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Depot-specific gene expression profiles during differentiation and transdifferentiation of bovine muscle satellite cells, and differentiation of preadipocytes

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ABSTRACT

We report a systematic study of gene expression during myogenesis and transdifferentiation in four bovine muscle tissues and of adipogenesis in three bovine fat tissues using DNA microarray analysis. One hundred hybridizations were performed and 7245 genes of known and unknown function were identified as being differentially expressed. Supervised hierarchical cluster analysis of gene expression patterns revealed the tissue specificity of genes. A close relationship in global gene expression observed for adipocyte-like cells derived from muscle and adipocytes derived from intramuscular fat suggests a common origin for these cells. The role of transthyretin in myogenesis is a novel finding. Different genes were highly induced during the transdifferentiation of myogenic satellite cells and in the adipogenesis of preadipocytes, indicating the involvement of different molecular mechanisms in these processes. Induction of CD36 and FABP4 expression in adipocyte-like cells and adipocytes may share a common pathway.

1. Introduction

Myogenic satellite cells (MSCs) are multi-potent muscle-derived stem cells. These cells are capable of differentiation into myotube formed cells (MFC) by dividing and fusing among themselves or with pre-existing myotubes [1]. In addition to the formation of myofibers, MSCs possess the multi-potential capacity to transdifferentiate into osteocytes, adipocyte-like cells and nerve cells [2]. Since myoblasts and adipocytes arise from the same mesodermal germ layer, it is possible to induce a direct conversion of myoblasts into adipocytes [3]. To date, mouse, human, rat, porcine and bovine MSCs have been transdifferentiated into adipocyte-like cells (ALCs) [4–7]. Many transcriptional and myogenic regulatory factors are involved in the differentiation of

these muscle-derived stem cells. Myogenic regulatory factor genes, including myogenin, MRF4 (MRF6), MYOD (MYOD1) and Myf5 are all expressed in anatomical- and time-dependent manners during differentiation [8], while the activation of the gene encoding peroxisome proliferator-activated receptor-gamma (PPARG) and the induction of fatty acid transporter genes involved in fatty acid storage and metabolism leads to the formation of ALCs [9]. Fat depots also contain a reservoir of potential adult progenitor cells known as preadipocytes. Preadipocyte differentiation and conversion into a viable adipocyte is a complex process which is accompanied by a coordinated change in cell morphology, hormone sensitivity, and gene expression [10].

Cellular and functional adipose depot-dependent traits are likely dictated by a combination of intrinsic and extrinsic factors [11,12]. Metabolic differences between intramuscular and subcutaneous fat depots have been reported [13]. To date, depot-specific differences in gene expression during adipogenesis have been studied in human, cattle and mouse adipocytes [12,14]. In mice, adipogenesis has been intensively studied using the 3T3-L1 preadipocyte cell line [15] and gene families such as PPAR and sterol regulatory element-binding proteins (SREBP) have been reported to play major roles during preadipocyte differentiation [16,17]. However, the expression of molecular markers and

interactions among genes during adipogenesis in livestock species are not necessarily similar to those of mouse 3T3-L1 cells [18]. Furthermore, there may be differences in the genetic characteristics among the MSCs and their differentiation and transdifferentiation from different muscle depots.

This study was performed to identify differentially expressed genes during differentiation and transdifferentiation of MSCs from four muscle tissues and the differentiation of preadipocytes in three fat depots in the bovine. Additionally, the gene expression profiles were compared across tissues to identify commonly up-regulated genes. Genes with known and unknown functions were identified as being differentially expressed, which furthers our understanding of the molecular mechanisms involved in these processes.

2. Materials and methods

2.1. Isolation and culture of MSCs and preadipocytes

Nine Hanwoo steers used for this study were managed in a feeding barn at the National Institute of Animal Science, Korea until they were sacrificed at 24-months-of-age. The animals were divided into three groups each of three animals with group I animals used for the beef shank (BS) studies, group II animals used for the BS and three fat depots study and group III animals used to study all four muscle and three fat depots. The animals were handled according to a protocol approved by the Animal Care and Concern Committee of the National Institute of Animal Science, Korea. Bovine muscle samples were collected from four different cut-specific depots: beef shank (BS), longissimus dorsi (LD), deep pectoral (DP), and semitendinosus (SE). Isolation and culture of MSCs were performed as previously described [19]. The culture medium was changed every second day and the condition of the cells was monitored. Separate differentiation induction cocktails were used to differentiate the myogenic cells into myotube forming cells and the preadipocytes into adipocytes.

To induce the transdifferentiation of MSCs into ALCs, culture medium was replaced with an adipogenic medium on the 14th day of culture when the MSCs reached approximately 80% confluence. The adipogenic medium was comprised of DMEM, 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.1% amphotericin along with 33 μM biotin (Sigma-Aldrich, St. Louis, MO, USA), 17 μM pantothenate (Sigma-Aldrich), 200 μM ascorbic acid (Sigma-Aldrich), 1 mM caprylic acid (Sigma-Aldrich), 10 mM acetic acid (Sigma-Aldrich), 10 μg/ml insulin (Sigma-Aldrich), 5 μM dexamethasone (Sigma-Aldrich), 0.5 mM 3-Isobutyl-1-methylxanthine (Sigma-Aldrich), and 10 μM thiazolidione (Merck, Hohenbrunn, Germany).

