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Transcriptional Profiling of Differentially Expressed Genes in Porcine Satellite Cell

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ABSTRACT

Muscle satellite cell (SC) is responsible for postnatal muscle growth, repair, and regeneration. Satellite cell is an important source of multi-potent stem cell process and differentiation into adipogenic, myogenic, and osteoblastogenic. The objective of this study was to identify alter of transcriptome during differentiation in porcine satellite cell and to elevated transcriptome at different stages of postnatal development to gain insight into the differences in differentiated PSC. We used RNA-seq technique to investigate the transcriptomes during differentiation in pig muscle. Sequence reads were obtained from Illumina HiSeq2000. Differentially expressed genes (DEG) were detected by EdgeR. Gene ontology (GO) terms are powerful tool for unification among representation genes or products. In study of GO biological terms, functional annotation clustering involved in cell cycle, apoptosis, extracellular matrix, phosphorylation, proteolysis, and cell signaling in differences stage. Taken together, these results would be contributed to a better understanding of muscle biology and processes underlying differentiation. Our results suggest that the source of DEGs could be better understanding of the mechanism of muscle differentiation and transdifferentiation.

(Key words : Microarray, Postnatal, Satellite cell, Muscle, Gene ontology)

INTRODUCTION

Muscle satellite cell is highly variable, which are influences meat mass and quality, growth, and maintenance during postnatal periods. Adipocyte, myoblast, and osteoblast arise from the same mesoderm layer of the embryos (Phillips *et al*., 2003; Chen *et al*., 2007) and early stages (Belu and Mizutani, 2011) and occur independently of cell during differentiation. The cell population in postnatal is maintained by resident stem cells present at specific sites in tissue. The multipotential capacity of resident muscle satellite cells (SC) to differentiate into myogenic, adipogenic, and osteogenic cells has been extensively investigated (Ding *et al*., 2013; Asakura *et al*., 2001). Porcine satellite cells (PSCs) have been differentiated into myoblast, adipocyte, or osteoblast. Previous studies investigated on the growth of neonatal skeletal muscle of pigs due to markedly increase protein synthesis according feeding and decrease development (Suryawan *et al*., 2001; Davis *et al*., 2000). However, the molecular mechanisms for postnatal growth of skeletal muscle have not yet been elucidated. Here, the relationship between postnatal growth (e.g. depend on differentiating time) and gene expression through the analysis of the transcriptome remain unknown. Whereas, the mechanisms regulation of muscle stem cell underlying muscle type composition remain rarely understood.

Microarray techniques used to compare differences in gene expression profiles between porcine mature adipocytes from isolated preadipocytes (Zhou *et al*., 2010).

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The differential protein expressions from adipose tissue have distinct adipogenic manners during differentiated preadipocytes (Tchkonia *et al*., 2007). Microarray is a powerful tool to study of transcriptome in human and animal. Microarray analyses serve transcription factors and enzymes as mechanism regulators for adipogenesis, myogenesis, and osteoblastogenesis.

Microarray analysis revealed a close relationship between gene expression profiles of different muscle and fat depots in both bovine and pig (Shi *et al*., 2010). Taken together, understood of basic platform for the functional genes following study of these known and unknown genes might be provided insight into common or specific signaling in myogenesis, adipogenesis, and osteoblatogenesis. Here, using microarray, we studied the transcription gene database of the many known genes affecting differentiation and identify differentially expressed genes in porcine satellite cell. The purpose of this study was to compare transcriptome at different stages of crossbred pig muscle tissue to gain insight into the differences of differentiated PSC.

