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Molecules and Cells

Transcriptional Alteration of p53 Related Processes As a Key Factor for Skeletal Muscle Characteristics in *Sus scrofa*

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The pig could be a useful model to characterize molecular aspects determining several delicate phenotypes because they have been bred for those characteristics. The Korean native pig (KNP) is a regional breed in Korea that was characterized by relatively high intramuscular fat content and reddish meat color compared to other western breeds such as Yorkshire (YS). YS grew faster and contained more lean muscle than KNP. We compared the KNP to Yorksire to find molecular clues determining muscle characteristics. The comparison of skeletal gene expression profiles between these two breeds showed molecular differences in muscle. We found 82 differentially expressed genes (DEGs) defined by fold change (more than 1.5 fold difference) and statistical significance (within 5% of false discovery rate). Functional analyses of these DEGs indicated up-regulation of most genes involved in cell cycle arrest, down-regulation of most genes involved in cellular differentiation and its inhibition, down-regulation of most genes encoding component of muscular-structural system, and up-regulation of most genes involved in diverse metabolism in KNP. Especially, DEGs in above-mentioned categories included a large number of genes encoding proteins directly or indirectly involved in p53 pathway. Our results indicated a possible role of p53 to determine muscle characteristics between these two breeds.

INTRODUCTION

The Korean native pig (KNP) is a popular breed for meat production in Korea. After western pig breeds such as Yorkshire (YS) and Berkshire were imported into Korea in 1900's, KNP had been random crossed with them to overcome its slow growth rate, low feed efficiency, and small litter size (Hwang et al., 2004; Kim et al., 2005a; 2005b). Recently, there has been a lot of effort to restore the original KNP based on morphological characteristics independently in Jeju island and Seonghwan on the mainland of Korea. The re-established KNP showed consistent molecular characteristics as a unique breed compared to other western breeds (Jeon et al., 2003; Kim et al., 2002; 2005a; 2005b).

There are clear differences in the character of KNP meat compared to western breeds such as YS. KNP meat color has a significantly higher redness and yellowness than that of YS meat (Kim et al., 2008). Meat color has been measured as one of important indicators for meat quality. Muscle-fat content and back-fat thickness were significantly higher in KNP, comparing to the YS breed (Kim et al., 2008). However, other factors such as water holding capacity (WHC) and pH were not significantly different between the two (Kim et al., 2008). Especially, due to these two breeds being selected for opposite reasons; fast and efficient growth for YS, and meat quality for KNP. We could hypothesize that the differences in muscle properties primarily would be due to the different rate of growth between these two breeds. Therefore comparing these two breeds could be useful in better understanding the molecular responses related to growth and muscle characteristics.

The p53 is a central molecule for the regulation of the cell cycle (Chumakov, 2007). It was first found as a 53 kD cellular protein that could associate with SV40 large T antigen, which controls its transforming effect in transformed mouse cells (Chang et al., 1979; Kress et al., 1979; Lane and Crawford, 1979; Linzer and Levine, 1979). Since then, the detailed pathways related to p53 have been accumulated and merged into two areas of biological consequences: cell cycle arrest and apoptosis. In addition, recent evidence showed that functional p53 is closely associated with cellular glucose metabolism through the regulation of several proteins such as TP53-induced glycolysis and apoptosis regulator (TIGAR) and phosphoglycerate mutase (PGM) (Bensaad and Vousden, 2007; Bensaad et al., 2006; Brand and Hermfisse, 1997). These results indicate potential role of p53 to link energy production to cell proliferation.

Previously, we determined fatty acid composition and gene expression profiles at backfat tissues from KNP and YS (Choi

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et al., 2008; Moon et al., 2009). The comparison of expression profiles revealed a possible relationship between arachidonic acid content and gene expression difference. In this study, we compared the gene expression profiles at *Longissimus dorsi* skeletal muscle between KNP and YS to find transcriptional evidence to explain the different muscle properties between the slow and fast grow breeds.

MATERIALS AND METHODS

Animals and tissue collection

Four male pigs of each KNP and YS breeds were used in this study. The pigs were raised under the same conditions. The details of conditions and feeding were fully described in the supplementary material of a previously published article (Moon et al., 2009). *Longissimus dorsi* muscle between the seventh and ninth ribs were collected and used for the microarray experiment. All tissue samples were quick frozen right after collection, and stored at -80°C until use.

RNA extraction, target preparation, and array hybridization

Three-five μg of total RNA was extracted from 0.2-1 g muslce using 2-6 ml TRIzo® Reagent (Invitrogen Inc, USA) by homogenization with Power Gen 125 S1 (Fisher Scientific, USA). RNA purity was measured around 1.5 as an A260/A280 ratio by UV spectrophotometer (Eppendorff AG, Germany). The integrity of total RNA was checked by electrophoresis on formaldehyde added 1% agarose gel. Ten μg of total RNA after treatment with DNase I (Invitrogen Inc., USA) were converted into double-stranded cDNAs using GeneChip® Expression 3′-Amplification One cycle cDNA synthesis kit, and cDNA was purified with the GeneChip® Sample Cleanup Module. Biotin labeled antisense cRNA was synthesized from the purified cDNA through in vitro transcription (IVT) using GeneChip® Expression 3′-Amplification Reagents for IVT Labeling kit. The cRNA was further purified and fragmented using the reagents in the GeneChip® Sample Cleanup Module, and the fragmented cRNA was hybridized onto the GeneChip® Porcine Genome array for 16 h. To remove extra cRNA fragments and non-specific hybridization, non-stringent (high salt) and stringent (low salt) washing steps were performed at GeneChip® Fluidics Station 450. Finally, the gene chip was stained with streptavidin-phycoerythrin (Invitrogen Inc., USA).

Image analysis and comparison

The microarray images were obtained by GeneChip® Scanner 3000 and analyzed by GeneChip® Operating Software 1.3 (GCOS, Affymetrix Inc., USA). Each transcript was detected by a probe set composed of 11 probe pairs, and each probe pair was consisted of a perfect match (PM) and a mismatch (MM) probe. The PM probe detects the matched RNA fragment from target mRNA, whereas MM probe estimates non-specific signals because the MM probe has a mismatch nucleotide at the middle position of the sequence of PM probe. Both the PM and MM signals were used to compute a discrimination score ([PM-MM]/[PM+MM]) to determine whether the transcript was detected as present (P) or absent (A) by the probe set. Weighted mean value of probe set using probe pairs was determined by one-step Tukey's biweight method. All signals from each array were normalized by global scaling. To compare the transcript differences between two arrays, the GCOS matches each probe pair and calculates 11 ratios in $\log_2$ scale. Again, the weighted mean difference value was determined from these 11 ratios by the one-step Tukey's biweight method. To implement this algorithm to compute the fold difference (KNP/YS ratio) between breeds, we conducted 16 pairs-wise comparisons between 4 KNP and 4 YS images. The data discussed in this study have been deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE18653.

