

# Cloning, Expression and Polymorphism Analysis of Porcine *SRPK3* Gene

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**Abstract** Protein SRPK3 acted as a crucial element of transcription pre-initiation complex, which play an important role in regulation procession of gene expression. In order to explore the genetic characteristic of *SRPK3* in pigs. *SRPK3* gene came from Yorkshire a pig was cloned by RT-PCR yet coding sequence was completed. The distribution determination of mRNA came from ten Yorkshire pigs and Duroc pigs in heart, muscle, liver, kidney, lung stomach, small and large intestine, spleen, brain was finished by Real-time PCR by age one day and 30 days. Expression test of gene *SRPK3* was implemented in a skeletal damage model during the period of skeletal muscles development. Sequence analysis of an mRNA fragment with a length of 1 708bp in gene *SRPK3* of a Yorkshire pig revealed a full coding region, 1 701bp, which coded 656 AAs including two S\_TKc domains. The sequence of the porcine protein SRPK3 shared high similarity with its homolog from human and bovine, and they were closed in the Phylogenetic tree. PCR-SSCP test shows: On the Sixth exon (CDS: 629, CDS: 653) get A→G, T→C, which change the amino as Pro → His, Ile→Thr, on the ninth exon (CDS: 1059) get a G → A, but no change of amino. Both breeds specific expression and tissues specific expression were detected by RT-PCR, however high expression was mainly detected in skeletal muscle and heart. The quantity of mRNA of gene *SRPK3* in the period of skeletal muscle destruction and repair, which showed gradually to be increased in different stage. Because of it's expressed in skeletal muscle and heart mainly, and gradually be increased in different stage of injury and repair process. SRPK3 may be related to skeletal muscle cell development.

**Key words** Pig SRPK3 Bioinformatics analysis PCR-SSCP Real-time PCR Skeletal muscle injury model

Serine/Arginine (SRPK) family has the function of phosphorylation of the RNA splicing factors with RS domain-containing<sup>[1]</sup>, which plays a very important role in the gene expression regulation. It was very significant for the porcine genetics research and breeding study to clarify the genetics function of *SRPK3* gene of clone porcine. The *SRPK3*, *SRPK2* and *SRPK3* of mammalian; specific

phosphorylation of SR protein splicing factors of three family members of SRPKs and its redistribution within the cell cycle<sup>[2-3]</sup>. Although there was some similarity on the structure and function among *SRPK3*, *SRPK2* and *SRPK3*, yet more obvious difference on their structural expression and action pathway, for instance: *SRPK1* and *SRPK2* were mainly expressed on gonad, but little expression on pancreas, brain and other tissue. During the development process from embryo to adult individual, specific expression of *SRPK3* was seen in the heart and skeletal muscle<sup>[4-5]</sup>. Osamu has reported that one

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downstream gene of MEF2 (skeleton muscle strengthening factor), *SRPK3*, as a new member of the SRPK family, the specific expression of this gene was under direct control of MEF2, centro nuclear myopathy will occur by lacking of this gene expression; While over-expression will lead to muscle fiber degradation and early embryos death<sup>[6]</sup>. Normal muscle growth and development balance needs the signal pathway of the regulation on *SRPK3* by MEF2. The *SRPK2* of human beings has also been cloned with sequencing analysis and chromosome location<sup>[7]</sup>. At present, there was not any related report about porcine *SRPK3*. These experiments have taken porcine as the experimental materials, and cloned the porcine *SRPK3* gene and utilize PCR-SSCP inspection technology to test the polymorphic loci within the *SRPK3* coding region. Real-time PCR method was applied to analyze the expressed alteration of *SRPK3* gene in different porcine tissue and skeletal muscle repair process. As a SR protein specific phosphorylation factors, *SRPK3* plays a very important and comprehensive biological function in the gene expression regulation. At present, six kinds of *SRPK3* gene have been cloned (six species such as human, mouse, cattle and etc), and some understanding for their function. But we need further research, as we do not have a thoroughly clear mind on the biological function of *SRPK3* protein. Research in recent years has proved the special function of *SRPK3* gene in the skeletal muscle development process, which offers a new way of thinking on its cause. And now, few reports can be seen on the porcine *SRPK3* gene. So the experiment takes Yorkshire, Duroc, northeastern min pig and northeastern wild boar as the research objective to carry on the preliminary research on the porcine *SRPK3* gene. Clone the gene of the porcine *SRPK3*, defining the expression status of this gene in different tissue, doing search on analyzing the SNPs polymorphism of part sequence and its function during the skeletal muscle repairing.

