Journal of Animal and Veterinary Advances 11 (2): 252-259, 2012
ISSN: 1680-5593

Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*RHOA*, *RCHY1* and *RSU1*

[1]Liu Yonggang and [2]Xia Xueshan
[1]College of Animal Science and Technology,
Yunnan Agricultural University, 650201 Kunming, China
[2]Faculty of Life Science and Technology,
Kunming University of Science and Technology, 650224 Kunming, China

Abstract: The complete coding sequences of three sheep genes-*RHOA*, *RCHY1* and *RSU1* were amplified using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The sheep *RHOA* gene encodes a protein of 193 amino acids that shares high homology with the ras homolog gene family, member A (RHOA) proteins of fifteen species- cattle (100%), rat (99%), human (100%), mouse (99%), chicken (99%), dog (99%), turkey (99%), sumatran orangutan (99%), poephila guttata (99%), Atlantic salmon (97%), green anole (96%), rainbow trout (96%), African clawed frog (96%), zebrafish (96%) and Western clawed frog (95%). The sheep *RCHY1* gene encodes a protein of 261 amino acids that shares high homology with the ring finger and CHY zinc finger domain containing 1 (RCHY1) proteins of nine species-cattle (94%), giant panda (92%), pig (91%), human (90%), white-tufted-ear marmoset (90%), chimpanzee (90%), rabbit (89%), mouse (86%) and rat (84%). The sheep *RSU1* gene encodes a protein of 277 amino acids that shares high homology with the ras suppressor protein 1 (RSU1) proteins of twelve species-cattle (99%), chimpanzee (98%), dog (99%), human (98%), giant panda (98%), horse (98%), rabbit (98%), chicken (96%), mouse (97%), African clawed frog (94%), Western-clawed-frog (94%) and zebrafish (91%). Finally, these three novel sheep genes were assigned to GeneIDs: 100302078, 100302546 and 100302548. The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle. Tissue expression profile analysis demonstrated that sheep *RHOA*, *RCHY1* and *RSU1* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, RHOA, RCHY1, RSU1, tissue expression, China

INTRODUCTION

Ras homolog gene family, member A (RHOA) belongs to the RhoA-like sub-family. The RhoA-like sub-family consists of RHOA, RHOB and RHOC. Latest researches demonstrated that RHOA promotes the formation of stress fibers and focal adhesions, regulating cell shape, attachment and motility (Su *et al*., 2006). RHOA is vital for muscle contraction in vascular smooth muscle cells, RHOA plays a key role in cell contraction, differentiation, migration and proliferation (Peyton *et al*., 2008). RHOA activities appear to be elaborately regulated in a time- and space-dependent manner to control cytoskeletal changes (Maekawa *et al*., 1999; Ohta *et al*., 1999; Saeki *et al*., 2005). Ring finger and CHY zinc finger domain containing 1 (RCHY1), a member of proteins containing the ring finger and CHY zinc finger domain has ubiquitin ligase activity. It mediates E3-dependent ubiquitination and proteasomal degradation of target proteins including TP53, HDAC1 and CDKN1B thus, regulating their levels and cell cycle progression (Wu *et al*., 2010; Yan *et al*., 2010; Shloush *et al*., 2011).

Ras Suppressor protein 1 (*RSU1*) gene encodes a protein that is involved in the ras signal transduction pathway, growth inhibition and nerve-growth factor induced differentiation processes as determined in mouse and human cell line studies. In mouse, the encoded protein was initially isolated based on its ability to inhibit v-Ras transformation. Multiple alternatively spliced transcript variants for this gene have been reported; one of these variants was found only in glioma tumors (Chunduru *et al*., 2002; Dougherty *et al*., 2005, 2008).

As mentioned before, *RHOA*, *RCHY1* and *RSU1* genes are three genes which have important functions. Until today, *RHOA*, *RCHY1* and *RSU1* genes had been reported in human and other animals but the sheep *RHOA*,

Corresponding Author: Xia Xueshan, Faculty of Life Science and Technology, Kunming University of Science and Technology, 650224 Kunming, China

RCHY1 and *RSU1* genes have not been reported yet. In present experiment, the researchers will isolate the coding sequences of sheep *RHOA*, *RCHY1* and *RSU1* genes based on the coding sequence information of *RHOA*, *RCHY1* and *RSU1* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform sequence and tissue expression profile analysis for these genes. These will establish the primary foundation of understanding these three sheep genes.

