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ABSTRACT: The objective of this study was to compare gene transcription profiles of LM between two pig breeds, Duroc and Taoyuan, which display dramatically different postnatal muscle growth. We isolated LM from neonatal pigs, and the Duroc muscle length and mass were greater ($P < 0.01$) than for Taoyuan pigs; however, insignificant differences in the muscle fiber area and the percentage of fiber types were found. A human high-density complementary DNA (cDNA) microarray consisting of 9,182 probes was used to compare gene transcription profiles of LM between the two breeds. The results showed that the transcription level of 73 genes and 44 genes in Duroc LM were upregulated and downregulated by at least 1.75-fold ($P < 0.05$) compared with Taoyuan, respectively. The strongly upregulated genes in Duroc pigs included those encoding the complex of myofibrillar proteins (e.g., myosin light and heavy chains, and troponin), ribosomal proteins, transcription regulatory proteins (e.g., skeletal muscle LIM protein 1 [SLIM1] and high-mobility group proteins), and energy metabolic enzymes (e.g., electron-transferring flavoprotein dehydrogenase, NADH dehydrogenase, malate dehydrogenase, and ATP synthases). The highly transcribed genes that encode energy metabolic enzymes indicate a more glycolytic metabolism in Duroc LM, thereby favoring carbohydrates rather than lipids for use as energy substrates in this tissue. The over-transcribed genes that encode skeletal muscle-predominant proteins or transcription regulators that control myogenesis and/or muscle growth suggest a general mechanism for the observed higher rate of postnatal muscle growth in Duroc pigs. The transcription of one such gene, SLIM1, was more highly transcribed ($P < 0.01$) in Duroc LM at birth and at postnatal d 7 than in Taoyuan. The transcription of SLIM1 increased ($P < 0.05$) in Duroc LM from neonate through 7 d of age, whereas its transcription remained essentially constant in Taoyuan during this period. These results suggest that SLIM1 may be useful for the development of markers associated with the postnatal muscle growth of pigs.

Key Words: Gene Transcription, Microarray, Neonate, Pig, Skeletal Muscle, Skeletal Muscle LIM Protein 1

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Introduction

Selection for efficiency of meat production and quality is of major priority in modern pig production. Skeletal muscle is a target tissue for identifying candidate genes for the traits (de Vries et al., 2000). Therefore, a better understanding of how genetic variations contribute to increased pork production strongly depends on identifying and studying genes transcribed in skeletal muscle (Malek et al., 2001; Eggen and Hocquette, 2003).

Recent developments in complementary DNA (**cDNA**) microarray technologies permit scientists to analyze the transcription of thousands of gene simultaneously in diverse biological systems (Schena et al., 1995). However, there have been few microarray-based gene transcription experiments reported for mammalian species other than human and rodents, primarily due to the limited availability of the relevant arrays. Although resources are being developed to facilitate production of microarrays for livestock species (Bai et al., 2003; Band et al., 2002; Cogburn et al., 2003), the resulting microarrays may not meet the needs of all researchers. One alternative to developing species-specific microarrays is to use commercially available human microarrays in cross-species hybridizations. Human microarrays have been used successfully in heterologous systems using RNA from pigs (Moody et al., 2002; Cogburn et al., 2003) and cattle (Dalbiés-Tran and Mermilhod, 2003; Sudre et al., 2003). Therefore, in the present study, we used human cDNA microarrays to characterize and compare gene transcription profiles in the LM of two pig breeds, Duroc and Taoyuan, which

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display dramatically different postnatal muscle growth. The identification of differentially transcribed genes may yield candidates for prognosis of postnatal muscle growth in pigs.

Materials and Methods

Experimental Animals

Twelve neonatal male pigs (six each of Duroc and Taoyuan) provided by the Animal Technology Institute of Taiwan or Taiwan Livestock Research Institute were used in the present study. The experimental protocol conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH, 1996) and was approved by the animal welfare committees of National Chiao Tung University and the Animal Technology Institute of Taiwan. We used six Duroc muscles and six Taoyuan muscles for muscle fiber typing and measurements of muscle mass as well as myofiber area. Total cellular RNA was extracted and mRNA was purified from all 12 muscle samples. Three mRNA samples for each breed were used for the cDNA microarray assay, and the remaining three Duroc and three Taoyuan mRNA samples were used for real-time reverse-transcriptase (**RT**) PCR analysis. Additionally, the mRNA of LM isolated from three Duroc and three Taoyuan pigs at postnatal d 7 were used to determine the gene transcription of skeletal muscle LIM protein 1 (**SLIM1**).

Muscle Sampling and Measurements

Before killing animals for muscle samples, the pigs were anesthetized by an intraperitoneal injection of 3 mL of buffer containing ketamine (49 mg/mL), xylazine (6.2 mg/mL), and acepromazine (2.0 mg/mL). Intact LM (approximately 3 g) at the 10th rib was removed within 10 min after slaughter and divided into three parts for use in muscle measurements, histochemical analysis, and RNA extraction. The mass, length, and cross-sectional area of the LM were immediately estimated after excision. Cross-sectional area was measured by tracing digitized images (Jandel video analysis, Jandel Scientific, San Rafael, CA), with the cortical area calculated as the difference. For histochemical analysis, a 0.5-cm^3 fragment of LM was trimmed and mounted on tongue depressors, frozen in 2-methylbutane (isopentane) prechilled with liquid N_2, and stored at –80°C until further processing. For RNA extraction, the excised LM were immediately and directly frozen in liquid N_2 and stored at –80°C until use.

Histochemical Analysis

Each frozen muscle sample was mounted on a cryostat chuck and sectioned (12 μm thick) with a microtome. Sections were treated using a combination of myofibrillar (acid) ATPase and succinic dehydrogenase staining procedures, as described by Solomon and Dunn (1988). The stained sections were photographed, and myofibers were identified as Type I, IIA, or IIBA according to the intensity of both ATPase and succinic dehydrogenase staining (Brooke and Kaiser, 1970; Ashmore and Doerr, 1971). Fiber type proportions were determined from the sections of 200 to 300 fibers from each muscle. The size of fibers in the LM was determined by the Bio-Quant software program (R&M Biometrics, Nashville, TN).

RNA Isolation

Approximately 0.2 g of LM was homogenized on ice in 3 mL of TRIzol reagent (Gibco BRL, Gaithersburg, MD) using a Polytron (Kinematica, Lucerne, Switzerland) at a setting of seven for three pulses of 15 s each. Cellular debris was removed by centrifugation for 5 min at 12,000 × *g*, and then cellular mRNA was extracted from the muscle using the Oligotex mRNA Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purified RNA was examined by agarose gel electrophoresis and quantified by determining A260/A280 using a spectrophotometer.