## 1 Materials and Methods

### 1.1 Materials

1.1.1 Animal Institute of Animal Sciences of Heilongjiang Academy of Agricultural Sciences offers

healthy Yorkshire (one day, one month), Duroc boar (one month) three pieces for each. After butchering, put their lung, liver, spleen, renal, heart, muscle, colorectal, intestinal tissue immediately into the liquid nitrogen for preservation as the experimental materials of gene clone expression. Harbin Sanyuan pig farm offers 12 pieces one month old Yorkshire, which half male and half female and weight 5 ~ 10kg, which can be used to make the repairing model of the skeletal muscle injury.

1.1.2 Reagent The Trizol reagent (Invitrogen), chloroform, isopropanol, 75 % ethanol made from 0.1 % DEPC and water, DEPC (Sigma separated loading), PrimeScript TMRT-PCR Kit, Taq DNA polymerase, DL2000, dNTP, pMD-18T vector, JM109, TE buffer, *Hind* III and *Eco*R I were all purchased from Dalian TaKaRa Co., Ltd. Gene primers were synthesized by Shanghai Shenggong Biology Engineering Technology Service Co., Ltd; Ethanol, agarose gel DNA purification kit, trace plasmid extraction kit were all purchased.

### 1.2 Methods

1.2.1 *SRPK3* gene cDNA clone According to the Trizol Kit method, the general RNA of one month old Yorkshire was extracted taken by 1 $\mu$ l of RT reaction and the first stand of cDNA was synthesized with oligo (dT) as the primers under the recommended method of the Primer-ScriptTMRT-PCR kit and the recommended method of the Prime ScriptTMRT-PCR kit and the primers C1-F: 5'-GATGAGCGCCAGCACGG-3'; C1-R: 5'-GCCCTCATCTTCTTCCTCTTGTT-3'; C2-F: 5'-GAGCTCATCGACGACTTCCG-3'; C2-R: 5'-CGGGGCCTAGGGATTGAGCC-3' according to the human *SRPK3* gene sequence NM 014370 in the GenBank. 20 $\mu$ l PCR reaction system: 14 $\mu$ l deionized water, 2 $\mu$ l 10  $\times$  PCR Buffer, 1.6 $\mu$ l dNTP, upstream-downstream primers 0.5 $\mu$ l (25 $\mu$ mol) for each, 0.2 $\mu$ l rTaq DNA polymerase, 1.2 $\mu$ l cDNA template. Reaction condition: 95  $^{\circ}$ C 10 min; 32 cycle: 95  $^{\circ}$ C 30s, (C1:63.4 $^{\circ}$ C, C2:67.2 $^{\circ}$ C) 30s, 72  $^{\circ}$ C 1 min; 72 $^{\circ}$ C 10 min; 4  $^{\circ}$ C 30min. It connects to the PMD18-T vector after the recovery-purification of 1% agarose gel electrophoresed products for altering *Escherichia coli* JM109, which was send to YingJun Biological Engineering Co. Ltd to test after and trains cultivation.

1.2.2 Sequence analysis DNASTar software was carried on sequence matching to get the longest sequence, and used BLASTn for nucleotide sequence homology analyzed<sup>①</sup> and established NJ phylogenetic tree. Use ORF finder to analyzed the open reading frame of nucleotide sequence<sup>②</sup>; to analyze the protein conservative structure domain use the Conserved Domain Architecture Retrieval Tool<sup>③</sup> of NCBI.

1.2.3 PCR-SSCP The ear tissue of four kinds of porcine was collected [min pig, wild crossbred pig (F1 generation), Yorkshire, wild pig] and extracted the DNA

of the gene group. Design primers and reaction conditions as table 1 according to the cloned CDS sequence (Fig 1). The PCR-SSCP process was as follows: Take 2  $\mu$ l PCR products for blending with 10 $\mu$ l degeneration buffer, then take ice bath for 10 minutes about 10 minutes after 98 $^{\circ}$ C penetration. Different primers should be applied on non-denaturing polyacrylamide gel with different density. Electrophoresis for 12 ~ 16 hours under 120V at room temperature; then silver staining after the electrophoresis. Choose homozygote fragment of different individuals with different gene type for cloning and sequence test.

