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Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*ARF3, ARF4* and *ARF5*

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Abstract: The complete coding sequences of three sheep genes-*ARF3*, *ARF4* and *ARF5* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The nucleotide sequences of these three genes revealed that sheep *ARF3* gene encodes a protein of 181 amino acids that shares high homology with the ADP-Ribosylation Factor 3 (ARF3) proteins of five species human (99%), zebrafish (99%), African clawed frog (99%), pig (99%) and atlantic salmon (98%). The sheep *ARF4* gene encodes a protein of 180 amino acids that shares high homology with the ADP-Ribosylation Factor 4 (ARF4) proteins of third species-cattle (100%), platypus (99%), green anole (99%), pig (99%), rabbit (98%), African clawed frog (98%), red jungle fowl (98%), mouse (98%), zebrafish (96%), human (97%), atlantic salmon (92%), rainbow trout (92%) and *Caligus rogercresseyi* (89%). The sheep *ARF5* gene encodes a protein of 180 amino acids that shares high homology with the ADP-Ribosylation Factor 5 (ARF5) proteins of seven species human (100%), mouse (100%), rat (100%), gray short-tailed opossum (99%), chicken (98%), channel catfish (93%) and zebrafish (93%). Finally, these three novel sheep genes were assigned to GeneIDs; 100302303, 100302304 and 100302305. The phylogenetic analysis indicated that the sheep *ARF3* gene has closer genetic relationship with the *ARF3* gene of human. The sheep *ARF4* gene has closer genetic relationship with the *ARF4* gene of cattle and the sheep *ARF5* gene has closer genetic relationship with the *ARF5* genes of human, mouse and rat. Tissue expression profile analysis was also carried out and results demonstrated that sheep *ARF3*, *ARF4* and *ARF5* genes were differentially expressed in detected tissues.

Key words: Sheep, ARF3, ARF4, ARF5, tissue expression, cattle, rat

INTRODUCTION

ADP-Ribosylation Factor 3 (ARF3), ADP-Ribosylation Factor 4 (ARF4) and ADP-Ribosylation Factor 3 (ARF5) are three members of the *ARF* gene family. These genes encode small guanine nucleotide-binding proteins that stimulate the ADP-ribosyltransferase activity of cholera toxin and play a role in vesicular trafficking and as activators of phospholipase D. The gene products include 6 ARF proteins and 11 ARF-like proteins and constitute 1 family of the RAS superfamily. The ARF proteins are categorized as class 1 (ARF1-ARF3), class 2 (ARF4 and ARF5) and class III (ARF6) and members of each class share a common gene organization (Bailey *et al*., 2010; Manolea *et al*., 2010; Woo *et al*., 2009; Kimura *et al*., 2006; Kim *et al*., 2003; Shiba *et al*., 2006; Brandenberger *et al*., 2004).

However, latest studies have shown that ARF3 plays a unique function at the Trans-Golgi Network (TGN) that likely involves recruitment by a specific receptor and is associated with protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration (Lim *et al*., 2006; Manolea *et al*., 2010). Recent researches also showed that ARF4 participates in the regulation of glioblastoma apoptosis through the inhibition of stress-mediated apoptotic signals (Woo *et al*., 2009; Kim *et al*., 2003). What's more, ARF5 had been identified to be involved in controlling tumor proliferation and metastasis (Boulay and Claing, 2009).

As mentioned before, *ARF3*, *ARF4* and *ARF5* genes are three genes which have important functions. Until today, *ARF3*, *ARF4* and *ARF5* genes had been reported in human and other animals but the sheep *ARF3*, *ARF4* and *ARF5* genes have not been reported yet.

In present experiment, we will isolate the coding sequences of sheep *ARF3*, *ARF4* and *ARF5* genes based on the coding sequence information of *ARF3*, *ARF4* and *ARF5* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform some necessary sequence analysis

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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: The 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 -8°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 *ARF3*, *ARF4* and *ARF5* genes and tissue expression profile analysis.

Isolation of the sheep *ARF3*, *ARF4* and *ARF5* genes: The primers for sheep *ARF3* gene isolation were designed based on the coding sequence information of human *ARF3* gene and its highly homologous sheep EST sequences: DY513665 and EE747569. Similarly, the primers for sheep *ARF4* gene isolation were designed based on the coding sequence information from human *ARF4* gene and its highly homologous sheep EST sequences: DY485622 and DY489964. The primers for sheep *ARF5* gene isolation were designed based on the coding sequence information from human and mouse *ARF5* genes and their highly homologous sheep EST sequences: EE794299 and FE033388. 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 (1U 1 μL^{-1}) and 9.5 μL sterile water. The PCR program initially started with a 94 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 to terminate the reaction. These PCR products for sheep *ARF3*, *ARF4* and *ARF5* 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.

RT-PCR for tissue expression profile analysis: RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009; Liu, 2009). We selected the housekeeping gene *β-actin* (Accession No.: NM_001009784) as a positive control.