Preparation of Fluorescently Labeled cDNA

Fluorescently labeled cDNA derived from mRNA was prepared by direct incorporation of fluorescence nucleotide analogs during a first-strand RT reaction. Each 40-μL labeling reaction consisted of 500 ng of mRNA, 2 m*M* oligo-dT primer, 0.5 m*M* each of dATP, dCTP and dGTP, 0.2 m*M* dTTP, 10 m*M* DTT, 0.5 U RT (Superscript II; Stratagene, La Jolla, CA) in the 1× reaction buffer provided by the manufacturer, and 2 nmol of either Cy3-dUTP (for Duroc subjects) or Cy5-dUTP (for Taoyuan subjects; Amersham Pharmacia Biotech, Piscataway, NJ). The mRNA and primers were preheated to 70°C for 5 min and snap-cooled in ice water before adding the remaining reaction components. The RT reaction preceded 10 min at 25°C followed by 2 h at 42°C. Buffer exchange, purification, and concentration of the cDNA products were accomplished by three cycles of diluting the reaction mixture in 450 μL of Tris-EDTA buffer and filtering through Microcon-30 micro-concentrators (Amicon, Charlotte, NC).

cDNA Microarray Hybridization

Both fluorescently labeled cDNA (Duroc muscle cDNA labeled with Cy3 and Taoyuan muscle cDNA labeled with Cy5) were competitively hybridized to the Incyte human uniGEM V2 (Incyte Genomics, Palo Alto, CA) cDNA microarray. Of the 9,182 probes, 8,556 unique human cDNA genes and expressed sequence tags (**EST**) were represented on the cDNA microarray for our study of gene transcription profiling. The positive and negative control probe sets also were included on the array. Arrays were prehybridized for 2 h at 42°C in MicroHyb hybridization solution containing denatured Cot-1 DNA and poly dA (ResGen, Carlsbad, CA).

Both of the fluorescently labeled cDNA were added, and hybridization was carried out in a humidified slide chamber for 12 h at 42°C. After hybridization, the arrays were washed twice for 15 min in 2× SSC/0.1% SDS at room temperature and again twice for 15 min in 0.1× SSC/0.1% SDS at 42°C. The slide was scanned at 532 nm (for Cy3) and 635 nm (for Cy5) using the Axon GenePix 4000B microarray scanner (Axon, Union City, CA). Microarray data were analyzed using with the Incyte GEMtools software (Incyte Genomics). Defective cDNA spots (signal/noise ratio <2.5 or <40% spot area compared with average) were eliminated from the data set. These criteria assured that the signal level was sufficiently high above background to be reliably read and that the reading was not the result of nonuniform noise emanating from the spotted DNA.

Analyses of Microarray Data

Three arrays were used for competitive hybridization with the Cy3- and Cy5-labeled cDNA. Data sets were first normalized for each array by multiplying the Cy5 channel with a balance coefficient that set its median gene signal value equal to that for the Cy3 channel. Analysis of microarray data was performed using an ANOVA approach (Kerr and Churchill, 2001; Walsh and Henderson, 2004). An initial model was fitted to normalize systematic effects common to all probes in a particular array. The model was as follows:

$$Y_{ijk} = \mu + A_i + D_j + P_k + (DP)_{jk} + \varepsilon_{ijk}$$

where Y_{ijk} is the logarithm of the local background-subtracted measurement (median pixel value) from array i (i = 1, 2, 3), dye (i.e., breed) j (j = Cy3, Cy5), and probe k (k = 1, …, 9182) for each breed, μ represents an overall mean value, A is the main effect for arrays, D is the main effect for dyes, P is the main effect for probes, DP is the interaction effect of the breed and probe where the spot was located, and ε is the stochastic error.

To identify differentially transcribed genes, the Cy5 and Cy3 signals of each array were subjected to a pairwise comparison (Chen et al., 2004). Each array hybridization result for Cy5 was compared with all three hybridization results for Cy3 (i.e., the ratio of Cy5 to Cy3), generating nine comparisons for each gene; comparisons were classified as an increase, marginal increase, no change, marginal decrease, or decrease. The transcription of a gene was considered to be increased when it was classified as increased in all nine data sets. The same criterion was applied to determine decreased genes.

Real-Time RT-PCR Measurement of Gene Transcription

A quantitative real-time RT-PCR-based approach was used to verify cDNA microarray hybridization data. The protocol, based on the use of the relative standard curve, was as previously described (da Costa et al., 2002; Bai et al., 2003). Eight sets of PCR primers, including sense, antisense, and TaqMan probe primers, were designed for randomly selected genes among those whose transcription was significantly different in Duroc or Taoyuan LM (Table 1). TaqMan probes and primer sequences were designed using Primer Express software (Applied Biosystems, Foster City, CA) and synthesized by Genset KK (Kyoto, Japan). The β-actin gene was included as internal control.

Each cDNA template for real-time RT-PCR was reverse-transcribed from mRNA extracted from 0.2 g of skeletal muscle. Complementary DNA was prepared using MultiScribe reverse transcriptase and TaqMan reverse transcription reagents (Applied Biosystems). Assays were performed using an ABI Prism 7700 real-time RT-PCR System with each specific primer set (Table 1) according to the manufacturer's recommendations (Applied Biosystems). Cycling conditions were as follows: one cycle at 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s.

Quantitative real-time RT-PCR measurement for each sample was performed in duplicate. Each quantification was performed based on six-point calibration curves. The transcription level of each gene was normalized according to the transcription level of the β-actin gene as a covariable (Hocquette and Brandstetter, 2002), and a relative fold-change in gene transcription (Duroc/Taoyuan or Duroc/Taoyuan) was obtained.

Statistical Analyses

All quantitative data for comparing traits on the muscle measurements and real-time RT-PCR between Taoyuan and Duroc pigs are expressed as mean ± SD. Differences between groups were evaluated by the Student's *t*-test. Probability values less than 0.05 were considered statistically significant.

Results

Features of the Longissimus Muscle

Body weight, LM length, and mass of neonatal Duroc pigs were greater ($P < 0.01$) than the values measured for Taoyuan pigs. The area of Duroc LM also tended to be greater ($P = 0.06$) than that of the Taoyuan pigs; however, this difference was not significant (Table 2), possibly because of the limited sample size (n = 6). There were no significant differences between Duroc and Taoyuan pigs with regard to the area of LM fibers or percentage of different fiber types (type I, IIA, and IIB).