MATERIALS AND METHODS

Animals and sample preparation: Five adult Yunnan local sheep were slaughtered. Spleen, skin, lung, fat, muscle, heart, liver, kidney and ovary 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). First-strand cDNA synthesis was performed as that described by Liu *et al*. (2004). These first-strand cDNA samples were used to perform RT-PCR for the isolation of sheep *RHOA*, *RCHY1* and *RSU1* genes and for the tissue expression profile analysis.

Isolation of the sheep *RHOA*, *RCHY1* and *RSU1* genes: The primers for sheep *RHOA* gene isolation were designed based on the coding sequence information of human *RHOA* gene and its highly homologous sheep EST sequences: DY503081 and EE758341. Similarly, the primers for sheep *RCHY1* gene isolation were designed based on the coding sequence information from human *RCHY1* gene and its highly homologous sheep EST sequences; DY519128 and EE754481. The primers for sheep *RSU1* gene isolation were designed based on the coding sequence information from human and mouse *RSU1* genes and their highly homologous sheep EST sequences; DY495018 and FE027712. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1. The RT-PCR was performed to isolate these three sheep genes using the pooled cDNAs from different tissues above. The 25 μL reaction system was; 2.0 μL cDNA, 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 PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of 94°C/50 sec, Ta°C/50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction (Table 1).

These PCR products for sheep *RHOA*, *RCHY1* and *RSU1* genes 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 sheep *RHOA*, *RCHY1*, *RSU1* and *beta-actin* genes and their annealing Temperature (Ta)

Genes	Primer sequence	Ta/°C
RHOA	Forward 5'- ATGGCTGCCATCCGGAAG-3'	
	Reverse: 5'-TCACAAGACAAGGCACCCAG-3'	61
RCHY1	Forward 5'-ATGGCTTCCTCGACGCTG-3'	
	Reverse: 5'-TCATTGCTGATCTAATGAAATTT-3'	61
RSU1	Forward: 5'-ATGTCCAAGTCTCTGAAA-3'	
	Reverse: 5'-TTATTTATTCTTGGCTGCC-3'	52
Beta-actin	Forward: 5'-CTTGATGTCACGGACGATTT-3'	
	Reverse: 5'- CACGGCATTGTCACCAACT-3'	56

RT-PCR for tissue expression profile analysis: 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) as a positive control. The primers of sheep *RHOA*, *RCHY1* and *RSU1* genes 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: 1 μL cDNA (100 ng μL^{-1}), 5 pmoles each oligonucleotide primer, 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 unit 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 25 cycles of 94°C/50 sec, Ta°C/50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction (Table 1).

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 BLAST tool 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

RT-PCR results for sheep *RHOA*, *RCHY1* and *RSU1* genes: Through RT-PCR with pooled tissue cDNAs, for sheep *RHOA*, *RCHY1* and *RSU1* genes, the resulting PCR products were 582, 786 and 834 bp (Fig. 1).

Sequence analysis: These cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these three genes were not homologous to any of the known sheep genes and they were then deposited into the GenBank database (Accession No.: FJ943984, FJ937958 and FJ937963). The sequence prediction was carried out