**Table 1 Polymorphism primers and the density of the non-denaturing polyacrylamide gel of SSCP**

Primers	Location	Length (bp)	Primer sequences (5'→3')	Annealing temperature (°C)	Concentration (%)
EK-1	Exon 6	121	F: TGCTGCACGCTCTGGATTACC R: ACTGCTGCCACTCTGTGGCTT	55.5	19
EK-2	Exon 9	190	F: GCGGCTCCACCTCCTCTTCA R: GTCTCCCGCTGCTTGACGA	61.5	17

1.2.4 The Expression Detection of SRPK3 gene tissue The RNA of lung, liver, spleen, renal, heart, muscle, colorectal, intestinal tissue of Yorkshire (one day, one month), Duroc (one month), was reversed transcription refer to 1.2.1. Take  $\beta$ -actin as the internal control,  $\beta$ -actin-F: 5'-GGACTTCGAGCAGGAGATGG-3',  $\beta$ -actin-R: 5'-GCACCGTGTGGCGTAGGG-3', use STRATAGEN E Mx3000p equipment and Real-time PCR Master Mix (ABI) kit for the expression level comparison of the porcine SRPK3 gene (B1) on different tissue. Primers such as B1-F: 5'-GGGATCCGAAGCTAGCTC-3'; B1-R: 5'-CCTCATCTTCTCTTG-3' was applied in the SRPK3 expression. General reaction system was 20 $\mu$ l: fluorescent dyes SYBRGreen Mix 10 $\mu$ l, (1 $\mu$ mol /L) 0.4 $\mu$ l for each upstream and downstream primers; tissue cDNA 1 $\mu$ l (dilute the 1 $\mu$ l RNA reverse transcription products for 10 times) deionized water 8.2 $\mu$ l. Real-time PCR reaction program was as follows: 95 $^{\circ}$ C predegeneration for 10 min, followed by 40 cycles of 95 $^{\circ}$ C for 30s and 60 $^{\circ}$ C for 1 min; a final soak at 4 $^{\circ}$ 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.

1.2.5 Establishment of Skeletal Muscle Injury Model Skeletal Muscle Injury Model of pig was established by Bupivacaine hydrochloride with Yorkshire (30 days) 12 pieces (half male and half female)<sup>[8,9]</sup>. Made 12 pigs into four groups with 3 pieces in each group; then carried on subcutaneous injection of Bupivacaine (3ml) into the hind limb left gastrocnemius under the same time and environment. Same volume normal saline was need to be injected into the same part of the right hind limb for comparison, then do butchering was done separately according to the function time 0.5h, 72h, 12h, 168h, then take destructed muscle tissue sample.

1.2.6 Dyeing of the paraffin sections The tissue sample was taken immediately divided into two parts, one be put into the liquid nitrogen, another being put into the 10% neutral formalin solution over 24h, treating, washing, dehydration, hyaline, embedding, normal paraffin sections, HE dyeing, optical lens observation and photography.

① <http://www.ncbi.nlm.nih.gov/blast/blast.cgi>

② <http://www.ncbi.nlm.nih.gov/gorf/gorf.html>

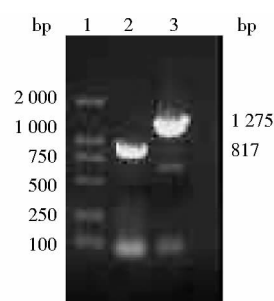
③ <http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.Shtml>

1. 2. 7 Data analysis The SPSS statistic analysis software was utilized in the date analysis, when  $P < 0.05$ , we regarded it as significant difference. SSCP test data was for separate statistical calculation of genotype frequency and gene frequency according to the phenotype displayed by the electrophoretogram. Fitness  $\chi^2$  test and independent test of the outcome was made for the observation of each group whether they were in Hardy-Weinberg balance and the diversity degree between species' polymorphism. Relative expression level of each gene in one tissue ( $\Delta Ct$ ) was calculated by:  $2^{-\Delta\Delta Ct}$  ( $\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{\beta\text{-actin gene}})_{\text{experimental group}} - (Ct_{\text{target gene}} - Ct_{\beta\text{-actin gene}})_{\text{control group}}$ ), expression form: Average  $\pm$  standard error.

