Molecular Characterization and Expression Patterns of Porcine Eukaryotic Elongation Factor 1 A

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ABSTRACT: The eukaryotic elongation factor 1 A (EEF1A) participates in protein synthesis by forming the eEF1A-GTP-tRNA complex to deliver aminoacyl-tRNA to the A site of ribosomes. This study described cDNA sequences and partial genomic structure of porcine EEF1A1. The porcine EEF1A1 gene encoded a protein with 462 amino acids, which shared complete homology with human, chimpanzee and dog. The temporal expression pattern showed the diversity of EEF1A1 level in mRNAs was relatively minor in prenatatal embryo skeletal muscle, however, the expression decreased during aging after birth in skeletal muscle of the Chinese Tongcheng pig. The spatial expression patterns indicated that the gene expressed in skeletal muscle, heart, lung, liver, kidney, fat and spleen. In addition, we assigned the gene to porcine chromosome 1 using a radiation hybrid panel. (Key Words: EEF1A1, Expression, Chromosome, Genome, Porcine)

INTRODUCTION

A variety of molecules have been identified in the complicated network system of protein synthesis, such as Guanine triphosphate (GTP), ribosome, initial factors, elongation factors and termination factors. In protein translational elongation process, elongation factor 1 A (EF1A) contributes to the binding of aminoacyl-tRNA to the A site of ribosomes at the presence of GTP and maintaining EF1A-GTP concentration in tRNA channeling which EF1βγ subunits were for exchange of GDP/GTP (Sanders et al., 1993). The elongation factor 2 promotes the translocation of tRNA with the nascent polypeptide from the A-site to the P-site of the ribosome (Joseph and Noller, 1998).

Eukaryotic EF1A has structurally been identified as a GTP-binding protein characterized consensus sequences including G-X4-G-K, D-X2-G and N-K-X-D sequences (Dever et al., 1987), which the three highly conserved motifs showed the important roles in the regulatory process of translational elongation by some previous mutation studies (Carr-Schmid et al., 1999; Knudsen et al., 2001; Chiron et al., 2005). Functions of EEF1A were also reviewed in cell process (Lamberti et al., 2004).

In this paper, we isolated the full-length cDNA of porcine EEF1A1 gene from a skeletal muscle cDNA library of porcine embryos, where the porcine eukaryotic translation termination factor 1 (ETF1) gene was also analyzed (Shao et al., 2005), characterized the genomic structure and patterns for temporal and spatial expression, identified the localization on porcine chromosome. All these will be the bases for a better understanding of EEF1A1 gene.

MATERIAL AND METHODS

Isolation of porcine EEF1A1 gene

The cDNA sequence of EEF1A1 gene was obtained from a porcine cDNA library of 55d-fetus skeletal muscle as methods described previously (Zhu et al., 2005), and shared higher homology with human EEF1A1 gene.

In order to analyze the genomic DNA sequence of EEF1A1 gene, the primers (Table 1) were designed in exons to amplify potential introns according to the comparing results of porcine cDNA and human DNA sequences of EEF1A1 gene in NCBI BLAST database (http://www.ncbi.nlm.nih.gov/blast/bl2seq/). Following optimization of all PCR conditions in PTC-100 (MJ research), every PCR fragment was purified and sequenced as described by Yang...
et al. (2005). Genomic DNA sequence of EEF1A1 was obtained through sequences assembling using DNAstar software. The genomic organization was identified and determined for the exon and intron size and junction phase of porcine EEF1A1. The information about genomic structure for porcine EEF1A1 gene was shown in Figure 1.

### Chromosome localization

Using the primers (Table 1), PCR was performed using the INRA-Minnesota Porcine Radiation Hybrid (IMpRH) panel in 10 μl reaction containing 1×PCR buffer, 20 ng panel DNA, 0.3 μM each primer, 75 μM each dNTP, 1.5 mM MgCl₂ and 1.0 U Taq DNA polymerase. In addition, the controls were designed containing pig, hamster genomic DNA and a blank control without DNA. The PCR protocol was 5 min at 94°C; 35 cycles of 20 s at 94°C, 20 s at 59°C, and 30 s at 72°C, and a final extension for 5 min at 72°C. The genotypes were electronically submitted and automatically analyzed by the IMpRH mapping program (Milan et al., 2000) available on the IMpRH web site (http://imprh.toulouse.inra.fr/).

### Analysis of expression patterns

Total RNA was extracted using TRIzol reagent kit (Promega) from seven tissues samples (skeletal muscle, heart, liver, lung, spleen, kidney and fat of Landrace), and skeletal muscle of six time points (prenatal 33 d-, 65 d-, 90 d-, postnatal 2 d-, 28 d- and adult Tongcheng pigs) and reverse transcript PCR (RT-PCR) was performed (Pan et al., 2003). PCR products were amplified as templates for cDNA in a volume of 20 μl containing 1×PCR buffer, 0.3 μM each primer, 75 μM each dNTP, 1.5 mM MgCl₂ and 2.0 U Taq DNA polymerase, which the program was 3 min at 94°C, 28 cycles of 20 s at 94°C, 20 s at 58°C, and 30 s at 72°C and a final 5-min extension at 72°C. Glycereraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference to standardize the targeted samples and the conditions were same above. The relative expression levels were analyzed using KODAK1D 3.6 software.

### RESULTS AND DISCUSSION

#### Analysis of full-length cDNA of porcine EEF1A1

A 1,783 bp sequence was obtained by double-direction sequencing and sequences assembling, and had 92% identity to both human and mouse EEF1A1 (GenBank Acc. No. NM_001402 and NM_010106). Open reading frame was predicted using ORF finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). As a result, the cDNA sequence contained 90 nucleotides of 5′ untranslation region, 1386 nucleotides of open reading frame and 307 nucleotides of 3′ untranslation region including polyA signal (ATTAAA) at nucleotide 1,744-1,750 upstream of a 19 bp polyA tail.

