Screening of Specific Genes Expressed in the Swine Tissues and Development of a Functional cDNA Chip

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ABSTRACT : To develop a functional cDNA chip, specific genes expressed in the tissues of swine *Kagoshima* Berkshire were screened. A total of 4,434 ESTs were obtained by constructing a cDNA library from total RNA isolated from the muscle and fat tissues, affirming their functions by investigating similarity of nucleotide sequences with the database at the NCBI. Among them, 1,230 ESTs were confirmed as novel genes, which, to date, have not been identified. Attaching the genes to a cDNA microarray slide revealed expression patterns of genes in muscle and fat according to the growth stages of swine. As specific genes expressed in the muscle tissues of swine with body weight of 30 kg, 60 genes including actin, myosin, tropomysin, transfer RNA-trp synthetase, Kel-like protein 23, KIAA0182 and COI, Foocen-m, etc were obtained. In addition, 18 novel genes were obtained. As specific genes expressed in fat tissues of swine with body weight of 30 kg, 47 genes including annexin II, Collagen, Fibronectin, Pleckstrin homology domain, serine protease, etc were obtained. 21 novel genes were also obtained. The genes specifically expressed in the muscle and fat tissues of swine affect contraction and relaxation of the muscle and the fat. However, studies on the expression mechanisms of the genes are insufficient. To reveal species of structural genes in swine muscle and fat tissue, interrelation studies in expression and function of genes by using the cDNA chip should be conducted. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 933-941)***

Key Words : Swine Tissues, cDNA Chip, EST, Microarray

INTRODUCTION

The Berkshire breed has proven unfavorable in the swine-raising industry, because it has thick back fat and a low ratio of feed efficiency, growth rate, and production rate. However, Berkshire consumption has recently increased due to its harder fat tissues, white fat, excellent texture, and abundant meat sap. Nevertheless, to date, very few genetic studies on meat quality or assays of genes associated with the meat quality of Berkshires have been carried out. In particular, the genetic traits associated with meat quality of Berkshires have not yet been studied sufficiently by reason of their complexity.

The major genes affecting swine meat quality that have been identified thus far are ryanodine receptor gene (Smith and Bampton, 1977; Webb, 1981; Fujii et al., 1991) and acid meat gene (Le Roy et al., 1990). Chromosome regions associated with meat quality by quantitative trait loci (QTL) analysis and various candidate genes have also been reported. Swine leucocytes antigen (SLA) complex exists on chromosome 7 (Geffrotin et al., 1984) and microsatellite markers S0064, S0066, S0102 or TNF, located on chromosome 7, which are associated with back fat thickness, sirloin cross-section, meat quality traits, and boar taint (Jung et al., 1989; Bidanel et al., 1996), respectively, have been described. It has also been revealed that QTL associated with back fat thickness and abdominal fat content exists in the location of microsatellite markers S0001-S0175 (Andersson et al., 1994). The pituitary-specific transcription gene (PIT1), known as a regulator factor for hormones, was reported to be associated with back fat thickness (Yu et al., 1995). Intramuscular fat content (IMF) largely affects tenderness, juiciness, and the taste of pork. Heart-fatty acid binding protein (H-FABP) gene affecting IMF (Gerbens et al., 1997) and the relationship between IMF and microsatellite SW1823-S0003 (74-79 cM) region existing on chromosome 6 has been investigated (Grindflek et al., 2001).

As locations of several QTLs mainly affecting meat quality traits were found on chromosomes 4, 6, and 7 (Clamp et al., 1992; Andersson et al., 1994; Renard et al., 1996; Rohrer and Keele 1998; de Koning et al., 1999;

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Gerbens et al., 2000; Ovilo et al., 2000), a large number of studies on development of markers related to meat quality, which are centered on these chromosomes, are in progress.

For the last several years, great efforts have been made to develop a genetic map containing unknown gene markers and well-known genes in connection with meat quality traits. Traditional methods of analyzing gene expression differences are not suitable for simultaneous analysis of gene expression. Upon this background, cDNA microarray has emerged as a new and strong means to study gene expression in various living entities. cDNA microarray technology has been applied to simultaneous expression of numerous genes and discovery of genes in a large scale, as well as genetic polymorphism screening and mapping of chromosomal DNA clones.

