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Full Length Research Paper

Cloning and expression of porcine SRPK1 gene

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Protein SRPK1 acts as a crucial element in the pre-initiation complex of transcription, which play an important role in the regulation procession of gene expression. This study was carried out in order to explore the genetic characteristic of *SRPK1* in pigs. *SRPK1* gene came from Yorkshire, a pig that was cloned by real time polymerase chain reaction (RT-PCR), yet coding sequence and partial 5'UTR sequence was completed. The distribution determination of mRNA taken from the heart, muscle, liver, kidney, lung stomach, small and large intestine, spleen and brain of ten Yorkshire and Duroc pigs was finished by real-time PCR by one day and 30 days old pigs. Expression test of gene *SRPK1* was implemented in a skeletal damage model during the period of skeletal muscles development. Sequence analysis of a DNA fragment with a length of 2499 bp in gene *SRPK1* of a Yorkshire pig revealed a full coding region that coded 656 AAs, yet including partial 5'UTR sequence. A total of 14 transcription binding sites were detected by bioinformatics analysis. Both the breeds' specific expression and the tissues' specific expression were detected by RT-PCR; however, high expression was mainly detected in the stomach, and the small and large intestines. The quantity of the mRNA of gene *SPRK1* was enhanced and it decreased repeatedly in the period of skeletal muscle destruction and repair. All the results in the foregoing indicated a few transcription binding sites, which showed that the relevant muscle growth protein lied in 5'UTR, and the positive regulation in the course of skeletal muscle repair revealed a potential association with skeletal muscle cells development or gene expression of other growth related factors.

Key words: Pig, SRPK1, real-time PCR, inverse PCR (I-PCR), bioinformatics analysis, skeletal muscle damage model.

INTRODUCTION

The serine/arginine (SRPK) family has the function of phosphorylation of the RNA splicing factors with RS domain-containing protein (Jian et al., 2006), which plays a very important role in the regulation of gene expression. It was very significant for the porcine genetics research and breeding study to clarify the genetics function of the SRPK1 gene of the cloned porcine. Human SRPK1 has been cloned and proven to belong to the regulating RNA splicing factors found in the nucleus of the cell cycle (Gui et al., 1994). SRPK1 was a kind of kinase that has a high specificity with RS sub-domain of RNA splicing; as such, it only identified arginine and phosphorylation of serine in the matrix (Gui et al., 1994). In order to further study its biological characteristics and attempt to explore whether or not SRPK1 relates with human diseases, SRPK1 probe was used to hybridize multiple sites of the mouse and humans, and the experiment found that SRPK1 has multiple hybridization signals in the mouse chromosomes (rat 17 autosomes and X chromosome) (Wang et al., 1999).

During the development process from embryo to adult individual, specific expression of SRPK3 was seen in the heart and skeletal muscle (Mylonis and Giannakouros, 2003; Bassel and Olson, 2006; Yongjie et al., 2010). Osamu et al. (2005) reported one downstream gene of MEF2 (skeleton muscle strengthening factor) as a new member of the SRPK family, and that the specific expression of this gene was under the control of MEF2 directly. As such, centronuclear myopathy will occur by a lack of this gene expression, while over-expression will

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lead to muscle fiber degradation and early embryos' death.

In addition, SRPK1 is very similar to SRPK3 in any foundational region, which is common in SRPKs family. At present, there is no related report about porcine SRPK1, yet. These experiments have taken porcine as the experimental materials, and have cloned the porcine SRPK1 gene, while the real-time PCR method was used to analyze the expression alteration of SRPK1 gene in different porcine tissue and skeletal muscle repair processes. For the fact that pig is not only an important livestock but also an experiment model organism with valuable and biomedical information, our data provided basic molecular information that is useful for the further investigation of the physiological functions of SRPK1 in pig model.

MATERIALS AND METHODS

Three samples each from a healthy Yorkshire (one month) and a Duroc boar (one month) were obtained from the Institute of Animal Sciences of Heilongjiang Academy of Agricultural Sciences, and 12 samples of Yorkshire (one month) were obtained from Harbin Sanyuan pig farm. This comprises six males and six females, weighing 5 to 10 kg, which can be used to make the repairing model of the skeletal muscle injury. After butchering, lung, liver, spleen, renal, heart, muscle, colorectal and intestinal tissues were immediately immersed in liquid nitrogen for preservation as the experimental relates to the gene clone expression.

Trizol reagent (Invitrogen), chloroform, isopropanol, 75% ethanol made from 0.1% diethyl pyrocarbonate (DEPC) and water, DEPC (Sigma separated loading), PrimeScriptTMRT-PCR Kit, *Taq* DNA polymerase, DL2000, dNTP, pMD-18T vector, JM109, TE buffer, HindIII, EcoRI and EaeI were all purchased from Dalian TaKaRa Co., Ltd. Gene primers were synthesized by Shanghai Shenggong Biology Engineering Technology Service Co., Ltd; while ethanol, agarose gel DNA purification kit and trace plasmid extraction kit were all purchased from Gaining Biological Engineering Co., Ltd. However, bupivacaine hydrochloride injection (5 mL: 37.5 mg) was purchased from Shanghai Zhaohui.

