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Full Length Research Paper

Isolation, sequence identification and tissue expression profile of a novel goat Gene-RAB7A

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The full-length cDNA sequence of one goat gene, RAB7A, was amplified using the rapid amplification of cDNA ends (RACE) method based on one goat EST sequence which was highly homologous to the coding sequence of human RAB7A gene. Sequence prediction analysis revealed that the open reading frame of this gene encodes a protein of 207 amino acids that has high homology with the RAB7A, member RAS oncogene family (RAB7A) of seven species—sheep (100%), human(99%), mouse(99%), Chinese hamster(99%), rat(99%), green anole(99%) and dog(99%)—so that it can be defined as goat RAB7A gene.. This novel goat gene was then deposited into NCBI database. The phylogenetic analysis revealed that the goat RAB7A gene has a closer genetic relationship with the RAB7A gene of sheep. Tissue expression analysis indicated that the goat RAB7A gene is differentially expressed in detected tissues including spleen, muscle, skin, kidney, lung, liver, fat and heart. Our experiment established the primary foundation for further research on the goat RAB7A gene.

Keywords: Goat, RAB7A, RACE, Tissue Expression Profile, Sequence Identification.

INTRODUCTION

RAB7A is a member of RAB oncogene family which are small, RAS-related GTP-binding proteins. This gene regulates vesicle traffic in the late endosomes and also from late endosomes to lysosomes(Wang *et al*., 2011). Researches showed that this gene is involved in the cellular vacuolation of the VacA cytotoxin of Helicobacter pylori (Papini *et al*., 1997; Daniele*et al*., 2011). Mutations at highly conserved amino acid residues in this gene had been identified to lead to some forms of Charcot-Marie-Tooth (CMT) type 2 neuropathies (Spinosa *et al*., 2008;McCray*et al*., 2010).As mentioned above, RAB7A gene is an important gene which has many biological functions. Until today, RAB7A gene has been reported in cattle, pig, human, dog, mouse, rat and other animals. The goat RAB7A has not been reported.

In the present experiment, we will clone the full-length cDNA sequence of the goat RAB7A gene, and further do necessary sequence analysis and tissue expression analysis. These will establish the primary foundation of understanding this goat gene.

MATERIALS AND METHODS

Animals and sample preparation

Two adult Fujian local goat (Capra hircus) were slaughtered. Spleen, muscle, skin, kidney, lung, liver, fat and heart samples were collected, frozen in liquid nitrogen and then stored at – 80 ℃. 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.

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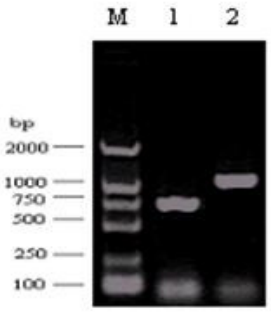

Figure 1 RACE results for goat RAB7A gene.
M, DL2000 DNA markers; 1, 5'-RACE product for RAB7A gene; 2, 3'-RACE product for RAB7A gene.