Microarray Analyses

Of the 9,182 genes and EST sequences analyzed by the human microarray, an average of 6,400 (approximately 70%) had signal intensities greater than back-

Table 1. List of TaqMan primers used in the study

Gene	Primer[a]	Sequence 5′ → 3′	Amplified length, bp[b]	GenBank Accession No.
	S	GCT ACA GCT TCA CCA CCA C		
β-Actin	A	CTC GTA GCT CTT CTC CAG G	134	U07786
	P	TGG ACT TCG AGC AGG AGA T		
Muscle, half LIM domains	S	CAT CAC ATC TGG AGG AAT CA		
1 (SLIM1)	A	TGT AGC AAT CCA CGC AGT A	137	AJ275967
	P	TCT AAG AAG CTG GCT GGG		
	S	ACC AGG GAA GCT ATG AAG AC		
Myosin light chain 1	A	CCT CTT TCA TCT TTT CAC CTA G		
	P	CAT GGT GAC TGA ACT TCG T	123	X94689
	S	CAA CAT GAA GAG CGT CGT G		
Heat shock 70-kD protein 1	A	TGT GCT CAA ACT CGT CCT T	143	M69100
	P	AGA AGA AGG TGC TGG ACA A		
	S	CTC TTC ATC GAC TCT CTG TTG		
Lipoprotein lipase	A	GTT GCA ACG GTT CTT TCT G	114	X62984
	P	CTA CCG GTG CAA TTC AAA G		
	S	CTT TAT TGG CTT CTC GTG AG		
Sarcolipin	A	GTC AGA AGT GCT ATG GGG T	149	Z98820
	P	GCT GTC TTC TGT ATG GCC T		
	S	TCG ACA GCT ATG AGA AGA AC		
Phosphoglucomutase 1	A	TTA GGT GAT GAC AGT GGG TAG	116	AF091608
	P	CCT TAT TTC CAT CGC TCT G		
	S	TCC TTC CAT GAG TCC AGA GT		
Glycogen phosphorylase	A	CTA GGC TGT TGT GTG GAT TC	118	AJ507153
	P	TAG ATT GTG CTC AGG CCA G		

[a]S = sense; A = antisense; P = TaqMan probe.
[b]Including the sequences of S and A primers.

ground by greater than 2.5-fold in both Cy3 and Cy5 channels (i.e., both Duroc and Taoyuan subjects). Figure 1 shows scatter plots of the hybridization results from one of the three arrays, and Figure 2 shows representative fluorescence images with pseudo coloring. To compare the reproducibility of Cy3 and Cy5 and the variability across each array, we calculated correlation coefficients for each channel using the results from the three hybridizations. Precise high correlation coefficients ($r^2 > 0.95$) were obtained across all assays, indicating that the cross-species microarray hybridization data were highly reproducible.

Table 2. Comparison of characteristics of longissimus muscles excised from Duroc and Taoyuan neonatal pigs

	Duroc	Taoyuan
No. of pigs	6	6
BW, kg	1.41 ± 0.09	0.98 ± 0.15**
Length of LM, cm	18.4 ± 0.7	15.3 ± 1.0**
Mass of LM, g	21.3 ± 1.8	17.2 ± 0.9**
10th-rib LM area, cm^2	2.63 ± 0.17	2.42 ± 0.22
Area of LM fiber, μm^2	1,635 ± 105	1,688 ± 148
Fiber type, %		
Type I	7.0 ± 1.5	8.2 ± 1.6
Type IIA	8.5 ± 2.4	9.5 ± 2.2
Type IIB	84.5 ± 3.1	82.2 ± 3.2

**$P < 0.01$.

We calculated and plotted the SD versus average transcription of each transcribed gene over three arrays (Figure 3). Only 210 genes or EST (i.e., 3.3% of sequences that were transcribed in the samples) had an SD value greater than twice that of the population from the microarray data.

Among the 6,400 transcribed genes we detected in LM, the transcription of 117 (1.8%) differed ($P < 0.05$) between the two pig breeds. The transcription of 65 genes and eight EST was elevated by ≥1.75-fold in Duroc pigs compared with Taoyuan pigs. A similar comparison showed that the transcription of 24 Duroc genes and 20 EST decreased by ≥1.75-fold. To analyze the global gene transcription profiles of porcine LM, we classified the differentially transcribed genes into eight functional groups (Tables 3 and 4), using a previously established method (Ashburner et al., 2000) and information from the Gene Ontology Consortium web site (http://www.geneontology.org/).

The microarray data showed that Duroc LM had relatively elevated transcription of several genes encoding myofibrillar proteins, including the complex of myosin heavy and light chains, troponin I, and T1. Seven genes encoding ribosomal proteins (**RP**), including RP-L3, RP-L6, RP-L9, RP-L17, RP-L19, RP-S24, and mitochondrial RP-L3, and seven genes encoding heat shock proteins (**Hsp**), including the Hsp70 complex, Hsp60, and DnaJ-like HSP also were highly transcribed in Duroc LM compared with Taoyuan muscle.

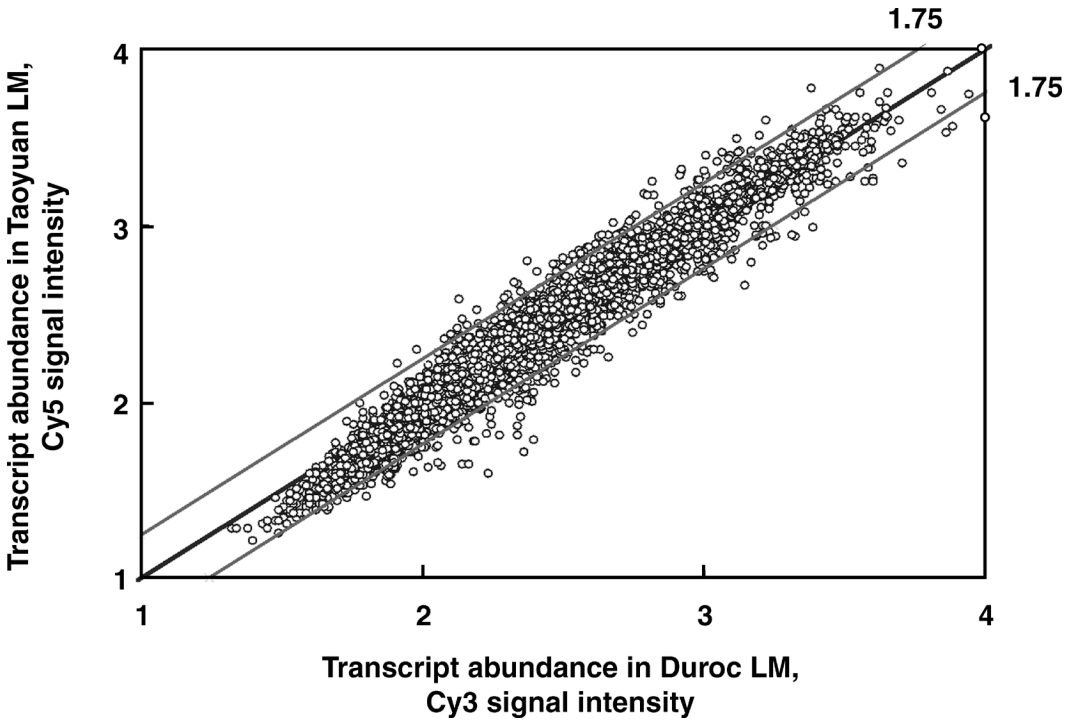

Figure 1. Scatter plots of fluorescence signal intensity from a competitive hybridization of muscular subjects on a human uniGEM V2 complementary DNA (cDNA) microarray. For each sequence, hybridization signal intensity is plotted logarithmically on the vertical axis for the Duroc LM (labeled with Cy3) and on the horizontal axis for the Taoyuan LM (labeled with Cy5). Relative differential transcription (Duroc/Taoyuan or Taoyuan/Duroc) is indicated from the line of unity. The majority of the transcription ratios are near the unity line. Points lying outside the 1.75 ratio diagonal lines indicate genes of potential importance for further study.

