag; ICDH, isocitrate dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; LMADH, liver mitochondrial aldehyde dehydrogenase; LPL, lipoprotein lipase; MCAD, medium-chain acyl-CoA dehydrogenase; M- and L-CPTI, muscle- and liver-type carnitine palmitoyltransferase; NADH-U, NADH dehydrogenase; UCP, uncoupling protein; WAT, white adipose tissue.

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in the expression levels of genes encoding several proteins that are involved in lipid or glucose metabolism in BAT by cold exposure [6]. In our most recent research, we demonstrated that the expression levels of genes encoding three subunits of NAD^+-dependent isocitrate dehydrogenase (NAD^+-ICDH) were remarkably high in BAT [7].

Recently, great progress has been made in techniques for comparing differentially expressed genes, i.e. differential display PCR, serial analysis of gene expression (SAGE), and microarrays. Above all, the development of the high-density cDNA or oligonucleotide microarrays enables the expression levels of tens of thousands genes to be simultaneously monitored. In the present study, we report on a global analysis of gene expression in BAT and WAT using a cDNA microarray to characterize unknown energy metabolism in BAT.

2. Materials and methods

2.1. Materials

A rat cDNA microarray (Part No. G4105A) was purchased from Agilent Technologies Inc. A cDNA synthesis kit was also obtained from Agilent Technologies Inc. (Part No. G2555).

2.2. Isolation of total RNA

BAT depots were obtained from interscapular regions of five male Wistar rats (4- to 5-week old), as described previously [6]. Since BAT preparation could be easily contaminated by its surrounding tissues, we paid maximum attention to eliminate the others especially, such as connective tissue and muscle tissue. WAT depots were obtained from epididymal region of above five rats. To avoid differences between individuals, RNA samples were prepared from pooled tissues. Total RNA samples of these tissues were prepared by the guanidine thiocyanate method as described previously [8], and their concentrations were determined using a Shimadzu spectrophotometer, model UV160.

2.3. Hybridization and microarray scanning

The reverse transcription labeling and hybridization essentially followed the protocol recommended by Agilent Technologies Inc. intended for Agilent microarray analysis. Briefly, a 20 μg aliquot of each total RNA sample was reverse transcribed into a cDNA probe with oligo(dT) primer and labeled nucleotides. The reaction was carried out in a solution containing 50 μM dATP/dGTP/dTTP, 25 μM dCTP, 25 μM cyanine 3 (Cy3)-dCTP (for WAT sample) or cyanine 5 (Cy5)-dCTP (for BAT sample) (Enzo Diagnostics, Inc.) and 400 U MMLV reverse transcriptase at 42° for 1 hr. The labeling reaction was terminated by incubating the reaction mixture at 70° for 10 min. The RNA was then degraded by adding 0.05 μg RNaseIA, followed by incubation at 37° for 30 min. Degraded RNA and unincorporated nucleotides were removed using a QIAquick PCR Purification Kit (Qiagen Inc.) according to the instructions of Agilent Technologies Inc. Hybridization was carried out in 22 μL of a hybridization mixture containing cDNA probes, the labeled orientation marker (Deposition Control SP300; Operon Technologies Inc.) and mouse Cot-1 DNA (Invitrogen Corporation) at 65° for 17 hr. The glass slides were then washed with 0.5 × SSC and 0.01% SDS at room temperature for 5 min, and with 0.06 × SSC at room temperature for 2 min. After immediately removing the wash buffer by centrifugation, the glass slides were scanned using GenePix 4000B (Axon Instruments, Inc.) containing a 532 nm laser for Cy3 measurement and a 635 nm laser for Cy5 measurement. Scans were made with a pixel resolution of 5 μm, a laser power of 100%, and a photomultiplier tube voltage of 600 V for the 532 nm laser and 520 V for the 635 nm laser.

2.4. Normalization and analysis of microarray data

Sixteen-bit TIFF images produced by the Axon scanner were analyzed using the GenePixPro 3.0 (Axon Instruments, Inc.) software package. After obtaining Cy3 and Cy5 grayscale images, each pseudo-color image was overlaid, and all spots in the ratio image were defined by accessing the gene list file that described the location of each gene on the microarray. The average of the signal intensity was subtracted from the median of background intensity and outputted with the UniGene and GenBank descriptors to a Microsoft Excel data spreadsheet. Relative expression levels were calculated by global normalization between two samples using all detected genes, after the exclusion of spots annotated as ‘‘Agilent QC’’, ‘‘Agilent Blank’’, and ‘‘Buffer’’.

2.5. Preparation of cDNA probes

cDNA fragments of rat UCP1 (nucleotide 214–1013 in Accession No. M11814), UCP2 (1–930 in AB005143), M-CPTI (1619–2614 in D43623), lipoprotein lipase (LPL; 839–1463 in L03294), ATPase epsilon-subunit (ATPase ε; 2–379 in AF010323), carnitine carrier (CC; 511–854 in X97831), liver mitochondrial aldehyde dehydrogenase (LMADH; 1320–1856 in X14977), dicarboxylate carrier (DIC; 1306–1898 in AJ223355), NADH dehydrogenase (ubiquinone) 1α subcomplex 5 (NADH-U; 1–533 in D86215) and for acetyl-CoA carboxylase β (ACC2; 4878–6097 in AB004329) were prepared by reverse transcription followed by the polymerase chain reaction (RT–PCR). After confirmation of the nucleotide sequences of the amplified cDNA fragments, they were radiolabeled and used as probes. Cross-hybridization of the probes used

in this study was negligible under the stringent conditions used for the hybridization (data not shown).

2.6. Northern blot analysis

Northern blot analysis was carried out essentially as described previously [9]. Briefly, a 10 μg aliquot of each total RNA sample was subjected to denatured 1% agarose gel electrophoresis. The separated RNAs were then transferred to membrane filters, and the transcription levels of various proteins were examined by hybridization with the radiolabeled probes.

