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A novel pig gene, *FASTK*, differentially expressed in the muscle tissues from Wujin and Large White pigs*

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The mRNA differential display technique was used to investigate gene expression differences in the *longissimus dorsi* muscle from Wujin and Large White pigs. A fragment of one differentially expressed gene was isolated and sequenced. A complete cDNA sequence of the gene was obtained using the rapid amplification of cDNA ends (RACE) method. The open reading frame of this gene encodes a protein of 542 amino acids, which is homologous with the Fas-activated serine/threonine kinase (FASTK) of seven species: cattle 92%), sumatran orangutan (91%), chimpanzee (90%), rhesus (85%), dog (92%), mouse (87%) and human (91%). This newly identified gene was respectively defined as the swine *FASTK* gene and has been assigned GeneID: 100233181. The phylogenetic tree analysis revealed that the porcine *FASTK* has a closer genetic relationship with the *FASTK* of cattle. The tissue expression analysis indicated that the *FASTK* gene is differentially expressed in various porcine tissues. The presented investigation is the first to establish the primary foundation for further research on the swine *FASTK* gene.

KEY WORDS: FASTK / mRNA differential display / muscle tissue /pig

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The mRNA differential display first described by Liang and Pardee [1992], remains an efficient tool for comparative profiling of gene expression under different experimental conditions. It has statistically been shown that 80-120 primer combinations would be sufficient to cover all the transcript populations in the cell [Liang *et al*. 1993]. Such assay has the following advantages: (1) it is based on well-established methods, (2) more than two samples can be compared simultaneously, and (3) only a small amount of starting material is needed [Yamazaki and Saito 2002].

Chinese indigenous pig breeds such as Wujin, Meishan, Erhualian and Tongcheng often have superabundant fat and too low lean meat content, while exotic breeds, such as Large White, Landrace and Duroc, have lower fat and higher content of lean of the carcass. Therefore, Chinese indigenous pigs are always named fat-type, while exotic pigs are always named the lean-type [Pan *et al*. 2004]. Given that phenotypic variances are mainly determined by the genetic differences, the identification of differentially expressed genes between Chinese indigenous and exotic pig breeds can give considerable promises for breeding.

The present study was carried out with the mRNA differential display technique to identify the differentially expressed genes in the muscle tissue from one Chinese indigenous Wujin breed and exotic Large White breed. Results are presented of the identification of earlier unrecognized porcine gene *FASTK*, which is differentially expressed in Large White *vs* Wujin pig skeletal muscle.

Material and methods

Samples collection

The *longissimus dorsi* (LD) muscle samples were collected from 120-day-old Large White (five males and five females) and Wujin (five males and five females) pigs for mRNA differential display and semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) analyses.The tissues including spleen, ovary, heart, small intestine, liver, lung, kidney, muscle and fat (from backfat) were collected from one adult Wujin × Large White crossbred pig for the later tissue expression profile analysis. Tissues were immediately frozen in liquid nitrogen and stored at -80°C. The total RNA was extracted from tissues using the total RNA extraction kit (GIBCO, Grand Island, NY, USA) in accordance with the manufacturer's recommendations. Before the first-strand complementary DNA (cDNA) synthesis, DNase I treatment of the total RNA was done.

Differential display

The differential display PCR amplification of each reverse transcription product was carried out with ten arbitrary and nine oligo (dT) primers [Liu *et al*. 2004, 2005ab]. The PCR products were then separated on the 8% non-denaturing polyacrylamide gel and displayed using the silver stain as described by Pan *et al*. [2003] and Liu *et al*. [2004].

