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Molecular cloning, sequence identification, and tissue expression profile analysis of three novel porcine genes: SDHB, SNRPA and CRYBB1

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Abstract The complete coding sequences of three porcine genes-SDHB, SNRPA and CRYBB1 were amplified using the reverse transcriptase polymerase chain reaction (RT-PCR) based on the conserved coding sequence information of the mouse or other mammals and highly homologous pig ESTs. These three genes were then deposited into GenBank database and assigned to GeneID: 100125544, 768109 and 780429. The phylogenetic tree analysis revealed that the swine SDHB and SNRPA have closer genetic relationships with the human SDHB and SNRPA, but swine CRYBB1 has a closer genetic relationship with the bovine CRYBB1.The tissue expression analysis indicated that that swine SDHB, SNRPA and CRYBB1 gene were differentially expressed in tissues including fat, lung, muscle, small intestine, kidney, large intestine, spleen and liver. Our experiment is the first to establish the primary foundation for further research on these three swine genes.

Keywords Pig · SDHB · SNRPA · CRYBB1 · Tissue expression analysis

Introduction

SDHB is one important membrane bound enzyme in the TCA cycle. It is implicated in converting succinate to fumarate as part of the TCA cycle and related to energy production and conversion [1, 2].It had been reported that defects in SDHB are the cause of hereditary paraganglioma 4 (PLG4), also known as familial non-chromaffin paraganglioma 4 tumor [3–5]. Recent researches found that defect of SDHB is associated with an increased level of mitochondrial hydrogen peroxide production and shortened lifespan in a Drosophila [6].

SNRPA is another important gene which had been reported to be functioned in binding stem loop II of U1 snRNA and may be involved in coupled pre-mRNA splicing and polyadenylation process [7–9]. Recently there had been many reports described that SNRPA is highly associated with tumor and apoptosis [10–13].

The product of CRYBB1gene, Beta crystallin B1, is the dominant structural component of the vertebrate eye lens. Specific cleavages in the N-terminal arm of CRYBB1 occuring during lens maturation and giving rise to truncated forms will lead to impaired oligomerization and protein insolubilization [14–17].

Based on above described of these three genes, it is necessary to isolate these three genes from pig for they are associated with energy metabolism, health and other important biological functions of animals. But until today the porcine SDHB, SNRPA and CRYBB1 have not been reported yet.

In present study we will isolate the coding sequences of porcine SDHB, SNRPA and CRYBB1 genes, subsequently perform some necessary sequence analyses and tissue expression profile analyses for these genes. These will establish the primary foundation of understanding these three porcine genes.

Materials and methods

Samples collection, RNA extraction and first-strand cDNA synthesis

The tissue samples of muscle, heart, liver, fat, kidney, lung, small intestine, large intestine, were derived from five

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180 days old Meishan pigs (A Chinese local pig breed). Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu et al. [18].

Isolation of the porcine SDHB, SNRPA and CRYBB1 genes

The RT-PCR was performed to isolate these three porcine genes using the pooled cDNAs from different tissues above. The 25 μl reaction system was: 2.0 μl cDNA (100 ng/μl), 2.5 μl 2 mM mixed dNTPs, 2.5 μl 10×Taq DNA polymerase buffer, 2.5 μl 25 mM $MgCl_2$, 2.0 μl 10 μM forward primer, 2.0 μl 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μl), and 9.5 μl sterile water.The primers for porcine SDHB gene isolation were designed based on the conserved CDS sequences information from human and mouse SDHB genes (GeneBank numbers NM_003000 and NM_023374) and their highly homologous pig EST sequences (GeneBank numbers BP170798 and CO989373). Similarly, the primers for porcine SNRPA gene isolation were designed based on the conserved CDS sequences information from human and mouse SNRPA genes (GeneBank numbers NM_004596 and BC094006) and their highly homologous pig EST sequences (GeneBank numbers CN158903 and CK464377). The primers for porcine CRYBB1 gene isolation were designed based on the conserved CDS sequences information from human and mouse CRYBB1 genes (GeneBank numbers HSU35340 and AF106853)and their highly homologous pig EST sequences (GeneBank numbers CK465525 and BE012508). These primer sequences and their annealing temperature for RT-PCR reaction were described in Table 1. The PCR program initially started with a 94°C denaturation for 4 min, followed by 35 cycles of 94°C/1 min, Ta°C/1 min, 72°C/1 min, then 72°C extension for 10 min, finally 4°C to terminate the reaction.