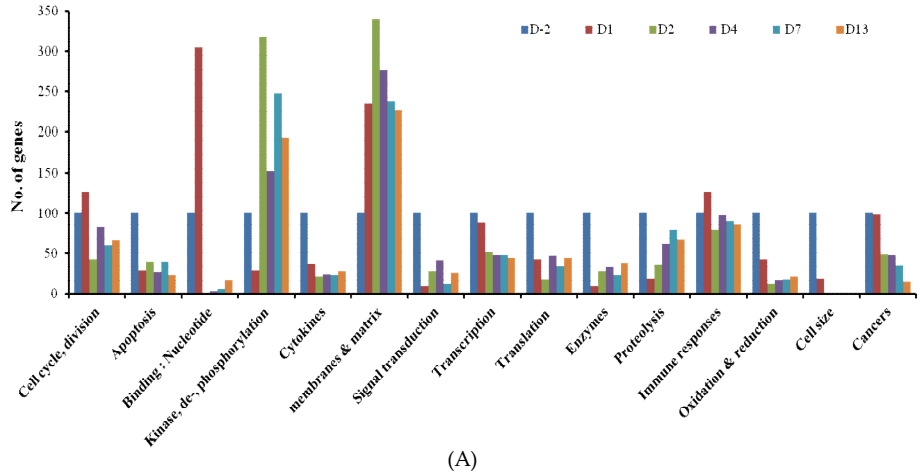
MATERIALS AND METHOS

Animals

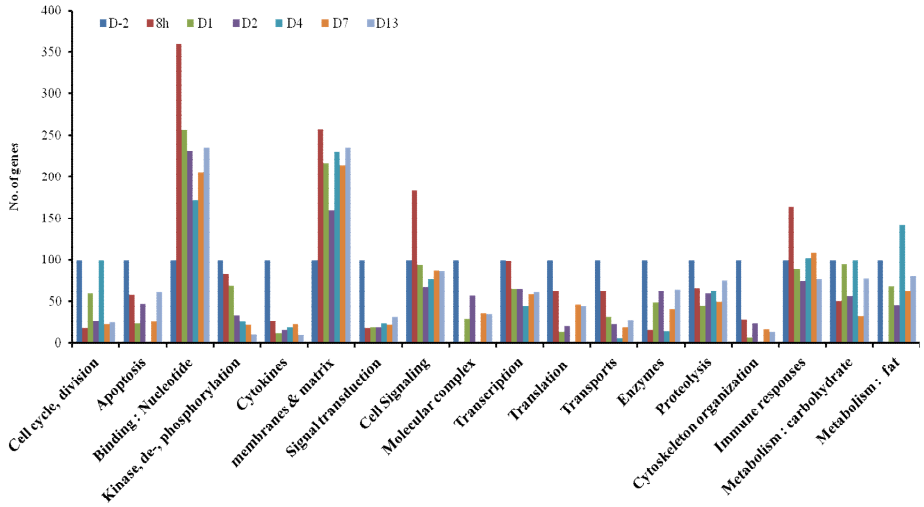
Swine (Landrace × Yorkshire × Duroc; LYD) were managed in the same feeding barn at National Institute of Animal Science (NIAS) under the high quality pork production program and slaughtered at 70～80 kg weight. All experimental procedures involving animals we-

Table 1. Selected genes altered the gene expression during adipogenesis

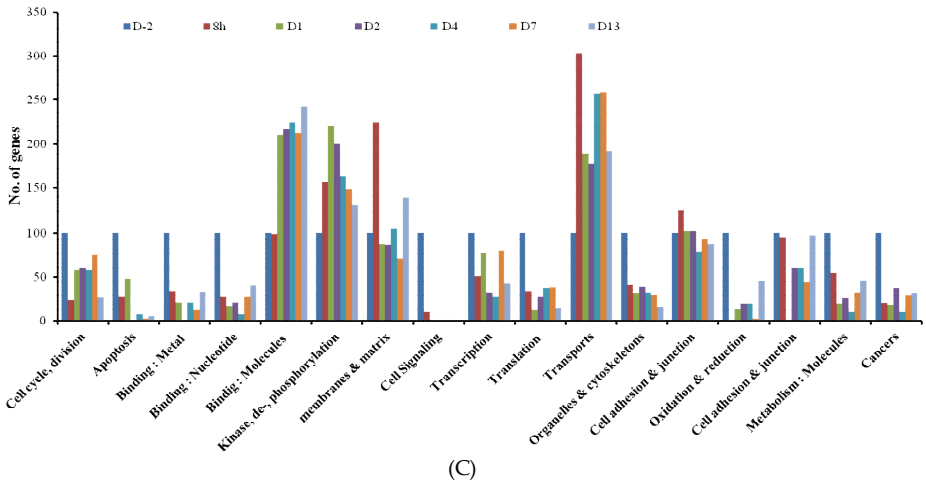
Unigene ID	Gene name	Fold	*P* value
Ssc.16648	Thioredoxin interacting protein	13.75	0.0037
Ssc.14182	Cyclin B2	9.28	0.0035
Ssc.17001	Cell division cycle 20 homolog (*S. cerevisiae*)	8.43	0.0024
Ssc.11630	Pituitary tumor-transforming 1	7.38	0.0056
Ssc.432	Baculoviral IAP repeat-containing 5	3.19	0.0025
Ssc.15749	Cyclin D2	2.18	0.0087
Ssc.49517	Regulator of G-protein signaling 2, 24kDa	2.18	0.0057
Ssc.3253	NAD(P) dependent steroid dehydrogenase-like	3.46	0.0053
Ssc.5712	Cytochrome P450, family 51, subfamily A	2.99	0.0016
Ssc.56656	Squalene epoxidase	2.95	0.0046
Ssc.18628	Sterol-C4-methyl oxidase-like	2.40	0.0009
Ssc.94	Myosin VI	5.32	0.0013
Ssc.18120	RAD18 homolog (*S. cerevisiae*)	2.58	0.0048
Ssc.226	High-mobility group box 2	2.31	0.0068
Ssc.21909	EPH receptor A4	4.57	0.0112
Ssc.5145	Heat shock protein 70.2	2.21	0.0009
Ssc.873	Cyclin-dependent kinase 1	10.20	0.0102
Ssc.4612	Minichromosome maintenance complex component 4	6.13	0.0157
Ssc.44027	Minichromosome maintenance complex component 3	5.27	0.0168
Ssc.73067	Cell division cycle 45 homolog (*S. cerevisiae*)	4.34	0.0148
Ssc.4785	CHK2 checkpoint homolog (*S. pombe*)	4.04	0.0126
Ssc.6966	Cyclin-dependent kinase inhibitor 1B	2.53	0.0185
Ssc.26665	DBF4 homolog (*S. cerevisiae*)	2.02	0.0165