2.7. Preparation of antiserums and Western analysis

Antiserums against UCP1, M-CPTI, CC, NADH-U, and ε-subunit of F_1-ATPase were raised as described previously [10] using peptides with amino acid sequences of QSHLHGIKPRYTGTYNAYRC, RYGSYGTPQTETLLSMVIC, GVTSLYKGFNAVMIRAFPAC, ELSLARKMLQWKPWEPLVEC, and ANAEKTSGTSIKTVKIKKEC, respectively. For Western analysis, mitochondrial fractions were prepared from BAT and WAT by differential centrifugation [11]. Samples corresponding to 20 μg mitochondrial protein were subjected to electrophoresis in SDS–polyacrylamide gel and following immunodetection. Immunoreactive protein bands were visualized with ECL kit (Amersham).

3. Results

3.1. Microarray analysis of gene expression profiles in WAT and BAT

After the experiments of microarray analysis, all hybridization spots on the image were quantified. When plotting the intensities of the positive spots using a BAT probe vs. those of the corresponding spots by a WAT probe after global normalization, the plot showed a widely scattered pattern (Fig. 1). This indicates that the hybridization levels of a number of clones by the BAT probes were different from those obtained by WAT probes. When color swap experiment was performed, green spot in one array appeared red spot in the other array, and *vice versa*, like mirror images, indicating high reproducibility and reliability of experimental results (data not shown). Tables 1 and 2 show the expression levels of the top 100 genes that are preferentially expressed in BAT and WAT, respectively, in the order of the values of the ratio of expression levels between BAT and WAT. The last cDNA listed in each table still showed a high ratio of 5.02 and a low ratio of 0.11, respectively, indicating the substantial differences in expression levels of protein species between BAT and WAT. In Table 1, UCP1 showed the highest ratio of 125, indicating the highly selective expression of this message in BAT. Furthermore, as the general characteristics of Table 1

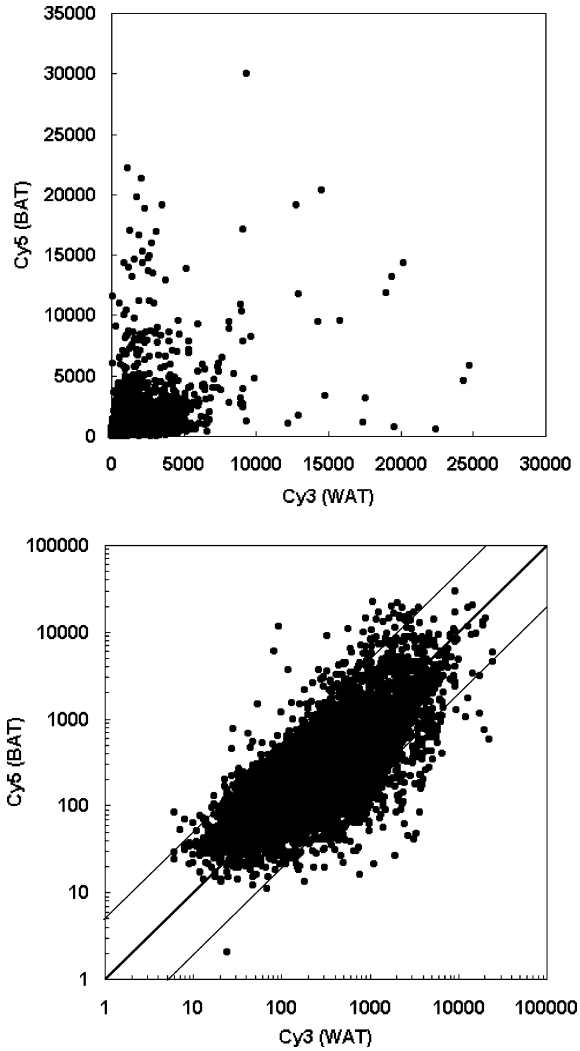

Fig. 1. Scatter plot for WAT vs. BAT. For each spot, the intensity of the hybridization signal by BAT was plotted against that by WAT directly (A) and on a logarithmic scale (B). On the plot B, the thick line denotes the line of identity (WAT equal to BAT) and positive clones (ratios of BAT to WAT higher than 5 or lower than 0.2) are outside of the two thin lines.

show, numerous genes encoding mitochondrial proteins are observed, indicating the higher cellular content of mitochondria in BAT than in WAT.

3.2. Relationship between the results obtained by microarray analysis and by Northern blotting

We next focused on the expression levels of several proteins that are involved in energy metabolism in BAT and WAT, and as summarized in Table 3, they were classified into several subclasses according to their physiological function. In our previous studies, the expression levels of several proteins, such as M-CPTI and ACC2 in BAT and WAT, were measured. In the present study, using the same RNA samples from BAT and WAT as those used for the microarray analysis, the expression levels of UCP1, M-CPTI, ACC2, CC, and LPL were further analyzed by Northern blotting. As a result, as shown in Fig. 2,