Fourteen genes encoding energy metabolic enzymes were differentially transcribed between Duroc and Taoyuan, of which seven had elevated transcription in Duroc muscle and seven had lower transcription. Among these differentially transcribed genes, the genes for sarcolipin (2.18-fold elevated transcription in Taoyuan pigs), phosphoglucomutase 1 (2.12-fold), and glycogen phosphorylase (1.95-fold) were selected for further analysis via real-time RT-PCR.

We also identified 11 genes involved in transcriptional regulation whose mRNA were significantly elevated in Duroc LM compared with Taoyuan. For instance, the relative mRNA abundance of SLIM1, high-mobility group protein 1 (**HMG1**), and high-mobility group protein A2 (**HMGA2**) in the Duroc pigs was 2.63-, 2.07-, and 1.95-fold compared with Taoyuan pigs, respectively.

Verification of Differentially Transcribed Genes by Quantitative Real-Time RT-PCR

To validate our cDNA microarray approach for identifying differentially transcribed genes, we performed quantitative real time RT-PCR (TaqMan) on the eight selected genes shown in Table 1. These genes were selected for three reasons: 1) their transcription level differed significantly between Duroc and Taoyuan, according to the microarray analysis; 2) their functions involve muscle maturation, myofiber switching or energy metabolism; and 3) their mRNA sequences were available in GenBank. Among the selected genes, four (SLIM1, myosin light chain 1, Hsp70, and lipoprotein lipase) were highly transcribed in Duroc LM, three (glycogen phosphorylase, phosphoglucomutase 1, and sarcolipin) were highly transcribed in Taoyuan pigs, and the β-actin gene was selected as a covariable. The relative transcription of these genes tended to be higher when measured by this technique, but the direction of the changes was consistent with that measured by the cDNA microarray (Figure 4). The transcription change of each selected gene achieved significance ($P < 0.05$ or 0.01). The quantitative real time RT-PCR results confirmed the array results for all selected genes, demonstrating that the microarray technique used in this study is accurate and reproducible.

SLIM1 Transcription in LD Muscles from Birth to 7 d of Age

The mRNA of LM was isolated from male Duroc and Taoyuan pigs at birth and at 7 d of age to evaluate SLIM1 gene transcription by quantitative real time RT-PCR. The transcription of SLIM1 in both neonatal and 7-d-old Duroc pigs was elevated compared with Taoyuan pigs ($P < 0.01$). Furthermore, in Duroc LM, postnatal SLIM1 transcription increased ($P < 0.05$) from birth

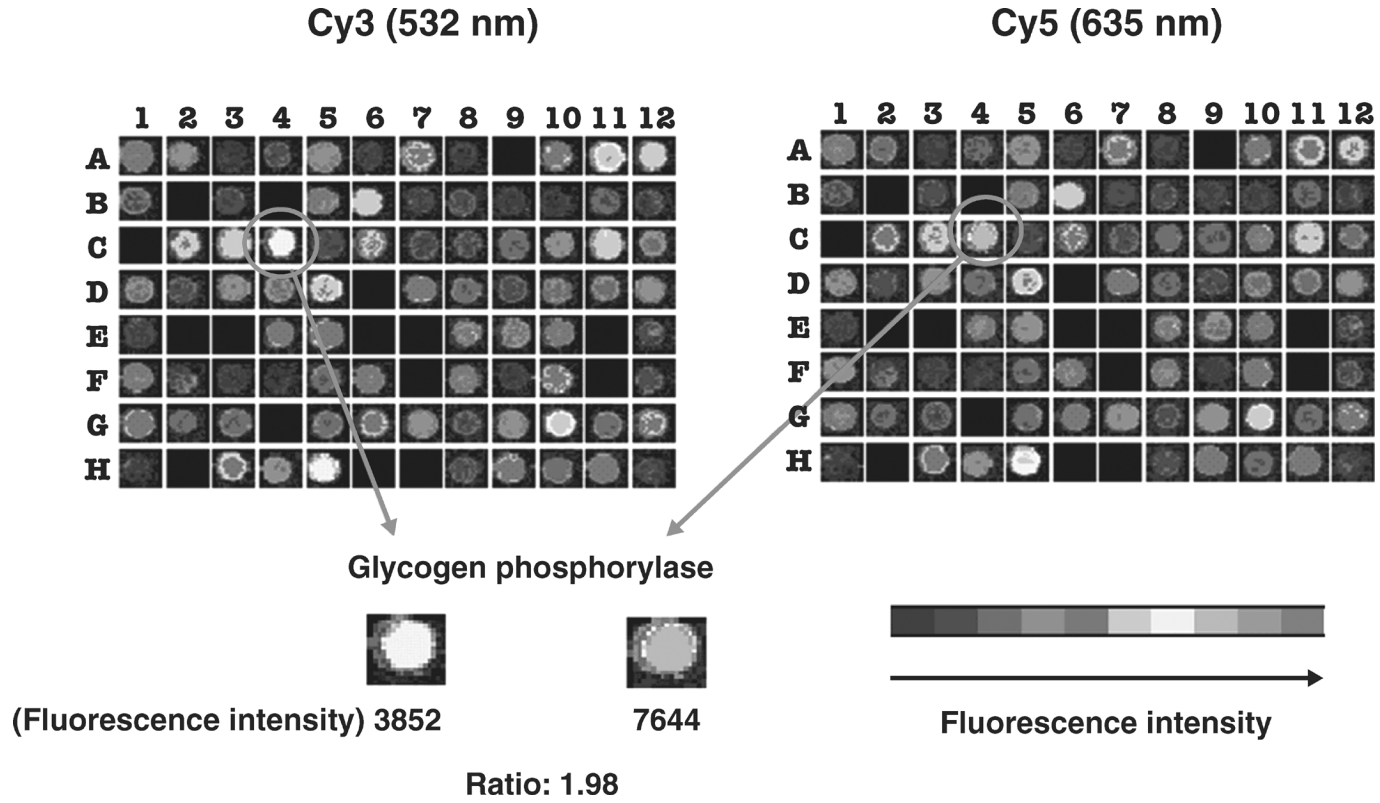

Figure 2. Illustration of representative raw images of differentially transcribed genes from one of the three arrays studied. Partially scanned images for Cy3 (532 nm) and Cy5 channel (635 nm) are shown. One gene, muscular glycogen phosphorylase, is transcribed at different ($P < 0.01$) levels between Duroc and Taoyuan LM according to the fluorescence intensity from the hybridization analysis.

to 7 d of age. By contrast, SLIM1 transcription in Taoyuan LM remained relatively constant during this period (Figure 5).