These PCR products for porcine SDHB, SNRPA and CRYBB1cDNAs were then cloned into PMD18-T vector and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced for every gene.

Table 1 Primers for porcine SDHB, SNRPA and CRYBB1 isolation and annealing temperature

Gene	Primer sequence	Ta/°C
SDHB	Forward: 5′-ATGGCGGCGGTGGTCGCG-3′	54
	Reverse: 5′-TCAGGCTGAAGCTTTCTTC-3′	
SNRPA	Forward: 5′-ATGGCAGTTCCCGAGACC-3′	55
	Reverse: 5′-CTACTTCTTGGCGAAGGAG-3′	
CRYBB1	Forward: 5′-ATGTCTCAGCCTGCGGTC-3′	56
	Reverse: 5′-TCACTTGGGGGGCTCAGC-3′	

RT-PCR for tissue expression profile analyses

RT-PCR tissue expression profile Analyses was performed as previously described elsewhere [19]. We selected the housekeeping gene G3PDH (glyceraldehyde-3-phosphate dehydrogenase) as the internal control. The control primers used were: 5′-ACCACAGTCCATGCCATCAC-3′ (G3PDH 5′ primer)and 5′-TCCACCACCCTGTTGCTGTA-3′ (G3P DH 3′ primer). The primers of porcine SDHB, SNRPA and CRYBB1 gene which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR above. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μl reaction system was: 2 μl pooled cDNA of each tissue (100 ng/μl), 5 pM each oligonucleotide primer, 2.5 μl 2 mM mixed dNTPs, 2.5 μl 10×Taq DNA polymerase buffer, 2.5 μl 25 mM $MgCl_2$, 1.0 units of Taq DNA polymerase, and finally add sterile water to volume 25μl. The PCR program initially started with a 94°C denaturation for 4 min, followed by 25 cycles of 94°C/1 min, Ta °C/1 min, 72°C/1 min, then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Sequence analysis

The cDNA sequence prediction was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conservedd Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://www.ebi.ac.uk/clustalw). The theoretical isoelectric point (pI) and molecular weight (Mw) of proteins was computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html).

Results and discussion

cDNA amplification of porcine SDHB, SNRPA and CRBB1

Through RT-PCR with pooled tissue cDNAs from muscle, heart, liver, backfat, kidney, lung, small intestine, large intestine, for porcine SDHB, SNRPA and CRYBB1 gene, the resulting PCR products were 843, 849 and 750 bp (Fig. 1).

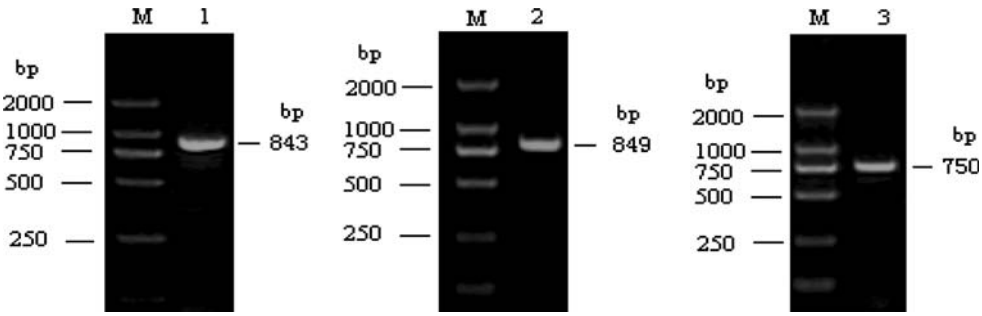

Fig. 1 RT-PCR results for porcine SDHB, SNRPA and CRYBB1. M, DL2000 DNA markers; 1, PCR product for porcine SDHB gene; 2, PCR product for porcine SNRPA gene; 3, PCR product for porcine CRYBB1 gene