Table 1
Top 100 genes that are preferentially expressed in BAT

Annotation	UniGene	GenBank	Expression level		Ratio
			WAT	BAT	
Rat gene for uncoupling protein (UCP)	Rn.10281	X12925	92	11529	125.32
Rat mRNA for cytochrome *c* oxidase (subunit VIII-h)	Rn.10325	X64827	81	6026	74.39
Rat glycerol-3-phosphate dehydrate dehydrogenase (mtGPDH) mRNA, 3′UTR	–	U83880	120	3609	30.08
Rat pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) mRNA, complete cds	Rn.30070	AF034577	327	9103	27.84
Unnamed protein product	–	BAB14647	28	758	27.06
Rat mRNA for DAPIT protein (dapit gene)	Rn.4281	AJ271158	565	10999	19.47
Mouse alpha-synuclein (Snca) gene, complete cds	–	AF163865	27	459	16.99
Syrian golden hamster androgen-dependent expressed protein mRNA, complete cds	–	M80427	42	686	16.33
Mouse ATP-specific succinyl-CoA synthetase beta-subunit (Scs) mRNA, partial cds	–	AF058955	908	14373	15.83
Hypothetical protein	–	CAC15000	266	3694	13.89
Unknown	–	AAF61275	6	83	13.81
Rat acyl-CoA dehydrogenase medium-chain mRNA, complete cds	Rn.6302	J02791	1253	17039	13.60
Mouse type-2 deiodinase mRNA, complete cds	–	AF096875	129	1552	12.03
Rat acyl-CoA dehydrogenase medium-chain mRNA, complete cds	Rn.6302	J02791	1177	13978	11.88
Rat glycerol-3-phosphate dehydrate dehydrogenase (mtGPDH) mRNA, 3′UTR	–	U83880	181	2145	11.85
Rat mRNA for cytochrome *c* oxidase subunit VIa	Rn.1745	X14208	1749	19788	11.31
myo-Inositol monophosphatase 2	–	AAD40683	227	2558	11.27
CG12140 gene product	–	AAF58873	598	6472	10.82
Hypothetical 15 kDa protein	–	AAF67006	328	3526	10.75
Rat parvalbumin mRNA, complete cds	Rn.2005	M12725	44	463	10.52
Mouse mRNA encoding epidermal keratin subunit	–	V00830	2050	21367	10.42
Unknown gene product	–	AAC23493	277	2822	10.19
Mouse cell death activator CIDE-A (Cide-a) mRNA, complete cds	–	AF041376	1041	10417	10.01
Rat small zinc finger-like protein DDP2 (Ddp2) mRNA, complete cds	Rn.2017	AF196315	300	2991	9.97
Chlordecone reductase	–	AAD14010	300	2989	9.96
DNAJ domain-containing protein MCJ	–	AAD38506	446	4161	9.33
Rat long-chain acyl-CoA dehydrogenase (LCAD) mRNA, complete cds	Rn.174	J05029	1433	13233	9.23
dJ257I20.3 (NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (14 kD, B14))	–	CAA17121	789	7121	9.03
Rat mRNA for carnitine/acylcarnitine carrier protein	Rn.3289	X97831	499	4424	8.87
Putative 4-hydroxyphenylpyruvate dioxygenase	–	CAB51008	23	203	8.83
dJ403L10.1 (SNX9 (Sorting Nexin 9))	–	CAB46196	61	527	8.64
Rat mRNA for carnitine palmitoyltransferase I-like protein (CPTI-like protein), complete cds	Rn.6028	D43623	521	4300	8.25
NADH dehydrogenase	–	CAA44895	529	4348	8.22
Mouse mRNA, complete cds, clone: 2–31	–	AB030192	2295	18844	8.21
Rat mRNA for cytochrome *c* oxidase assembly protein COX17, complete cds	Rn.19207	AB032178	997	8171	8.20
CG14407 gene product	–	AAF48392	361	2801	7.76
Mouse peroxisomal phytanoyl-CoA alpha-hydroxylase (PAHX) mRNA, complete cds	–	AF023463	1071	8280	7.73
NADH dehydrogenase-ubiquinone 30 kDa subunit	–	AAG17541	302	2331	7.72
NADH dehydrogenase	–	CAA44894	1065	8180	7.68
Putative ATPase	–	AAA91360	195	1496	7.67
IL-1 receptor-associated-kinase-M; IRAK-M	–	AAD40879	17	130	7.67
PRO1580	–	AAF69602	891	6799	7.63
Rat NAD(P)H:menadione oxidoreductase mRNA, complete cds	Rn.11234	J02608	355	2663	7.50
Mouse NOCTURNIN (Nocturnin) mRNA, complete cds	–	AF199491	133	981	7.38
Rat mRNA for very long-chain acyl-CoA synthetase, complete cds	Rn.3608	D85100	218	1575	7.22
Rat troponin I mRNA, complete cds	Rn.9924	M73701	91	632	6.95
Unnamed protein product	–	BAA91575	474	3242	6.84
CG11095 gene product	–	AAF48325	201	1369	6.81
Rat F_1-ATPase epsilon-subunit mRNA, nuclear gene encoding mitochondrial protein, complete cds	Rn.3454	AF010323	849	5745	6.77
Rat apoptosis-regulating basic protein mRNA, complete cds	Rn.3718	AF304429	2162	14368	6.65
gb|AAF24540.1∼gene_id:K15I22.14∼similar to unknown protein	–	BAB09322	200	1322	6.61
Rat AMP deaminase isoform C (AMPD3) mRNA, complete cds	Rn.11106	U90888	79	516	6.53
Rat mRNA for 70 kDa tumor-specific antigen, partial	Rn.13808	Y15054	492	3186	6.48
DERP2	–	BAA93049	895	5775	6.45
Neogenin	–	AAC59662	1049	6760	6.44
Rat mRNA for NADH: ubiquinone oxidoreductase, complete cds	Rn.29882	D86215	1566	9750	6.23
Unnamed protein product	–	BAA91285	24	149	6.19
KIAA0404	–	BAA23700	16	96	6.00

Table 1 (*Continued*)