## 2 Results

### 2.1 The Result of cDNA clone

After the 1.0% agarose gel electrophoresis, the PCR product of porcine *SRPK3* has been enlarged to 2 bands of 1 275bp, 817b fragment (Fig. 1). *SRPK3* gene sequence 1 708bp was gotten after sample sequence test, which includes the CDS were a 1 701bp, and get the login number GQ428209 after submitting the sequence to the GenBank. Analyzing the protein molecular weight of the target gene was 61. 9kDa with DNASTar software, isoelectric point was about 6.81.



**Fig. 1 Agarose gel electrophoresis images of cloning *SRPK3* CDS area**

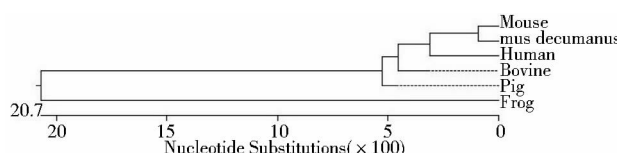
1: DL2000 Marker; 2: Clone product of C1;  
3: Clone products of C2

### 2.2 Sequence analysis and the establishment of the molecular phylogenetic tree

Human (AAH92416), mouse (CAM22458), frog (NP\_001158810), bovine (AAI23798), mus decumanus (NP\_908934) was Searched through Swiss-Prot. The

homology comparison and the molecular phylogenetic tree were made by the CLUATAL W program of the DNASTar Software. Compared the *SRPK3* protein of porcine with human being, mouse, bovine, their amino consistency were 95%, 96% and 94%. Gene and protein amino homology analysis has proved that, *SRPK3* was relatively a conservative protein during the evolution process.

Phylogenetic analysis on *SRPK3* with DNASTar has pointed out that, porcine has the closest genetic relationship with human being and the bovine, and can be classified to one category, while biggest difference was seen by frogs, the evolution relationship was shown in Fig. 2. Two S\_TKc kinase domains (shown in Fig. 3) have been found out after comparison between the porcine *SRPK3* protein and human *SRPK3* in the Structure data base of NCBI.



**Fig. 2 Homology comparison of the *SRPK3* protein coding area of 6 species**



**Fig. 3 Analysis of *SRPK3* protein sequence domain**

### 2.3 Result of PCR-SSCP Analysis

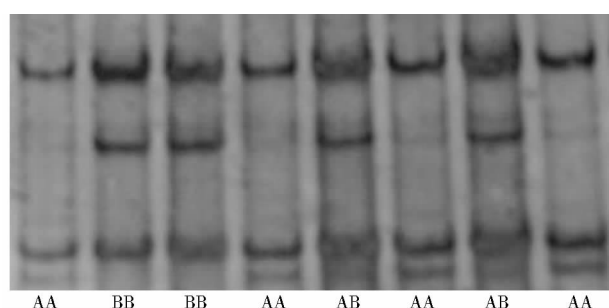
All the designed primers PCR amplification have led to good outcome, the fragment length was the same size as the designed amplified fragment, there was no non-specific band, SSCP analysis can be carried on as shown in Table 2. SSCP analysis result showed the PCR products of EK-1, EK-2 have tested three gene type (AA, BB and AB), the electrophoresis result was shown in Fig. 4 and Fig. 5. Sequence test result has shown that the polymorphism of EK-1 was caused by two nucleotide mutation on the sixth exon. The change of C629  $\rightarrow$  A629, T653  $\rightarrow$  C653 was related to the amino acid change of Porches, Ile  $\rightarrow$  Thr. The polymorphism of EK-2 was one nucleotide mutation on the ninth exon, G1059  $\rightarrow$  A1059, and there was no change of amino acid. Define the same sequence with the one in the GenBank (GQ428209) as AA. The polymorphism of

the EK-2 primers was caused by one nucleotide mutation in one fragment. The genotype frequency and gene frequency of different pig breeds was shown in Table 3. The outcome of Fitness  $\chi^2$  test showed that, the EK-2 was in the Hardy-Weinberg unbalanced status ( $P < 0.05$ ) in the wild, wild

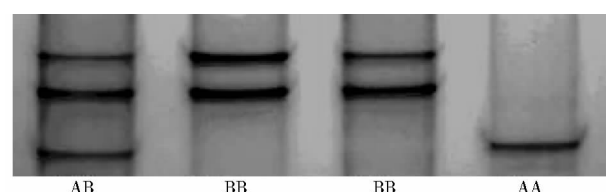
crossbred pig and the Yorkshire; EK-2 was in Hardy-Weinberg unbalanced status ( $P < 0.05$ ) between Yorkshire and wild. SRPK3 gene was in light polymorphic status, an allele of EK-1 and EK-2 takes advantage.