(A)



(B)



(C)

Fig. 1. DEGs based on gene ontology (GO) biological process terms during myogenesis, adipogenesis, and osteoblastogenesis. (A), the DEGs of 6590 are expressed in PSC myogenic differentiation. (B), the DEGs of 11180 are expressed in PSC adipogenic differentiation. (C), the DEGs of 8727 are expressed in PSC osteoblastogenic differentiation. These results are summarized in biological process and cellular component and function. X axis indicates functional groups. Y axis indicates number of genes.

re approved by the National Institute of Animal Science Institutional Animal Use and Care Committee (NIASIAUCC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines provided by NIASIAUCC.

Total RNA Extraction and Gene Ontology (GO) Analysis of DEGs

In our previous study, to investigate the DEGs at a functional level, DAVID (Database for Annotation, Visualization and Integrated Discovery) was used to cluster the genes according to the Gene Ontology (GO) categories of cellular component, biological process and molecular function (Lee *et al.*, 2013). Briefly, Total RNA of porcine satellite cells was extracted using TRIzol reagent (Molecular Research Center) and an RNeasy RNA purification kit with DNase treatment (Qiagen) according to the manufacturer's instructions. Total RNA was quantified by absorbance at 260 nm, and the integrity of total RNA was checked by 1.2% agarose gel electrophoresis. mRNA was isolated from the total RNA using oligo-dT beads and was reverse transcribed into double strand cDNA fragments. To RNA-seq Library Preparation and Sequencing Analysis, quality of RNA-seq reads from PSC was checked using FastQC. Constructing and sequencing RNA-seq library for each sample were carried out based on protocols of Illumina HiSeq-2000 to generate 90 pair-end reads.

RESULTS AND DISCUSSION

DEGs during Differentiated Porcine Satellite Cell

PSC undergoing adipogenic, myogenic, and osteoblstogenic differentiation reflected in the cell transcriptome. During differentiated PSCs, there is an exquisitely coordinated alteration in gene expression that regulates the conversion of satellite cell into multipotent cell. To identify gene ontology and signaling pathway changes during porcine satellite cell differentiation and to compare whether the change is origin specific, In this study, the same conditions were used to stimulate of differentiation and RNA-seq analysis was conducted to create a comprehensive view of the global DEGs during differentiation. These results showed different number of DEGs based on biological process, cellular component and function during PSC differentiation (Supplementary Tables 1～5). For study of GO biological terms, a lower DEGs (6590) were detected during myogenic differentiation (Fig. 1A; Supplementary Tables 8～11). The number of GOs increased in membrane, matrix related genes, kinase, and phosphorylation path-

Table 2. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D-2 during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	67	5.21	0.00
Cell growth	10	0.77	0.03
Apoptosis	41	3.18	0.55
Metal binding	118	9.17	0.28
Nucleotide binding	93	7.23	0.69
Molecules binding	60	4.66	0.86
Kinase, de-, phosphorylation	30	2.33	0.49
Cytokines	17	1.32	0.68
Membranes & matrix	97	7.54	0.93
Signal transduction	29	2.25	0.99
Cell signaling	95	7.38	0.23
Molecular complex	9	0.69	0.61
Transcription	54	4.19	0.22
Translation	4	0.31	0.38
Enzymes	5	0.38	0.56
Proteolysis	44	3.42	0.49
Transports	2	0.15	0.70
Cytoskeleton organization	3	0.23	0.91
Organelles & cytoskeletons	115	8.94	0.95
Immune responses	46	3.57	0.32
Oxidation & reduction	19	1.47	0.46
Cell adhesion & junction	33	2.56	0.78
Carbohydrate metabolism	34	2.64	0.80
Fatty acid metabolism	22	1.71	0.42
Catabolism	43	3.34	0.19
Molecules metabolism	60	4.66	0.25
Cell size	6	0.46	0.30
Disease	42	3.26	0.86
Cancers	81	6.29	0.00

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

ways. DEGs (8727) were detected during osteoblastogenic differentiation (Fig. 1C; Supplementary Tables 12 ~15). The number of GOs showed higher in membrane, matrix related genes, and nucleotide pathways. Whereas, a higher number of total DEGs (11180) were existed during adipogenic differentiation (Fig. 1B; Supplementary Tables 1~7). In study of DEGs based on time-course, a lower DEGs (5901) were detected during myogenic differentiation (Fig. 2A). DEGs (8175) were

Table 3. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D8h during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	8	0.91	0.30
Apoptosis	26	2.98	0.76
Metal binding	48	5.51	0.96
Nucleotide binding	163	18.71	0.33
Kinase activity	38	4.36	0.11
Cytokines	12	1.37	0.78
Membranes & matrix	117	13.43	0.30
Signal transduction	8	0.91	0.77
Cell signaling	83	9.52	0.02
Transcription	45	5.16	0.71
Translation	28	3.21	0.50
Transports	28	3.21	0.99
Proteolysis	30	3.44	0.39
Enzymes	7	0.80	0.68
Organization	13	1.49	0.05
Organelles & cytoskeletons	30	3.44	0.73
Immune responses	74	8.49	0.06
Cell adhesion	13	1.49	0.69
Catabolism	10	1.14	0.11
Metabolism	23	2.64	0.04
Cancer	36	4.13	0.00
Disease	15	1.72	0.73

