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Molecular cloning, sequence characteristics analysis and tissue expression profiles of three novel genes *RhoB*, *RhoF* and *RhoH* from the Black-boned sheep (*Ovis aries*)*

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ABSTRACT

The complete coding sequences of three of the Black-boned sheep (*Ovis aries*) genes - *RhoB*, *RhoF* and *RhoH* were amplified using the reverse transcriptase polymerase chain reaction (RT-PCR) according to the conserved sequence information of the mouse or other mammals and known highly homologous sheep ESTs. The Black-boned sheep *RhoB* gene encodes a protein of 196 amino acids which contains the conserved putative *RhoA*_like domain and has high homology with the RhoB proteins of six species-cattle (100%), goat (100%), human (100%), pig (100%), mouse (100%) and chicken (97%). The Black-boned sheep *RhoF* gene encodes a protein of 215 amino acids that contains the conserved putative *Rho4*_like domain and has high homology with the RhoF proteins of five species-bovine (100%), human (94%), pig (93%), mouse (92%) and chicken (58%). The Black-boned sheep *RhoH* gene encodes a protein of 191 amino acids that contains the conserved putative *Rho* domain and has high homology with the RhoH proteins of four species-bovine (99%), human (96%), pig (96%) and mouse (96%). The phylogenetic tree analysis demonstrated that the Black-boned sheep RhoB, RhoF and RhoH proteins share a common ancestor. The tissue expression analysis indicated that the three genes were expressed in a range of tissues

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including small intestine, large intestine, liver, muscle, fat, lung and spleen. Our experiment is the first to provide the primary foundation for further insight into these three sheep genes.

KEY WORDS: Black-boned sheep, *RhoB*, *RhoF*, *RhoH*, tissue expression analysis

INTRODUCTION

RhoB (ras homolog gene family, member B), is an important Rho GTPase. It is a regulator of protein signalling and trafficking. It plays a pivotal role in the dynamic regulation of the actin cytoskeleton and is involved in intracellular protein trafficking of a number of proteins (Ridley, 2001; Huang et al., 2007). *RhoB* is an inhibitor of cancer progression. It affects cell adhesion and growth factor signalling in transformed cells and its deletion increases tumor formation initiated by Ras mutation (Mazieres et al., 2004). Furthermore, it is a modulator of cancer cell apoptosis which promotes proapoptotic signalling of regulators involved in cell cycle checkpoints, cell adhesion, vesicle trafficking, MAPK signalling, transcription and immunity. It mediates apoptosis in neoplastically-transformed cells after DNA damage. It is one of the targets of farnesyltransferase inhibitors which are currently under investigation as cancer therapeutics (Liu et al., 2000; Wherlock et al., 2004).

The *RhoF* gene is also a plasma membrane-associated small GTPase which alternates between an active GTP-bound and an inactive GDP-bound form. It also causes the formation of thin, actin-rich surface projections and increases the diversity of actin-based morphology by cooperating with CDC42 and Rac to generate additional structures (Nobes and Hall, 1995; Ellis and Mellor, 2000).

RhoH is GTPase deficient and does not switch between GTP- and guanosine diphosphate (GDP)-bound forms. It also regulates proliferation, survival, migration, and engraftment of haematopoietic progenitor cells (Lahousse et al., 2004; Gu et al., 2005b). It has a negative impact on cell proliferation and F-actin polymerization (Gu et al., 2005a),

Surprisingly, we reported the discovery of the Black-boned sheep (*Ovis aries*) in a population of sheep found in Nanping County of Yunnan Province (Deng et al., 2006, 2007, 2008). These sheep had dark coloured (black) tissues, compared to the reddish colouration for normal sheep (*Ovis aries*), and the colouration was shown to be due to the presence of excessive melanin, as in the silky fowl. The trait for dark colouration in sheep has been found to be inherited in cross-breeding studies (Mao et al., 2005).

The preceding description of the functions of *RhoB*, *RhoF* and *RhoH* genes and the association of the genes with growth, health, cell morphology and other important functions that are highly related or potentially related to melanocyte distribution in the Black-boned sheep, justifies their cloning in the Black-boned sheep.

