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Proteomic Analysis of Bovine Muscle Satellite Cells during Myogenic Differentiation

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ABSTRACT : The aim of this study was to analyze the proteome expression of bovine satellite cells from longissimus dorsi (LD), deep pectoral (DP) and semitendinosus (ST) muscle depots during *in vitro* myogenic differentiation. Proteomic profiling by two-dimensional gel electrophoresis and mass spectrometry of differentiating satellite cells revealed a total of 38 proteins that were differentially regulated among the three depots. Among differentially regulated proteins, metabolic proteins like lactate dehydrogenase (LDH), malate dehydrogenase (MDH) were found to be up regulated in ST, while alpha-enolase (NNE) in LD and DP depot satellite cells were down regulated. Also, our analysis found that there was a prominent up regulation of cytoskeletal proteins like actin, actin-capping protein and transgelin along with chaperone proteins like heat shock protein beta 1 (HSPB 1) and T-complex protein 1 (TCP-1). Among other up regulated proteins, LIM domain containing protein, annexin 2 and Rho GDP-dissociation inhibitor 1 (Rho GDI) are observed, which were already proven to be involved in the myogeneis. More interestingly, satellite cells from ST depot were found to have a higher myotube formation rate than the cells from the other two depots. Taken together, our results demonstrated that, proteins involved in glucose metabolism, cytoskeletal modeling and protein folding plays a key role in the myogenic differentiation of bovine satellite cells. (**Key Words :** Bovine Satellite Cells, Depot, Proteome, Myogenesis)

INTRODUCTION

Skeletal muscle is a tissue of major economic importance for meat production. The physiochemical properties of muscle tissue affecting beef taste and palatability are generally known to depend on their depot origins. And much researche are focused on determining the influence of bovine muscle characteristics on meat quality. However, the factors for depot specific features in bovine muscle are not yet fully understood. To investigate the factors responsible for muscle depot traits, we compared the protein expression patterns of Hanwoo bovine muscle satellite cells from three depots, longissimus muscle dorsi (LD), deep pectoral muscle (DP), semi tendinosus (ST) muscle, which are selectively used in Korean cuisine according to their unique muscle traits .

The number of muscle fibers in meat-producing animals is essentially fixed at birth, thus postnatal muscle growth results from hypertrophy of existing muscle fibers (Morgan and Partridge, 2003; Kamanga-Sollo et al., 2008). This fiber hypertrophy requires an increase in the number of myonuclei present in the fibers. However, the nuclei present in muscle fibers are unable to divide, so the nuclei must come from outside the fiber (Campion, 1984). The source of the nuclei needed to support muscle hypertrophy is mononucleated myogenic precursor cells, satellite cells, located on the periphery of the fiber. Further it was shown that muscle satellite cells play an essential role in the postnatal muscle growth by fusing with existing muscle fibers (Moss and Leblond, 1971; Campion, 1984). A better understanding of the involvement of satellite cells in postnatal muscle hypertrophy represents a powerful key to improve the efficiency of muscle growth in meat-producing animals.

Large mammals such as pigs and cattle offer an advantageous model for the study of myogenesis *in vitro*, as individual muscles can be identified and reliably dissected from different stages of growth. In addition, livestock represent useful models for muscle hypertrophy and other muscle traits, as these have been subject for intensive

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research due to their impact on meat quality (Lehnert et al., 2007). Numerous investigations have been focused on understanding the involvement of satellite cells in postnatal myogenesis (Allen and Rankin, 1990; Cooper et al., 1999; Bhasin et al., 2001; Halevy et al., 2001). Apart from these, many investigations have successfully explored the differential proteomic features from different skeletal muscles during various stages of development (Jia et al., 2006; Houbak et al., 2008; Kim et al., 2008, 2009; Laville et al., 2009). But most of these studies gave attention either on changes in post-mortem muscle or muscle tissue of live stock. However, results from such studies did not fetch much information in understanding the proteins that play role in myogenesis. Bouley et al. (2005) reported a study involving the proteomic changes during bovine semitendinosus muscle hypertrophy. On the other hand, no study has attempted to understand the changes in the proteomic features during myogenic differentiation of bovine satellite cells from different depots. Previously in our lab, we have isolated the bovine muscle satellite cells and were able to induce myogenic differentiation successfully (Kook et al., 2006; Rajesh et al., 2009). Hence, the present study was undertaken to improve our knowledge to understand the changes on the differentially regulated/ expressed proteome of bovine muscle satellite cells from longissimus muscle dorsi (LD), deep pectoral muscle (DP), semi tendinosus (ST) muscle depots during myogenic differentiation.

MATERIALS AND METHODS

Animals

Five heads of Hanwoo (Korean cattle) steers were fed and managed in the feeding barn at National Institute of Animal Science under the high quality beef production program (1997) and slaughtered at 24 months old. All experimental procedures and the care of animals were conducted in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC) of the National Institute of Animal Science, Korea.

Cell preparation

Animal experimentation was approved by the animal care and concern committee of the National Institute of Animal Science, in Korea.

Satellite cells were isolated from the three bovine muscle depots (LD, DP and ST) as described by (Doumit and Merkel, 1992). Briefly, the muscles were excised, trimmed of visible connective tissue and minced with fine sharp scissors. One gram of minced muscle was incubated for 50 min at 37°C in PBS with protease (4 mg/ml; Sigma P34245). After enzymatic digestion, the cells were separated from the tissue fragments by repeated centrifugation at 1,200 and 300×g for 15 and 5 min respectively, followed by filtration using mash nylon cloth. Thereafter, the filtrate was further centrifuged at 1,200×g for 15 min and the pellet was collected and reconstituted with DMEM medium supplemented with 10% Fetal Bovine Serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) and seeded in 10 cm petri-dish at a density of approximately 1×10^4 cells/cm^2. The cells were incubated at 37°C in air containing 5% CO_2. The medium was changed every second day allowing the cells to proliferate until confluence (about 9 days). Myogenic differentiation of the cells was induced by transfer to fresh DMEM medium supplements with 2% horse serum, 100 U/ml pencillin, 2 mM L-glutamine at 70% or 80% confluency and maintained at 37°C in air containing 5% CO_2 for 10 days post-confluence. Cells from each site and within each animal were kept and analyzed separately.

Protein preparation for 2-dimentional electrophoresis (2-DE)

Proteins from stromal vascular cells of three depots on day 0 and 8 of differentiation were extracted in 0.5 ml of lysis buffer (7 M Urea, 2 M Thiourea, 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 100 mM Dithiothreitol (DTT), protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). Following 15 min centrifugation at 15,000 rpm, the supernatant was collected and precipitated with either one or three volumes of acetone at -20°C for 2 h. The pellets were collected by centrifugation and then completely dried using speed-vac. Dried samples were re-dissolved in the 2-dimensional electrophoresis sample buffer (7 M Urea, 2 M Thiourea, 2% CHAPS, 100 mM DTT, 0.5% pH3-10NL IPG (Immobilized pH gradient) buffer for isoelectric focusing (IEF). The concentration of total protein in the sample was determined by Bradford's protein assay method.

2-DE and image analysis : Five hundreds μg of protein was loaded onto the immobiline dry strips pH 3-10 NL (GE Health care). The rehydrated strips were focused on IEF system (AP Biotech, Sweden) for ~80 kVh at a maximum of 8,000 V in a rapid ramping mode with maximum current per strip of 50 μA. Equilibration of the immobilized pH gradient strips was performed in two steps: reduction followed by alkylation (Ahmed and Bergsten, 2005). The second dimension was run on 12.5% polyacrylamide sodium dodecyl sulphate gels (26×20 cm) with a constant voltage of 100 V for 30 min, 250 V for 6hr using the EttanDALT II system (Amersham Bioscience, Piscataway, USA). The proteins were visualized using a Coomassie Brilliant Blue (CBB) G-250 staining method.

The CBB-stained gels were scanned using a GS-800 scanner (Bio-Rad) at an optical resolution of 300 dpi. Spot detection, quantification and matching were performed

using Image Master Version 7.0 (GE healthcare). Further, to identify the differentially expressed proteins, a set of 5 gels were produced from each experiment (each depot) and all the gels associated with the same collection were completely super imposable. Each spot detected by the Image Master (ver 7.0) was assigned a unique number to identify spots in a gel matching process. A match set, undifferentiated vs. differentiated, consisted of three images and each one was created from one muscle depot. To correct for variability due to CBB staining and reflect the quantitative variations of protein spot, the individual spot volumes were normalized by dividing their optical density (OD) values by the total OD values of all the spots present in the gel. The significance of the difference in protein expression between undifferentiated and differentiated satellite cells in each muscle depot was estimated by student's t-test, $p<0.05$ by using Image Master (ver 7.0) software.