Subcutaneous fat (SF) and intramuscular fat (IMF) were isolated from between ribs six and seven. Abdominal fat (AF) was taken from the lesser curvature of the abomasum. Collected tissues were kept in sterile saline solution (0.154 M NaCl, 37 °C) for the recovery of stromal vascular cells as previously described [20]. Tissues were sliced and digested in Krebs Ringer Bicarbonate (KRB) buffer (1.22 mM CaCl$_2$) for 1 h and filtered through nylon mesh to release the cells. The filtrate was centrifuged at 2500 rpm for 5 min and the obtained pellet was twice washed with Hank's Balanced Salt Solution (HBSS) at 2500 rpm for 5 min. The pellet was resuspended in M199 culture medium supplemented with 10% FBS and 1% penicillin/streptomycin, and the cells were then seeded in a 100 mm-diameter culture dish at a density of 2.5×10^3 cells/cm^2. The cells were allowed to grow until 70% confluence (about 10 days) at 37 °C in 5% CO$_2$ with medium changes on alternate days. Cells were then differentiated by treatment with a differentiation-induction cocktail consisting of triiodothyronine (2 nM, Sigma-Aldrich), insulin (5 ng/ml, Sigma-Aldrich), dexamethasone (10 nM mix, 0.1 μM, Sigma-Aldrich), lipids (10 μl/ml), L-ascorbic acid (50 μM, Sigma-Aldrich), biotin (10 nM, Sigma-Aldrich), and pantothenic acid 100 μM (Sigma-Aldrich) in DMEM-F12 supplemented with 10% FBS and 1%

penicillin/streptomycin. The culture medium was changed every second day and the condition of the cells was monitored.

2.2. Preparation of RNA

Total RNA was isolated from the cells by using Trizol reagent (Invitrogen, Carlsbad, CA, USA) as previously described [21]. Concentration of the RNA was measured by a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the RNA was stored in diethyl pyrocarbonate-treated water at −80 °C until use.

2.3. DNA microarray hybridization

The bovine oligonucleotide microarray contained 24,000 70-mer probes. The probes targeted the transcripts of 16,341 genes, which represent about 70% of the total number of genes in the bovine genome [22]. DNA microarray analysis with a total of 100 hybridizations was performed to identify differentially expressed genes in MSC vs MFC, MSC vs ALC, and preadipocyte vs adipocyte differentiation (Table 1). DNA microarrays were hybridized as previously described [22]. RNA samples from MSC, MFC, and ALC isolated from the four muscle depots were hybridized as MSC vs MFC, and MSC vs ALC. Similarly, RNA samples from preadipocytes harvested from the three different fat depots were hybridized as preadipocyte vs adipocyte. For control MSCs or preadipocytes and test RNAs [MFC, ALCs, or adipocyte] the synthesis of target cRNA probes was performed using a Low RNA Input Linear Amplification kit (Agilent Technologies, Palo Alto, CA, USA) according to the manufacturer's instructions.

2.4. Identification of differentially expressed genes

An AXON GenePix4000B scanner (Axon Instruments, CA) was used to image the hybridized microarrays and images were analyzed using the GenePix Pro 6.0 program (Axon Instruments GeneSpring GX 7.3 software (Agilent Technologies)) for data normalization and the identification of fold differences. Lowess (locally weighted scatter plot smoothing) normalization was performed to remove intensity dependent dye bias effects. The averages of normalized ratios were calculated by dividing the average of normalized signal channel intensity by the average of normalized control channel intensity. Functional annotation of genes was performed according to Gene Ontology™ Consortium (http://www.geneontology.org/index.shtml) by GeneSpringGX 7.3.1. Gene classification was based on searches performed using BioCarta (http://www.biocarta.com/), GenMAPP (http://www.genmapp.org/), DAVID (http://david.abcc.ncifcrf.gov/), and NCBI (National Center for

Table 1
Microarray analysis during myogenesis, ALC formation in different muscles, and adipogenesis in different fat tissues.

Tissue	Cuts	Analysis	Replications		
			Biological	Technical	Total
Muscle	BS	MSC vs MFC	9	2	18
		MSC vs ALC	6	2	12
	LD	MSC vs MFC	3	2	6
		MSC vs ALC	3	2	6
	DP	MSC vs MFC	3	2	6
		MSC vs ALC	3	2	6
	SE	MSC vs MFC	3	2	6
		MSC vs ALC	3	2	6
Adipose	IMF	Preadipocyte vs adipocyte	5	2	10
	AF	Preadipocyte vs adipocyte	6	2	12
	SF	Preadipocyte vs adipocyte	6	2	12
Total					100

Footnote: BS: Beef shank, LD: Longissimus dorsi, DP: Deep pectoral, SE: Semitendinosus, IMF: Intramuscular fat, AF: Abdominal fat, SF: Subcutaneous fat, MSC: Myogenic satellite cells, MFC: Myotube formed cells and ALC: Adipocyte-like cells. Biological replicates indicate the number of bovine individuals and technical replicates stand for repetition of DNA microarray analysis on each individual.