Protein characterization was analyzed in the website (http://kr.expasy.org/tools/). The protein predicted encoded 462 amino acids with molecular weight 50.14 kDa and theoretical isoelectric point 9.10. The predicted results in the PROSITE database showed the protein contained the typical structure of GTP-binding protein for the three consensus sequences, GHVDSGK was localized at 13-19 amino acids and DAPG localized at 100-104 amino acids for 58 amino acids upstream of NKMD (Figure 1).

Alignment of EEF1A1 amino acids sequences among different species was performed in NCBI BLAST database (http://www.ncbi.nlm.nih.gov/blast/bl2seq/). The results indicated the degree of homology is very high (up to 100%) between pig and human, Chimpanzee, Macaque and dog, but relatively low (99%, 99%, 96% and 91% respectively) between pig and mouse, rat, chicken and zebrafish. Therefore, we suggested the gene might derive from a common gene and be very conserved in evolution according to high homology among species and its central roles in various cell processes. Simultaneously, the results supported the closer relationship is between pig and human, which provides further foundation for organ xenotransplantation (pig to human).

#### Analysis of expression profiles

In order to observe expression alternations of EEF1A1 gene during skeletal muscle development, six important periods for muscle development of pigs were chosen and the gene was considered to be up-regulated when the intensity ratio between EEF1A1 and GAPDH was ≥1.8. Analysis of semi-quantity reverse transcriptase PCR (RT-PCR) assay showed the EEF1A1 expression trend was basically invariable during skeletal muscle development.

Table 1. Amplification primers for porcine EEF1A1

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5′-3′)</th>
<th>Annealing temperature (°C)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1L</td>
<td>CTACACATCACATCGTGTTC</td>
<td>59</td>
<td>693</td>
</tr>
<tr>
<td>P1R</td>
<td>CCGTTCTTGGAAATACCTG</td>
<td>58</td>
<td>638</td>
</tr>
<tr>
<td>P2L</td>
<td>TGACTACACCCTGGTACACA</td>
<td>58</td>
<td>638</td>
</tr>
<tr>
<td>P2R</td>
<td>CTTCATAGGTGGTCATT</td>
<td>58</td>
<td>900</td>
</tr>
<tr>
<td>P3L</td>
<td>GTGGTATATTGTACAGTCCC</td>
<td>58</td>
<td>900</td>
</tr>
<tr>
<td>P3R</td>
<td>GAAACAGTTTCATGAGACCGT</td>
<td>58</td>
<td>900</td>
</tr>
</tbody>
</table>

**Note:** Primers of chromosome mapping.
before birth, but the expression was decreased with age after birth in Tongcheng pigs. It was demonstrated that the different expression of *EEF1A1* was existed between prenatal (33 day- and 65 day-) and postnatal muscle in Tongcheng pigs (Figure 2). The previous study showed the *EEF1A1* gene was detected down-regulation expression from 75 day- fetal to 1 week- postnatal muscle in pig using cDNA macroarray method (Zhao et al., 2003). Another study showed *EEF1A1* expression level was decreased with aging in human fibroblasts (Cavallius et al., 1986).

**Figure 1.** Nucleotide and the predicted partial protein sequences of porcine *EEF1A1*. The exons are showed in uppercase and the introns are showed in lowercase. 5’ splice donor and 3’ splice acceptor (gt and ag) are in boldface. The stop codon (TGA) is indicated in underlined type. The polyadenylation signal (ATTAAA) is boxed. The three consensus sequences (GHVDSGK, DAPG and NKMD) are in shadow.
Therefore, our results were consistent with the previous studies. As we know, the increase of muscle mass was mainly due to the increase of muscle fibre number before birth and that of muscle fibre size after birth. So it was suggested that \textit{EEF1A1} may play an important role in formation of muscle fibres.

The result of spatial expression profile showed \textit{EEF1A1} expressed in every tissue detected, and higher in fat than in skeletal muscle, liver, heart, spleen, lung and kidney (Figure 3). It was thought that the wide expression was related to the important functions of \textit{EEF1A1} in protein elongation.

\textbf{Chromosome localization}

The result of chromosome physical mapping showed \textit{EEF1A1} was assigned to porcine chromosome 1 using 90 of 118 clones of IMpRH panel. Two-point analysis revealed the gene was closely linked with microsatellite marker \textit{SW1514} at a distance of 85 cR with LOD score 3.07. Based on comparative genome information, our mapping result was agreed with that of human \textit{EEF1A1} gene on chromosome 6q14.1 using fluorescence in situ hybridization (FISH) and somatic cell hybrid panel (SCHP) methods (Lund et al., 1996).

\textbf{Analysis of genomic structure}

The genomic organization of porcine \textit{EEF1A1} was described (Figure 1). We obtained all intron sequences except for the first intron. The sequence assembled spanned 2,362 bp length containing seven exons (132, 180, 297, 151, 257, 235, 435 bp, respectively) and six introns (195, 121, 83, 88, 88, 100 bp, respectively) and conformed to the GT-AG rule for the splice donor and acceptor sites. The structure is similar with that of human and mouse \textit{EEF1A1}. Information of the genomic sequence will provide a good way to polymorphism detection and functional analysis.

\textbf{CONCLUSION}

In summary, we obtained the full-length cDNA sequence and analyzed the characterization of porcine \textit{EEF1A1} gene. The temporal and spatial expression profiles, chromosome localization and partial genomic organization were described in this study. All will give a good pathway to study the function of \textit{EEF1A1} gene.

\textbf{ACKNOWLEDGEMENT}

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\textbf{REFERENCES}