In the current experiment, we have cloned the coding sequences of the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes according to the conserved sequence information of cattle or other mammals and highly homologous sheep ESTs sequence information. We have also conducted sequence analysis of established nucleotide sequences, some necessary function analysis, and finally examined the expression of these genes in a range of Black-boned sheep tissues. The information provides a foundation for further research on these three sheep genes.

MATERIAL AND METHODS

Sample collection, RNA extraction and first-strand cDNA synthesis

Samples of small intestine, large intestine, liver, muscle, fat, lung and spleen were collected from six adult Black-boned sheep (*Ovis aries*). Total RNA extraction and first-strand cDNA synthesis were conducted as described by Liu et al. (2004, 2008).

Isolation of the RhoB, RhoF and RhoH genes

The sequences published on GenBank for *RhoB* from human (Accession No. NM_004040), cattle (Accession No. NM_001077922), pig (Accession No. DQ917637) and their highly homologous sheep EST sequences: DY498093 and DY497633 were selected to design a primer pair by using Primer Premium 5.0 software for amplifying the complete coding sequence of sheep *RhoB*. Similarly, another two pairs primers for sheep *RhoF* and *RhoH* gene isolation were designed according to the conserved coding sequences from cattle and their highly homologous sheep EST sequences.

RT-PCR was performed to isolate the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes using the cDNAs from different tissues above. The 25 μl reaction system was: 2.0 μl cDNA, 2.0 μl 10 mM mixed dNTPs, 2.5 μl 10×Taq DNA polymerase buffer, 0.8 μl 10 pM forward primer, 0.8 μl 10 pM reverse primer, 0.4 μl Taq DNA polymerase (5 U/μl), and 16.5 μl sterile water. These primer sequences and their annealing temperature for RT-PCR were showed in Table 1.

Table. 1 Primers for sheep *RhoB*, *RhoF* and *RhoH* isolation and their annealing temperatures

Genes	Primer sequences	Ta / °C
RhoB	Forward 5'- ATGGCGGCCATCCGCAAG -3' Reverse 5'- TCATAGCACCTTGCAGCAG -3'	57
RhoF	Forward 5'-ATGGACGCTGCTGGGGCC -3' Reverse 5'-TCAGAGCAGCAAGCAAAGCCG -3'	52
RhoH	Forward 5'-ATGCTGAGTTCCATCAAG -3' Reverse 5'-TTAGAGGATCTTGCACTC -3'	54

Then, PCR products for the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes were cloned into PMD18-T vector and sequenced bidirectionally.

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously presented elsewhere (Fehr et al., 2000; Daigo et al., 2003; Liu et al., 2005). To eliminate the effect of cDNA concentration, we repeated the RT-PCR five times using 1, 2, 3, 4 and 5 μl cDNA as templates. Furthermore, the housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was selected as the internal control. The control primers used were: 5'-AAGTTCAACGGCACAGTCA-3' (GAPDH 5' primer) and 5'-TCATAAGTCCCTCCACGAT-3' (GAPDH 3' primer). To ensure that no false positive PCR fragments were generated from pseudogenes in the contaminating genomic DNA, GAPDH primers were derived from different exons in the same gene. PCR primer combinations were tested using sheep genomic DNA as a negative control and an approximately 364 bp PCR fragment was amplified when cDNA was contaminated by genomic DNA. The primers of sheep *RhoB*, *RhoF* and *RhoH* genes which were used to perform the semi-quantitative RT-PCR for tissue expression profile analysis were the 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.

Bioinformatic analysis

The cDNA sequence prediction was conducted using the GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction, alignment and phylogenetic 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). Secondary structures of deduced amino acid sequences were predicted by SOPMA (http://npsa-pbil.ibcp.fr/).

RESULTS AND DISCUSSION

RT-PCR result for the Black-boned sheep RhoB, RhoF and RhoH genes. Through RT-PCR with pooled cDNAs from different tissues, for the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes, the resulting PCR products were 591, 648 and 576 bp, respectively (Figure 1).