Protein identification : The CBB-stained protein spots were excised from gels using a punch and placed in 500 μl Eppendorf tubes. The proteins were digested in-gel with trypsin as described elsewhere (Hellmann, 1995). Briefly, each spot was destained with 50 μl 50% acetonitrile (ACN) in 50 mM NH_4HCO_3, incubated at 37°C for 30 min and repeated once. Then the gels were reduced and alkylated. The gel pieces were digested overnight with trypsin (20 μg/μl) in 50 mM NH_4HCO_3 containing 10% ACN. The digest was then vortexed for 30 min and dried using speed vac. The dried extracted peptides were resuspended in 1μl solution containing pure water:ACN:trifluoracetic acid (TFA) (93:5:2).

Solution-phase nitrocellulose target preparation was used according to the method reported earlier (Landry, 2000). α-cyano-4-hydroxycinnanic acid (CHCA) (40 mg/ml) and nitrocellulose (20 mg/ml) were prepared separately in acetone and mixed with 2-propanol at a ratio of 2:1:1. The matrix solution was mixed with the sample at a ratio of 1:1, 0.5-0.3 μl was spotted onto the target and dried. The dried immobilized samples were washed with 1% formic acid twice and dried prior to the MALDI-TOF-MS/MS analysis.

Sample peptide masses were obtained using the Applied Biosystems 4700 Proteomics analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems) in the positive ion reflector mode. MS/MS analysis was performed on the 5 most abundant ions and the proteins identified by searching the SWISS-PROT and National Center for Biotechnology Information databases using the Mascot programs (Matrix Science, London, UK). Mass accuracy was considered to be within 50 ppm for peptide mass analysis and within 100 ppm for MS/MS analysis. For protein identification, known contamination peaks such as those of keratin and autoproteolytic products were removed. Molecular weight and protein score were came from MS/MS analysis result and pI was predicted from gel.

RNA isolation and real-time RT-PCR analysis

Total RNA was isolated from the cells using TRIzol reagent (Invitrogen Co., Carlsbad) based on the manufacturer's description. The first strand of cDNA was synthesized using 2 μg of total RNA as a template, oligo-dT primer and reverse transcriptase (Invitrogen Co.) according to the manufacturer's instructions. Measurement of the relative quantity of the cDNA of interest was carried out using SYBR Green PCR Master Mix (Applied Biosystems), 300 nM of the appropriate forward and reverse primers, and 1 μl of the cDNA mixture. The conditions of the PCR reactions were 28 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min, followed by a final amplification step of 72°C for 10 min. The sequence of forward and reverse primers used for detection of MyoD, Myf5, Myogenin, desmin, and many of the differentially expressed proteins from our proteomic analysis are given in Table 1. The housekeeping glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a load control. The PCR products were resolved on 2% agarose gels stained with ethidium bromide.

RESULTS

Myogenic differentiation of satellite cells

Satellite cells were isolated from bovine LD, DP and ST muscle depots and cultured under myogenic differentiation conditions. After being cultured for 5 days, satellite cells appeared in the medium (Figure 1A). Further to induce myogenesis, the confluence reached cells were placed into fresh DMEM medium containing 2% horse serum, and the cells were incubated until 10 days post-confluence. After myogenesis induction multinucleated myotubes appeared on the day 5 of their incubation in the differentiation medium (Figure 1A). On comparing the rate of myotube formation among the cells from three different muscle depots, ST muscle satellite cells were showing higher rate than the cells from other two depots (Figure 1C and 1D). We examined expression of the myoblast markers, namely MyoD, Myf5, myogenin and desmin. All the four myogenic markers mRNA expression was found to be increased on 10th day of incubation, when compared to day 0 (Figure 2). Also, we observed that MyoD, Myf5, myogenin expression levels were high in ST cells than in other two muscle depots.

Two-dimensional electrophoresis and mass spectrometry

Our image analysis of 2-DE gels showed 260 spots across three depots during differentiation and using MALDI-TOF mass spectrometry, we identified 34 differentially expressed proteins from all the three muscle

Table 1. Sequence of primers used in RT-PCR

Gene name	Forward primer	Reverse primer	Product size (bp)
MY5	ctcaggaatgccatccgctacattgaga	atccaagctggataaggagcttttatccg	216
MyoD	atcctgcgcaacgccatccgctatatcga	ctcgcgctgtagtaagtgcggtcgtagcagt	203
Myogenin	gagaagcgcagactcaagaaggtgaatga	tctgtagggtccgctgggagcagatgatc	314
Desmin	aggcctactcgtccagccagcgcg	cataggatggcgctcgggtggtcc	185
GAPDH	gggtcatcatctctgcacct	ggtcataagtccctccacga	176
EF-Tu	tcgagcatgtggtggtgat	tgtccacagcatccagtagc	216
Aldolase	attgtccctttgggcttct	gcaagaggccacatttcatt	206
MDH	tgccaaagtgaaactgcaag	ggagttgccatcggagataa	241
Collagen 2	cttcatggtgactcggtcct	ttccccatccagatcttcag	219
ACTB	actgggacgacatggaaag	catctccagagtccagcaca	240
NNE	gagctccgggacaatgataa	tgttccatccatctcgatca	159
HSPB 1	agctcagcagtggggtctc	tatttgcgagtgaagcaacg	188
LDB	ccaacccagtggacattgtt	aaacacctgccacattcaca	219
LASP-1	tccagcttgttttgccaact	ggatgtgaggggagagaaca	215
SD	aattgctggaagccatcaaac	agcagggggattaagacctgt	192
TAGLN	aacagcctgtaccctgatgg	cggtagtgcccatcattctt	239
VIM	gcagctctacgaggaggaga	tcaaggctaactggcgtctt	238
Septin-2	ggactgaagccactggatgt	gatgctggccttgagaagtc	231
PURH	acagtggcttgtccaggtgt	tcaaggctaactggcgtctt	205
TAGLN 2	gacgcgagaacttccagaac	acacaggccatgttctttcc	232
Protease type3	accgggtatgatctgtcagc	tgccaacaaacctgctactg	234
Rho GDI 1	cagagaacgaggaggacgag	ttagtcgggtcaccacaaca	180
TCP-1	cattctggagccaaagtcgt	ttcacactggtctggatgga	158

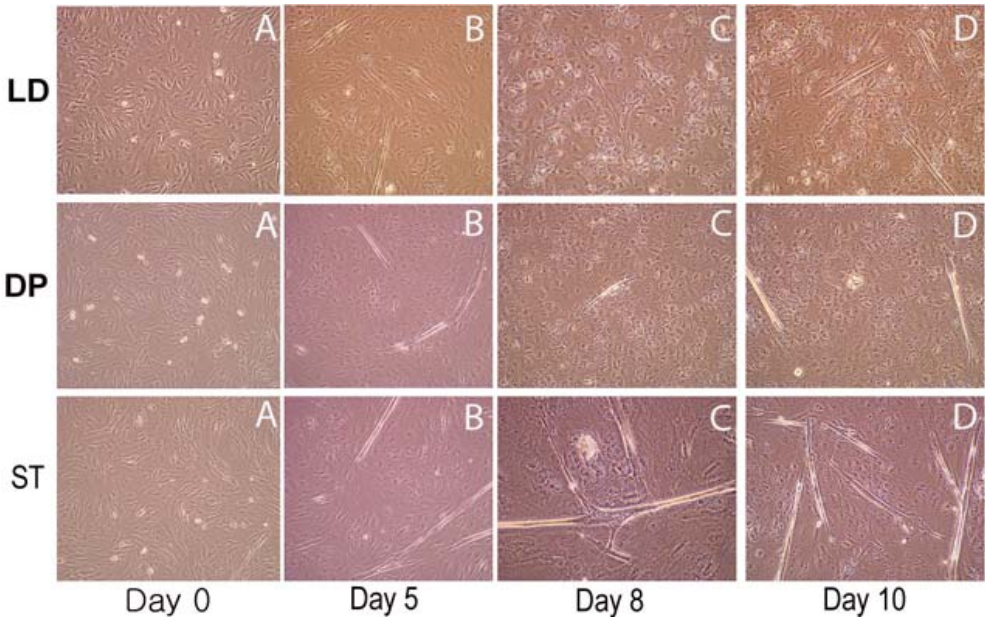


Figure 1. Myogenic differentiation of bovine satellite cells from LD, DP and ST muscle depots. Confluent satellite cells were induced to differentiate and maintained in the differentiation medium for 10 days. Note the formation of myotubes from the day 5 (B). Also, satellite cells from ST showed greater potential for myotube formation (D from ST).