Biotechnology Information) (http://www.ncbi.nlm.nih.gov/). The statistical relevance of gene expression differences was confirmed by SAM (Standard University, Palo Alto, CA, USA). For SAM analysis, the significance cut-off was set at a median false discovery rate <5%.

2.5. Data base construction for microarray analysis

To analyze the bovine microarray data, microarray-related information including bovine reference nucleotide sequence identification, amino acid sequence identification, and annotation data were downloaded from NCBI. Based on this initial information, we constructed a web-based sequence database (http://edunabi.com/~bgrb/chip) to facilitate analysis of the bovine microarray data.

2.6. Real time RT-PCR analysis

Real time RT-PCR was performed to confirm the microarray results. One microgram of RNA in 20 μl total volume was primed with oligo (dT)$_{20}$ primers (Bioneer, Daejeon, Korea), and reverse transcribed in a PTC-100 thermal cycler (MJ Research Inc, MA, USA) at 42 °C for 50 min and 72 °C for 15 min. Subsequently, 2 μl of the 10× diluted cDNA product and 10 pmol of each gene-specific primer were used to perform PCR using a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Power SYBR® Green PCR Master Mix (Applied Biosystems) was used as the fluorescence source. Primers were designed with Primer 3 software (http://frodo.wi.mit.edu) using sequence information listed at the National Center for Biotechnology Information (Primer information is provided in Supplemental Table S1).

2.7. KOG analysis

Queries of the NCBI eukaryotic Orthologous Group (KOG) database were performed using a local server (stand-alone BLAST system, http://www.ncbi.nlm.nih.gov/COG/grace/kognitor.html) for the functional classification of the reference sequences. Differentially expressed genes with a minimum 2-fold up-regulation and higher statistical significance (p<0.0001), were categorized into 25 functional groups by KOG database and the Blastx program (<1×e^{-10}) [23].

2.8. Statistical analysis

The normalized expression means were compared using Tukey's Studentized Range (HSD) to detect significances in gene expression between ALCs and adipocyte. A nominal p-Value less than 0.05 were considered statistically significant. All RT-PCR data were analyzed by one-way ANOVA using PROC GLM in SAS package ver. 9.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Microarray analysis of gene expression

MSCs from four different muscle tissues were differentiated into MFC or ALC and preadipocytes from three fat depots were differentiated into adipocytes. DNA microarray analysis involving a total of 100 hybridizations was performed to identify differentially expressed genes in MSC vs MFC, MSC vs ALC, and preadipocytes vs adipocytes (Table 1). During myogenesis, ALC formation and adipogenesis, a large number of genes with known and unknown functionalities were identified as being differentially expressed. Table 2 summarizes the number of statistically significant (p<0.0001) up- and down-regulated genes with at least 2-fold differences in expression that are involved in these cellular processes. In total, 1074 and 2056 genes were found to be up-regulated and 842 and 1420 genes were found to be down-regulated, by more than 2-fold in myogenesis and ALC formation, respectively. Some genes encoding proteins involved in different functions, such as tropoelastin, transthyretin, slow cardiac myosin light chain 2, and ryanodine receptors were up-regulated during myogenesis. During adipogenesis, 195 and 71 genes were up- and down-regulated, respectively, in all muscle depots. Among the up-regulated genes, 13 and 195 were expressed in all muscle tissues during MFC and ALC formation, respectively. However, 133 genes were up-regulated in all three fat tissues during adipogenesis.

3.2. Hierarchical clustering and KOG analyses

The genes identified as being differentially expressed during differentiation in MFC, ALC, and adipocytes from the different tissue depots were subjected to correlation based clustering. During the differentiation of MSCs, the DP and SE gene expression profiles were found to be very similar, while the profile for BS differed from those for the other depots (Fig. 1A). When compared in ALC, LD and DP possessed similar gene expression profiles which differed from that for BS. During adipogenesis, similar genes were found to be expressed in SF and AF. However, gene expression in IMF more resembled ALCs produced from muscle tissues (Fig. 1B) than from SF or AF. Differentially expressed genes with a minimum of a 2-fold up-regulation and high statistical significance (p<0.0001), were categorized into 25 functional groups by KOG analysis. In LD, DP, and SE, approximately the same number of genes were found to be involved in amino acid transport and metabolism, and extracellular structure during myogenesis. Genes responsible for signal transduction were more predominantly expressed in the four muscle tissues. Among ALCs, genes involved in signal transduction were enriched in expression, regardless of tissue specificity. Lipid transport and metabolism related genes were expressed to about the same degree during adipogenesis within all fat tissues, and expression was higher when compared to ALCs or MFCs (Table 3). The complete data from the KOG analysis are provided in Supplemental Table S2.

Table 2
Differentially expressed genes during myogenesis, ALC formation, and adipogenesis (Genes differentially expressed by at least 2-fold, p <0.0001).