Sequence analysis

These cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these genes were not homologous to any of the known porcine genes and they were then deposited into the GenBank database (Accession number: DQ915498, DQ972960, DQ915497). The sequence prediction was carried out using the GenScan software and results showed that the 843, 849 and 750 bp cDNA sequences represent three single genes which encoded 280,282,249 amino acids, respectively. The theoretical isoelectric point (pI) and molecular weight (Mw) of these deduced proteins of these three swine genes were computed using the Compute pI/Mw Tool. The pI of porcine SDHB, SNRPA and CRYBB1 are 5.08, 5.02 and 5.06. The molecular weights of these three putative proteins are 69345.50, 70951.19 and 61390.07 respectively. The complete CDS of these genes and the encoded amino acids were presented in Figs. 2, 3, 4.

Further BLAST analysis of these proteins revealed that porcine SDHB has high homology with the succinate dehydrogenase (SDHB) from sixteen species-human (96%), bovine (95%), mouse (92%), fruit fly (DROME)(73%), candida glabrata (CANGA)(69%), ustilago maydis (USTMA)(74%), ashbya gossypii (ASHGO)(70%), yeast (72%), caenorhabditis elegans (CAEEL)(64%), uromyces viciae-fabae (UROFA)(69%), reclinomonas americana (RECAM) (72%), paracoccus denitrificans (PARDE) (68%), rickettsia conorii (RICCN)(71%), rickettsia bellii RML369-C (RICPR)(70%), mycosphaerella graminicola (MYCGR)(67%), and fission yeast (SCHPO)(67%).The porcine SNRPA gene has high homology with the U1 small

Fig. 2 The complete CDS of porcine SDHB gene and its encoding amino acids * indicates the stop codon