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

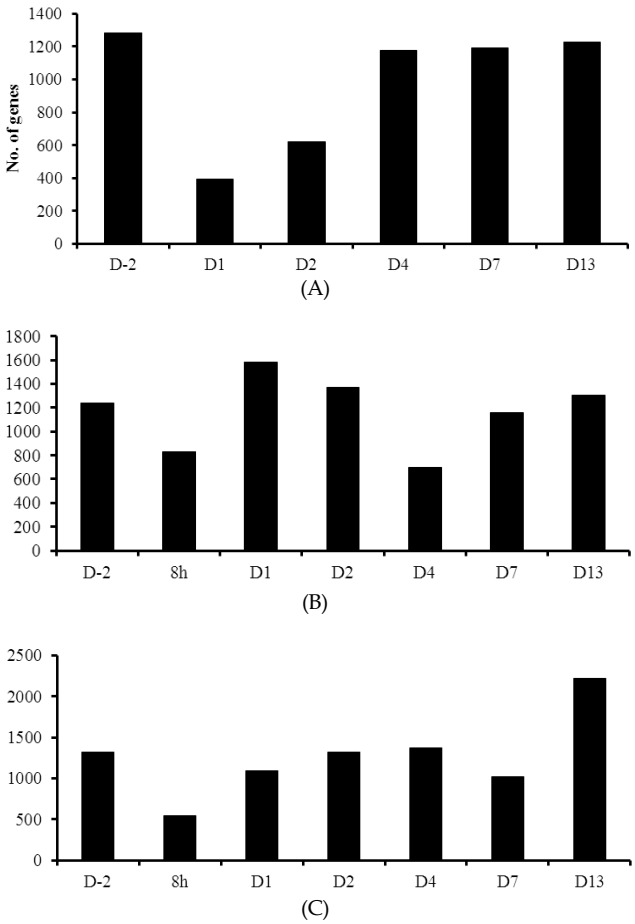


Fig. 2. Number of differentially expressed genes in porcine satellite cell compared to time-course using a microarray. (A) total number of 5901 DEGs are expressed in PSC myogenic differentiation. (B) total number of 8175 DEGs are expressed in PSC adipogenic differentiation. (C) total number of 8889 DEGs are expressed in PSC osteoblastogenic differentiation. The differentially expressed genes with ≥2-fold up- or downregulation were counted.

detected during adipogenic differentiation (Fig. 2B). Whereas, a higher number of total DEGs (8889) were existed during osteoblastogenic differentiation (Fig. 2C). The number of GO increased in transport related genes. Finally, these results might be explained as process was different during differentiation.

Functional Analysis of DEGs

In this study, the different process affected the different number of DEGs and gene ontology terms during differentiation of PSCs. DEGs were classified according to biological process ($p<0.05$). This analysis revealed cellular changes including regulation of cellular transport, protein localization, the regulation of protein transport, and the regulation of cytoplasmic transport. These changes affected translation and post transla-

Table 4. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D1 during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	62	3.05	0.02
Apoptosis	25	1.23	0.35
Metal binding	171	8.41	0.25
Nucleotide binding	272	13.38	0.03
Macromolecules binding	79	3.88	0.02
Cytoskeleton binding	6	0.29	0.81
Kinase & phosphorylation	73	3.59	0.04
Cytokines	12	0.59	0.84
Membranes & matrix	229	11.27	0.34
Signal transduction	20	0.98	0.97
Cell signaling	99	4.87	0.02
Molecules complex	31	1.52	0.35
Transcription	69	3.39	0.46
Translation :localization	14	0.68	0.86
Enzymes	52	2.55	0.04
Proteolysis	48	2.36	0.79
Transport	34	1.67	0.99
Cytoskeletons organization	6	0.29	0.45
Organelles & cytoskeletons	129	6.34	0.49
Stress responses	7	0.34	0.02
Oxidation-reduction	23	1.13	0.31
Cell adhesion & junction	44	2.16	0.41
Catabolism & metabolism : carbohydrate	71	3.49	0.68
Catabolism & metabolism ; DNA	100	4.92	0.02
Catabolism & metabolism : fat	55	2.70	0.64
Catabolism & metabolism : Protein	72	3.54	0.50
Catabolism & metabolism : macromolecules	45	2.21	0.03
Disease	24	1.18	0.93
Cancer	58	2.85	0.07