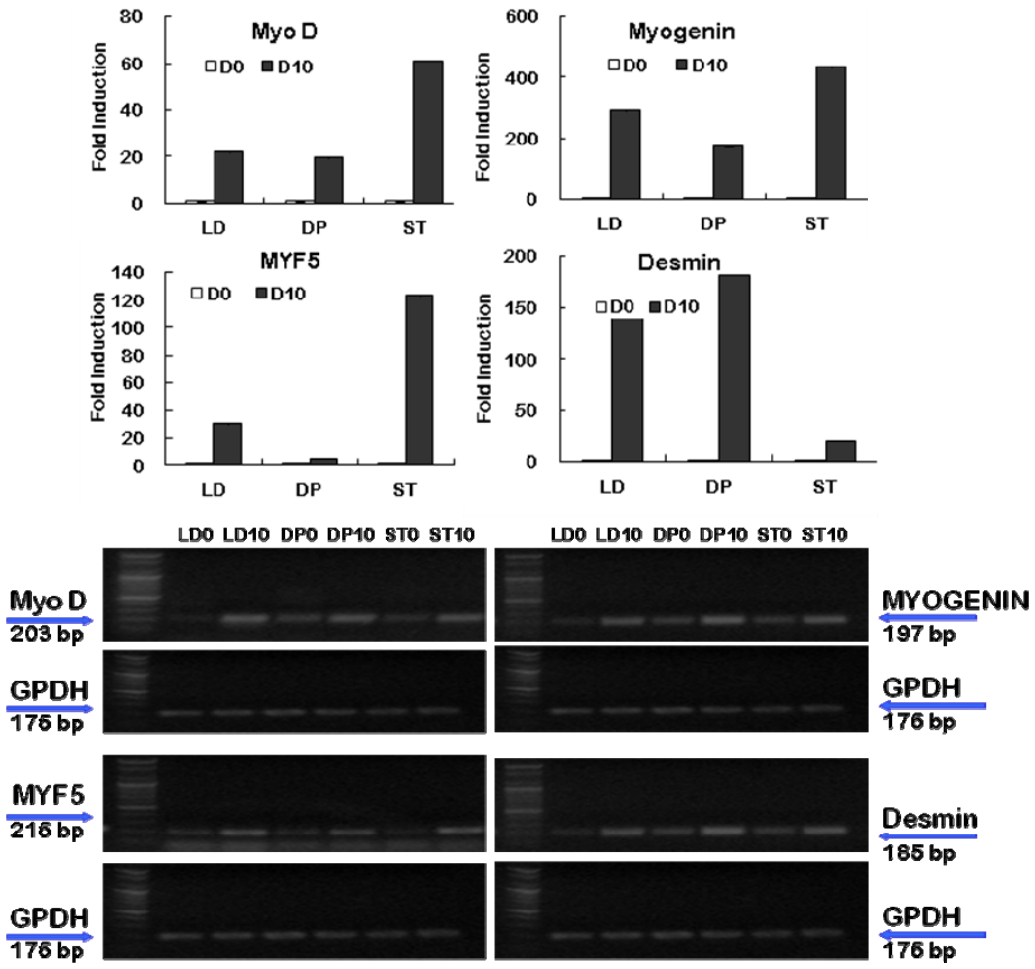


Figure 2. The relative mRNA levels of My5, Desimin, MyoD and Myogenin were compared for the undifferentiated and differentiating satellite cells in LD, DP and ST muscle depots using real-time PCR. The expression levels represented the average values of three independent culture experiments (Mean±SED).

depots during *in vitro* myogenic differentiation of satellite cells. Further, our differential protein expression analysis identified 12, 13 and 13 different proteins from LD, DP and ST muscle depots respectively. Out of 34 differentially expressed proteins, 9 proteins were either common to three depots or two depots (Tables 2, 3, and 4; Figure 3). Many of the differentially expressed proteins evidenced in all the three muscle depots display the modulation in the protein expression of three different functional categories: metabolism, cytoskeleton and heat shock proteins/ chaperones.

Five proteins that are differentially expressed during differentiation were associated with glucose metabolism: Fructose-biphosphate aldolase A (aldolase A), Alpha-enolase (NNE), Malate dehydrogenase (MDH), L-lactate dehydrogenase (LDH) and Glyceraldehyde-3-phosphate dehydrogensae (GAPDH). Both MDH and LDH, which were identified in all three depots had up-regulated along with NNE identified only in LD and DP depots. Aldolase A, detected in all the three depots was down-regulated during myogenic differentiation of satellite cells. Also GAPDH showed down regulation, but only in ST muscle depot.

Other six different proteins differentially expressed were identified as cytoskeletal proteins: collagen-binding protein 2 (Collagen 2), alpha-actin capping protein, actin (ACTB), vimentin (VIM), transgelin-2 (TAGLN 2) and transgelin (SM22). Both collagen 2 and alpha-actin capping protein identified only in LD depot were up-regulated during differentiation. ACTB was up-regulated both in LD and ST depots and VIM and TAGLN 2 proteins expressed in DP depot only were up-regulated during myogenic differentiation. SM 22 was up- and down-regulated in DP and ST depots respectively.

In the category of heat shock proteins/chaperones, we identified two different proteins: Heat shock protein beta 1 (HSPB1) and T- complex protein 1 (TCP-1). HSPB1 was up regulated in LD and DP depots during myogenic differentiation, whereas TCP-1 was identified in ST depot and its protein expressions were up regulated. In the list of other proteins, LIM and SH3 domain protein (LASP-1),

Table 2. List of differentially expressed proteins during myogenic differentiation of satellite cells from bovine longissimus dorsi muscle

Spot No	Accession number	Protein name	Protein (MW)	Protein (PI)	Sequence coverage (%)	Total ion score C.I. %	Fold change*
1681	P00883	Fructose-bisphosphate aldolase A	36.28	6.15	17	90.3	0.6
2078	P14315	Alpha-actin capping protein subunit beta isoform 1	47.17	6.37	26	100.0	1.7
3116	P49410	Elongation factor Tu, mitochondrial precursor (EF-Tu)	49.37	6.72	30	100.0	1.7
2306	Q5E9B1	L-lactate dehydrogenase B chain (LDH-B)	29.98	6.15	34	100.0	1.7
2959	Q29561	UMP-CMP kinase (EC 2.7.4.14) (Cytidylate kinase)	22.26	7.88	55	99.99	1.7
1459	Q9XSJ4	Alpha-enolase (NNE)	47.17	6.35	51	100.0	2.0
2049	Q3T145	Malate dehydrogenase, cytoplasmic	36.28	6.15	30	100.0	2.0
1141	P50454	Collagen-binding protein 2 precursor	39.19	8.41	32	99.3	2.2
2956	Q3T149	Heat-shock protein beta-1 (HSPB1)	36.28	6.15	70	100.0	2.2
453	P04272	Annexin A2 (Annexin II)	38.45	6.9	58	100.0	2.4
2068	Q61792	LIM and SH3 domain protein 1 (LASP-1)	46.41	8.75	25	99.9	3.0
3308	Q71FK5	Actin, cytoplasmic 1 (Beta-actin)	41.79	5.29	37	100.0	4.0

* Negative sign indicates decreased fold change.

Table 3. List of differentially expressed proteins during myogenic differentiation of satellite cells from bovine deep pectoral muscle

Spot No	Accession number	Protein name	Protein (MW)	Protein (PI)	Sequence coverage (%)	Total ion score C.I. %	Fold change*
569	P00883	Fructose-bisphosphate aldolase A	39.19	84.12	17	99.4	0.6
532	P31939	Bifunctional purine biosynthesis protein PURH	64.59	6.27	20	95.6	0.4
1967	Q5RA66	Septin-2	41.46	6.15	37	100	0.4
1151	P41976	Superoxide dimutase	41.46	6.15	29	100	1.2
2306	Q3T145	Malate dehydrogenase, cytoplasmic (MDH)	36.28	6.15	30	100.0	1.7
990	Q5E9F5	Transgelin-2	62.24	5.95	40	97.0	1.7
451	P02544	Vimentin	53.57	5.06	37	100.0	1.7
1677	Q9XSJ4	Alpha-enolase (NNE)	47.17	6.35	51	100.0	2.0
946	Q61792	LIM and SH3 domain protein 1 (LASP-1)	46.41	8.75	25	99.9	2.0
448	Q9TS87	Transgelin (smooth muscle protein22-alpha)	36.28	6.15	62	100.0	2.1
1741	Q3T149	Heat-shock protein beta-1 (HSPB1)	36.28	6.15	70	100.0	2.5
1048	Q58DU5	Proteasome subunit alpha type 3 (EC 3.4.25.1)	36.29	6.15	31	100.0	2.5
988	Q5E9B1	L-lactate dehydrogenase B chain (LDH-B)	29.98	6.15	34	100.0	4.0

* Negative sign indicates decreased fold change.