In this study, we generate a functional cDNA chip composed of only specific genes by searching expression profiles of genes specifically expressed in the muscle and fat tissues of *Kagoshima* Berkshire using cDNA microarray technology. We further attempt to apply the cDNA chip in establishing high quality seed-swine and evaluating meat quality according to varieties of swine and tissues of pork.

MATERIALS AND METHODS

Breeds and tissues

Total RNA for array fabrication was extracted from the muscle and fat tissues of the longissimus dorsi of *Kagoshima* Berkshire from the Sung Chook farm with body weights of 30 kg and 90 kg, respectively.

For preparation of target DNA to screen the muscle specific genes expressed in the muscle and fat tissues of swine, tissue samples were collected from the longissimus dorsi area of *Kagoshima Berkshires* with body weights of 30 kg and 90 kg, respectively. The muscle and fat tissues were cut into 5-8 mm length, frozen with liquid nitrogen, and stored at -70°C.

Array fabrication

A probe DNA comprised of cDNA amplified by PCR was prepared and attached to a slide glass. Total RNA was extracted using a RNA extraction kit (Qiagen, Germany) according to the manual and mRNA was extracted using an oligo (dT) column. The extracted mRNA sample was subjected to RT-PCR using SP6, T3 forward primer, and T7 reverse primer (Amersham Pharmacia Biotech, England) to synthesize cDNA. The total volume of each PCR reactant was 100 μl. 100 pM of forward primer and reverse primer were each transferred to a 96-well PCR plate (Genetics, England). Each well contained 2.5 mM dNTP, 10×PCR buffer, 25 mM $MgCl_2$, 0.2 μg of DNA template, and 2.5 units of Taq polymerase. PCR was performed in a GeneAmp PCR system 5700 (AB Applied BioSystem, Canada) under the following conditions: total 30 cycles of 30 seconds at 94°C, 45 seconds at 58°C, 1 minute at 72°C. The size of the amplified DNA was identified by agarose gel electrophoresis. The PCR product was precipitated with ethanol in a 96-well plate, dried, and stored at -20°C.

A total of 4,434 cDNAs (ESTs), prepared as described above, were cloned to analyze the nucleotide sequences of the genes, and their genetic information was identified from the database at NCBI. The genes having information were isolated and purified by PCR. The genetic locus and map for the total 4,434 cDNAs (ESTs) were constructed. The total 4,434 cDNAs (ESTs) and 90 yeast controls were arrayed in an area of 1.7 cm^2. The probe DNA was then spotted on a slide glass for analysis under a microscope (produced by Corning), and coated with CMT-GAPSTM aminosilane using Microgrid II (Biorobotics). The pin apparatus was approached to a well in a microplate to inject the solution into the slide glass (1 to 2 nL). After printing of the probe DNA, the slide was dried. The spotted DNA and the slide were then UV cross-linked at 90 mJ using a Stratalinker TM (Stratagene, USA), washed twice with 0.2% SDS at room temperature for 2 minutes, and washed once with third distilled water at room temperature for 2 minutes. After washing, the slide was dipped in a water tank at 95°C for 2 minutes and then blocked for 15 minutes by adding a blocking solution (a mixture of 1.0 g $NaBH_4$ dissolved in 300 mL of pH 7.4 phosphate buffer and 100 mL of anhydrous ethanol). The slide was subsequently washed three times with 0.2% SDS at room temperature for 1 minute and once with third distilled water at room temperature for 2 minutes and dried in air. Printed slides were stored in a light-tight box in a bench-top desiccator at room temperature until used for hybridization (Cheung et al., 1999).

Target DNA preparation and hybridization

Total RNAs were isolated from 0.2 to 1.0 g of the experimental group and the control group according to the manual of the Trizol™ kit (Life Technologies, Inc.) to prepare the target DNA. Trizol™ was added to the tissue in an amount of 1 mL of Trizol™ per 50 to 100 mg of tissue and disrupted using a glass-Teflon or Polytron homogenizer. The disrupted granules were centrifuged at 4°C at a speed of 12,000 g for 10 minutes and 1 mL of the supernatant was aliquoted. 200 μl of chloroform was added to each aliquot, which was then voltexed for 15 seconds, placed on ice for 15 minutes and centrifuged at 4°C at a speed of 12,000 g for 10 minutes. Chloroform of the same amount was again added thereto, voltexed for 15 seconds, placed on ice for 15 minutes, and centrifuged at 4°C at a speed of 12,000 g for 10 minutes. The supernatant was then transferred to a new tube. 500 μl of isopropanol was added to the tube, voltexed, and placed on ice for 15 minutes, centrifuged at 4°C at a