Statistical analysis and selection of DEGs

The signals were transformed into log scale to stabilize data variation (Nadon and Shoemaker, 2002) in testing the statistical significance of differences between two breeds. Additionally, to remove array-to-array variation, all the array data were normalized by quantile normalization. The data was further corrected using the batch effect function in Partek software (Partek Incorporated, USA). The signals were compared by false discovery rate (FDR), and DEGs were defined when *P* values were within 5% FDR. The Affymetrix Porcine Annotation Revision 5 (http://www4.ncsu.edu/~stsai2/annotation/) was used for the annotation information of pig probes (Tsai et al., 2006).

Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

To synthesize single-stranded cDNA, 12.5 μg of total RNA was reverse-transcribed in a mixture of 2 μl of anchored oligo$(dT)_{20}$ primer (Invitrogen Inc., USA) and 2.5 μl of 200 U SuperScript II reverse transcriptase (Invitrogen Inc., USA). The cDNA reaction mixture was diluted 3 times. One μl of the diluted cDNA reaction mixture was used as the template for each qPCR reaction. qPCR was conducted using CyberGreen reagent in a rotary type real-time analyzer, Rotor-Gene 6000 (Corbett Research, Australia). All primer sequences were designed based on corresponding consensus expressed sequences extracted from NetAffx Analysis Center (http://www.affymetrix.com/analysis/index/affx) using PrimerQuest software (USA). To minimize technical error, at least three technical replicates of each qPCR were conducted. As a result of the qPCR, the C_t value for each sample was determined by averaging three technical replicates. The difference (ΔC_t) between average C_t values of KNP and YS breeds were used to calculate fold differences as $2^{\Delta Ct}$.

RESULTS

Array data overview and selection of DEGs

Using the high density porcine oligonucleotide microarray chip (23,935 probe sets implemented), 9,272 genes (38.7%) and 7,841 (32.8%) genes were detected as P in all 4 skeletal muscle samples from KNP and YS, respectively. More genes were able to be detected in samples from KNP than from YS, which was consistent in other tissues such as liver and backfat. This may indicate existence of genetic complexity in KNP and genetic simplification in YS to achieve a single goal, such as a fast growth rate by artificial selection. The 9,630 probe sets detected transcripts as P from all 4 muscle samples of at least one breed were defined these genes as the porcine skeletal transcriptome. To select DEGs from the porcine skeletal transcriptome, we used both significance level and fold difference. Eighty-two DEGs were found to satisfy both FDR (5%) and fold difference (> 1.5) (Supplementary Table 1). After a massive literature search to define the role of DEGs, the DEGs were found to fall into four major functional categories: p53-related process, signaling for cell proliferation/differentiation, muscular system and several metabolic processes.

Up-regulated DEGs indicated cell cycle arrest mediated by p53

Among DEGs, we found nine genes encoding proteins that

Table 1. DEGs that are classified in p53-related processes

FC	Probe set ID	Symbol	Gene title	Bit Score	E-value	*p* value
2.4	Ssc.30871.1.A1_at	TP53INP1	Tumor protein p53 inducible nuclear protein 1	93.7	2E-18	3.8E-04
1.7	Ssc.29750.1.A1_at	TP53INP1	Tumor protein p53 inducible nuclear protein 1	268.1	5.8E-71	6.4E-04
1.9	Ssc.9522.1.A1_at	RNF144B	RNF144B ring finger 144B	137.3	3E-31	8.5E-05
1.6	Ssc.4271.1.S1_at	HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	987.7	0	1.5E-04
1.6	Ssc.6154.1.S1_a_at	SLC6A6	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6	44.1	0.042	1.1E-03
1.6	Ssc.6154.1.S1_at	SLC6A6	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6	44.1	0.042	1.1E-04
1.6	Ssc.14009.1.A1_at	MED17	Mediator complex subunit 17	1895.6	0	2.2E-04
1.5	Ssc.5695.1.A1_at	CKS1B	CDC28 protein kinase regulatory subunit 1B	42.1	0.139	5.9E-05
1.5	Ssc.26274.1.S1_at	ETS1	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	40.1	0.473	2.8E-05
-2.1	Ssc.16937.1.A1_at	TP53INP2	Tumor protein p53 inducible nuclear protein 2	274.1	1.9E-72	1.5E-03
-2.0	Ssc.11281.1.A1_at	PENK	Proenkephalin	506.0	4E-142	3.8E-04

FC: fold change between KNP and YS
Probe set ID: identification number used in Affymetrix gene chip
Bit score and E value indicate similarity between pig and human orthologs (Tsai et al., 2006)

either directly or indirectly related to p53. Most of these genes were up-regulated in KNP. The up-regulated genes included *Tumor protein p53 inducible nuclear protein 1* (*TP53INP1*), *RNF144B ring finger 144B* (*RNF144B*), *HIV-1 Tat interactive protein 2, 30 kDa* (*HTATIP2*), *Solute carrier family 6* (*neurotransmitter transporter, taurine*), *member 6* (*SLC6A6*), *Mediator complex subunit 17* (*MED17*), *v-ets erythroblastosis virus E26 oncogene homolog 1* (*avian*) (*ETS1*) and *CDC28 protein kinase regulatory subunit 1B* (*CKS1B*). The *TP53INP1* gene encodes two stress-induced protein (SIP) isoforms. They activate p53 through physically interacting with homeodomain-interacting Protein Kinase-2 (HIPK2) in response to cellular stress, and the expression of *TP53INP1* depends on p53 (Tomasini et al., 2003). *RNF144B* encodes an E3 ubiquitin ligase, which is inducible by p53, and can target p21 for degradation (Huang et al., 2006). *HTATIP2* encodes a tumor suppressor protein that increases p53 expression, which eventually inhibits cell growth. Indeed, ectopic expression of *HTATIP2* induced the expression of p53 (Zhao et al., 2006). *SLC6A6* encodes a transporter protein that uptakes taurine, and the expression of *SLC6A6* is suppressed by p53 (Han et al., 2000). The protein encoded in *MED17* also is known to interact with p53 during transcriptional regulation (Mahajan et al., 2007; Malik and Roeder, 2000). *ETS1* encodes an Ets family transcription factor that is involved in several biological processes including development of hematopoietic cells, tumorigenesis, adaptive angiogenesis and p53 mediated apoptosis (Dittmer, 2003). p53 suppresses the expression of *ETS1* (Dittmer, 2003; Iotsova et al., 1996) and directly regulates the transcription of *TP53INP1* and *SLC6A6*. All of these DEGs in p53 pathway supported the overall suppression of cell proliferation in KNP comparing to YS breed.