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 5. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D2 during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	28	1.36	0.19
DNA repairs	13	0.63	0.15
Apoptosis	51	2.48	0.34
Metal binding	157	7.64	0.37
Nucleotide binding	247	12.02	0.19
Macromolecules binding	78	3.79	0.21
Cytoskeleton binding	14	0.68	0.51
Kinase	36	1.75	0.17
Cytokines	17	0.82	0.45
Membranes & matrix	171	8.32	0.35
Signal transduction	20	0.97	0.99
Cell signaling	72	3.50	0.34
Molecules complex	61	2.96	0.53
Transcription	70	3.40	0.57
Translation	22	1.07	0.89
Enzymes	66	3.21	0.83
Proteolysis	63	3.06	0.77
Transport	24	1.16	1.00
Cytoskeletons organization ; organelle	25	1.21	0.45
Organelles & cytoskeletons	188	9.14	0.77
Immunological responses	80	3.89	0.99
Oxidation-reduction	34	1.65	0.61
Cell adhesion & junction	47	2.28	0.60
Metabolism : general	24	1.16	0.78
Metabolism : carbohydrate	60	2.92	0.68
Metabolism : fat	49	2.38	0.63
Metabolism : protein	75	3.65	0.13
Metabolism : nucleotide	73	3.55	0.92
Metabolism : molecules	48	2.33	0.00
Disease	21	1.02	0.93
Cancer	56	2.72	0.04

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 6. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D4 during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	43	5.19	0.03
DNA repair	13	1.57	0.11
Metal binding	91	11.00	0.28
Nucleotide binding	74	8.94	0.73
Macromolecules binding	51	6.16	0.94
Kinase	11	1.33	0.74
Cytokines	8	0.96	0.75
Membranes & matrix	99	11.97	0.16
Signal transduction	10	1.20	0.98
Cell signaling	33	3.99	0.01
Transcription	19	2.29	0.82
Proteolysis	27	3.26	0.43
Organelles & cytoskeletons	77	9.31	0.07
Immunological responses	44	5.32	0.82
Oxidation-reduction	19	2.29	0.35
Metabolism : carbohydrates	43	5.19	0.28
Metabolism : fat	61	7.37	0.24
Metabolism : protein	15	1.81	0.40
Metabolism : DNA	16	1.93	0.08
Cancer	24	2.90	0.25
Disease	9	1.08	0.27

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

tional modifications, such as phosphorylation of amino acid, translation, and biogenesis. The aim of the present work was to develop a model based on muscle satellite cell culture to further investigate the regulation of transcriptome during differentiation in pig skeletal muscle.

Adipocyte differentiation required the process a variety of biological groups (Gregoire *et al*., 1998). Differentiation characterized through alters in cell morphology and complex molecular from hormone signaling (Samulin *et al*., 2008). Accordingly, GO term analysis was

Table 7. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D7 during adipogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	18	1.18	0.35
DNA repair	13	0.85	0.28
Apoptosis	20	1.31	0.28
Metal binding	177	11.66	0.28
Nucleotide binding	163	10.73	0.58
Macromolecules binding	44	2.89	0.56
Kinase	17	1.11	0.68
Cytokines	18	1.18	0.70
Membranes & matrix	169	11.13	0.30
Signal transduction	17	1.11	0.99
Cell signaling	69	4.54	0.02
Molecule complex	28	1.84	0.52
Transcription	46	3.03	0.77
Translation	37	2.43	0.63
Enzymes	32	2.10	0.01
Proteolysis	39	2.56	0.81
Transport	15	0.98	0.70
Organelles	116	7.64	0.70
Immunological responses	86	5.66	0.09
Oxidation-reduction	20	1.31	0.19
Cell adhesion & junction	33	2.17	0.11
Metabolism : carbohydrate	67	4.41	0.52
Metabolism : DNA	26	1.71	0.10
Metabolism : fat	64	4.21	0.02
Metabolism : protein	49	3.22	0.59
Metabolism : molecules	27	1.77	0.48
Cancer	21	1.38	0.71
Disease	27	1.77	0.49

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 8. Selected genes altered the gene expression during myogenesis

Unigene ID	Gene name	Fold	*P* value
Ssc.6966	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	2.24	0.0136
Ssc.43394	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	2.52	0.0125
Ssc.20585	Growth arrest and DNA-damage-inducible, gamma	3.35	0.0189
Ssc.27593	Transforming growth factor, beta 3	7.89	0.0124
Ssc.53924	Lipoprotein lipase	7.98	0.0402
Ssc.41844	Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	6.54	0.0415
Ssc.5549	Fatty acid binding protein 5 (psoriasis-associated)	3.66	0.0435
Ssc.16159	Stearoyl-CoA desaturase (delta-9-desaturase)	2.85	0.0405
Ssc.53771	Carnitine palmitoyltransferase 1A (liver)	3.86	0.0479
Ssc.15878	Protein phosphatase 3, catalytic subunit, alpha isozyme	2.24	0.0068
Ssc.7701	Stathmin 1	2.05	0.0165
Ssc.1577	Similar to Serum response factor (SRF)	2.28	0.0223
Ssc.16243	Brain-derived neurotrophic factor	2.67	0.0265
Ssc.9781	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	2.58	0.0028
Ssc.873	Cyclin-dependent kinase 1	6.53	0.0369
Ssc.373	Matrix Gla protein	3.74	0.0472
Ssc.54157	Low density lipoprotein receptor	3.16	0.0035
Ssc.20	Interleukin 18 (interferon-gamma-inducing factor)	2.94	0.0419
Ssc.719	Alveolar macrophage-derived chemotactic factor-II	2.67	0.0203
Ssc.6080	Secretory leukocyte peptidase inhibitor	2.53	0.0269

