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Research Paper

Differential transcriptional analysis between red and white skeletal muscle of Chinese Meishan pigs

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Abstract

In order to better understand and elucidate the major determinants of red and white muscle phenotypic properties, the global gene expression profiling was performed in white (longissimus doris) and red (soleus) skeletal muscle of Chinese Meishan pigs using the Affymetrix Porcine Genechip. 550 transcripts at least 1.5-fold difference were identified at $p < 0.05$ level, with 323 showing increased expression and 227 decreased expression in red muscle. Quantitative real-time PCR validated the differential expression of eleven genes (*α-Actin*, *ART3*, *GATA-6*, *HMOX1*, *HSP*, *MYBPH*, *OCA2*, *SLC12A4*, *TGFB1*, *TGFB3* and *TNX*). Twenty eight signaling pathways including ECM-receptor interaction, focal adhesion, TGF-beta signaling pathway, MAPK signaling pathway, Wnt signaling pathway, mTOR signaling pathway, insulin signaling pathway and cell cycle, were identified using KEGG pathway database. Our findings demonstrate previously unrecognized changes in gene transcription between red and white muscle, and some potential cascades identified in the study merit further investigation.

Key words: Affymetrix; Differential transcriptional analysis; Longissimus doris; Pig; Soleus

1. Introduction

Skeletal muscle is the most abundant human tissue comprising almost 50% of the total body mass, exhibiting major metabolic activity by contributing up to 40% of the resting metabolic rate in adults and serving as the largest body protein pool [1]. Skeletal muscle is a very heterogeneous tissue that is composed of a large variety of functionally diverse fiber types [2]. Traditionally, skeletal muscle can be distinguished as red (type I and IIa) and white (type IIb) fibers. Red skeletal muscles, such as the soleus and psoas in the pig, have a higher percentage of capillaries, myoglobin, lipids and mitochondria than white skeletal muscles such as the gastrocnemius and longissimus doris [3]. In meat animal production, favorable meat traits such as color and, in the pig in particular, tenderness have been found to closely associate with the greater abundance of red or highly oxidative fibres [4-9]. In addition, individuals with muscles that are abundant in oxidative type I fibres are associated with favorable metabolic health, and are less likely to predispose to obesity and insulin resistance [10]. Collectively, understanding the molecular processes that govern the expression of specific fiber types and the phenotypic characteristics of muscles is very important in agricultural and medical fields.

Microarray technology can simultaneously measure the differential expression of a large number of genes in a given tissue and may identify the genes responsible for the relevant phenotype [11]. Campbell et al. identified 49 differentially expressed mRNA sequences between the white quad (white muscle)

and the red soleus muscle (mixed red muscle) of female mice using Affymetrix Mu11K SubB containing 6516 probe sets [12]. Bai et al. profiled the differential expression of genes between the psoas (red muscle) and the longissimus dorsi (white muscle) of a 22-week-old Berkshire pig using porcine skeletal muscle cDNA microarray comprising 5500 clones [13]. The tremendous rise in porcine transcriptomic data has occurred with the development of pig cDNA microarray in the past decade. The Affymetrix porcine genome array showed particularly superior performance for swine transcriptomics [14]. In this study, a genome-wide investigation of the porcine differential expression between red (soleus) and white (longissimus dorsi) muscle was conducted using the Affymetrix GeneChip® Porcine Genome Array containing oligonucleotides representing approximately 23937 transcripts from 20201 porcine genes.

2. Materials and methods

2.1 Animals and tissue sampling

Three Meishan gilts from the same litter were slaughtered at 150 days by electrical stunning and exsanguination, in compliance with national regulations applied in commercial slaughtering. Immediately after slaughter, two muscles with different locations, functions, and biochemical properties were sampled: the longissimus doris at the last rib level, a fast twitch glycolytic muscle involved in voluntary movements of the back, and the deep portion of the soleus, a oxidative muscle. Samples were frozen by liquid nitrogen, and stored at -80℃ until further analysis.

2.2 Total RNA preparation and microarray hybridization

Six microarrays were used in the experiment, corresponding to the RNAs from longissimus doris and soleus of three sibling gilts. Total RNA was isolated using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. Twenty micrograms total RNA was suspended in RNase-free water with a final concentration of 1.5μg/μl. The RNA labelling and Affymetrix Gene Chip microarray hybridization were conducted according to the Affymetrix Expression Analysis Technical Manual (CapitalBio Corporation, Beijing, China). Array scanning and data extraction were carried out following the standard protocol.

2.3 Identification and bioinformatic analyses of differentially expressed transcripts

The probe-pair (PM-MM) data were used to detect the expression level of transcripts on the array (present call, marginal call, and absent call) by MAS 5.0 (Wilcoxon signed rank test). The signals from the probe pairs were used to determine whether a given gene was expressed and to measure the gene expression level. Raw data from .CEL files were converted to gene signal files by MAS 5.0 (Ver.2.3.1). The expression data from three pigs were loaded into GeneSpring GX 10.0 software (Agilent Technologies) for data normalisation and filtering. Differentially expressed transcripts between longissimus doris and soleus were identified by cutoff of fold-change (FC) ≥ 1.5 and p-value < 0.05 using unpaired t-test. Mean FC is the mean of three biological replicates. Molecular function of differentially expressed genes was classified according to MAS (molecule annotation system) 3.0 (http://bioinfo.capitalbio.com/mas3/). Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used for signaling pathway analysis on differentially expressed genes. Microarray expression data have been deposited in the Gene Expression Omnibus database (GEO, National Center for Biotechnology Information) under accession number GSE19975.