Discussion

Several studies have used cDNA microarrays to gain a greater understanding of the changes in gene transcription in skeletal muscle that accompanies aging, differences in muscle fiber type, or physical activity level (reviewed in Reecy et al., 2003). The present study represents one of the first applications of cDNA microarrays to evaluate differences in the global transcriptome in skeletal muscle between pig breeds. Yao et al. (2002) generated a cDNA library for porcine skeletal muscle that contains 782 nonredundant EST. Unfortunately, such a library represents only a small percentage of the genes transcribed in skeletal muscle. Bai et al. (2003) recently developed a porcine skeletal muscle microarray containing 5,500 EST, but only 10% of the clones have been identified by DNA sequencing. Complementary DNA microarrays are more useful for profiling gene transcription in porcine skeletal muscle for studying the phenotypic determination of pigs. Although resources are being developed that will facilitate production of microarrays for pigs (Yao et al., 2002; Bai et al., 2003), these microarrays may not be available in the near future or meet the needs of all researchers.

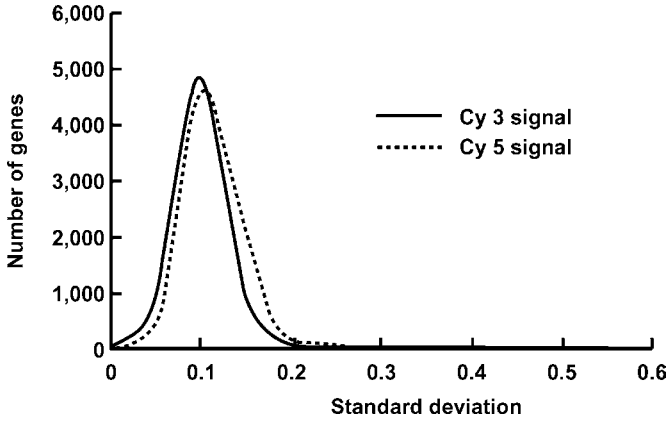

Figure 3. The distribution of standard deviations for each gene calculated from the median-normalized and log-transformed data of three microarrays.

Human microarrays have been used successfully in heterologous systems using RNA from pigs (Moody et al., 2002; Gladney et al., 2004) and cattle (Dalbiés-Tran and Mermilhod, 2003; Sudre et al., 2003), and hybridization conditions must be modified to retain cross-species hybridization. For example, in our study the tem-

Table 3. List of genes more highly transcribed in the skeletal muscle of Duroc (D) than those in Taoyuan (T) pigs[a]

Gene name	D/T transcript abundance, fold difference	Accession No.
Cytoskeleton-related protein		
Myosin, light polypeptide 2, regulatory	3.07 ± 0.15	S69022
Troponin I, skeletal, slow	2.57 ± 0.10	NM_003281
Myosin, heavy polypeptide 7	2.52 ± 0.36	M57965
Myosin, light polypeptide 1	2.38 ± 0.09	M31211
Crystallin, alpha B	2.22 ± 0.11	S45630
Troponin T1, skeletal, slow	2.07 ± 0.16	S69208
Myosin, light polypeptide 3, skeletal, slow	1.93 ± 0.33	NM_000258
Transgelin	1.91 ± 0.20	AF013711
Myosin, heavy polypeptide 11	1.89 ± 0.22	AB020673
Myosin, heavy polypeptide 9	1.84 ± 0.12	Z82215
Myosin, light polypeptide 6	1.83 ± 0.13	M22918
Cell matrix protein		
Cyclophilin B	1.96 ± 0.14	BE386706
Acidic protein rich in leucines	1.83 ± 0.30	AI887908
Collagen, type IV, α1	1.79 ± 0.37	M26576
Membrane, receptor, and transport proteins		
Ras-GTPase-activating protein SH3-domain-binding protein	2.32 ± 0.26	U32519
Annexin A2	1.96 ± 0.40	BE293414
Short form of βII spectrin	1.95 ± 0.10	AW028717
Kell blood group precursor	1.94 ± 0.33	Z32684
ATP synthase, H^+ transporting	1.94 ± 0.26	BE383477
KIAA0598	1.90 ± 0.32	AB011170
ATP synthase, H^+ transporting	1.79 ± 0.08	AI138629
Protein catabolism		
Ribosomal protein L17	2.30 ± 0.36	X53777
Ribosomal protein S24	2.12 ± 0.11	AA564880
Protease, serine, 15, ATP binding/nucleotide binding	1.96 ± 0.30	D88674
Ribosomal protein L9	1.89 ± 0.19	AA602007
Proteasome subunit, beta, 3	1.88 ± 0.32	AI028114
Ribosomal protein, mitochondrial, L3	1.86 ± 0.10	AA676597
Ribosomal protein L3	1.83 ± 0.32	BE297658
Ribosomal protein L6	1.81 ± 0.12	AW675430
Ribosomal protein L19	1.78 ± 0.08	AA599084
Transcription-related protein		
Four and a half LIM domains 1, muscle	2.63 ± 0.27	AA725097
Zinc finger protein 267	2.24 ± 0.13	X78925
TTK protein kinase	2.08 ± 0.13	AI973225
High-mobility group protein 1	2.07 ± 0.10	BE266776
Zinc finger protein 184 (Kruppel-like)	2.02 ± 0.16	AL021918
Transcription factor 17	1.96 ± 0.25	D89928
High-mobility group protein 2	1.95 ± 0.19	BE252235
Splicing factor proline/glutamine rich	1.92 ± 0.11	X70944
Choline kinase-like	1.91 ± 0.16	AB029886
Zinc finger protein 7	1.87 ± 0.37	NM_003416
Calreticulin	1.82 ± 0.13	M84739
Nucleolar phosphoprotein p130	1.79 ± 0.18	NM_004741
Metabolism		
Electron-transferring-flavoprotein dehydrogenase	2.19 ± 0.14	S69232
Coenzyme A hydratase	2.06 ± 0.20	BE295827
Lipoprotein lipase	2.03 ± 0.11	NM_000237
Malate dehydrogenase 1, NAD	2.03 ± 0.08	AA021037
Aconitase 2, mitochondrial	1.99 ± 0.26	AL023553
Electron-transfer-flavoprotein, alpha polypeptide	1.93 ± 0.16	W19485
NADH dehydrogenase	1.91 ± 0.13	BE311640
Stress protein		
Heat shock 70-kD protein 5	2.63 ± 0.29	AL043206
Heat shock 70-kD protein 6	2.54 ± 0.39	X51757
Heat shock 70-kD protein 10	2.33 ± 0.41	AW249010
Heat shock 70-kD protein 1	2.32 ± 0.24	M59828
Heat shock 60-kD protein 1	2.06 ± 0.37	AJ250915
Heat shock protein, DNAJ-like 2	2.03 ± 0.27	NM_001539
DnaJ-like heat shock protein 40	2.01 ± 0.32	NM_007034
Others and unknown		
Vitamin D binding protein	2.07 ± 0.27	S67527
Adenine nucleotide translocator 2	1.85 ± 0.31	AW163616
Monooxygenase activation protein	1.82 ± 0.20	BE315169
CTG-B43a	1.82 ± 0.33	L10378
Tumor rejection antigen (gp96) 1	1.81 ± 0.26	AW905119
FK506-binding protein 9	1.81 ± 0.19	AF089745
Ewing sarcoma breakpoint region 1	1.77 ± 0.22	NM_005243