GCTTGAAGGATGACCTCGAGGAAGAAAGTGCTGCTGAAGGTGATCATCCTGGGAGACTCTGGAGTCGGGAAGACCTCGCTTATGAACCAG
M T S R K K V L L K V I I L G D S G V G K T S L M N Q
TACGTGAACAAGAAGTTCAGTAACCAGTACAAAGCCACGATAGGCGCGGACTTCCTGACGAAGGAGGTGATGGTGGACGACAGGCTCGTG
Y V N K K F S N Q Y K A T I G A D F L T K E V M V D D R L V
ACCATGCAGATCTGGGACACGGCGGGACAGGAGCGGTTCCAGTCCCTGGGCGTGGCCTTCTACAGAGGCGCCGACTGCTGTGTCCTGGTG
T M Q I W D T A G Q E R F Q S L G V A F Y R G A D C C V L V
TTCGACGTGACCGCCCCCAACACGTTCAAGACCCTTGACAGCTGGAGAGACGAGTTTCTCATCCAGGCCAGTCCCCGGGACCCCGAGAAC
F D V T A P N T F K T L D S W R D E F L I Q A S P R D P E N
TTCCCCTTCGTCGTGCTGGGAAACAAGATTGACCTCGAAAACAGACAGGTGGCCACCAAGCGGGCTCAGGCCTGGTGCTACAGCAAAAAC
F P F V V L G N K I D L E N R Q V A T K R A Q A W C Y S K N
AACATCCCCTACTTCGAGACCAGCGCCAAGGAGGCCATCAACGTGGAGCAGGCCTTCCAGACGATCGCCCGGAACGCGCTCAAGCAGGAA
N I P Y F E T S A K E A I N V E Q A F Q T I A R N A L K Q E
ACGGAAGTGGAGCTGTACAACGAGTTCCCCGAGCCCATCAAACTGGACAAGAATGACCGCCCCAAGGCCTCGGCCGAGAGCTGCAGCTGC
T E V E L Y N E F P E P I K L D K N D R P K A S A E S C S C
TGAAGAGGCAGAGAGCAGAGCACACAGTCCTCCGCGACCGAGAACACACGTGCAGGCCTTCTTCCACGAGCCCCTCCTCCCACAGACAG
TCATCTCTCGCCCAGCTGCCCGGAGAGCCCAGCACACCGCCGACCCCCGCACACGCCCCACAGACCCACCGCCCGCCCCGCGGGCTCCAC
GGCAGCAGCAAGGCCGGAGGCGCACACCCACCTGGGTGGAGTCTGCGCTGGAAGCTTCCTTTTGTCCACTGGAGAGAGAACTGTTTACAG
TTAATCTGTGTCTAATTAGTTCTGATTTTTTTAACCGTCTTGTGGTCTTTTTACCCACCCCCTCCTGTGAAGGCCACCACGTGGGAAGGC
TGGTGCCCTGCGCTCCATCACAGGCTCACACCCAGTCTGATCAGGCTGAGTTTTGTATGTATCTGTTAATGCTTGTTACTTTTAACTAAT
CAGATCTTTTTACAGTATCCATTTATTATGTAATGCTTCGTAGAAAAGAATCTTATAGTACATGTTAATATATGCAACCAATTAAAATGT
ATAAATCAGTGTAAGAAATTCTTGGGTTATGTGTTTAAGTCCTGTGATGCAGGCCTAGAGGCAGAGGGCTGAACCCGGTCTGGACCGCCG
CGTGTTCAGCATCTCAAGAGGTGAGAAGTCCAGCAGGAGGCAGTATTCTGTACAGTAGACACGAGAATCATGTACGCCTTTTATCAAAGA
CTTCAGAGCCAAAAAGCTTTGCATCTCTCCAGGGGGAGGCTGTCCAGTTCCCTCTGCATCTAAATTTTCCAAGAGGCGATTTGCATAATA
CTTTGGTGTGTGCAATGGAGAAACAGCTGTTTCACAAATTAAAACTCTCATTTTCCCTTTTTTTCTTTCCTGCTCCACACTTTTAAAACT
CCCGTTAGATTCGCATCCACGTCCAAGAGGGAGAGGAGGCCCTCCCAGACCTGTGCTAGCGACGGTACCTTTGTTCTAGACGGCGCTCCT
CTCGGGGTGTGGCGTCCTCGGTGAGCACACCTTCCCCAGCCCCGACTCCCCAGTGTAGCCCGCGGCCCCCCCACACTGTAGCCTGCTTCA
CAGAGCTCCCCTCTGAGGGCCTGTGTCCCCGGGTGTGGGCCAGGTTCTTCTGTAAAGAGACGAACGTGATGCCAATAAAATGTACCAAGA
ACAAAAAAAAAAAAAAAAAAAAAAAA

Figure 2. The complete cDNA sequence and encoded amino acids of goat RAB7A gene (GenBank accession number: JQ417405). ATG: start codon; TGA: stop codon.