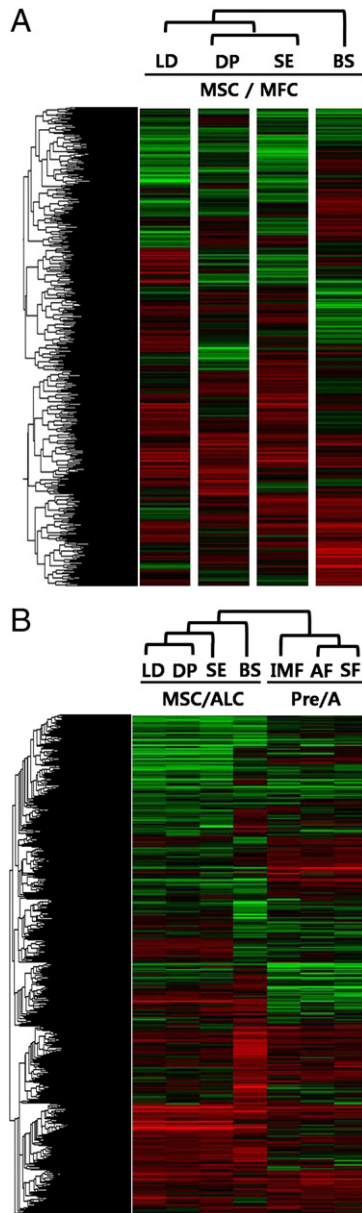| Muscle tissue	Myogenesis		ALC formation		Adipose tissue	Adipogenesis	
	Down	Up	Down	Up		Down	Up
BS	486	370	224	361	IMF	272	285
LD	158	272	430	593	AF	359	293
DP	69	166	361	527	SF	272	273
SE	129	266	405	575	Only in IMF	6	6
Only in BS	476	275	125	117	Only in AF	12	4
Only in LD	1	83	4	1	Only in SF	3	1
Only in DP	0	18	0	0	All in three	119	133
Only in SE	0	22	0	0			
In all four muscles	0	13	71	195			

Fig. 1. Hierarchical cluster analysis of gene expression profiles. (A). Green indicates genes up-regulated in MSCs and preadipocytes and red indicates genes up-regulated in MFC, ALC and adipocytes, respectively (B). Footnote: The lines at the top of the figures represent correlations among muscle and fat depots.

3.3. Comparative analysis of gene expression between transdifferentiation and adipogenesis

A comparative study of genes up-regulated with a minimum of a 4-fold difference in ALCs and adipocytes was performed. Ten and seven genes were up-regulated in all muscle and fat depots during ALC and adipocyte formation, respectively, and four genes found to be expressed in all seven tissues at high significance (p<0.0001) were validated by real time RT-PCR (Table 4A-C). *EREG*, *DLX5*, and *CD163* genes were up-regulated only in ALC. However, *CSF1*, *BACE2*, and *FABP5* genes were also expressed in adipocytes in addition to ALCs. This agreed with the results of the microarray analysis. In adipocytes, *EXP1*, *THRSP*, *P-protein*, and *GPNMB* genes were highly expressed in all three fat depots and no induction was found in muscle tissues. When genes expressed in both ALCs and adipocytes were examined, *CD36* expression was consistent with the microarray data (Figs. 2A–C).

4. Discussion

Genomic studies have added a new chapter in the understanding of MSCs and adipocytes. Several reports have described the genes involved in the differentiation and transdifferentiation of MSCs and adipogenesis [24–26]. We report here for the first time a comparative DNA microarray study of genes differentially expressed during myogenesis and ALC formation in different muscle depots and adipogenesis in fat depots. Primary bovine cells offer an advantage over cell lines, as they closely mimic the *in vivo* cellular state and generate physiologically more relevant data. Furthermore, we speculate that the use of serum from the same genus that was used in cell culture will help protect the genotypic and phenotypic characteristics of the primary cells. The high number of biological and technical replicates utilized in this study is directly related to the reliability of the analysis. During myogenesis, large numbers of differentially expressed genes with known and unknown functions were identified. Among them, 1074 and 842 genes were found to be up- and down-regulated, respectively, by more than a 2-fold difference in expression. Genes encoding proteins involved in different functions, such as tropoelastin [27] and transthyretin [28], were up-regulated during myogenesis. We have previously reported that tropoelastin shows tissue specific induction during the myogenesis of MSCs, and is highly expressed in BS [29]. However, the role of transthyretin in myogenesis is a novel finding, since the protein has previously been reported as a systemic precursor to deposition and amyloid fibril formation [28]. Similarly, slow cardiac myosin light chain 2, a myogenic marker [30] and ryanodine receptors [31] were highly up-regulated during myogenesis. We are currently studying the role of transthyretin in muscle progenitor lineage, which may provide a detailed understanding of developmental and evolutionary mechanism for anatomical muscle formation and may form a foundation in the development of stem cell therapies to repair diseased and damaged muscles.