**Table 2 Polymorphism primers and the density of the non-denaturing polyacrylamide gel of SSCP**

Primers	Location	Length (bp)	Primer sequences (5'→3')	Annealing temperature (°C)	Concentration (%)
EK-1	Exon 6	121	F: TGCTGCACGCTCTGGATTACC R: ACTGCTGCCACTCTGTGGCTT	55.5	19
EK-2	Exon 9	190	F: GCGGCTCCACCTCCTCTTCA R: GTCTCCCGCTGTTGGACGA	61.5	17



**Fig. 4 SSCP electrophoretic outcome of the primers EK-1**



**Fig. 5 SSCP electrophoretic outcome of the primers EK-2**

SRPK3 gene genotype frequencies and gene frequencies of different porcine

**Table 3 Allele frequencies and genotype frequencies of SRPK3 among four swine breeds**

Primers	Breeds	Number	AA	AB	BB	A	B	H	PIC	$\chi^2$
EK-1	Yorkshire	60	1	0	0	1	0	0	0	0 *
	Min pig	30	0.70	0.167	0.133	0.783	0.217	0.341	0.208	7.778 9
	Wild boar	10	0.90	0	0.10	0.90	0.10	0.180	0.164	0.555 6 *
	Crossbred pig	80	0.85	0.15	0	0.925	0.075	0.139	0.129	0.566 8 *
EK-2	Yorkshire	60	0.883	0.117	0	0.941 5	0.058 5	0.111	0.104	24.626
	Min pig	30	0.533	0.167	0.30	0.617	0.383	0.361	0.472	0.506 7 *
	Wild boar	10	0.90	0.10	0	0.95	0.05	0.090	0.095	0 *
	Crossbred pig	80	0.85	0.15	0	0.925	0.075	0.129	0.138	22.826 4

Note: H is level of heterozygosis, PIC is polymorphism Information content, \* means significant difference ( $P < 0.05$ )

## 2.4 Research on the expression profiles

Taking housekeeping gene  $\beta$ -actin as reference, the expression analysis between different tissue has been made by utilizing gene specific primers. The result showed that the expression of SRPK3 gene was low in kidney, lung, colorectal, intestine, heart and liver of one day old porcine, but high in the spleen, stomach and the brain. The expression of SRPK3 gene was low in kidney, lung, colorectal, spleen and liver, while high in the muscle, intestine and the stomach for one month old Yorkshire. The expression of SRPK3 gene was low in kidney, intestine, and spleen, liver, while high in the muscle, heart, and stomach and colorectal for one month old Duroc

(Fig 6).

## 2.5 The muscle tissue expression status of the SRPK3 gene for different breed pig in different age

The muscle tissue expression status of the SRPK3 gene for different breed pig in different age was shown in Fig. 7. The expression level of the SRPK3 gene of different breed pig has obvious difference ( $P < 0.05$ ) among the myocardium, dorsal muscle and the thigh muscle. The dorsal muscle SRPK3 expression of one day old Yorkshire was little higher than the one month old Yorkshire, while expression in myocardium and thigh muscle was lower than one month old Yorkshire. The SRPK3 gene of one month old Duroc has with relatively

high expression in the three skeletal muscles, but less expression volume in the thigh muscle compared with the Yorkshire of the same age.

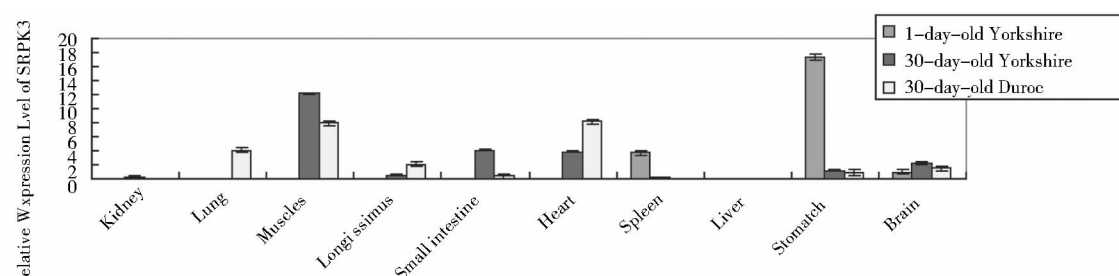


Fig. 6 Issue expression profile of 1-day-old and 30-day-old Yorkshire and 30-day-old Duroc