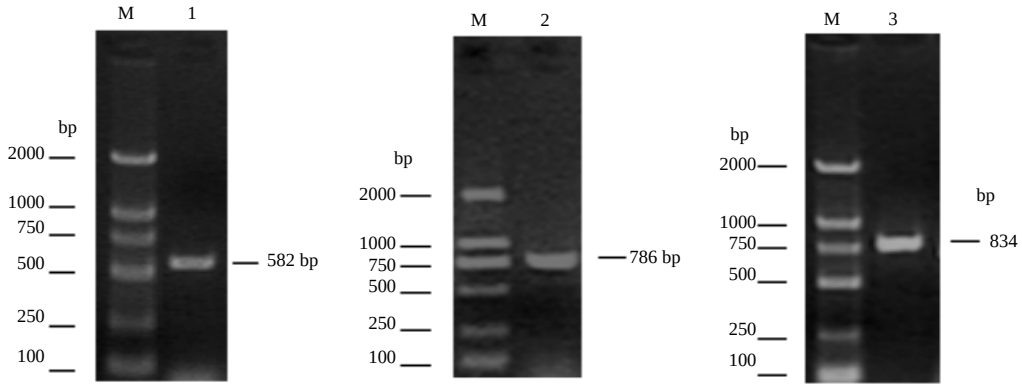

Fig. 1: RT-PCR results for sheep *RHOA*, *RCHY1* and *RSU1* genes. M: DL 2000 DNA markers; 1: PCR product for sheep *RHOA* gene; 2: PCR product for sheep *RCHY1* gene; 3: PCR product for sheep *RSU1* gene

using the GenScan software and results showed that the 582, 786 and 834 bp cDNA sequences represent three single genes which encoded 193, 261 and 277 amino acids, respectively. Finally, these three novel sheep genes were assigned to GeneIDs; 100302078, 100302546 and 100302548.

Further BLAST analysis of these proteins revealed that the sheep RHOA protein has high homology with the ras homolog gene family, member A (RHOA) proteins of fifteen species-cattle (Accession No.: NP_788818; 100%), rat (Accession No.: NP_476473; 99%), human (Accession No.: NP_001655; 100%), mouse (Accession No.: NP_058082; 99%), chicken (Accession No.: NP_990035; 99%), dog (Accession No.: NP_001003273; 99%), Turkey (Accession No.: ADX97247; 99%) sumatran orangutan (Accession No.: NP_001124755; 99%), poephila guttata (Accession No.: ACH45076; 99%), Atlantic salmon (Accession No.: ACI33725; 97%), green anole (Accession No.: XP_003216698; 96%), rainbow trout (Accession No.: NP_001158532; 96%), African clawed frog (Accession No.: AD40671; 96%), zebrafish (Accession No.: NP_998515; 96%) and Western clawed frog (Accession No.: CAJ81715; 95%) (Fig. 2).

The sheep RCHY1 protein has high homology with the ring finger and CHY zinc finger domain containing 1 (RCHY1) proteins of nine species-cattle (Accession No.: NP_001077223; 94%), giant panda (Accession No.: XP_002919200; 92%), pig (Accession No.: NP_001090959; 91%), human (Accession No.: NP_056251; 90%), white-tufted-ear marmoset (Accession No.: XP_002745761; 90%), chimpanzee (Accession No.: XP_517222; 90%), rabbit (Accession No.: XP_002717057; 89%), mouse (Accession No.: NP_0808337; 86%) and rat (Accession No.: NP_001007619; 84%) (Fig. 3).

The sheep RSU1 protein has high homology with the ras suppressor protein 1 (RSU1) proteins of twelve species-cattle (Accession No.: NP_001035691; 99%), chimpanzee (Accession No.: XP_001151460; 98%), dog (Accession No.: XP_535177; 99%), human (Accession No.: NP_036557; 98%), giant panda (Accession No.: XP_002918673; 98%), horse (Accession No.: XP_001498103; 98%), rabbit (Accession No.: XP_002717469; 98%), chicken (Accession No.: NP_001186520; 96%), mouse (Accession No.: NP_033131; 97%), African clawed frog (Accession No.: NP_001085943; 94%), Western-clawed-frog (Accession No.: NP_001015695; 94%) and zebrafish (Accession No.: XP_002666722; 91%) (Fig. 4).

Based on the results of the alignment of RHOA, RCHY1 and RSU1 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7. The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle.

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes are all generally but differentially expressed in tissues including spleen, lung, muscle, kidney, ovary, skin, liver, heart and fat (Fig. 8).

