cDNA Microarray Analysis of the Gene Expression Profile of Swine Muscle

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ABSTRACT : By screening specific genes related to the muscle growth of swine using cDNA microarray technology, a total of 5 novel genes (GF (growth factor) I, II, III, IV and V) were identified. Results of southern blotting to investigate the number of copies of these genes in the genome of swine indicated that GF I, GF III, and GF V existed as one copy and GF II, and GF IV existed as more than two copies. It was suggested that there are many isoforms of these genes in the genome of swine. Also, results of northern blotting to investigate whether these genes were expressed in grown muscle, using GF I, III, and V indicated that all the genes were much more expressed in the muscle of swine with body weight of 90 kg. Expression patterns of these genes in other organs, namely muscle and propagation and fat tissues, were investigated by extracting RNA from the tissues. These genes were not expressed in the propagation and fat tissues, but were expressed in the muscle tissue. To determine the mechanism of muscle growth, further studies should be preceded using the 3 specific genes related to muscle growth, that is GF I, III, and V. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1080-1087)***

Key Words : Muscle Genes, Swine, Expression Profiles, cDNA Microarray

INTRODUCTION

For creation of value in swine raising farms, acquisition of foreign currencies, and raising the competitiveness of the domestic swine-raising industry, which depends on foreign countries for feed and swine supplies, it is necessary to obtain a swine breed of excellent quality. To this end, we have screened meat quality-related specific genes in swine and made a cDNA chip using prominent meat quality of *Kagoshima Berkshire*. In the production of recombinant swine using such specific genes, the analysis of functions of the swine genes is an indispensable step.

During the last several years, studies of the linkage map and physical maps of the swine genome have advanced remarkably. The PiGMaP Project was initiated in Europe and now involves 18 European labs and a total of 7 other labs from the US, Japan, and Australia. At present, nearly 1,800 markers and genes have been mapped in swine (Archibald 1994; Marklund et al., 1996; Rohrer et al., 1996). The physical genetic map in swine currently consists of over 600 genes. Several quantitative trait loci (QTL) scans and location of candidate genes were found on chromosomes while major genes associated with economic traits in swine were identified. Genes related to growth and back fat exist on chromosomes 3, 4, 5, 6, 7, 8, 13 and 14, genes related to meat quality exist on chromosomes 2, 3, 4, 6, 7, 12, and 15, and genes related to reproduction traits exist on chromosomes 4, 6, 7 and 8. In addition, ESR and PRLR, candidate genes related to litter size, FUT1, a gene for disease resistance, SLA, NRAMP, and KIT, genes for coat color, and MSHR were identified.

Growth was one of major economic traits of swine. Investigating the main traits, genes related to growth were analyzed using a Wild Boar and Large White three-generation family. The analysis revealed major QTL accounting for 20% of the phenotypic variance for back fat and abdominal fat on chromosome 4. A QTL for growth rate was found on chromosome 13, accounting for 7% to 12% of the phenotypic variation. By candidate gene analysis, Andersson et al. (1994) found that PRT1 is associated with back fat and birth weight and maps in the center of chromosome 13. The swine MHC is positioned on chromosome 7 (Genet et al., 2001). Associations between MHC haplotypes and several traits have been reported over the years. These have been confirmed, in part, using MHC class DNA probes. Recently, QTL related to growth and back fat traits was found on chromosome 7 in a Chinese breed. The QTL for back fat and birth weight are shown to

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be located near the region of the TNFA and SO102. The overall results to date suggest that at least one growth and back fat QTL exists in this region. Other results have included a growth trait QTL on chromosome 6, but it seems to be associated with an effect of the RYR1 gene, causing malignant hyperthermia, or other unknown genes around RYR1 (Harbitz et al., 1996). Some similar associations have been reported for chromosomes 3, 6, 8, and 14. Additionally, according to Gerbens and Tepas et al. (2001), it was reported that the fatty acid absorbing protein in the heart and major genetic factors are associated with average daily gain. Other candidate genes, including Leptin, CCK and CCKAR, have been mapped and may prove to be associated with appetite, fatness, and growth traits.

