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Identification of Differentially Expressed Genes Between Preadipocytes and Adipocytes Using Affymetrix Bovine Genome Array

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ABSTRACT

Adipocytes are differentiated from preadipocytes and have large capacity for storing fats inside cells. In cattle, intramuscular fat (IMF) content is one of the major determinants for meat quality and also highly affects market prices, especially in Japan and Korea. In order to profiling differentially expressed genes between intramuscular fibroblast-like cells (preadipocytes) and their differentiated adipocytes, we have established intramuscular fibroblast-like cells from *M. longissimus thoracis* in Korean cattle (Hanwoo). The differentially expressed genes were selected by comparing these two types of cells ug thecommercially available 23kese two types of cells ug theco. The results indan ced that 206 arecomelements were differentially expressed. Of these, 67 and 94 ks wn genes were up and d wn regulaced, respectively, in adipocytes ug ng both 2-fold difference and Welch's t-test as the cut-off points. The differentially expressed genes identified in this study can be used as good markers for improving meat quality traits with further verification of their biological functions, especially IMF contents in cattle.

(**Key words :** Adipocytes, Bovine genome array, Differentially expressed genes, Intramuscular fat, Korean cattle)

INTRODUCTION

Adipogenesis is the differentiation processes from the primitive mesoderm stem cells to preadipocytes and ultimately become adipocytes. This process occurs at both prenatal and postnatal states. The adipose tissues are very important because they can store fats as energy sources. Recently, researches on these cells have been growing because the basic mechanisms of the adipose tissue development can give the solutions for obesity, type II diabetes, hypertension and coronary heart diseases (Zalesin et al., 2008). The basis of these researches was started by making immortal preadipocyte cell lines (Green and Kehinde, 1975). Later on, molecular regulation of adipocyte differentiation has been investigated using immortalized cell lines such as 3T3-L1, Ob1771, and 3T3-F442A. The results were confirmed by *in vivo* studies including knock out mice and/or transgenic mice (Rosen and Spiegelman, 2000). These results indicated that adipocyte differentiation was controlled by a number of adipogenic transcription factors, including CCAAT-enhancer binding protein (C/EBP), peroxisome proliferator activated receptor-γ (PPARr) families, and adipocyte determination and differentiation dependent factor 1 (ADD1) (Rosen and Spiegelman, 2000; Rangwala and Lazar, 2000). Also, adipocyte differentiation was regulated by differentiation-inducing agents including insulin, dexamethasone and methyl-isobutylxanthine (MIX; a phosphodiesterase inhibitor) (Girard et al., 1994; Wu et al., 1999; Reusch et al., 2000).

In case of cattle, many researches already have been carried out for identifying underline mechanisms for intramuscular fat deposition. The fat deposition inside muscles is called marbling and this trait is one of the important factors for determining meat quality in cattle (Wood et al., 1999). However, meat quality is very complex trait because it includes all meat related factors containing tenderness, flavor, and juiciness. Aso et al. (1995) firstly established intramuscular preadipocyte (BIP) cell line in cattle and they suggested that adipose tissue metabolism in ruminants was different from that of non-ruminants. In addition, they found that extracellular matrix (ECM) of adipose tissue

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during adipogenesis was newly organized and alternations of this extracellular matrix were associated with ECM proteins (collagen, laminin and fibronectin) as well as caveolin-1 and cytoskeletal proteins (actin and vimentin), which play important roles in intracellular accumulation of lipid droplets (Aso et al., 1995; Nakajima et al., 1998; Inoue-Murayama et al., 2000; Nakajima et al., 2002; Tahara et al., 2004; Takenouchi et al., 2004). However, these results have limitations for only investigation of extracellular matrix. Recently, Taniguchi et al. (2007) profiled differentially expressed genes during early adipogenic differentiation of bovine perimuscular fat (PMF) preadipocytes.

In this study, we investigated differentially expressed genes between intramuscular fibroblast-like cells and their differentiated adipocytes using 23k bovine genome array (Affymetrix, USA) in order to identify important genes for adipogenesis and ultimately give the basic information for identification of genes for the IMF contents in cattle.

MATERIALS AND METHODS

1. Preparation and Differentiation of intramuscular fibroblast-like cells

The primary intramuscular fibroblast like cells were prepared and differentiated using the method described by Jeong et al. (2008). In order to confirm the cell differentiation, the cells were stained with Oil Red O. The triglycerides (TG) in the differentiated cells were quantified using Triglyceride G Test Kit (Wako, USA). All experiments were performed in triplicate.

2. Total RNA preparation

In order to isolate total RNA from intramuscular fibroblast-like cells and their differentiated adipoctyes, 0.8 ml Tri Reagent (Molecular Research Center, USA) was added to the cultured cells in 60 mm dish and the cells were lysed by repetitive pipettings. The homogenates were stored at room temperature for 5 min for the complete dissociation of nucleoprotein complexes. Then it was vigorously mixed with 0.2 volume of chloroform and centrifuged at 12,000 rpm for 15 min at 4°C. The aqueous phase was transferred to a fresh tube and 0.5 volume of isopropanol was added. Then, the samples were left at room temperature for 10 min, and centrifuged at 12,000 rpm for 8 min at 4°C. In order to obtain the RNA pellet, supernatant was removed and RNA pellet was washed with 1 volume of 75% ethanol and briefly air-dried. Total RNA was subjected to RNA clean up by RNeasyTM kit (QIAGEN, Germany). Total RNA integrity was monitored by denaturing 1% agarose gel electrophoresis. RNA concentrations and purities were measured by spectrophotometer (Amersham Pharmacia Biotech, UK).