2.4 Quantitative real time RT-PCR (qRT-PCR)

The primer sequences, melting temperature and product sizes of analyzed genes were shown in Table 1. The correct fragment sizes of the PCR products were confirmed using agarose gel electrophoresis (1.5%). Each primer set amplified a single product as indicated by a single peak during melting curve analyses. Both longissimus doris and soleus RNA prepared for microarray were also included for qRT-PCR. Total RNA were treated with DnaseI and reverse transcribed by the M-MLV Reverse Transcriptase (Promega, Madison, USA) according to the manufacturer's instructions. qRT-PCR was performed on the ABI 7300 real-time PCR thermal cycle instrument (Applied Biosystems, Foster City, CA, USA) using SYBR® Green Realtime PCR Master Mix (Toyobo Co., Ltd, Japan). The reactions contained 1× SYBR Green real-time PCR Master Mix, 1μl diluted cDNA template and each primer at 200 nM in a 25 μl reaction volume. After an initial denaturation at 95℃ for 3 min amplification was performed with 40 cycles of 95℃ for 15 s, 61℃ for 15 s, 72℃ for 20 s; plate read; melting curve from 55℃ to 95℃, read every 0.2℃, hold for 1 second. For each sample, reactions were set up in triplicate to ensure the reproducibility of the results. At the end of the PCR run, melting curves were generated and analyzed to confirm non-specific amplification, then the mean value of each triplicate was used for further calculation. Gene expression level was quantitated relative to the expression of the reference gene (*HPRT*: hypoxanthine phosphoribosyl transfe-

rase) by employing the $2^{-\Delta\Delta Ct}$ value models [15]. For each gene, the sample with the largest ΔCt value was set as control. The expression data were calculated using the SigmaPlot version 9.0 software (Systat software Inc., USA). Expression difference of target genes between two muscles was analyzed using t-test. The $p < 0.05$ was deemed to be significant and $p < 0.01$ highly significant.

Table 1. Specific primer sequences for qRT-PCR

Gene symbol	Description	Reference sequence	Primer sequence (5′-3′)	Tm (℃)	Product size (bp)
α-Actin	α-Actin	Ssc.1901	F: GATGGCGTAACCCACAAC	61	194
			R: AGGGCAACATAGCACAGC		
FHL1C	Four and a half LIM domains 1 protein, isoform C	Ssc.14463	F: GCTGTGGAGGACCAGTATTA R: CCAGATTCACGGAGCATT	61	175
HMOX1	Heme oxygenase (decycling) 1	Ssc.115	F: CACTCACAGCCCAACAGCA R: GTGGTACAAGGACGCCATCA	61	162
TNX	Tenascin-X	Ssc.28161	F: GCTGACAGCGACCGACATAA	61	197
			R: CGAGCCCATACAGGACGAAT		
MYBPH	Myosin binding protein H	Ssc.20879	F: CGTCAGGTGGGAGAAGCAA R: GAGCGGATGAAGAGGATGG	61	149
TGFB3	Transforming growth factor, beta 3	Ssc.27593	F: TTCCGCTTCAACGTGTCG R: CGCTGCTTGGCTATGTGC	61	158
TGFB1	Transforming growth factor, beta 1	Ssc.76	F: GCTGCTGTGGCTGCTAGTG R: TCGCGGGTACTGTTGTAAAG	61	216
HSP	Heat shock protein 20kDa	Ssc.13823	F: CTACCGCCCAGGTGCCAA	61	96
			R: CGCCAACCACCTTGACGG		
SLC12A4	Solute carrier family 12 (potassium/chloride transporters), member 4	Ssc.4097	F: CAGCACAAGGTTTGGAGGAA R: CGTAGGTGGTACAGGAAGAT	61	110
GATA-6	Transcription factor GATA-6	Ssc.2258	F: CAGAAACGCCGAGGGTGAA R: GAGGTGGAAGTTGGAGTCAT	61	216
OCA2	Oculocutaneous albinism 2	Ssc.15775	F: CTGCCATCATCGTAGTAGTC R: CTCCAATCAGTGTCCCGTTA	61	192
ART3	ADP-ribosyltransferase 3	Ssc.15864	F: ATGTCTATGGCTTCCAGTTCA R: CTGGCTTATGCTATACACCAC	61	110
HPRT	Hypoxanthine phosphoribosyl transferase	Ssc.4158	F: GGACTTGAATCATGTTTGTG R: GTTTGGAAACATCTG	61	91
MyHCI	Myosin heavy chain, type I	Ssc.1544	F: CGACACACCTGTTGAGAAG R: AGATGCGGATGCCCTCCA	61	233
MyHCIIa	Myosin heavy chain, type IIa	Ssc.15909	F: GGGCTCAAACTGGTGAAGC R: AGATGCGGATGCCCTCCA	61	249
MyHCIIb	Myosin heavy chain, type IIb	Ssc.56948	F: GTTCTGAAGAGGGTGGTAC R: AGATGCGGATGCCCTCCA	61	234
MyHCIIx	Myosin heavy chain, type IIx	Ssc.56721	F: CTTCACTGGCGCAGCAGGT R: AGATGCGGATGCCCTCCA	61	257