Annotation	UniGene	GenBank	Expression level		Ratio
			WAT	BAT	
Rat mRNA for 70 kDa tumor-specific antigen, partial	Rn.13808	Y15054	387	2320	6.00
Rat membrane interacting protein of RGS16 (Mir 16) mRNA, complete cds	Rn.3731	AF212861	424	2495	5.88
Mouse mRNA for NADH dehydrogenase	–	Y07708	1897	11163	5.88
NADH dehydrogenase	–	CAA44896	1248	7237	5.80
Mouse mbgt-5 mRNA for beta-1,4-galactosyltransferase V, complete cds	–	AB004786	31	178	5.74
Rat (Sprague–Dawley) cytochrome *c* pseudogene, clone Ch4A-RC9	–	K03238	2788	15959	5.72
ISCU2	–	AAG37428	778	4446	5.71
NADH dehydrogenase (ubiquinone) 39 kDa subunit	–	CAA42054	1510	8623	5.71
Mouse Int-3 mRNA, complete cds	–	M80456	39	222	5.70
Rat DD6C4-4 mRNA, partial sequence	–	AF034249	973	5509	5.66
Rat mRNA for acyl-CoA synthetase 5, complete cds	Rn.3061	AB012933	743	4165	5.61
Rat mRNA for cytochrome *c* oxidase subunit VIIa (EC 1.9.3.1)	Rn.6686	X54080	2660	14892	5.60
Rat mRNA for small heterodimer partner homologue, complete cds	Rn.10712	D86580	114	637	5.59
Mouse pantothenate kinase 1 beta (panK1beta) mRNA, complete cds	–	AF200357	264	1469	5.56
Rat gamma-crystallin gene cluster, encoding gamma-A, B, C, D and E crystallins, complete cds	Rn.64733	M19359	119	660	5.54
Mouse untranslated RNA G90	–	AJ132433	172	950	5.52
Mouse cyclin ania-6b gene, partial sequence	–	AF185591	18	99	5.50
Rat liver cytochrome oxidase subunit VIc (COX-VIc) mRNA, complete cds	Rn.846	M27466	3500	19106	5.46
GTP-binding protein Sara	–	AAD40372	371	2016	5.43
HSPC119	–	AAF29083	2532	13706	5.41
Mouse BALB/c2-oxoglutarate dehydrogenase E1 component mRNA, partial cds	–	U02971	1047	5658	5.40
Mouse chromosome 10 clone rp21-12n20 strain 129S6/SvEvTac, complete sequence	–	AC009361	3139	16909	5.39
Rat phosphoenolpyruvate carboxykinase (GTP) gene, exons 1–3	–	K03243	1371	7376	5.38
Similar to YBS4_YEAST (P38244) hypothetical 47.8 kD protein in HSP26-SEC18 intergenic region	–	AAA62526	24	128	5.35
Acylphosphatase	–	CAA58988	122	651	5.33
DNb-5	–	AAD27583	45	239	5.32
Rat mRNA for ATP synthase subunit d, complete cds	Rn.80	D13120	179	952	5.32
Rat Eker rat-associated intracisternal-A-particle element	–	U23776	657	3484	5.30
Mouse mRNA for calcium channel gamma 4 subunit (CACNG4 gene)	–	AJ272045	40	211	5.28
Rat mitochondrial fumarase mRNA, complete cds	Rn.29782	J04473	469	2460	5.24
Unnamed protein product	–	BAB15618	521	2726	5.23
Rat pituitary adenylate cyclase-activating polypeptide precursor protein mRNA, complete cds	Rn.37400	M63006	26	135	5.21
Unknown	–	AAF76523	733	3765	5.14
Mouse mRNA for hypothetical protein (ORF1), 1975 BP	–	AJ278735	556	2854	5.13
Tetraspanin Tspan-5	–	AAF28869	145	744	5.13
Mouse zic mRNA for Zic protein, complete cds	–	D32167	55	282	5.12
Mouse DXImx38e protein (DXImx38e) mRNA, complete cds	–	AF229635	181	927	5.12
Rat interleukin-6-dependent binding protein (IL-6DBP) mRNA, complete cds	Rn.6479	M57235	976	4990	5.11
KIAA1183 protein	–	BAA86497	24	122	5.09
Rat electron transfer flavoprotein (ETF) alpha-subunit DNA, 3′ end	Rn.32496	M22030	1578	8030	5.09
Rat mRNA for mitochondrial 3–2-*trans*-enoyl-CoA isomerase	Rn.4005	X61184	934	4740	5.07
Rat cytochrome P450-LA-omega (lauric acid omega-hydroxylase) mRNA, complete cds	Rn.5721	M14972	36	181	5.02

The top 100 genes that are preferentially expressed in BAT are shown in the order of the values of the ratio of expression levels between BAT and WAT. Forty-two 'Incyte ESTs' were ranked higher than the gene at the lowest position (rat cytochrome P450-LA-omega mRNA). However, they were all removed from the list since they have not been well annotated.

expression levels of UCP1, M-CPTI, ACC2, and CC were significant in BAT but very weak in WAT, and that of LPL was more significant in WAT than in BAT. These results were essentially the same as those obtained in the previous studies, as well as those obtained by the microarray analysis described above, indicating that the expression profiles of these proteins in BAT and WAT are highly reproducible over different RNA preparations and that the results obtained by microarray analysis are highly reliable.

3.3. Differences in the expression of mitochondrial proteins between BAT and WAT do not simply reflect differences in mitochondrial content between these tissues

As described in the above section, various mitochondrial proteins were highly expressed in BAT, but not in WAT. These results suggest that differences in expression levels of various proteins in BAT and WAT, as revealed by microarray analysis, can be explained based on differences in the mitochondrial content of these tissues.