SRPK1 gene cDNA clone

RNA extraction was carried out using Trizol Kit method (Invitrogen). The general RNA of one month old Yorkshire was extracted and 1 µl was taken for RT reaction (the first strand synthesis of the cDNA was generated by using a clamped gT primer, which is designed to be attached to the poly-A junction of the RNA). The first stand of cDNA was synthesized with oligo (dT) as the primers under the recommended method of the Primer-ScriptTMRT-PCR kit. The primers are SE-1F: 5'- R: ACCTTGGATGCCCAATAGCC -3'; SE-1R: 5'- CAGGATCAGACACTTACGGAACG -3'; SE-2F: 5'-TTCCGTAAGTGTCTGATCCTGGTG -3'; SE-2R: 5'-GAAAATCGGAGAATCTATTCAATGGG -3'; SE-3F: 5'-TGGCGGTTACCGGTCTCGC -3'; and SE-3R: 5'-AGGAAGCCCTTGATAATTGGACTTG- 3' according to the human SRPK3 gene sequence NM 014370 in the GenBank. The 20 µl PCR reaction system consisted of: 14 µl deionized water, 2 µl 10 ×PCR Buffer, 1.6 µl dNTP, 0.5 µl upstream-downstream primers (25 µmol) for each sequence, 0.2 µl rTaq DNA polymerase and 1.2 µl cDNA template. The reaction conditions were: 95°C for 10 min; 32 cycle at 95°C for 30 s, (SE-1: 56°C, SE-2: 58°C, SE-3: 64°C, 72°C for 1 min; 72°C for 10 min; and 4°C for 30 min. The system was then connected to the PMD18-T vector after the recovery-purification of 1% agarose gel electrophoresed products for altering Escherichia coli JM109, after which it was sent to YingJun Biological Engineering Co., Ltd for sequence test after strains' cultivation.

Cloning the SRPK1 of a part of 5'UTR by inverse PCR

Inverse-PCR was used to clone the SRPK1 of 5'UTR (Ling et al., 2010). According to the EaeI Kit (TaKaRa) of enzyme total cDNA, the total reaction volume was 40 µl. The SRPK1 of 5'UTR was soaked in 37°C water overnight, after which the produce was put in 65°C water for 15 min to make a variety (hybridize) of restriction endonucleases inactivation. According to T_4-DNA ligase Kit (TaKaRa), the reaction system for 400 µl: 316 µl ddH_2O, 40 µl T4 DNA ligase buffer, 40 µl enzymed (enzyme) product and 4 µl T4 DNA ligase (20°C) was used to connect the produce to a ring overnight, and then precipitated by ethanol and 70% alcohol elution. It was later dried and deionized (dilute) by 40 µL ddH_2O, after which the template for the PCR reaction was obtained. The primer of FY-1F: 5'- CTGGATTATTTACACACCAAGTGCCG -3' and FY-1R: 5'-:CGCTCCCATGGCGAGACCGG-3' for the PCR reaction system included: 2 µl 2 × GC Buffer, 1.6 µl dNTP, upstream and downstream of each primer (0.5 µl), 0.2 µl L-Taq DNA polymerase, 1.2 µl template and 3 µl ddH_2O; whereas the reaction conditions were: 95°C for 10 min; 32 cycles at 95°C for 30 s, FY-1: 62.5°C for 30 s, 72°C for 1 min, 72°C for 10 min and 4°C for 30 min. The PCR product was purified and connected to PMD18-T vector, which was transformed into E. coli JM109, by picking the positive clones randomly. After evacuation of the bacterial by shaking, the sequences were sent to Chun Ying Biotechnology Co.

Sequence analysis

Sequences were aligned and compared using DNAstar software and the longest sequence was obtained. BLASTn was used for nucleotide sequence homology analysis (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) and neighbor-joining (NJ) phylogenetic tree was established. Open reading frame (ORF) finder was used to analyze the open reading frame of nucleotide sequence (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), while the Conserved Domain Architecture Retrieval Tool (http: //www. ncbi.nlm.nih.gov/Structure/ cdd /cdd.html) of NCBI was used to analyze the protein conservative structure domain. However, the transcription factor binding site was analyzed by TF SEARCH-line program analysis (http: ‖mbs.cbrc.jp/research/db/TFSEARCH. html).

SRPK1 gene tissue expression detection

RNA extractions of the lung, liver, spleen, renal, heart, muscle, colorectal and intestinal tissue of Yorkshire (one day, and one month) and Duroc (one month) were obtained by the reverse transcription referred to as 1.2.1. β-Actin was taken as the internal parameter of β-actin-F:5'- GGACTTCGAGCAGGAGATGG-3' and β-actin-R:5'-GCACCGTGTTGGCGTAGGG-3'; while STRATAGENE Mx3000P equipment and real-time PCR Master Mix(ABI) kit were used for the expression level comparison of the porcine SRPK1 gene (B1) on different tissues. Primers such as B1-F: GAACGTGGTGTAGAGGGTGG- 3' and B1-F: 5'-TGCTTTCTTGAAGTTGGCTG-3' were applied in the SRPK1 expression. The general reaction system was 20 µl: fluorescent dyes SYBRGreen Mix 10 µl (1 µmol /L), 0.4 µl for each upstream