A total of 2056 and 1420 genes were up- and down-regulated, respectively, during the formation of ALCs. However, 195 and 71 genes were up- and down-regulated, respectively, in all muscle depots. CD163 antigen, a member of the scavenger receptor cysteine-rich (*SRCR*) super family class B [32], distal-less homeobox 5 (*DLX5*) responsible for proliferation of tumor cells [33] and insulin-like growth factor binding protein 5 (*IGFBP5*) isoform 2 found in breast cancer [34] were also identified in ALC formation. Thyroid hormone-responsive protein *(THRSP)* previously reported in adipogenesis [35,36] and retinoic acid receptor RXR-gamma associated with familial combined hyperlipidemia [37] were identified during adipogenesis. *FABP4* and *CD36* which encode lipid transporters [38,39] were up-regulated in all tissues. Continuing the comparative gene study with adipogenesis, we analyzed the differentially expressed genes in preadipocytes and adipocytes in all three fat depots. We found that 119 genes were down-regulated and 133 genes were up-regulated in all three fat depots. Besides the genes involved in adipogenesis, serum amyloid A protein precursor and microsomal glutathione S-transferase 3 were found to be highly expressed.

To examine relationships between the genes involved in myogenesis in the four muscle tissues, a hierarchical cluster analysis was performed. The gene expression profile of BS was least similar to LD, DP, and SE which had similar profiles. The cluster analysis for ALC and adipocyte gene expression revealed a relationship with IMF and muscle depots. A previous study reported that interscapular brown fat, but not white fat, and muscle cells arise from a population of Engrailed-1-expressing cells in the dermomyotome [40]. It is tempting to speculate that ALCs and adipocytes within muscle share a common origin. This is supported by the role of *PRDM16* in the transdifferentiation of MSCs to brown adipose cells [41] and in the co-activation of the transcriptional functions of *PPARG* during adipogenesis [42]. Functional analyses of differentially expressed genes were confirmed by KOG and were categorized into 25 different classes. The KOG analysis was preferred over GO as it allows a simple and straightforward search for a conserved core of largely

Table 3
KOG functional analysis of differentially expressed genes up-regulated by more than 2-fold (Genes differentially expressed by at least 2-fold, p <0.0001).

Gene function	Myogenesis				ALC formation				Adipogenesis		
	BS	LD	DP	SE	BS	LD	DP	SE	IMF	AF	SF
RNA processing and modification	1	2	2	1	1	2	1	1	1	2	1
Chromatin structure and dynamics	1	1	4	0	1	4	4	3	1	0	1
Energy production and conversion	3	1	2	2	5	8	7	5	1	13	6
Cell cycle control, cell division, chromosome partitioning	1	1	4	1	2	1	1	1	1	1	1
Amino acid transport and metabolism	1	9	12	7	6	4	5	5	3	3	2
Nucleotide transport and metabolism	2	1	0	2	1	1	1	1	1	1	1
Carbohydrate transport and metabolism	1	5	2	6	1	3	2	2	1	2	1
Coenzyme transport and metabolism	1	0	0	0	0	1	0	1	1	1	1
Lipid transport and metabolism	3	7	7	5	6	7	8	8	15	18	17
Translation, ribosomal structure and biogenesis	1	1	2	2	2	0	1	1	0	1	1
Transcription	5	6	11	3	6	4	4	4	5	2	6
Replication, recombination and repair	1	1	2	1	1	0	0	1	0	0	0
Cell wall/membrane/envelope biogenesis	0	1	2	0	1	2	2	2	3	1	1
Cell motility	0	0	0	0	0	0	0	0	1	1	1
Posttranslational modification, protein turnover, chaperones	14	15	9	17	9	12	12	14	9	8	6
Inorganic ion transport and metabolism	1	2	4	4	5	4	4	5	3	3	2
Secondary metabolites biosynthesis, transport and metabolism	5	5	2	3	1	3	3	2	2	2	3
General function prediction only	10	11	14	12	14	11	12	2	22	16	19
Function unknown	3	4	0	3	8	7	7	12	8	6	7
Signal transduction mechanisms	15	13	18	12	16	14	14	15	18	13	18
Intracellular trafficking, secretion, and vesicular transport	0	3	0	3	2	2	2	1	0	1	1
Defense mechanisms	1	1	4	3	1	2	2	2	1	1	1
Extracellular structures	19	4	2	3	9	6	6	7	3	4	3
Nuclear structure	0	0	0	0	0	0	0	0	0	0	0
Cytoskeleton	10	5	2	9	5	2	2	3	3	1	2
	100	100	100	100	100	100	100	100	100	100	100

Footnote: Numbers indicate the percentage of genes in each category.

Table 4
Microarray analysis; List of genes with at least a 4 fold up-regulation during ALC formation of MSCs and preadipocyte differentiation.

A) Genes up-regulated only in ALCs.

