

Full Length Research Paper

Isolation, sequence identification and tissue expression profile of a novel goat gene-RAP1B

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The full-length cDNA sequence of one goat gene, RAP1B, 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 RAP1B gene. Sequence prediction analysis revealed that the open reading frame of this gene encodes a protein of 184 amino acids that shares high homology with the RAP1B, member of RAS oncogene family(RAP1B) of nine species—human(100%), cattle(100%), mouse(100%), dog(99%), rat(99%), umatran orangutan(99%), blue catfish(98%), giant panda(98%) and common carp(98%)—so that it can be defined as goat RAP1B gene. This novel goat gene was then deposited into NCBI database. The phylogenetic analysis revealed that the goat RAP1B gene has a closer genetic relationship with the RAP1B genes of human, cattle and mouse. Tissue expression analysis indicated that the goat RAP1B gene is generally and 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 RAP1B gene.

Keywords: Goat, RAP1B, RACE, Tissue Expression Profile, Sequence Identification.

INTRODUCTION

RAP1B, member of RAS oncogene family (RAP1B) is a gene encodes a member of the RAS-like small GTP-binding protein superfamily. Members of this family regulate multiple cellular processes including cell adhesion and growth and differentiation. This protein localizes to cellular membranes and has been shown to regulate integrin-mediated cell signaling. This protein also plays a role in regulating outside-in signaling in platelets(Matsuse *et al.*, 2009; Carmona *et al.*, 2009; Yan *et al.*, 2008; Malchinkhuu *et al.*, 2009). As mentioned above, RAP1B gene is an important gene which has many biological functions. Until today, RAP1B gene has been reported in cattle, pig, human, dog, mouse, rat and other animals. The goat RAP1B gene has not been reported.

In the present experiment, we will clone the full-length cDNA sequence of the goat RAP1B 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 °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.

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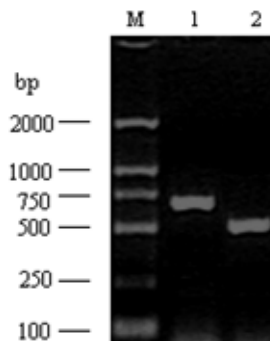


Figure 1. RACE results for goat RAP1B gene.

M, DL2000 DNA markers; 1, 5'-RACE product for RAP1B gene; 2, 3'-RACE product for RAP1B gene.

5'- and 3'-RACE

5'- and 3'-RACE were performed to isolate the full-length cDNA for goat RAP1B gene as the instructions of BD SMART[™] RACE cDNA Amplification Kit (BD science, USA). For the goat RAP1B 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 RAP1B gene: EV443023. The Gene-Specific Primers (GSPs) were: 5'-RACE GSP: 5'-CCTTCCCAGGCACTGGAGTTTTTCT-3', 3'-RACE GSP: 5'-AGAAAACTCCAGTGCCTGGGAAGG-3'.

RACE touchdown PCRs were carried out with 5 cycles of 94°C / 30 s and 72°C / 3 min, followed by 5 cycles of 94°C / 30 s, 70°C / 30 s and 72°C / 3 min, finally with 30 cycles of 94°C / 30 s, 68 °C / 30 s, 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.

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 beta-actin gene primers used were: 5'- TGGCATTGTCATGGAAGCT-3' (forward primer 1) and 5'-CCGTGGTGGTGAAGCT-3' (reverse primer1). The PCR product was 164-bp in length. The following RAP1B gene specific primers were used to perform the RT-PCR for tissue expression profile analysis:

5'- TTCCGATGATTCTGGTTG-3' (forward primer 2) and 5'- TCCGCCTCTTACAGGGTA-3' (reverse primer2). The PCR product is 365-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 MgCl₂, 1.0 units of Taq DNA polymerase, and finally add sterile water to volume 25µl. The PCR program initially started with a 94°C denaturation for 4min, followed by 30 cycles of 94°C / 50s, 55°C / 50s, 72°C / 50s, 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

RACE results for goat RAP1B gene

Through 5'-RACE, one PCR product of 691-bp was obtained. The 3'-RACE product was 514-bp. These products were then cloned to T-vector and sequenced. Taken together, a 1170-bp cDNA complete sequence was