3. GeneChip array analysis and data analysis

Microarray analyses were performed in two replicates according to the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, USA). First strand cDNA was synthesized from reaction containing 10 μg total RNA, 50 μM T7-Oligomer, 1× first-strand reaction buffer, 100 mM DTT, 500 μM dNTP, and 200 U/μl Superscript II RTase (Gibco BRL, USA) at 42°C for 1 hour. To synthesis second strand of DNA, the first strand DNA was added in the solution containing 5× second-strand reaction buffer, 200 μM dNTP, 10 unit *E. coli* DNA ligase, 40 unit *E. coli* DNA polymerase I, and *E. coli* RNase H and reaction was carried out at 16°C for 2 hour. Double-stranded cDNA was purified using Sample Cleanup Module (Affymetrix, USA) and labeled Biotin by *in vitro* transcription using Enzo Bioarray (Affymetrix, USA). The cDNA was hybridized with the solution containing 50 pM Oligo B2 (3 nM), 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 1× hybridization buffer, and 10% DMSO at 45°C for 16 hours. After hybridization, the arrays were washed in a GeneChip Fluidics station and stained with a streptavidin-phycoerythrin complex. The intensities of chip elements were determined with a GeneChip scanner. After scanning, data acquisition and image processing were carried out with the GeneChip Operating Software (GCOS) and normalization of the data was done with MA-plot. Finally, significant test was performed using Welch's t-test and Volcano Plot. In order to identify differentially expressed genes in duplicate experiments, multiple test correction was also performed. The classification of gene ontology was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/).

4. Quantitative real-time PCR

Single-stranded cDNA was synthesized from 1 μg total RNA of intramuscular fibroblast-like cells and their differentiated adipoctyes. Initially, RNA was denatured at 70ºC

for 10 min and reverse transcription was performed in reactions containing 5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton® X-100, 1 mM each dNTP, 0.5 unit Recombinant RNasin® Ribonuclease Inhibitor, 15 unit AMV Reverse Transcriptase (Promega, USA) and 500 ng Oligo $(dT)^{15}$ primer at 42ºC for 1 hour.

Quantitative real-time PCR was performed using Rotor Gene 2000 PCR machine and analyzed using Rotor Gene 2000 program (Corbett Research, Australia) on five selected bovine genes showing differential expression in two samples examined: matrix metalloproteinase 1 (MMP1), caveolin 1 (CAV1), caveolin 2 (CAV2), transthyretin (TTR), dual specificity phosphatase 10 (DUSP10). Two expression control genes, namely, bovine β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were used for the RT-PCR reaction. The thermal profiles included 25 cycles of denaturation at 94 ºC for 30 sec, annealing at 56 ºC for 30 sec and extension at 72 ºC for 3 min.

RESULTS AND DISCUSSION

1. Preparation and differentiation of intramuscular fibroblast-like cells

In order to profile differentially expressed genes between intramuscular fibroblast-like cells and their differentiated adipocytes, intramuscular fibroblast-like cells were prepared from sirloin tissues of 12-month-old Korean cattle (Hanwoo) and differentiated adipocytes were obtained by inducing for 18 days with differential medium contained insulin, dexamethasone, 1-methy-3-isobutylxanthine and acetic acid. In order to confirm the adipocytes differentiation, Oil red O staining and triglyceride assays were performed. As the results, differentiated adipocytes gave strong Oil red O positive and significantly increased the amount of triglycerides in induced cells for 18 days of differentiation (Jeong et al., 2008). Asakura et al. (2001) suggested that muscle satellite cells for postnatal growth and regeneration have multipotential stem cell activity. Therefore, these cells also have the ability for adipocyte formation. Recently, Kook et al. (2006) reported that bovine primary satellite cells in skeletal muscle can differentiate into adipocytes in adult cattle. Based on the evidence in the previous publications and the increase of triglycerides, we can conclude that the preadipocytes in the bovine skeletal muscle were successfully differentiated into adipocytes in this study.