Next, in connection with meat quality traits, it has been reported that PSE pork is caused by RYR1 on chromosome 6. This has been demonstrated to be associated with several meat quality traits related to PSE in an F2 population originating from a Pietrain background. Focus has also centered on Hampshires for the RN gene, which is associated with increased glycogen content and lower pH in the meat. The RN gene has now been mapped to chromosome 15 and is located between flanking markers (Mariani et al., 1996). Andersson et al. have conducted one of the most complete QTL scans for meat quality using 234 markers on 191 F2 animals for mapping. QTL for several meat quality traits (pH, water holding capacity, and pigmentation) were found to be on chromosomes 2 and 12. Rothschild et al. (1998) reported that growth and performance traits are associated with regions on chromosomes 4 and 7. Additional associations with meat quality traits have been reported on chromosome 7 and for a number of muscle fibers on chromosome 3. The activity of Malic enzyme, a lipogenic enzyme in muscle, has been shown to be associated with the SLA complex on chromosome 7. Furthermore, a major QTL for androstenone level, which is associated with boar taint in the region of the SLA complex, was found. Among candidate genes investigated for muscle quality, heart-fatty acid-binding protein (H-FABP) gene may be associated with intramuscular fat. Many genes were also found for myogenesis.

For reproduction traits, since larger resource families and time are required to obtain information thereon, thus making the study difficult, results of QTL scan for these traits are limited. Wilkie et al. (1999) reported QTL for uterine length and ovulation rate, through in different chromosomal positions. Rathje et al. (1997) reported a QTL related to ovulation rate on chromosome 8, but Wilkie et al. observed some differences from the ovulation-related QTL. In France, a QTL experiment by Milan et al. (1998), a QTL for increasing litter size of one piglet was found in the same location on chromosome 8 as Rathje et al. The large ovulation rate-related QTL on chromosome 8 is of interest as it mapped to the same region to the Booroola gene in sheep. Interestingly, significant effects of this locus were also found for litter size in commercial lines. Limited chromosome QTL analyses for reproductive QTL have been conducted on chromosomes 4, 6, 7, 13, and 15. It has been clearly demonstrated that the estrogen gene is significantly associated with litter size. Though genetic effects vary according to the breeds, the increase is 1.15 swine/litter in Meishan synthetics and 0.42 swine/litter in Large White lines. More recent results have demonstrated that the prolactin receptor locus is significantly associated with litter size.

Finally, for disease resistance and immune response traits, to date, QTL scans for disease resistance or immune response have been limited. Some immunity related QTL have been identified. Also, a QTL for cortisol level, which may be related to stress and perhaps immune response, has been mapped to the end of chromosome 7. Two alpha genes FUT1 and FUT2 on porcine chromosome 6 have been identified. Vogeli et al. (1997) have disclosed a marker showing a polymorphism that is closely linked to ECF18R gene in Large White, Landrace, Hampshire, Duroc, and Pietrain swine and it could be a good marker for marker assisted selection (MAS) of *E. coli* F18 adhesion resistant animals in these breeds. Recently, it has been reported that the SLA complex on chromosome 7 is associated with resistance to infections with *Trichinella spiralis* but not resistance to toxoplasmosis. The NRAMP1 gene, known to be associated with resistance to Salmonella challenge in mice, has been recently mapped to swine chromosome 15 (Sun et al., 1998). Genes associated with human disorders, which have been identified in swine, include clotting factor IX (Sarkar et al., 1990) and the hypercholesterolaemia gene (Grunwald et al., 1999).

We made efforts to find candidate genes for genetic improvement of economic traits in swine, that is, for development of swine with excellent growth performance, meat quality, disease resistance, and reproductive performance. Thus far, several technologies to analyze gene expression at mRNA level such as northern blotting, differential display, sequential analysis of gene expression, and dot blot analysis have been used to examine genetic differences in swine. However, these methods have disadvantages that make them unsuitable for simultaneous analysis of a plurality of expressed products. Recently, the new technology of cDNA microarray has been developed to overcome these drawbacks. The cDNA microarray has become one of the strongest means to study gene expression in various living organisms. This technology is applied to simultaneous expression of numerous genes and the

discovery of genes on a large scale, as well as genetic polymorphism screening and mapping of genetic DNA clones. In this study, we identify novel specific genes related to the growth of muscle in swine using cDNA microarray technology and analyze functions of these genes with the aim of identifying swine that grow more quickly.

MATERIALS AND METHODS

Breeds and tissues

Total RNA for array fabrication was extracted from the muscle and fat tissue of the longissimus dorsi of *Kagoshima Berkshire* from the SUNG-CHOOK farm with body weight of 30 kg and 90 kg, respectively.