3. Results and discussion

3.1 Myosin heavy chain expression analysis

MyHC isoforms are generally considered as the molecular markers of different muscle fiber types. In postnatal growing pigs, type I, IIb, IIa and IIx *MyHC* are all expressed in skeletal muscle, which are encoded by a distinct gene [16, 17]. In this study, *MyHCI* (oxidative fiber) and *MyHCIIa* (intermediate fiber) expressions in soleus were significantly higher than their counterparts in longissimus doris, while *MyHCIIb* (glycolytic fiber) expression in soleus was significantly lower than that in longissimus doris (Figure 1). In particular, the mRNA level of type IIb in longissimus doris was nearly 11 times greater than that in soleus. Therefore, the longissimus doris was composed of more glycolytic type of muscle fibers than fiber composition of soleus. The increasing percentages of type IIb fiber, and decreasing percentages of types I and IIa fibers, are related to increases in drip loss and lightness, which are deteriorative to pork quality [18].

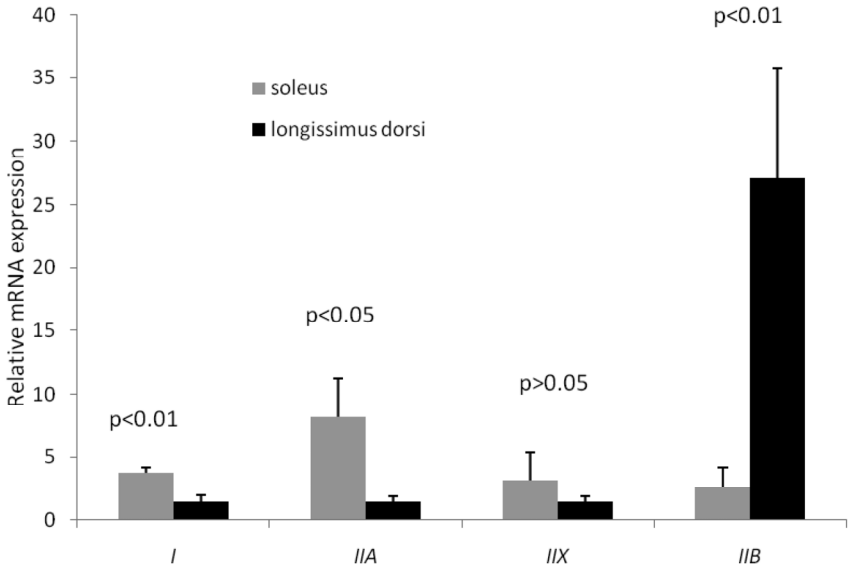


Figure 1. Expression of four *MyHC* isoforms in longissimus doris and soleus mRNA by qRT-PCR. The data presented in Y-axis were calculated using the expression values of $2^{-\Delta\Delta C_t}$ of three pigs and expressed as means ± s.d.

3.2 Identification of differentially expressed transcripts between white and red skeletal muscle

The transcriptome analysis indicated that 13241 and 14433 probe sets were expressed in porcine longissimus doris and soleus, respectively. The global expression profile of longissimus doris was compared with that of the soleus group. After quantile normalization and statistical analyses, 550 transcripts with at least 1.5-fold difference were identified at the $p < 0.05$ significance level ($p < 0.05$, FC≥1.5). Compared with the expression of transcripts in longissimus doris, a set of 323 transcripts belonged to the up-regulated group, and another set of 227 transcripts belonged to the down-regulated group in soleus. Taking the FC of two or greater as the criteria ($p < 0.05$, FC≥2), a total of 159 transcripts showed differential expression, of which 107 transcripts were up-regulated and 52 down-regulated in soleus. The differentially expressed transcripts were involved in many functions related to contractile structure and cytoskeleton, extracellular matrix, energy metabolism, stress, transcription regulation and so on (Table 2). The microarray results confirmed several differentially expressed genes between red and white skeletal muscle in the previous studies, such as *MyHCIIb, a-actin, HSP20, PGM*, fibronectin and muscle LIM protein encoding genes [3, 12, 13]. As expected, the expression levels of energy metabolism enzyme genes, cathepsin, collagen protein, oxygenase and slow-type muscle protein encoding genes, were significantly higher in red muscle than in white muscle, which could contribute to the better meat quality of red muscle. In addition, some important transcription factors including *GATA-6*, *TGFB1*, *TGFB3*, *MEF2C*, *EGF* and *HMOX1* that were not previously known to be expressed in a fiber type manner, were identified as differential expression in microarray analysis. It is interesting as the newly identified factors might be candidates for transcriptional regulation of the specificity of the metabolic and contractile characteristics of different fiber types.