used to explore the function of DEGs involved in the significant profiles. As expected, we found DEGs, which is comprised functional groups, such as metabolic and regulation of biological process during differentiation. The general functional groups may be essential to the conversion from PSC to multipotent cell. Moreover, cellular analysis indicated that extracellular matrix (ECM) and extracellular regions were significantly enriched in among adipogenic, myogenic, and osteoblastogenic progression. The extracellular environment of cells originating from distinct cell may markedly affect the differentiation of PSC. This is a novel study comparing DEGs of PSC during differentiation. Our study produced abundant data for the analysis of adipogenic, myogenic, and osteoblastogenic differentiation. A lot of DEGs were found to be involved during differentiation. GO terms results indicated that DEGs related to multipotent differentiation were clustered into the same mechanism in PSC. DEGs were regulated by transcription factors or differentiation via the distinct signaling pathways. Additionally, the extracellular matrix might an important in the differentiation of PSC. In conclusion, DEGs profiling and different cell models have potentially involved in the regulation of cell fate. The gene expression profiles and further study insights into the molecular mechanisms of the cell process during differentiation.

Table 9. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D1 during myogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	27	6.60	0.19
Cell growth	4	0.97	0.12
Apoptosis	6	1.46	0.26
Metal binding	47	11.49	0.58
Nucleotide binding	65	15.89	0.51
Macromolecules binding	13	3.17	0.50
Kinase & phosphorylation	5	1.22	0.82
Cytokines	8	1.95	0.12
Membranes & matrix	50	12.22	0.02
Signal transduction	2	0.48	0.98
Signal signaling	23	5.62	0.32
Transcription	19	4.64	0.68
Translation & modification	9	2.20	0.50
Enzymes	2	0.48	0.75
Proteolysis	4	0.97	0.59
Organelles & cytoskeletons	34	8.31	0.41
Immunological responses	27	6.60	0.36
Oxidation-reduction	9	2.20	0.35
Cell adhesion & junction	2	0.48	0.71
Catabolism & metabolism : carbohydrate	2	0.48	0.32
Catabolism & metabolism : fat	4	0.97	0.26
Catabolism & metabolism : protein	2	0.48	0.36
Catabolism & metabolism : DNA	6	1.46	0.55
Catabolism & metabolism : molecules	6	1.46	0.69
Cell size	4	0.97	0.14
Cancer	21	5.13	0.06
Disease	6	1.46	0.12

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 10. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D4 during myogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle, division	55	4.30	0.00
Cell growth	12	0.93	0.38
Apoptosis	18	1.40	0.87
Metal binding	124	9.69	0.83
Nucleotide binding	101	7.89	0.91
Molecules binding	38	2.97	0.80
Kinase & phosphorylation	10	0.78	0.92
Cytokine	16	1.25	0.27
Membranes & matrix	184	14.38	0.89
Signal transduction	28	2.18	0.05
Cell signaling	57	4.45	0.60
Transcription	32	2.50	0.92
Translation	31	2.42	0.77
Enzymes	22	1.72	0.22
Proteolysis	41	3.20	0.85
Transport	42	3.28	0.70
Cytoskeleton organization	16	1.25	0.70
Organelles & cytoskeletons	114	8.91	0.40
Immunological responses	65	5.08	0.97
Cell adhesion & junction	28	2.18	0.81
Catabolism & metabolism : carbohydrate	23	1.79	0.49
Catabolism & metabolism : nucleotide	50	3.90	0.49
Catabolism & metabolism : fat	13	1.01	0.60
Catabolism & metabolism : protein	31	2.42	0.64
Catabolism & metabolism : macromolecules	32	2.50	0.91
Disease	37	2.89	0.90
Cancer	32	2.50	0.42

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 11. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D13 during myogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	40	3.42	0.02
Cell growth	4	0.34	0.37
DNA repair	10	0.85	0.47
Apoptosis	14	1.19	0.90
Metal binding	121	10.36	0.53
Nucleotide binding	118	10.11	0.95
Molecules binding	55	4.71	0.95
Kinase & phosphorylation	14	1.19	0.76
Cytokine	17	1.45	0.34
Membranes & matrix	138	11.82	0.56
Signal transduction	16	1.37	0.97
Cell signaling	63	5.39	0.05
Transcription	27	2.31	0.91
Translation	31	2.65	0.73
Enzymes	23	1.97	0.20
Proteolysis	41	3.51	0.67
Transport	27	2.31	0.94
Organelles & cytoskeletons	134	11.48	0.26
Immunological responses	52	4.45	0.96
Oxidation-reduction	13	1.11	0.57
Cell adhesion & junction	24	2.05	0.15
Catabolism & metabolism : carbohydrate	30	2.57	0.84
Catabolism & metabolism : DNA	54	4.62	0.82
Catabolism & metabolism : fat	10	0.85	0.20
Catabolism & metabolism : proteins	14	1.19	0.78
Catabolism & metabolism : molecules	25	2.14	0.08
Disease	32	2.74	0.48
Cancer	9	0.77	0.87