For the preparation of target DNA to screen the muscle specific genes expressed in the muscle and fat tissues of swine, tissue samples were collected from same swine as RNA preparation. 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 composed 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, and then dried and stored at -20°C.

A total of 4,434 cDNAs (ESTs) prepared as described above were cloned to analyze nucleotide sequences of 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. Then, the probe DNA was spotted on a slide glass for a microscope (produced by Corning), and subsequently coated with CMT-GAPSTM aminosilane using Microgrid II (Biorobotics). The probe DNA was printed onto a Microgrid II using a split pin. The pin apparatus was approached to the 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 was 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). Then, the slide was washed three times with 0.2% SDS at room temperature for 1 minute and once with 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 use 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 a Trizol TM kit (Life Technologies, Inc.) to prepare the target DNA. TrizolTM was added to the tissue in an amount of 1 ml of TrizolTM 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, 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 transferred to a new tube. 500 μl of isopropanol was added to the tube, voltexed, and placed on ice for 15 minutes. The ice was cooled and centrifuged at 4°C at a 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 into 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-18 mer 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

Table 1. Identifying nucleotide sequences of growth factor-related genes using cDNA microarray

GF (Growth factor) I gene:
gagaccagca aatactatgt gaccatcatt gatgccccag gacacagaga cttcatcaaa 60
aacatgatta caggcacatc ccaggctgac tgtgctgtcc tgattgttgc tgctggtgtt 120
ggtgaatttg aagctggtat ctccaagaac gggcagaccc gcgagcatgc tcttctggct 180
tacaccctgg gtgtgaaaca gctgattgtt ggtgtcaaca aaatggattc caccgagcca 240
ccatacagtc agaagagata cgaggaaatc gttaaggaag tcagcaccta cattaagaaa 300
cttggctaca accctgagac agtagcattt gtgccaattt ctggttggaa tggtgagaac 360
ctgctggagc caagtgctaa attgccttgg ttcaagggat ggaaagtcac ccgcaaagat 420
ggcagtgcca gtggcaccac gctgctggaa gctttggatt gtatcctacc accaactagt 480
ccaactgaca agcctctgcg actgcccctc caggatgtct ataaaattgg aggcattggc 540
actgtccctg tgggccgagt ggagactggt gttctcaaac ctggcatggt ggttaccttt 600
gcctcagtca atgtaacaac tgaagtcaag tctgttgaaa tgcaccatga agctttgagt 660
GF (Growth factor) II gene:
gctgactgat cgggagaatc agtctatctt aatcaccgga gaatccgggg caggaaagac 60
tgtgaacacg aagcgtgtca tccagtactt tgccacaatc gccgtcactg gggagaagaa 120
gaaggaggaa cctactcctg gcaaaatgca ggggactctg gaagatcaga tcatcagtgc 180
caaccccctg ctcgaggcct ttggcaacgc caagaccgtg aggaacgaca actcctctcg 240
ctttggtaaa ttcatcagga tccacttcgg taccactggg aagctggctt ctgctgacat 300
cgaaacatat cttctagaga agtctagagt cactttccag ctaaaggcag aaagaagcta 360
ccacattttt tatcagatca tgtctaacaa gaagccagag ctcattgaaa tgctcctgat 420
caccaccaac ccatctgact acgccttcgt cagtcaaggg gagatcactg tccccagcat 480
tgatgaccaa gaggagctga tggccacaga tagtgccatt gaaatcctgg 530