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ATGGCGGCGGTGGTCGCGGTCTCCTTGAAACGCTGGTTCCCGGCCACAACCCTTGGCGGAGCCTGC
 M  A  A  V  V  A  V  S  L  K  R  W  F  P  A  T  T  L  G  G  A  C
CTGCAGGCCTGCCGTGGGGCCCAGACAGCTGCAGCCACAGCTCCCCGAATCAAGAAATTTGCCATC
 L  Q  A  C  R  G  A  Q  T  A  A  A  T  A  P  R  I  K  K  F  A  I
TATCGATGGGACCCAGACAAGACTGGAGATAAACCTCATATGCAAACTTATGAAATTGATTTGAAT
 Y  R  W  D  P  D  K  T  G  D  K  P  H  M  Q  T  Y  E  I  D  L  N
AACTGTGGTCCTATGGTGTTGGATGCGTTAATCAAGATTAAGAATGAAATTGATTCTACCTTGACC
 N  C  G  P  M  V  L  D  A  L  I  K  I  K  N  E  I  D  S  T  L  T
TTCCGAAGATCGTGTAGAGAAGGCATCTGCGGCTCCTGCGCCATGAACATCAACGGAGGCAACACT
 F  R  R  S  C  R  E  G  I  C  G  S  C  A  M  N  I  N  G  G  N  T
CTGGCTTGCACCCGAAGAATTGACACCAACCTCGACAAAGTTTCAAAAATCTACCCTCTTCCACAT
 L  A  C  T  R  R  I  D  T  N  L  D  K  V  S  K  I  Y  P  L  P  H
ATGTATGTGATAAAGGATCTTGTTCCTGATTTGAGCAATTTCTATGCTCAGTACAAATCCATCGAG
 M  Y  V  I  K  D  L  V  P  D  L  S  N  F  Y  A  Q  Y  K  S  I  E
CCTTATCTAAAGAAGAAGGATGAATCCCAGGAAGGCAAGCAGCAGTACCTGCAGTCCATAGAGGAG
 P  Y  L  K  K  K  D  E  S  Q  E  G  K  Q  Q  Y  L  Q  S  I  E  E
CGCGAGAAACTGGATGGGCTGTACGAGTGTATTCTTTGCGCCTGCTGCAGCACCAGCTGCCCGAGC
 R  E  K  L  D  G  L  Y  E  C  I  L  C  A  C  C  S  T  S  C  P  S
TACTGGTGGAATGGAGACAAGTACCTGGGACCCGCAGTCCTCATGCAGGCCTATCGCTGGATGATC
 Y  W  W  N  G  D  K  Y  L  G  P  A  V  L  M  Q  A  Y  R  W  M  I
GACTCCAGAGATGACTTCACGGAGGAGCGCCTGGCCAAGCTGCAGGACCCGTTCTCTCTGTACCGC
 D  S  R  D  D  F  T  E  E  R  L  A  K  L  Q  D  P  F  S  L  Y  R
TGCCACACCATCATGAACTGCACGGGGACCTGTCCCAAGGGGCTGAATCCAGGGAAAGCTATTGCT
 C  H  T  I  M  N  C  T  G  T  C  P  K  G  L  N  P  G  K  A  I  A
GAAATCAAGAAAATGATGGCAACCTACAAGGAGAAGAAAGCTTCAGCCTGA
 E  I  K  K  M  M  A  T  Y  K  E  K  K  A  S  A  *
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nuclear ribonucleoprotein A (SNRPA) from three species-human (98%), mouse (96%) and xenopus laevis (XENLA)(81%). The porcine CRYBB1 gene has high homology with the beta crystallin subunit beta B1(CRYBB1)from five species-bovine (91%), mouse (88%), rat (88%), human (82%), and chicken (66%). The porcine SDHB, SNRPA and

Fig. 5 The alignment of the protein encoded by porcine SDHB gene and other sixteen kinds of SDHB from human (HUMAN), bovine (BOVINE), mouse (MOUSE), DROME (fruit fly), CANGA (candida glabrata), USTMA (ustilago maydis), ASHGO (ashbya gossypii), yeast (YEAST), CAEEL (caenorhabditis elegans), UROFA (uromyces viciae-fabae), RECAM (reclinomonas americana), PARDE (paracoccus denitrificans), RICCN (rickettsia conorii), RICPR (rickettsia bellii RML369-C), MYCGR (mycosphaerella graminicola), and SCHPO (fission yeast) ▶