In the current study, the researchers firstly get the coding sequences of sheep *RHOA*, *RCHY1* and *RSU1* genes by RT-PCR. With the development of modern bioinformatics, establishment of specific sheep NCBI EST database and different convenient analysis tools, researchers can easily find the useful ESTs which were highly homologous to the coding sequences of human

```
Rat                     MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Mouse                   MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Sheep                   MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Cattle                  MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Human                   MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Sumatran orangutan      MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Dog                     MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Poephila guttata        MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Chicken                 MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Turkey                  MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Zebrafish               MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
Green anole             MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Rainbow trout           MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
Atlantic salmon         MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
African clawed frog     MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDGKQVELALWDT
Western clawed frog     MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENYVADIEVDSKQVELALWDT
                        **************************************************.**********

Rat                     AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Mouse                   AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Sheep                   AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Cattle                  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Human                   AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Sumatran orangutan      AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Dog                     AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Poephila guttata        AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLQNIPEKWTPEVKHFCPNVPIILVGNKKD
Chicken                 AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Turkey                  AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Zebrafish               AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Green anole             AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Rainbow trout           AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Atlantic salmon         AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
African clawed frog     AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
Western clawed frog     AGQEDYDRLRPLSYPDTDVILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKD
                        ********************************:*************************

Rat                     LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Mouse                   LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Sheep                   LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Cattle                  LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Human                   LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Sumatran orangutan      LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Dog                     LRNDEHTRRELAKMKQEPVKPTEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Poephila guttata        LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Chicken                 LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Turkey                  LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQ
Zebrafish               LRNDEHTRRELAKMKQEPVKPEEGRDMANRINAFGYLECSAKTKDGVREVFEMATRAALQ
Green anole             LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYLECSAKTKDGVREVFEMATRAALQ
Rainbow trout           LRNDEHTRRELAKMKQEPVKPEEGRDMANRISAFGYMECSAKTKDGVREVFEMATRAALQ
Atlantic salmon         LRNDEHTRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKSKDGVREVFEMATRAALQ
African clawed frog     LRNDEHTRRELTKMKQEPVKPEEGRDMANRISAYAYMECSAKTKDGVREVFELATRAALQ
Western clawed frog     LRNDEHTRRELTKMKQEPVKPEEGRDMANRISAYGYMECSAKTKDGVREVFELATRAALQ
                        ***********:********* *********.*:.*:*****:*********:*******

Rat                     ARRGKKKSGCLIL
Mouse                   ARRGKKKSGCLIL
Sheep                   ARRGKKKSGCLVL
Cattle                  ARRGKKKSGCLVL
Human                   ARRGKKKSGCLVL
Sumatran orangutan      ARRGKKKSGRLVL
Dog                     ARRGKKKSGCLVL
Poephila guttata        ARRGKKKSGCLFL
Chicken                 ARRGKKKSGCLLL
Turkey                  ARRGRKKSGCLLL
Zebrafish               ARKRGKKSGCLLL
Green anole             AKRGRKKTSCQLL
Rainbow trout           ARRGKPRNKCLLL
Atlantic salmon         ARRGKKSNKCLLL
African clawed frog     ARRGKKKPRCLLI
Western clawed frog     ARRGKKKTTCLLI
                        *::        .:
```