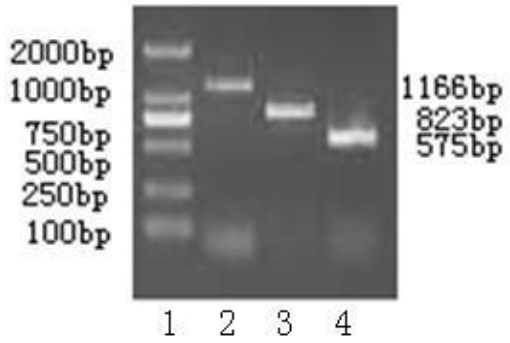

Figure 1. One percent agarose gel electrophoresis images of cloning SRPK1CDS area. 1, DL2000 marker; 2, 3 and 4, SE-1, SE-2 and SE-3 cloned product.

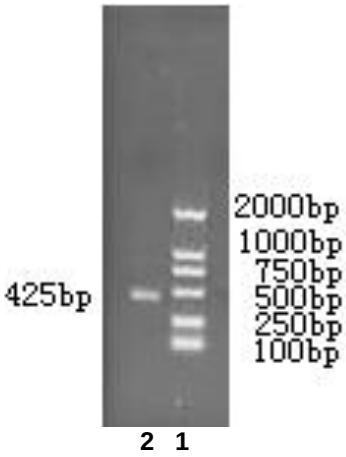

Figure 2. One percent agarose gel electrophoresis of I-PCR (FY-1) product. 1, DL2000; 2, product of FY-1.

and downstream primers, and 1 µl tissue cDNA (the 1 µl RNA reverse transcription products were diluted 10 times in 8.2 µl deionized water). The real-time PCR reaction program was as follows: 95°C predegeneration for 10 min, followed by 40 cycles of 95°C for 30 s and 60°C for 1 min; after which a final soak at 4°C was also incorporated. All of the samples were measured in duplicate. Three measurements of each tissue sample were averaged for further analysis. The comparative Ct method was used to calculate the relative gene expression level across the tissues.

Establishment of skeletal muscle injury model

The skeletal muscle damaged model of pig was established by Bupivacaine hydrochloride with Yorkshire (30 days) in 12 pieces (half male and half female) (Nonaka et al., 1983; Akiyama et al., 1983; Zeng et al. 2004). Pigs were divided into four groups with three pieces in each group; then Bupivacaine (3 ml) was injected subcutaneously into the hind left limb gastrocnemius muscle under the same time and environment. Same volume of normal saline was injected to the same part of the right hind limb for comparison. Butchering was done separately according to the function time (0.5, 72, 12 and 168 h). Then the samples of injured muscle tissue were taken for analysis.

Dyeing of the paraffin sections

The tissue samples were divided into two parts: one part was immediately put into liquid nitrogen and the other part was put into 10% neutral formalin solution for 24 h. This was followed by treating, washing, dehydration, hyaline, embedding, normal paraffin sections, HE dyeing, optical lens observation and photography.

Analysis of data

The relative expression level of each gene in one tissue (ΔCt) was calculated by: 2-ΔΔCt [ΔΔCt = (Ct target gene-Ct β-actin gene)] in the experimental (Ct target gene-Ct β-actin gene) and control groups. The expression form was: Average ± standard error, and the different levels of SRPK1 were compared for both groups by variance analysis and SNK method. However, it was analyzed using ANOVA of SAS 9.13.

RESULTS

Pig cloning and sequence analysis of SRPK1

After the 1.0% agarose gel electrophoresis, the PCR product of porcine SRPK1 was enlarged to three bands of 1166, 823 and 575 bp fragment (Figure 1). By inverse PCR, we got partly 5'UTR of the pigs' SRPK1 gene sequence as shown in Figure 2 and the sequenced results as shown in Figure 3. The SRPK1 gene sequence was gotten after the sample sequence test, which included the CDS area of 1968 bp, and the GeneBank accession number (FJ476290). The result of the analysis of the protein molecular weight of the target gene with DNAstar software was 74.35KDa, and the isoelectric point was about 5.89. A 320 bp length of 5'UTR sequence was obtained by I-PCR, which was predicted by transcription factor binding site prediction tools online to find a number of features of the specific binding sites of transcription, such as: HSF1, HSF2, Ik-1, IK-2, SRY, SP1 MyoD, USF, E47 , p300, CP2, RREB-1, E2F and AP-1.