AGCCGGAGCGGCCAGCGCGCGCGGTAGCAGCAGCAGCAGCAGCAGCGGTAGCCGGAGGACCCCGGTGGCCGCCGGGACGAGCAGCAGCAGCGGAGGGAGCGTGAGGCGACGCTGGCTGCGGGCACCCGGTGACAGCGCAGAGGTTACTAGCTTTTACAAGTTTGACCAATGCGTGAGTAT
M R E Y
AAGCTAGTCGTTCTTGGCTCGGGAGGCGTTGGAAAACTGCTCTGACTGTACAGTTTGTTC AAGGAATTTTGTGTA AAAATATGATCCT
K L V V L G S G G V G K S A L T V Q F V Q G I F V E K Y D P
ACGATAGAAGATTCTTATAGGAAGCAAGTTGAAGTAGATGCACAGCAGTGATGCTTGAATCTTGGATACTGCAGGAACGGAACAATTC
T I E D S Y R K Q V E V D A Q Q C M L E I L D T A G T E Q F
ACAGCAATGAGGGATTGTACATGAAAAATGGACAAGGCTTGCATTAGTTTATTCCATCACAGCACAGTCCACATTTAATGATTACAA
T A M R D L Y M K N G Q G F A L V Y S I T A Q S T F N D L Q
GATCTGAGAGAACAGATTCTTCGAGTTAAAGACCGGATGATGTTCCGATGATTCTGGTTGGTAATAGTGTGACTTGG AAGATGAAAGA
D L R E Q I L R V K D T D D V P M I L V G N K C D L E D E R
GTTGTAGGAAAGGAACAAGGTCAAAATCTAGCACGACAGTGGAACTGTGCATTCTTAGAATCCTCTGCAAAATCAAAAATAAATGTT
V V G K E Q G Q N L A R Q W N N C A F L E S S A K S K I N V
AATGAGATCTTTTATGACCTAGTGCACAAATTAACAGAAAACTCCAGTGCTGGGAAGGCCCGCAAAAAGTCGTATGTCAGCTGCTT
N E I F Y D L V R Q I N R K T P V P G K A R K K S S C Q L L
TAAATACTAAATGCATTGTAGCTTTGAGCCAGGTCTGAAGAACTGTTGCCCAATTCAACAGTGCCAGCATTCACACTTTGTTAAACCT
ACCAACATCTTAAATGGACTTTCTGTGGTGTACCTGTAAAGAGCGGATGAAACTACTACATCAGTTTGACATTTTAACTACTTTCC
AGTATCACAGAGAGATTTTACTTATATAATAGTCTAGAGTATGCAGCTGGTAAACCAGAGGCTACAATCCAGTATTACTGCTAAGAG
ACATTTTTCATCCACCAATGTTGTACATGTATGAAATGGTGTACTGTATACTTTAACACACCCATACTTTGTATTGGGGAGTACAATA
ATGTAATCCTAAAAGCACCCTATTTTAGCATAATAAAAGAAAGTCCAAAGAGCTCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
A

Figure 2. The complete cDNA sequence and encoded amino acids of goat RAP1B gene (GenBank accession number: JQ407045) . ATG: start codon; TAA: stop codon.

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Dog
Giant_panda
Goat_Human_Cattle_Mouse
Rat
Sumatran_orangutan
Blue_catfish
Common_carp
-MREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDAQ
-MREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDAQ
-MREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDAQ
-MREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDAQ
MMREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDGQ
-MREYKLVVLGSGGGVGKSALTIVQFVQGI FVEKYDPTIEDSYRKQVEVDGQ
*****:*****.****.*

Dog
Giant_panda
Goat_Human_Cattle_Mouse
Rat
Sumatran_orangutan
Blue_catfish
Common_carp
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
QCMLEILDAGTEQFTAMRDLYMKNGQG FALVYSITAQSTFNDLQDLREQ
*****:*****.*****

Dog
Giant_panda
Goat_Human_Cattle_Mouse
Rat
Sumatran_orangutan
Blue_catfish
Common_carp
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
ILRVKDTDDVPMILVGNKCDLEDERVVGKEQGQN LARQWNNCAFLSSAK
*****:*****.*****

Dog
Giant_panda
Goat_Human_Cattle_Mouse
Rat
Sumatran_orangutan
Blue_catfish
Common_carp
SKINVNEIFYDLVRQINRKT FVPGKARKKSTCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSTCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSSCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSSCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSSCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSSCQLL
SKINVNEIFYDLVRQINRKT FVPGKARKKSTCQLL
*****:*****.*****

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Figure 3. The alignment of the protein encoded by RAP1B gene from goat and nine other kinds of RAP1B proteins from human, cattle, mouse, dog, rat, sumatran orangutan, blue catfish, giant panda and common carp.