Fig. 2: The alignment of the protein encoded by sheep *RHOA* gene and fifteen other kinds RHOA proteins

```
Human                          MAATAREDGASGQERGQRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
White-tufted-ear marmoset      MAATAREDGASGQERVQRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Chimpanzee                     MAATAREDGAGGQERGQRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Sheep                          MASSTLEDGARGQAQRRRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Cattle                         MAFSALEDGARGQAQRRRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Pig                            MAASAREDGGRGQEHGRRGCEHYDRGCLLKAPCCDKLYTCRLCHDSNEDH
Giant panda                    MAASAREDGARSQKQGRRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Rabbit                         MAATAREDGARSRQRGQRGCEHYDRGCLLKAPCCDKLYTCRLCHDNNEDH
Mouse                          MAATAREDGVRNLAQGPRGCEHYDRACLLKAPCCDKLYTCRLCHDTNEDH
Rat                            MAATAQEDGVRSPAPGPRGCEHYDRACLLKAPCCDKLYTCRLCHDTHEDH
                               ** :: ***  .     ********.******************.:***

Human                          QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCDICHLFDKDKKQY
White-tufted-ear marmoset      QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCDICHLFDKDKKQY
Chimpanzee                     QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCDICHLFDKDKKQY
Sheep                          QLDRFKVKEVQCINCEKIQRAQQTCEECSTLFGEYYCSVCHLFDKDKKQY
Cattle                         QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCSVCHLFDKDKKQY
Pig                            QLDRFKVKEVQCINCEKIQHAQQICEECSTLFGEYYCSICHLFDKDKKQY
Giant panda                    QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCSICHLFDKDKKQY
Rabbit                         QLDRFKVKEVQCINCEKIQHAQQTCEECSTLFGEYYCNICHLFDKDKKQY
Mouse                          QLDRFKVKEVQCINCEKLQHAQQTCEDCSTLFGEYYCSICHLFDKDKRQY
Rat                            QLDRFKVKEVQCINCEKLQHAQQTCEDCSTLFGEYYCSICHLFDKDKKQY
                               *****************:*:*** **:**********.:********:**

Human                          HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGRHKCIENVSRQNCPICLE
White-tufted-ear marmoset      HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGRHKCIENVSRQNCPICLE
Chimpanzee                     HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGRHKCIENVSRQNCPICLE
Sheep                          HCENCGICRIGPKEDYFHCLKCNLCLAVNLQGKHKCIENVSRQDCPICLE
Cattle                         HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGKHKCIENVSRQNCPICLE
Pig                            HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGKHKCIENVSRQNCPICLE
Giant panda                    HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGKHKCIENVSRQNCPICLE
Rabbit                         HCENCGICRIGPKEDFFHCLKCNLCLAMNLQGKHKCIENVSRQNCPICLE
Mouse                          HCESCGICRIGPKEDFFHCLKCNLCLTTNLRGKHKCIENVSRQNCPICLE
Rat                            HCESCGICRIGPKEDFFHCLKCNLCLAMTLRGKHKCIENVSRQNCPICLE
                               ***.***********:**********: .*:*:**********:******

Human                          DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
White-tufted-ear marmoset      DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Chimpanzee                     DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Sheep                          DIHTSRIVARVLPCGHLLHKTCYEDMLKEGYRCPLCMHSALDMSRHWRQL
Cattle                         DIHTSRIAAQVLPCGHLLHRTCYEDMLKEGYRCPLCMRSALDMSRSWRQR
Pig                            DIHTSHIVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Giant panda                    DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Rabbit                         DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Mouse                          DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
Rat                            DIHTSRVVAHVLPCGHLLHRTCYEEMLKEGYRCPLCMHSALDMTRYWRQL
                               *****::.*:*********:****:************:*****:* ***

Human                          DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCKICESYNTAQ
White-tufted-ear marmoset      DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCKICESYNTAQ
Chimpanzee                     DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCKICESYNTAQ
Sheep                          DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCNICESYNTTQ
Cattle                         DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCNICESYNTAQ
Pig                            DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCNICESYNTAQ
Giant panda                    DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCNICESYNTAQ
Rabbit                         DDEVAQTPMPSEYQNMTVDILCNDCNGRSTVQFHILGMKCKICDSYNTAQ
Mouse                          DTEVAQTPMPSEYQNVTVDILCNDCNGRSTVQFHILGMKCKLCDSYNTAQ
Rat                            DIEVAQTPMPSEYQNVTVDILCNDCNGRSTVQFHILGMKCKLCDSYNTAQ
                               * *************:************************::*:****:*

Human                          AGGRRISLDQQ
White-tufted-ear marmoset      AGGRRISLDQQ
Chimpanzee                     AGGRRISLDQQ
Sheep                          AGRYKISLDQQ
Cattle                         AGKYRISIDQQ
Pig                            AGGCRISLDQQ
Giant panda                    AGGCRISLDQQ
Rabbit                         AGGRRISLDHQ
Mouse                          AGGRRVPVDQQ
Rat                            AGGRTVSMDEQ
                               **   :.:*.*
```