3.3 Validation of microarray data by qRT-PCR

Among the differentially expressed transcripts identified by microarray, twelve known genes were selected for validation by qRT-PCR. These genes included three down-regulated genes (*ART3*, *MYBPH* and *OCA2*) and nine up-regulated genes (*a-actin*, *FHL1C*, *GATA-6*, *HMOX1*, *HSP*, *SLC12A4*, *TGFB1*, *TGFB3* and *TNX*) in soleus. Except for *FHL1C*, all the other selected genes showed significant ($p < 0.05$ or 0.01) differential expression between two muscles in the qRT-PCR results. Remarkably, qRT-PCR showed significant correlation with microarray analysis, with all the genes being the similar expression patterns in both methods (Pearson correlation coefficient ranged from 0.612 to 0.946) (Figure 2). The fold changes ob-

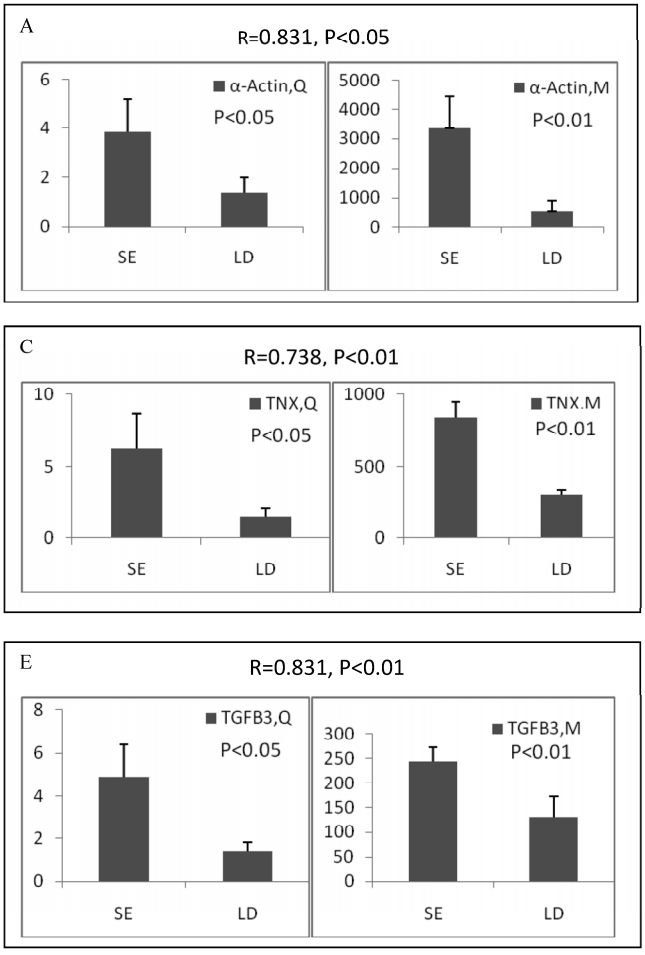
tained by qRT-PCR were much more or less than those obtained in the microarray. This may be due to the greater accuracy of quantitation provided by qRT-PCR in comparison to microarrays, the differences in the dynamic range of the two techniques, and the lack of specificity in the primers designed to discriminate gene family members at the level of primary screening by DNA arrays [19]. However, the trends were same between the results of two methods, showing the reliability of the microarray analysis.

Table 2. List of some differential expressed genes between red and white muscle of Meishan pigs

Gene title	Fold change	P value	Structure and function	Unigene
Muscle contraction and cytoskeleton genes				
myosin heavy chain IIb	-1.51	0.023	striated muscle contraction, actin binding	Ssc.56948
α-actin	7.52	0.007	striated muscle contraction	Ssc.1901
filamin A, alpha (actin binding protein 280)	1.83	0.009	striated muscle contraction	Ssc.55452
filamin B, beta (actin binding protein 278)	1.84	0.030	striated muscle contraction	Ssc.6691
tubulin, beta 2B	2.50	0.004	microtubule subunit protein, bind to colchicine,vincristine	Ssc.55842
tubulin, beta 6	2.02	0.046	microtubule subunit protein, bind to colchicine,vincristine	Ssc.58401
α-actinin	2.22	0.030	regulate the length of actin	Ssc.5941
integrin, beta 3	1.76	0.029	cell adhesion, integrin-mediated signaling pathway, regulation of cell migration	Ssc.44
catenin (cadherin-associated protein), alpha 1	1.61	0.025	bind to cadherin	Ssc.58861
myosin binding protein C, slow type isoform 3	2.28	0.006	bind to myosin	Ssc.13955
myosin binding protein H	-2.84	0.035	bind to myosin	Ssc.20879
Extracellular matrix genes				
fibromodulin	3.12	0.013	protein binding	Ssc.56133
fibronectin	2.51	0.011	extracellular region	Ssc.16743
tenascin-X	2.94	0.001	signal transduction	Ssc.28161
tenascin-C	2.66	0.001	cell adhesion, signal transduction	Ssc.16209
ankyrin 1 isoform 5	-1.51	0.006	attach to cytoskeleton, membrane-associated protein	Ssc.21745
collagen, type I, alpha 1	3.10	0.008	phosphate transport, cell adhesion	Ssc.46811
collagen, type V, alpha 1	2.54	0.016	phosphate transport, cell adhesion	Ssc.54853
Metabolic enzyme genes				
pyruvate dehydrogenase kinase, isozyme 3	1.9	0.012	phosphorylate pyruvate dehydrogenase	Ssc.19740
heme oxygenase (decyclizing) 1	3.25	0.025	heme oxidation	Ssc.115
phosphoglucomutase	-1.58	0.005	phosphotransferases, carbohydrate metabolic process	Ssc.4307
fructose 1,6-bisphosphatase 2	2.12	0.022	carbohydrate metabolic, gluconeogenesis	Ssc.5127
creatine kinase	1.65	0.020	transferring phosphorus-containing groups	Ssc.9914
phosphofructokinase, platelet, partial	2.31	0.012	6-phosphofructokinase activity	Ssc.862
glutathione S-transferase omega	-1.55	0.029	glutathione transferase activity	Ssc.183
ADP-ribosyltransferase 3	-2.68	0.004	protein amino acid ADP-ribosylation	Ssc.15864
AXL receptor tyrosine kinase	2.29	0.010	regulates tyrosine phosphorylation in cellular signal transduction	Ssc.6566
protein tyrosine phosphatase 4a2	-2.01	0.014	dephosphorylation in cellular signal transduction, cell growth control	Ssc.54932
Stress protein genes				
heat shock protein 2	1.91	0.005	response to stress	Ssc.7654
heat shock protein 20kDa	2.15	0.032	response to stress	Ssc.13823
Transport protein genes				
solute carrier family 12 (potassium/chloride transporters), member 4	2.42	0.016	ion transport	Ssc.4097
aquaporin 3	-3.66	0.026	water reabsorption	Ssc.3832
oculocutaneous albinism 2	-12.6	0	citrate transmembrane transport	Ssc.15775
Transcription factor genes				
transforming growth factor, beta induced	2.9	0.040	binds to type I, II, IV, VI collagens and fibronectin	Ssc.16671
transforming growth factor, beta 3	1.99	0.027	cell differentiation, embryogenesis and development	Ssc.27593
transforming growth factor, beta 1	1.85	0.003	immune, regulation of cell proliferation and differentiation	Ssc.76
transcription factor GATA-6	2.23	0.040	positive regulation of transcription	Ssc.2258
general transcription factor IIE, polypeptide 2, beta 34kDa	1.69	0.003	regulation of transcription initiation	Ssc.3369
homeobox protein A10	2.27	0.001	regulation of transcription, DNA-dependent	Ssc.26254