In contrast, there were two down-regulated genes in KNP, *Tumor Protein p53 inducible nuclear protein 2* (*TP53INP2*) and *Proenkephalin* (*PENK*). Both *TP53INP1* (2.4 fold change) and *TP53INP2* (-2.1 fold change) were expressed in the opposite direction in KNP compared to YS. The function of *TP53INP2* gene has not been characterized, but its expression was very active during mouse embryogenesis (Bennetts et al., 2007). *TP53INP2* was also suggested as an oncogene for bladder cancer (Guo et al., 2004). *PENK* has been found to be involved in stress-induced apoptosis. PENK physically interacts with p53 to suppress the expression of *Microtubule-associated protein 4* (*MAP-4*) gene that encodes an anti-apoptotic protein (McTavish et al., 2007; Murphy et al., 1996).

Other DEGs for cell proliferation and differentiation

We found thirteen genes encoding proteins involved in the signaling for cell proliferation and differentiation (Table 2). Among them, four genes were up-regulated and nine genes were down-regulated in KNP. The up-regulated genes included *NMDA receptor regulated 2 (NARG2), Insulin-like growth factor 1 receptor (IGF1R), Adrenergic, beta, receptor kinase 2 (ADRBK2)*, and *Makorin, ring finger protein, 1 (MKRN1). NARG2* is transcriptionally active in dividing and immature cell types, and inactive in terminally differentiated cell types (Sugiura et al., 2004). *IGF1R* gene encodes an IGF1 receptor that transmits IGF signaling through phosphatidylinositol 3-kinase (PI3K)-AKT and/or extracellular-signal regulated kinases (ERKs), which eventually regulate cell proliferation, survival and growth (Casa et al., 2008; Chitnis et al., 2008; Kim and Lee, 2009). *ADRBK2* encodes a protein that acts on desensitizing agonist-dependent G-protein coupled receptors (GPCRs) such as β-adrenergic receptors, lysophosphatidic acid (LPA) receptors and thrombin receptors (Daaka et al., 1997). These receptors commonly mediate signaling that can promote cell proliferation (Lynch and Ryall, 2008; Moolenaar, 1995; Walsh et al., 2008). *MKRN1* encodes a RING zinc finger protein that acts as a repressor of the proto-oncogene c-Jun transcriptional activity (Omwancha et al., 2006).

Nine genes in this category were down-regulated in KNP (Table 2). Most of these genes participate in cell proliferation and differentiation. *cAMP responsive element modulator* (*CREM*) encodes a basic leucine zipper protein that controls differentiation. *Integrin beta 1 binding protein 3* (*ITGB1BP3*) encodes a protein that blocks the progression of terminal myogenic differentiation (Li et al., 2003). *Transforming growth factor, beta 2* (*TGFB2*) encodes a protein that promotes proliferation and delays differentiation of C2C12 myoblasts (Schabort et al., 2009). *Nephroblastoma overexpressed* (*NOV*) gene encodes a secreted matrix-associated protein that inhibits myogenic differ-

Table 2. Cell proliferation & differentiation signaling DEGs

FC	Probe set ID	Symbol	Gene title	Bit score	E-value	*p* value
1.9	Ssc.9883.1.A1_at	ADRBK2	Adrenergic, beta, receptor kinase 2	46.1	0.013	4.2E-04
1.7	Ssc.20175.1.S1_at	IGF1R	Insulin-like growth factor 1 receptor	450.5	3E-125	1.4E-05
1.7	Ssc.6849.1.A1_at	NARG2	NMDA receptor regulated 2	63.9	6.1E-09	1.2E-03
1.6	Ssc.11539.1.A1_at	MKRN1	Makorin, ring finger protein, 1	151.2	1.1E-35	5.5E-04
-4.5	Ssc.2841.1.S1_at	CREM	cAMP responsive element modulator	521.9	9E-147	1.9E-04
-3.7	Ssc.2841.2.S1_a_at	CREM	cAMP responsive element modulator	521.9	6E-147	8.5E-06
-4.2	Ssc.18764.1.A1_at	ITGB1BP3	Integrin beta 1 binding protein 3	654.7	0	7.1E-07
-2.0	Ssc.92.1.S1_at	TGFB2	transforming growth factor, beta 2	1790.6	0	6.0E-04
-2.0	Ssc.16434.1.A1_at	NOV	Nephroblastoma overexpressed gene	210.6	4.8E-53	4.2E-04
-1.9	Ssc.17238.1.A1_at	MUSTN1	Musculoskeletal, embryonic nuclear protein 1	280.0	2E-74	9.5E-05
-1.9	Ssc.20906.1.A1_at	TAOK1	TAO kinase 1	63.9	9.1E-09	1.7E-04
-1.8	Ssc.10952.1.S1_at	DLG1	Discs, large homolog 1 (Drosophila)	44.1	0.016	4.5E-04
-1.8	Ssc.16910.2.S1_at	ROD1	ROD1 regulator of differentiation 1 (*S. pombe*)	2698.5	0	5.6E-04
-1.5	Ssc.9766.2.S1_at	AK2	Adenylate kinase 2	1094.8	0	1.2E-03