Table 1. Classification according to functions and analysis of the obtained ESTs

Description	Redundancy (%)	
Gene/protein expression	1,358	30.6
Cell structure and mobility	792	17.9
Cell division	106	2.4
Cell signaling and cell communication	240	5.4
Metabolism	350	7.9
Membrane transport	67	1.5
Growth factor	35	0.8
Immune response	66	1.5
ETC (Not classified)	190	4.3
No match	1,230	27.7
Total	4,434	100

Table 2. Classification according to organs and analysis of the obtained ESTs

Category	Number of clones (%)
Homology to previously characterized gene	3,014 (68%)
Human	1,593 (35.9)
Swine	384 (8.7)
Other species	1,037 (23.4)
Homology to uncharacterized ESTs from other database	190 (4.3%)
Human	13 (0.3)
Swine	159 (3.6)
Other species	18 (0.4)
No match	1,230 (27.7%)
Total	4,434 (100%)

speed of 12,000 g for 5 minutes. The supernatant was removed, mixed with 1 mL of 75% cold ethanol, and centrifuged at 4°C at a speed of 12,000 g for 5 minutes. The supernatant was removed, freeze-dried on a clean bench for 30 minutes, and placed in 20 μl of RNase-free water or DEPC water to dissolve RNA. The total DNA concentration was set to 40 μg/17 μl for electrophoresis.

The target DNA was prepared according to the standard first-strand cDNA synthesis. Briefly, according to the method described by Schuler (1996), 40 μg of total RNA and oligo dT-18mer primer (Invitrogen Life Technologies) were mixed, heated at 65°C for 10 minutes, and cooled at 4°C for 5 minutes. Then, 1 μl of a mixture of 25 mM dATP, dGTP and dTTP, 1 μl of 1 mM dCTP (Promega) and 2 μl of 1 mM cyanine 3-dCTP or 2 μl of 1 mM cyanine 5-dCTP, 20 units of RNase inhibitor (Invitrogen Life Technology), 100 units of M-MLV RTase, and 2 μl of 10×first strand buffer were added thereto. The reaction mixture was incubated at 38°C for 2 h and the non-bound nucleotides were removed by ethanol precipitation. Here, DEPC treated sterile water was used.

The slide, prepared as above, was pre-hybridized with a hybridization solution (5×SSC, 0.2% SDS, 1 mg/mL herring sperm DNA) at 65°C for 1 h. The target DNA labeled with cyanine 3 (Cy-3) and cyanine 5 (Cy-5) was re-suspended in 20 μl of the hybridization solution at 95°C and denatured for 2 minutes. Then, the slide was hybridized with the solution at 65°C overnight. The hybridization was performed in a humidity chamber covered with a cover glass (Grace Bio-Lab).

After hybridization, the slide was washed 4 times with 2×SSC, 0.1% SDS at room temperature for 5 minutes while vigorously stirred in a dancing shaker. Then the slide was washed twice with 0.2×SSC for 5 minutes and 0.1×SSC for 5 minutes at room temperature.

The slid was scanned on a Scanarray 5,000 (GSI Lumonics Version 3.1) with a pixel size of 50 μm. The target DNA labeled by cyanine 3-dCTP was scanned at 565 nm and the target DNA labeled by cyanine 5-dCTP was scanned at 670 nm. Linear scanning of cyanine 3-dCTP- and cyanine 5-dCTP-labeled spots standardized two fluorescence intensities. The slide was again scanned on a Scanarray 4,000XL with a pixel size of 10 μm. The resulting TIFF image files were analyzed on Quantarray software version 2.1 and the background was automatically subtracted. The intensity of each spot was put into Microsoft Excel from Quantarray.