Table 4. List of differentially expressed proteins during myogenic differentiation of satellite cells from bovine semitendinosus muscle

Spot No	Accession number	Protein name	Protein (MW)	Protein (PI)	Sequence coverage (%)	Total ion score C.I. %	Fold change*
3454	P00883	Fructose-bisphosphate aldolase A	39.19	8.41	17	99.4	0.6
1137	P10096	Glyceraldehyde-3-phosphate dehydrogenase	35.71	8.52	55	100.0	0.6
4805	P49773	Histidine triad nucleotide-binding protein 1	36.28	6.15	30	100.0	0.6
3599	Q9TS87	Transgelin (Smooth muscle protein 22-alpha)	36.28	6.15	62	100.0	0.6
1313	P61163	Alpha-centractin (Centractin) (ARP1)	42.59	6.19	27	100.0	0.4
1262	Q2NKZ1	T-complex protein 1 subunit eta (TCP-1)	41.46	6.15	28	100.0	1.7
3986	P41976	Superoxide dimutase	41.46	6.15	29	100.0	1.9
398	P84336	Actin, cytoplasmic 1	41.79	5.29	37	100.0	2.1
4690	P19803	Rho GDP-dissociation inhibitor 1 (Rho-GDI alpha)	23.2	5.12	51	100.0	2.1
4788	P49410	Elongation factor Tu, mitochondrial precursor (EF-Tu)	49.37	6.72	35	100.0	2.5
3921	Q3T145	Malate dehydrogenase, cytoplasmic (MDH)	36.28	6.15	30	100.0	3.5
3572	Q5E9B1	L-lactate dehydrogenase B chain (LDH-B)	29.98	6.15	34	100.0	4.0
2323	Q61792	LIM and SH3 domain protein 1 (LASP-1)	29.98	6.6	28	100.0	5.0

* Negative sign indicates decreased fold change.

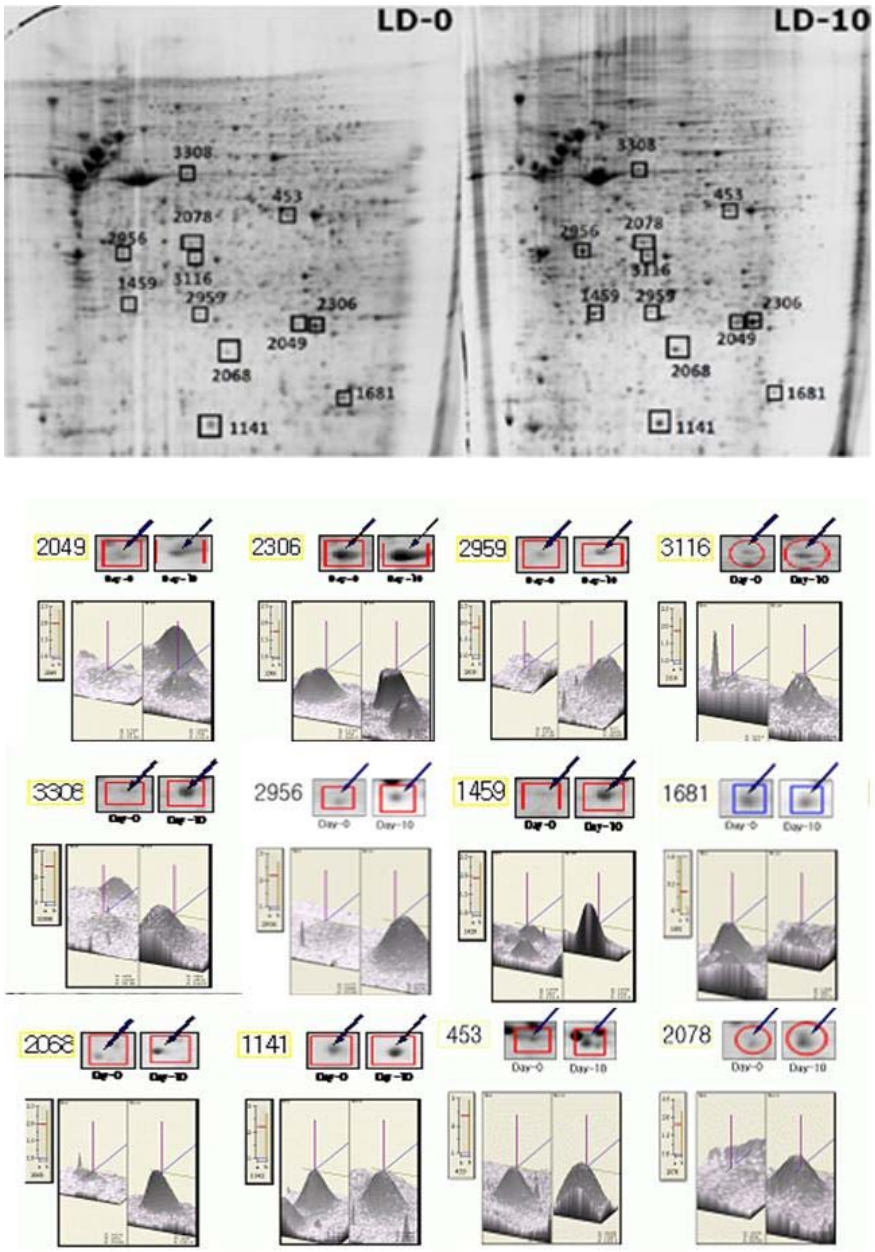


Figure 3. (A) Representative 2-DE protein profiles of differentiating bovine muscle satellite cells. LD, DP and ST muscle depots. Proteins (500 μg) from differentiating bovine muscle satellite cells at day 0 (LD-0; DP-0; ST-0) or day 10 (LD-10; DP-10; ST-10) was separated by 2-DE over the pIs range on the 3-10 NL, 12.5% acrylamide gels and stained with coomassie blue G-250. The positions of differentially regulated proteins are annotated by open squares and numbered by their spot numbers as given in Table 2 for LD, Table 3 for DP and Table 4 for ST.