Table 3. Muscular-structural system DEGs

FC	Probe set ID	Symbol	Gene title	Bit score	E-value	*p* value
1.7	Ssc.1290.1.S1_at	MYLK	Myosin, light chain kinase	44.1	0.026	5.2E-04
1.7	Ssc.13859.1.A1_at	UNC45B	Unc-45 homolog B (*C. elegans*)	176.9	2.1E-43	7.2E-05
-7.2	Ssc.761.1.S1_at	NR4A3	Nuclear receptor subfamily 4, group A, member 3	1854.0	0	2.6E-04
-2.8	Ssc.1876.1.S1_at	MYH10	Myosin, heavy chain 10, non-muscle	807.3	0	1.8E-03
-2.1	Ssc.10392.1.A1_at	MYH11	Myosin, heavy chain 11, smooth muscle	708.2	0	1.3E-03
-1.9	Ssc.1664.1.A1_at	NRN1	Neuritin 1	1370.3	0	1.1E-04
-1.7	Ssc.8574.1.A1_at	TMOD1	Tropomodulin 1	561.5	2E-158	1.5E-04

entiation (Calhabeu et al., 2006). *Musculoskeletal, embryonic nuclear protein 1* (*MUSTN1*) encodes a specific differentiation marker in musculoskeletal system, which is co-regulated with other common myogenic marker genes including MyoD and myogenin (Liu et al., 2007). *TAO kinase 1* (*TAOK1*) encodes a kinase which activity is elevated in mitosis, and known to be required for check point signaling and for proper chromosome segregation (Draviam et al., 2007). *Discs, large homolog 1* (*Drosophila*) (*DLG1*) is known as a tumor suppressor gene. DLG1 protein can be phosphorylated by CDK1 and CDK2, and shows cell cycle-dependent localization (Caruana and Bernstein, 2001; Iizuka-Kogo et al., 2007; Narayan et al., 2009). *ROD1 regulator of differentiation 1* (*S. pombe*) (*ROD1*) is a mammalian homologue of fission yeast differentiation regulator Nrd1 that blocks differentiation (Yamamoto et al., 1999). *Adenylate kinase 2* (*AK2*) is involved in cell motility, differentiation and transduction of mechanoelectrical signal (Dzeja and Terzic, 2009). Adenylate kinase activity has been correlated with muscle exercise performance, which suggests its importance for maintaining cellular energetic homeostasis (Linossier et al., 1997). *AK2* gene is located in a quantitative trait locus for body weight and abdominal fat weight (Aksu et al., 2007).

DEGs responsible for muscular structure

There were seven DEGs related to muscular structure (Table 3). Among them, five genes were down-regulated in KNP including *Myosin, heavy chain 10, non-muscle* (*MYH10*), *Myosin, heavy chain 11, smooth muscle* (*MYH11*), *Nuclear receptor subfamily 4, group A, member 3* (*NR4A3*), *Neuritin 1* (*NRN1*) and *Tropomodulin 1* (*TMOD1*). MYH10 was known as a non-sarcomeric MYH, but it was found at the Z-line of sarcomeres (Takeda et al., 2000). Although *MYH11* is known to encode a smooth muscle component, its transcript was consistently detected in all skeletal muscle samples. *TMOD1* encodes an actin-capping protein that prevents actin assembly and disassembly (Fischer and Fowler, 2003; Weber et al., 1994). *NR4A3* might be required for the remodeling of actin filaments (Qi et al., 2004). *NRN1* encodes a protein that plays a role in neurite outgrowth (Nedivi et al., 1998). *NRN1* expression is markedly increased in regenerating muscle (Seale et al., 2004).

On the other hand, there were two up-regulated genes in KNP (Table 3). *Unc-45 homolog B* (*C. elegans*) (*UNC45B*) encodes a protein that forms a complex with Hsp90. The UNC45B-Hsp90 complex specifically associates with unfolded myosin motor domain to induce its folding (Srikakulam et al., 2008). *Myosin, light chain kinase* (*MYLK*) encodes a key regulator for muscle contraction process (Flores et al., 2007).

DEGs for other metabolic processes

There were 24 DEGs involved in various metabolic processes

Table 4. Metabolic DEGs

FC	Probe set ID	Symbol	Gene title	Bit score	E-value	*p* value
Glucose metabolism						
2.0	Ssc.8241.1.A1_at	PPM2C	Protein phosphatase 2C, magnesium-dependent, catalytic subunit	178.9	1.2E-43	8.4E-04
1.7	Ssc.26463.1.S1_at	KLF15	Kruppel-like factor 15	393.0	3E-108	1.0E-03
1.7	Ssc.8506.1.A1_at	PHKB	Phosphorylase kinase, beta	40.1	0.386	6.3E-04
1.5	Ssc.4307.1.A1_at	PGM3	Phosphoglucomutase 3	1195.9	0	3.2E-06
Lipid metabolism						
1.7	Ssc.2874.1.S1_at	LIPH	Lipase, member H	44.1	0.049	5.4E-06
1.6	Ssc.21972.1.A1_at	DGKG	Diacylglycerol kinase, gamma 90 kDa	44.1	0.044	1.3E-03
Protein metabolism						
2.0	Ssc.29341.1.A1_at	FBXL4	F-box and leucine-rich repeat protein 4	73.8	1.8E-12	1.9E-03
1.6	Ssc.13572.1.A1_at	CPN1	Carboxypeptidase N, polypeptide 1	58.0	5E-06	1.0E-05
1.6	Ssc.13262.1.S1_at	EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1	490.1	2E-137	1.7E-03
1.6	Ssc.3669.1.A1_at	TRSPAP1	tRNA selenocysteine associated protein 1	470.3	2E-131	3.1E-04
1.6	Ssc.3669.1.A1_a_at			470.3	2E-131	2.1E-05
1.6	Ssc.4805.1.S1_at	RPS27	Ribosomal protein S27 (metallopanstimulin 1)	490.1	3E-137	1.9E-03
-1.8	Ssc.4538.1.S1_at	PRSS23	Protease, serine, 23	1104.7	0	4.6E-05
Others						
1.7	Ssc.22641.3.S1_at	RDH16	Retinol dehydrogenase 16 (all-trans)	589.3	6E-167	3.7E-04
1.7	Ssc.4571.1.A1_at	GNPTG	N-acetylglucosamine-1-phosphate transferase, gamma subunit	617.0	4E-175	1.3E-04
-2.4	Ssc.16041.1.S1_at	DHDH	Dihydrodiol dehydrogenase (dimeric)	773.6	0	1.5E-03
-1.7	Ssc.11186.1.S1_at	NUDT7	Nudix (nucleoside diphosphate linked moiety X)-type motif 7	186.8	5.1E-46	4.7E-04
Transcription/RNA metabolism						
Transcription factor & cofactor						
2.0	Ssc.24043.1.S1_at	LCORL	Ligand dependent nuclear receptor corepressor-like	60.0	1.6E-06	4.5E-04
1.7	Ssc.2836.1.S1_at	POLR1D	Polymerase (RNA) I polypeptide D, 16 kDa	638.8	0	1.2E-05
1.5	Ssc.6157.1.A1_at	ZNF521	Zinc finger protein 521	313.7	9.1E-85	1.8E-07
RNA processing						
2.2	Ssc.4676.1.A1_at	RAVER2	Ribonucleoprotein, PTB-binding 2	123.4	4.2E-27	6.8E-04
1.9	Ssc.7191.1.A1_at	LSM14A	LSM14A, SCD6 homolog A (*S. cerevisiae*)	60.0	4.2E-08	2.6E-04
1.7	Ssc.14456.1.S1_at	PCBP2	Poly(rC) binding protein 2	963.9	0	2.2E-05
1.6	Ssc.14431.1.S1_at	FTSJ2	FtsJ homolog 2 (*E. coli*)	440.6	3E-122	5.3E-07
Other						
-1.6	Ssc.25399.1.S1_at	FBXL11	F-box and leucine-rich repeat protein 11	44.1	0.044	7.9E-04