2. Identification of differentially expressed genes

The differentially expressed genes between intramuscular fibroblast-like cells and their differentiated adipocytes were investigated using 23k Bovine Genome array (Affymetrix, USA). In order to identify differentially expressed genes between the two samples, both 2-fold expression differences and p-value 0.05 in Welch's t-test were applied. As the results, 2,747 chip elements were identified as significantly differentially expressed genes. Of these, 1,484 elements were up-regulated and 1,263 were down-regulated in the adipogenesis. However, large number of chip elements are currently unknown ESTs (Expressed Sequence Tags) and hard to estimate their functions, even though the bovine genome sequence has been released. Therefore excluding the functionally unknown transcripts, we found 927 elements were up-regulated and 1,028 were down-regulated in the development of adipocytes based on the BLAST search results. Also, we found that only 652 elements were significantly differentially expressed genes based on Welch's t-test ($P<0.05$). Of these, 476 chip elements are known genes. Based on the two statistical methods, 206 chip elements were identified as differentially expressed, which includes 67 up-regulated and 94 down-regulated chip elements, respectively, in differentiated adipocytes (Table 1 and 2). In order to find more statistically differentially expressed genes, multiple test correction was also performed. However, we could not identify any significant genes using the multiple test correction, indicating high variability of the duplicate chip experiments. This also indicates the differentially expressed genes in this study need to be confirmed using other methods including quantitative real time PCR.

The 161 differentially expressed chip elements were subjected to three main functional classifications, namely, biological process, molecular function and cellular component, using the web-based DAVID program (Table 3). The results indicated that 38 up-regulated and 39 down-regulated genes were the known genes for their functions. The up-regulated chip elements belong to ion binding in molecular function (26.3%), proteinaceous extracellular matrix (7.9%) and organelle membrane in cellular function (10.5%). The down-regulated chip elements were related with extracellular space in cell component (7.7%), protein binding in molecular function (33.3%) and nitrogen compound metabolic process in biological process (7.7%). Especially, 23 differentially expressed genes (29.9%) were related with ion and protein binding. Therefore, these results suggested that the cell properties

Table 1. List of up-regulated genes in differentiated adipocytes

UniGene ID	Gene name	Symbol	Fold change
Bt.19813	Similar to delta/notch-like EGF-related receptor	LOC540552	654.4
Bt.22693	Transcribed locus	–	138
Bt.5401	Transglutaminase 2	TGM2	92.1
Bt.594	Uterine milk protein precursor	LOC286871	79.5
Bt.96035	Fibroblast growth factor receptor 2	FGFR2	74.8
Bt.1171	Smoothelin-like2	SMTNL2	60.5
Bt.24823	Transcribed locus	–	58.1
Bt.8088	Fibroblast growth factor receptor 2	FGFR2	53.6
Bt.16059	Distal-less homeobox 5	DLX5	48.3
Bt.64947	phosphoglucomutase 5	PGM5	42
Bt.25236	Matrix-remodelling associated 5	MXRA5	41.9
Bt.91101	Metallothionein 1E	MT1E	39.1
Bt.29868	Cytochrome c oxidase subunit VIb testes-specific isoform	COX6B2	34.2
Bt.11436	Transcribed locus	–	33.5
Bt.13670	Alkaline phosphatase, liver/bone/kidney	ALPL	28.1
Bt.15658	Transcribed locus	–	27.3
Bt.3595	Matrix Gla protein	MGP	23.3
Bt.23505	Pyruvate dehydrogenase kinase, isozyme 4	PDK4	22.2
Bt.5530	Dehydrogenase/reductase (SDR family) member 3	DHRS3	17.5
Bt.74139	T-cell lymphoma invasion and metastasis 1	TIAM1	17.1
Bt.36109	ATPase, aminophospholipid transporter (APLT), Class I, type 8A, member 1	ATP8A1	15
Bt.13108	ADAM metallopeptidase with thrombospondin type 1 motif, 5	ADAMTS5	13.2
Bt.87249	S100 calcium binding protein A9	S100A9	11.3
Bt.24413	Similar to transmembrane and tetratricopeptide repeat containing 2	LOC781126	11
Bt.53247	Sushi domain containing 2	SUSD2	10.3
Bt.46188	Similar to cAMP-dependent protein kinase inhibitor beta	LOC781283	9.8
Bt.3946	Similar to Coiled-coil domain-containing protein 3	LOC509875	9.2
Bt.96928	Transcribed locus	–	8.62
Bt.92589	Roundabout, axon guidance receptor, homolog 1 (Drosophila)	ROBO1	8.48
Bt.8644	Potassium voltage-gated channel, Shal-related subfamily, member 1	KCND1	8.35
Bt.13057	Calcium channel, voltage-dependent, T type, alpha 1H subunit	CACNA1H	6.45
Bt.16951	Frizzled homolog 2 (Drosophila)	FZD2	6.33
Bt.12314	6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3	PFKFB3	6.2
Bt.14241	Transcribed locus	–	6.12
Bt.21766	Myeloid leukemia factor 1	MLF1	6.12
Bt.92908	Transcribed locus	–	6.02
Bt.11489	Similar to histone cluster 1, H3f	LOC523214	5.77
Bt.16951	Matrix metalloproteinase 1	MMP1	5.72
Bt.27611	Similar to Zinc finger protein ZIC 2	LOC514433	5.71
Bt.96693	Transcribed locus	–	5.69
Bt.42710	Rho guanine nucleotide exchange factor (GEF) 11	ARHGEF11	5.47
Bt.64960	Teashirt zinc finger homeobox 1	TSHZ1	5.4
Bt.3138	Transcribed locus	–	5.33