Fig. 3: The alignment of the protein encoded by sheep *RCHY1* gene and nine other kinds of RCHY1 proteins

```
African clawed frog     MSKSLKKIVEESREKNVPDIDMCDRGIANMLDVPGLFTLSHITQLILSHNKLTTVPPNIA
Western clawed frog     MSKSLKKIVEESREKNVPDIDMCDRGIANMLDVPGLFTLSHITQLILSHNKLTTVPPNIA
Sheep                   MSKSLKKLVEESREKNQPEVDMSDRGISNMLDVNGLFTLSHITQLVLSHNKLTTVPPNIA
Cattle                  MSKSLKKLVEESREKNQPEVDMSDRGISNMLDINGLFTLSHITQLVLSHNKLTTVPPNIA
Giant panda             MSKSLKKLVEESREKNQPEVDMSDRGISNMLDVNGLFSLSHITQLVLSHNKLTTVPANIA
Horse                   MSKSLKKLVEESREKNQPEVDMSDRGISNMLDVNGLFSLSHITQLVLSHNKLTTVPANIA
Dog                     MSKSLKKLVEESREKNQPEVDMSDRGISNMLDVNGLFSLSHITQLVLSHNKLTTVPPNIA
Mouse                   MSKSLKKLVEESREKNQPEVDMSDRGISSMLDVNGLFSLAHITQLVLSHNKLTTVPPNVA
Rabbit                  MSKSLKKLVEESREKNQTEVDMSDRGISNMLDVNGLFTLSHITQLVLSHNKLTTVPPNIA
Chimpanzee              MSKSLKKMVEESREKNQPEVDMSDRGISNMLDVNGLFTLSHITQLVLSHNKLTTVPPNIA
Human                   MSKSLKKLVEESREKNQPEVDMSDRGISNMLDVNGLFTLSHITQLVLSHNKLTMVPPNIA
Chicken                 MSKSLKKIVEESREKNQPEVDMCDRGISNMLDVPGLFTLSHITQLVLSHNKLTTVPANIA
Zebrafish               MSKSLKKIVEESRDKNLPEVDMCDRGISNLLDIPGLFSLSSITQLVLSHNKLSAVPPNIA
                        *******:*****:** .::**.****:.:**: ***:*: ****:******: **.*:*

African clawed frog     DLKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNGLPRGFGSLPALEVLDLTYNN
Western clawed frog     DLKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNSLPRGFGSLPALEVLDLTYNN
Sheep                   ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Cattle                  ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Giant panda             ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Horse                   ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Dog                     ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Mouse                   ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Rabbit                  ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Chimpanzee              ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Human                   ELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Chicken                 DLRNIEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLNTLPRGFGSLPALEVLDLTYNN
Zebrafish               DLKNLEVLNMFNNQIEELPTQISSLQKLKHLNLGMNRLSTLPRGFGSLPALEVLDLTYNN
                        :*:*:****:****************************. ********************

African clawed frog     LNENSLSGNFFYLTTLRALYLSDNDFETLPPDIGKLTKLQIISLRDNDLISLPKEVGELT
Western clawed frog     MNENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQIISLRDNDLISLPKEIGDLT
Sheep                   LNENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Cattle                  LNENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Giant panda             LSENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Horse                   LNESSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Dog                     LNENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Mouse                   LNEHSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Rabbit                  LNENSLPGNFFYLTTLRALYLSDNDFEILPADIGKLTKLQILSLRDNDLISLPKEIGELT
Chimpanzee              LSENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Human                   LSENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Chicken                 LNENSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLTKLQILSLRDNDLISLPKEIGELT
Zebrafish               LNESSLPGNFFYLTTLRALYLSDNDFEILPPDIGKLAKLQILSLRDNDLISLPREIGDLT
                        :.* **.******************** **.*****:****:***********:*:*:**

African clawed frog     QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQVGVSHVFEYIRSE
Western clawed frog     QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQVGVSHVFEYIRSE
Sheep                   QLKELHIQGNRLTVLPPELGNLDLTGQKQIFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Cattle                  QLKELHIQGNRLTVLPPELGNLDLTGQKQIFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Giant panda             QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Horse                   QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Dog                     QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Mouse                   QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Rabbit                  QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Chimpanzee              QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Human                   QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Chicken                 QLKELHIQGNRLTVLPPELGNLDLTGQKQVFKAENNPWVTPIADQFQLGVSHVFEYIRSE
Zebrafish               QLKELHIQGNRLTVLPPELGNLDLTGPKQVFKAENNSWVTPIADQFQLGISHVFEYIRSE
                        ************************* **:******.**********:*:**********

African clawed frog     TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNK
Western clawed frog     TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNK
Sheep                   TYKYLYGRHMQANPEPPKKNNDKSKRISRKPLAAKNK
Cattle                  TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNK
Giant panda             TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLTAKNK
Horse                   TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLTAKNK
Dog                     TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLTAKNK
Mouse                   TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNK
Rabbit                  TYKYLYGRHMQANPEPPRKNNDKSKKISRKPLTAKNK
Chimpanzee              TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNR
Human                   TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAAKNR
Chicken                 TYKYLYGRHMQANPEPPKKNNDKSKKISRKPLAARNK
Zebrafish               TYKYLYGRHMQANPEAPKKNADKSKKISRKPLAAKNK
                        ***************.*:** ****:******:*:*:
```

