

## Isolation, Sequence Identification and Tissue Expression Profile of a Novel Sheep Gene-*SERPINF1*

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**Abstract:** The full-length cDNA sequences of one sheep gene, *SERPINF1* was amplified using the Rapid Amplification of cDNA Ends (RACE) method based on one pig EST sequence which was highly homologous to the coding sequence of human *SERPINF1* gene. Sequence prediction analysis revealed that the open reading frame of this gene encodes a protein of 416 amino acids that has high homology with the serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (SERPINF1) of twelve species-bovine (96%), pig (91%), human (88%), chimpanzee (88%), horse (88%), crab-eating macaque (87%), dog (89%), domestic guinea pig (84%), mouse (85%), rat (83%), red jungle fowl (63%) and western clawed frog (55%) so that it can be defined as sheep *SERPINF1* gene. This novel sheep gene was assigned to GeneID: 100192425. The phylogenetic analysis revealed that the sheep *SERPINF1* gene has a closer genetic relationship with the *SERPINF1* gene of bovine. Tissue expression analysis indicated that the sheep *SERPINF1* gene is differentially expressed in detected tissues including spleen, muscle, skin, kidney, lung, liver, fat and heart. The experiment is the first to establish the primary foundation for further research on the sheep *SERPINF1* gene.

**Key words:** Sheep, SERPINF1, RACE, tissue expression profile, sequence identification, China

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### INTRODUCTION

SERPINF1 is a member of the serpin family although, it does not display the serine protease inhibitory activity shown by many of the other serpin family members. The encoded protein is secreted and strongly inhibits angiogenesis. In addition this protein is a neurotrophic factor involved in neuronal differentiation in retinoblastoma cells (Gvritshvili *et al.*, 2010; Konson *et al.*, 2011; Ho *et al.*, 2010; Chen *et al.*, 2010). However, other studies have demonstrated that *SERPINF1* gene was also involved in biological processes and metabolic processes such as cell proliferation, aging, kidney development, multicellular organismal development, negative regulation of angiogenesis, negative regulation of endopeptidase activity, negative regulation of epithelial cell proliferation involved in prostate gland development, negative regulation of inflammatory response, positive regulation of neurogenesis, regulation of proteolysis, response to glucocorticoid stimulus, response to retinoic acid and short-term memory (Pignolo *et al.*, 1993; Simonovic *et al.*, 2001; Becerra *et al.*, 1993; Wagsater *et al.*, 2010).

*SERPINF1* gene is an important gene which has many biological functions. Until today, *SERPINF1* gene

has been reported in bovine, pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl, western clawed frog and other animals. The sheep SERPINF1 has not been reported.

In the present experiment, the researchers will clone the full-length cDNA sequence of the sheep *SERPINF1* gene and further do necessary sequence analysis and tissue expression analysis. These will establish the primary foundation of understanding this sheep gene.

### MATERIALS AND METHODS

**Animals and sample preparation:** Five adult Yunnan local sheep were slaughtered. Spleen, muscle, skin, kidney, lung, liver, fat and heart samples were collected, frozen in liquid nitrogen and then stored at -80°C. The total RNA was extracted using the Total RNA Extraction Kit (Gibco, USA). These RNA samples were used to perform RACE PCR and tissue expression profile analysis.

**5'- and 3'-RACE:** The 5'-and 3'-RACE were performed to isolate the full-length cDNA for sheep *SERPINF1* gene as the instructions of BD SMART™ RACE cDNA Amplification Kit (BD science, USA). For the sheep