Table 1. Continued

UniGene ID	Gene name	Symbol	Fold change
Bt.11850	Phosphatidic acid phosphatase type 2A	PPAP2A	5.32
Bt.5523	Golgi membrane protein 1	GOLM1	4.94
Bt.4067	Myristoylated alanine-rich protein kinase C substrate	MARCKS	4.41
Bt.19745	Elongation factor, RNA polymerase II, 2	ELL2	4.41
Bt.89975	Transcribed locus	–	4.29
Bt.51574	Caveolin 1, caveolae protein, 22kDa	CAV1	4.18
Bt.16628	Transcribed locus	–	4.03
Bt.11856	Transcribed locus	–	3.63
Bt.23265	Sprouty-related, EVH1 domain containing 1	SPRED1	3.56
Bt.26635	Frizzled homolog 1 (Drosophila)	FZD1	3.54
Bt.13989	Caveolin 2	CAV2	3.51
Bt.43663	Similar to Transcription factor COE4 (Early B-cell factor 4)	LOC506470	3.48
Bt.96693	Transcribed locus	–	3.45
Bt.16738	Iroquois homeobox 3	IRX	3.4
Bt.16087	Nidogen 2 (osteonidogen)	NID2	3.4
Bt.26682	Transcribed locus	–	3.32
Bt.5292	Period homolog 1 (Drosophila)	PER1	3.24
Bt.16125	Ring finger protein, transmembrane 1	RNFT1	3.23
Bt.14853	RAB3A interacting protein (rabin3)	RAB3IP	3.21
Bt.19081	Laminin, gamma 1 (formerly LAMB2)	LAMC1	3.09
Bt.100290	Transcribed locus	–	3.01
Bt.10146	Similar to E3 ubiquitin-protein ligase MARCH5	LOC788125	3
Bt.18938	Similar to BCL-6 interacting corepressor	LOC541088	2.87
Bt.10882	Tissue inhibitor of metalloproteinase 3	TIMP3	2.84
Bt.40036	Transmembrane protein 135	TMEM135	2.75
Bt.21959	transmembrane protein 64	TMEM64	2.64
Bt.96928	Transcribed locus	–	2.48
Bt.25998	Similar to Glioma tumor suppressor candidate region gene 1 protein	LOC531868	2.48
Bt.12578	Origin recognition complex, subunit 5-like (yeast)	ORC5L	2.46
Bt.19382	Transcribed locus	–	2.43
Bt.29527	STARD3 N-terminal like	STARD3NL	2.43
Bt.52756	Prostaglandin F2 receptor negative regulator	PTGFRN	2.38
Bt.18938	Similar to BCL-6 interacting corepressor	LOC541088	2.38
Bt.10147	Pyruvate carboxylase	PC	2.38
Bt.24324	Amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	APBB2	2.29
Bt.24150	Hepatitis A virus cellular receptor 1 N-terminal domain containing protein	MGC137099	2.28
Bt.20496	Oxysterol binding protein-like 2	OSBPL2	2.19
Bt.92182	Transcribed locus	–	2.17
Bt.55266	Similar to procollagen alpha 2(V)	LOC782818	2.15
Bt.26139	Transcribed locus	–	2.14
Bt.6993	Non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase)	NME7	2.12
Bt.64611	Hypothetical LOC513716	MGC143285	2.1
Bt.21079	Similar to solute carrier family 35, member F2	LOC533008	2.1
Bt.11304	Paroxysmal nonkinesigenic dyskinesia	PNKD	2.04

