Journal of Animal and Veterinary Advances 10 (19): 2593-2598, 2011
ISSN: 1680-5593

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

[1]Jiang Caode and [2]Liu Yonggang
[1]School of Life Science, Southwest University, 400715 Chongqing, China
[2]College of Animal Science and Technology, Yunnan Agricultural University, 650201 Kunming, China

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

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 (Gvritishvili *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

Corresponding Author: Liu Yonggang, College of Animal Science and Technology, Yunnan Agricultural University, 650201 Kunming, China

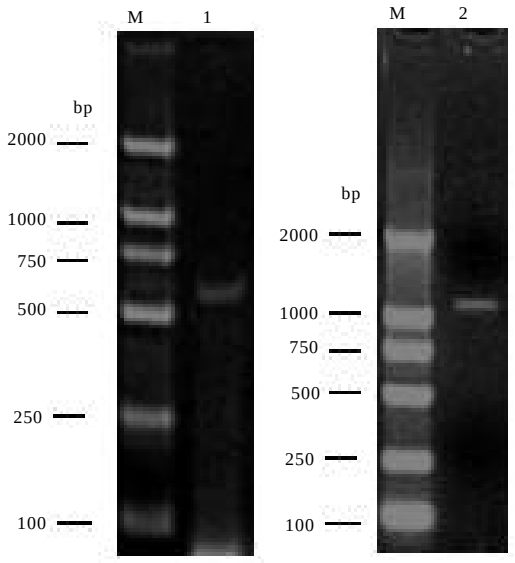

Fig. 1: RACE results for sheep *SERPINF1* gene. M, DL2000 DNA markers 1, 5'-RACE product for *SERPINF1* gene; 2, 3'-RACE product for *SERPINF1* gene