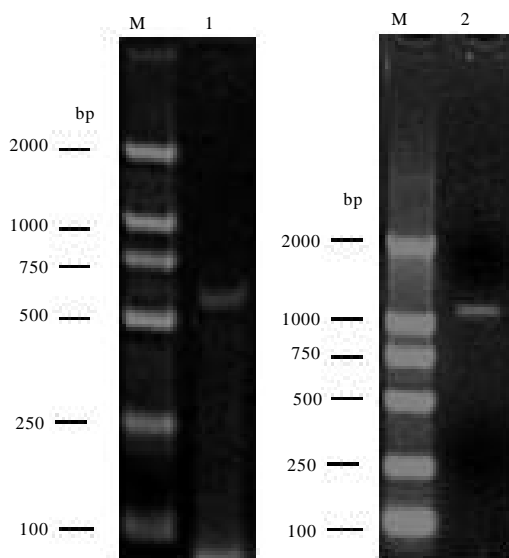


Fig. 1: RACE results for sheep *SERPINF1* gene. M, DL2000 DNA markers 1, 5'-RACE product for *SERPINF1* gene; 2, 3'-RACE product for *SERPINF1* gene

*SERPINF1* gene, the Gene-Specific Primers (GSPs) were designed based on one sheep EST sequence whose sequence is highly homologous to the coding sequence of human *SERPINF1* gene: EE758330. The Gene-Specific Primers (GSPs) were: 5'-RACE GSP: 5'-GCCTGCACCCA GTTGTTAATCTCCT-3', 3'-RACE GSP: 5'-CCGGGCTCT GTACTACGACCTGATC-3'. RACE touchdown PCRs were carried out with 5 cycles of 94°C/30 sec and 72°C/3 min followed by 5 cycles of 94°C/30 sec, 67°C/30 sec and 72°C/3 min, finally with 30 cycles of 94°C/30 sec, 67°C/30 sec, 72°C/3 min to terminate reaction. The RACE PCR products were then cloned into pMD18-T vector (TaKaRa, Dalian, China) and sequenced bidirectionally with the commercial fluorometric method (SHENGGONG, Shanghai, China). At least five independent clones were sequenced for each PCR product (Fig. 1).

**Semi-quantitative RT-PCR:** RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009; Yonggang and Shizheng, 2009; Liu, 2009). The researchers selected the housekeeping gene  $\beta$ -actin (Accession no: NM\_001009784) was performed as a positive control. The control primers used were: 5'-ATCACCATCG-GCAATG AGC-3' (forward primer1) and 5'-CCGTGTTGGCGTAGA GGT-3' (reverse primer1). The PCR product is 151 bp in length. The following *SERPINF1* gene specific primers were used to perform the RT-PCR for tissue expression profile analysis: 5'-ACATCCACG-GCACCTACA-3' (forward primer2) and 5'-AACTTTCACGGTCCTCCC-3'

(reverse primer2). The PCR product is 374 bp in length. The 25  $\mu$ L reaction system was: 2  $\mu$ L cDNA (100 ng), 5 pmoles each oligonucleotide primer (forward primer 1 and reverse primer1 or forward primer and reverse primer2), 2.5  $\mu$ L 2 mmol L<sup>-1</sup> mixed dNTPs, 2.5  $\mu$ L 10 $\times$ Taq DNA polymerase buffer, 2.5  $\mu$ L 25 mmol L<sup>-1</sup> MgCl<sub>2</sub>, 1.0 units of Taq DNA polymerase and finally add sterile water to volume 25  $\mu$ L. The PCR program initially started with a 94°C denaturation for 4 min, followed by 30 cycles of 94°C/50 sec, 54°C/50 sec, 72°C/50 sec 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 conserved 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>).

## RESULTS AND DISCUSSION

**ACE results for sheep *SERPINF1* gene:** Through 5'-RACE, one PCR product of ~600 bp was obtained. The 3'-RACE product was ~1.1 kb. These products were then cloned to T-vector and sequenced. Taken together, a 1422 bp cDNA complete sequence was finally obtained.