Fig. 4: The alignment of the protein encoded by sheep *RSU1* gene and twelve other kinds of RSU1 proteins

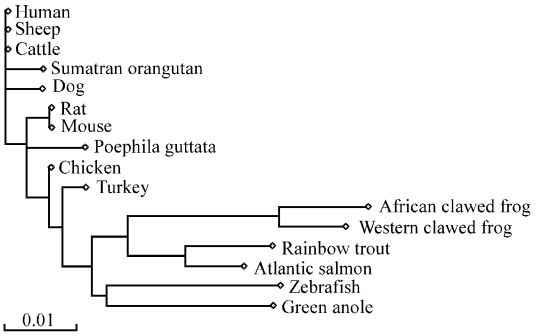

Fig. 5: The phylogenetic analysis for sixteen kinds of *RHOA* genes

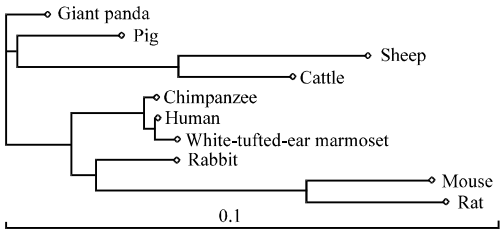

Fig. 6: The phylogenetic analysis for ten kinds of *RCHY1* genes

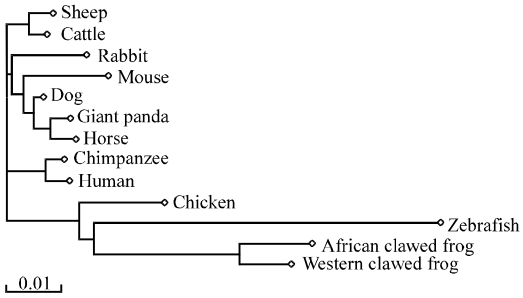

Fig. 7: The phylogenetic analysis for thirteen kinds of *RSU1* genes

genes. Based on these sheep EST sequences, the researchers can obtain the complete coding sequences of some novel sheep genes through the some experimental methods such as RT-PCR. From the clone and sequence analysis of sheep *RHOA*, *RCHY1* and *RSU1* genes, it could be seen that this is an effective method to isolate some novel sheep genes.

Through sequence analysis, the researchers found that the encoding protein of the sheep *RHOA*, *RCHY1* and *RSU1* genes are highly homologous with RHOA, RCHY1 and RSU1 proteins of human and some other animals. This implied that the *RHOA*, *RCHY1* and *RSU1* genes were highly conserved in some species and the sheep *RHOA*, *RCHY1* and *RSU1* genes will have similar

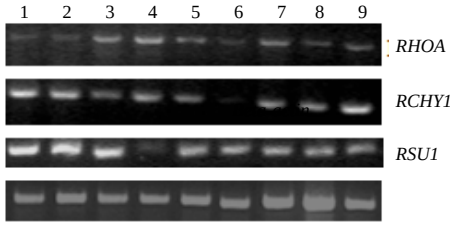

Fig. 8: Tissue expression distribution of sheep *RHOA*, *RCHY1* and *RSU1* genes. The beta-actin expression is the internal control. 1: muscle; 2: heart; 3: lung; 4: spleen; 5: skin; 6: fat; 7: liver; 8: kidney; 9: ovary

functions as the *RHOA*, *RCHY1* and *RSU1* genes of human and other animals. The researchers also found that the sheep RHOA, RCHY1 and RSU1 proteins do not show complete identity to some animals. This implied that the sheep *RHOA*, *RCHY1* and *RSU1* genes will have some differences in functions to those of other animals.