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 *β-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 μL reaction system was: 2 μL cDNA (100 ng), 5 pmoles each oligonucleotide primer (forward primer 1 and reverse primer1 or forward primer and reverse primer2), 2.5 μL 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL 25 mmol L^{-1} $MgCl_2$, 1.0 units of Taq DNA polymerase and finally add sterile water to volume 25 μL. 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).

```
GGCTGGGCGTGGAGCGGCGGTGCACCCACAGGCCCCGAGATGCAGGCCCTCGTGCTACTCCTCTGG
                                          M  Q  A  L  V  L  L  L  W
ACTGGAGCCCTCCTTGGGTTTGGCCACTGTCAGAACGCCGGCCCGGAGGCGGGCTCCCTGGCCCCT
 T  G  A  L  L  G  F  G  H  C  Q  N  A  G  P  E  A  G  S  L  A  P
GAGAGCACAGGGGCACCCGTGGAGGAAGAGGATCCCTTCTTCAAGGTCCCCGTGAACAAGCTGGCG
 E  S  T  G  A  P  V  E  E  E  D  P  F  F  K  V  P  V  N  K  L  A
GCAGCCGTCTCCAACTTCGGCTACGACCTGTACCGCGTGAGATCTGGCGAGAGCCCCACCACCAAC
 A  A  V  S  N  F  G  Y  D  L  Y  R  V  R  S  G  E  S  P  T  T  N
GTGCTGCTGTCTCCGCTCAGCGTGGCCACGGCGCTCTCTGCCCTGTCGCTGGGTGCGGAACAGCGG
 V  L  L  S  P  L  S  V  A  T  A  L  S  A  L  S  L  G  A  E  Q  R
ACAGAATCCAGCATTCACCGGGCTCTGTACTACGACCTGATCAGTAACCCAGACATCCACGGCACC
 T  E  S  S  I  H  R  A  L  Y  Y  D  L  I  S  N  P  D  I  H  G  T
TACAAGGACCTCCTTGCCTCCGTCACTGCCCCCCAGAAGAACCTTAAAAGTGCTTCCCGGATTATC
 Y  K  D  L  L  A  S  V  T  A  P  Q  K  N  L  K  S  A  S  R  I  I
TTTGAGAGGAAGCTGCGGATAAAAGCCAGCTTCGTCCCACCCCTCGAGAAGTCATATGGGACCAGG
 F  E  R  K  L  R  I  K  A  S  F  V  P  P  L  E  K  S  Y  G  T  R
CCCAGAATCCTGACCGGCAACTCTCGAATAGACCTTCAGGAGATTAACAACTGGGTGCAGGCCCAG
 P  R  I  L  T  G  N  S  R  I  D  L  Q  E  I  N  N  W  V  Q  A  Q
ATGAAAGGGAAAATTGCTAGATCCACACGGGAAATACCCAGTGGAATCAGCATTCTCCTTCTTGGT
 M  K  G  K  I  A  R  S  T  R  E  I  P  S  G  I  S  I  L  L  L  G
GTGGCTTACTTCAAGGGGCAGTGGGTAACAAAGTTTGACTCCAGGAAGACTTCCCTGGAGGATTTC
 V  A  Y  F  K  G  Q  W  V  T  K  F  D  S  R  K  T  S  L  E  D  F
CACTTGGATGAGGGGAGGACCGTGAAAGTTCCCATGATGTCAGACCCTAAGGCCGTTTTACGGTAC
 H  L  D  E  G  R  T  V  K  V  P  M  M  S  D  P  K  A  V  L  R  Y
GGCTTGGATTCTGATCTCAACTGCAAGATCGCCCAGCTGCCCTTGACCGGGAGCACAAGTATCATC
 G  L  D  S  D  L  N  C  K  I  A  Q  L  P  L  T  G  S  T  S  I  I
TTCTTCCTGCCTCAGAAAGTGACCCAGAACTTGACCTTGATAGAAGAGAGCCTCACCTCTGAGTTC
 F  F  L  P  Q  K  V  T  Q  N  L  T  L  I  E  E  S  L  T  S  E  F
ATTCATGACATAGACCGAGAACTGAAGACTGTTCAGGCAGTCCTGACCATTCCCAAGCTGAAGCTG
 I  H  D  I  D  R  E  L  K  T  V  Q  A  V  L  T  I  P  K  L  K  L
AGTTATGAAGGCGAACTCACGAAGTCTGTGCAGGAGCTGAAGCTACAATCCCTGTTTGATGCACCA
 S  Y  E  G  E  L  T  K  S  V  Q  E  L  K  L  Q  S  L  F  D  A  P
GACTTTAGCAAGATCACAGGCAAACCTATCAAACTTACTCAAGTGGAACATCGCATCGGATTCGAG
 D  F  S  K  I  T  G  K  P  I  K  L  T  Q  V  E  H  R  I  G  F  E
TGGAATGAGGATGGGGCGGGTACTAACTCCAGCCCAGGGGTCCAGCCTGCCCGCCTCACCTTCCCT
 W  N  E  D  G  A  G  T  N  S  S  P  G  V  Q  P  A  R  L  T  F  P
CTGGACTATCACCTTAACCAACCTTTCATCTTTGTACTGAGGGACACAGACACAGGGGCCCTTCTC
 L  D  Y  H  L  N  Q  P  F  I  F  V  L  R  D  T  D  T  G  A  L  L
TTCATAGGCAAAATTCTGGACCCCAGAGGCACTTAATACTCAACTTAATGTTCAAATACCCCAGAAGA
 F  I  G  K  I  L  D  P  R  G  T  *
AAAAAACACTAGCGGGATGGCAGATTATATATTATATGAAGGCTGCCCCTACGTTTCAATGTATACTTTG
CAATAAAAGTGCTTTCTCCTTAAAAAAAAA
```