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as described by Liu and Xiong [2009a, 2009b]. To avoid the influence of cDNA concentration on semi-quantitative RT-PCR, repeated were PCR amplifications using 100, 200, 300, 400 and 500 ng cDNA as template. The housekeeping gene GAPDH was selected as the internal control. The control gene primers used were: 5'-ACCACAGTCCATGCCATCAC-3' (forward primer 1) and 5'- TCCACCACCCTGTTGCTGT -3' (reverse primer 1). The 420-bp PCR product was verified by sequencing. The following primers were used to perform the RT-PCR for identification and tissue expression profile analysis of the *FASTK* gene: 5'- GGATATAGTAGCAGAGGG-3' (forward primer 2) and 5'-CCGCAGCGTCAGCACCAC-3' (reverse primer 2). The PCR product was 220 bp in length (verified by sequencing). The 25 µl reaction system contained 2 µl cDNA (100-500 ng), 5 pmoles each oligonucleotide primer (forward primer 1 and 2, reverse primer 1 and 2), 2.5 µl 2 mmol/l mixed dNTPs, 2.5 µl 10×Taq DNA polymerase buffer, 2.5 µl 25 mmol/l $MgCl_2$ and 2 U of Taq DNA polymerase. The PCR programme initially started with a 94°C) denaturation for 4 min, followed by 25 cycles of 94°C/50 s, 60°C/50 s, 72°C/ 50 s, then 72°C extension for 10 min, finally 4°C to terminate the reaction.

PCR products were analysed in the linear range of amplification by agarose gel electrophoresis and intensity of bands was estimated using GLYCO BandScan Software (PROZYME®, San Leandro, CA, USA). The ratio of FASTK to GAPDH was calculated using EXCEL programme. Significance of difference of ratios of FASTK to GAPDH was verified with the least squares method (GLM procedure, SAS version 8.0).

Rapid amplification of cDNA ends (5'- and 3'-RACE)

The 5'- and 3'-RACE were performed following the instructions of BD SMART™ RACE cDNA Amplification Kit (BD Science, USA).

The Gene-Specific Primers (GSPs) were:

5'-RACE GSP: 5'- CACCCTTTGGGCAGGGTCTTGGGTT-3', and 3'-RACE GSP:5'- CTTCCTGCCATATCCTCCCAGGTCC-3'.

RACE touchdown PCRs were carried out with 5 cycles of 94°C / 30 s and 72°C / 3 min, followed by 5 cycles of 94°C / 30 s, 70°C / 30 s and 72°C / 3 min, finally with 30 cycles of 94°C / 30 s, 67 °C / 30 s, 72°C / 3 min to terminate reaction. The RACE PCR products were then cloned into pMD18-T vector (TaKaRa, Dalian, China) and sequenced bidirectionally with the commercial fluorometric method (SHENGGONG, Shanghai, China). At least five independent clones were sequenced for each PCR product.

Sequence analysis

The cDNA sequence prediction was conducted using GenScan software ttp:// genes.mit.edu/GENSCAN.html). Protein sequence prediction and analysis were

performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the National Centre for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and ClustalW software (http://www.ebi.ac.uk/ clustalw).

Results and discussion

The mRNA differential display

From the mRNA differential display, one band, nominated as the band 132, later identified as a fragment of the *FASTK* gene, was found to be predominantly expressed in the LD muscle of Large White, while it was barely visible in the band pattern of the LD of Wujin pigs (Photo 1).

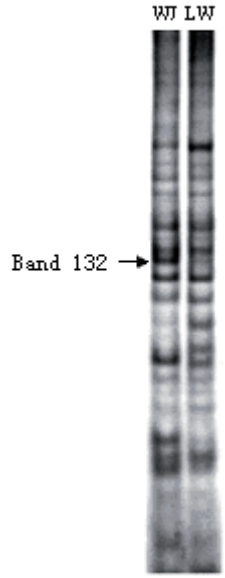

Photo 1. Representative band pattern on mRNA differential display analysis showing upregulated band 132 (arrow, about 400 bp) in Large White (LW) *vs*. Wujin (WJ) LD muscles.

Semi-quantitative RT-PCR

The differentially expressed band was recovered from gel and used as the template for the re-amplification, which was performed with the corresponding oligo(dT) and arbitrary primers used in the mRNA differential display assay. The resulting PCR product was 424 bp long being consistent with that of the differential display (Photo 1). The purified PCR product was then cloned into the pMD18-T vector and the recombinant plasmid was sequenced. Semi-quantitative RT-PCR was conducted and the results (Photo 2) indicated that the band 132 (FASTK) is predominantly expressed in the LD muscle of Large White pigs.