including energy metabolism and RNA metabolism (Table 4). Most of these genes were up-regulated in KNP. These DEGs suggest that KNP showed more diverse and active metabolic activity or capacity than YS. Other hand, YS might be optimized by sacrificing these various metabolic processes to focus on the fast growth of lean muscle.

Validation of DEGs by quantitative real time PCR analysis

Randomly selected 21 DEGs were validated independently using quantitative PCR analysis (Table 5). Most qPCR results were consistent with microarray results in a single trial. Few were not significant in a single trial, but an additional trial immediately increased the significance (P value < 0.05). The fold change trends were strongly correlated between these two methods (Fig. 1).

DISCUSSION

The YS has been bred to improve its economical value for lean meat production and rapid growth. In contrast, the KNP has been restored and maintained because of specific meat quality for local market favoring slightly fatty meat trading off with slow growth. The DEGs reflecting these opposite meat characters strongly indicated the importance of the balance between cell proliferation and differentiation in determining meat qualities. Most genes responsible for cell cycle arrest were up-regulated in KNP compared to YS (Fig. 2). Especially, most of the up-regulated DEGs in KNP associated with p53 were either directly or indirectly involved in cell cycle arrest. These DEGs are involved in up-stream or down-stream of the p53 pathway. This suggests that p53 may be located in a central position determining phenotypic differ-

Table 5. Quantitative RT-PCR primer information and results for 21 DEGs with a reference gene

Probe set ID	Gene symbol	Forward	Reverse	Average Ct ± SD	Average Ct ± SD	FC	*P* value
				KNP	YS		
p53-related process							
Ssc.11281.1.A1_at	PENK	TTACTGTCCTGTGCTGCTTGCCTT	ACACAGGACCTGAAAGGACAGCTT	26.8 ± 0.56	25.9 ± 0.19	-1.8	0.0490
Ssc.16937.1.A1_at	TP53INP2	AGGTGCAATGAGGACCCTATCAGA	AGGTGGCAGAACAAGAGGGAAGAT	24.9 ± 0.31	23.5 ± 0.90	-2.6	0.0456
Ssc.9522.1.A1_at	RNF144B	GGTCCCTGAATGGATGCCTTTATGTG	CATCTGAGGCATTCTGAAACTGTC	30.3 ± 0.65	32.0 ± 0.42	3.2	0.0067
Cell proliferation/Differentiation signaling							
Ssc.2841.1.S1_at	CREM-1	CTGACTCCCACATGTACAGTTGCT	CTACGTACAGTGAAGCAGTTTGGG	21.1 ± 1.06	18.4 ± 0.77	-7.1	0.0060
Ssc.2841.2.S1_a_at	CREM-2	CTGGTGACATGCCGACTTATCAGA	TGTTGCCTCCTCTGCTAGTTGCT	24.5 ± 1.06	21.7 ± 0.72	-6.5	0.0076
Ssc.18764.1.A1_at	ITGB1BP3	ACACTGCGTGACCATTTCCCTTTG	GCCACCGTTGAGGTTACAGAAACA	23.2 ± 1.82	20.4 ± 0.90	-7.1	0.0447
Ssc.17238.1.A1_at	MUSTN1	TGGCCAAGAACCAGGAGATCAAGT	GTGTGCACTTCTCAGCCAAAGACA	20.4 ± 0.87	18.8 ± 0.50	-2.9	0.0288
Muscular system							
Ssc.10392.1.A1_at	MYH11	ACGGTTAACTAAGCCCTGGCAACT	TCTCGGTGAACTGTGTGCATCTGA	17.3 ± 7.73	17.1 ± 0.13	-1.2	0.0417
Ssc.761.1.S1_at	NR4A3	ACCAGCTGTTAATGGAGAGTGCCT	AAGAGCAACGCTGTTAGAGGAGCA	26.4 ± 1.69	22.5 ± 0.43	-14.6	0.0165
Metabolism							
Ssc.16041.1.S1_at	DHDH	AGGTAGCTTGCTAGACCTTGGCAT	ATTGGAGAGCTGGGAAGTGATGCT	16.2 ± 1.14	14.2 ± 0.77	-3.7	0.0376
Ssc.29341.1.A1_at	FBXL4	TGGTGGGAATGTCTTTGTATCCCA	GAGCTACTCACACATTCTTAAAGTGC	25.8 ± 0.39	26.8 ± 0.41	2.0	0.0124
Ssc.4538.1.S1_at	PRSS23	CACAGGATTTCAACGTGGCCGTTA	TGCAACCAGGAAGGAACACCATGT	17.9 ± 0.85	16.5 ± 0.46	-2.7	0.0364
Ssc.14456.1.S1_at	PCBP2	TTACCATCACTGGATCTGCTGCCA	AGATGGATCATGGGTGGTGGTGAA	20.7 ± 0.49	21.4 ± 0.25	1.7	0.0442
Miscellaneous							
Ssc.24153.1.S1_at	ADSSL1	CGTCGGCAAATCCAGAGATTCGAT	GGGAACACGATTGTGGCTGTTTGT	22.3 ± 0.60	21.0 ± 0.56	-2.5	0.0198
Ssc.12492.1.A1_at	SORT1	CACCACATGCTGTTTCTCCTGAGT	GTTGTCATTACAGGCAAAGAGCCC	17.6 ± 0.80	16.1 ± 0.58	-2.9	0.0223
Ssc.12989.1.A1_at	YIPF6	TGGGTGACAACCAGTGACTCAAGA	AGCTGCACCCTGAACTGGTATTCT	18.5 ± 0.91	16.8 ± 0.71	-3.2	0.0303
Ssc.2429.1.S1_at	ROCK2	GATGAGAGCAAGTCCCTTCTGAGT	TACGGTCTCTTCACGATACAGGGA	14.5 ± 0.69	13.2 ± 0.68	-2.3	0.0467
Ssc.23484.1.A1_a_at	SCARB2	ACCCAGAATGGGAAATGGGCTTTG	AAGCGCCTTTGGCAGAACTCTCAT	24.9 ± 0.24	25.6 ± 0.31	1.7	0.0078
Ssc.3909.1.A1_at	SLC16A9	CCTCATTCTTTGGTCACTGTCAAGG	TAAACACAGTGAAGACGCACCTGG	24.3 ± 1.15	22.4 ± 0.76	-3.7	0.0400
Unknown							
Ssc.21490.1.S1_a_at	C12orf45	ACAGTACCTCTTCCGAAGGCGAAA	ACTGTCCAGAACCTCTATCTTGCC	26.8 ± 0.53	25.5 ± 0.40	-2.5	0.0093
Ssc.10752.1.A1_at	TMEM65	TTCACCACTTCCCTGAAGTCCACA	AGAGGAGTTAGCATGTGGAAGGCT	21.4 ± 0.42	20.5 ± 0.52	-1.9	0.0303
Reference							
Ssc.947.1.S1_s_at	RPS28	TCCATCATCCGAAACGTGAAAGGC	ATATCCAGGACCCAGCCACAACTT	10.3 ± 0.08	10.4 ± 0.10	1.0	0.9071