[a]Data are means ± SEM of three independent Taoyuan/Duroc determinations of microarray analysis. Fold changes in the LM of Duroc are related to the LM of Taoyuan. The identifier denoted the GenBank Accession No. Genes with different ($P < 0.05$) mRNA abundance representing fold changes ≥1.75 were listed in this table, but not expressed sequence tags.

perature of the array washes was set at 42°C, a stringency that was decreased from the recommended 50 to 55°C to preserve heterologous hybridization in the cDNA microarray, as reported previously (Moody et al., 2002; Sudre et al., 2003). Moreover, to increase the sensitivity of hybridization, we used mRNA instead of total RNA, and a total of 500 ng of mRNA was used to prepare Cy3- or Cy5-labeled cDNA. Compared with the more common experiments involving homologous cDNA microarrays, heterologous systems present additional limitations. Cross-species hybridization is expected to yield decreased sensitivity, higher background, and a higher false-positive rate (Gladney et al., 2004). In our array results, out of 9,182 spotted cDNA, a total of 550 and 150 false positives would be expected for a *P*-value of 0.05 and 0.01, respectively; however, we did not evaluate the effects of changes on false positive rate under different hybridization and washing conditions. Thus, our results should be interpreted with caution, and future confirmation of individual transcription differ-

Table 4. List of genes more highly transcribed in the skeletal muscle of Taoyuan (T) than those in Duroc (D) pigs[a]

Gene name	T/D transcript abundance, fold difference	Accession No.	Gene name	T/D transcript abundance, fold difference	Accession No.
Cytoskeleton-related protein			Metabolism		
Myosin-binding protein C, fast-type	2.37 ± 0.13	X73113	Enolase 3 (beta, muscle)	2.34 ± 0.22	X56832
			Pancreatic lipase-related protein	2.26 ± 0.32	M93283
Cell matrix protein			Phosphoglucomutase 1	2.12 ± 0.11	NM_002633
Parvalbumin	3.15 ± 0.35	AI022812	Enolase 2 (gamma, neuronal)	2.10 ± 0.10	M22349
T54 protein	1.80 ± 0.24	AW250209	Glycogen phosphorylase	1.95 ± 0.13	U94777
Membrane, receptor, and transport proteins			Protein phosphatase 1, regulatory subunit 1A	1.82 ± 0.09	U48707
Sarcolipin	2.18 ± 0.12	U96094	Carboxyl ester lipase	1.76 ± 0.07	H97908
T-cell receptor, alpha	1.84 ± 0.28	X64643			
Solute carrier family 22	1.80 ± 0.08	NM_007105	Others and unknown		
			Cryptochrome 1	2.62 ± 0.25	D83702
Transcription-related protein			Lymphocyte antigen 75	2.07 ± 0.28	AF011333
Proline-rich protein with nuclear targeting signal	2.03 ± 0.13	BE169071	CD44 antigen	1.90 ± 0.09	X55150
Transcription factor IIH, polypeptide 2	2.00 ± 0.08	AW148977	Neuro-d4 (rat) homolog	1.90 ± 0.30	NM_004647
GRO1 oncogene	1.95 ± 0.13	NM_001511	IT1 mRNA	1.81 ± 0.29	AF040964
Ring finger protein 3	1.83 ± 0.07	AA403225	KIAA0414 protein	1.81 ± 0.34	AB007874

[a]Data are means ± SEM of three independent Taoyuan/Duroc determinations of microarray analysis. Fold changes in the LM of Taoyuan are related to the LM of Duroc. The identifier denoted the GenBank Accession No. Genes with different ($P < 0.05$) mRNA abundance representing fold changes ≥1.75 were listed in this table, but not expressed sequence tags.

ences is required to differentiate between results caused by genetic differences between breeds and variations among individuals.

For each experimental treatment applied in studies using high-density microarrays, multiple arrays yield reliable gene transcription data (Piétu et al., 1996,