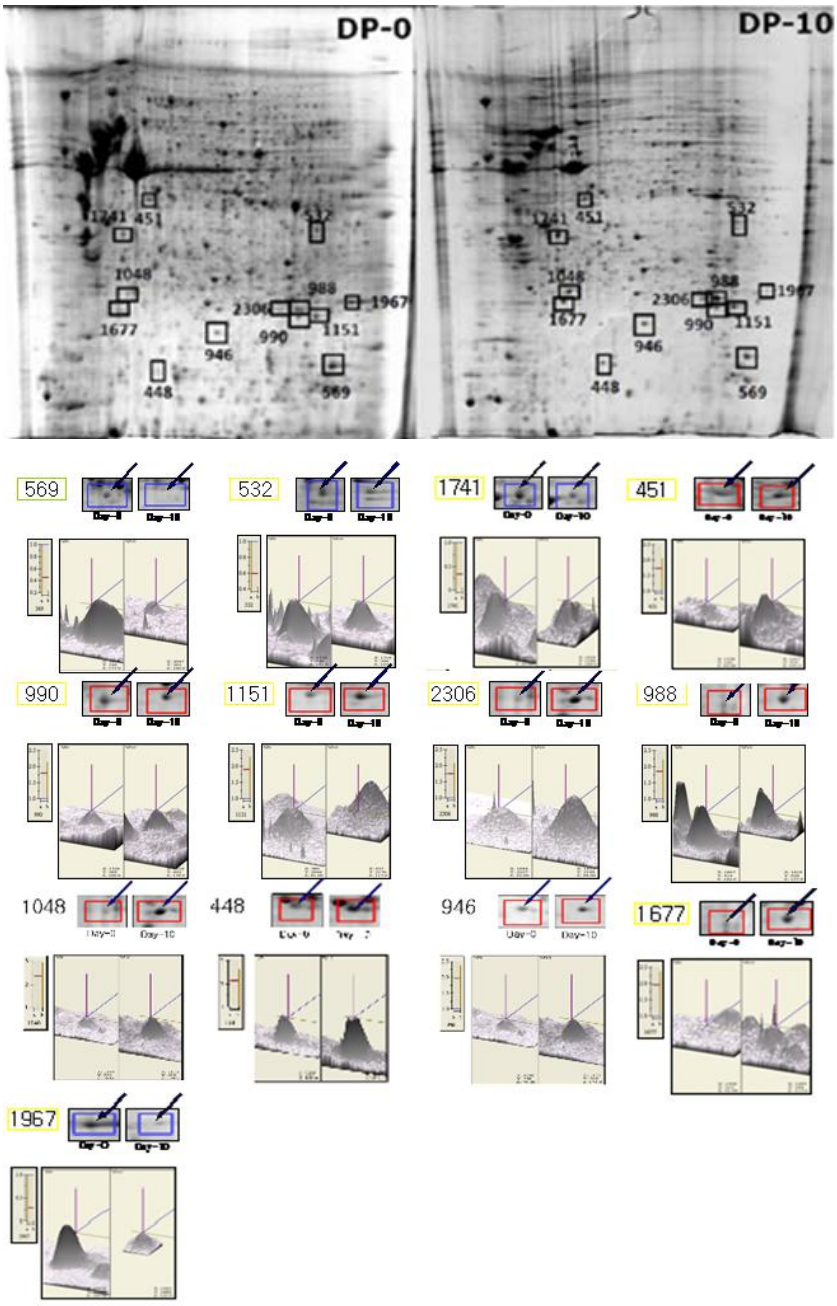


Figure 3. (B) Representative 2-DE protein profiles of differentiating bovine muscle satellite cells. LD, DP and ST muscle depots. Proteins (500 μg) from differentiating bovine muscle satellite cells at day 0 (LD-0; DP-0; ST-0) or day 10 (LD-10; DP-10; ST-10) was separated by 2-DE over the pIs range on the 3-10 NL, 12.5% acrylamide gels and stained with coomassie blue G-250. The positions of differentially regulated proteins are annotated by open squares and numbered by their spot numbers as given in Table 2 for LD, Table 3 for DP and Table 4 for ST (Continued).

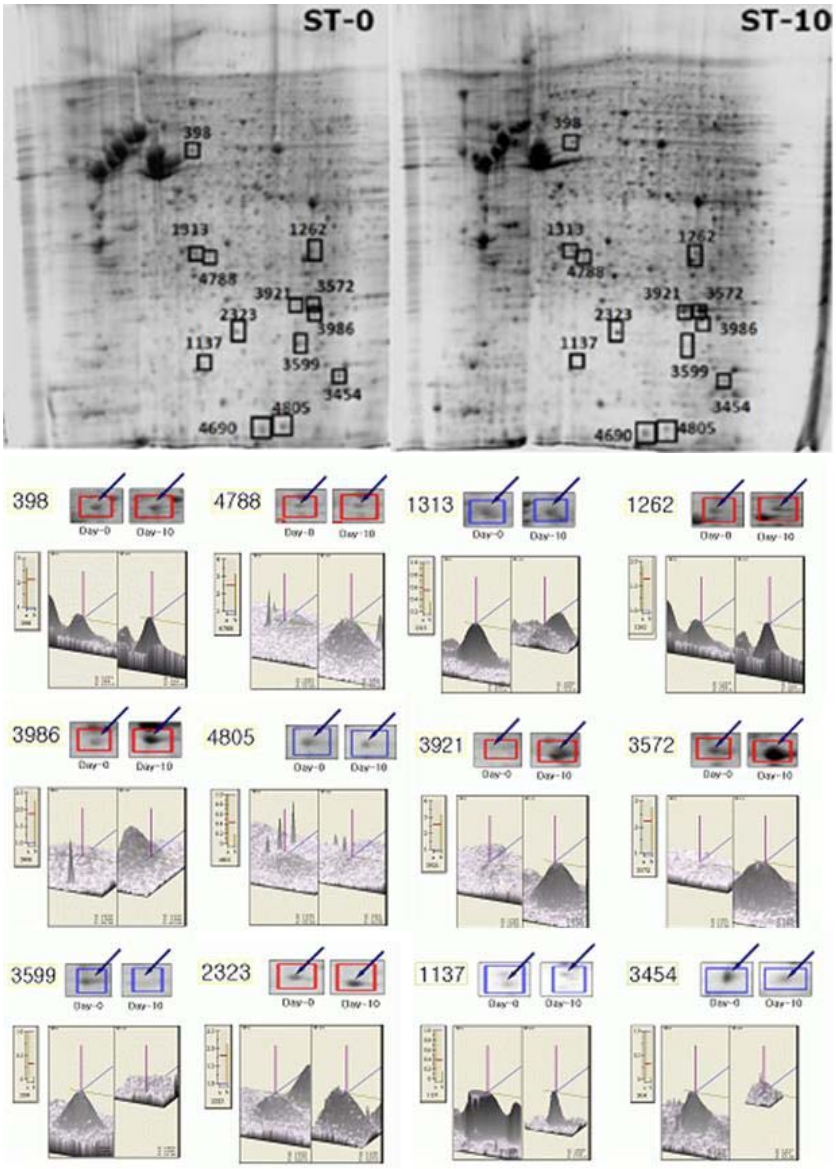


Figure 3. (C) Representative 2-DE protein profiles of differentiating bovine muscle satellite cells. LD, DP and ST muscle depots. Proteins (500 μg) from differentiating bovine muscle satellite cells at day 0 (LD-0; DP-0; ST-0) or day 10 (LD-10; DP-10; ST-10) was separated by 2-DE over the pIs range on the 3-10 NL, 12.5% acrylamide gels and stained with coomassie blue G-250. The positions of differentially regulated proteins are annotated by open squares and numbered by their spot numbers as given in Table 2 for LD, Table 3 for DP and Table 4 for ST (Continued).

which were spotted in all the three depots, were up regulated. Elongation factor (EF-Tu), that was spotted in LD and ST depots also showed increase in its protein expressions. Proteins like Annexin 2, Proteasome subunit alpha and Rho GDP-disassociation inhibitor 1 were up regulated and spotted in LD, DP and ST depots respectively during myogenic differentiation of satellite cells. Apart from our proteomic analysis, we have also measured the mRNA levels of differentially expressed proteins by RT-PCR (Figure 4). Our mRNA expression analysis complemented our proteomic results, further confirming the proteome expression changes during *in vitro* myogenic differentiation of bovine satellite from three different muscle depots.

DISCUSSION

Previous works have demonstrated that myogenic cell line such as C2C12, primary myoblasts isolated from ovine or satellite cells from adult skeletal muscle display potential myogenic differentiation with dynamic proteome changes (Kislinger et al., 2005; Hamelin et al., 2006; Kook et al., 2006). In a recent review by Picard et al. (2010) on proteomics in livestock, the role of proteomics as an interesting tool to enhance the knowledge on the quantity and quality of the meat produced were emphasized. The