Bovine ID		Fold difference (p-Value)				Annotation
		BS	LD	DP	SE	
Only in ALC						
Up-regulation	CK730120.1	15 (1.2E−14)	13 (8.9E−09)	13 (3.3−09)	15 (6.9E−07)	Monoamine oxidase B (MAOB)
(MSC<ALC)	CV975243.1	4 (2.9E−07)	9 (4.4E−07)	10 (5.7E−05)	11 (1.1E−06)	Colony stimulating factor 1 (CSF1)
"	CX950612.1	7 (2.1E−07)	10 (6.4E−06)	11 (1.4E−06)	19 (4.8E−07)	Epiregulin (EREG)
"	NM_001075313.1	6 (1.3E−05)	14 (3.5E−06)	13 (2.6E−06)	21 (3.7E−08)	Distal-less homeobox 5 (DLX5)
"	NM_001076257.1	5 (3.9E−06)	5 (9.4E−06)	4 (3.3E−04)	5 (2.3E−05)	Arrestin domain containing 3 (ARRDC3)
"	NM_173891.2	5 (8.4E−01)	9 (1.7E−06)	9 (2.4E−07)	7 (5.0E−07)	Brain ribonuclease (RNASE1, ANG, BRB)
"	NM_174315.3	4 (3.9E−04)	9 (1.0E−05)	6 (2.7E−07)	4 (2.2E−05)	Fatty acid binding protein 5 (FABP5)
"	XM_600908.3	4 (7.7E−04)	4 (1.7E−06)	4 (3.0E−05)	6 (5.1E−05)	Insulin-like growth factor binding protein 5 (IGFBP5)
"	XM_613380.3	42 (3.7E−09)	19 (3.7E−07)	18 (2.6E−06)	20 (2.8E−06)	CD163
"	XM_614655.3	8 (1.7E−12)	5 (2.2E−06)	5 (1.4E−05)	8 (8.2E−05)	Beta-site APP-cleaving enzyme 2 (BACE2)

B) Genes up-regulated only in adipocytes.

Bovine ID		Fold difference (p-Value)			Annotation
		IMF	AF	SF	
Only in adipocytes					
Up-regulation	XM_001254707.1	9 (2.1E−11)	9 (2.2E−0.9)	8 (6.5E−12)	Extracellular proteinase inhibitor (EXPI)
(Preadipocyte<Adipocyte)	NM_001040533.1	4 (8.8E−09)	4 (9.4E−05)	7 (4.0E−05)	Thyroid hormone-responsive protein (THRSP)
"	NM_001075408.1	5 (1.4E−06)	5 (1.2E−06)	6 (1.4E−11)	Retinoic acid X receptor gamma (RXRG)
"	CX950197.1	5 (1.3E−07)	5 (7.6E−05)	5 (4.4E−07)	Unknown
"	XM_001254244.1	6 (5.2E−15)	5 (3.3E−08)	5(8.1E−09)	P protein
"	NM_001038065.1	4 (9.8E−08)	5 (5.6E−09)	4 (7.6E−11)	Glycoprotein nmb (GPNMB)
"	CK727505.1	4 (1.5E−07)	5 (3.0E−09)	4 (4.6E−09)	Unknown

C) Genes up-regulated in both ALCs and adipocytes.

Bovine ID	Annotation	Fold difference (p-Value)						
		BS	LD	DP	SE	IMF	AF	SF
2807_CL1Contig1	Platelet glycoprotein IV (CD36)	9 (4.6E−11)	16 (2.3E−07)	17 (9.1E−08)	18 (1.3E−06)	9 (1.3E−12)	6 (3.2E−10)	7 (5.7E−11)
NM_173959.3	Stearoyl-CoA desaturase (SCD)	4 (1.8E−04)	19 (7.4E−09)	12 (9.2E−09)	12 (2.9E−08)	17 (1.5E−12)	15 (3.3E−12)	14 (1.7E−13)
2607_CL1Contig1	Fatty acid binding protein 4 (FABP4)	11 (4.6E−10)	22 (2.9E−07)	23 (3.4E−08)	25 (8.0E−07)	11 (3.8E−10)	12 (7.5E−14)	10 (5.8E−15)
CK727063.1	Unknown	4 (1.2E−07)	11 (7.8E−07)	7 (9.9E−06)	8 (4.6E−05)	10 (1.2E−11)	8 (2.1E−14)	6 (5.0E−09)

essential eukaryotic genes as well as indicating the major diversification and innovation that has arisen in the evolution of the eukaryotic genome [27]. During myogenesis, gene functionality was mostly tissue-specific. Genes responsible for extracellular structure showed a higher level of expression in BS than in the other muscle depots, which could be correlated with the most active movements of BS among muscle depots. However, genes involved in posttranslational modifications were more abundantly expressed in LD and SE. Approximately the same percentage of genes related to signal transduction, protein modification and turnover, and extracellular structure were found to be up-regulated in ALCs and adipocytes, indicating a close relationship between the two processes. Among all of the depots, most of the up-regulated genes were related to signal transduction and lipid transport which indicates a similarity in the molecular mechanisms involved in adipogenesis and the trandifferentiation of MSCs, and a coordinated function among fatty acid transporter proteins. The coordinated function of fatty acid transporters was validated by real time RT-PCR, which revealed the higher expression of CD36 and FABP4 in ALCs and adipocytes. In addition, some genes with unknown function were up-regulated during myogenesis, transdifferentiation, and adipogenesis. The gene with

bovine ID CK727063.1 was expressed in both ALCs from MSCs and adipocytes. Further work is required to elucidate the function of this unknown gene.