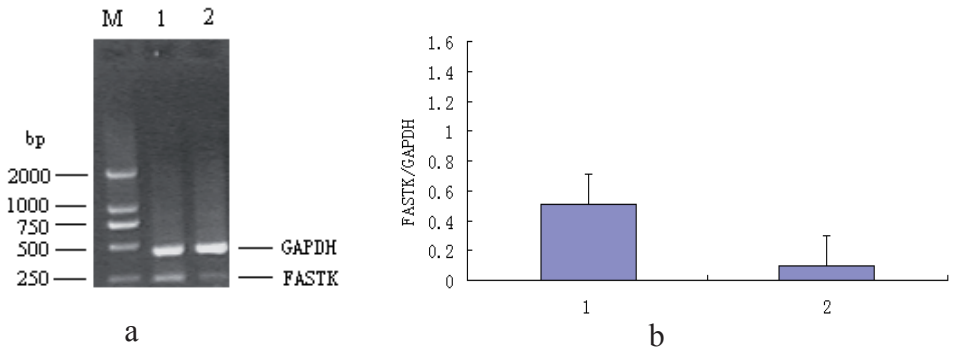

Photo 2. Representative (a) and calculated (b) FASTK mRNA expression levels in Large White (1) *vs.* Wujin (2) LD muscle samples (RT-PCR, n=10).

The 5'- and 3'-RACE and sequence analysis

Through 5'-RACE, one PCR product of 1455 bp was obtained. The 3'-RACE product was 395 bp. These products were then cloned to T-vector and sequenced. The alignment of these 61-bp overlapping sequences yielded a 1789-bp cDNA sequence (Fig.1). The nucleotide analysis, using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST), revealed that a 1789-bp cDNA sequence was not homologous to any of the known porcine genes, and it was then deposited into the GenBank database (Accession number: FJ438466). The sequence prediction was carried out using the GenScan software. An open reading frame (ORF) encoding 542 amino acids was found in the 1789-bp cDNA sequence. The complete cDNA sequence of the gene and the encoded amino acids are shown on Figure 1.

GGTTCCGGGGGCTGCTGGGAAGATGGCGGACTCGGTGGCCCGCCGATGAGGAGGCCGCGGGGGGAG
M R R P R G E
CCCGGCTCTCGGGCCCCGAGACCGACTGAGGGAGCGATCTGCGCGGGGCCCGGGGAGTCATGGTCT
P G S R A P R P T E G A I C A G P G E S W S
CCATCACCCAACTCCATGCTTCGAGTCCTGCTCTCTGCCCAGGCCTCCAGCGCTCGGCTCTCTGGC
P S P N S M L R V L L S A Q A S S A R L S G
CTGCTGCTGCTCCCGCCAGTACAGCCCTACTGTCTGGGGCCCAGCAAGTGGGGGGACCGGCCTCCT
L L L L P P V Q P Y C L G P S K W G D R P P
GGAGGAGGCTCCCATGTAGGCCCTGTGCAGGGGCTGCAGCGGCTTCTGGAACAGGCAAGGAGCCCT
G G G S H V G P V Q G L Q R L L E Q A R S P
GGGGAGCTGCTGCGCTGGCTGGGCCAGAACCCCACCAAGGTCCACGCCCACCACTACCTTGTGGCA
G E L L R W L G Q N P T K V H A H H Y L V A