Fig. 3 The complete CDS of porcine SNRPA gene and its encoding amino acids * indicates the stop codon

```
ATGGCAGTTCCCGAGACCCGTCCCAATCACACTATTTATATCAACAATCTCAACGAGAAGATCAAG
 M  A  V  P  E  T  R  P  N  H  T  I  Y  I  N  N  L  N  E  K  I  K
AAGGATGAGCTGAAGAAGTCCCTGTACGCCATCTTCTCCCAGTTTGGCCAGATCCTGGATATCCTGGTC
 K  D  E  L  K  K  S  L  Y  A  I  F  S  Q  F  G  Q  I  L  D  I  L  V
TCACGAAGCCTGAAGATGAGGGGCCAGGCCTTTGTCATCTTCAAGGAGGTCAGCAGCGCCACCAAT
 S  R  S  L  K  M  R  G  Q  A  F  V  I  F  K  E  V  S  S  A  T  N
GCCCTGCGCTCCATGCAGGGTTTCCCCTTCTACGACAAGCCCATGCGCATCCAGTACGCCAAGACC
 A  L  R  S  M  Q  G  F  P  F  Y  D  K  P  M  R  I  Q  Y  A  K  T
GACTCGGATATCATCGCCAAGATGAAGGGCACCTTCGTGGAGCGGGACCGCAAGCGGGAGAAGAGG
 D  S  D  I  I  A  K  M  K  G  T  F  V  E  R  D  R  K  R  E  K  R
AAGCCCAAGAGCCAAGAGACCCCGGCCTCCAAGAAGGCCGTGCAGGGCGGGGCAGCCGCCCCCGTG
 K  P  K  S  Q  E  T  P  A  S  K  K  A  V  Q  G  G  A  A  A  P  V
GTGGGCGCCGTTCAGGGTCCTGTCCCGGGCATGCCGCCGATGACTCAGACACCCCGCATCATGCAC
 V  G  A  V  Q  G  P  V  P  G  M  P  P  M  T  Q  T  P  R  I  M  H
CACATGCCGGGCCAGCCTCCCTACATGCCGCCCCCTGGCATGATCCCGCCTCCAGGCCTCGCGCCC
 H  M  P  G  Q  P  P  Y  M  P  P  P  G  M  I  P  P  P  G  L  A  P
GGCCAGATCCCGCCCGGGGCCATGCCCCCCCAGCAGCTTATGCCTGGGCAGATGCCACCTGCCCAG
 G  Q  I  P  P  G  A  M  P  P  Q  Q  L  M  P  G  Q  M  P  P  A  Q
CCTCTTTCAGAAAACCCACCAAATCACATCTTGTTCCTCACCAACCTGCCGGAGGAGACCAACGAG
 P  L  S  E  N  P  P  N  H  I  L  F  L  T  N  L  P  E  E  T  N  E
CTCATGCTTTCCATGCTTTTCAACCAGTTCCCTGGCTTCAAGGAGGTCCGGCTGGTCCCTGGGCGG
 L  M  L  S  M  L  F  N  Q  F  P  G  F  K  E  V  R  L  V  P  G  R
CACGACATCGCCTTTGTGGAGTTTGACAACGAGGTGCAGGCGGGGGCCGCTCGCGATGCCCTGCAG
 H  D  I  A  F  V  E  F  D  N  E  V  Q  A  G  A  A  R  D  A  L  Q
GGCTTCAAGATCACCCAGAACAACGCCATGAAGATCTCCTTCGCCAAGAAGTAG
 G  F  K  I  T  Q  N  N  A  M  K  I  S  F  A  K  K  *
```

Fig. 4 The complete CDS of porcine CRYBB1 gene and its encoding amino acids * indicates the stop codon