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                   MQALVLLLWTGALLGHGSSQNVPSSSEGSPVPDSTGEPVEEED-PFFKVPVNKLAAAVSN
Rat                     MQTLVLLLWTGALLGHGSSQNVPDSSQDSPAPDSTGEPVVEEDDPFFKAPVNKLAAAVSN
Sheep                   MQALVLLLWTGALLGFGHCQN--AGPEAGSLAPESTGAPVEEEDPFFKVPVNKLAAAVSN
Bovine                  MQALVLLLWTGALLGFGRCQN--AGQEAGSLTPESTGAPVEEEDPFFKVPVNKLAAAVSN
Pig                     MQALVLLLWTGALLGSGSCQN--AGPEEGSPAPDTVGAPVEEEDPFFKVPVNKLAAAVSN
Horse                   MQALMLLLWTGALLGHGSCQNNAGGPEEGSPDPDITGAPVEEEDPFLKVPVNKLAAAVSN
Chimpanzee              MQALVLLLCIGALLGHSSCQNPASPPEEGSPDPDSTGALVEEEDPFFKVPVNKLAAAVSN
Human                   MQALVLLLCIGALLGHSSCQNPASPPEEGSPDPDSTGALVEEEDPFFKVPVNKLAAAVSN
Crab-eating macaque     MQALVLFLCFAALLGHSSCQSLASGPEEGSPDPDSTGALVEEEDPFFKVPVNKLAAAVSN
Dog                     ----------------------MRAAPKDSPAPDATGVPVEEEDPFFRVPVNKLAAAISN
Domestic guinea pig     MQVLVLLLWTGALLGRGSCQDIASNPED-SPSPESTGEPVEEEDPFFKVPVNKLAAAISN
Red jungle fowl         MQIPAVLLLLGLLTIPSKSQN--SPAGQNSPTTDGTVGEVEEEDPFYKTPINKLAAAVSN
Western clawed frog     MKIYLALLFTGSFLSYTSAQN----------AADEVPTEVEEEDPFYKSPINRLASSASN
                                                      .      **: ** : *:*:**:: **

Mouse                   FGYDLYRLRSSASPTGNVLLSPLSVATALSALSLGAEHRTESVIHRALYYDLITNPDIHS
Rat                     FGYDLYRLRSGAVSTGNILLSPLSVATALSALSLGAEQRTESVIHRALYYDLINNPDIHS
Sheep                   FGYDLYRVRSGESPTTNVLLSPLSVATALSALSLGAEQRTESSIHRALYYDLISNPDIHG
Bovine                  FGYDLYRVRSGESPTANVLLSPLSVATALSALSLGAEQRTESNIHRALYYDLISNPDIHG
Pig                     FGYDLYRVRSSESPTANVLLSPLSVATALSALSLGAEQRTESSLHRALYYDLISNPDLHG
Horse                   FGYDLYRAKSSMSPTANVLLSPLSVATALSALSLGAEQRTESSIHLALYYDLIKNPDIHG
Chimpanzee              FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSLGAEQRTESIIHRALYYDLISSPDIHG
Human                   FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSLGAEQRTESIIHRALYYDLISSPDIHG
Crab-eating macaque     FGYDLYRVRSSMSPTTNVLLSPLSVATALSALSLGAEQRTESVIHRALYYDLISSPDIHG
Dog                     FGYDLYRVRSSFSPAANVLLSPLSVATALSALSLGAEQRTESTIHRALYYDLISNPDIHS
Domestic guinea pig     FGYDLYRVRSIESPTTNVLLSPLSVATALSALSLGAEQRTEATIHRALYYDMISNPDIHS
Red jungle fowl         FGYDLYRQQSSRTATANVLLSPFSLATALSGLSLGAGERTEDVISRALFYDLLNKAEVHN
Western clawed frog     FGYDLYRMQANKNPNSNIIISPLSIATSLSSLSLGGGQRTESLIQRSLYYDLLNDPEVHA
                        ******* ::   .  *:::**:*:**:**.****. .***  :  :*:**::...::*