RESULTS

Classification according to function of the selected ESTs

The annotation of each EST was confirmed indirectly by tests on similarity of amino acid sequences (Blastx), nucleotide sequences (Blastn), and EST (dbEST). In the case of swine, the number of gene resources accessible in GeneBank as of November 1, 2003 was 103,321, which is smaller than that of other living organisms. Therefore, the total 4,434 EST clones obtained in this study will be an important reference. The 4,434 ESTs were analyzed according to gene function by GeneBank data and performed clustering. On the basis of the searching results in GeneBank, the genes were classified according to protein diversities. The number of unique EST was 1,230 excluding overlapped genes (Table 1). Genes having no similarity with known genes or ESTs were obtained and 1,420 genes without matches, which were discovered newly in swine, will be used as a basic reference to apply for a patent for genes or production on a commercial scale. Table 1 provides classification of the obtained ESTs according to gene function. As shown here, genes related to gene/protein expression and genes related to cell structure and mobility, which are expressed highly for formation of muscle and fat, are widely distributed.

As shown in Table 1, the number of no match genes is 1,230, 27.7% of the whole, and the number of genes related to gene/protein expression is 1,358, 30.6% of the whole. The number of cell structure and mobility-related genes, which affect formation and quality of muscle, is 792, 17.9%.