Table 2. List of down-regulated genes in differentiated adipocytes

UniGene ID	Gene name	Symbol	Fold change
Bt.69254	Transcribed locus	–	0.01
Bt.57510	Adhesion molecule with Ig-like domain 2	AMIGO2	0.01
Bt.207	Transthyretin	TTR	0.01
Bt.62498	Similar to subtilisin-like proprotein convertase PACE4 precursor, splice form A	LOC524684	0.02
Bt.26772	Calcium homeostasis modulator 2	CALHM2	0.02
Bt.34795	Fas apoptotic inhibitory molecule 2	FAIM2	0.04
Bt.42349	Transcribed locus	–	0.05
Bt.89962	Ankyrin repeat domain 29	ANKRD29	0.05
Bt.33139	Selenium binding protein 1	SELENBP1	0.05
Bt.44938	La ribonucleoprotein domain family, member 6	LARP6	0.06
Bt.64516	Renin binding protein	RENBP	0.07
Bt.47512	Platelet-derived growth factor receptor-like	PDGFRL	0.07
Bt.28164	Transcribed locus	–	0.07
Bt.15886	acyl-CoA synthetase long-chain family member 5	ACSL5	0.07
Bt.3686	Interleukin 6 (interferon, beta 2)	IL6	0.07
Bt.11576	Spermidine/spermine N1-acetyltransferase family member 2	SAT2	0.08
Bt.17884	Pirin (iron-binding nuclear protein)	PIR	0.10
Bt.21071	Transcribed locus	–	0.11
Bt.29580	Transcribed locus	–	0.11
Bt.2070	Dual specificity phosphatase 10	DUSP10	0.11
Bt.24813	Receptor transporter protein 4	RTP4	0.11
Bt.7724	Septin 11	SEP11	0.12
Bt.28110	Transcribed locus	–	0.12
Bt.4040	Platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit (29kD)	PAFAH1B3	0.13
Bt.57742	Transmembrane protein 140	TMEM140	0.13
Bt.59240	SP140 nuclear body protein	SP140	0.15
Bt.16118	Similar to C-type lectin domain family 2, member h	LOC504309	0.15
Bt.2408	Chemokine (C-C motif) ligand 2	CCL2	0.15
Bt.49579	Solute carrier family 29 (nucleoside transporters), member 1	SLC29A1	0.17
Bt.28855	Transcribed locus	–	0.17
Bt.20267	Glutamate-cysteine ligase, modifier subunit	GCLM	0.18
Bt.14163	Chloride intracellular channel 2	CLIC2	0.19
Bt.16053	Similar to plexin D1	LOC781625	0.20
Bt.4204	Alpha-N-acetylglucosaminidase	NAGLU	0.20
Bt.57860	Membrane bound O-acyltransferase domain containing 1	MBOAT1	0.21
Bt.49270	Receptor accessory protein 4	REEP4	0.21
Bt.64813	Rho GTPase activating protein 22	ARHGAP22	0.22
Bt.7142	Similar to Absent in melanoma 1 protein	LOC526200	0.22
Bt.46096	Acyl-CoA synthetase long-chain family member 1	ACSL1	0.23
Bt.53697	Proteasome subunit, beta type 8	PSMB8	0.23
Bt.63482	Mesenchyme homeobox 2	MEOX2	0.23
Bt.59240	SP140 nuclear body protein	SP140	0.24
Bt.20910	Microspherule protein 1	MCRS1	0.24
Bt.3913	Vascular endothelial growth factor C	VEGFC	0.24
Bt.101334	choline phosphotransferase 1	CHPT1	0.24
Bt.996	Transcribed locus	–	0.24
Bt.32841	HECT domain containing 3	HECTD3	0.25
Bt.52452	Proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)	PSME1	0.25
Bt.9728	ATPase, Na^+/K^+ transporting, beta 3 polypeptide	ATP1B3	0.26
Bt.24736	Similar to sperm associated antigen 1	LOC530104	0.27
Bt.26919	Netrin 4	NTN4	0.27
Bt.3726	Development and differentiation enhancing factor 2	DDEF2	0.27
Bt.54952	Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 4	ELOVL4	0.28
Bt.22036	Transcribed locus	–	0.28

Table 2. Continued

UniGene ID	Gene name	Symbol	Fold change
Bt.53493	Gelsolin (amyloidosis, Finnish type)	GSN	0.29
Bt.49466	Optineurin	OPTN	0.29
Bt.17770	Glycogenin 2	GYG2	0.3
Bt.28338	Endoplasmic reticulum aminopeptidase 1	ERAP1	0.3
Bt.22655	GTF2I repeat domain containing 2	GTF2IRD2	0.31
Bt.56475	Transcribed locus	−	0.31
Bt.21731	Drebrin 1	DBN1	0.32
Bt.20432	Serpin peptidase inhibitor, clade B (ovalbumin), member 8	SERPINB8	0.32
Bt.53943	Ectodermal-neural cortex (with BTB-like domain)	ENC1	0.33
Bt.4873	Cytochrome b5 reductase 3	CYB5R3	0.34
Bt.66339	Interleukin 17D	IL17D	0.35
Bt.5986	fms-related tyrosine kinase 3 ligand	FLT3LG	0.36
Bt.9004	Branched chain aminotransferase 2, mitochondrial	BCAT2	0.36
Bt.11418	RYK receptor-like tyrosine kinase	RYK	0.36
Bt.53789	Aconitase 1, soluble	ACO1	0.37
Bt.63052	RNA binding motif protein 19	RBM19	0.37
Bt.6989	Transmembrane BAX inhibitor motif containing 1	TMBIM1	0.37
Bt.13543	Chromosome 1 open reading frame 21 ortholog	H16C1ORF21	0.38
Bt.5857	TRNA methyltransferase 6 homolog (S. cerevisiae)	TRMT6	0.38
Bt.76883	Transcribed locus	−	0.38
Bt.7058	Transcribed locus	−	0.38
Bt.89664	Transcribed locus	−	0.39
Bt.58583	Ash1 (absent, small, or homeotic)-like (Drosophila)	ASH1L	0.39
Bt.64949	Transcribed locus	−	0.39
Bt.101068	Oxysterol binding protein-like 1A	OSBPL1A	0.39
Bt.28705	Myotubularin related protein 2	MTMR2	0.39
Bt.44278	Galactosidase, beta 1-like	GLB1L	0.39
Bt.44212	Transcribed locus	−	0.39
Bt.27957	Poly (ADP-ribose) polymerase family, member 8	PARP8	0.39
Bt.24470	Tripartite motif-containing 37	TRIM37	0.4
Bt.60919	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NFKB1	0.4
Bt.91711	THAP domain containing 7	THAP7	0.4
Bt.35232	Vacuolar protein sorting 41 homolog (S. cerevisiae)	VPS41	0.42
Bt.16348	HERPUD family member 2	HERPUD2	0.42
Bt.26917	Arginyl aminopeptidase (aminopeptidase B)-like 1	RNPEPL1	0.42
Bt.98161	Transcribed locus	−	0.42
Bt.6685	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	MTHFD2	0.42
Bt.21055	MAP kinase interacting serine/threonine kinase 1	MKNK1	0.43
Bt.64884	Transcribed locus	−	0.44
Bt.97014	TBC1 domain family, member 24	TBC1D24	0.44
Bt.43257	Transcribed locus	−	0.45
Bt.42764	Prosaposin	PSAP	0.45
Bt.20323	Hypothetical LOC513740	LOC513740	0.46
Bt.65044	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	MAFF	0.46
Bt.3689	Chromosome 10 open reading frame 35 ortholog	C28H10ORF35	0.46
Bt.94187	Zinc finger protein 75 (D8C6)	ZNF75	0.47
Bt.91525	Transcribed locus	−	0.47
Bt.7572	EGF-containing fibulin-like extracellular matrix protein 2	EFEMP2	0.48
Bt.77226	K(lysine) acetyltransferase 5	KAT5	0.48
Bt.15231	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	ARHGEF6	0.49
Bt.1726	TBC1 domain family, member 17	TBC1D17	0.49
Bt.49556	SERTA domain containing 1	SERTAD1	0.49
Bt.7636	Transcribed locus	−	0.49
Bt.9289	Similar to carnitine O-palmitoyltransferase	LOC506812	0.5