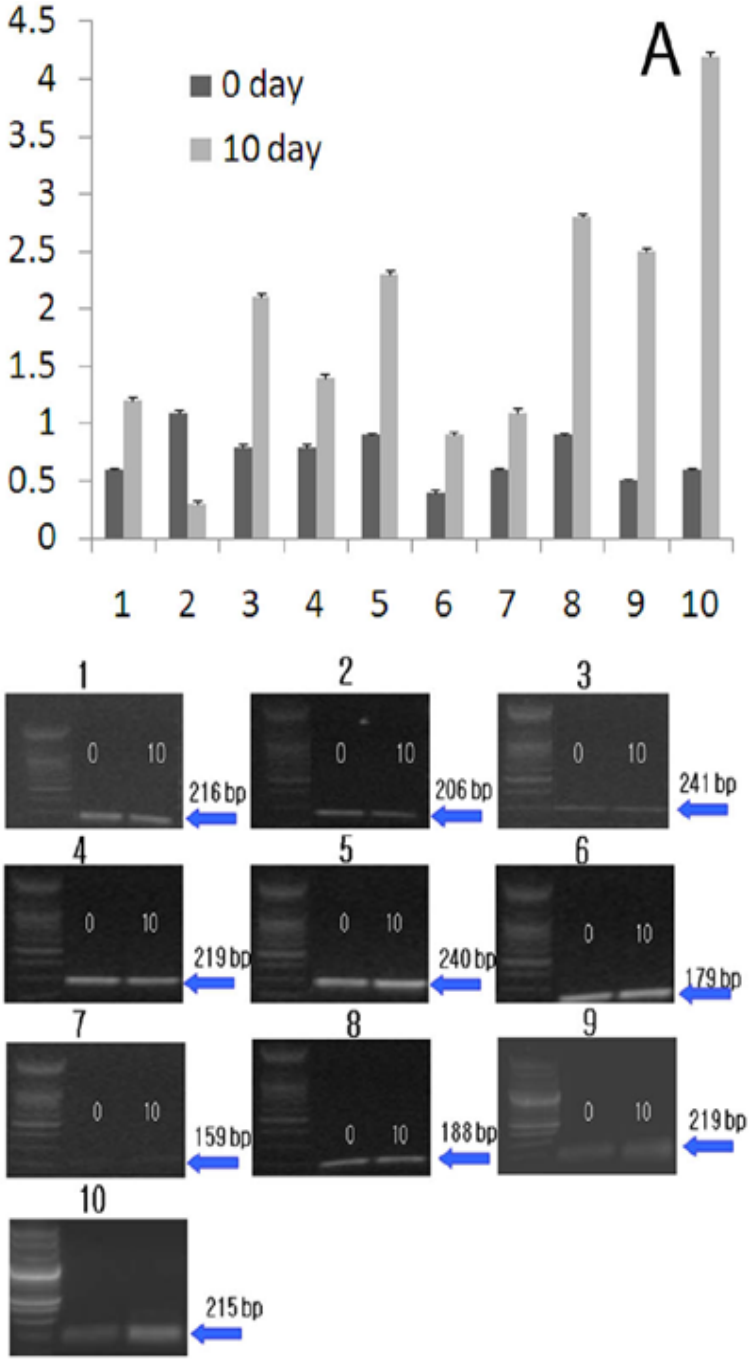
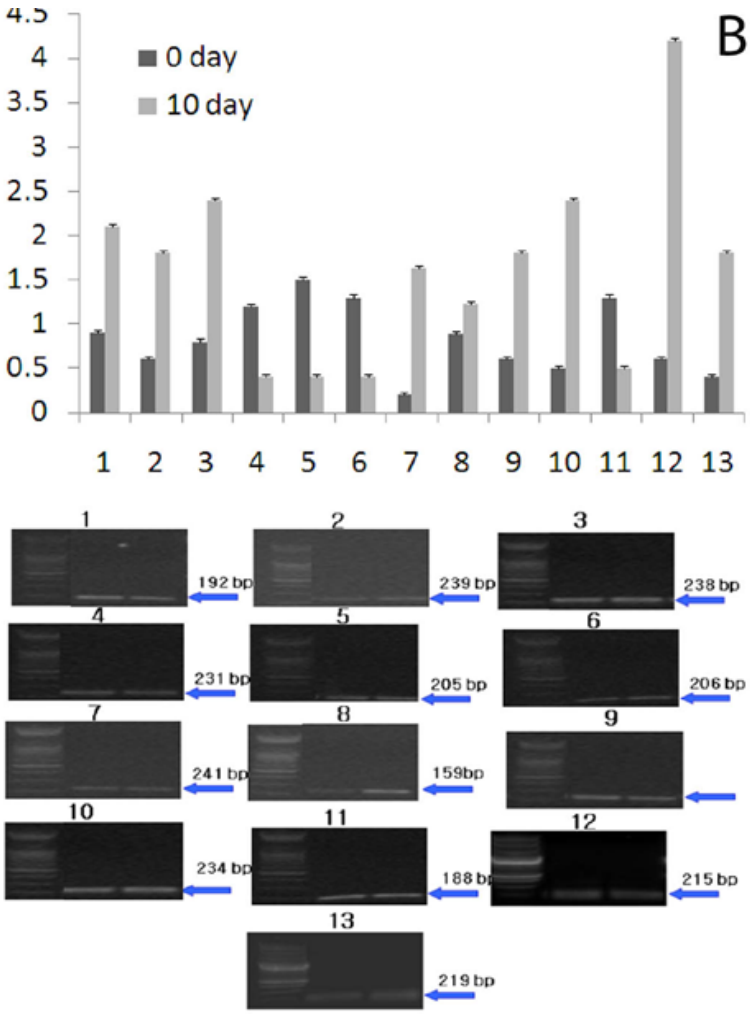


Figure 4. RT-PCR and relative mRNA levels of differentially expressed proteins during myogenic differentiation of bovine satellite cells from LD, DP and ST muscle depots. Total RNA was isolated from differentiating bovine satellite cell cultures at day 0 and 10 from three depots and their mRNA expression levels was analysed using RT-PCR. (A) LD: 1) Elongation factor Tu; 2) Fructose-bisphosphate aldolase A; 3) Malate dehydrogenase; 4) Collagen-binding protein; 5) Alpha-actin capping protein; 6) Actin; 7) Alpha-enolase; 8) Heat-shock protein beta-1; 9) L-lactate dehydrogenase; 10) LIM and SH3 domain protein 1. (B) DP: 1) Superoxide dismutase; 2) Transgelin; 3) Vimentin; 4) Septin-2; 5) Bifunctional purine biosynthesis protein; 6) Fructose-bisphosphate aldolase A; 7) Malate dehydrogenase; 8) Alpha-enolase; 9) Transgelin-2; 10) Proteasome subunit alpha type 3; 11) Heat-shock protein beta-1; 12) LIM and SH3 domain protein 1; 13) L-lactate dehydrogenase. (C) ST: 1) Rho GDP-dissociation inhibitor 1; 2) Elongation factor Tu; 3) Malate dehydrogenase; 4) Superoxide dismutase; 5) Fructose-bisphosphate aldolase A; 6) T-complex protein 1; 7) Transgelin 8) Actin; 9) Glyceraldehyde-3-phosphate dehydrogenase; 10) LIM and SH3 domain protein 1; 11) L-lactate dehydrogenase. The data points in the figure are from individual assays and represents the average of mRNA levels obtained from triplicate culture experiments (Mean±SED).



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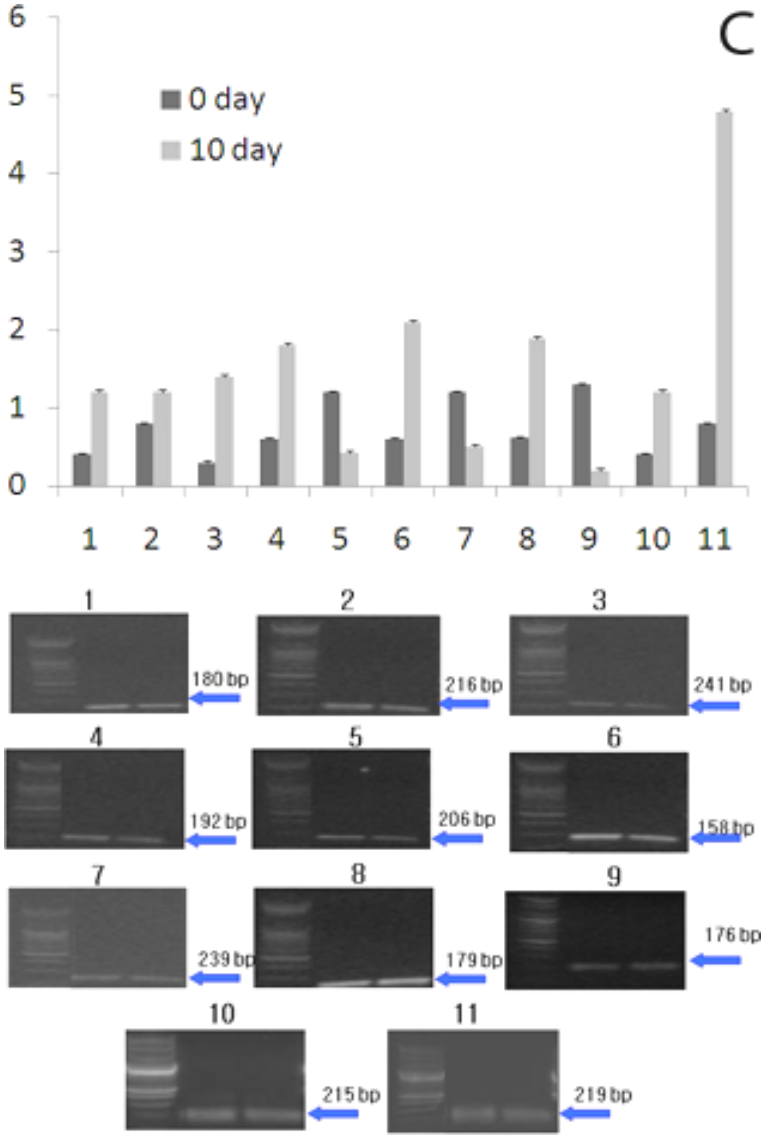

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proteomic studies reported till now involved either using muscle tissue as such (Kim et al., 2008) or during *in vivo* myogenesis in cattle (Chaze et al., 2008). However, information on proteomic changes during bovine satellite cells differentiation into myocytes is obscure. In this work, we clearly demonstrate i) bovine muscle satellite cells isolated from LD, DP and ST depots can give rise to skeletal myocytes *in vitro* ii) also, we provide information about the changes in proteomic features during *in vitro* myogenic differentiation of such bovine satellite cells from different depots.