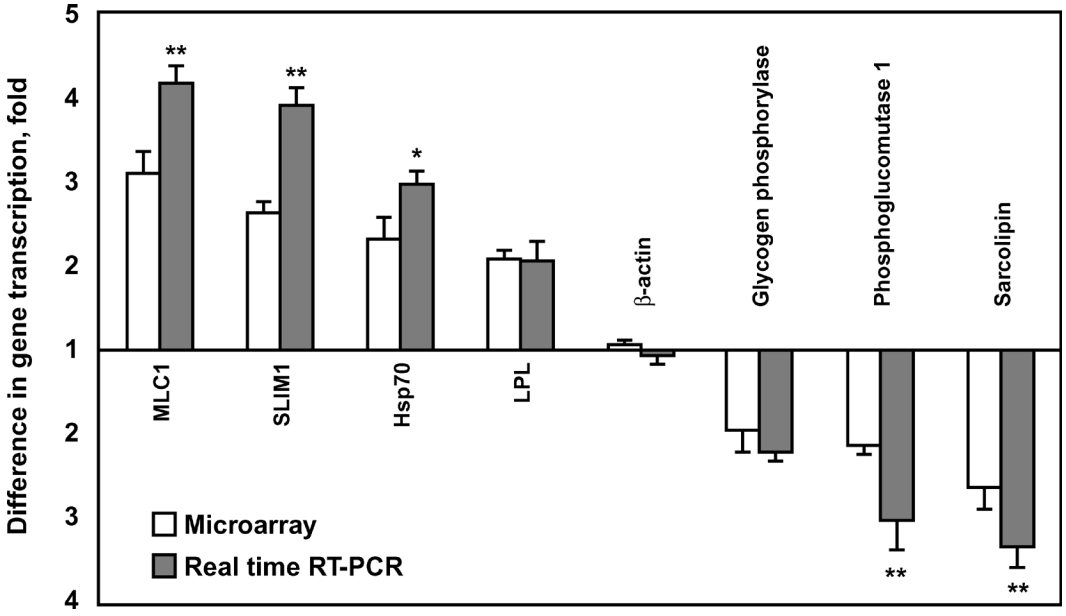

Figure 4. Comparison of differential mRNA abundance in the skeletal muscle of Taoyuan and Duroc pigs using complementary DNA (cDNA) microarrays (n = 3) and real-time reverse-transcriptase (RT) PCR (n = 3). Relative of the fold-change in gene transcription (Duroc/Taoyuan in the upper panel and Taoyuan/Duroc in the lower panel) were determined for the indicated genes by cDNA microarray and by quantitative real time RT-PCR. For the quantitative real time RT-PCR analysis, β-actin gene transcription was used as an internal control. MLC1 = myosin light chain 1, SLIM1 = skeletal muscle LIM protein 1, heat shock 70-kD protein = Hsp70, and LPL = lipoprotein lipase. Values are means ± SEM. *$P < 0.05$; **$P < 0.01$ vs. cDNA microarray.

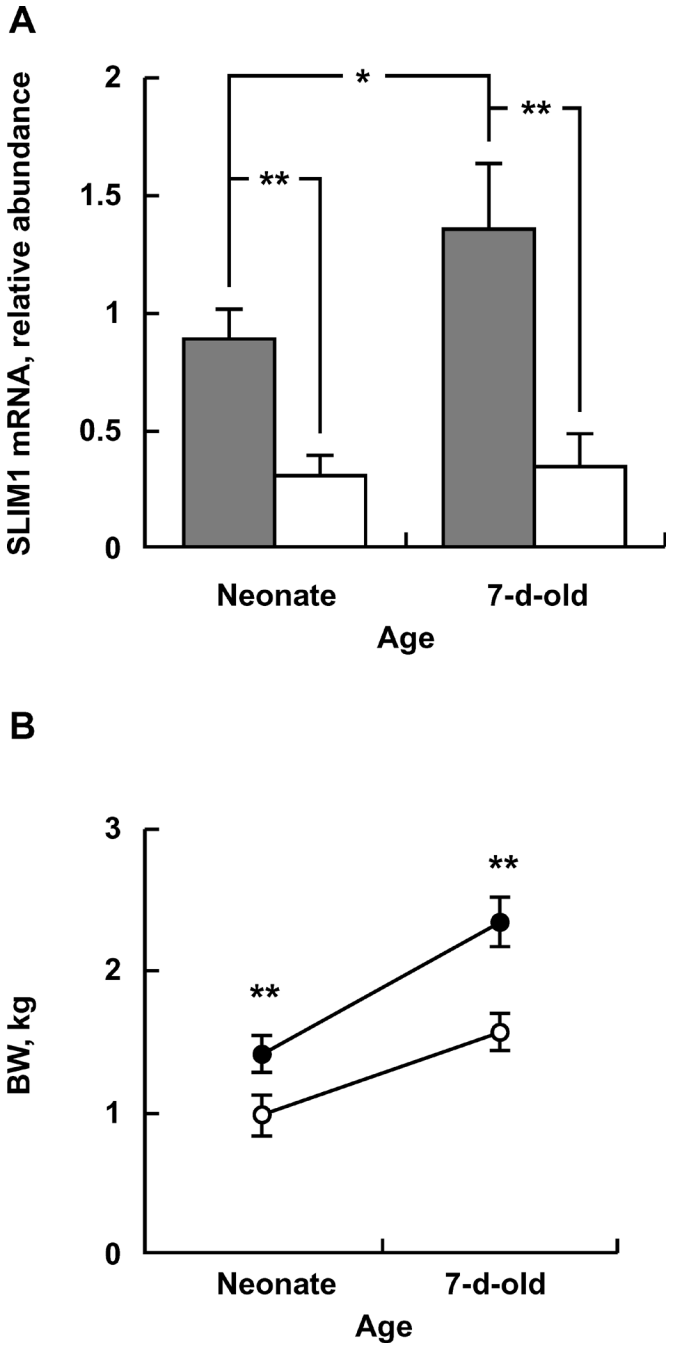

Figure 5. The gene transcription of skeletal muscle LIM protein 1 (SLIM1) in LM of neonatal and 7-d-old Duroc (shaded bars) and Taoyuan (open bars) pigs (A), and the corresponding BW (B; open bars = Duroc, and open circles = Taoyuan). Data are means ± SEM, where n = 3 for each value. *$P < 0.05$; **$P < 0.01$.

1999; Lee et al., 2000). Accordingly, we performed three pairwise comparisons of gene transcription profiles in LM between Taoyuan and Duroc pigs. The results combined the correlation coefficients from three competitive hybridizations, and real-time RT-PCR showed that our array data were highly reproducible for the majority of the genes tested. In addition, approximately 70% of genes from pig muscle were detected in this cross-species hybridization. This proportion compares reasonably well with previous reports involving same-species hybridizations in human or mouse Affymetrix or glass microarrays (Dempsey et al., 2001; Gu et al., 2002; Saito-Hisaminato et al., 2002). Moreover, the estimated proportion of transcribed genes in pig LM in this study was more than that achieved with cross-species hybridization of pig mRNA to a human nylon microarray (Moody et al., 2002). We speculate that more transcribed genes could be detected in the LM in the present study due to the low background noise of the fluorescence signals, which is a positive attribute of glass arrays (Cheung et al., 1999).

Neonatal pigs were chosen for our array study because neonates deposit protein at very high rates and efficiently utilize dietary AA for protein deposition in skeletal muscle compared with older animals (Davis et al., 2002a). Additionally, neonatal pig skeletal muscle is highly sensitive to circulating IGF-I and insulin, which act on cellular signaling components to stimulate protein synthesis (Brunetti et al., 2001; Davis et al., 2002b). The HMG1 and HMGA2 bind to the insulin response element within the IGFBP-1 promoter and regulate the transcription of this gene. The HMGI also is required for proper transcription of insulin receptor gene (Brunetti et al., 2001). Hence, by regulating the IGF and/or their signaling pathway, both HMG1 and HMGA2 are involved in muscle growth regulation (Powell et al., 1995; Brants et al., 2004). We confirmed that two genes, HMG1 and HMGA2, are more highly transcribed in neonatal LM of Duroc compared with Taoyuan. The result may indicate that the transcription level of HMG1 and/or HMGA2 represent the candidate marker in pig, which has a relationship with skeletal muscle growth.