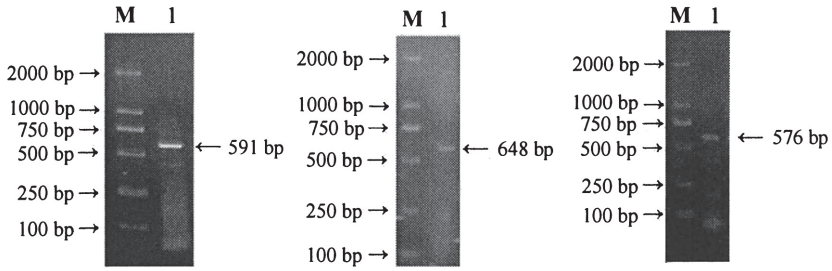

Figure 1. RT-PCR results for the Black-boned sheep *RhoB* (left), *RhoF* (middle) and *RhoH* (right) genes. M, DL2000 DNA Marker

Sequence analysis. This cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) showed that these three gene were not homologous to any of the known sheep genes and it was then deposited into the GenBank database (Accession Nos: EU664600, EU626204, EU626206). The sequences prediction were carried out using the GenScan software and results indicated that the 591, 648 and 576 bp cDNA sequences represent three single genes which encoded 196, 215 and 191 amino acids. The theoretical isoelectric point (pI) and molecular weight (Mw) of the three putative proteins of the sheep genes were also computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html). The pI of sheep *RhoB*, *RhoF* and *RhoH* were 5.096, 8.61 and 9.19. The molecular weights of the three putative proteins were 22076.33, 23840.51 and 21294.42, respectively.

These putative proteins were also blasted using the Conserved Domain Architecture Retrieval Tool of Blast at the NCBI server (http://www.ncbi. nlm.nih.gov/BLAST) and their conserved domains were identified as *RhoA*_like, *Rho4*_like and *RhoH*, respectively (Figure 2).

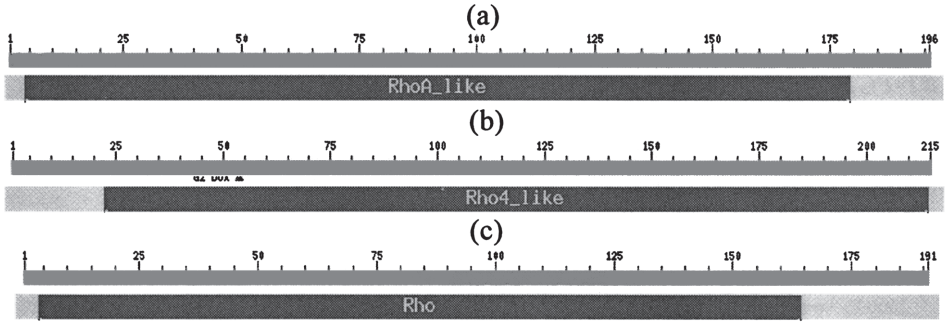

Figure 2. Putative domains of the protein encoded by the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes. (a) *Rho*A_like domain of RhoB; (b) *Rho4*_like domain of *RhoF*; (c) Rho domain of *RhoH*

Further BLAST analysis revealed that the Black-boned sheep *RhoB* has high homology with the *RhoB* (ras homolog protein family, member B) proteins of six species-bovine (100%), goat (100%), human (100%), pig (100%), mouse (100%) and chicken (97%) (Figure 3). The Black-boned sheep RhoF has high homology with the RhoF (ras homolog protein family, member F) proteins of five species-bovine (100%), human (94%), pig (93%), mouse (92%) and chicken

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Black-boned_sheep       MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Cattle                  MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Human                   MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Pig                     MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Goat                    MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Mouse                   MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
Chicken                 MAAIRKKLVVVGDGACGKTCLLIVFSKDEFPEVYVPTVFENYVADIEVDGKQVELALWDT
                        ************************************************************