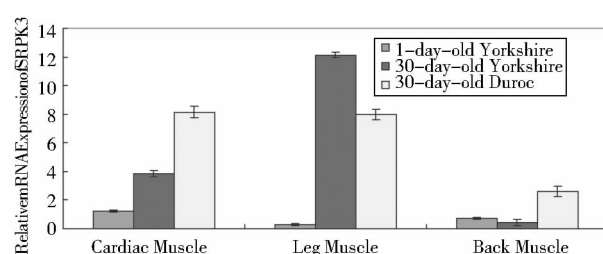


Fig. 7 The *SRPK3* gene expression result in myocardium, dorsal muscle and the thigh muscle of different type of pigs in different age in days

## 2.6 Skeletal muscle injury and expression in the muscle different process

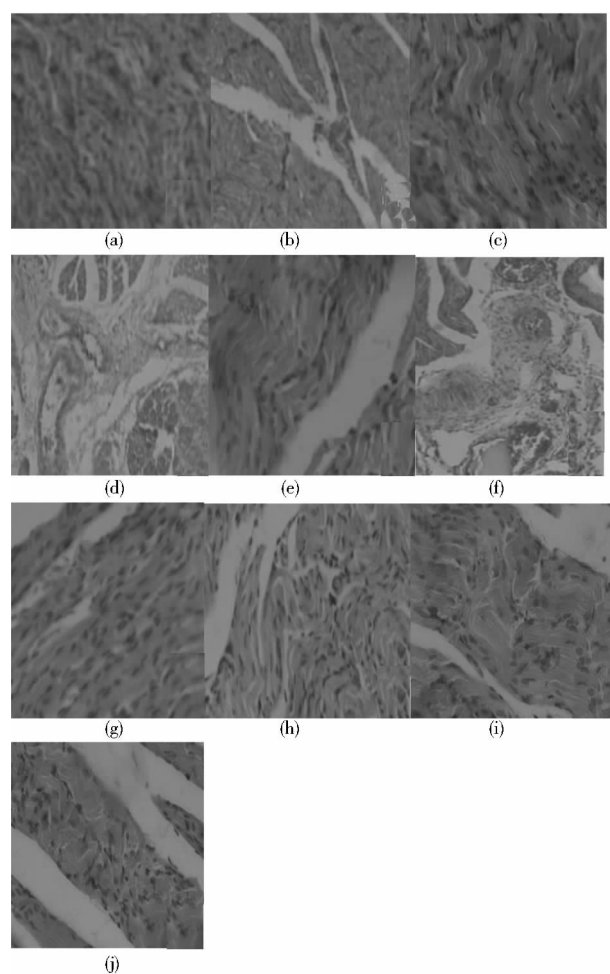
**2.6.1 Pathology symptom** Their behavior changed after the injection of bupivacaine hydrochloride to one month old Yorkshire. After the injection of bupivacaine hydrochloride to part of the peroneus longus of Yorkshire, the muscle on this side turned to be spongy immediately, when creeping, we saw obvious dragging status and that was similar to the related report [10]. This kind of dragging could be seen even after 24 hours, creeping slowly with 48 hours later and went normal about 64 hours later.

**2.6.2 Pathological outcome** Muscle injury pathological section was shown that 0.5 hours, after the injection, was the peak function time of bupivacaine hydrochloride, section showed that the muscle cells nucleus gathers inconstantly, and loose gap shows between the cells, nucleus array in disorder within the cell and a large number of cell show death symptom, (Fig. 8b, 8c) with little *SRPK3* expression. 72 hours after the injection shows muscle cell was still in injury status and partial vascular hemorrhage. (Fig. 8d), at the same time, eosinophilic