In the present study, we undertook a global assessment of the genes whose transcription level in LM differs between Duroc and Taoyuan neonatal pigs. The majority of the highly transcribed genes in Duroc, including myosin light and heavy chains, as well as troponin, are involved in myofiber synthesis. Mathialagan et al. (2002) demonstrated that the genes encoding troponin and tropomyosin are upregulated in the skeletal muscle of a high-lean group of pigs compared with a low-lean group. These results suggest that the transcribed level of these myofibrillar protein-encoding genes correlates positively with the muscle growth potential of pigs. Our results confirm that postnatal Duroc pigs are markedly more efficient with respect to muscle growth than Taoyuan pigs (Baile et al., 1983; Cheng et al., 2000).

Besides the genes encoding myofibrillar proteins, some of the known glycolytic metabolism genes were highly transcribed in Duroc LD compared with Taoyuan. The genes encode enzymes such as malate dehydrogenase 1, electron-transferring-flavoprotein dehydrogenase, NADH dehydrogenase, and ATP synthases (H^+ transporting, mitochondrial F_1 or F_0). These differences indicate that Duroc LM rely more on glycolytic

metabolism, using more carbohydrates and less lipids as fuel, relative to Taoyuan muscle. Our results support the hypothesis that the intensive selection for lean muscle growth in modern pigs has, over time, induced a shift in muscle metabolism toward a more glycolytic and less oxidative fiber type (Lefaucheur et al., 2004). Moreover, our results also demonstrate that glycogen phosphorylase and phosphoglucomutase 1 are downregulated in Duroc muscle (Figure 4), implying that the Duroc LM must have relatively higher glycogen content. Glycogen enrichment in Duroc skeletal muscle supports the above proposal that these pigs carry more glycolytic-type muscle fibers. The muscle metabolic profile is a determinant for meat quality in meat-producing animals. Glycolytic-type fibers generally contain less intramuscular fat and are implicated in meat aging after slaughter, yielding meat with greater tenderness (Hocquette et al., 1998).

Duroc LM also transcribed at greater levels of a few genes encoding cellular growth factors and proteins associated with muscle hypertrophy or dystrophy (Haslett and Kunkel, 2002) compared with Taoyuan. Campbell et al. (2001) reported several genes that are differentially transcribed in white muscle compared with red muscle. This phenomenon was not observed in our study because the composition of Duroc and Taoyuan LM types is similar. Myogenic factors, such as myogenin and MyoD, are important for regulating genes related to skeletal muscle growth and metabolism (Cooper et al., 1999; Yablonka-Reuveni and Paterson, 2001); however, we found that these genes are transcribed at similar levels in the LM of both pig breeds. This result is consistent with our previous report in which a myogenic factor, myostatin, was transcribed similarly in the skeletal muscle of neonatal Duroc, Landrace, Taoyuan, and Small-ear pigs (Lin et al., 2002).

A number of RP genes, including RP-L4, RP-L9, RP-L10a, RP-L15, RP-L29, RP-L37a, RP-L42, RP-S7, and RP-S20, are rapidly and significantly downregulated from prenatal d 75 to postnatal d 7 in pigs (Zhao et al., 2003). Several results have shown that the levels of most RP are lower in differentiated cells compared with undifferentiated cells (Agrawal and Bowman, 1987). The phenomenon of decreased transcription of muscular RP during muscle development may be associated with decreasing protein utilization and deposition in skeletal muscle during the neonatal period. Seven genes encoding RP, including RP-L3, RP-L6, RP-L9, RP-L17, RP-L19, RP-S24, and mitochondrial L3, were highly transcribed in Duroc LM compared with Taoyuan (Table 3). The high transcription levels of RP found in Duroc neonatal pigs indicate that myoblast differentiation in pigs is potentially programmed during early postnatal growth. We suggest that the high ribosome concentration may be associated with the rapid rate of protein synthesis in LM of neonatal Duroc.

The SLIM1 contains half LIM domains and is highly transcribed in skeletal muscle of Duroc pigs compared with Taoyuan (Figures 4 and 5). Elevated SLIM1 mRNA abundance during postnatal skeletal muscle growth suggests an important role for this protein during the early stages of skeletal muscle differentiation, specifically in $\alpha_5\beta_1$-integrin-mediated signaling pathways (McGrath et al., 2003). Over-expression of muscle LIM protein promotes myogenesis by enhancing the activity of the muscle-specific transcription factor, MyoD (Arber et al., 1994; Kong et al., 1997). Elevated SLIM1 mRNA abundance has been associated with postnatal skeletal muscle growth (Morgan et al., 1995; Loughna et al., 2000). Sudre et al. (2003) also have reported that transcription of the gene encoding LIM-related protein in bovine skeletal muscle is higher in the adult compared with 260 d after conception. The high levels of SLIM1 mRNA in postnatal skeletal muscle of all species examined suggest a significant role for the protein synthesis in muscle growth (Morgan et al., 1995; Morgan and Madgwick, 1999). The function of SLIM1 during skeletal muscle differentiation remains unclear; however, these observations suggest a link between increased SLIM1 transcription and skeletal muscle growth.

Heat shock proteins (Hsp70, Hsp60, and DnaJ-like proteins), which are molecular chaperones and indicators of cellular stress (Liu and Steinacker, 2001), were highly transcribed in Duroc LM. It has been reported that Hsp70 contributes to the remodeling response of skeletal muscle tissue, including muscle regeneration and contraction (Neufer et al., 1996; Duguez et al., 2003). The Hsp genes are upregulated during human muscle hypertrophy and muscle type switching (Locke et al., 1994; Carson et al., 2002), as well as in glycolytic-type muscles of adult animals (Bai et al., 2003). In our study, seven genes encoding Hsp70 complex, Hsp60, and DnaJ-like HSP, were highly transcribed in Duroc LM compared with Taoyuan muscle; however, it is unclear whether the high transcription of Hsp genes is related to postnatal muscle growth.

We have used cDNA microarray assays to identify genes that are differentially transcribed between Taoyuan and Duroc pigs. Several genes were selected for real-time RT-PCR analysis to further confirm the differential transcription indicated by the microarray analysis. The identification of these genes should help to determine the mechanisms that regulate skeletal muscle growth, and candidate genes may be used to develop genetic or serum markers associated with postnatal growth potential in pigs.

Implications

We used cross-species microarray hybridization to differentiate skeletal muscle gene transcription between European (Duroc) and Asian (Taoyuan) pigs. Several genes in the skeletal muscle of Taoyuan and Duroc neonatal pigs displayed significantly different transcription for a complex trait, muscle growth. A combination of microarray and real-time polymerase chain reaction results indicated that differential transcription of

those genes is caused mainly by biological differences rather than animal variability. These genes, including those encoding the complex of myofibrillar proteins, ribosomal proteins, energy metabolic enzymes, and transcription regulators coding genes, showed greater messenger ribonucleic acid abundance in Duroc than in Taoyuan longissimus muscle. Proteins or enzymes encoded by these genes play important roles in skeletal myocytes growth and metabolism, suggesting that the differences in transcription relate to the genetic architecture underlying muscle growth potential.

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