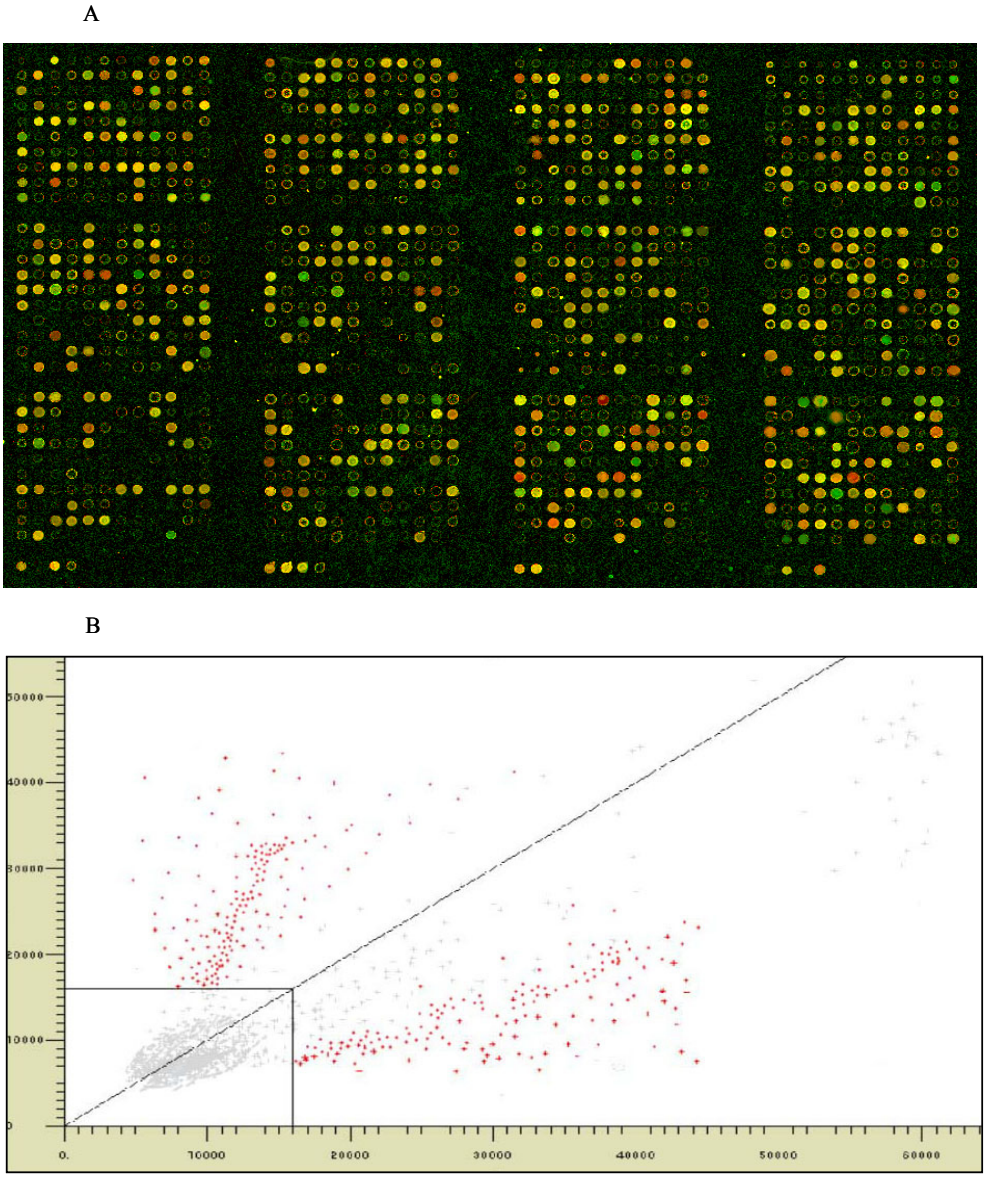

Figure 1. Chip image was hybridized to cy3 and cy5 probes from muscle at 30 kg and 90 kg, respectively. A. A 4,434-gene procine cDNA microarray was printed by the GeneMachines microarray and imaged using the Scanarray 4,000. B. Gene expression differences in 30 kg (cy3) and 90 kg (cy5) by scatter plot.

Also, occurrence number according to organisms of the 4,434 ESTs genes was confirmed (Table 2). Among the 4,434 ESTs, the number of ESTs similar to human genes was 1,598, 36% of the whole. And the number of ESTs similar to the swine gene was 384, 8.7% of the whole. However, studies on swine genes are insufficient compared with studies on human genes, particularly following completion of the human genome project. As such, more genetic studies may be performed in the future to develop new drugs for human genetic disease treatment or new improved varieties using swine.

Table 2 presents classification of the ESTs, which were obtained from the muscle and fat tissues of *Kagoshima* Berkshire, according to species. As shown in Table 2, many ESTs have homology with human genes; however, only 8.7% and 3.6% of the ESTs have homology with previously characterized swine genes and uncharacterized swine genes, respectively. This suggests that analysis of swine genes is not yet sufficient.

Patterns of gene expression between muscle and fat tissues of early growth stage of swine with body weight of 90 kg

To discover major genes and mechanisms in the formation of muscle and fat in swine, patterns of gene expression between muscle and fat in the early growth stage of swine with body weight 30 kg were investigated using cDNA microarray (Figure 1). The genes were very well

Table 3-i). Information of candidate genes using expression patterns of chip between the muscle and fat of early growth stage of swine with body weight of 30 kg

ESTs No.	Accession No.[1]	Description[2]	Ratio of gene expression ESF (30)/ESM (90)
Cell structure and motility			
SM2149	CAB56598	1-alpha dynein heavy chain	-2.1
SM781	NP_033891	19 kDa-interacting protein 3-like	+2.2
SM1068	AAF20165	Actin	+4.5
SM635	BAB19361	Actin	+2.6
SM106	P53506	Actin	+4.9
SM768	X52815	Actin	+2.4
SM363	B25819	Actin	+3.7
SM713	AAA51586	Actin	+5.6
SMk77	NM_001100	Actin, alpha 1	+4.5
SM128	NP_033740	Actin, gamma 2	+3.9
SM1091	JC5971	Alpha-b crystallin	+2.1
SM902	BC001748	Annexin A2	-4.2
SM846	P81287	Annexin V	-3.5
SM653	P04272	Annexin II	-2.3
SMk340	U75316	Beta-myosin heavy chain mRNA	+2.2
SM1807	AAF99682	Calpain large polypeptide L2	+2.7
SM541	NP_000079	Collagen	-4.9
SM715	L47641	Collagen	-5.2
SM1023	Q9XSJ7	Collagen alpha 1	-4.6
SM758	CGHU1S	Collagen alpha 1	-4.3
SM62	CGHU2V	Collagen alpha 2	-4.4
SM949	O46392	Collagen alpha 2	-3.2
SM410	CAA28454	Collagen(alpha V)	-2.3
SM1121	NM_000393	Collagen, type V, alpha 2	-2.8
SM53	NP_000384	Collagen, type V, alpha 2	-2.5
SM1651	XM_039583	Discs, large (Drosophila) homolog 5	-8.6
SM1050	AAA30521	Fibronectin	-3.1
SM381	FNHU	Fibronectin precursor	-2.6
SM122	P07589	Fibronectin(FN)	-2.5
SM1573	XM_044160	Lamin A/C	+2.1
SMk55	NP_006462	Myosin	+3.6
SMk168	AB025261	Myosin heavy chain	+5.0
SM1732	NP_004678	Myotubularin related protein 4	+4.7
SM690	NP_003109	Secreted protein, acidic	-5.2
SM1043	P06469	Tropomyosin alpha chain	+8.6
SMk173	X66274	Tropomysin	+2.2
SMk19	P02587	Troponin C	+6.9
SMk57	AAA91854	Troponin-C	+7.1
SMk50	Y00760	Troponin-C	+9.0
SM1535	P02554	Tubulin beta chain	+3.3
SM1063	P20152	Vimentin	-5.1
SM730	CAA69019	Vimentin	-3.2