CTTCGTCGCCTGGGTCAGCTCTTGGGGTCCCAGCCACGGCCCCCTCCCGTGGAGCAGGCCACACTA
L R R L G Q L L G S Q P R P P P V E Q A T L
CAGGACTTGAGTCAGCTCATCATCCGAAACTGCCCCTCCTTTGACATTCACACCATCCACGTGTGT
Q D L S Q L I I R N C P S F D I H T I H V C
CTGCACCTCGCAGTCTTACTTGGCTTCCCATCAGATGGGCCCCTGGTGCGTGCCCTGGAACAGGAG
L H L A V L L G F P S D G P L V R A L E Q E
CGCAGGTGCCGCCTCCATTCGAAGCCGCCTCCCTCTCCGCAACCTGTCCTTCATGGTGGGCAAAGG
R R C R L H S K P P P S P Q P V L H G G Q R
TTGGAAGCTGCTTTGAGCTGCCCCCGTTTCCTGCGGCATCCACAGCAGCACCTCATCCGCAGCCTG
L E A A L S C P R F L R H P Q Q H L I R S L
GCAGAGGCCAGGCCAGAGGAACTGACTCCCCAAGTGATGGTGCTCCTGGCCCAGCACCTGGCCCGC
A E A R P E E L T P Q V M V L L A Q H L A R
CACCGGTTGCGGGAACCCCAGCTTCTGGAAGCTATTGCTCACTTTTTGGTGGTCCAGGAAGCCCAG
H R L R E P Q L L E A I A H F L V V Q E A Q
CTCAACAGCAAGGTGGTCCAGAAGTTGGTCCTGCCCTTTGGGCGGCTGAACTACCTGCCTCTGGAA
L N S K V V Q K L V L P F G R L N Y L P L E
GAGCAGTTTATGCCCTGCCTCGAGAGGATCCTGGCTCGGGAAGCAGGGGTGGCCCCCCTGGCAACT
E Q F M P C L E R I L A R E A G V A P L A T
GTCAACATCTTGATGTCACTGTGCCAGCTGCGGTGCCTGCCCTTCCGAGCCCTGCGCTTTGTCTTT
V N I L M S L C Q L R C L P F R A L R F V F
TCCCCAGGTTTCATCAATCACATCAGTGGCACCCCTCACGCCCTGATCATGCGGCGCTACCTCTCC
S P G F I N H I S G T P H A L I M R R Y L S
CTGCTGGACACAGCCGTGGAGCTGGAGCTCCCGGGATACCGGGGTCCCCGCCTTCCCCGAAGGCAG
L L D T A V E L E L P G Y R G P R L P R R Q
CAAGTGCCCATCTTCCCCCAGCCACTCATCACCGACCGCGCCCGCTGCAAGTACAGCCACAAGGAT
Q V P I F P Q P L I T D R A R C K Y S H K D
ATAGTAGCAGAGGGGCTGCGCCAGCTGCTGGGGGAGGAGAAGTACCGGCAGGACCTGACCGTGCCT
I V A E G L R Q L L G E E K Y R Q D L T V P
CCAGGCTACTGCACAGACTTCCTGCTGTGTGTCAGCAGCTCTGGTGCTGTGCTTCCTGTGAGAACC
P G Y C T D F L L C V S S S G A V L P V R T
CAGGACCCCTTCCTGCCATATCCTCCCAGGTCCTGTCCCCACGCAACCCAAGACCCTGCCCAAAGG
Q D P F L P Y P P R S C P H A T Q D P A Q R
GTGGTGCTGACGCTGCGGGAACGCTGGCATTTTTGCCGGGATGGCAGAGTGCTGCTGGGCTCCCGG
V V L T L R E R W H F C R D G R V L L G S R
GCCCTGCGGGAGCGGCACCTGGGCCTGATGGGCTACCAGCTCCTGCCGCTCCCCTTCGAGGAACTG
A L R E R H L G L M G Y Q L L P L P F E E L
GAGTCCCAGAGAGGCCTGCCCCAACTCAAGAACTACCTGAGGCAGAAGCTCCAGGCCCTGGGCCTC
E S Q R G L P Q L K N Y L R Q K L Q A L G L

CGCTGGGGGCCGGAAGGGGGGTGAGGGGGTGCACACGGGGTCCATGATGGCCCCCCCCATGGGGGGC
R W G P E G G *
GGACAATTTGCACTTTGACTCTCTGTTTTGATTTGTCATTAAAGCCCCCTTCTTCCCCCAAAAAAAAAA
AAAA

Fig. 1. The cDNA and amino acid sequence of the pig gene containing band 132 (GenBank accession number: FJ436390). ATG, start codon; TGA, stop codon (* – stop codon).