**Sequence analysis:** The cDNA nucleotide sequence analysis using the BLAST software revealed that this 1422 bp cDNA sequence was not homologous to any of the known sheep genes and it was then deposited into the GenBank database (Accession number: FJ211198). The sequence prediction was carried out using the GenScan software and results showed that this 1422 bp cDNA sequence represented one single gene which encoded 416 amino acids. The theoretical isoelectric point (pI) and Molecular weight (Mw) of this deduced protein were computed using the Compute pI/Mw tool. The pI is 7.73. The molecular weight of this putative protein is 45957.77. This novel sheep gene was assigned to GeneID: 100192425. The complete cDNA sequence of this gene and the encoded amino acids were shown in Fig. 2.

Further BLAST analysis of this deduced protein revealed that this protein has high homology with the serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (*SERPINF1*) of twelve species-bovine (96%), pig (91%), human (88%), chimpanzee (88%), horse (88%), crab-eating macaque (87%), dog (89%), domestic guinea pig (84%), mouse (85%), rat (83%), red jungle fowl (63%) and western clawed frog (55%) (Fig. 3).

GGCTGGGCGTGGAGCGGCGGTGCACCCACAGGCCCCGAGATGCGAGGCCCTCGTGC TACTCCTCTGG  
 ACTGGAGCCCTCC TTGGGTTGGCCACTGTCAGAACGCCGCCGGAGGGCGGGCTCCCTGGCCCT  
 T G A L L G F G H C C Q N A G P E A G S L A A  
 GAGACACAGGGGCACCCGTGGAGGAAGAGGATCCCTTCAAGGTCGCCG TGAACAAGCTGGCG  
 E S T G A P V E E E D P F F K V P V N K L A  
 GCAGCCGCTCCAAC TCGGCTACGACCTGACCGCGTGAGATCTGGCGAGAGCCCCACCACCAAC  
 A A V S N F G Y D L Y R V R S G E S P T T N  
 GTGCTGCTGCTCCGCTACGCGTGGCCACGGCGCTCTGCCCCGTGCTGGGTGGCGAACAGCGG  
 V L L S P L S V A T A L S A L S L G A E Q R  