Table 3. Functional Annotation of differentially expressed genes between preadipocyte and its differentiated adipocyte cells using DAVID program

	Category	Term	Genes	Count	%	P-value
Up-regulated genes	Molecular function	Ion binding	LOC540552, ATP8A1, PC, NME7, ALPL, ADAMTS5, MGP, TGM2, MMP1, KCND1	10	26.3	3.5E-2
	Cellular component	Organelle membrane	PC, CAV1, COX6B2, CAV2	4	10.5	9.7E-2
	Cellular component	Proteinaceous extracellular matrix	ADAMTS5, MMP1, TIMP3	3	7.9	3.6E-2
Down-regulated genes	Molecular function	Protein binding	TTR, CCL2, PAFAH1B3, MAFF, IL6, NFKB1, VEGFC, GCLM, DBN1, AMIGO2, FLT3LG, ATP1B3, PSME1	13	33.3	1.9E-2
	Cellular component	Extracellular space	CCL2, FLT3LG, IL6	3	7.7	4.0E-2
	Biological component	Nitrogen compound metabolic process	GCLM, TTR, BCAT2	3	7.7	8.1E-2

have been changed during the process of differentiation. Particularly, we found that hypothetical protein, LOC540552, was the most highly expressed chip element for adipocyte differentiation, which is unknown gene in cattle. Further investigation of this chip element indicated that the nucleotide sequence of LOC540552 has similarity to Delta/Notch-like EGF-related receptor (DNER) gene based on the BLAST search result (http://www.ncbi.nlm.nih.gov/BLAST/). DNER was known to bind Notch1 gene at cell-to-cell contact and it has a function as an activator in Notch1 signaling. Garces et al. (1997) suggested that expression of Notch1 plays a key role in adipocyte differentiation of 3T3-L1 cells in mouse. Moreover, DNER was located on BTA3, where the quantitative trait loci (QTL) for marbling score was located (Casas et al. 2001; 2004). In monkey and human cell lines, Transglutaminase 2 (TGM2) was expressed dependently by binding all-trans-retinoic acid (RA) into retinoic acid receptor-retinoid X receptor (RAR · RXR) heterodimeric complexes (Nagy et al., 1996; Ma et al., 2003). Therefore, we can expect that RA can increase the TGM2 expression in relation to the differentiation of adipocytes. However, until now, no known relationship was identified between TGM2 expression and adipogenesis.

3. Validation of expression by semi-quantitative real-time PCR

To validate differentially expressed genes from the bovine genome array data, five genes were selected, namely matrix metalloproteinase 1 (MMP1), Calveolin 1 (CAV1), Calveolin 2 (CAV2), Transthyretin (TTR) and Dual specificity phosphatase 10 (DUSP10) and confirmed with semi-quantitative real-time PCR assays. Based on bovine genome array results, MMP1, CAV1 and CAV2 genes were up-regulated and TTR and DUSP10 genes were down-regulated in differentiated adipocytes (Fig. 1). The mRNA expression patterns of selected five genes were highly consistent with the bovine genome array results. MMPs block cholesterol efflux in macrophage foam cells (Lindstedt et al., 1999) and have a function as modulator of adipogenesis (Chavey et al., 2003). Therefore, we assumed that MMP1 of the fibroblast type collagenase, which associated with alteration of cell membrane according to adipogenesis of intramuscular fibroblast-like cells. Up-regulated CAV1 and CAV2 genes play an important role in cholesterol homeostasis as major structural component of caveolae *in vivo* (Martin and Parton, 2005). CAV1 gene induced adipogenesis in 3T3-L1 cells (Scherer et al., 1994) as well as increased during adipocyte differentiation of bovine intramuscular preadipocyte (BIP) cells (Takenouchi et al., 2004). Moreover, CAV2 protein increased expression in 3T3-L1 adipocytes (Scherer et al., 1997). Therefore, we can conclude that CAV1 and CAV2 genes are highly related with adipogenesis. Down-regulated TTR gene was reported that it can bind to plasma membrane and related to change of membrane fluidity (Hou et al., 2005). DUSP10 gene was included as a subclass of protein tyrosine phosphatase (PTP) gene superfamily, which has important roles for control of Mitogen-activated protein (MAP) kinase functions (Camps et al., 2000). Therefore, we assumed that expression changes of these genes can ultimately give the different cell properties