Sequence analysis and the establishment of the molecular phylogenetic tree

The sequences of human (NM_003137), house mouse (NM_016795), Norway rat (NM_001025726), cattle (XM_590178), dog (XM_845237), chimpanzee (XM_001172595), sumatran orangutan (NM_001131683) and zebra fish (NM_199650) were obtained from Swiss-Prot. The homology comparison and the molecular phylogenetic tree were made by the CLUATAL W program of the DNAstar Software (Figure 4). Gene and

TACACACCAAGTGCCGTATCATCCACACTGACATTAAACCAGAAAACATCTTAT

TGTCAGTGAATGAGCAGTACATCCGGAGGCTG*GGCCACGCTCTTCGCCATTCCTCAGAA*

GCAACCGCTCGAAAATAGAACAAAGCTGATCTTCCCAAGCTTCCAAGTTTGGTTCTCAAACA

CCCCGGGAACATTCACCCTAGGCGTTCTTTCCTCCCCTTCCTCCCCTATTCTCTACGCCCCC

CCCCCCGTCTCGGGAGCAGGTGGTAGGCTCCGCGCTCAACGCGCAGGCGCGCGCCGTCG

TGGGCGGGGGCGGGGCGGGAACTGGGGGGCGGGGTGTGCGCGGGCTTGGTTTGGGGC

*CGCGGCGGGAGCGGGAGTCACCGCCACTCCAGTGCGCAGGCGCCTGGCGGTTA***CCGGT**

CTCGCCATGGAGCG

Figure 3. 5'UTR sequence of pigs with SRPK1 by I-PCR. Bold parts are the primers, underlined parts are the 5'UTR sequences; and the other non-underlined regions are the sequences of exon 8

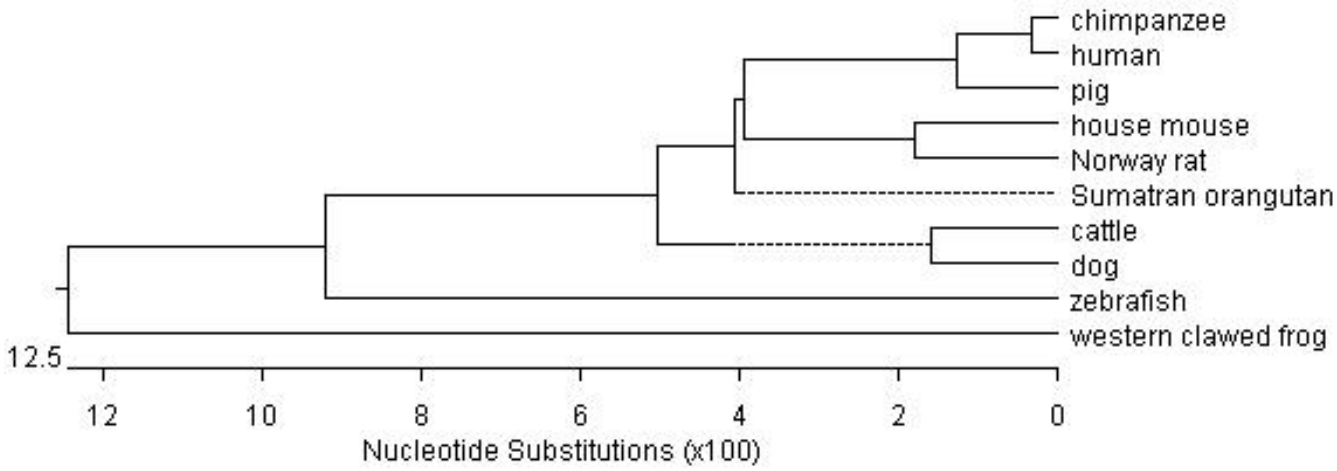

Figure 4. Homology comparison of the SRPK1 protein coding area of 10 species.

protein amino homology proved that SRPK1 was relatively a conservative protein during the evolution process.

Phylogenetic analysis on SRPK1 with DNAstar pointed out that porcine has the closest genetic relationship with human beings and the bovine, and can be classified into one category, while the biggest difference was seen with frogs, in which the evolution relationship is shown in Figure 5. Two S_TKc kinase domains (Figure 5) were found after comparison between the porcine SRPK1 protein and human SRPK1 in the structure data base of NCBI.

Research on the expression profiles

Taking housekeeping gene β-actin as the internal control, the expression analysis between different tissues was made by utilizing gene specific primers (Figure 6). The result shows that the expression of SRPK1 gene was low in the kidney, lung, large intestine, small intestine, heart,

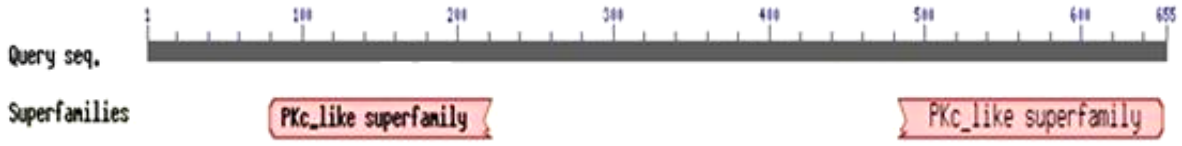

Figure 5. The structure domain analysis of SRPK1.