 ACAGAATCCAGCATTACCGGGCTCTGTACTACGACCTGATCAGTAACCCAGACATCCACGGCACC  
 T E S S I H R A L Y Y D L I S N P D I H G T  
 TACAAGGACCTCCCTTGCCTCCGTCACCTGCCCCAGAAAGACCTTAAAAGTGC TCCCGGATTATC  
 Y K D L L A S V T A P Q K N L K S A S R I I  
 TTTGAGAGAAAGCTGGGATAAAAGCCAGCTTCGTCACCCCTCGAGAAAGTCAATGGGACCAAG  
 F E R K L R I K A S F V P P L E K S Y G T R  
 CCCAGAATCCGACCGGCAACTCGAATAGACCTTCAGGAGATTAAACAAC TGGGTGCAGGCCGAG  
 P R I L T G N S R I D L Q E I N N W V Q A A Q  
 ATGAAAGGAAAATTGCTAGATCCACA CGGAAATAACCCAGTGAATCAGCATTCTCCTTCTTGGT  
 M K G K I A R S T R E I P S G I S I L L L G  
 GTGGCTTACTCAAGGGGAGTGGGTAACAAGTTGACTCCAGGAAGACTTCCCTGGAGGATTC  
 V A Y F K G Q W V T K F D S R K T S L E D F  
 CACTGGATGAGGGAGGACCGTGAAGTTC CATGATCTCAGACCCTAAGGCCGTTTACGGTAC  
 H L D E G R T V K Y P M S D P K A V L R Y  
 GGCTTGGATTCTGATCTCAACTGCAAGATCGCCAGCTGCCCTGACCGGGAGCACAAGTATCATC  
 G L D S D L N C K I A Q L P L T G S T S I I  
 TTCTTCCGCTCAGAAAGTGACCCAGAACTTGACCTTGATAGAAGAGAGCCTCACCTCTGAGTTC  
 F L P Q K V T Q N L T L I E E S L T S E F  
 ATTATGACATAGACCGAGAAC TGAAGACTGTTACGGCAGTCC TGACCATTCCAAGCTGAAGCTG  
 I H D I D R E L K T V Q A V L T I P K L K A  
 AGTTATGAAGGCGAACTCAGAAAGCTGTCAGGAGCTGAAGCTACAATCCCTGTTGATGCACCA  
 S Y E G E L T K S V Q E L K L Q S L F D A P  
 GACTTACGAAATCAGGCAAACTATCAAACTTACTCAAGTGAACATCGCATCGGATTCGAG  
 D F S K I T G K P I K L T Q V E H R I G F E  
 TGAATGAGGATGGGGCGGTA CTAAGTCCAGCCAGGGGTCCAGCCTGCCCGCTCACCTTCCCT  
 W N E D G A G T N S S P G V Q P A R L T F P  
 CTGGACTACCTTAACCAACCTTTCATCTTGTACTGAGGGACACAGACACAGGGGCCCTTCTC  
 L D Y H L N Q P F I F V L R D T D T G A L L  
 TTCATAGGCAAAATCTGGACCCAGAGGCAC TAACTACTCAACTTAATGTTCAAATACCCAGAA  
 F I G K I L D P R G T \*  
 AAAAAACACTAGCGGATGGCAGATTATATATGAAGGCTGCCCTACGTTTCAATGTATACTTTG  
 CAATAAAGTGCTTCTCCTTAAAAA