GF (Growth factor) I gene:
gagaccagca aatactatgt gaccatcatt gatgccccag gacacagaga cttcatcaaa 60
aacatgatta caggcacatc ccaggctgac tgtgctgtcc tgattgttgc tgctggtgtt 120
ggtgaatttg aagctggtat ctccaagaac gggcagaccc gcgagcatgc tcttctggct 180
tacaccctgg gtgtgaaaca gctgattgtt ggtgtcaaca aaatggattc caccgagcca 240
ccatacagtc agaagagata cgaggaaatc gttaaggaag tcagcaccta cattaagaaa 300
cttggctaca accctgagac agtagcattt gtgccaattt ctggttggaa tggtgagaac 360
ctgctggagc caagtgctaa attgccttgg ttcaagggat ggaaagtcac ccgcaaagat 420
ggcagtgcca gtggcaccac gctgctggaa gctttggatt gtatcctacc accaactagt 480
ccaactgaca agcctctgcg actgcccctc caggatgtct ataaaattgg aggcattggc 540
actgtccctg tgggccgagt ggagactggt gttctcaaac ctggcatggt ggttaccttt 600
gcctcagtca atgtaacaac tgaagtcaag tctgttgaaa tgcaccatga agctttgagt 660
GF (Growth factor) II gene:
gctgactgat cgggagaatc agtctatctt aatcaccgga gaatccgggg caggaaagac 60
tgtgaacacg aagcgtgtca tccagtactt tgccacaatc gccgtcactg gggagaagaa 120
gaaggaggaa cctactcctg gcaaaatgca ggggactctg gaagatcaga tcatcagtgc 180
caaccccctg ctcgaggcct ttggcaacgc caagaccgtg aggaacgaca actcctctcg 240
ctttggtaaa ttcatcagga tccacttcgg taccactggg aagctggctt ctgctgacat 300
cgaaacatat cttctagaga agtctagagt cactttccag ctaaaggcag aaagaagcta 360
ccacattttt tatcagatca tgtctaacaa gaagccagag ctcattgaaa tgctcctgat 420
caccaccaac ccatctgact acgccttcgt cagtcaaggg gagatcactg tccccagcat 480
tgatgaccaa gaggagctga tggccacaga tagtgccatt gaaatcctgg 530

GF (Growth factor) IV gene:
catttatgag ggctacgcgc tgccgcacgc catcatgcgc ctggacctgg cgggccgcga 60
tctcaccgac tacctgatga agatcctcac tgagcgtggc tactccttct gaccacagct 120
gagcgcgaga tcgtgcgcga catcaaggag aagctgtgct acgtggccct ggacttcgag 180
aacgagatgg cgacggccgc ctcctcctcc tccctggaaa agagctacga gctgccagac 240
gggcaggtca tcaccatcgg caacgagcgc ttccgctgcc cggagacgct cttccagccc 300
tccttcatcg gtatggagtc ggcgggcatt cacgagacca cctacaacag catcatgaag 360
tgtgacatcg acatcaggaa ggacctgtat gccaacaacg tcatgtcggg gggacaccac 420
GF (Growth factor) V gene:
tatatagaac cgaatcacgt acactgggcc tgaccaagca gggccaaaac aaggcaacct 60
aggaggttat aaaataggta tacgcgcgct gacacataca tactcactac ccgaacgcgg 120
ggacaactag ggctccgcca taagccatcc tttcctggtc gtcgatgttg cgggctgcag 180
ttatagggct gccaaccgcc atacacacct taccagccac ttattaagtt acatccacga 240
gggctctgta ccacccctaa gcagtggcag tggtagccgc tgcccgctta ccctgcgcag 300
tgttggtgct agctccgtcc taagcttccc cgatagccgc cgctttttac acaccatcgg 360
cggactagac accgttggtt gcagcgtaag cgtctatggt agcagctgcg gcgaccgccg 420
tgtagccagc ttactacatg ttagtttcag caaccaccct gccaataccc gtgttcccta 480
ctccaactct gtcggtttca gccgcag 507

GF I: Mus musculus eukaryotic translation elonggation factor1 alfa1.
GF II: Sus scrofa mRNA for myosin heavy chain.
GF III: Homo sapiens HCV NS3-transactivated protein 1.
GF IV: Homo sapiens actin, alpha 1, skeletal muscle.
GF V: Mus musculus chromosome 18.

units of RNase inhibitor (Invitrogen Life Technology), 100 units of M-MLV RTase, and 2 μl of 10×first strand buffer were added thereto and mixed with a pipette. 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 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 slide was scanned on a ScanArray 5000 (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 4000XL 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

Comparison of patterns of gene expression in muscle tissue of swine according to growth stage

A cDNA microarray was prepared with the 4,434 genes obtained from the muscle tissue of swine to identify genes related to muscle growth. The entire expression patterns of genes expressed in the early growth stage muscle (ESM) of swine with body weight of 30 kg were compared with those of genes expressed in the adult growth stage muscle (ASM) of swine with body weight of 90 kg using the cDNA microarray.