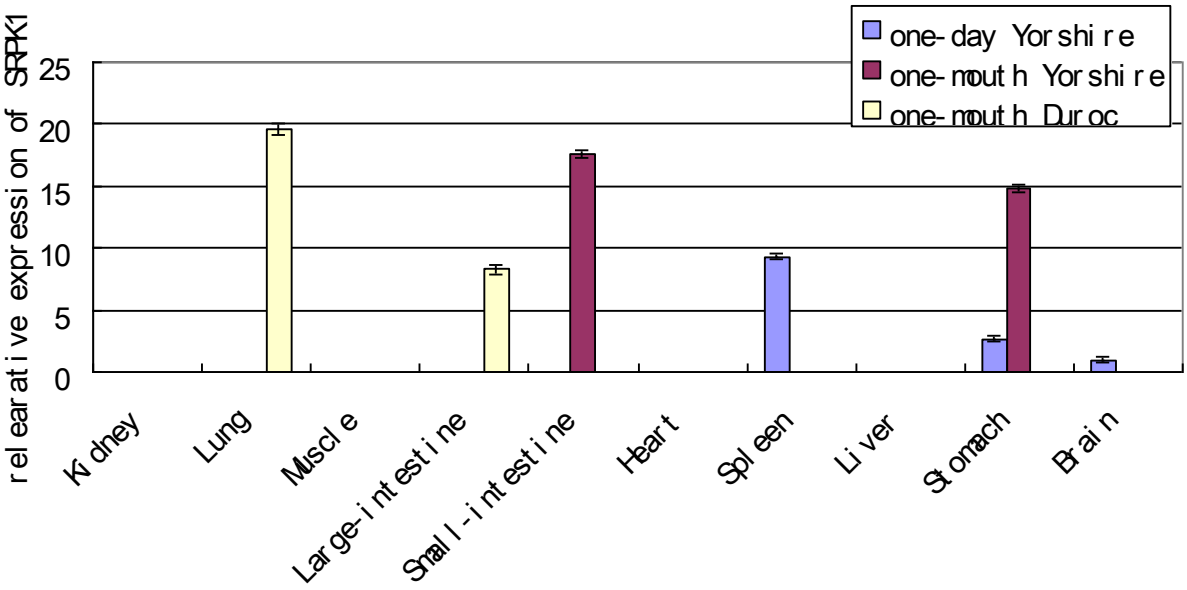

Figure 6. The tissue distribution of porcine SRPK1 mRNA assessed by real time PCR. The values shown in this figure are the averages of three independent experiments. Error bars represent the SE (n = 3) of relative mRNA expression levels of SRPK1 normalized to β-actin.

liver and muscle of one-day old porcine, but high in the spleen, stomach and brain of the same porcine. Also, the expression of SRPK1 gene was low in the kidney, lung, colon, spleen, liver, muscle and large intestine of one month old Yorkshire, while it was high in the small-intestine and stomach of the same Yorkshire. Additionally, the expression of SRPK1 gene was low in kidney, small intestine, spleen, stomach, heart, liver and muscle of one month old Duroc, while it was high in the lung and large intestine of the same Duroc.

The muscle tissue expression status of the SRPK1 gene for different pig breeds in different ages

The result of homoscedasticity is shown in Table 1. Also, it was shown that the three groups were adequate for the condition of homogeneity of the variance, and each one was provided with statistical significance. So, the mean of SRPK1 was compared between different groups by variance and SNK method, which showed that each group has a statistical significant difference. However, the muscle tissue expression status of the SRPK1 gene for different pig breeds in different ages is shown in Figure 7. In Figure 7, the expression level of the SRPK1 gene of different pig breeds had obvious differences ($P \square 0.001$) among the myocardium, dorsal muscle and the thigh muscle. The cardiac muscle SRPK1 expression of one day old Yorkshire was lower than that of one month old Yorkshire and Duroc, while the expression in leg muscle was higher than one month old Yorkshire and Duroc. However, the SRPK1 gene of one month old Duroc had relatively higher expression in the back muscles.

Pathological outcome

The muscle injury pathological section of pigs showed the peak function time of bupivacaine hydrochloride at 0.5 h, after it was injected. It illustrated that the nucleus of the muscle cells irregularly gathered together, and showed the loose gap between the cells. As such, the nucleus array was in disorder within the cell and a large number of cells showed death symptoms (Figures 9B and C) which, to an extent, were a bit higher than those of the control group for SRPK1 expression. At 72 h after the