Black-boned_sheep       AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Cattle                  AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Human                   AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Pig                     AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Goat                    AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Mouse                   AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
Chicken                 AGQEDYDRLRPLSYPDTDVILMCFSVDSPDSLENIPEKWVPEVKHFCPNVPIILVANKKD
                        ************************************************************

Black-boned_sheep       LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Cattle                  LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Human                   LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Pig                     LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Goat                    LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Mouse                   LRSDEHVRTELARMKQEPVRTDDGRAMAVRIQAYDYLECSAKTKEGVREVFETATRAALQ
Chicken                 LRNDEHVRNELARMKQEPVRTEDGRAMAIRIQAYDYLECSAKTKEGVREVFETATRAALQ
                        **.*****.************:******:**********************************

Black-boned_sheep       KRYGSQNGCINCCKVL
Cattle                  KRYGSQNGCINCCKVL
Human                   KRYGSQNGCINCCKVL
Pig                     KRYGSQNGCINCCKVL
Goat                    KRYGSQNGCINCCKVL
Mouse                   KRYGSQNGCINCCKVL
Chicken                 KRYGTQNGCINCCKVL
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Figure 3. The alignment of the protein encoded by the Black-boned sheep *RhoB* gene and other six types of *RhoB* from cattle (NP_001071390), goat (EU599087), pig (NP_001116661), human (NP_004031), mouse (NP_031509) and chicken (NP_990240)

(58%) (Figure 4). The Black-boned sheep RhoH has high homology with the RhoH (ras homolog protein family, member H) proteins of four species-bovine (99%), human (96%), mouse (96%) and pig (96%) (Figure 5). The sheep RhoB, RhoG, and RhoH have common conserved domains with highly homologous proteins from other mammals. Moreover, these three proteins share the similar conserved domain, Rho. This result agrees with the Rho family proteins being defined by the presence of a Rho-type GTPase-like domain (Wennerberg and Der, 2004).

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Black-boned_sheep      -----MDAAGAPAPAPAPPAAPGSGRKELKIVIVGDGGCGKTSLLMVYSQGSFPEHYAPS
Cattle                 -----MDAAGAPAPAPAPPAAPGSGRKELKIVIVGDGGCGKTSLLMVYSQGSFPEHYAPS
Pig                    ---------MDTPGAPAPTAAPGPGRKELKIVIVGDGGCGKTSLLMVYSQGSFPEHYAPS
Human                  ---------MDAPGALAQTAAPGPGRKELKIVIVGDGGCGKTSLLMVYSQGSFPEHYAPS
Mouse                  ---------MDAPGAPAPAAAPSSARKELKIVIVGDGGCGKTSLLMVYCQGSFPEHYAPS
Chicken                MEAANGAVPDGAAGGKGGSAAAPSGKKEVKMVIVGDGGCGKTSLLMVYAKGAFPEQYAPS
                                  .. . . .**. ..:**:*:******************.:*:***:****

Black-boned_sheep      VFEKYTASVTVGSKEVTLNLYDTAGQEDYDRLRPLSYQNTHLVLICYDVMNPTSYDNVLI
Cattle                 VFEKYTASVTVGSKEVTLNLYDTAGQEDYDRLRPLSYQNTHLVLICYDVMNPTSYDNVLI
Pig                    VFEKYTARVTVGSKEVTLNLYDTAGQEDYDRLRPLSYQNTHLVLICYDVMNPTSYDNVLI
Human                  VFEKYTASVTVGSKEVTLNLYDTAGQEDYDRLRPLSYQNTHLVLICYDVMNPTSYDNVLI
Mouse                  VFEKYTASVTVGNKEVTLNLYDTAGQEDYDRLRPLSYQNTHLVLICYDVMNPTSYDNVLI
Chicken                VFEKYATSITIGKKEVILNLYDTAGQEDYDRLRPLSYQNTNVVLICYDVMNPTSYDNVAD
                       *****:: :*:*.*** ************************::****************