Mouse                   TYKELLASVTAPEKNLKSASRIVFERKLRVKSSFVAPLEKSYGTRPRILTGNPRVDLQEI
Rat                     TYKELLASVTAPEKNFKSASRIVFERKLRVKSSFVAPLEKSYGTRPRILTGNPRIDLQEI
Sheep                   TYKDLLASVTAPQKNLKSASRIIFERKLRIKASFVPPLEKSYGTRPRILTGNSRIDLQEI
Bovine                  TYKDLLASVTAPQKNLKSASRIIFERKLRIKASFIPPLEKSYGTRPRILTGNSRVDLQEI
Pig                     TYKELLAAVTAPQKNLKSASRIIFEKKLRIKASFVAPLEKSYGTRPRILTGNSRLDLQEV
Horse                   TYKELLASVTAPNKNFKSASRIIFEKKLRIKSSFVAPLEKSYGTRPKILTGNSRTDLQEI
Chimpanzee              TYKELLDTVTAPQKNLKSASRIVFEKKLRIKSSFVAPLEKSYGTRPRVLTGNPRLDLQEI
Human                   TYKELLDTVTAPQKNLKSASRIVFEKKLRIKSSFVAPLEKSYGTRPRVLTGNPRLDLQEI
Crab-eating macaque     TYKELLGTVTAPQKNLKSASRIVFEKKLRIKSSFVAPLEKSYGTRPRVLTGNPRLDLQEI
```

Fig. 3: Continue

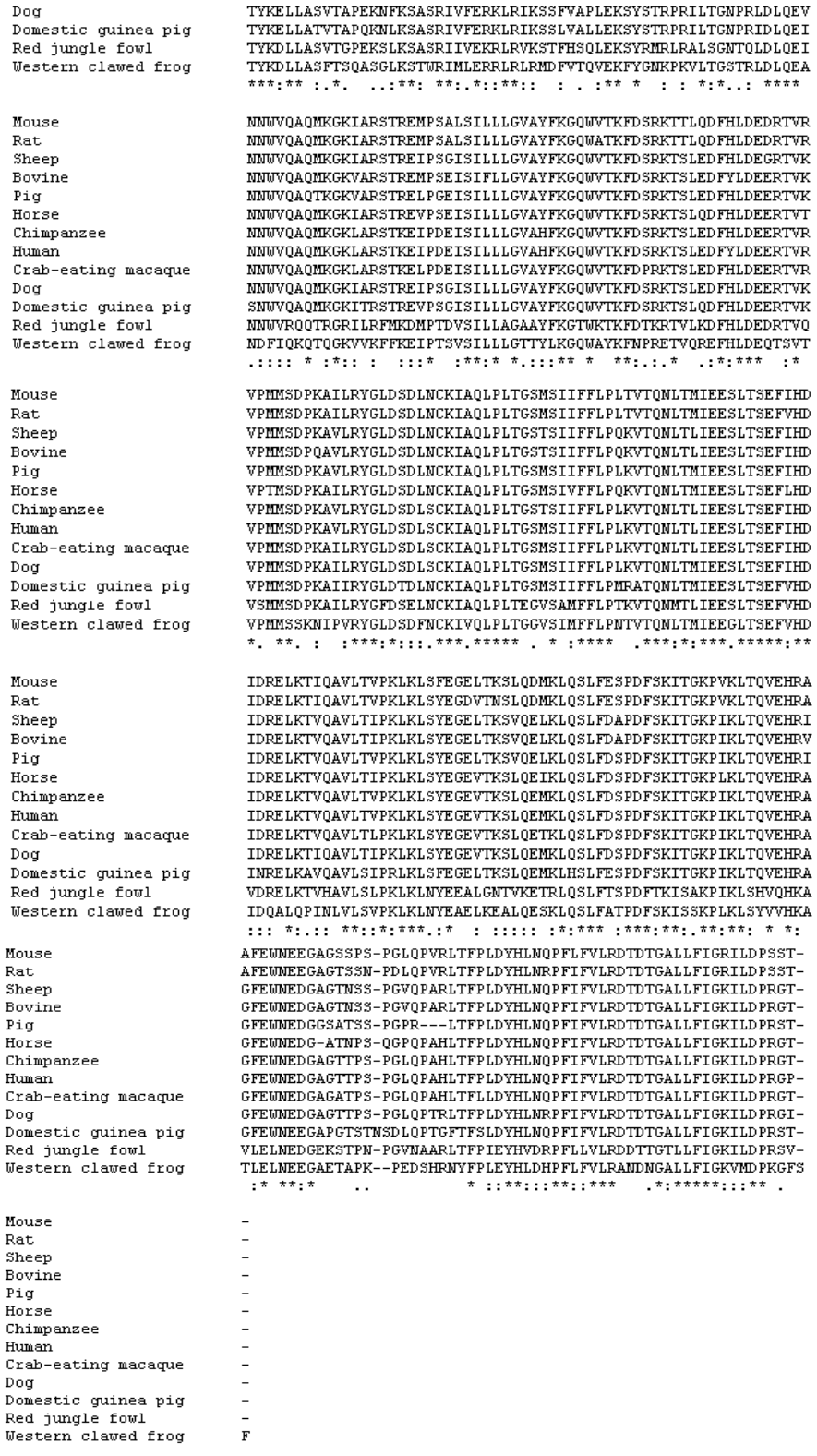

Fig. 3: The alignment of the protein encoded by sheep *SERPINF1* gene and twelve other kinds of SERPINF1 proteins from bovine, pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl and western clawed frog

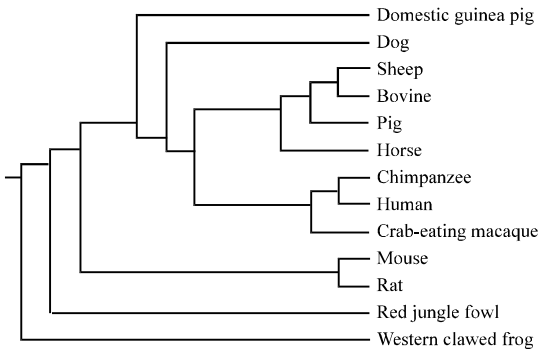

Fig. 4: The phylogenetic analysis for thirty kinds of SERPINF1 from sheep, bovine, pig, human, chimpanzee, horse, crab-eating macaque, dog, domestic guinea pig, mouse, rat, red jungle fowl and western clawed frog

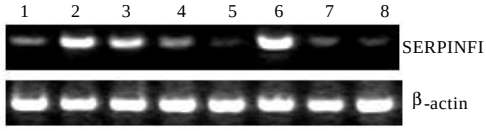

Fig. 5: Tissue expression profile analysis of the sheep *SERPINF1* gene on the agarose gel of 1% stained with ethidium bromide. The β-actin expression is the control. M, DL2000 marker; 1, spleen; 2, muscle; 3, skin; 4, kidney; 5, lung; 6, liver; 7, heart; 8, fat

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.

REFERENCES

Becerra, S.P., I. Palmer, A. Kumar, F. Steele, J. Shiloach, V. Notario and G.J. Chader, 1993. Overexpression of fetal human pigment epithelium-derived factor in *Escherichia coli*. A functionally active neurotrophic factor. J. Biol. Chem., 268: 23148-23156.