Specifically expressed columns among the 4,434 ESTs get cDNA microarray (data not shown). Hybridization in the cDNA microarray was shown well and specifically expressed genes were well distributed. Also, twenty genes showed a 5 times higher expression level in ASM, as compared to ESM (Table 1). 18 genes showed a 5 to 10 times higher expression level in ESM, as compared to ASM. Putative muscle growth-related genes, that is, genes promoting relaxation and contraction of muscle and meat

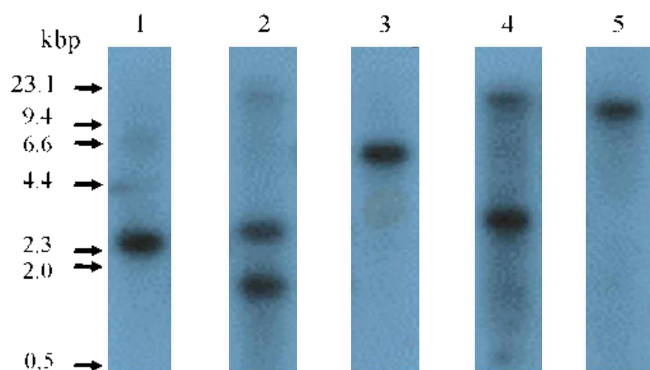

Figure 1. Genomic southern blot analysis. Ten micrograms of DNA were digested *Eco*R I; fractionated on a 0.8% agarose gel; and transferred onto a nylon membrane. The membranes were probed with the full-length of each gene. P[32]-labeled swine muscle cDNA. Size markers are shown on the left in kilobase pairs. Confirming existences and copy number of the swine genes. obtained using cDNA microarray. Lane 1: GF I. Lane 2: GF II. Lane 3: GF III. Lane 4: GF IV. Lane 5: GF V.

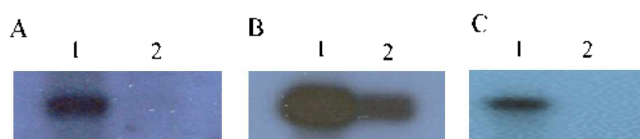

Figure 2. Expression patterns of the muscle *GF* (Growth factor) genes of swine in the RNA level according to the muscle tissues of swine growth stage. A: GF I; B: GF III; C: GF V. Lane1: 90 kg muscle. Lane 2: 30 kg muscle. * Actin are shown as a control.

quality-related genes, were expressed in a high ratio. Glycyl-tRNA synthase, which is a gene affecting meat quality and energy metabolism, showed a high expression level. Glycyl-tRNA synthase is an enzyme known to promote synthesis of glucose when new energy or glucose is needed after glycolysis. It is thus considered to express higher in ASM (90 kg) than ESM (30 kg) and affect the formation and quality of ASM (90 kg) to supply sufficient energy for formation and quality of swine muscle. Actin and collagen, which have a strong effect on contraction and relaxation of muscle, showed a high expression level. The gene known as Elongation factor in human showed a high expression level in swine. Galectin, which is known to be associated with meat quality, also showed a difference in expression patterns. Accordingly, it is expected that a basal model for study on the growth mechanism in humans may be presented using the genes showing differences in expression patterns. Considering the genes expressed high in the muscle tissue of the swine with body weight of 30 kg, it was observed clearly with the naked eye that the expression level of the genes available to be used as energy source to formation of muscle was higher than that of the genes affecting contraction and relaxation of muscle, namely, meat quality. Fructose-1, 6-bisphosphatese, and NADH play a key role in formation of ATP and are suggested to supply sufficient energy source for growth of muscle. COT, LIM domain 1 protein, Reticulum Protein, Kel-like protein 23 and the like were already demonstrated as energy sources for the formation of bone structure or muscle in other organs.

Sequencing of specific genes related to muscle growth of swine

To identify specific genes related to muscle growth of swine using the cDNA microarray prepared with the 4434 clones that have been obtained from the muscle tissue of swine, we investigated the expression pattern of the genes by hybridizing 4.4 kb cDNA. This was obtained from RNA isolated from the muscle tissues of swine with body weight of 30 kg and swine with body weight of 90 kg, on a microarray slide.

As shown in Table 2, 5 specific genes and their information were obtained from the expression pattern of the chip. Considering information obtained from the sequencing that was performed before preparing the chip, the 5 genes are suggested to be unknown genes, and even nucleotide sequences. Therefore, in this study, sequencing was carried out again to accurately identify their nucleotide sequences. The results are shown in Table 2. The 5 genes are judged as novel genes by search with the database at NCBI.