myocyte enhancer factor 2C	1.58	0.011	regulation of transcription, DNA-dependent	Ssc.34788
four and a half LIM domains 1 protein, isoform C	1.53	0.027	metal ion binding	Ssc.14463
epidermal growth factor	-1.57	0.040	calcium ion binding, integral to membrane	Ssc.87
Hormone genes				
parathyroid hormone-like hormone	1.77	0.003	hormone activity	Ssc.9991
Others				
calponin 1	1.67	0.015	actomyosin structure organization and biogenesis, actin and calmodulin binding	Ssc.9013
calcyclin binding protein isoform 1	-1.76	0.012	ubiquitin-mediated degradation of beta-catenin	Ssc.10299
cathepsin B	1.59	0.045	proteolysis	Ssc.53773
cathepsin H	1.83	0.018	proteolysis	Ssc.3593
cathepsin Z	1.65	0.016	proteolysis	Ssc.16769
mitochondrial ribosomal protein S26	-2.12	0.033	catalytic function in reconstituting biologically active ribosomal subunits	Ssc.12554
p53 protein	1.64	0.028	control of cell proliferation	Ssc.16010
p55 TNF receptor superfamily, member 1A	1.51	0.008	cell surface receptor linked signal transduction	Ssc.4674
interleukin 15	-1.59	0.031	stimulating the proliferation of T-lymphocytes	Ssc.8833
cytochrome P450, family 27, subfamily A, polypeptide 1	1.73	0.012	biosynthesis of steroids, fatty acids and bile acids	Ssc.3804

"+" and "-" indicated the up- and down- regulated expression in soleus group, respectively.