5'- and 3'-RACE

5'- and 3'-RACE were performed to isolate the full-length cDNA for goat RAB7A gene as the instructions of SMART™ RACE cDNA Amplification Kit (Clontech, USA). For the goat RAB7A gene, the Gene-Specific Primers (GSPs) were designed based on one goat EST sequence whose sequence is highly homologous to the coding sequence of human RAB7A gene: EV441941. The Gene-Specific Primers (GSPs) were: 5'-RACE GSP: 5'-GCGAGAGATGACTGTCTGTGGGAGG-3', 3'-RACE GSP: 5'- GAGAACACACGTGCAGGCCTTCTTC-3'.
RACE touchdown PCRs were carried out with 5 cycles of 94℃ / 30 s and 72℃ /3 min, followed by 5 cycles of 94℃ / 30 s, 70℃ / 30 s and 72℃ / 3 min, finally with 30 cycles of 94℃ / 30 s, 68 ℃ / 30 s, 72℃ / 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.

RT-PCR for tissue expression profile analysis

RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009a, b; Liu, 2009).We selected the housekeeping gene beta-actin (Accession no: AF481159) was performed as a positive control. The control gene primers used were: 5'- TGGCATTGTCATGGACTC-3' (forward primer 1) and 5'-CCGTGGTGGTGAAGCT-3' (reverse primer1). The PCR product is 164-bp in length. The following RAB7A gene specific primers were used to perform the RT-PCR for tissue expression profile analysis: 5'- GTGGATGCGAATCTAACG-3' (forward primer 2) and 5'-TCCAGCAGGAGGCAGTAT-3' (reverse primer2). The PCR product is 262-bp in length. The 25 µl reaction system was: 2 µl cDNA (100 ng), 5 pmoles each oligonucleotide primer(forward primer 1 and reverse primer1or forward primer and reverse primer2), 2.5 µl 2 mmol/l mixed dNTPs, 2.5 µl 10×Taq DNA polymerase buffer, 2.5µl 25 mmol/l $MgCl_2$, 1.0 units of Taq DNA polymerase, and finally add sterile water to volume 25µl. The PCR program initially started with a 94□ denaturation for 4min, followed by 30 cycles of 94□/ 50s, 56□ /50s, 72□/50s, then 72□extension for 10 min, finally 4□ 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

RACE results for goat RAB7A gene

Through 5'-RACE, one PCR product of 730-bp was obtained. The 3'-RACE product was 1156-bp. These products were then cloned to T-vector and sequenced. Taken together, a 1826-bp cDNA complete sequence was finally obtained.

Sequence analysis

The nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that this gene was not homologous to any of the known goat genes and it was then deposited into the GenBank database (Accession number: JQ417405). The sequence prediction was carried out using the GenScan software. An open reading frame encoding 207 amino acids was found in the1826-bp cDNA sequence. Further BLAST analysis of this protein revealed that this protein has high homology with the phosphoglycerate kinase 1 (RAB7A) of seven species—sheep (accession number:NP_001119836; 100%), human (accession number:NP_004628; 99%), mouse (accession number:NP_033031; 99%), Chinese hamster(accession number: XP_003503603; 99%), rat (accession number:NP_076440; 99%), green anole(accession number:XP_003217771; 99%), dog(accession number:NP_001003316; 99%). The complete cDNA sequence of this gene and the encoded amino acids were shown in Figure 2.

From the sequencing and structural results described, this gene can be defined as the goat RAB7A gene. Based on the results of the alignment of different species of RAB7A proteins, a phylogenetic tree was constructed using the Clustal W software (http://www.ebi.ac.uk/clustalw), as shown in Figure 4. The phylogenetic tree analysis revealed that the goat RAB7A gene has a closer genetic relationship with the sheep RAB7A gene than with those of human, mouse, Chinese hamster, rat, green anoleand dog.