Black-boned_sheep      KWFPEVTHFCRGIPMVLIGCKTDLRKDKEQLRKLRAAQLEPITYTQGQSACEQIRAALYL
Cattle                 KWFPEVTHFCRGIPMVLIGCKTDLRKDKEQLRKLRAAQLEPITYTQGQSACEQIRAALYL
Pig                    KWFPEVTHFCRGTPTVLIGCKTDLRKDKEQLRKLRAAQLEPITYMQGQSACEQIRAALYL
Human                  KWFPEVTHFCRGIPMVLIGCKTDLRKDKEQLRKLRAAQLEPITYMQGLSACEQIRAALYL
Mouse                  KWFPEVTHFCRGIPTVLIGCKTDLRKDKEQLRKLRAAQLEPITYTQGLNACEQMRGALYL
Chicken                KWYPEVNHFCQGVPLVLIGCKTDLRKDKEQLRKLRASKQEPITYNQVSISAFIVLFSLPK
                       **:***.***:* * ********************:: ***** *   :.  :  :*

Black-boned_sheep      ECSAKFRENVEDVFREAAKVALSALKKAQRQKQHRLCLLL
Cattle                 ECSAKFRENVEDVFREAAKVALSALKKAQRQKQHRLCLLL
Pig                    ECSAKFRENVEDVFREAAKVALGALKKAQRQKKLRLCLLL
Human                  ECSAKFRENVEDVFREAAKVALSALKKAQRQKKRRLCLLL
Mouse                  ECSAKFRENVEDVFREAAKVALSALKKAQRQKKHRICLLL
Chicken                ----------------------------------------
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Figure 4. The alignment of the protein encoded by the Black-boned sheep *RhoF* gene and other five types of RhoF from cattle (NP_001035692), pig (NP_001116662), human (NP_061907), mouse (NP_780301) and chicken (NP_001025831)

The prediction of secondary structure by SOPMA (Combet et al., 2000) indicated that the deduced *RhoB* contained 53 α-helices, 29 extended strands, and 114 random coils. Furthermore, the deduced RhoF consisted of 62 α-helices, 57 extended strands and 96 random coils compared to RhoH having 23 α-helices, 67 extended strands and 101 random coils (Figure 6).

Based on the results of the alignment of *RhoB*, *RhoF* and *RhoH*, the phylogenetic tree was constructed using the ClustalW software (http://www.ebi. ac.uk/clustalw), as shown in Figure 7.

The phylogenetic tree analysis showed that sheep RhoF and RhoH have close genetic relationships with cattle *RhoF* and *RhoH*, but sheep *RhoB* has a closer genetic relationship with the bovine, human, pig, goat and mouse *RhoB*. Furthermore, these three proteins share a common ancestor. This agrees with the previous studies that Rho subfamily remained conserved during evolution (Wennerberg and Der, 2004).

Gene expression profiles analysis. Gene expression profile analysis was conducted and results showed that the Black-boned sheep *RhoB* gene was

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Black-boned_sheep       MLSSIKCVLVGDSAVGKTSLLVRFTSETFPEAYKPTVYENTGVDVFMDGIQISLGLWDTA
Cattle                  MLSSIKCVLVGDSAVGKTSLLVRFTSETFPEAYKPTVYENTGVDVLMDGIQISLGLWDTA
Human                   MLSSIKCVLVGDSAVGKTSLLVRFTSETFPEAYKPTVYENTGVDVFMDGIQISLGLWDTA
Mouse                   MLSSIKCVLVGDSAVGKTSLLVRFTSETFPEAYKPTVYENTGVDVFMDGIQISLGLWDTA
Pig                     MLSSIKCVLVGDSAVGKTSLLVRFTSETFPEHYKPTVYENTGVDVFMDGIQISLGLWDTA
                        ******************************** *************:**************