[1] Term: cooperation of a set of genes.
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[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 12. Selected genes altered the gene expression during osteoblastogenesis

Unigene ID	Gene name	Fold	*P* value
Ssc.15932	Iintegrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	2.96	0.0002
Ssc.33914	Iintegrin, alpha 5 (fibronectin receptor, alpha polypeptide) /// zinc finger protein 385A	2.43	0.0003
Ssc.924	Thrombospondin 1	2.26	0.0003
Ssc.12323	Syndecan 2	2.00	0.0002
Ssc.28161	Tenascin-X	2.28	0.0026
Ssc.44	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	4.07	0.0024
Ssc.12068	Collagen, type VI, alpha 3	4.29	0.0036
Ssc.16209	Tenascin C	5.25	0.0006
Ssc.27593	Transforming growth factor, beta 3	9.54	0.0013
Ssc.20585	Growth arrest and DNA-damage-inducible, gamma	4.19	0.0026
Ssc.20913	Ggrowth arrest and DNA-damage-inducible, alpha	2.05	0.0052
Ssc.74811	Anaphase promoting complex subunit 5	2.08	0.0042
Ssc.15749	Cyclin D2	2.11	0.0036
Ssc.73067	Cell division cycle 45 homolog (*S. cerevisiae*)	3.44	0.0046
Ssc.873	Cyclin-dependent kinase 1	7.58	0.0043
Ssc.14182	Cyclin B2	10.25	0.0029
Ssc.18628	Sterol-C4-methyl oxidase-like	2.90	0.0046
Ssc.56656	Squalene epoxidase	2.56	0.0076
Ssc.3253	NAD(P) dependent steroid dehydrogenase-like	2.56	0.0089
Ssc.5712	Cytochrome P450, family 51, subfamily A	2.06	0.0005
Ssc.48473	Rreplication factor C (activator 1) 5, 36.5kDa	2.14	0.0013
Ssc.44027	Minichromosome maintenance complex component 3	3.24	0.0046
Ssc.4612	Minichromosome maintenance complex component 4	4.79	0.0025
Ssc.11071	Yinculin	5.23	0.0038
Ssc.15932	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	2.96	0.0082
Ssc.924	Thrombospondin 1	2.26	0.0046
Ssc.54244	Myosin, light chain 9, regulatory	2.24	0.0072

Table 13. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D8h during osteoblastogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	7	1.24	0.18
Cell growth & development	8	1.42	0.22
Cell physiology	10	1.77	0.51
Apoptosis	8	1.42	0.65
Metal binding	29	5.15	0.97
Nucleotide binding	46	8.17	0.31
Macromolecules binding	66	11.72	0.30
Cytoskeleton binding	3	0.53	0.31
Kinase, phosphorylation	15	2.66	0.50
Cytokine activity	10	1.77	0.08
Membranes & matrix	89	15.81	0.19
Signal transduction	12	2.13	0.85
Cell signaling	37	6.57	0.66
Transcription	28	4.97	0.19
Translation & modification	16	2.84	0.16
Proteolysis	11	1.95	0.72
Transport	6	1.06	0.46
Cytoskeletons organization	28	4.97	0.83
Organelles & cytoskeletons	3	0.53	0.72
Immunological responses	5	0.88	0.48
Oxidation-reduction	2	0.35	0.99
Cell adhesion & junction	24	4.26	0.51
Catabolism & metabolism	8	1.42	0.26
Catabolism & metabolism : protein	17	3.02	0.22
Catabolism & metabolism : DNA	8	1.42	0.25
Catabolism & metabolism : molecules	8	1.42	0.53
Cancer	9	1.59	0.09
Disease	2	0.35	0.33

[1] Term: cooperation of a set of genes.
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[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 14. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D2 during osteoblastogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	51	3.11	0.00
Apoptosis	18	1.09	0.89
Metal binding	185	11.29	0.44
Nucleotide binding	171	10.43	0.47
Molecules binding	74	4.51	0.56
Kinase & de-, phosphorylation	28	1.70	0.49
Cytokines	23	1.40	0.76
Membranes & matrix	152	9.27	0.69
Signal transduction	33	2.01	0.00
Cell signaling	87	5.30	0.63
Molecule complex	17	1.03	0.14
Transcription	51	3.11	0.44
Translation & modification	22	1.34	0.42
Enzymes	32	1.95	0.51
Proteolysis	37	2.25	0.35
Cytoskeleton organization	16	0.97	0.32
Organelles & cytoskeletons	159	9.70	0.17
Immunological responses	71	4.33	0.92
Oxidation-reduction	20	1.22	0.19
Cell adhesion & junctions	68	4.14	0.11
Catabolism & metabolism	68	4.14	0.27
Catabolism & metabolism : DNA	42	2.56	0.27
Catabolism & metabolism : fat	25	1.52	0.23
Catabolism & metabolism : protein	45	2.74	0.04
Catabolism & metabolism : molecules	50	3.05	0.02
Disease	41	2.50	0.99
Cancer	30	1.83	0.14