Fig. 2: The complete cDNA sequence and encoded amino acids of sheep *SERPINF1* gene (GenBank accession number: FJ211198). ATG, start codon; TAA, stop codon. \* indicates the stop codon

Mouse	MQALVLLWTGALLGHGSSQNVPSSESGSPVPDSTGEPVEEED-PFFKVPVNKLAIAAVSN
Rat	MQTLVLLWTGALLGHGSSQNVPSQSDSPADSTGEPVVEEDPFFKAPVNKLAIAAVSN
Sheep	MQALVLLWTGALLGFHGCQN--AGPEAGSLAPESTGAPVEEEDPFFKVPVNKLAIAAVSN
Bovine	MQALVLLWTGALLGFGRQCN--AGQEAGSLTPESTGAPVEEEDPFFKVPVNKLAIAAVSN
Pig	MQALVLLWTGALLGSGCQCN--AGPEEGSPADTVGAPVEEEDPFFKVPVNKLAIAAVSN
Horse	MQALMLLWTGALLGHGSCQNMAGPPEEGSPDPDITGAPVEEEDPFLKVPVNKLAIAAVSN
Chimpanzee	MQALVLLLCIGALLGHSSCQNPASPEEGSPDPDSTGALVEEEDPFFKVPVNKLAIAAVSN
Human	MQALVLLLCIGALLGHSSCQNPASPEEGSPDPDSTGALVEEEDPFFKVPVNKLAIAAVSN
Crab-eating macaque	MQALVFLCFAALLGHSSCQSLASGPEEGSPDPDSTGALVEEEDPFFKVPVNKLAIAAVSN
Dog	-----MRAAPKDSPADATGVPVEEEDPFFRVVNKLAIAAISN
Domestic guinea pig	MQVLVLLWTGALLGRGSCQDIASNPED-SFSPSTGEPVEEEDPFFKVPVNKLAIAAISN
Red jungle fowl	MQIPAVLLLGLLTIPTSKSQN--SPAGQNSPTDGTGVEEEDPFYKTPINKLAIAAVSN
Western clawed frog	MKIYLALLFTGFSFLSYTSAQN-----AADEVTEVEEEDPFYKSPINRLASSASN
	** : ** : ** : ** : **
Mouse	FGYDLYRLRSSASPTGNVLLSPLSVATALSALSGLGAEHRTESVIHRALYYDLINPDIHS
Rat	FGYDLYRLRSGAVSTGNILLSPLSVATALSALSGLGAEQRTESVIHRALYYDLINPDIHS
Sheep	FGYDLYRVRSGESPTTNVLLSPLSVATALSALSGLGAEQRTESSIHRALYYDLISNPDIHG
Bovine	FGYDLYRVRSGESPTANVLLSPLSVATALSALSGLGAEQRTESSNIHRALYYDLISNPDIHG
Pig	FGYDLYRVRSGESPTANVLLSPLSVATALSALSGLGAEQRTESSLHRALYYDLISNPDIHG
Horse	FGYDLYRAKSSMSPTANVLLSPLSVATALSALSGLGAEQRTESSIHLALYYDLIKNPDIHG
Chimpanzee	FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSGLGAEQRTESSIHRALYYDLISSPDIHG
Human	FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSGLGAEQRTESSIHRALYYDLISSPDIHG
Crab-eating macaque	FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSGLGAEQRTESSVIHRALYYDLISSPDIHG
Dog	FGYDLYRVRSSFSPTANVLLSPLSVATALSALSGLGAEQRTESSIHRALYYDLISNPDIHS
Domestic guinea pig	FGYDLYRVRSESPTTNVLLSPLSVATALSALSGLGAEQRTEATHRALYYDMI SNPDIHS
Red jungle fowl	FGYDLYRQQSSRTATANVLLSPLS LATALSGLGAEQRTEVLSRALFYDLLNKAIEVHN
Western clawed frog	FGYDLYRMOANKNPNSNIIISPLS IATLSLSLGGGQRTESLIQRSLYYD L LMDPEVHA
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Mouse	TYKELLASVTAPEKNLKSASRIVFERKLRVKSFFVAPLEKSYGTRPRILTGMPRLDQEI
Rat	TYKELLASVTAPEKNLKSASRIVFERKLRVKSFFVAPLEKSYGTRPRILTGMPRLDQEI
Sheep	TYKDLLASVTAPEKNLKSASRIIFERKLRVKSFFVAPLEKSYGTRPRILTGMSRLDQEI
Bovine	TYKDLLASVTAPEKNLKSASRIIFERKLRVKSFFVAPLEKSYGTRPRILTGMSRLDQEI
Pig	TYKELLAAVTAPEKNLKSASRIIFERKLRVKSFFVAPLEKSYGTRPRILTGMSRLDQEI
Horse	TYKELLASVTAPEKNLKSASRIIFERKLRVKSFFVAPLEKSYGTRPRILTGMSRLDQEI
Chimpanzee	TYKELLDTVTAPEKNLKSASRIVFERKLRVKSFFVAPLEKSYGTRPRVLTGMPRLDQEI
Human	TYKELLDTVTAPEKNLKSASRIVFERKLRVKSFFVAPLEKSYGTRPRVLTGMPRLDQEI
Crab-eating macaque	TYKELLDTVTAPEKNLKSASRIVFERKLRVKSFFVAPLEKSYGTRPRVLTGMPRLDQEI