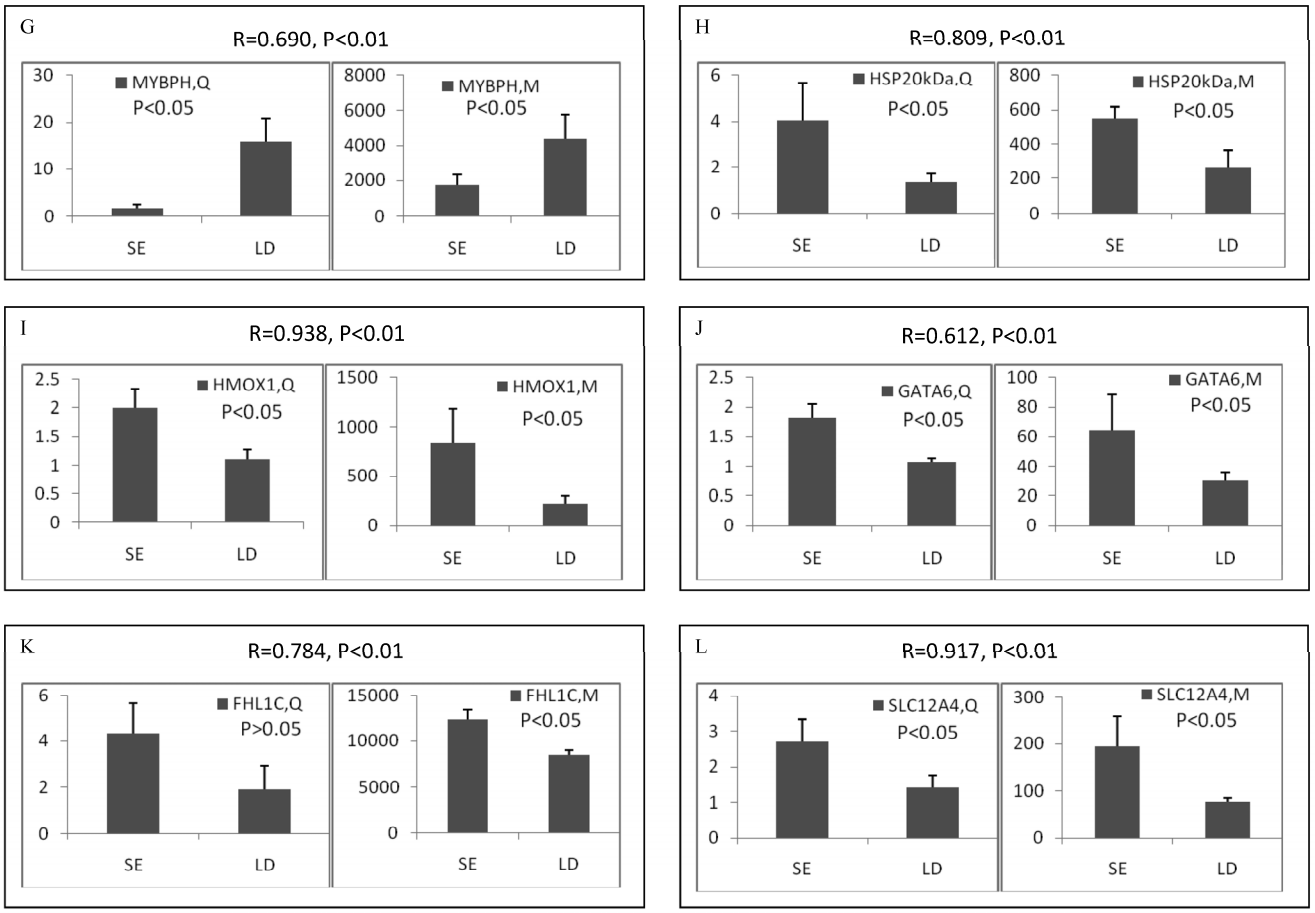


Figure 2. Validation of differentially expressed genes between longissimus doris (LD) and soleus (SE) by qRT-PCR. The data presented in Y-axis indicated the relative mRNA expression of both microarray (M) and qRT-PCR (Q) and expressed as means of three pigs ± s.d. The correlation coefficient (R) and the corresponding significance value (P) were shown above their respective columns.

3.4 Gene Ontology (GO) analysis

To elucidate the relationship between gene differential expression pattern and phenotypic difference of red and white muscle, we examined the functional bias of 550 differentially expressed transcripts according to Gene Ontology classifications. These differentially expressed transcripts were grouped into 404 GO terms based on biological process GO terms. The most enriched GO terms included cellular biopolymer metabolic process, protein metabolism and cellular protein metabolism (Table 3). Analyses of GO also indicated that there were 108 GO terms identified by cellular component classification, and 64 GO terms identified by molecular function classification.

Table 3. List of the top 20 enriched Gene Ontology (GO) terms based on GO classifications

Biological process	Count	Percent	Molecular function	Count	Percent	Cellular component	Count	Percent
cellular biopolymer metabolic process	41	3%	pyrophosphatase activity	6	6%	intracellular organelle	53	10%
protein metabolism	23	2%	G-protein coupled receptor activity	5	5%	intracellular organelle part	38	7%
cellular protein metabolism	19	2%	cation transporter activity	4	4%	cytoplasm	33	6%
biopolymer biosynthesis	14	1%	transcription coactivator activity	3	3%	cytoplasmic part	32	6%
cellular macromolecule biosynthetic process	14	1%	symporter activity	3	3%	intracellular membrane-bound organelle	31	6%
cellular biopolymer biosynthetic process	14	1%	phosphoric monoester hydrolase activity	3	3%	intracellular non-membrane-bound organelle	27	5%

DNA metabolism	13	1%	iron ion binding	2	2%	cytoskeleton	14	3%
regulation of cellular metabolism	13	1%	carbohydrate kinase activity	2	2%	nucleus	13	2%
organ morphogenesis	13	1%	protein kinase activity	2	2%	nuclear part	12	2%
regulation of macromolecule metabolic process	13	1%	cysteine-type peptidase activity	2	2%	cytoskeletal part	11	2%
biopolymer modification	12	1%	exopeptidase activity	2	2%	chromosome	11	2%
negative regulation of cellular physiological process	12	1%	phosphofructokinase activity	2	2%	chromosomal part	9	2%
cytoskeleton organization and biogenesis	12	1%	anion transporter activity	2	2%	actin cytoskeleton	8	1%
RNA metabolism	11	1%	protein methyltransferase activity	2	2%	intracellular organelle lumen	7	1%
transcription	11	1%	S-adenosylmethionine-dependent methyltransferase activity	2	2%	chromatin	7	1%
regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	11	1%	peptide receptor activity, G-protein coupled	2	2%	organelle envelope	6	1%
intracellular signaling cascade	11	1%	double-stranded DNA binding	2	2%	contractile fiber	6	1%
protein modification	11	1%	P-P-bond-hydrolysis-driven transporter activity	2	2%	endoplasmic reticulum	5	1%
cell morphogenesis	11	1%	phosphorylase activity	2	2%	contractile fiber part	5	1%
intracellular transport	10	1%	copper ion binding	1	1%	intrinsic to membrane	5	1%