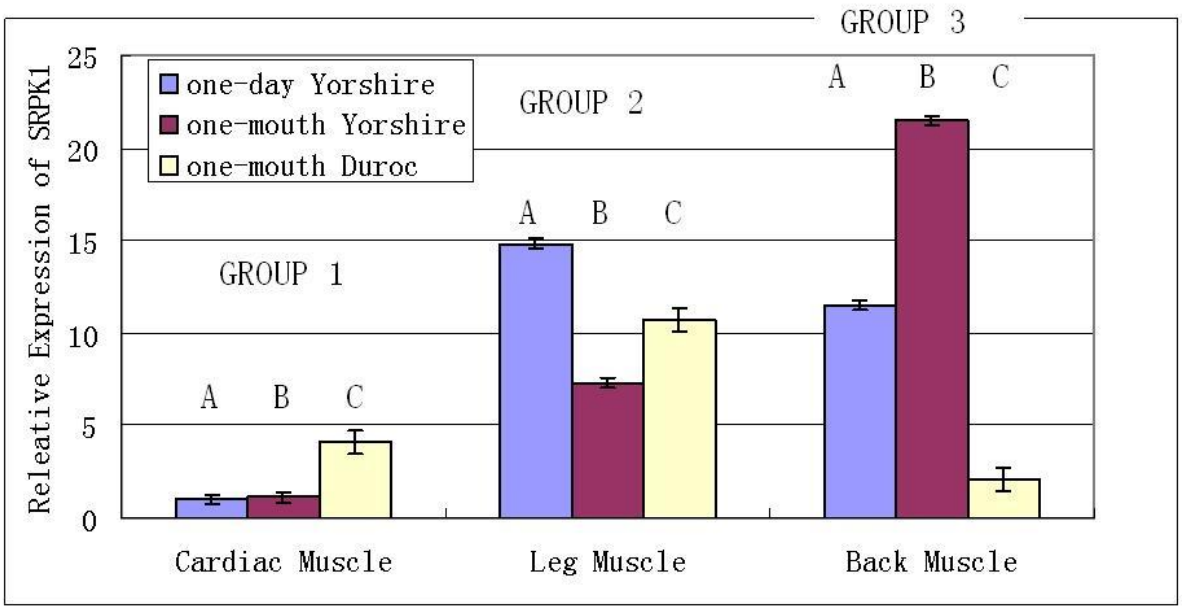

Figure 7. The SRPK1 gene expression in the skeletal muscle from different stages of Yorshire and Duroc pigs by real time PCR. The expression level was normalized to β-actin and measured with 2^ (-DDCt) value. Results are averaged from three independent biological replicates during all stages. Data are shown as the mean ± SE of three independent replicates. There is significant difference (P (SNK)) in each group (A, B and C, respectively) between Yorshire (one-day and one-month) and large Duroc. Cardiac muscle is the first group; Leg muscle is the second group; Back muscle is the third group.

Table 1. The result of the statistic analysis relative expression level of SRPK1 gene in different intramuscular types with different ages by variance analysis.

Group	Tissue	F value	P value	P value(SNK)	SNK Grouping
	One-day Yorkshire			<0.001	A
1	One-month Yorkshire	1.45	0.3055	<0.001	B
	One-month Duroc			<0.001	C
	One-day Yorkshire			<0.001	A
2	One-month Yorkshire	2.32	0.1792	<0.001	B
	One-month Duroc			<0.001	C
	One-day Yorkshire			<0.001	A
3	One-month Yorkshire	0.97	0.4322	<0.001	B
	One-month Duroc			<0.001	C

P (SNK) values within a vertical column followed by different letters is significantly different (P<0.001).

injection, it was shown that muscle cell was still in injury status and partial vascular hemorrhage (Figure 9D). At the same time, eosinophilic macrophage moved to the necrosis muscle fiber (Figure 9E) and SRPK1 expression increased quickly. At 120 h after the injection, the necrosis fiber and the external gap of the cell were infiltrated by a large amount of monocytes, followed by a large amount of regenerated muscle fiber with central nucleus in myotube shape. At the same time, the vascular hemorrhage can be seen in Figures 9F and G, in which the SRPK1 appeared down trend. At 168 h after the injection, most necrosis muscle fibers were replaced by newborn muscle fibers (Figures 9H, I and J). Due to the limitation of the capability of the newborn muscle fiber, many connective tissues proliferate to fill the gap between the muscle cells. At this time, the SRPK1 rapidly increased and exceeded the previous highest level; but due to the observation time limitation, the peak time of

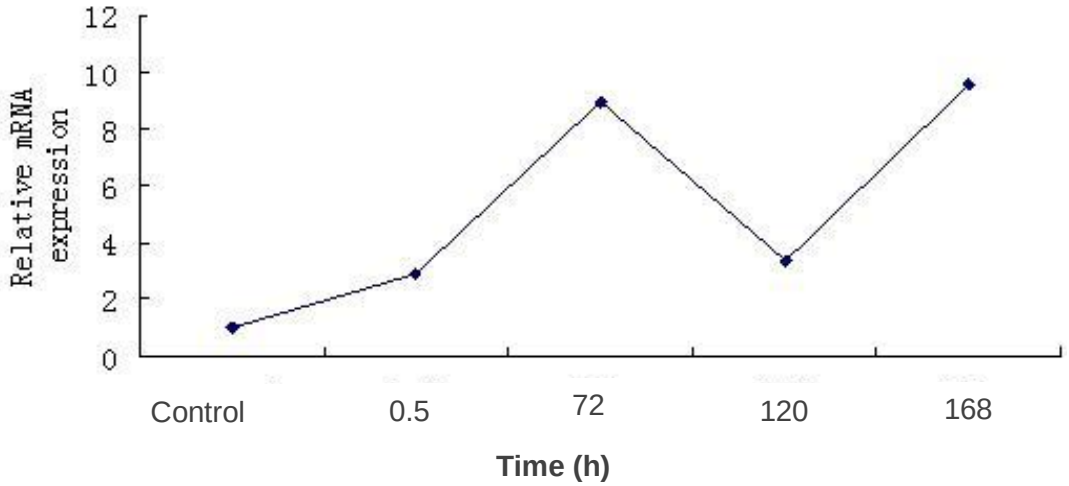

Figure 8. Expression of SRPK1 in the developmental stages of the skeletal muscle.