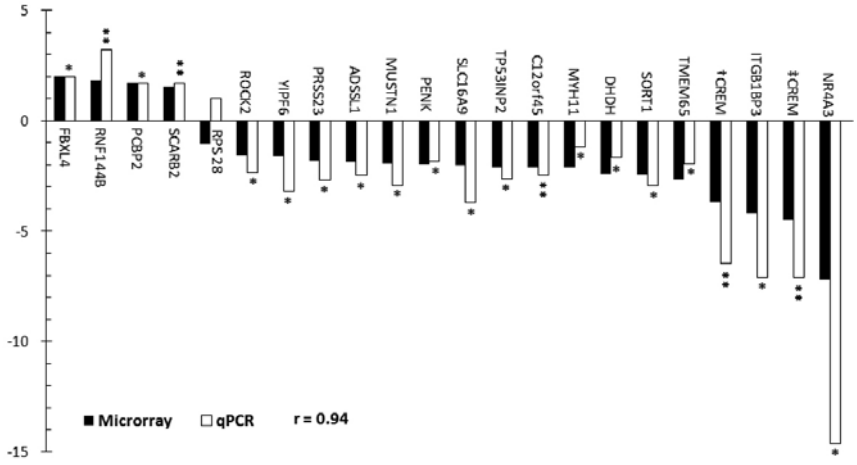

Fig. 1. Comparison of fold change as determined by microarray and qPCR results. The genes were ordered by fold change of microarray (black bar). *RPS 28* was used as the reference gene. There are two probe sets for detecting CREM in the array (†Ssc.2841.2.S1_a_at and ‡Ssc.2841.1.S1_at). Our qPCR primers were designed to distinguish between probe sets. Statistical significance of qPCR result are displayed by asterisks (**$P < 0.01$; *$0.01 \leq P < 0.05$). The r value indicates the Pearson correlation coefficient presenting a strong linear relationship between the methods.

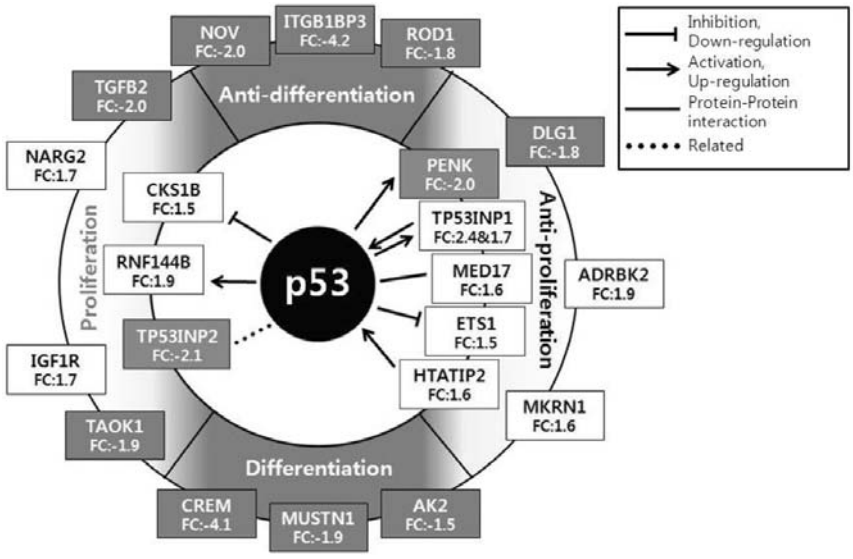

Fig. 2. A proposed functional network based on the major DEGs. This network indicated that the KNP breed tended to slower skeletal proliferation by up-regulation of genes in cell cycle arrest than YS. Interestingly, all proliferation and anti-proliferation genes at inner circle were directly or indirectly related to p53, which indicated the importance of p53 in phenotypic differences between the two breeds.

ences, not only cell proliferation of muscle fiber but also taste of meat between KNP and YS breeds.

In summary, we found 82 DEGs from the comparison between KNP and YS breeds, which showed clear difference in growth rate and muscle characters. Therefore, these DEGs might be useful to explain molecular characters of muscle and to provide expression markers in the aspect of leanness and complexity. Indeed, the functional analyses of the DEGs indicated that leanness of YS primarily came from avoiding p53 related cell cycle arrest and intensifying other cell proliferation signaling. In contrast, the complexity of KNP muscle primarily came from opposite direction of these DEGs and intensification of other various metabolic capabilities.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERECES

Aksu, S., Koczan, D., Renne, U., Thiesen, H.J., and Brockmann, G.A. (2007). Differentially expressed genes in adipose tissues of high body weight-selected (obese) and unselected (lean) mouse lines. J. Appl. Genet. *48*, 133-143.

Bennetts, J.S., Rendtorff, N.D., Simpson, F., Tranebjaerg, L., and Wicking, C. (2007). The coding region of TP53INP2, a gene expressed in the developing nervous system, is not altered in a family with autosomal recessive non-progressive infantile ataxia on chromosome 20q11-q13. Dev. Dyn. *236*, 843-852.