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

Table 15. KEGG pathways or GO terms that changed expression according to each condition (D0 vs. D7 during osteoblastogenesis)

Term[1]	Count[2]	Percentage[3]	EASE score[4]
Cell cycle	55	3.90	0.02
Cell growth	9	0.63	0.17
Apoptosis	20	1.41	0.26
Binding : metal, ion	156	11.07	0.02
Binding : nucleotide	109	7.73	0.88
Binding : molecules	52	3.69	0.69
Kinase, de-, phosphorylation	59	4.18	0.01
Cytokines	28	1.98	0.28
Membranes & matrix	190	13.48	0.24
Signal transduction	22	1.56	0.00
Cell signaling	68	4.82	0.59
Transcription	32	2.27	0.93
Translation & modification	24	1.70	0.93
Enzymes	20	1.41	0.16
Proteolysis	35	2.48	0.05
Transports	21	1.49	1.00
Organelles & cytoskeletons	88	6.24	0.76
Immunological responses	50	3.54	0.88
Oxidation-reduction	15	1.06	0.54
Cell adhesion & junctions	54	3.83	0.17
Catabolism & metabolism : Carbohydrate	57	4.04	0.22
Catabolism & metabolism : DNA	15	1.06	0.81
Catabolism & metabolism : fat	25	1.77	0.09
Catabolism & metabolism : protein	65	4.61	0.75
Catabolism & metabolism : molecules	59	4.18	0.08
Disease	31	2.27	0.89
Cancer	28	1.98	0.02

[1] Term: cooperation of a set of genes.
[2] Count: number of genes involved in this annotation category.
[3] Percentage: abundance in set of genes, involved genes/total genes.
[4] EASE score: modified Fisher Exact *p*-value.
The EASE score in each cluster is identical to the meaning/value of the *p*-value (Fisher Exact/EASE score).

REFERENCES

1. Asakura A, Komaki M, Rudnicki M (2001): Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation 68:245-253.
2. Belu M, Mizutani CM (2011): Variation in mesoderm specification across Drosophilids is compensated by different rates of myoblast fusion during body wall musculature development. LoS One 6:e28970.
3. Chen TL, Shen WJ, Qiu XW, Li T, Hoffman AR, Kraemer FB (2007): Generation of novel adipocyte monolayer cultures from embryonic stem cells. Stem Cells Dev 16:371-380.
4. Davis TA, Nguyen HV, Suryawan A, Bush JA, Jefferson LS, Kimball SR (2000): Developmental changes in the feeding-induced stimulation of translation initiation in muscle of neonatal pigs. Am J Physiol Endocrinol Metab 279:E1226-34.
5. Ding K, Yang Z, Zhang YL, Xu JZ (2013): Injectable thermosensitive chitosan/β-glycerophosphate/collagen hydrogel maintains the plasticity of skeletal muscle satellite cells and supports their *in vivo* viability. Cell Biol Int 37:977-987.
6. Gregoire FM, Smas CM, Sul HS (1998): Understanding adipocyte differentiation. Physiol Rev 78:783-809.
7. Lee HJ, Jang M, Kim H, Kwak W, Park W, Hwang JY, Lee CK, Jang GW, Park MN, Kim HC, Jeong JY, Seo KS, Kim H, Cho S, Lee BY (2013): Comparative transcriptome analysis of adipose tissues reveals that ECM-receptor interaction is involved in the Depot-specific adipogenesis in cattle. PLoS One 8(6):e662-67.
8. Phillips BW, Vernochet C, Dani C (2003): Differentiation of embryonic stem cells for pharmacological studies on adipose cells. Pharmacol Res 47:263-268.
9. Samulin J, Lien S, Grindflek E, Berget I, Ruyter B (2008): Depot specific differences during adipogenesis of porcine stromal-vascular cells. Cell Biol Int 32:525-531.
10. Shi F, Miao J, Zhang L, Tao H, Lü J, Ruan Z, Zong H (2010): Detection of bovine, goat, pig and chicken derived ingredients in animal products with universal PCR-microarray method. Sheng Wu Gong Cheng Xue Bao 26:823-829.
11. Suryawan A, Nguyen HV, Bush JA, Davis TA (2001): Developmental changes in the feeding-induced activation of the insulin-signaling pathway in neonatal pigs. Am J Physiol Endocrinol Metab 281(5):E908-915.
12. Tchkonia T, Lenburg M, Thomou T, Giorgadze N, Frampton G, Pirtskhalava T, Cartwright A, Cartwri-

ght M, Flanagan J, Karagiannides I, Gerry N, Forse RA, Tchoukalova Y, Jensen MD, Pothoulakis C, Kirkland JL (2007): Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. Am J Physiol Endocrinol Metab 292:E298-307.

13. Zhou G, Wang S, Wang Z, Zhu X, Shu G, Liao W, Yu K, Gao P, Xi Q, Wang X, Zhang Y, Yuan L, Jiang Q (2010): Global comparison of gene expression profiles between intramuscular and subcutaneous adipocytes of neonatal landrace pig using microarray. Meat Sci 86(2):440-450.

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