Black-boned_sheep       GNDAFRSIRPLSYQQADVVLMCYSVANHNSFLNLKNKWIGEVRSNLPCTPVLVVATQTDQ
Cattle                  GNDAFRSIRPLSYQQADVVLMCYSVANHNSFLNLKNKWIGEVRSNLPCTPVLVVATQTDQ
Human                   GNDAFRSIRPLSYQQADVVLMCYSVANHNSFLNLKNKWIGEIRSNLPCTPVLVVATQTDQ
Mouse                   GNDAFRSIRPLSYQQADVVLMCYSVANHNSFLNLKNKWISEIRSNLPCTPVLVVATQTDQ
Pig                     GNDAFRSIRPLSYQQADVVLMCYSVANYNSFLNLKNKWIGEVRNNLPCTPVLVVATQTDQ
                        ****************************:************.*:*.*****************

Black-boned_sheep       REVGPHRASCVNAIEGKRLAQDVRAKGYLECSALSNRGVQQVFECAVRTAVNQARRRNRR
Cattle                  REVGPHRASCVNAIEGKRLAQDVRAKGYLECSALSNRGVQQVFECAVRTAVNQARRRNRR
Human                   REMGPHRASCVNAMEGKKLAQDVRAKGYLECSALSNRGVQQVFECAVRTAVNQARRRNRR
Mouse                   REVGPHRASCINAIEGKRLAQDVRAKGYLECSALSNRGVQQVFECAVRTAVNQARRRNRR
Pig                     REVGPHRASCVSAVEGKRLAQDVRAKGYLECSALSNRGVQQVFECAVRTAVNQARRRNRR
                        **:********:.*:***:*********************************************

Black-boned_sheep       RFFSINECKIL
Cattle                  RFFSINECKIL
Human                   RLFSINECKIF
Mouse                   KLFSINECKIF
Pig                     RFFPLNECKIL
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Figure 5. The alignment of the protein encoded by the Black-boned sheep *RhoH* gene and other four types of RhoH from cattle (NP_001071592), pig (AK233362), human (NP_004301) and mouse (NP_001074574)

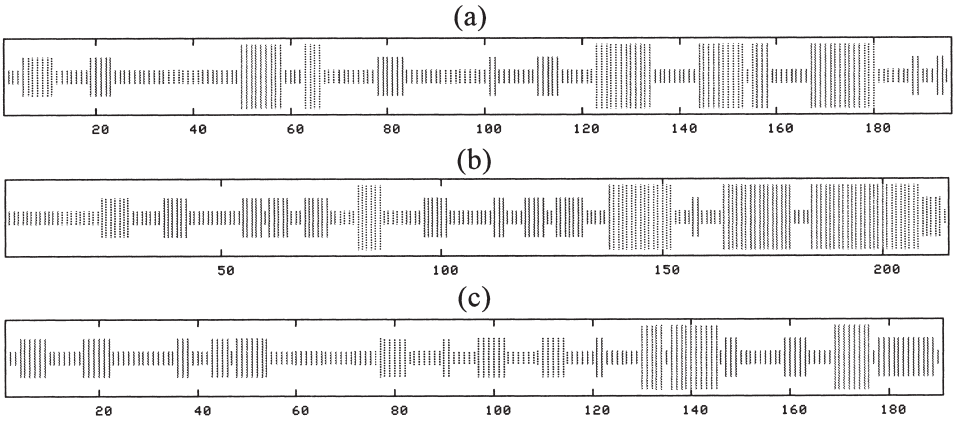

Figure 6. Predicted secondary structure of the Black-boned sheep *RhoB* (a), *RhoF* (b) and *RhoH* (c) proteins by SOPMA. Helices, strands and coils are indicated, respectively, with the longest, the middle and the shortest vertical lines

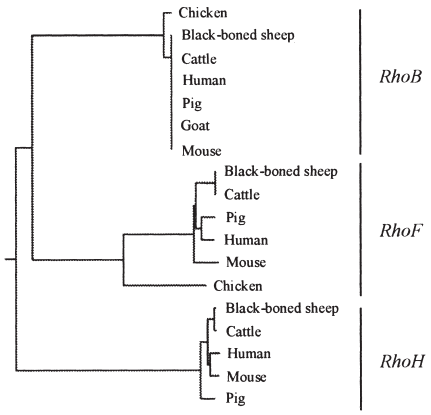

Figure 7. The phylogenetic tree for different kinds of *RhoB*, *RhoF* and *RhoH* proteins

moderately expressed in large intestine, lung and spleen, and weakly expressed in small intestine, liver and fat. There was almost no expression in muscle. The Black-boned sheep *RhoF* gene was almost weakly expressed in all measured tissues besides the large intestine (Figure 8). Furthermore, the Black-boned sheep *RhoH* gene was moderately expressed in liver, muscle and lung, weakly expressed in large intestine and spleen, with hardly any expression in small intestine and fat.