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ATGTCTCAGCCTGCGGTCAAGGCCTCGGCCACAGCTGCGGTGAACCCAGGGCCTGATGGGAAGGGG
 M  S  Q  P  A  V  K  A  S  A  T  A  A  V  N  P  G  P  D  G  K  G
AAGGGGGCCCCGCCCCCCGGACCAGCCCCGGGCTCCGGCCCCGCCCAGGCTCCAGCCCAGCCAATG
 K  G  A  P  P  P  G  P  A  P  G  S  G  P  A  Q  A  P  A  Q  P  M
CCCGCTGCCAAAGGGGACCTGCCTCCCGGGAGCTACAAGCTGGTGGTCTTTGAGCAAGAGAACTTC
 P  A  A  K  G  D  L  P  P  G  S  Y  K  L  V  V  F  E  Q  E  N  F
CAGGGCCGGCGGGTGGAATTCTCCGGGGAGTGCTTGAACCTGGGAGACCGGGGCTTTGACCGAGTG
 Q  G  R  R  V  E  F  S  G  E  C  L  N  L  G  D  R  G  F  D  R  V
CGCAGCATCATTGTCACCTCAGGACCCTGGGTCGCCTTTGAGCAGTCCAACTTCCGGGGCGAGATG
 R  S  I  I  V  T  S  G  P  W  V  A  F  E  Q  S  N  F  R  G  E  M
TTCATCCTGGAGAAGGGCGAGTACCCGCGATGGGACACATGGTCGAGCAGCTACCGCAGCGACCGG
 F  I  L  E  K  G  E  Y  P  R  W  D  T  W  S  S  S  Y  R  S  D  R
CTCATGTCCTTCCGGCCCATCAGGATGGATGCCCAGGAACACAAGCTCTGCCTGTTTGAAGGTGCC
 L  M  S  F  R  P  I  R  M  D  A  Q  E  H  K  L  C  L  F  E  G  A
AACTTCAAGGGCAACACCATGGAGATCCAGGAGGATGACGTGCCCAGTCTCTGGGTCTATGGCTTC
 N  F  K  G  N  T  M  E  I  Q  E  D  D  V  P  S  L  W  V  Y  G  F
TGTGACCGTGTGGGCAGCGTGAGGGTCTCCAGTGGAACCTGGGTCGGCTATCAGTATCCTGGCTAC
 C  D  R  V  G  S  V  R  V  S  S  G  T  W  V  G  Y  Q  Y  P  G  Y
CGCGGGTACCAGTACCTCCTGGAGCCTGGTGACTTCCGGCACTGGAACGACTGGGGGGCCTTCCAG
 R  G  Y  Q  Y  L  L  E  P  G  D  F  R  H  W  N  D  W  G  A  F  Q
CCCCAGATGCAGGCTGTGCGCCGTCTGCGTGACAGACAGTGGCACCGTGAAGGCTGCTTCCCCGTC
 P  Q  M  Q  A  V  R  R  L  R  D  R  Q  W  H  R  E  G  C  F  P  V
CTGGCTGCTGAGCCCCCCAAGTGA
 L  A  A  E  P  P  K  *
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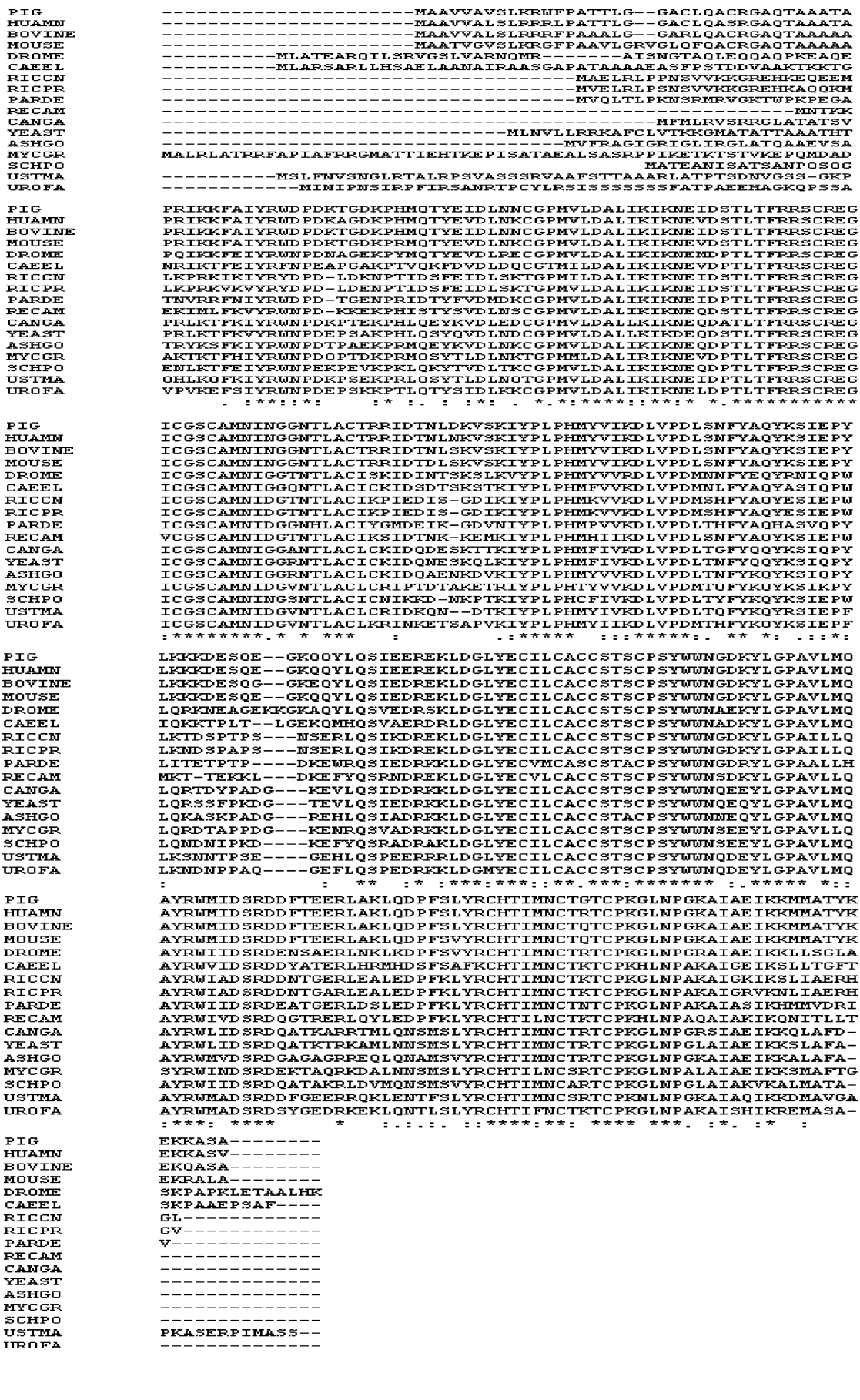
PIG
HUAMN
BOVINE
MOUSE
DROME
CAEEL
RICCN
RICPR
PARDE
RECAM
CANGA
YEAST
ASHGO
MYCGR
SCHPO
USTMA
UROFA