Proliferating satellite cell-derived from all the three muscle depots expressed several myoblast markers, such as MyoD, Myf5, myogenin and desmin, indicating that isolated satellite cells are already committed to the myogenic cell lineage (Grounds et al., 1992; Smith et al.,

1994; Cornelison and Wold, 1997; Seale et al., 2000; and this work). Further, our mRNA expression results of all the four markers revealed higher expressions of Myf5, Myo D and Myogenin in ST depot, while desmin was showing lower expressions. The myogenic regulatory genes encode a family of transcription factors comprising four members, MyoD, Myf5, myogenin and desmin. The function of these myogenic factors *in vivo* has been investigated by determining their expression during development and also by analyzing mice with mutations in these genes (Cooper et al., 1991). Myf5 and MyoD are expressed in proliferating myoblasts prior to differentiation, whereas Myogenin is expressed in terminally differentiating cells (Grounds et al., 1992; Smith et al., 1994). Mice lacking either MyoD or Myf5 show no severe muscle abnormalities but double mutants lacking both MyoD and Myf5 have no differentiated skeletal muscle, and furthermore the myoblast precursor cell population is absent (Rudnicki et al., 1993). This suggests that not only do MyoD and Myf5 act at an early stage of muscle cell determination but that they also have functionally overlapping roles. The Myf5 gene is, however, activated prior to MyoD during development suggesting that these genes may also have discrete and possibly complementary functions during muscle formation (reviewed by Tajbakhsh and Cossu, 1997). Hence, the expressions of Myf5 and MyoD along with myogenin are important for satellite differentiation. However, role of higher expressions of different myogenic genes in greater myotube formation as observed in our report is a question to be answered.

Our proteomic analysis revealed differential expression of five proteins that are associated with glucose metabolism. Carbohydrate metabolism plays a very important role during myogenic differentiation (Bracha1 et al., 2010). The greater abundance of enzymes involved in glucose metabolism in our study was in accordance with previous reports (Kislinger et al., 2005; Hamelin et al., 2006). Up regulation of LDH and MDH as in ST depot were also reported during myogenic differentiation of C2C12 cells (Kislinger et al., 2005; Hamelin et al., 2006). In fact, LDH is one of the important marker for myogenic differentiation in rabbit muscle cells (Barjoti et al., 1998). These results confirm that ST depot specific satellite cells are active in carbohydrate metabolism during differentiation, and this may further enhance the rates of myotube formation. NNE, an enzyme involved in glycolysis was seen up regulated in LD and DP depots, but was not detected in SD depot. Hence, LDH and MDH in ST and NNE in LD and DP depots can act as differentiating marker enzymes for depot specific myogenic differentiation. Also NNE was reported to be up regulated in primary myoblast cells during neonatal development and further, it was defined as muscle-specific myogenic marker (Peterson et al., 1992). On the other hand, glycolytic enzymes like aldolase A and GAPDH were down regulated in our study. So, the energy requirements for the myogenic differentiation of bovine satellite cells are derived from citric acid cycle rather than glycolytic cycle, as MDH and LDH enzymes are key proteins in citric acid cycle.

Initiation of differentiation in myoblast cells leads to profound changes in the transduction of locomotion and cell shape controlling proteins that precede the fusion of myoblasts into multinucleated muscle cells and depends on the reorganization of their cytoskeleton and plasma membranes (Schmidt and Hall, 1998). Temporal changes in the steady-state levels of a number of key proteins known to regulate cell architecture seen in our study are consistent with this scenario. Furthermore, up regulation of collagen, actin, actin-capping protein, vimentin, transgelin and trangelin-2 during myogenic differentiation of satellite cells *in vitro* underscore the role of cell shape changes during this process. Up regulation of actin, actin-capping protein and transgelin was reported during the myogenic differentiation of C2C12 cells. Further, they have reported that C2C12 derived myotubes accumulate tissue-specific actin and its related proteins (Tannu et al., 2001). Skeletal myogenesis is a highly ordered process that allows the differentiation of proliferating myoblasts into multinucleated myotubes expressing the contractile apparatus. A coordinate induction of muscle-specific gene products occurs concomitantly with the cell morphological changes.

The HSPB1 and TCP-1 are chaperone proteins that promote cell survival during physiological stress (Sternlicht et al., 1993; Thompson et al., 2003). In our study, HSPB1 in LD and DP, TCP-1 in ST were up regulated during myogenic differentiation. It was reported that proteins involved in apoptosis are expressed abundant during bovine myogenesis (Chaze et al., 2008). Up regulation of HSPB1 and TCP-1 in LD, DP and ST depots respectively would explain the cell survival process during myogenic differentiation of bovine satellite cells from different depots. Also, HSPB1 has been reported to play a central role in the structural and functional organization of the 3-dimensional intermediate filament structure and the actin microfilament system (Fischer et al., 2002) and it may also act as a molecular chaperone having a major role in protein folding renaturation. The chaperonin containing TCP-1 is a hetero-oligomeric particle needed to fold the cytoskeletal proteins tubulin and actin (Sternlicht et al., 1993). Some of the other proteins that were up regulated in our proteomic analysis like LIM domain containing protein, annexin 2 and Rho GDI are already proven to be involved in the myogenesis. Among three depots, LIM protein was found to have its higher expression in ST depot than LD and DP. Moreover, it was shown that LIM protein promotes myogenesis by enhancing the activity of MyoD (Kong et al., 1998). So, high expressions of LIM protein could complement in

enhancing the MyoD activity in the ST depot satellite cells during differentiation. It was also shown that over expression of LIM in C2C12 myoblasts enhances skeletal myogenesis, whereas inhibition of LIM activity blocks terminal differentiation (Kong et al., 1998). In our study, we found Rho GD1 protein was up regulated in ST depot satellite cells; this result was in agreement with earlier study by Chaze et al., 2008. They reported Rho GD1 protein was up regulated during *in vivo* myogenic differentiation of bovine ST muscle. Up regulation of this protein at early stages of growth was attributed to the function of cytoskeleton reorganization during cell proliferation. Rho GD1 protein is also known to bind and alter *in vivo* expression of most of the RhoGTPase proteins (Dovas and Couchman, 2005). These RhoGTPase proteins are the crucial regulators of skeletal myogenesis (Bryan et al., 2005). Annexin 2 which was up regulated in LD satellite cells was reported to mediate myogeneis by interacting with dysferlin proteins (Lennon et al., 2003).

In conclusion, 2-DE and MALDI TOF/ TOF identified several proteins, which showed differential expressions during myogenic differentiation of bovine satellite cells from three depots. To our best knowledge, this study is first of its kind to provide the proteomic details during the myogenic differentiation of bovine muscle satellite cells from different depots. Satellite cells from ST depot were found to have the higher rates of myotube formation than from the corresponding depots. Also, up regulation of metabolic proteins like LDH, MDH along with other proteins like LIM and Rho GD1 could not only differentiate the depot specificity, but also could play an important role in myogenic differentiation of satellite cells from ST depot. Also, up-regulation of cyotskeletal proteins like actin, actin-capping protein, vimentin along with chaperone proteins like HSPB 1 and TCP-1could explain the structural changes and cell survival strategies adapted by the bovine satellite cells during *in vitro* myogenic differentiation.

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REFERENCES

Ahmed, M. and P. Bergsten. 2005. Glucose-induced changes of multiple mouse islet proteins analysed by two-dimensional gel electrophoresis and mass spectrometry. Diabetologica 48:477-485.

Allen, R. E. and L. L. Rankin. 1990. Regulation of satellite cells during skeletal muscle growth and development. Proc. Soc. Exp. Biol. Med. 94:81-86.

Barjoti, C., V. Laplace-Marieze, L. Gannoun-Zaki, G. Mckoy, M. Le Briand, P. Vigneroni and F. Bacou. 1998. Expression of lactate dehydrogenase, myosin heavy chain and myogenic regulatory factor genes in rabbit embryonic muscle cell cultures. J. Muscle Res. Cell. Motil. 19:343-351.

Bhasin, S., L. Woodhouse, R. Casaburi, A. B. Singh, D. Bhasin and N. Berman. 2001. Testosterone dose-response relationships in healthy young men. Am. J. Physiol. Endocrinol. Metab. 281:E1172-1181.

Bouley, J., B. Meunier, C. Chambon, S. De Smet, J. F. Hocquette and B. Picard. 2005. Proteomic analysis of bovine skeletal muscle hypertrophy. Proteomics 5:490-500.