Bensaad, K., and Vousden, K.H. (2007). p53: new roles in metabolism. Trends Cell. Biol. *17*, 286-291.

Bensaad, K., Tsuruta, A., Selak, M.A., Vidal, M.N., Nakano, K., Bartrons, R., Gottlieb, E., and Vousden, K.H. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell *126*, 107-120.

Brand, K.A., and Hermfisse, U. (1997). Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB J. *11*, 388-395.

Calhabeu, F., Lafont, J., Le Dreau, G., Laurent, M., Kazazian, C., Schaeffer, L., Martinerie, C., and Dubois, C. (2006). NOV/CCN3 impairs muscle cell commitment and differentiation. Exp. Cell Res. *312*, 1876-1889.

Caruana, G., and Bernstein, A. (2001). Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation

in the discs large gene. Mol. Cell. Biol. *21*, 1475-1483.
Casa, A.J., Dearth, R.K., Litzenburger, B.C., Lee, A.V., and Cui, X. (2008). The type I insulin-like growth factor receptor pathway: a key player in cancer therapeutic resistance. Front Biosci. *13*, 3273-3287.
Chang, C., Martin, R.G., Livingston, D.M., Luborsky, S.W., Hu, C.P., and Mora, P.T. (1979). Relationship between T-antigen and tumor-specific transplantation antigen in simian virus 40-transformed cells. J. Virol. *29*, 69-75.
Chitnis, M.M., Yuen, J.S., Protheroe, A.S., Pollak, M., and Macaulay, V.M. (2008). The type 1 insulin-like growth factor receptor pathway. Clin. Cancer Res. *14*, 6364-6370.
Choi, K.Y., Moon, J.K., Choi, S.H., Kim, K.S., Choi, Y.I., Kim, J.J., and Lee, C.K. (2008). Differential expression of cytochrome P450 genes regulate the level of adipose arachidonic acid in Sus scrofa. Asia-Aust. J. Anim. Sci. *21*, 967-971.
Chumakov, P.M. (2007). Versatile functions of p53 protein in multicellular organisms. Biochemistry *72*, 1399-1421.
Daaka, Y., Pitcher, J.A., Richardson, M., Stoffel, R.H., Robishaw, J.D., and Lefkowitz, R.J. (1997). Receptor and G betagamma isoform-specific interactions with G protein-coupled receptor kinases. Proc. Natl. Acad. Sci. USA *94*, 2180-2185.
Dittmer, J. (2003). The biology of the Ets1 proto-oncogene. Mol. Cancer *2*, 29.
Draviam, V.M., Stegmeier, F., Nalepa, G., Sowa, M.E., Chen, J., Liang, A., Hannon, G.J., Sorger, P.K., Harper, J.W., and Elledge, S.J. (2007). A functional genomic screen identifies a role for TAO1 kinase in spindle-checkpoint signalling. Nat. Cell Biol. *9*, 556-U136.
Dzeja, P., and Terzic, A. (2009). Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing. Int. J. Mol. Sci. *10*, 1729-1772.
Fischer, R.S., and Fowler, V.M. (2003). Tropomodulins: life at the slow end. Trends Cell Biol. *13*, 593-601.
Flores, C., Ma, S.F., Maresso, K., Ober, C., and Garcia, J.G. (2007). A variant of the myosin light chain kinase gene is associated with severe asthma in African Americans. Genet. Epidemiol. *31*, 296-305.
Guo, Z., Linn, J.F., Wu, G., Anzick, S.L., Eisenberger, C.F., Halachmi, S., Cohen, Y., Fomenkov, A., Hoque, M.O., Okami, K., et al. (2004). CDC91L1 (PIG-U) is a newly discovered oncogene in human bladder cancer. Nat. Med. *10*, 374-381.
Han, X., Budreau, A.M., and Chesney, R.W. (2000). Identification of promoter elements involved in adaptive regulation of the taurine transporter gene: role of cytosolic Ca^{2+} signaling. Adv. Exp. Med. Biol. *483*, 535-544.
Huang, J., Xu, L.G., Liu, T., Zhai, Z., and Shu, H.B. (2006). The p53-inducible E3 ubiquitin ligase p53RFP induces p53-dependent apoptosis. FEBS Lett. *580*, 940-947.
Hwang, I., Park, B., Cho, S., Kim, J., Choi, Y., and Lee, J. (2004). Identification of muscle proteins related to objective meat quality in Korean native black pig. Asian-Australasian J. Anim. Sci. *17*, 1599-1607.
Iizuka-Kogo, A., Ishidao, T., Akiyama, T., and Senda, T. (2007) Abnormal development of urogenital organs in Dlgh1-deficient mice. Development *134*, 1799-1807.
Iotsova, V., Crepieux, P., Montpellier, C., Laudet, V., and Stehelin, D. (1996) TATA-less promoters of some Ets-family genes are efficiently repressed by wild-type p53. Oncogene *13*, 2331-2337.
Jeon, J.T., Park, E.W., Jeon, H.J., Kim, T.H., Lee, K.T., and Cheong, I.C. (2003). A large-insert porcine library with sevenfold genome coverage: a tool for positional cloning of candidate genes for major quantitative traits. Mol. Cells *16*, 113-116.
Kim, H.J., and Lee, W.J. (2009). Insulin-like growth factor-I induces androgen receptor activation in differentiating C2C12 skeletal muscle cells. Mol. Cells *28*, 189-194.
Kim, K.S., Yeo, J.S., and Kim, J.W. (2002). Assessment of genetic diversity of Korean native pig (Sus scrofa) using AFLP markers. Genes Genet. Syst. *77*, 361-368.
Kim, T.H., Choi, B.H., Chang, G.W., Lee, K.T., Lee, H.Y., Lee, J.H., Kim, K.S., Park, C.K. and Moran, C. (2005a). Molecular characterization and chromosomal mapping of porcine adipose differentiation-related protein (ADRP). J. Anim. Breed Genet. *122*, 240-246.
Kim, T.H., Kim, K.S., Choi, B.H., Yoon, D.H., Jang, G.W., Lee, K.T., Chung, H.Y., Lee, H.Y., Park, H.S., and Lee, J.W. (2005b). Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. J. Anim. Sci. *83*, 2255-2263.
Kim, N., Lim, J., Song, M., Kim, O., Park, B., Kim, M., Hwang, I., and Lee, C. (2008). Comparisons of longissimus muscle metabolic enzymes and muscle fiber types in Korean and western pig breeds. Meat Sci. *78*, 455-460.
Kress, M., May, E., Cassingena, R., and May, P. (1979). Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. J. Virol. *31*, 472-483.
Lane, D.P., and Crawford, L.V. (1979). T antigen is bound to a host protein in SV40-transformed cells. Nature *278*, 261-263.
Li, J., Rao, H., Burkin, D., Kaufman, S.J., and Wu, C. (2003). The muscle integrin binding protein (MIBP) interacts with alpha7beta1 integrin and regulates cell adhesion and laminin matrix deposition. Dev. Biol. *261*, 209-219.
Linossier, M.T., Dormois, D., Perier, C., Frey, J., Geyssant, A., and Denis, C. (1997). Enzyme adaptations of human skeletal muscle during bicycle short-sprint training and detraining. Acta Physiol. Scand. *161*, 439-445.
Linzer, D.I., and Levine, A.J. (1979). Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell *17*, 43-52.
Liu, C., Kronenberg, M., Jiang, X., Rowe, D., and Hadjiargyrou, M. (2007). Characterization of Mustn1 PRO-GFPtpz transgenic mice, pp. 13-14.
Lynch, G.S., and Ryall, J.G. (2008). Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. Physiol. Rev. *88*, 729-767.
Mahajan, M.A., Murray, A., Levy, D., and Samuels, H.H. (2007). Nuclear receptor coregulator (NRC): mapping of the dimerization domain, activation of p53 and STAT-2, and identification of the activation domain AD2 necessary for nuclear receptor signaling. Mol. Endocrinol. *21*, 1822-1834.
Malik, S., and Roeder, R.G. (2000). Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. Trends Biochem. Sci. *25*, 277-283.
McTavish, N., Copeland, L.A., Saville, M.K., Perkins, N.D., and Spruce, B.A. (2007). Proenkephalin assists stress-activated apoptosis through transcriptional repression of NF-kappaB- and p53-regulated gene targets. Cell Death Differ. *14*, 1700-1710.
Moolenaar, W.H. (1995). Lysophosphatidic acid, a multifunctional phospholipid messenger. J. Biol. Chem. *270*, 12949-12952.
Moon, J.K., Kim, K.S., Kim, J.J., Choi, B.H., Cho, B.W., Kim, T.H., and Lee, C.K. (2009). Differentially expressed transcripts in adipose tissue between Korean native pig and Yorkshire breeds. Anim. Genet. *40*, 115-118.
Murphy, M., Hinman, A., and Levine, A.J. (1996). Wild-type p53 negatively regulates the expression of a microtubule-associated protein. Genes Dev. *10*, 2971-2980.
Nadon, R., and Shoemaker, J. (2002). Statistical issues with microarrays: processing and analysis. Trends Genet. *18*, 265-271.
Narayan, N., Massimi, P., and Banks, L. (2009). CDK phosphorylation of the discs large tumour suppressor controls its localisation and stability. J. Cell Sci. *122*, 65-74.
Nedivi, E., Wu, G.Y., and Cline, H.T. (1998) Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. Science *281*, 1863-1866.
Omwancha, J., Zhou, X.F., Chen, S.Y., Baslan, T., Fisher, C.J., Zheng, Z., Cai, C., and Shemshedini, L. (2006). Makorin RING finger protein 1 (MKRN1) has negative and positive effects on RNA polymerase II-dependent transcription. Endocrine *29*, 363-373.
Qi, R.Z., Ching, Y.P., Kung, H.F., and Wang, J.H. (2004) alpha-Chimaerin exists in a functional complex with the Cdk5 kinase in brain. FEBS Lett. *561*, 177-180.
Schabort, E.J., van der Merwe, M., Loos, B., Moore, F.P., and Niesler, C.U. (2009). TGF-beta's delay skeletal muscle progenitor cell differentiation in an isoform-independent manner. Exp. Cell Res. *315*, 373-384.
Seale, P., Ishibashi, J., Holterman, C., and Rudnicki, M.A. (2004). Muscle satellite cell-specific genes identified by genetic profiling of MyoD-deficient myogenic cell. Dev. Biol. *275*, 287-300.
Srikakulam, R., Liu, L., and Winkelmann, D.A. (2008). Unc45b forms a cytosolic complex with Hsp90 and targets the unfolded myosin motor domain. PLoS ONE *3*, e2137.
Sugiura, N., Dadashev, V., and Corriveau, R.A. (2004). NARG2 encodes a novel nuclear protein with (S/T)PXX motifs that is ex-