Fig. 3: Continue

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Dog          TYKELLASVTAPEKNFKSASRIVFERKLRKIKSSFVAPLEKSYSTRPRILTGNPRDLQEV
Domestic guinea pig TYKELLATVTAQPNLKSASRIVFERKLRKIKSSLVALLEKSYSTRPRILTGNPRDLQEI
Red jungle fowl    TYKDLLASVTGPEKSLKSASRIIVEKRLRVKSTFHSQLEKSYRMRRLRALSNGNTQLDLQEI
Western clawed frog TYKDLLASFTSQASGLKSTWRIMLERLRRLRMDFVTQVEKFFYGMKPKVLTGSTRDLQEA
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Mouse       NNWVQAQMKGKIARSTREMP SALSILLGVAYFKGQWVTKFD SRKTTLQD FHLDEDRIVR
Rat         NNWVQAQMKGKIARSTREMP SALSILLGVAYFKGQWVTKFD SRKTTLQD FHLDEDRIVR
Sheep       NNWVQAQMKGKIARSTREI P S G S I L L G V A Y F K G Q W V T K F D S R K T S L E D F H L D E G R I V K
Bovine      NNWVQAQMKGKVARSTREMP SEISIFLLGVAYFKGQWVTKFD SRKTSLED FYLDEERTVK
Pig         NNWVQAQTKGKVARSTREL PGEISILLGVAYFKGQWVTKFD SRKTSLED FHLDEERTVK
Horse       NNWVQAQMKGKIARSTREVP SEISILLGVAYFKGQWVTKFD SRKTSLQD FHLDEERTVT
Chimpanzee NNWVQAQMKGKIARSTKEI PDEISILLGVAYFKGQWVTKFD SRKTSLED FHLDEERTVR
Human       NNWVQAQMKGKIARSTKEI PDEISILLGVAYFKGQWVTKFD SRKTSLED FYLDEERTVR
Crab-eating macaque NNWVQAQMKGKIARSTKEL PDEISILLGVAYFKGQWVTKFD PRKTSLED FHLDEERTVR
Dog         NNWVQAQMKGKIARSTREI P S G S I L L G V A Y F K G Q W V T K F D S R K T S L E D F H L D E E R T V K
Domestic guinea pig SNWVQAQMKGKITRSTREVP S G S I L L G V A Y F K G Q W V T K F D S R K T S L Q D F H L D E E R T V K
Red jungle fowl    NNWVRQQTGRILRFMKDMPDVSILLGAAAYFKGTWTKFDTKRTVLKDFHLDEDRIVQ
Western clawed frog  NDFIQKQTGKVVKFFKEIPTSVSILLGTTYLKGGWAYKFNPRETVQRE FHLDEQTSVHT
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Mouse       VPMMSDPKAILRYGLDSDLNCKIAQLPLTGSM S I I F F L P L T V T Q N L T M I E E S L T S E F I H D
Rat         VPMMSDPKAILRYGLDSDLNCKIAQLPLTGSM S I I F F L P L T V T Q N L T M I E E S L T S E F V H D
Sheep       VPMMSDPKAVLRYGLDSDLNCKIAQLPLTG S T S I I F F L P Q K V T Q N L T L I E E S L T S E F I H D
Bovine      VPMMSDPQAVLRYGLDSDLNCKIAQLPLTG S T S I I F F L P Q K V T Q N L T L I E E S L T S E F I H D
Pig         VPMMSDPKAVLRYGLDSDLNCKIAQLPLTG S M S I I F F L P L K V T Q N L T M I E E S L T S E F I H D
Horse       VPTMSDPKAILRYGLDSDLNCKIAQLPLTG S M S I V F F L P Q K V T Q N L T M I E E S L T S E F I H D
Chimpanzee VPMMSDPKAVLRYGLDSDL SCKIAQLPLTG S T S I I F F L P L K V T Q N L T L I E E S L T S E F I H D
Human       VPMMSDPKAVLRYGLDSDL SCKIAQLPLTG S M S I I F F L P L K V T Q N L T L I E E S L T S E F I H D
Crab-eating macaque VPMMSDPKAILRYGLDSDL SCKIAQLPLTG S M S I I F F L P L K V T Q N L T L I E E S L T S E F I H D
Dog         VPMMSDPKAILRYGLDSDL SCKIAQLPLTG S M S I I F F L P L K V T Q N L T M I E E S L T S E F I H D
Domestic guinea pig VPMMSDPKAILRYGLDSDLNCKIAQLPLTG S M S I I F F L P M R A T Q N L T M I E E S L T S E F V H D
Red jungle fowl    VSMMSDPKAILRYGDFSELNCKIAQLPLTE GVSAMFFLPKVTQNM TLIEE S L T S E F V H D
Western clawed frog  VPMSSKNIPVRYGLDSDFNCKIVQLPLTG GVSIMFPLPNTVTQ N L T M I E E G L T S E F V H D