[1] Agreed Accession no. [2] Information agreed to the database. No match: No information agreed to the database; novel EST.
ESM: early stage muscle (body weight 30 kg), ESF: early stage fat (body weight 30 kg). SM: swine.

hybridized in cDNA microarray and genes specifically expressed in the muscle and fat tissues were widely distributed. Collagen, known to affect the formation of muscle, and elongation factor, known to affect muscle growth, were expressed very highly in the muscle tissue (Table 3). Genes used as an energy source in the formation or growth of muscle, such as calpain, troponin C, and sugar-related genes, were expressed highly in the fat tissues. In particular, troponin C was expressed little in the muscle tissue, but very highly in fat tissue.

DISCUSSION

In this study, we found that formation of muscle and fat

Table 3-ii). Information of candidate genes using expression patterns of chip between the muscle and fat of early growth stage of swine with body weight of 30 kg

ESTs No.	Accession No.[1]	Description[2]	Ratio of gene expression ESF (30)/ESM (90)
Metabolism			
SMk344	NM_012839	Cytochrome C	+2.4
SM800	AAG53955	Cytochrome C oxidase subunit I	+2.9
SMk151	CAA06313	Fructose-1,6-bisphosphatase	+4.2
SMk254	231300	Glycogen Phosphorylase b	+2.6
SM2070	P00339	L-lactate dehydrogenase M chain	+10.6
SM928	O79874	NADH-ubiquinone oxidoreductase chain 1	+3.2
SMk81	O19094	Octanoyltransferase(COT)	+3.9
SM295	AB006852	Phosphoarginine phosphatase	+2.3
SMk346	M97664	Phosphoglucomutase isoform 2 mRNA	+3.3
SM36	TVMVRR	Protein-tyrosine kinase	+2.6
SM723	P52480	Pyruvate kinase	+7.5
SM698	S64635	Pyruvate kinase	+6.6
SM887	P11980	Pyruvate kinase	+6.3
SM1594	AAA62278	Superoxide dismutase	-3.2
SM1033	XM_018138	Tyrosine phosphatase type IVA	+2.2
Gene/protein expression			
SM75	U09823	Elongation factor 1 alpha	-3.7
SM1989	AAH05660	Elongation factor 1 alpha 1	-3.8
SMk120	AJ275968	LIM domains 1 protein	+9.9
SMk91	AAC48501	Reticulum protein	+2.1
SM2083	NP_003083	Ribonucleoprotein polypeptide B	+3.2
SM21	NP_000994	Ribosomal	+2.2
SM1784	228176	Ribosomal protein P0	+5.5
SM1820	BC014277	Tissue inhibitor of metalloproteinase 3	-2.6
SM1801	AAA30799	Transfer RNA-Trp synthetase	+5.7
SM997	51077272	Translation initiation factor eif1	+2.3
Cell signaling/communication			
SM464	AJ002189	Complete mitochondrial DNA	+2.7
Immune response			
SMk1	AAG52886	Kel-like protein 23	+4.6
EST			
SM1776	XM_050494	KIAA0182	+3.2
SM1556	XP_043678	KIAA1096 protein	+4.5
Unknown			
SM2152	BI327422	AR078G01iTHYEG01S	-5.5
SMk3	AL13277	Chromosome 14 DNA sequence	+2.3
SM908	AAG28205	COI	+2.2
SM1738	CAA19420	DJ466P17.1.1(Laforin)	+3.5
SM1007	AAD31021	Foocen-m	+3.0
SM1724	XP_016035	Hypothetical protein	-2.6
SMk137	XP_002275	Hypothetical protein	+10.0
SM1972	XP_039195	Hypothetical protein	+2.8

[1] Agreed Accession no. [2] Information agreed to the database. No match: No information agreed to the database; novel EST.
ESM: early stage muscle (body weight 30 kg), ESF: early stage fat (body weight 30 kg). SM: swine.

of swine greatly affects the growth as well as taste of pork. Obtaining related genes can help the swine-raising industry economically by controlling the growth of swine via genes that enhance muscle and fat formation in a short period. Further, it will help studies on formation of muscle in the human body by revealing related mechanisms between muscle and fat. Differences in gene expression patterns of muscle and fat in the early growth stage of swine with body weight of 30 kg were investigated using a cDNA chip of swine in order to examine genes specifically expressed in swine muscles and fat (Table 3).