macrophage moved to the necrosis muscle fiber. (Fig. 8e) and begin to express. 120 hours after the injection, necrosis fiber and the external gap of the cell have been infiltrated by a large amount of monocytes, follows by a large amount of regenerated muscle fiber with central nucleus in myotube shape, at the same time, vascular hemorrhage can be seen (Fig. 8f, 8g), the *SRPK3* expression volume amplified. 168 hours after the injection, most necrosis muscle fiber has been replaced by newborn muscle fiber (Fig. 8h, 8i, 8j), as the limitation of the newborn capability of muscle fiber, many connective tissue proliferates to fill the gap between muscle cell, at this time, the expression volume of *SRPK3* has came to its maximum. Due to the observation time limitation, whether around 168 hours was the peak time of *SRPK3* expression could not be concluded. After the injection of normal saline (Positive control), there was no muscle fiber denaturation, necrosis, inflammation filtration, and its muscle satellite cells arrayed closely, nucleus was well-distributed and on the side wall of the cell. (Fig. 8a); the expression of *SRPK3* was a little higher than 0.5 hours later after the injection of bupivacaine hydrochloride. The expression tendency of *SRPK3* was shown in Fig. 9, in the process of muscle cellular differentiation after the injury, the expression volume of *SRPK3* kept on increased.

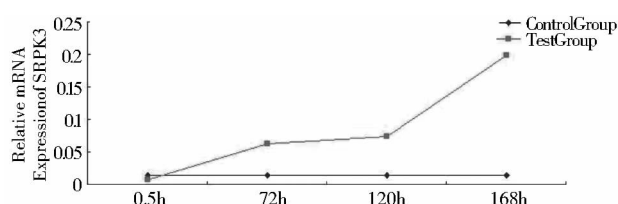
## 3 Discussion

Research by Jumma and etc has found out, *SRp20* exists at the early stage of the mice ova and embryo development; the mice fertilized eggs without *SRp20* could hardly form blastocyst, and died in the morula stage [11].



**Fig. 8 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 0.5h later after the injection of bupivacaine hydrochloride (d) and (e) The skeletal muscle characteristics 72h later after the injection of bupivacaine hydrochloride (f) (g) and (h) The skeletal muscle characteristics 120h later after the injection of bupivacaine hydrochloride (i) and (j) The skeletal muscle characteristics 168h later after the injection of bupivacaine hydrochloride



**Fig. 9 Expression relationship of SRPK3 in the skeletal muscle development stage**

Inhibition of RNAi (RNA interference) on the SR gene

CeSF2/ ASF would lead to the death of advanced nematode embryo. If the two or more SR protein function were inhibited, it would lead to more death of nematode or other type of developmental defects<sup>[12]</sup>. The research on tumor by Skickeler and etc. has shown that, before the function of tumor, SR protein expression type was different in different tissue cells, generally one subtype in one family, but with the development of the tumor, SR protein expression type increased, and the protein expression type of malignant tumor became complex<sup>[13]</sup>. Research on the cells of different tissue by Zahler and etc<sup>[14]</sup> has found out that, each SR protein had its own expression type and regulated the choosing of the splicing sites, which decided the specificity of the tissue. As SR protein specific kinase, SRPK3 gene brought new research perspective and starting point on the research of the relationship between related regulation factors, formation process of the muscle and the tumor cell. In this study, the writers have precisely cloned the nucleotide sequence (including the open reading frame) of the porcine SRPK3, and carried on preliminary analysis like as speculate and analyze its amino acid sequence and its characteristics. Which setted an important reference to the clone expression appraisal in other species for this gene, lays a foundation on the establishment of high conservative tag sequence between species, and enriched the foundation of its function research. The research focused on the analysis of the tissue expression of SRPK3, researched on mice and Meishan Pig showed the SRPK3 gene mainly expressed in muscle and heart, and weak in the womb and ovary<sup>[15-16]</sup>, which was almost the same as the result of this research. The difference was the latter research objective was the SRPK3 expression particularity in this experiment caused by the interspecific difference between the mice and Meishan Pig. The research has found out that, SRPK3 was under direct regulation of MEF2, lacking of this gene expression will lead to centronuclear myopathy, 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, differentiation of the skeletal muscle somatic.

The regeneration of the skeletal muscle was one physiological reaction after the injury of grown skeletal muscle, mainly activate based on the stimulating signal of the muscle satellite cell in the skeletal muscle tissue, and proliferated in the cell cycle, then differentiated and blend to form the new muscle fiber<sup>[17]</sup>. And now, it is well known that many grow factors have participated in the regulation of the differentiation and regeneration of the skeletal muscle, these growing factors expressed highly in the formation of the embryos muscle and regeneration of the destruction skeletal muscle, which could stimulate the development and differentiation, mature of the muscle precursor.