3.5 Pathway analysis

Twenty eight signaling pathways were identified using KEGG pathway database (Figure 3). The genes could be assigned into numerous subcategories including the extracellular matrix (ECM)-receptor interaction (*COL5A1*, *COL1A2*, *TNC*, *COL1A1* and *FN1*), focal adhesion (*COL5A1*, *COL1A2*, *TNC*, *FLNB*, *FLNA*, *COL1A1* and *FN1*), TGF-beta signaling pathway (*TGFB1* and *TGFB3*), MAPK signaling pathway (*p53*, *EGF*, *TNFRSF1A*, *TGFB1* and *TGFB3*) , cytokine-cytokine receptor interaction (*CCR1*, *IL15*, *EGF* and *TNFRSF1A*), regulation of actin cytoskeleton (*ITGB3* and *EGF*), mTOR signaling pathway (*VEGFA*), JAK-STAT signaling pathway (*IL15*), cell cycle (*p53*) and so on. There were cross-talks among these pathways, as one gene might participate in several signaling pathways.

The ECM-receptor interaction, focal adhesion and cell communication pathways accounted for a large part of the involved differentially expressed genes. The major constituents of the ECM are collagens, proteoglycans, and adhesive glycoproteins. In addition to being responsible for the strength and form of tissues, each collagen type has specific sequences providing them with special features such as flexibility and the ability to interact with other matrix molecules and cells [20]. Specific interactions between cells and ECM mediated by transmembrane molecules or other cell-surface-associated components, lead to a direct or indirect control of cellular activities such as adhesion and migration. Focal adhesions are large, dynamic protein complexes through which the cytoskeleton of a cell connects to the ECM. They actually serve for not only the anchorage of the cell, but can function beyond that as signal carriers (sensors), which inform the cell about the condition of the ECM and thus affect their behavior [21]. Collagen is an abundant connective tissue protein and is a contributing factor to variation in meat tenderness and texture. Although collagen constitutes <2% of most skeletal muscles, it is associated with background toughness and can be quite resistant to physical breakdown during cooking [22]. No significant difference in total amount of glycosaminoglycans (GAGs) was found, but a significant difference in the ratio of GAG/collagen was found between the tough (m. semitendinosus) and tender (m. psoas major) muscles [23]. The higher expressions of some collagen encoding genes were detected in red muscle than in white muscle in this study, reflecting the composition difference of collagens in two types of muscles.