The phylogenetic analysis revealed that the sheep *RHOA*, *RCHY1* and *RSU1* genes all have a closer genetic relationship with the *RHOA*, *RCHY1* and *RSU1* genes of cattle. This implied that we can use cattle as a model organism to study the sheep *RHOA*, *RCHY1* and *RSU1* genes or use sheep as a model organism to study the cattle *RHOA*, *RCHY1* and *RSU1* genes.

From the tissue distribution analysis in the experiment, it can be seen that the sheep *RHOA*, *RCHY1* and *RSU1* genes were obviously differentially expressed in some tissues. As the researchers did not study functions at protein levels yet there might be many possible reasons for differential expression of sheep *RHOA*, *RCHY1* and *RSU1* genes. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of sheep *RHOA*, *RCHY1* and *RSU1* genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep *RHOA*, *RCHY1* and *RSU1* genes and performed necessary sequence and tissue expression profile analysis. This established the primary foundation for further insight into these novel sheep genes.

ACKNOWLEDGEMENTS

This research was supported by the Candidates of the Young and Middle Aged Academic and Technical Leaders of Yunnan province.

REFERENCES

Chunduru, S., H. Kawami, R. Gullick, W.J. Monacci, G. Dougherty and M.L. Cutler, 2002. Identification of an alternatively spliced RNA for the Ras suppressor RSU-1 in human gliomas. J. Neurooncol., 60: 201-211.

Dougherty, G.W., C. Jose, M. Gimona and M.L. Cutler, 2008. The Rsu-1-PINCH1-ILK complex is regulated by Ras activation in tumor cells. Eur. J. Cell Biol., 87: 721-734.

Dougherty, G.W., T. Chopp, S.M. Qi and M.L. Cutler, 2005. The Ras suppressor Rsu-1 binds to the LIM 5 domain of the adaptor protein PINCH1 and participates in adhesion-related functions. Exp. Cell Res., 306: 168-179.

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.

Liu, G.Y., Y.Z. Xiong, C.Y. Deng, B. Zuo and J.H. Zhang, 2004. Comparison of gene expression patterns in *Longissimus dorsi* of pigs between the high-parent heterosis cross combination Landrace×Large White and the mid-parent heterosis cross combination Large White×Meishan. Asian-Aust. J. Anim. Sci., 17: 1192-1196.

Maekawa, M., T. Ishizaki, S. Boku, N. Watanabe and A. Fujita *et al*., 1999. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science, 285: 895-898.

Ohta, Y., N. Suzuki, S. Nakamura, J.H. Hartwig and T.P. Stossel, 1999. The small GTPase RalA targets filamin to induce filopodia. Proc. Natl. Acad. Sci. USA., 96: 2122-2128.

Peyton, S.R., P.D. Kim, C.M. Ghajar, D. Seliktar and A.J. Putnam, 2008. The effects of matrix stiffness and RhoA on the phenotypic plasticity of smooth muscle cells in a 3-D biosynthetic hydrogel system. Biomaterials, 29: 2597-2607.

Saeki, N., H. Tokuo and M. Ikebe, 2005. BIG1 is a binding partner of myosin Ixb and regulates its Rho-GTPase activating protein activity. J. Biol. Chem., 280: 10128-10134.

Shloush, J., J.E. Vlassov, I. Engson, S. Duan and V. Saridakis *et al*., 2011. Structural and functional comparison of the RING domains of two p53 E3 ligases, Mdm2 and Pirh2. J. Biol. Chem., 286: 4796-4808.

Su, W., P. Chardin, M. Yamazaki, Y. Kanaho and G. Du, 2006. RhoA-mediated phospholipase D1 signaling is not required for the formation of stress fibers and focal adhesions. Cell Signal, 18: 469-478.

Wu, G., M. Sun, L. Zhang, J. Zhou, Y. Wang and K. Huo, 2010. A novel hPirh2 splicing variant without ubiquitin protein ligase activity interacts with p53 and is down-regulated in hepatocellular carcinoma. FEBS Lett., 584: 2772-2778.

Yan, J., D. Zhang, Y. Di, H. Shi, H. Rao and K. Huo, 2010. A newly identified Pirh2 substrate SCYL1-BP1 can bind to MDM2 and accelerate MDM2 self-ubiquitination. FEBS Lett., 584: 3275-3278.

Yonggang, L. 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.