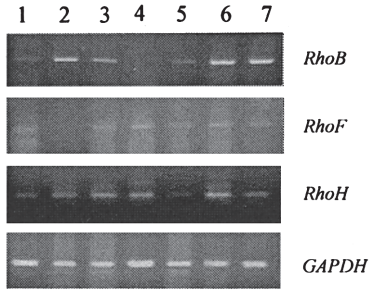

Figure 8. Tissue expression distribution of the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes. M, DL2000 marker; 1. small intestine; 2. large intestine; 3. liver; 4. muscle; 5. fat; 6. lung; 7. spleen. The GAPDH expression is the internal control

Comparative genomics embodies the relationship of genome structure and function among different biological species or strains. Researchers have learned a great deal about the function of human genes by examining their counterparts in completed reference sequences of the human and mouse genomes and some results have shown that virtually all (99%) of the protein-coding genes in humans

align with homologs in mouse, and over 80% are clear 1:1 orthologs (Hardison, 2003; Liu et al., 2007, 2008). This extensive conservation in protein-coding regions implies that the same sequences could be expected in different mammals such as pigs, dogs, cats, rabbits, monkeys and cattle. This provides us with a useful method to identify the functional regions of different genes for sheep according to the conserved sequence information for the mouse, human, cattle or other mammals and to predict what those functions are.

With the development of modern bioinformatics, many more specific databases such as NCBI sheep EST database were established along with different convenient analysis tools and these make it much easier to search the sheep EST sequences which are highly homologous to some coding sequences of the mouse, human or other mammals. This implies that when we can clone the functional regions of different genes for sheep based on the conserved sequence information of the mouse, human or other mammals we also can refer to this highly homologous EST sequence information.

In this experiment, the complete coding sequences of the sheep *RhoB*, *RhoF* and *RhoH* genes were isolated based on the sequence information of the pig, cattle or other mammals and some referenced sheep ESTs. Sequence identification further validated that the comparative genomics method is a useful tool for cloning the unknown genes especially the conserved coding region of genes for sheep. From our results we know that sheep *RhoB*, *RhoF* and *RhoH* are highly homologous with *RhoB*, *RhoF* and *RhoH* of mouse or other mammals and sheep *RhoB*, *RhoF* and *RhoH* also have the same domains with their corresponding highly homologous proteins from mouse or other mammals. This implies sheep *RhoB*, *RhoF* and *RhoH* will have similar functions as *RhoB*, *RhoF* and *RhoH* of mouse or other mammals. We also find that sheep RhoF and RhoH do not show complete identity to mouse or other mammals. This implies that they will have some differences in functions to those of mouse or other mammals.

The phylogenetic tree analysis revealed that the sheep proteins - *RhoB*, *RhoF* and *RhoH*, have closer genetic relationships with those of other species. This implies that different genes have different evolutionary models even though they are in one individual or in one species. But we still could find these sheep proteins have a close relationship with those of other mammals. This supported our methods used in this experiment to clone the sheep encoding regions based on the conserved encoding region information from other mammals.

In our experiment, we not only cloned the complete coding sequence of the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes but also conducted the sequence analysis and tissue expression profiles analysis. From the tissue profile analysis it can be seen that these genes were obviously differentially expressed in different tissues. The suitable explanation for this is that at the same time those biological

activities associated with the functions of these genes were presented diversely in different tissues.

In conclusion, this is the first reporting of the cloning of the Black-boned sheep *RhoB*, *RhoF* and *RhoH* genes and the conducting of the necessary functional analysis and tissue expression profiles. This information provides the primary foundation for further insight into these three sheep genes.

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