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Mouse       IDRELKTIQAVLTVPKLKL SFE G E L T K S L Q D M K L Q S L F E S P D F S K I T G K P V K L T Q V E H R A
Rat         IDRELKTIQAVLTVPKLKL S Y E G D W T N S L Q D M K L Q S L F E S P D F S K I T G K P V K L T Q V E H R A
Sheep       IDRELKTVQAVLTI P K L K L S Y E G E L T K S V Q E L K L Q S L F D A P D F S K I T G K P I K L T Q V E H R I
Bovine      IDRELKTVQAVLTI P K L K L S Y E G E L T K S V Q E L K L Q S L F D A P D F S K I T G K P I K L T Q V E H R V
Pig         IDRELKTVQAVLTI P K L K L S Y E G E L T K S V Q E L K L Q S L F D S P D F S K I T G K P I K L T Q V E H R I
Horse       IDRELKTVQAVLTI P K L K L S Y E G E V T K S L Q E I K L Q S L F D S P D F S K I T G K P L K L T Q V E H R A
Chimpanzee IDRELKTVQAVLTI P K L K L S Y E G E V T K S L Q E M K L Q S L F D S P D F S K I T G K P I K L T Q V E H R A
Human       IDRELKTVQAVLTI P K L K L S Y E G E V T K S L Q E M K L Q S L F D S P D F S K I T G K P I K L T Q V E H R A
Crab-eating macaque IDRELKTVQAVLTI P K L K L S Y E G E V T K S L Q E T K L Q S L F D S P D F S K I T G K P I K L T Q V E H R A
Dog         IDRELKTIQAVLTI P K L K L S Y E G E V T K S L Q E M K L Q S L F D S P D F S K I T G K P I K L T Q V E H R A
Domestic guinea pig INRELKAVQAVLSI P R L K L S F E G E L T K S L Q E M K L H S L F E S P D F S K I T G K P I K L T Q V E H R A
Red jungle fowl    VDRELKTVHAVLSL P K L K L N Y E E A L G N T V K E T R L Q S L F T S P D F T K I S A K P I K L S H V Q H K A
Western clawed frog  IDQALQPINLVL S V P K L K L N Y E A E L K E A L Q E S K L Q S L F A T P D F S K I S S K P L K L S Y V V H K A
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Mouse       AFEWNEEGAGSSPS-PGLQPVRLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G R I L D P S S T
Rat         AFEWNEEGAGTSSN-PDLQPVRLTFPLDYHLNR P F I F V L R D T D T G A L L F I G R I L D P S S T
Sheep       GFEWNEDGAGTNSN-PGVQPARLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G T
Bovine      GFEWNEDGAGTNSN-PGVQPARLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G T
Pig         GFEWNEDGGSATSS-PGPR--LTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R S T
Horse       GFEWNEDG-ATNPS-OGPQAHLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G T
Chimpanzee GFEWNEDGAGTTPS-PGLQPAHLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G T
Human       GFEWNEDGAGTTPS-PGLQPAHLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G P
Crab-eating macaque GFEWNEDGAGATPS-PGLQPAHLTFPLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R G T
Dog         GFEWNEDGAGTTPS-PGLQPTLRFPLDYHLNR P F I F V L R D T D T G A L L F I G K I L D P R G I
Domestic guinea pig GFEWNEEGAGPTSTNSDLQPTGFTFSLDYHLNQ P F I F V L R D T D T G A L L F I G K I L D P R S T
Red jungle fowl    VLELNEDEKSTPN-PGVNAARLTFPIEYHVDRPFLVLRDDTTGTL L F I G K I L D P R S V
Western clawed frog  TLELNEEGAEATPK--PEDSHRNYFPLE Y H L D H P F L V L R A M D M G A L L F I G K V M D P K G F S
:* **:* .. * :***:***:*** .*:*****:*** .