pressed during development. Eur. J. Biochem. *271*, 4629-4637.
Takeda, K., Yu, Z.X., Qian, S., Chin, T.K., Adelstein, R.S., and Ferrans, V.J. (2000). Nonmuscle myosin II localizes to the Z-lines and intercalated discs of cardiac muscle and to the Z-lines of skeletal muscle. Cell Motil. Cytoskeleton *46*, 59-68.
Tomasini, R., Samir, A.A., Carrier, A., Isnardon, D., Cecchinelli, B., Soddu, S., Malissen, B., Dagorn, J.C., Iovanna, J.L., and Dusetti, N.J. (2003). TP53INP1s and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. J. Biol. Chem. *278*, 37722-37729.
Tsai, S., Cassady, J.P., Freking, B.A., Nonneman, D.J., Rohrer, G.A., and Piedrahita, J.A. (2006). Annotation of the Affymetrix porcine genome microarray. Anim. Genet. *37*, 423-424.
Walsh, C.T., Radeff-Huang, J., Matteo, R., Hsiao, A., Subramaniam, S., Stupack, D., and Brown, J.H. (2008). Thrombin receptor and RhoA mediate cell proliferation through integrins and cysteine-rich protein 61. FASEB J. *22*, 4011-4021.
Weber, A., Pennise, C.R., Babcock, G.G., and Fowler, V.M. (1994). Tropomodulin caps the pointed ends of actin-filaments. J. Cell Biol. *127*, 1627-1635.
Yamamoto, H., Tsukahara, K., Kanaoka, Y., Jinno, S., and Okayama, H. (1999). Isolation of a mammalian homologue of a fission yeast differentiation regulator. Mol. Cell. Biol. *19*, 3829-3841.
Zhao, J., Zhang, X., Shi, M., Xu, H., Jin, J., Ni, H., Yang, S., Dai, J., Wu, M., and Guo, Y. (2006). TIP30 inhibits growth of HCC cell lines and inhibits HCC xenografts in mice in combination with 5-FU. Hepatology *44*, 205-215.