Real time RT-PCR results confirmed the high expression of CSF1, EREG, DLX5, CD163 and BACE2 in ALCs from all muscle depots. However, FABP5 involved in lipid metabolism and transport [38,43] was highly expressed in all muscle tissues except for SE. The presence of FABP5 in ALC suggests its important role in the transdifferentiation of MSCs. During adipogenesis CD163, BACE2 and FABP5 were highly expressed in IMF and SF compared to AF. The difference in gene expression may be speculated to be associated with morphological and biochemical differences between SF and AF. Furthermore RNA and protein expression of fatty acid binding proteins have been found to be proportional to the higher rates of basal lipolysis in adipocytes isolated from subcutaneous compared to abdominal adipose tissues [44]. Similarly, EXP1, THRSP, P protein, and GPNMB were highly expressed during adipogenesis. We found a lower expression of CD36 in SF compared to IMF and AF, which may be due to the higher metabolic rate and higher turnover of triglycerides (TG) in internal fat depots than in SF. The higher rate of lipogenesis is regulated by a set of transcription factors such as PPARG
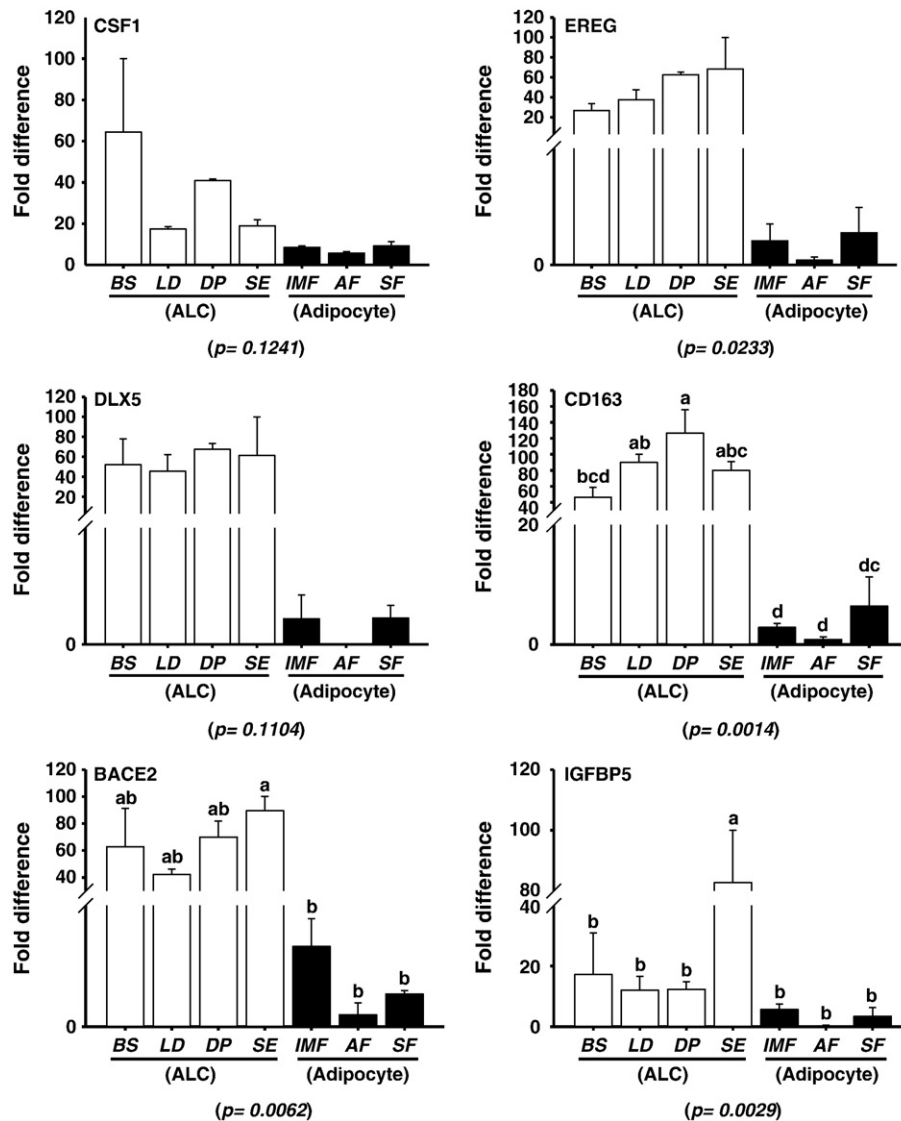