SRPK1 expression at around 72 and 168 h could not be concluded. After the injection of normal saline (positive control), there was no muscle fiber demodulation, necrosis and inflammation filtration, but instead its muscle satellite cells arrays were closely brought together and the nucleus was well-distributed on the side wall of the cell (Figure 9A). Consequently, the expression of SRPK1 was lower at 0.5 h after the injection of bupivacaine hydrochloride. The expression tendency of SRPK1 is shown in Figure 8, in the process of muscle cellular differentiation after the injury, the expression volume of SRPK1 kept on increasing.

DISCUSSION

In the research of Jumma et al. (1980), it was found that SRp20 exists at the early stage of the mice ova and embryo development; however, the mice which fertilized their eggs without SRp20 can hardly form blastocyst, thus they die in the morula stage (Jumma et al., 1999). Consequently, inhibition of RNAi (RNA interference) on the SR gene CeSF2/ ASF will lead to the death of advanced nematode embryo. If two or more SR protein functions are inhibited, it would lead to more death of nematode or other types of developmental defects (Longman et al., 2000). The research on tumor by Skickeler et al. (1999) showed that before the formation of tumor, SR protein expression type varies in different tissues, generally by one subtype in one family but with the development of the tumor, the SR protein expression type increased, and the protein expression type of malignant tumor became complex. The research on the cells of different tissues by Zahler et al. (1993) found out that each SR protein has its own expression type and regulates the choosing of the splicing sites, which decide the specificity of the tissue. As an SR protein specific kinase, SRPK1 gene brought a new research perspective and was used as the starting point of the research of the relationship between related regulation factors, formation process of the muscle and the tumor cell. We have precisely cloned the nucleotide sequence (including the open reading frame) of the porcine SRPK1, and have carried on preliminary analyses and speculation of its amino acid sequence and its characteristics.

In this study, we cloned the partly 5'UTR of SRPK1 by inverse PCR. Inverse PCR was simpler, avoiding the screening from library, and sub-cloning as well as other cumbersome steps. The test conditions did not ask for much, but spent less, without a large number of costly and synthesis nucleotide connector to overcome the trouble to produce non-specific products. In expression of the tissue, it was found that the digestive system of older (one-month-old) pigs, such as stomach, large intestine and small intestine, had higher expression of SRPK1 gene, even in specific areas. Therefore, we speculated that SRPK1 may play a certain role in the digestive function and nutrient absorption and utilization. A research was conducted to prove that SP1 protein in the region of obesity QTL could play a role in controlling fat (Fu¨rbass et al., 2006); moreover, we found a number of SP1 transcription binding sites in partly 5'UTR of SRPK1, so we speculated that it may be related to obesity of pig. A research on SRPK3 under direct regulation of MEF2, showed that lack of this gene expression will lead to centronuclear myopathy and over expression in the skeletal muscle would lead to muscle fiber degradation and death of early embryo. The development of skeletal muscle in the embryo stage was a complicated process including orderly regulation in time and space for the transferring, proliferation and differentiation of the skeletal muscle somatic. The regeneration of the skeletal muscle is one physiological reaction after the injury of the grown skeletal muscles, and is mainly activated based on the stimulating signal of the muscle satellite cell in the skeletal muscle tissue. This regeneration is proliferated in