Chen, C., A.W. Tso, L.S. Law, B.M. Cheung and K.L. Ong *et al*., 2010. Plasma level of pigment epithelium-derived factor is independently associated with the development of the metabolic syndrome in Chinese men: A 10-year prospective study. J. Clin. Endocrinol. Metab., 95: 5074-5081.

Gvritishvili, A.G., K.W. Leung and J. Tombran-Tink, 2010. Codon preference optimization increases heterologous PEDF expression. PloS One, 5: 15056-15056.

Ho, T.C., S.L. Chen, S.C. Shih, J.Y. Wu and W.H. Han *et al*., 2010. Pigment epithelium-derived factor is an intrinsic antifibrosis factor targeting hepatic stellate cells. Am. J. Pathol., 177: 1798-1811.

Konson, A., S. Pradeep, C.W. D'Acunto and R. Seger, 2011. Pigment epithelium-derived factor and its phosphomimetic mutant induce JNK-dependent apoptosis and p38-mediated migration arrest. J. Biol. Chem., 286: 3540-3551.

Liu, G.Y. and S.Z. Gao, 2009. Molecular cloning, sequence identification and tissue expression profile of three novel sheep (*Ovis aries*) genes-BCKDHA, NAGA and HEXA. Biol. Res., 42: 69-77.

Liu, G.Y., 2009. A novel HADHA gene differentially expressed in muscle and other tissues from black-boned vs. ordinary sheep. Anim. Sci. Pap. Rep., 27: 127-137.

Pignolo, R.J., V.J. Cristofalo and M.O. Rotenberg, 1993. Senescent WI-38 cells fail to express EPC-1, a gene induced in young cells upon entry into the G0 state. J. Biol. Chem., 268: 8949-8957.

Simonovic, M., P.G. Gettins and K. Volz, 2001. Crystal structure of human PEDF, a potent anti-angiogenic and neurite growth-promoting factor. Proc. Natl. Acad. Sci. USA., 98: 11131-11135.

Wagsater, D., S. Lofgren, N. Zar, A. Hugander and J. Dimberg, 2010. Pigment epithelium-derived factor expression in colorectal cancer patients. Cancer Invest., 28: 872-877.

Yonggang, June 24, 2011L. and G. Shizheng, 2009. A novel sheep gene, MMP7, differentially expressed in muscles from black-boned sheep and local common sheep. J. Applied Genet., 50: 253-256.