Mouse       -
Rat         -
Sheep       -
Bovine      -
Pig         -
Horse       -
Chimpanzee -
Human       -
Crab-eating macaque -
Dog         -
Domestic guinea pig -
Red jungle fowl -
Western clawed frog F
    
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Fig. 3: The alignment of the protein encoded by sheep *SERPINF1* gene and twelve other kinds of SERPINF1 proteins from bovine, pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl and western clawed frog

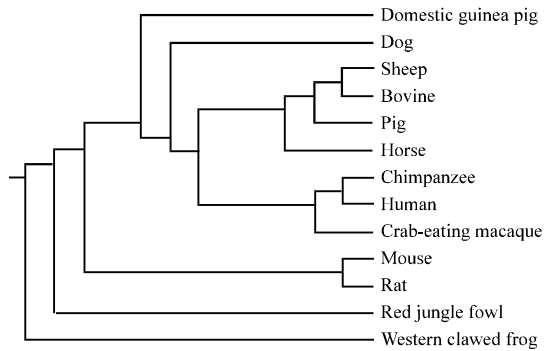


Fig. 4: The phylogenetic analysis for thirty kinds of SERPINF1 from sheep, bovine, pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl and western clawed frog

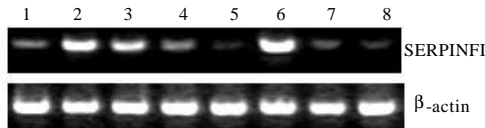


Fig. 5: Tissue expression profile analysis of the sheep SERPINF1 gene on the agarose gel of 1% stained with ethidium bromide. The β-actin expression is the control. M, DL2000 marker; 1, spleen; 2, muscle; 3, skin; 4, kidney; 5, lung; 6, liver; 7, heart; 8, fat

From the sequencing and structural results described, this gene can be defined as the sheep SERPINF1 gene. Based on the results of the alignment of thirty different species of SERPINF1, a phylogenetic tree was constructed using the ClustalW software (<http://www.ebi.ac.uk/clustalw>) as shown in Fig. 4. The phylogenetic analysis revealed that the sheep SERPINF1 gene has a closer genetic relationship with the bovine SERPINF1 gene than with those of pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl and western clawed frog.

**Tissue expression profile:** The RT-PCR analysis of the tissue expression profile was carried out using the pooled tissue cDNAs as the templates. The tissue expression analysis indicated that the sheep SERPINF1 gene is highly expressed in muscle and liver and moderately expressed in skin but weakly expressed in spleen, kidney, lung, heart and fat (Fig. 5).

In the current study, we firstly get the full length of sheep SERPINF1 gene cDNA by using 5'- and 3'-RACE. With the development of modern bioinformatics and specific sheep NCBI EST database was established along

with different convenient analysis tools make researchers much easier to find the useful ESTs which was highly homologous to the coding sequence of human genes. Based on these sheep EST sequences, the researchers can obtain the complete coding sequences of some novel sheep genes through the some modern experimental methods such as Rapid Amplification of cDNA Ends (RACE) method. From the clone and sequence analysis of sheep SERPINF1 gene, it could be seen that this is an effective method to isolate some novel pig genes.

Through sequence analysis, we found that the encoding protein of the sheep SERPINF1 gene is highly homologous with SERPINF1 proteins of human, mouse and other mammals. This implied that the SERPINF1 genes were highly conserved in some mammals and the sheep SERPINF1 gene will have similar functions as the SERPINF1 genes of human, mouse and other mammals. The researchers also found that the sheep SERPINF1 protein does not show complete identity to human, mouse or other mammals. This implied that the sheep SERPINF1 gene will have some differences in functions to those of human, mouse or other mammals. From phylogenetic analysis we found that sheep SERPINF1 gene has a closer genetic relationship with the SERPINF1 gene of bovine, this implied that we can use bovine as a model organism to study the sheep SERPINF1 gene.

From the tissue distribution analysis in the experiment it can be seen that the sheep SERPINF1 gene was obviously differentially expressed in some tissues. As we did not study functions at protein levels yet there might be many possible reasons for differential expression of sheep SERPINF1 gene. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of sheep SERPINF1 gene were presented diversely in different tissues.

## CONCLUSION

In this study, the researchers first isolated the sheep SERPINF1 gene and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into this novel sheep gene.

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