The cDNA microarray constructed in this study makes it easy to discover patterns of gene expression in the muscle, fat, and breed tissues of swine. Using the cDNA microarray,

Table 3-iii). Information of candidate genes using expression patterns of chip between the muscle and fat of early growth stage of swine with body weight of 30 kg

ESTs No.	Accession No.[1]	Description[2]	Ratio of gene expression
			ESF (30)/ESM (90)
SM787	AF192528	Integrin beta-1 subunit	+2.0
SM1474	BG384994	MARC 1PI	+2.8
SM1676	BG548727	NIH_MGC_77	+2.3
SM1650	BI337009	Peripheral blood cell cDNA library	+7.3
SM1774	BAB30715	Putative	+5.1
Unknown			
SM1064	BAB28119	Putative	+3.0
SM1690	BF864360	Reinhardtii CC-1690	+2.5
SM96	M17733	Thymosin beta-4 mRNA	-3.9
SM1922	AAH03026	Unknown	+4.7
No match			
SMk58		No match	+2.9
SM717		No match	-4.4
SMk6		No match	+2.4
SMk68		No match	+3.2
SMk80		No match	+4.3
SMk112		No match	+2.1
SM1639		No match	-2.8
SMk148		No match	+2.9
SM1665		No match	+9.8
SMk95		No match	+2.1
SMk152		No match	+6.4
SM1897		No match	+2.6
SMk138		No match	+3.1
SM796		No match	-2.2
SMk342		No match	+3.9
SMk181		No match	+4.4
SM904		No match	-2.7
SMk262		No match	+2.7
SM9		No match	+2.9
SM1964		No match	+2.6
SMk335		No match	+3.8

[1] Agreed Accession no. [2] Information agreed to the database. No match: No information agreed to the database; novel EST.
ESM: early stage muscle (body weight 30 kg), ESF: early stage fat (body weight 30 kg). SM: swine.

candidate genes (Table 3) acting in the muscle and fat tissues of swine with a body weight of 30 kg can also be profiled. This will be used to identify many more genes affecting the growth of muscles and fat as well as mechanisms between them.

Considering the patterns of gene expression in muscle tissue, genes related to formation and growth of muscle were expressed more highly than meat quality-related genes in the early growth stage of swine. Therefore, developing feed to make muscles grow rapidly or breeding seed-swine with the trait of rapid muscle growth using these genes would make it possible to create an economical advantage in the swine-raising industry and breed swine with excellent competitive power by changing the ratio of swine rotation from a 2.5 cycle to 3 cycle. Genetic diseases with the trait of slow growth, such as short stature, may also be treated more easily with genetic drugs by discovering the growth mechanism in swine muscle and applying it to humans.

Genes used as energy sources in the formation or growth of muscles such as calpain, troponin C, and sugar-related genes were expressed mainly in the fat tissues of the early growth stage of swine. In particular, troponin C was little expressed in the muscle tissue, but highly expressed in fat tissue. This suggests that genes highly expressed in fat tissues act as a temporary bridge to help the formation or growth of muscle, and thus the relation between the growth of muscle and growth of fat is symbiotic. That is, first the genes to support genes affecting the development of muscles are expressed from fat tissues, and then, formation or growth of muscles occurs using the same, and finally, growth of fat occurs.

We are presently carrying out function analyses of the obtained genes to determine the mechanisms connected to muscle and fat in the early stage of growth. The benefit of

finding the mechanisms is that they can be applied to the development of new seed-swine. Also, the cDNA chip prepared in this study can provide a foundation for the comparison of gene expression according to varieties and tissues of swine, gene mutation screening, genetic polymorphism interpretation, and the development of new drugs for disease treatment and disease diagnosis.

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