Confirming existence of the genes in the genome of swine and analyzing expression pattern of the genes

Southern blotting was performed to investigate whether the genes, discovered above, exist in the genome of swine. First, genomic DNA was extracted from the blood of the swine, digested with *Eco*RI, and used to make a blot. Blotting was then carried out using the blot and probe, which was prepared with the 5 genes showing a high expression level.

As the result, all of the 5 genes were found to exist in the genome of pig. Among them, 3 genes existed as one copy and 2 genes existed as 2 copies. We are currently carrying out an analysis of molecular biological experiments for the 3 genes. Regarding the other 2 genes, we are conducting experiments to determine the mechanism between them, since the genes are considered to have peculiar isoforms, respectively.

Comparison between *in vivo* expression patterns of growth factor genes in swine muscle

Expression patterns of the 3 genes, which exist as 1 copy in the genome of swine, among the 5 growth factor genes were investigated at the RNA level. The expression of the 3 genes showed the same pattern with the expression ratio in the experiment using the cDNA microarray. The expression pattern of the 3 genes in the muscle tissues of

Figure 3. Expression patterns of the *GF* genes according to the other tissues of swine. A: GF I; B: GF III; C: GF V. Lane 1: 90 kg muscle, Lane 2: 30 kg muscle, Lane 3: uterus, Lane 4: ovary. * Actin are shown as a control.

the swine with body weight of 30 kg and the swine with body weight of 90 kg were also investigated to assess whether the genes are only associated with formation and quality of muscle, respectively. As shown in Figure 1, the 3 genes were expressed more highly in the muscle tissue of swine with body weight of 90 kg.

To discover the expression pattern of the 3 genes in other tissues, we extracted RNA from the reproductive tissue and investigated their expression pattern (Figure 3). The 3 genes were also expressed more highly in the muscle tissue of swine with body weight of 90 kg.

From the above results, we found that the 3 genes were not expressed in other tissues, namely, propagation tissue, and are specific genes only related to muscle growth of swine.

DISCUSSION

Recently, there have been many studies using human chip after completion of the human genome project. Research on cancer in humans constitutes the greater part of this work, involving efforts to find cancer-inducing genes and to reveal functions of the genes by analyzing expression patterns between cancer cells and normal cells.

A study on inhibiting proliferation of live cancer by finding a promoter of genes related to the growth of liver cell and growth process of the genes in human is in progress (Park et al., 2003).

Current investigation has demonstrated that insulin-like growth factor-I gene acts as an intermediate enzyme informing the growth of a myosin heavy chain in the mechanism of myosin heavy chain during growth of human muscle and MHC, IIA/IIX family also promotes formation and growth of muscle in human by catalyzing the insulin growth factor-1 gene (Flint et al., 2004).

In addition, the mechanism of mobility of vascular smooth muscle cells has been elucidated in recent research (Wang, 2004). The research has reported that thrombin-induced gene dl p38 MAPKdp activates the mobility of vascular smooth muscle cells and diphenyleneiodonium (DPI) inhibits the mobility of vascular smooth muscle cells. This suggests that promoting the function of the vascular smooth muscle cell activates development or function of the muscle while inhibiting the function of the vascular smooth muscle cell yields myasthenia or muscle cancer.

As outline above, many factors are used for studies on mechanisms involved in muscle or growth of human. However, generally mice or rabbits are used as experimental animals in these studies, and as such it remains uncertain whether the mechanism can be applied to humans practically. Therefore, it is expected that a complete analysis of the genes identified in the present study will provide useful information to elucidate the mechanism of growth of human or cancer cells.

Therefore, we found that expression patterns of genes in swine with body weight of 30 kg were considerably different from those of genes in swine with body weight of 90 kg. That is, in the early growth stage of swine, the genes available to be used as energy sources for formation and sustenance of muscle are expressed much more highly. In addition, many genes were used for tissue formation of muscle rather than elongation of muscle; however, in the adult growth stage of swine, genes related to quality, contraction, relaxation, and growth of muscle were more highly expressed.

Further study on the functions of these genes in various ways would be useful to develop a new breed of swine with the trait of rapid muscle growth and to treat short stature patients or genetic disease patients.

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