Table 2
Top 100 genes that are preferentially expressed in WAT

Annotation	UniGene	GenBank	Expression level		Ratio
			WAT	BAT	
Rat fibrillin-1 mRNA, complete cds	Rn.12759	AF135059	3135	40	0.01
dJ688G8.3 (similar to chelonianin (basic protease inhibitor))	–	CAC18560	1932	26	0.01
Thioesterase B	–	AAA49223	3317	48	0.01
Rat gene encoding tyrosine aminotransferase	Rn.9947	AJ010709	2707	44	0.02
Rat fibrillin-1 mRNA, complete cds	Rn.12759	AF135059	1107	21	0.02
Rat Sprague–Dawley Golgi apparatus sialoglycoprotein MG-160 mRNA, complete cds	Rn.10507	U08136	771	16	0.02
Actin	–	CAA39280	3711	83	0.02
Rat carbonic anhydrase III (CA3) mRNA, complete cds	Rn.1647	AF037072	22381	579	0.03
Rat mRNA for osteonectin	Rn.31991	Y13714	19554	739	0.04
Rat late gestation lung protein 1 (Lgl1) mRNA, complete cds	Rn.4346	AF109674	1429	56	0.04
Unknown	–	AAF02423	2266	90	0.04
Rat mRNA for vascular alpha-actin	–	X06801	3818	159	0.04
Rat mRNA for E-cadherin, complete cds	Rn.1303	AB017696	703	30	0.04
Mouse mRNA for monoglyceride lipase	–	AJ001118	1961	85	0.04
Unnamed protein product	–	BAA91528	3947	174	0.04
Rat cytosolic inhibitor of Nrf2 (Inrf2) mRNA, complete cds	Rn.23467	AF304364	499	22	0.04
Rat Sprague–Dawley Golgi apparatus sialoglycoprotein MG-160 mRNA, complete cds	Rn.10507	U08136	745	33	0.04
Rat protein phosphatase inhibitor-1 protein mRNA, complete cds	Rn.9756	J05592	2289	103	0.05
Rat LL5 mRNA	Rn.11128	X74226	665	30	0.05
Mouse mRNA for laminin alpha 4	–	Y09827	2380	112	0.05
Rat mRNA for MHC class II-associated invariant chain	–	X14254	3665	184	0.05
Rat mRNA for liver mitochondrial aldehyde dehydrogenase	Rn.2300	X14977	3985	204	0.05
Mouse nid gene (exon 17)	–	X83091	2134	114	0.05
Rat mRNA for transferrin, complete cds	Rn.2514	D38380	3244	176	0.05
Rat aldehyde dehydrogenase mRNA, complete cds	Rn.74044	M23995	1296	73	0.06
BAF60b	–	BAA24106	467	26	0.06
KIAA0631 protein	–	BAA31606	1341	78	0.06
Mouse factor B mRNA, complete cds	–	M57890	4614	273	0.06
Rat gene encoding tyrosine aminotransferase	Rn.9947	AJ010709	6622	398	0.06
Mouse cysteine protease inhibitor (MS1) mRNA sequence	–	M92417	2382	143	0.06
Rat mRNA for mitochondrial dicarboxylate carrier	Rn.3631	AJ223355	1486	90	0.06
Rat mRNA for sensory neuron synuclein	Rn.10421	X86789	3738	226	0.06
Rat TRPM-2 mRNA, complete cds	Rn.1780	M64723	4270	260	0.06
Rat mRNA for alternatively spliced smooth muscle myosin heavy chain (clone RAMHC21) Rn.1239	–	X16262	2166	133	0.06
Rat fibrillin-1 mRNA, complete cds	Rn.12759	AF135059	3657	230	0.06
Rat phospholipase C type IV mRNA, complete cds	Rn.9751	J05155	304	19	0.06
Rat mRNA for EGP-314 protein homologue	Rn.24930	AJ001044	967	62	0.06
Rat folate-binding protein mRNA, complete cds	Rn.6912	AF219904	599	38	0.06
Rat nonmuscle caldesmon mRNA, complete cds	Rn.33965	U18419	2020	130	0.06
Rat GTP-binding protein (G-alpha-i2) mRNA, complete cds	Rn.3036	M17528	977	65	0.07
Rat mRNA for collagen alpha1 type-I	Rn.2953	Z78279	17403	1160	0.07
Rat glutathione *S*-transferase Yb2 subunit mRNA, 3′ end	Rn.625	M13590	1416	98	0.07
Rat Bcl-2-related ovarian killer protein (Bok) mRNA, complete cds	Rn.44461	AF027954	184	13	0.07
Rat gamma-enteric smooth muscle actin isoform mRNA, complete cds	Rn.958	M22323	2474	186	0.08
Rat adenylyl cyclase type VI mRNA, complete cds	Rn.3313	L01115	1182	89	0.08
C3F	–	AAC36007	1398	109	0.08
Mouse mRNA for chondroitin 4-*O*-sulfotransferase (C4S-1 gene)	–	AJ289133	400	31	0.08
Ribosome receptor	–	CAA60676	2827	222	0.08
Mouse mRNA for desmoyokin, partial	–	X65157	764	63	0.08
Desmoplakin	–	AAA35766	861	71	0.08
Mouse complement component 6 (C6) mRNA, complete cds	–	AF184900	755	63	0.08
Rat (Wistar) B7 mRNA for B7 antigen	Rn.34155	X76697	1953	167	0.09
Rat Lot1 mRNA, complete cds	Rn.6977	U72620	1466	125	0.09
Mouse glutathione *S*-transferase class mu (GST1-1) mRNA, complete cds	–	J04632	2245	192	0.09
Rat mRNA for ad1-antigen	Rn.11068	X61654	5027	431	0.09
Endogenous vascular elastase = serine protease adipsin homologue [rats, Sprague–Dawley, pulmonary arteries, mRNA, 846 nt]	–	S73894	12189	1052	0.09
Rat putative eps protein (MGEPS) mRNA, partial cds	–	AF081251	3899	339	0.09
Rat mRNA for steroidogenic acute regulatory protein, complete cds	Rn.11399	AB001349	497	43	0.09

Table 2 (*Continued*)