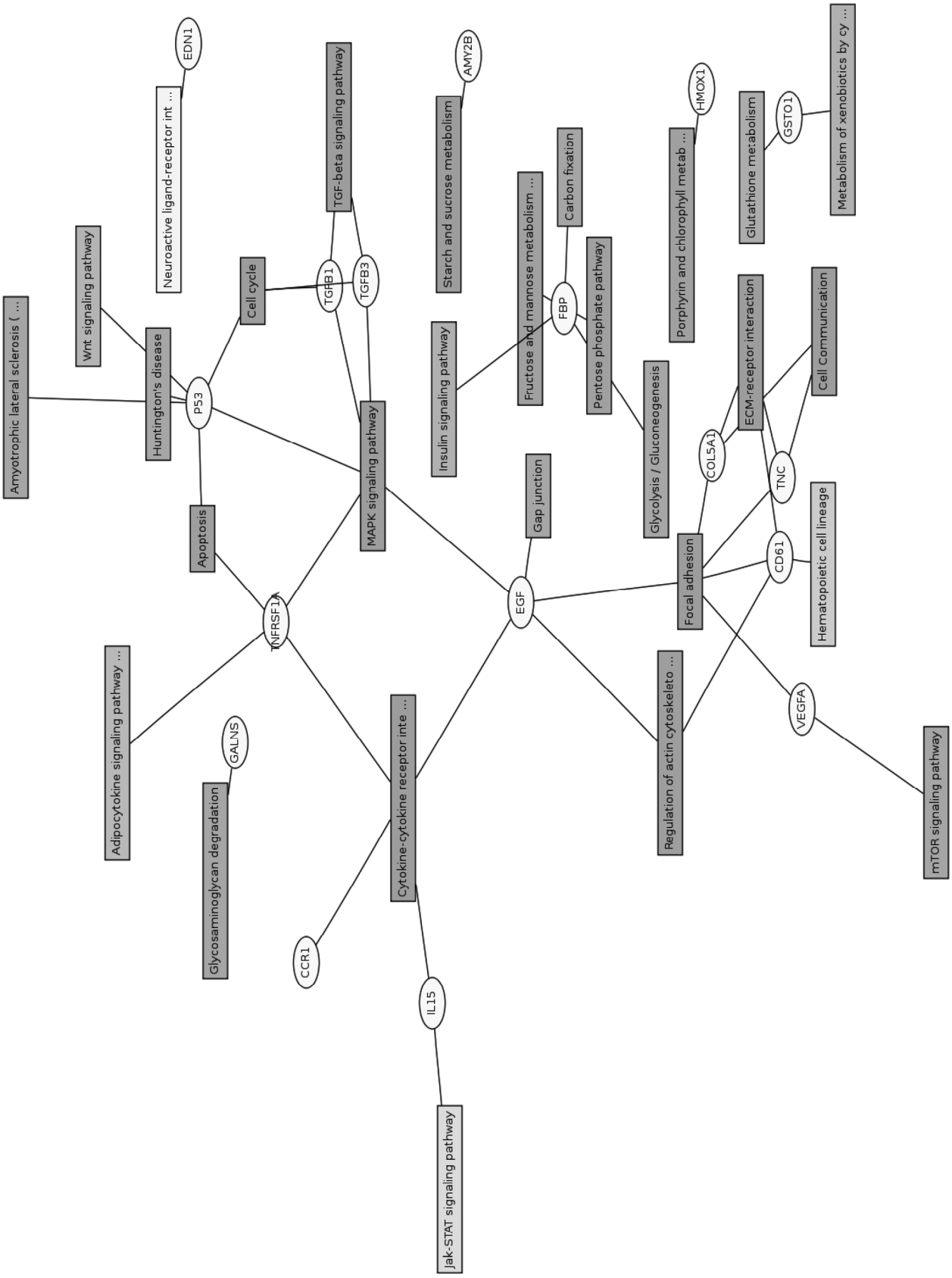


Figure 3. Gene pathway network about the differential expressed genes. The differential expressed genes and the corresponding pathways were shown in the circles and boxes, respectively.

Other significant signaling pathways contained TGF-beta signaling pathway, cytokine-cytokine receptor interaction, MAPK signaling pathway, mTOR Signaling pathway and JAK-STAT signaling pathway. Two genes of the TGFB signaling pathway (*TGFB1* and *TGFB3*) which also participated in the MAPK signaling pathway, were up-regulated in soleus. TGFB1 plays an important role in controlling the immune system, and shows different activities on different types of cell, or cells at different developmental stages. Most immune cells (or leukocytes) secrete TGFB1 [24]. TGFB3 is a type of protein, known as a cytokine, which is involved in cell differentiation, embryogenesis and development [25]. During skeletal muscle development, TGFB1 is a potent inhibitor of muscle cell proliferation and differentiation, as well as a regulator of extracellular matrix (ECM) production [26]. TGFB1 induces an incomplete shift from a slow to a fast phenotype in regenerating slow muscles and that conversely, neutralization of TGFB1 in regenerating fast muscle leads to a transition towards a less fast phenotype [27]. TGFB1 is also able to induce synthesis of connective tissue growth factor (CTGF) in myoblasts and myotubes. CTGF induced several ECM constituents such as fibronectin, collagen type I and α 4, 5, 6, and β1 integrin subunits in myoblasts and myotubes [28]. Stimulation with TGFB1 caused a 14.8-fold increase in collagen I, alpha 1 mRNA and a fourfold increase in fibronectin mRNA abundance in Human Tenon Fibroblasts [29]. In this study, the expression levels of collagen I, alpha 1 and fibronectin were more 3.1- and 2.51-fold in soleus than in longissimus doris, while the expression levels of *TGFB1* and *TGFB3* were more 1.85- and 1.99-fold in soleus than in longissimus doris. Thus, the correlation between their expression trends was positive, which was consistent with their roles in regulating ECM production. Moreover, since TGFB1 influences some aspects of fast muscle-type patterning during skeletal muscle regeneration [27], it will be worthwhile in further investigation to determine at the cellular level how TGFB1 influences fibre type formation and characteristics.

Besides the above identified pathways, *GATA-6* is another important differentially expressed transcription factor that might affect the expression of specific fiber types. GATA proteins are a family of transcription factors with two zinc fingers that directly bind DNA regulatory elements containing a consensus (A/T)GATA(A/G) motif. To date, six mammalian members of the GATA family have been identified that can be divided, on the basis of sequence and expression similarities, into two subgroups [30]. The GATA-4/5/6 subfamily is expressed within various mesoderm- and endoderm-derived tissues including the heart, liver, lung, gonads, and small intestine [31]. During development GATA-6 becomes the only member of the family expressed in vascular smooth muscle cells and has been linked to the differentiated phenotype of these cells [32]. Overexpression of GATA-6 significantly decreased endogenous telokin and 130-kDa MLCK expression in A10 vascular smooth muscle cells. In contrast, expression of the 220-kDa MLCK and calponin were markedly increased. GATA-6 has been shown to bind directly to the telokin and 130-kDa MLCK promoters at consensus binding sites [33, 34]. Knockdown of endogenous GATA-6 in primary human bladder smooth muscle cells led to decreased mRNA levels of the differentiation markers: α-smooth muscle actin, calponin, and smooth muscle myosin heavy chain [35]. In the present study, compared with these in white muscle, the expressions of *GATA-6*, calponin and α-actin were all up-regulated in red muscle. Therefore, it can be inferred that GATA-6 also possibly regulates the expression of myosin light chain kinase, calponin and actin in skeletal muscle cells.

In summary, we have identified the global changes of gene expression in porcine red and white muscle. The results indicated distinguishable trends in ECM structure, contractile structure and cytoskeleton, collagen, focal adhesion, immune response and energy metabolism between two muscles. Some potential cascades identified in the study merit further investigation at the cellular level in the function of controlling the fibre type formation and characteristics. Although the work was limited to three animals in each group and to a single time point, the present microarray analysis provided new information that increased our understanding of governing the expression of specific fiber types.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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