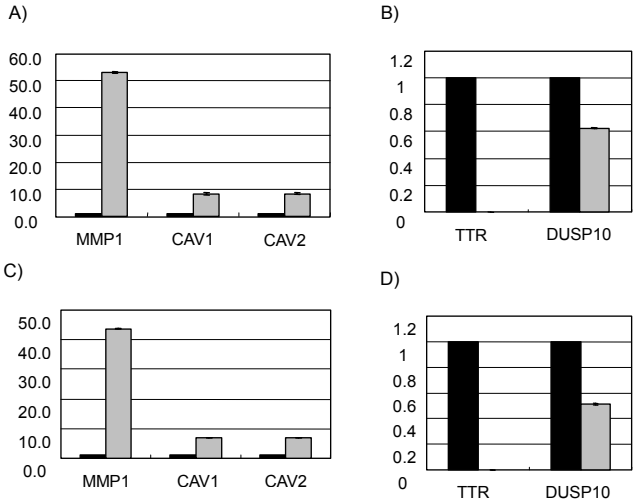

Fig. 1. The mRNA expression patterns based on the semi-quantitative real time PCR for the five selected genes among differentially expressed genes in bovine genome array. Black bars indicate intramuscular fibroblast-like cells and gray bars are their differentiated adipocytes. The numbers in vertical line indicate fold differences. The gene expression levels of each gene were normalized to the expression level of beta-actin (A, B) and GAPDH (C, D).

between fibroblast-like cells and differentiated adipocytes.

Based on this experiment, we found that the large number of differentially expressed chip elements was currently unknown functions and enormous efforts were needed for the further verification of the differentially expressed genes.

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REFERNECES

1. Asakura, A., Komaki M. and Rudnicki, M. 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation. 68:245-253.
2. Aso, H., Abe, H., Nakajima, I., Ozutsumi, K., Yamaguchi, T., Takamori, Y., Kodama, A., Hoshino, F. B. and Takano. S. 1995. A preadipocyte clonal line from bovine intramuscular adipose tissue: nonexpression of GLUT-4 protein during adipocyte differentiation. Biochem. Biophys. Res. Commun. 213:369-375.
3. Camps, M., Nichols, A. and Arkinstall, S. 2000. Dual specificity phosphatases: a gene family for control of MAP kinase function. FASEB J. 14:6-16.
4. Casas, E., Stone, R. T., Keele, J. W., Shackelford, S. D., Kappes, S. M. and Koohmaraie, M. 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. J. Anim. Sci. 79:854-860.
5. Casas, E., Keele, J. W., Shackelford, S. D., Koohmaraie, M. and Stone, R. T. 2004. Identification of quantitative trait loci for growth and carcass composition in cattle. Anim. Genet. 35:2-6.
6. Chavey, C., Mari, B., Monthouel, M. N., Bonnafous, S., Anglard, P., Van Obberghen, E. and Tartare-Deckert, S. 2003. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J. Biol. Chem. 278:11888-11896.
7. Garces, C., Ruiz-Hidalgo, M. J., Font de Mora, J., Park, C., Miele, L., Goldstein, J., Bonvini, E., Porras, A. and Laborda, J. 1997. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. J. Biol. Chem. 272:29729-29734.
8. Girard, J., Perdereau, D., Foufelle, F., Prip-Buus, C. and Ferre, P. 1994. Regulation of lipogenic enzyme gene expression by nutrients and hormones. FASEB J. 8:36-42.
9. Green, H. and Kehinde, O. 1975. An established preadipose cell line and its differentiation in culture II. Factors affecting the adipose conversion. Cell. 5:19-27.
10. Hou, X., Richardson, S. J., Aguilar, M. I. and Small, D. H. 2005. Binding of amyloidogenic transthyretin to the plasma membrane alters membrane fluidity and induces neurotoxicity. Biochemistry. 44:11618-11627.
11. Inoue-Murayama, M., Sugimoto, Y. Niimi, Y. and Aso, H. 2000. Type XVIII collagen is newly transcribed during bovine adipogenesis. Differentiation. 65:281-285.
12. Jeong, Y. H., Lee, S. M., Kim, H. M., Park, H. Y., Yoon, D., Moon, S. J., Hosoda, A., Kim, D. H., Saeki, S. and Kang, M. J. 2008. Cloning, expression and regulation of bovine cellular retinoic acid-binding protein II (CRABP-II) during adipogenesis. Asian-Aust. J. Anim. Sci. 21:1551-1558.
13. Kook, S. H., Choi, K. C., Son, Y. O., Lee, K. Y., Hwang, I. H., Lee, H. J., Chang, J. S., Choi, I. H. and Lee, J. C. 2006.