Bracha1, A. L., A. Ramanathan, S. Huang, D. EIngber and S. L. Schreiber. 2010. Carbon metabolism-mediated myogenic differentiation. Nat. Chem. Biol. 6:202-204.

Bryan, B. A., D. Li, X. Wu and M. Liu. 2005. The Rho family of small GTPases: Crucial regulators of skeletal myogenesis. Cell. Mol. Life Sci. 62:1547-1555.

Campion, D. R. 1984. The muscle satellite cell: a review. Int. Rev. Cytol. 87:225-251.

Chaze, T., B. Meunier, C. Chambon, C. Jurie and B. Picard. 2008. *In vivo* proteome dynamics during early bovine myogenesis. Proteomics 8:4236-4248.

Cooper, R. N., S. Tajbakhsh, V. Mouly, G. Cossu, M. Buckingham and G. S. Butler-Browne. 1999. *In vivo* satellite cell activation via Myf5 and MyoD in regenerating mouse skeletal muscle. J. Cell Sci. 112:2895-2901.

Cornelison, D. D. and B. J. Wold. 1997. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev. Biol. 191:270-283.

Doumit, M. E. and R. A. Merkel. 1992. Conditions for the isolation and culture of porcine myogenic satellite cells. Tissue Cell 24:253-262.

Dovas, A. and J. R. Couchman. 2005. RhoGDI: Multiple functions in the regulation of Rho family GTPase activities. Biochem. J. 390:1-9.

Fischer, D., J. Matten, J. Reimann, C. Bonnemann and R. Schroder. 2002. Expression, localization and functional divergence of alphaB-crystallin and heat shock protein 27 in core myopathies and neurogenic atrophy. Acta Neuropathol. 104:297-304.

Grounds, M. D., K. L. Garrett, M. C. Lai, W. E. Wright and M. W. Beilharz. 1992. Identification of skeletal muscle precursor cells *in vivo* by use of MyoD1 and myogenin probes. Cell Tissue Res. 267:99-104.

Halevy, O., A. Krispin, Y. Leshem, J. P. MCMurthy and S. Yahav. 2001. Early-age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281:R302-R309.

Hamelin, M., T. Sayd, C. Chambon, J. Bouix, B. Bibé, D. Milenkovic, H. Leveziel, M. Georges, A. Clop, P. Marinova and E. Laville. 2006. Proteomic analysis of ovine muscle hypertrophy. J. Anim. Sci. 84:3266-3276.

Hellmann, U., C. Wemstedt, J. Gonez and C. H. Heldin. 1995. Improvement of an “In-gel” digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. Anal. Biochem. 224:451-455.

Houbak, M. B., P. Ertbjerg and M. Therkildsen. 2008. *In vitro* study to evaluate the degradation of bovine muscle proteins post-mortem by proteasome and μ-calpain. Meat Sci. 79:77-85.

Jia, X., K. Hollung, M. Therkildsen, K. I. Hildrum and E. Bendixen. 2006. Proteome analysis of early post-mortem changes in two bovine muscle types: *M. longissimus dorsi* and *M. semitendinosus*. Proteomics 6:936-944.

Kamanga-Sollo, E., M. E. White, M. R. Hathaway, K. Y. Chung, B. J. Johnson and W. R. Dayton. 2008. Roles of IGF-1 and the estrogen, androgen and IGF-1 receptors in estradiol-17β and trenbolone acetate-stimulated proliferation of cultures bovine satellite cells. Domest. Anim. Endocrinol. 35:88-97.

Kim, N. K., S. Cho, S. H. Lee, H. R. Park, C. S. Lee, Y. M. Cho, Y. H. Choy, D. Yoon, S. K. Im and E. W. Park. 2008. Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. Meat Sci. 80:1068-1073.

Kim, N. K., S. H. Lee, Y. M. Cho, E. S. Son, K. Y. Kim, C. S. Lee, D. Yoon, S. K. Im, S. J. Oh and E. W. Park. 2009. Proteome analysis of the m. longissimus dorsi between fattening stages in Hanwoo steer. BMB Rep. 42:433-438.

Kislinger, T., A. O. Gramolini, Y. Pan, K. Rahman, D. H. MacLennan and A. Emili. 2005. Proteome dynamics during C2C12 myoblast differentiation. Mol. Cell Proteomics 4:887-901.

Kong, Y., M. J. Flick, A. J. Kudla and S. F. Konieczny. 1996. Muscle LIM protein promotes myogenesis by enhancing the activity of MyoD. Mol. Cell. Biol. 17:4750-4760.

Kook, S. H., K. C. Choi, Y. O. Son, K. Y. Lee, I. H. Hwang, H. J. Lee, J. S. Chang, I. H. Choi and J. C. Lee. 2006. Satellite cells isolated from adult Hanwoo muscle can proliferate and differentiate into myoblasts and adipose-like cells. Mol. Cells 22:239-245.

Landry, F., C. R. Lombardo and J. W. Smith. 2000. A method for application of samples to Matrix-Assisted Laser Desorption Ionization Time-of-Flight targets that enhances peptide detection. Anal. Biochem. 279:1-8.

Laville, E., T. Sayd, M. Morzel, S. Blinet, C. Chambon, J. Lepetit, G. Renand and J. F. Hocquette. 2009. Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. J. Agric. Food Chem. 57:10755-10764.

Lehnert, S. A., A. Reverter, K. A. Byrne, Y. Wang, G. S. Nattrass, N. J. Hudson and P. L. Greenwood. 2007. Gene expression studies of developing bovine longissimus muscle from two different beef cattle breeds. BMC Dev. Biol. 7:95-108.

Lennon, N. J., A. Kho, B. J. Bacskai, S. L. Perlmutter, B. T. Hyman and R. H. Brown Jr. 2003. Dysferlin Interacts with Annexins A1 and A2 and Mediates Sarcolemmal Wound-healing. J. Biol. Chem. 278:50466-50473.

Moss, F. P. and C. P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421-435.

Peterson, C. A., M. Cho, F. Rastinejad and H. M. Blau. 1992. β-Enolase is a marker of human myoblast heterogeneity prior to differentiation. Dev. Biol. 151:626-629.

Picard, B., C. Berri, L. Lefaucheur, C. Molette, T. Sayd and C. Terlouw. 2010. Skeletal muscle proteomics in live stock. Brief Funct Genomic Proteomic bfg.oxfordjournals.org/cgi/content/full/elq005v1

Rajesh, R. V., S. K. Kim, M. R. Park, M. A. Park, E. J. Jang, S. G. Hong, J. S. Chang, D. Yoon, T. H. Kim and H. J. Lee. 2009. Differential proteome expression of *in vitro* proliferating bovine satellite cells from *Longissimuss dorsi*, Deep pectoral and *Semitendinosus* muscle depots in response to hormone deprivation and addition. Korean Journal of Animal Science and Technology 51:459-470.

Rudnicki, M. A., P. N. J. Schnegelsberg, R. H. Stead, T. Braun, H. H. Arnold and R. Jaenisch. 1993. MyoD or Myf5 is required for the formation of skeletal muscle. Cell 75:1351-1359.

Schmidt, A. and M. N. Hall. 1998. Signaling to the actin cytoskeleton. Annu. Rev. Cell Dev. Biol. 14:305-338.

Seale, P. and M. A. Rudnicki. 2000. A new look at the origin, function, and ‘‘Stem-Cell’’ status of muscle satellite cells. Dev. Biol. 218:115-124.

Shibata, M., K. Matsumoto, M. Oe, M. Ohnishi-Kameyama, K. Ojima, I. Nakajima, S. Muroya and K. Chikuni. 2009. Differential expression of the skeletal muscle proteome in grazed cattle. J. Anim. Sci. 87:2700-2708.

Smith, C. K., M. J. Janney and R. E. Allen. 1994. Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal muscle satellite cells. J. Cell. Physiol. 159:379-385.

Sternlicht, H., G. W. Farr, M. L. Sternlicht, J. K. Driscoll, K. Willison and M. B. Yaffe. 1993. The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin *in vivo*. Proc. Natl. Acad. Sci. USA. 90:9422-9426.

Tajbakhsh, S., D. Rocancourt and M. Buckingham. 1996. Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice. Nature 384:266-270.

Tannu, N. S., V. K. Rao, R. M. Chaudhary, F. Giorgianni, A. E. Saeed, Y. Gao and R. Raghow. 2004. Comparative proteomes of the proliferating C2C12 myoblasts and fully differentiated myotubes reveal the complexity of the skeletal muscle differentiation program. Mol. Cell Proteomics 3:1065-1082.

Thompson, H. S., E. B. Maynard, E. R. Morales and S. P. Scordilis. 2003. Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. Acta Physiol. Scand. 178:61-72.