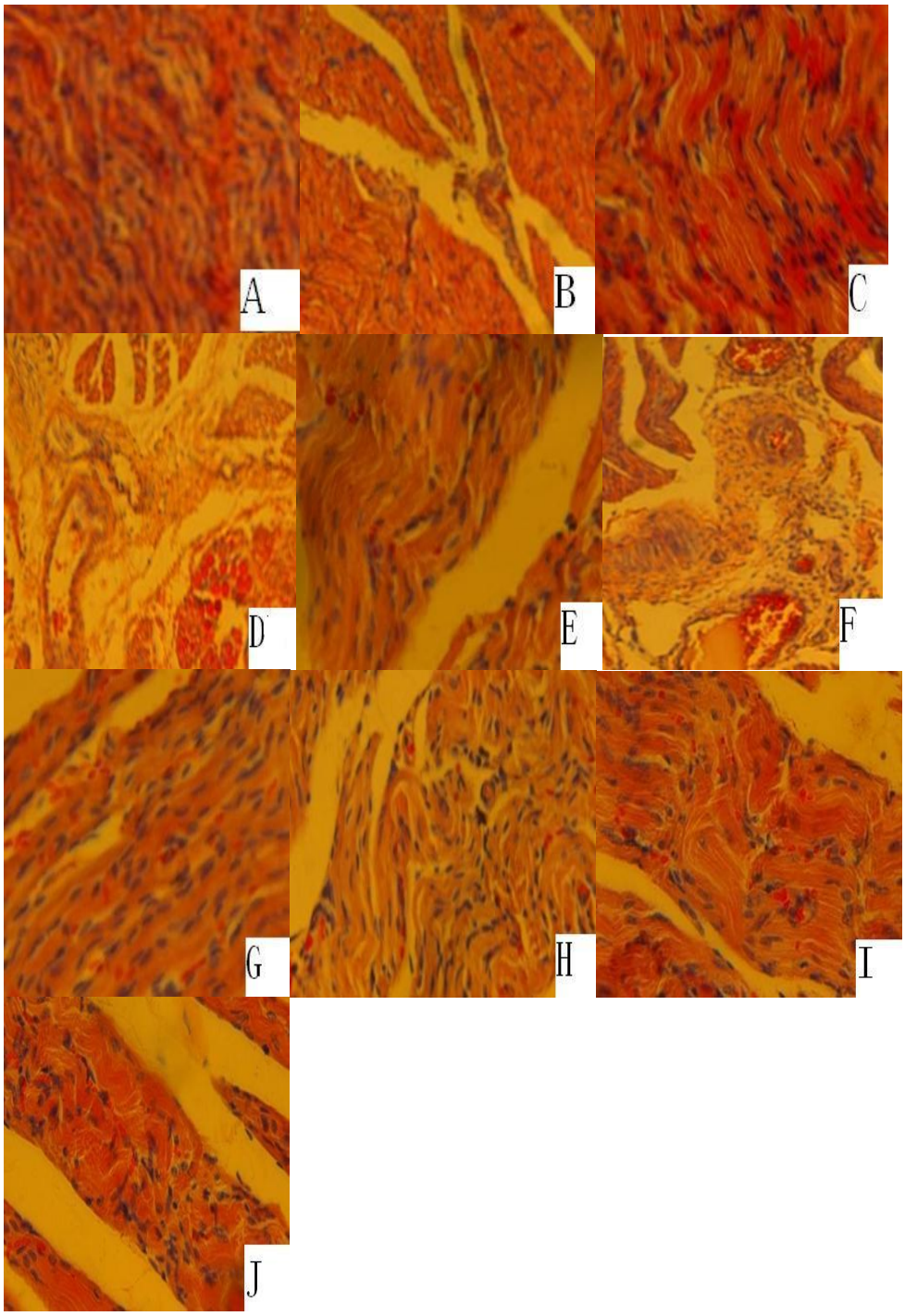

Figure 9. Porcine skeletal injury modeling paraffin (He dyeing) section and its TEM (40×). A, The control group with the same injection dose of normal saline; B and C, the skeletal muscle characteristics at 0.5 h after the injection of bupivacaine hydrochloride; D and E, the skeletal muscle characteristics at 72 h after the injection of bupivacaine hydrochloride; F, G and H, the skeletal muscle characteristics at 120 h after the injection of bupivacaine hydrochloride; I and J, the skeletal muscle characteristics at 168 h after the injection of bupivacaine hydrochloride.

the cell cycle, after which it differentiates and blends to form the new muscle fiber. At present, we now know that many growth factors have participated in the regulation of the differentiation and regeneration of the skeletal muscle, thus, these growth factors are expressed highly in the formation of the embryos muscle and regeneration

of the injured skeletal muscle, which could stimulate the development, differentiation and maturity of the muscle precursor. This experiment was tested on the mRNA expression level of SRPK1 gene on heart muscle, leg muscle and cardiac muscle of different aged Yorkshire and Duroc. The result showed much difference in SRPK1 expression between different pigs with different ages in days. This proved that the SRPK1 gene availability is different for different pigs, which may be related to the intermediate shape difference. At the same time, from the expression difference in different times of Yorkshire and Duroc, in which there is a higher expression, we could see that the expression of SRPK1 in the leg muscle of pigs accelerates, and we could particularly speculate its participation in the leg development of Yorkshire. So the porcine skeletal muscle injury repairing module was established for further research of its mechanism on the thigh muscle fiber. Skeletal muscle injury model replays the development process of the skeletal muscle under human management. We found out through the expression status of SRPK1 gene in this model that the expression of SRPK1 in the control remained stable, while SRPK1 gene in the experiment showed related change according to the different development stages of the muscle cell, which offers feasible evidence for the establishment of the porcine skeletal muscle injury repairing model. So we used this method for the research of SRPK1 expression status during the skeletal muscle development process. However, it was shown that at 0.5 h after the injection of cardio toxin, the SRPK1 expression increased, and this high expression was maintained till the seventh day after the injury; so we speculated that the expression characteristic of SRPK1 gene was higher in normal skeletal muscle tissue, and not only in the quiescent muscle satellite cell. Consequently, it will increase after large amount of muscle satellite cell activation, proliferation and differentiation of the myotube. The SRPK1 expression volume of 0.5 h after the injection on the control was lower than the experiment group (0.5 h); so it proved that SRPK1 gene did not put any auxo-action on the muscle cell injury. The expression law of SRPK1 was almost the same as the myoblast specific differentiation marker MyoD, Myogenin. Judging from the expression profile of the SRPK1 gene, it probably participated in the regulation process on the proliferation and differentiation of the muscle satellite cell (Jiao et al., 2001).

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