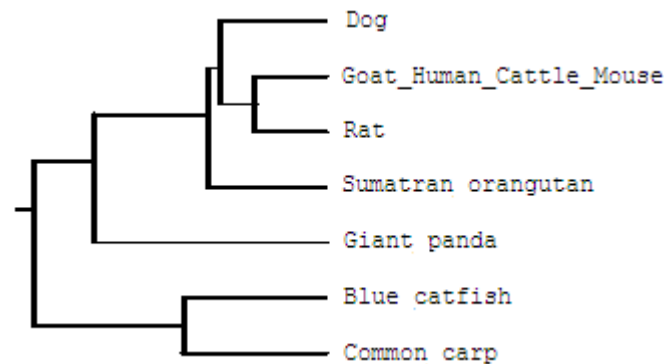


Figure 4. The phylogenetic tree for seven kinds of RAP1B from goat, human, cattle, mouse, dog, rat, sumatran orangutan, blue catfish, giant panda and common carp.

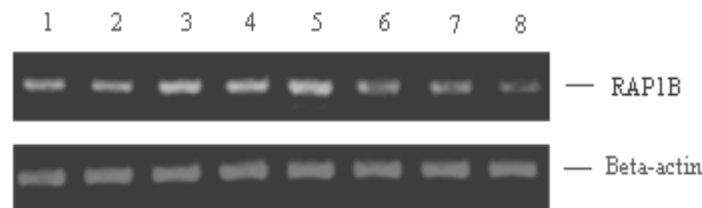


Figure 5. Tissue expression profile analysis of the goat RAP1B gene on the agarose gel of 1% stained with ethidium bromide. The beta-actin expression is the control. 1, skin; 2, kidney; 3, spleen; 4, heart; 5, fat; 6, lung; 7, liver; 8, muscle

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: JQ417404). The sequence prediction was carried out using the GenScan software. An open reading frame encoding 184 amino acids was found in the 1170-bp cDNA sequence. Further BLAST analysis of this protein revealed that this protein has high homology with the phosphoglycerate kinase 1

(RAP1B) of nine species—human (accession number:NP_056461; 100%), cattle (accession number:NP_787018; 100%), mouse (accession

number:NP_077777; 100%), dog (accession number:XP_851250; 99%), rat (accession number:NP_599173; 99%), sumatran orangutan (accession number: CAH91916; 99%), blue catfish (accession number:ADO27927; 98%), giant panda (XP_002918366; 98%) and common carp (accession number:Q9YH37; 98%). 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 RAP1B gene. Based on the results of the alignment of different species of RAP1B 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 RAP1B gene has a closer genetic relationship with the RAP1B genes of human, cattle and mouse than with those of dog, rat, sumatran orangutan, blue catfish, giant panda and common carp.

carried out using the tissue cDNAs as the templates. The tissue expression analysis indicated that the goat RAP1B gene is generally and differentially expressed in detected tissues including spleen, muscle, skin, kidney, lung, liver, fat and heart.

Tissue expression profile

The RT-PCR analysis of the tissue expression profile was

DISCUSSION

Through sequence analysis, we found that the encoding protein of the goat RAP1B gene is highly homologous with RAP1B proteins of human, mouse and other mammals. This implied that the RAP1B genes were highly conserved in some mammals and the goat RAP1B gene will have similar functions as the RAP1B genes of human, mouse and other mammals. We also found that the goat RAP1B protein does not show complete identity to rat or other mammals. This implied that the goat RAP1B gene will have some differences in functions to those of rat or other mammals. From phylogenetic analysis we found that goat RAP1B gene has a closer genetic relationship with the RAP1B genes of human, cattle and mouse, this implied that we can use human, cattle and mouse as model organisms to study the goat RAP1B gene.

From the tissue distribution analysis in our experiment it can be seen that the goat RAP1B 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 RAP1B 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 RAP1B gene were presented diversely in different tissues.

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CONCLUSION

In conclusion, we first isolated the goat RAP1B 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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