Annotation	UniGene	GenBank	Expression level		Ratio
			WAT	BAT	
Rat betaglycan mRNA, complete cds	Rn.9953	M77809	827	73	0.09
Rat betaglycan mRNA, complete cds	Rn.9953	M77809	827	73	0.09
Rat ribosomal DNA external transcribed spacer 1 (ETS1)	–	X16321	917	82	0.09
Rat partial mRNA for conjugate export pump protein (MRP1 gene)	Rn.10495	AJ277881	441	39	0.09
Mouse A–X actin mRNA, complete cds	–	J04181	5822	529	0.09
Rat 21-hydroxylase mRNA, complete cds	Rn.36545	U56853	2233	205	0.09
Rat basement membrane-associated chondroitin proteoglycan Bamacan mRNA, complete cds	Rn.11074	U82626	493	46	0.09
Rat gelatinase A mRNA, complete cds	Rn.6422	U65656	2415	229	0.09
Mouse KRAB-A interacting protein mRNA, complete cds	–	U67303	965	92	0.10
Mouse *myo*-inositol 1-phosphate synthase A1 (IsynA1) mRNA, complete cds	–	AF288525	658	64	0.10
Rat prostaglandin-H-2 D-isomerase gene, complete cds	Rn.11400	M94134	365	35	0.10
Insulin-like growth factor I {exon 6} [rats, genomic/mRNA, 7224 nt]	–	S43941	4425	431	0.10
Mouse core promoter-binding protein mRNA, partial cds	–	AF072403	1204	118	0.10
Mouse EIG 180 mRNA for ethanol induced gene product, complete cds	–	AB023957	1001	100	0.10
Rat DOC-2 p82 isoform mRNA, complete cds	Rn.14763	U95177	3980	401	0.10
Rat nucleolin gene	–	M55015	3046	309	0.10
Rat steroid sensitive gene-1 protein (SSG-1) mRNA, complete cds	Rn.2193	AF223677	3929	399	0.10
Unnamed protein product	–	BAA91573	643	66	0.10
Rat tensin (Tns) mRNA, partial cds	–	U26310	1313	134	0.10
Rat mRNA for protein disulfide isomerase (PDI; EC 5.3.4.1)	Rn.4234	X02918	2002	207	0.10
Rat gene for desmin	Rn.1657	X73524	300	31	0.10
Rat Y-b3 glutathione *S*-transferase mRNA, complete cds	Rn.6036	J02744	1716	182	0.11
Mouse p53 apoptosis-associated target (Perp) mRNA, complete cds	–	AF249870	485	52	0.11
Rat mRNA encoding alpha-tubulin	Rn.54749	V01227	5545	591	0.11
Rat mRNA for type-I thyroxine deiodinase	Rn.42914	X57999	303	32	0.11
Microsomal glutathione *S*-transferase 2	–	AAC51768	2149	230	0.11
Rat mRNA for ficolin-A, complete cds	Rn.20051	AB026057	687	74	0.11
Mouse carbonic anhydrase VB mRNA, complete cds; nuclear gene for mitochondrial product	–	AF192978	3416	368	0.11
Rat CaM-kinase II inhibitor alpha mRNA, complete cds	Rn.3551	AF271156	814	88	0.11
Rat kidney protein phosphatase 1 myosin-binding subunit mRNA, partial cds	–	U50185	522	57	0.11
Hypothetical protein	–	CAB94873	1146	124	0.11
HP1-BP74 protein	–	AAF14871	428	46	0.11
Rat mRNA for channel integral membrane protein 28	Rn.1618	X67948	1637	179	0.11
Rat mRNA for tropomyosin isoform 6	Rn.24727	X72859	553	61	0.11
Rat liver delta-5 desaturase mRNA, complete cds	Rn.28161	AF320509	1235	135	0.11
Rat mRNA for membrane-type metalloprotease	Rn.10371	X91785	1105	121	0.11
Rat insulin-like growth factor I (IGF-I) mRNA, complete cds	Rn.6282	M15481	3339	367	0.11
Mouse fibroblast growth factor inducible gene 14 (FIN14) mRNA, complete cds	–	U42386	699	77	0.11
Rat developmentally regulated intestinal protein (OCI-5) mRNA, complete cds	Rn.9717	M22400	2049	226	0.11
Rat Sprague–Dawley Golgi apparatus sialoglycoprotein MG-160 mRNA, complete cds	Rn.10507	U08136	591	66	0.11
Mouse mRNA for complement subcomponent C1Q alpha-chain	–	X58861	408	45	0.11
Rat RNA helicase with arginine-serine-rich domain mRNA, complete cds	Rn.3436	U25746	704	79	0.11
Rat follistatin-related protein precursor mRNA, complete cds	Rn.2979	U06864	1122	126	0.11

The top 100 genes that are preferentially expressed in WAT are shown in the order of the values of the ratio of expression levels between BAT and WAT. Ten 'Incyte ESTs' ranked higher than the gene at the lowest position (rat follistatin-related protein precursor mRNA) were removed from the list.

To examine whether this interpretation is true, we further inspected the expression levels of several genes. Of Table 3, five cDNAs were selected for this experiment. Two cDNAs of NADH-U and ATPase ε were found to be expressed in BAT at levels that were 6.2- and 6.8-fold higher than in WAT, as evidenced by microarray analysis. On the contrary, UCP2, a homologue of UCP1, was expressed at slightly higher levels in WAT than in BAT, and LMADH and DIC were significantly more highly (about 20-fold) expressed in WAT than in BAT. All of these five proteins are mitochondrial proteins, but the latter three were expressed at significantly higher levels in WAT. In support of this, as shown in Fig. 2, Northern analysis gave essentially the same expression profiles for these five cDNAs, as was obtained by microarray analysis.

3.4. Western analysis of five proteins between BAT and WAT

In the above sections, we evaluated the expression level of proteins by measuring their transcript levels. However, expression levels of messages are not always identical with those of proteins. Thus, to examine the relationship between the expression levels messages and proteins, we

Table 3
Expression levels of representative genes involved in energy metabolism in rat adipose tissues