Fig. 2. Real-time RT-PCR analysis for differentially expressed genes. Real-time RT-PCR results for CSF1, EREG, DLX5, CD163, BACE2 and FABP5 in ALCs (A). EXP1, RHRSP, P protein, and GPNMB during adipogenesis (B). CD36 and FABP4 in both ALCs and adipocytes (C).
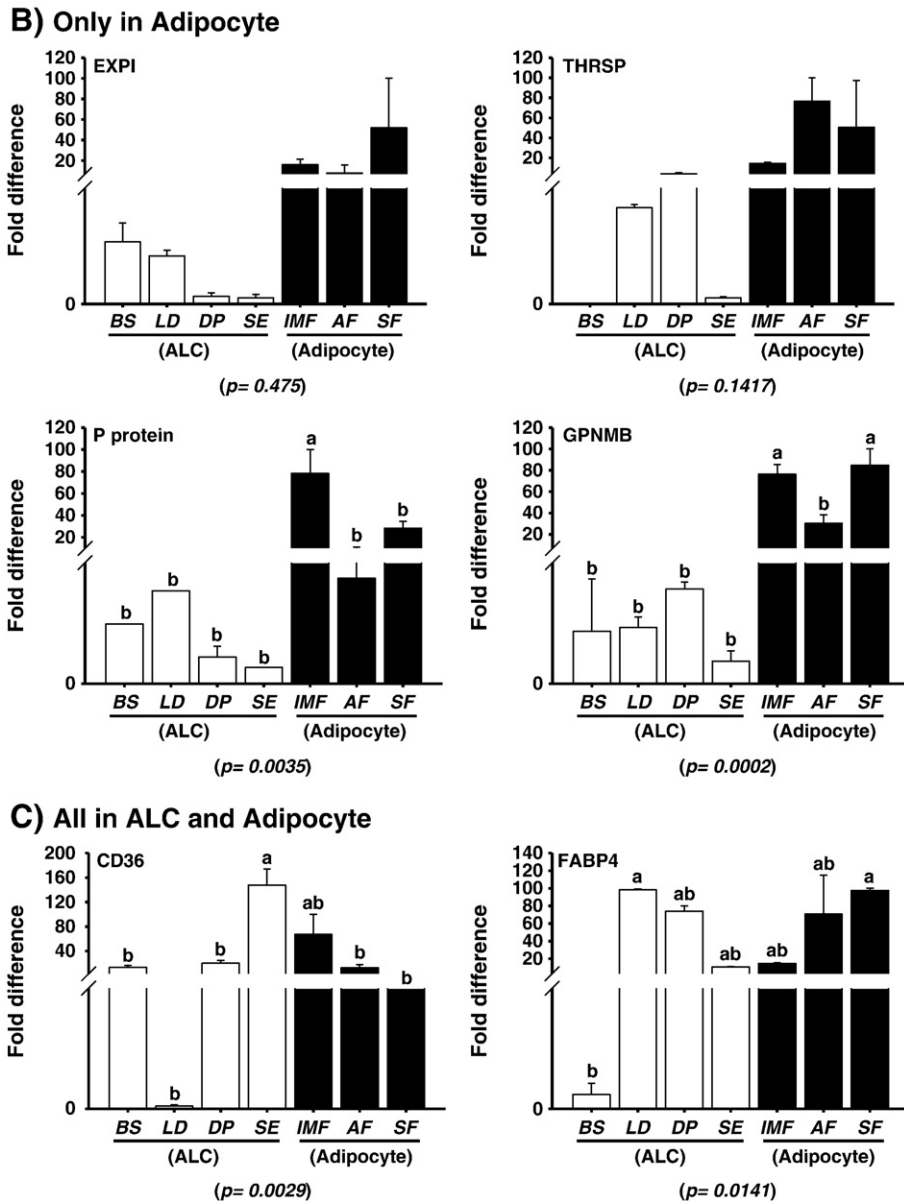
B) Only in Adipocyte

Fig. 2 (continued).

and their downstream target genes, including *CD36* [45,46]. In addition, *CD36* helps in TG synthesis and storage in adipose tissue along with insulin activated glucose transporter (SLC2A4) and LP4 enzyme, which catalyze the hydrolysis of circulating TG to fatty acids-by providing substrates (glucose and fatty acids) [47]. The higher expression of *CD36* during transdifferentiation of MSCs may be due to the transportation of fatty acids from intracellular depots to the cell surface, which occurs with muscle contraction [48]. Fatty acid transporters *FABP4* [38] and *CD36* [39] induced during ALC and adipocyte formation may share a common pathway. However, different genes were identified as being highly induced during the transdifferentiation of MSCs and adipogenesis, indicating that different molecular mechanism(s) are involved in these processes.

5. Conclusions

The study was performed to evaluate the relationship between gene expression profiles of tissue-specific differentiated and trans-differentiated MSCs and adipocytes. Additionally, genes expressed in ALCs and adipocytes from different muscle and fat sites were also compared. Based on the detected gene expression profiles, the pathways which stimulate MFC, ALC, and adipocyte formation from their corresponding precursor cells were found to be tissue-specific. However, a close relationship was found between IMF and ALC which may be related to the mechanism of formation of brown adipose tissue. This opens a new avenue of research into the transdifferentiation of MSCs. Several genes with known and unknown functions were identified as being up-regulated during these cellular processes and were classified into different cellular functionalities. Further study of these genes will be helpful for understanding the pathways involved in MFC, ALC, and adipocyte formation, and may lead to the identification of biomarker genes.

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