CRYBB1 have common conservedd domains with their corresponding highly homologous proteins (Figs. 5, 6, 7).

Based on the results of the alignment of SDHB, SNRPA and CRYBB1, the phylogenetic trees were constructed using the ClustalW software (http://www.ebi.ac.uk/clustalw), as shown in Figs. 8, 9, 10.

The phylogenetic tree analysis revealed that the swine SDHB and SNRPA have closer genetic relationships with

Fig. 6 The alignment of the protein encoded by porcine SNRPA gene and other three kinds of SNRPA from HUMAN (human), MOUSE (mouse) and XENLA (xenopus laevis)

Fig. 7 The alignment of the protein encoded by porcine CRYBB1 gene and other five kinds of CRYBB1 from BOVINE (bovine), MOUSE (mouse), RAT (rat), HUMAN (human), and CHICKEN (chicken)

the human SDHB and SNRPA, but swine CRYBB1 has a closer genetic relationship with the bovine CRYBB1.

Tissue expression profile

Tissue expression profile analysis was carried out and results revealed that, compared to G3PDH expression, the swine SDHB gene was over-expressed in spleen, weakly in kidney, and hardly expressed in small intestine, large intestine muscle, fat, liver, and lung. The swine SNRPA gene was moderately expressed in fat, weakly in muscle, and hardly expressed in small intestine, large intestine, spleen, liver, lung, and liver. The swine CRYBB1 gene was moderately expressed in small intestine, large intestine fat, lung, muscle,spleen and kidney, and weakly expressed in liver (Fig. 11).

Comparative genomics is the analysis and comparison of genomes from different species. Researchers have learned a great deal about the function of human genes by examining their counterparts in simpler model organisms such as the mouse and some results has revealed that virtually all (99%) of the protein-coding genes in humans align with homologs in mouse, and over 80% are clear 1:1 orthologs [20]. This extensive conservation in protein-coding regions implied that this conservation of protein-coding sequences may be expected in different mammals including pigs. From the isolation of swine SDHB, SNRPA and CRYBB1 genes, we can find that swine SDHB, SNRPA and CRYBB1 are highly homologous with SDHB, SNRPA and CRYBB1of human, mouse and other mammals.This further validated that comparative genomics method is one useful tool to isolate the unknown genes especially the conserved coding region of genes for pigs.