Tissue expression profile

The RT-PCR analysis of the tissue expression profile was carried out using the tissue cDNAs as the templates. The tissue expression analysis indicated that the goat RAB7A

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Green anole          MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
Dog                  MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
Rat                  MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
Human_Mouse          MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
Goat_sheep           MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
Chinese hamster      MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQ
                     ************************************************************

Green anole          IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
Dog                  IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
Rat                  IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
Human_Mouse          IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
Goat_sheep           IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
Chinese hamster      IWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPF
                     ************************************************************

Green anole          VVLGNKIDLENRQVTTKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
Dog                  VVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
Rat                  VVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
Human_Mouse          VVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
Goat_sheep           VVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
Chinese hamster      VVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEV
                     **************:*********************************************

Green anole          ELYNEFPEPIKLDKNDRAKASAESCSC
Dog                  ELYNEFPEPIKLDKNDRAKTSAESCSC
Rat                  ELYNEFPEPIKLDKNERAKASAESCSC
Human_Mouse          ELYNEFPEPIKLDKNDRAKASAESCSC
Goat_sheep           ELYNEFPEPIKLDKNDRPKASAESCSC
Chinese hamster      ELYNEFPEPIKLDKNDRVKASAESCSC
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Figure 3. The alignment of the protein encoded by RAB7A gene from goat and seven other kinds of RAB7A proteins from sheep, human, mouse, Chinese hamster, rat, green anole and dog.

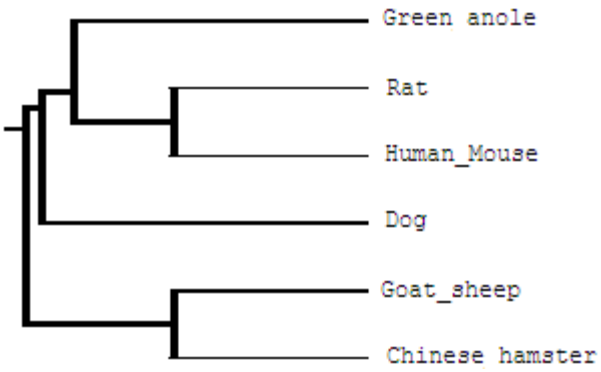

Figure 4. The phylogenetic tree for seven kinds of RAB7A genes from goat, sheep, human, mouse, Chinese hamster, rat, green anole and dog.

Figure 5. Tissue expression profile analysis of the goat RAB7A gene on the agarose gel of 1% stained with ethidium bromide. The beta-actin expression is the control.
1, skin; 2, kidney; 3, spleen; 4, heart; 5, fat; 6, lung; 7, liver; 8, muscle

gene is generally differentially expressed in detected tissues including spleen, muscle, skin, kidney, lung, liver, fat and heart.

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DISCUSSION

Through sequence analysis, we found that the encoding protein of the goat RAB7A gene is highly homologous with RAB7A proteins of human, mouse and other mammals. This implied that the RAB7A genes were highly conserved in some mammals and the goat RAB7A gene will have similar functions as the RAB7A genes of human, mouse and other mammals. We also found that the goat RAB7A protein does not show complete identity to human, mouse or other mammals. This implied that the goat RAB7A gene will have some differences in functions to those of human, mouse or other mammals. From phylogenetic analysis we found that goat RAB7A gene has a closer genetic relationship with the RAB7A gene of sheep, this implied that we can use cattle as a model organism to study the goat RAB7A gene.

From the tissue distribution analysis in our experiment it can be seen that the goat RAB7A 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 goat RAB7A gene. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of goat RAB7A gene were presented diversely in different tissues.

CONCLUSION

In conclusion, we first isolated the goat RAB7A gene and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into this novel goat gene.

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