Annotation	UniGene	GenBank	Expression level		Ratio
			WAT	BAT	
UCPs and other solute carriers					
Rat gene for uncoupling protein (UCP1)	Rn.10281	X12925	92	11529	125.32
Rat mRNA for UCP2, complete cds	Rn.13333	AB010743	511	298	0.58
Rat UCP3 mRNA, complete cds	Rn.9902	U92069	120	216	1.80
Rat mRNA for mitochondrial adenine nucleotide translocator (ANT2)	Rn.3746	D12771	3741	6640	1.77
Rat mitochondrial proton/phosphate symporter mRNA, complete cds (PiC)	Rn.3606	M23984	2403	738	0.31
Rat mRNA for mitochondrial dicarboxylate carrier (DIC)	Rn.3631	AJ223355	1486	90	0.06
ATPase					
Rat mitochondrial H^+-ATP synthase alpha-subunit mRNA, complete cds	Rn.40255	J05266	2998	8472	2.83
Rat nuclear-encoded mitochondrial ATP synthase beta-subunit mRNA, 5′ end	–	M25301	15768	9538	0.60
Rat (clone gamma-3) ATP synthase gamma-subunit (ATP5c) mRNA, 3′ end cds	Rn.9723	L19927	3952	8650	2.19
Rat F_1-ATPase epsilon-subunit mRNA, nuclear gene encoding mitochondrial protein, complete cds (ATPase ε)	Rn.3454	AF010323	849	5745	6.77
Rat P1 mRNA for ATP synthase subunit c, complete cds	Rn.3357	D13123	1597	249	0.16
Rat P2 mRNA for ATP synthase subunit c, complete cds	Rn.29258	D13124	2450	1161	0.47
Rat mRNA for ATP synthase subunit d, complete cds	Rn.80	D13120	179	952	5.32
Complex I					
Rat mRNA for NADH:ubiquinone oxidoreductase, complete cds (NADH-U)	Rn.29882	D86215	1566	9750	6.23
Rat NADH ubiquinone oxidoreductase subunit (IP13) gene, complete cds	Rn.6166	L38437	439	1790	4.08
Complex IV					
Rat cytochrome *c* oxidase subunit IV (COXIV) gene, complete cds	Rn.2528	J05425	4207	7089	1.68
Rat DNA for cytochrome *c* oxidase subunit Vb, complete cds	Rn.6686	D10951	2837	13520	4.77
Rat mRNA for cytochrome *c* oxidase subunit VIa	Rn.1745	X14208	1749	19788	11.31
Rat mRNA for heart cytochrome *c* oxidase subunit VIa	Rn.5119	X12554	86	166	1.93
Rat mRNA for cytochrome *c* oxidase subunit VIIa (EC 1.9.3.1)	Rn.6686	X54080	2660	14892	5.60
Rat mRNA for cytochrome *c* oxidase (subunit VIII-h)	Rn.10325	X64827	81	6026	74.39
Fatty acid metabolism					
Rat lipoprotein lipase mRNA, complete cds (LPL)	Rn.3834	L03294	24734	5808	0.23
Rat fatty acid transport protein mRNA, complete cds	Rn.1047	U89529	1797	1208	0.67
Rat mRNA for very long-chain acyl-CoA synthetase, complete cds	Rn.3608	D85100	218	1575	7.22
Rat mRNA for acetyl-CoA carboxylase, complete cds (ACC2)	Rn.44359	AB004329	123	291	2.37
Rat fatty acid synthase mRNA, complete cds	Rn.9486	M76767	248	139	0.56
Rat mRNA for long-chain acyl-CoA synthetase (EC 6.2.1.3)	Rn.6215	D90109	4515	6982	1.55
Rat mRNA for carnitine palmitoyltransferase I-like protein, complete cds (M-CPTI)	Rn.6028	D43623	521	4300	8.25
Rat carnitine palmitoyltransferase I mRNA, complete cds (L-CPTI)	Rn.2856	L07736	362	186	0.51
Rat mRNA for carnitine/acylcarnitine carrier protein (CC)	Rn.3289	X97831	499	4424	8.87
Rat mitochondrial carnitine palmitoyltransferase II (CPT II) mRNA, complete cds	Rn.11389	J05470	422	1182	2.80
Rat acyl-CoA dehydrogenase medium-chain mRNA, complete cds (MCAD)	Rn.6302	J02791	1177	13978	11.88
Rat long-chain acyl-CoA dehydrogenase (LCAD) mRNA, complete cds	Rn.174	J05029	1433	13233	9.23
Others					
Rat brain glucose-transporter protein mRNA, complete cds (GLUT1)	Rn.3205	M13979	153	43	0.28
Rat glucose transporter-3 (GLUT3) gene, complete cds	Rn.44400	U17978	49	39	0.80
Rat mRNA for glucose transporter, GLUT4	Rn.1314	D28561	1412	705	0.50
HKII = hexokinase II [rats, epididymal fat pad, mRNA partial, 1456 nt, segment 2 of 2]	–	S56464	552	1469	2.66
Rat mRNA for glucokinase, alternatively spliced GK2 (EC 2.7.1.1)	Rn.10447	X53588	90	230	2.56
Rat mRNA for liver mitochondrial aldehyde dehydrogenase (LMADH)	Rn.2300	X14977	3985	204	0.05
Rat mRNA for mitochondrial enoyl-CoA hydratase (EC 4.2.1.17)	Rn.6847	X15958	1889	1175	0.62
Rat mRNA for mitochondrial malate dehydrogenase (EC 1.1.1.37)	Rn.1011	X04240	4312	3321	0.77
Rat mRNA for sarcomeric mitochondrial creatine kinase	–	X59736	60	120	2.00

next analyzed expression levels of five proteins for which messages are remarkable in BAT. As shown in Fig. 3, UCP1, M-CPTI, and CC were significantly expressed in BAT in comparison in WAT. On the contrary, expression levels of NADH-U and ε-subunit of F_1-ATPase were not markedly different between BAT and WAT. Possible interpretations of these results are discussed in the following section.

4. Discussion

The ultimate objective of the present study was to determine if additional genes were expressed at different levels in BAT vs. WAT. This would advance our knowledge of the gene expression profile that might lead to a better understanding of the metabolic differences between these