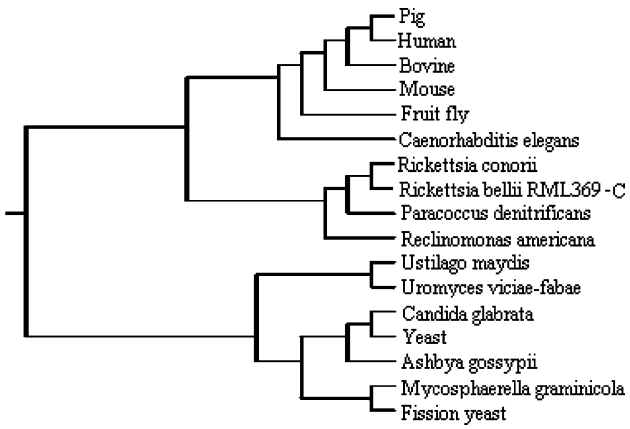

Fig. 8 The phylogenetic tree for fifteen kinds of SDHB

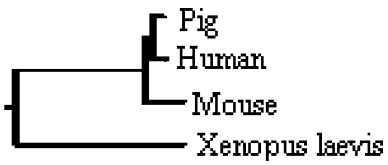

Fig. 9 The phylogenetic tree for four kinds of SNRPA

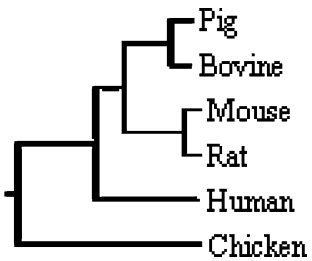

Fig. 10 The phylogenetic tree for six kinds of CRYBB1

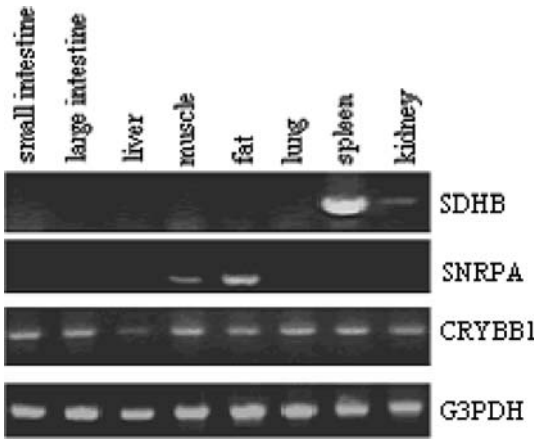

Fig. 11 Tissue expression distribution of swine SDHB, SNRPA and CRYBB1 gene. The G3PDH expression is the internal control

From the alignment anlyses for swine SDHB, SNRPA and CRYBB1 proteins we found that swine SDHB, SNRPA and CRYBB1 proteins were not identity to the SDHB, SNRPA and CRYBB1 proteins of other spieces. This implied that swine SDHB, SNRPA and CRYBB1will have some differences in functions to those of human, mouse and other spieces.

The phylogenetic tree analysis revealed that the swine SDHB and SNRPA have closer genetic relationships with human SDHB and SNRPA. These implied that pig should be a better model animal to study these two genes of human than others. Similarly, pig also should be a good model animal to study the bovine CRYBB1 gene.

We also noticed that human and mouse SDHB, SNRPA and CRYBB1 genes had been found to be expressed in most of tissues (http://www.ncbi.nlm.nih.gov/UniGene). From the tissue expression profile analysis in our experiment it can be seen that these genes were obviously differentially expressed in some tissues and there were no expression in some tissues. As we did not study functions nor protein levels yet, there might be many possible

reasons for differential expression of these three porcine genes. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of these genes were presented diversely in different tissues.

In conclusion, we first isolated the porcine SDHB, SNRPA and CRYBB1 gene and performed necessary functional analysis and tissue expression profile analysis. This established the primary foundation for further research on these three swine genes.

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