Satellite cells isolated from adult Hanwoo muscle can proliferate and differentiate into myoblasts and adipose-like cells. Mol. Cells. 22:239-245.

14. Lindstedt, L., Saarinen, J., Kalkkinen, N., Welgus, H. and Kovanen, P. T. 1999. Matrix metalloproteinases-3, -7, and -12, but not -9, reduce high density lipoprotein-induced cholesterol efflux from human macrophage foam cells by truncation of the carboxyl terminus of apolipoprotein A-I. Parallel losses of pre-beta particles and the high affinity component of efflux. J. Biol. Chem. 274:22627-22634.
15. Ma, Y., Koza-Taylor, P. H., DiMattia, D. A., Hames, L., Fu, H., Dragnev, K. H., Turi, T., Beebe, J. S., Freemantle, S. J. and Dmitrovsky, E. 2003. Microarray analysis uncovers retinoid targets in human bronchial epithelial cells. Oncogene. 22:4924-4932.
16. Martin, S. and Parton, R. G. 2005. Caveolin, cholesterol, and lipid bodies. Semin. Cell. Dev. Biol. 16:163-174.
17. Nakajima, I., Muroya, S., Tanabe, R. and Chikuni, K. 2002. Extracellular matrix development during differentiation into adipocytes with a unique increase in type V and VI collagen. Biol. Cell. 94:197-203.
18. Nakajima, I., Muroya, S., Tanabe, R. and Chikuni, K. 2002. Positive effect of collagen V and VI on triglyceride accumulation during differentiation in cultures of bovine intramuscular adipocytes. Differentiation. 70:84-91.
19. Nakajima, I., Yamaguchi, T., Ozutsumi, K. and Aso, H. 1998. Adipose tissue extracellular matrix: newly organized by adipocytes during differentiation. Differentiation. 63:193-200.
20. Nagy, L., Saydak, M., Shipley, N., Lu, S., Basilion, J. P., Yan, Z. H., Syka, P., Chandraratna, R. A., Stein, J. P., Heyman, R. A. and Davies, P. J. 1996. Identification and characterization of a versatile retinoid response element (retinoic acid receptor response element-retinoid X receptor response element) in the mouse tissue transglutaminase gene promoter. J. Biol. Chem. 271:4355-4365.
21. Rangwala, S. M. and Lazar, M. A. 2000. Transcriptional control of adipogenesis. Annu. Rev. Nutr. 20:535-559.
22. Reusch, J. E., Colton, L. A. and Klemm, D. J. 2000. CREB activation induces adipogenesis in 3T3-L1 cells. Mol. Cell. Biol. 20:1008-1020.
23. Rosen, E. D. and Spiegelman, B. M. 2000. Molecular regulation of adipogenesis. Annu. Rev. Cell Dev. Biol. 16:145-171.
24. Scherer, P. E., Lisanti, M. P., Baldini, G., Sargiacomo, M., Mastick, C. C. and Lodish, H. F. 1994. Induction of caveolin during adipogenesis and association of GLUT4 with caveolin-rich vesicles. J. Cell Biol. 127:1233-1243.
25. Scherer, P. E., Lewis, R. Y., Volonte, D., Engelman, J. A., Galbiati, F., Couet, J., Kohtz, D. S., van Donselaar, E., Peters, P. and Lisanti, M. P. 1997. Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex *in vivo*. J. Biol. Chem. 272:29337-29346.
26. Tahara, K., Aso, H., Yamasaki, T., Rose, M. T., Takasuga, A., Sugimoto, Y., Yamaguchi, T., Tahara, K. and Takano, S. 2004. Cloning and expression of type XII collagen isoforms during bovine adipogenesis. Differentiation. 72:113-122.
27. Takenouchi, T., Miyashita, N., Ozutsumi, K., Rose, M. T. and Aso, H. 2004. Role of caveolin-1 and cytoskeletal proteins, actin and vimentin, in adipogenesis of bovine intramuscular preadipocyte cells. Cell Biol. Int. 28:615-623.
28. Taniguchi, M., Guan, L. L., Zhang, B., Dodson, M. V., Okine, E. and Moore, S. S. 2007. Gene expression patterns of bovine perimuscular preadipocytes during adipogenesis. Biochem. Biophys. Res. Commun. 366:346-351.
29. Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I. and Sheard, P. R. 1999. Manipulating meat quality and composition. Proc. Nutr. Soc. 58:363-370.
30. Wu, Z., Rosen, E. D., Brun, R., Hauser, S., Adelmant, G., Troy, A. E., McKeon, C., Darlington, G. J. and Spiegelman, B. M. 1999. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol. Cell. 3:151-158.
31. Zalesin, K. C., Franklin, B. A., Miller, W. M., Peterson, E. D. and McCullough, P. A. 2008. Impact of obesity on cardiovascular disease. Endocrinol. Metab. Clin. North Am. 37: 663-684.

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