Further BLAST analysis revealed that the protein sequence is characterized by a high homology with that of the Fas-activated serine/threonine kinase (FASTK) of cattle (accession number: NP_001030249, 92%), sumatran orangutan (accession number: NP_001124735, 91%),chimpanzee (accession number: XP_528000, 90%), rhesus monkey (accession number: XP_001103566, 85%), dog (accession number: NP_001028683, 92%), mouse (accession number: NP_075718, 87%), and human (accession number: NP_006703, 91%).

Based upon the present results it is assumed that the gene in question can be defined as the pig *FASTK* gene. The results of the alignment of six known FASTK proteins mentioned allowed the phylogenetic tree to be constructed using the ClustalW software (Fig. 2). The pig *FASTK* gene is genetically closer to cattle than to human, rhesus monkey, mouse and dog *FASTIC* genes.

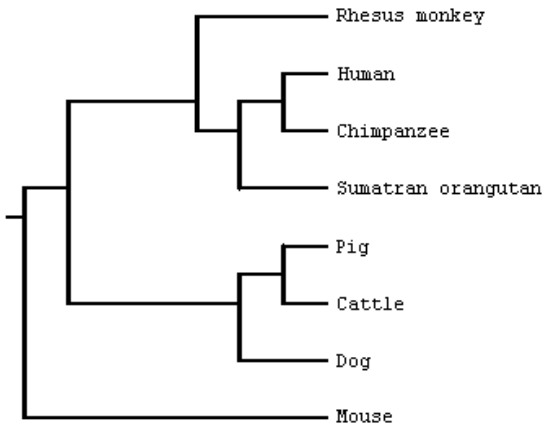

Fig. 2. The phylogenetic tree for the FASTK gene.

Tissue Expression

The RT-PCR profiling of tissue expression of the pig *FASTK* gene was carried out using samples from one adult crossbred of Wujin × Large White. The pig *FASTK* gene is moderately expressed in liver, muscle and heart, weakly in spleen and lung, and strongly in small intestine, fat and kidney (Photo 3).

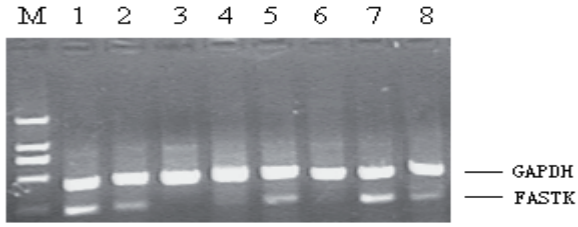

Photo 3. Photo RT-PCR profiling of the pig FASTK gene expression. M DL2000 marker kit (marker size the same as in Figure 1); 1 – liver; 2 – lung;, 3 – fat; 4 – small intestine; 5 – muscle; 6 – kidney; 7 – heart; 8 – spleen.

FASTK gene encodes the Fas-activated serine/threonine kinase which is a BCL-X(L)-associated mitochondrial protein. The kinase was shown to be activated rapidly during Fas-mediated apoptosis in Jurkat cells and synergizes with TIA1/TIAR proteins to regulate Fas alternative splicing. The encoded protein is a strong inducer of lymphocyte apoptosis [Izquierdo *et al.* 2007, Simarro *et al.* 2005, Li *et al.* 2004, Tian *et al.* 2005]. To this date, the *FASTK* gene was identified and characterized in cattle, sumatran orangutan, chimpanzee, rhesus monkey, dog, mouse, human and other animals; the porcine *FASTK* has not been reported yet. The present results show that the *FASTK* gene is differentially expressed in the LD muscle being a more abundant in Large White than in Wujin pigs. Wujin is a fat-type breed, the carcass of which contains much more body fat than lean meat/muscle. On the other hand, Large White is a typical lean-type pig, presenting the opposite phenotype to that described for the Wujin breed. The two pig breeds used in this study differ in lean meat percentage. It is, therefore, interesting that the expression of the porcine *FASTK* gene in the LD muscle shows the trend of a higher expression in Large White than in Wujin pigs. A major question is the extent to which such predominant expression could be developmentally or metabolically significant in terms of acquiring of any phenotypic change in favour of a higher lean-type rate. Clearly, this merits further study.

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