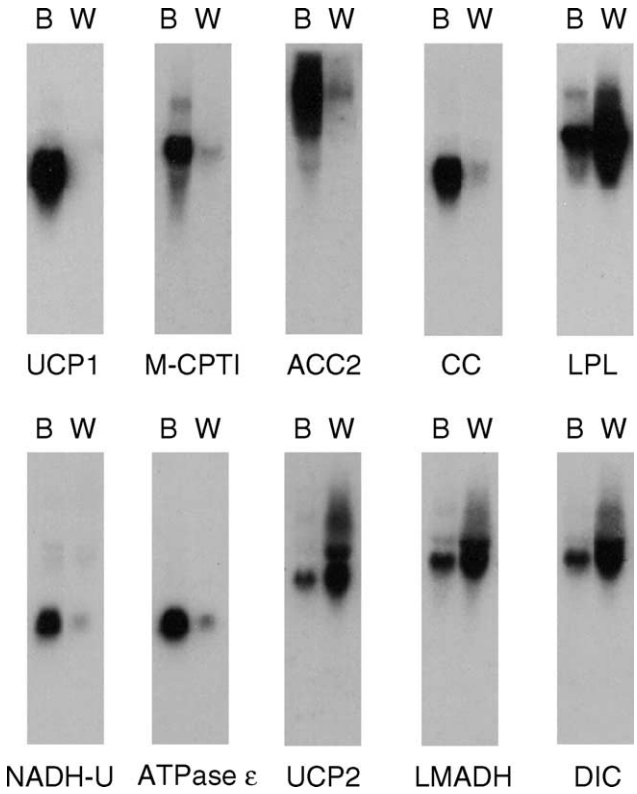

Fig. 2. Verification of microarray results with Northern blotting. Ten micrograms of aliquot of each total RNA sample obtained from BAT or WAT of rats was subjected to denatured agarose gel (1%) electrophoresis. The separated RNAs were then transferred to nitrocellulose membrane filters, and hybridized with the cDNA probes, as described under Section 2; W and B represent RNA samples of WAT and BAT. M-CPTI, muscle-type carnitine palmitoyltransferase; ACC2, acetyl-CoA carboxylase β; CC, carnitine carrier; LPL, lipoprotein lipase; NADH-U, NADH dehydrogenase (ubiquinone) 1α subcomplex 5; ATPase ε, epsilon-subunit of F_1-ATPase; LMADH, liver mitochondrial aldehyde dehydrogenase; DIC, dicarboxylate carrier.

two adipose tissues. The microarray analysis used in the present study confirmed previous reviews of the differential expression of genes encoding UCP1 in BAT [1–4]. In addition, hundreds of genes that were not previously known to be expressed were identified as differentially expressed by microarray analysis.

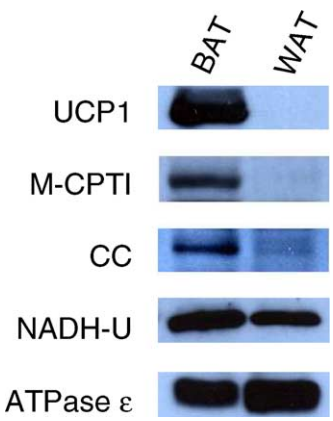

Fig. 3. Comparison of expression levels of five proteins between BAT and WAT. Samples corresponding to 20 μg mitochondrial protein were subjected to electrophoresis, and protein bands of UCP1, M-CPTI, CC, NADH-U, and ATPase ε were immunologically detected. For more details, see Section 2.

We previously attempted to identify genes that are differentially expressed in BAT using a differential screening, cDNA subtraction method or individual comparisons of the expression level of each gene. As a result, we showed that genes encoding M-CPTI [5], ACC2, CC, medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCAD), 3-ketoacyl-CoA thiolase (KACT), UCP3 [6], three subunits of NAD^+-ICDH [7] were preferentially expressed in BAT, while genes encoding LPL, liver-type carnitine palmitoyltransferase (L-CPTI), type-1 glucose transporter (GLUT1), type-I hexokinase (HKI), UCP2, CCAAT enhancer-binding protein α (C/EBPα) [6] were preferentially expressed in WAT. A comparison of the results of the current microarray experiment (Table 3) with previously reported differential gene expression obtained by Northern analysis showed a good agreement in the expression levels of LPL, ACC2, M-CPTI, L-CPTI, CC, MCAD, LCAD, UCP2, and UCP3. Thus, for these genes, the microarray analysis, as performed here, confirmed the previously reported differences in gene expression between BAT and WAT. Furthermore, most of the results of Northern blotting for representative genes in this study (Fig. 2) were consistent with the microarray results. These results improved the reliability of the values for gene expression levels obtained from the present study using the Agilent cDNA microarray.

Most genes that encode proteins involved in mitochondrial oxidative phosphorylation were shown to be expressed at relatively high levels in BAT. Recently, we determined the cellular content of mitochondria in adipose tissues by comparing the amount of mitochondrial DNA in various tissues and the findings indicated that the cellular content of mitochondria in BAT was about 4-fold higher than that in WAT.[1] Therefore, it is possible that the results shown in Table 3 may partially reflect the higher cellular content of mitochondria in BAT. However, UCP1 and several other genes were shown to be expressed at high levels in BAT independent of the mitochondrial content. Interestingly, several genes encoding mitochondrial proteins were found to be expressed at lower levels in BAT contrary to the mitochondrial content. Of these, the expression levels of LMADH and DIC genes were both less than about 20-fold in BAT. Further studies to clarify the roles of these proteins and others that are indicative of marked differences in gene expression levels between the two types of adipose tissues and to identify the upstream transcriptional regulation mechanisms of these genes may provide much more information for use in characterizing energy metabolism in BAT.

In this study, we further examined the expression levels of proteins which showed higher transcript levels in BAT. As a result, UCP1, M-CPTI, and CC were significantly

[1] Kajimoto *et al.*, submitted for publication.

expressed even in protein levels. However, expression levels of two proteins of NADH-U and ε-subunit of F_1-ATPase in BAT were not markedly different from those in WAT. The exact reason for the latter discrepancy between the transcript levels and protein levels is uncertain. However, it should be noteworthy that both of latter proteins are one each subunits of redox complex and ATPase complex, respectively. Therefore, as a probable interpretation, regulations of expression levels of these proteins would be dependent on expression levels of the other subunits of the complexes. To examine the validity of this interpretation, further experiments are in progress.

In the present study, we performed microarray analysis using tissues of brown and white fat. However, it should be noted that these tissues are not consisted of homogeneous adipocytes and may contain cells showing the other gene expression profiles. To overcome this problem, analysis using homogenous cells or single cell would be necessary. In addition, recently, Boeuf *et al*. reported the microarray analysis of gene expression profiles between precursor cells of brown and white adipocytes [12]. They tried to identify genes differently expressed between white and brown preadipocytes using combination of representational difference analysis (RDA) and DNA microarray screening. They identified seven genes with higher expression levels in brown preadipocytes, of which three are structural genes implicated in cell adhesion and cytoskeleton organization and four that might function in gene transcription and protein synthesis. This study is very important to understand the key genes determining the fate of precursor cells of white and brown adipocytes, and thus we are also performing more exhaustive analysis.

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