The experiment tests on the quantity of mRNA expression level of *SRPK3* gene on heart muscle, thigh muscle, and dorsal muscle of different aged Yorkshire and Duroc. The result showed the *SRPK3* expression differs a lot between different pigs with different age in days. This proved that, the *SRPK3* gene availability was different for different pigs, which may relate to the intermediate shape difference. At the same time, from the expression difference in different time of Yorkshire, we could see the expression of *SRPK3* in thigh muscle accelerates, and the writers speculated its particular participation in the thigh development of Yorkshire. So the writers have established porcine skeletal muscle injury repairing modeled 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 have found out through the expression status of *SRPK3* gene in this model, the expression of *SRPK3* in the control stays stable, while *SRPK3* gene in the experiment showed related change according to the different development stage of the muscle cell, which offered feasible evidence for the establishment of the porcine skeletal muscle injury repairing model. So using this method for the research on the *SRPK3* expression status during the skeletal muscle development process and it showed, 0.5 hours after the injection of cardiotoxin, the *SRPK3* expression was increased, and this high expression maintains to the seventh day after the injury, so researchers could speculate that, the expression characteristic of *SRPK3* gene was

lower in normal skeletal muscle tissue, only in the quiescent muscle satellite cell; while, it would increase after large amount of muscle satellite cell activation, proliferation, and differentiate to the myotube. The *SRPK3* expression volume of 0.5 hours after the injection on the Control was higher than the experiment group (0.5h), so it proved that, *SRPK3* gene did not put any auxo-action on the muscle cell injury. The expression law of *SRPK3* was almost the same as the myoblast specific differentiation maker MyoD, Myogenin. Judge from the expression profile of the *SRPK3* gene, it probably participated in the regulation process on the proliferation and differentiation of the muscle satellite cell.

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## 猪精氨酸-丝氨酸蛋白激酶3 (SRPK3) 基因的克隆、表达及多态性分析\*

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**摘要** 通过对猪 *SRPK3* 基因初步的研究,为猪分子遗传育种提供基础分子生物学信息,为猪的遗传育种提供分子标记。以大白猪为实验材料,采用 RT-PCR 方法克隆了精氨酸-丝氨酸蛋白激酶3 (serine/arginine-rich specific kinase 3, SRPK3) 的全长基因 CDS 区;采用生物信息学方法分析了 *SRPK3* 基因核苷酸序列并对其所编码的蛋白序列进行了预测与分析编码蛋白序列的结构特点;采用 PCR-SSCP 方法对大白猪,野猪,民猪及野家杂交猪的 *SRPK3* 基因的多态性进行了检验;采用实时荧光定量 PCR (Real-time) 方法检测了 *SRPK3* 在 1 日龄和 30 日龄大白猪及杜洛克的心脏、肌肉、脾脏、肝脏、肾脏、肺脏、胃、小肠、大肠、脑的表达情况;采用皮下注射的方式构建猪骨骼肌损伤模型用于研究在骨骼肌修复过程中 *SRPK3* 基因表达特性。经拼接所得到的 1708bp 核苷酸片段,涵盖了 *SRPK3* 基因的全长 CDS (1701bp),该基因编码含 567 个氨基酸片段;蛋白存在两个 S<sub>2</sub>TKc 结构域,猪 *SRPK3* 蛋白序列与人和牛的相似性较高。PCR-SSCP 检测发现第 6 外显子上 A<sup>629</sup>→G<sup>629</sup>, T<sup>653</sup>→T<sup>653</sup> 的突变,氨基酸变化为 Pro→His, Ile→Thr;第 9 外显子处的 G<sup>1059</sup>→A<sup>1059</sup>,氨基酸无突变。利用荧光定量 PCR 研究发现,表达结果显示该基因表达具有组织和种间特异性。*SRPK3* 基因的表达在整个骨骼肌细胞损伤修复过程中逐渐升高。*SRPK3* 基因主要在肌肉和心肌内表达,骨骼肌损伤修复过程中伴随骨骼肌细胞分化 *SRPK3* 的表达持续升高,推测其可能与骨骼肌细胞发育相关。

**关键词** 猪 精氨酸/丝氨酸特异蛋白激酶3 生物信息学分析 PCR-SSCP 实时荧光定量 PCR 骨骼肌损伤模型

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