Table 1: Primers for sheep *ARF3*, *ARF4*, *ARF5* and *β-actin* genes and their annealing temperatures

Genes	Primer sequences	Ta/°C
ARF3	Forward: 5'-ATGGGTAACATCTTTGGA-3'	
	Reverse: 5'-TCACTTCTTGTTTTTGAGC-3'	50
ARF4	Forward: 5'-ATGGGCCTCACCATCTCC-3'	
	Reverse: 5'-TTAACGTTTTGAAAGCTC ATTT-3'	55
ARF5	Forward: 5'-ATGGGCCTCACCGTGTCC-3'	
	Reverse: 5'-CTAGCGCTTTGACAGCTCG-3'	62
β-actin	Forward: 5'-CTTGATGTCACGGACGATTT-3'	
	Reverse: 5'-CACGGCATTGTCACCAACT-3'	56

The primers of sheep *ARF3*, *ARF4* and *ARF5* 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 denaturation for 4 min, followed by 25 cycles of 94/50, Ta/50, 72/50 sec 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 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 *ARF3*, *ARF4* and *ARF5* genes: Through RT-PCR with pooled tissue cDNAs for sheep *ARF3*, *ARF4* and *ARF5* genes, the resulting PCR products were 546, 543 and 543 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.: FJ969413, FJ969400 and FJ969399). The sequence prediction was carried out using the GenScan software and results showed that the 546, 543 and 543 bp cDNA sequences represent three single genes which encoded 181, 180 and 180 amino acids, respectively. Finally, these three novel sheep genes were assigned to GeneIDs; 100302303, 100302304 and 100302305.

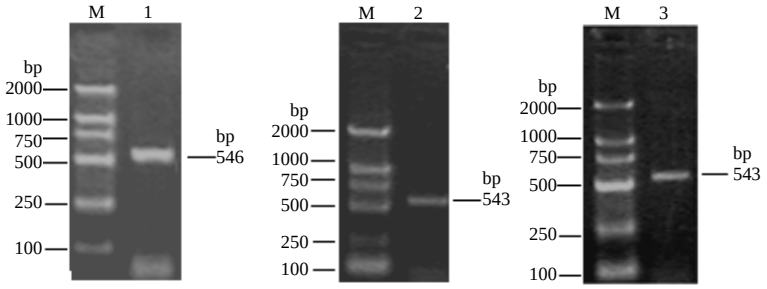

Fig. 1: RT-PCR results for sheep *ARF3*, *ARF4* and *ARF5* genes. M: DL2000 DNA Markers; 1: PCR product for sheep *ARF3* gene; 2: PCR product for sheep *ARF4* gene and 3: PCR product for sheep *ARF5* gene

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Sheep                MGNIFGNLLKSLIGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKN
Human                MGNIFGNLLKSLIGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKN
Pig                  MGNIFGNLLKSLIGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKS
Zebrafish            MGNIFGNLLKSLIGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKN
Atlantic salmon      MGNIFGNLLKSLLGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKN
African clawed frog  MGNIFGNLLKSLLGKKEMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKN
                     ***********:**********************************************.

Sheep                ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
Human                ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
Pig                  ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
Zebrafish            ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
Atlantic salmon      ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
African clawed frog  ISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAEDELRDAV
                     ************************************************************

Sheep                LLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQAPCATSGDGLYEGLDWLANQLKNK
Human                LLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLANQLKNK
Pig                  LLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLANQLKNK
Zebrafish            LLIFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLANQLKNK
Atlantic salmon      LLIFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLANQLKNK
African clawed frog  LLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLANQLKNK
                     **:*********************************.**********************

Sheep                K
Human                K
Pig                  K
Zebrafish            K
Atlantic salmon      K
African clawed frog  K
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Fig. 2: The alignment of the protein encoded by sheep *ARF3* gene and five other kinds ARF3 proteins

Further BLAST analysis of these proteins revealed that the sheep ARF3 protein has high homology with the ADP-Ribosylation Factor 3 (ARF3) proteins of five specie-human (Accession No.: NP-001650; 99%), zebrafish (Accession No.: NP-001003441; 99%), African clawed frog (Accession No.: NP-001086694; 99%), pig (Accession No.: NP-001153898; 99%) and Atlantic salmon (Accession No.: NP-001133306; 98%) (Fig. 2).

The sheep ARF4 protein has high homology with the ADP-Ribosylation Factor 4 (ARF4) proteins of 3rd species-cattle (Accession No.: NP-001029702; 100%), platypus (Accession No.: XP-001511094; 99%), green anole (Accession No.: XP-003217762; 99%), pig (Accession No.: NP-001041537; 99%), rabbit (Accession No.: XP-002713309; 98%), African clawed frog (Accession No.: NP-001082540; 98%), red jungle fowl (Accession No.: XP-001232784; 98%), mouse (Accession No.: NP-031505; 98%), zebrafish (Accession No.: NP-956170; 96%), human (Accession No.: NP-001651; 97%), Atlantic salmon (Accession No.: ACM08502; 92%), rainbow trout (Accession No.: NP-001154084; 92%) and caligus rogercresseyi (Accession No.: ACO10868; 89%) (Fig. 3).

The sheep ARF5 protein has high homology with the ADP-Ribosylation Factor 5 (ARF5) proteins of seven species-human (Accession No.: NP-001653; 100%), mouse (Accession No.: NP-031506; 100%), rat (Accession No.: NP-077063; 100%), gray short-tailed opossum (Accession No.: XP-001366170; 99%), chicken (Accession

```
Atlantic salmon           MGLTISSIFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Rainbow trout             MGLTISSIFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Zebrafish                 MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Sheep                     MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Cattle                    MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Platypus                  MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Pig                       MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Mouse                     MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Rabbit                    MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Red jungle fowl           MGLTISSLFSRLFGKNQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Human                     MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Green anole               MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
African clawed frog       MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Caligus rogercresseyi     MGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
                          *******:*******:**********************************

Atlantic salmon           FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Rainbow trout             FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Zebrafish                 FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Sheep                     FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Cattle                    FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Platypus                  FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Pig                       FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Mouse                     FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Rabbit                    FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Red jungle fowl           FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Human                     FNVETVEYKNICFTVWDVGGQDRIRPLWKHYFQNTQGLIFVVDSNDRERI
Green anole               FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
African clawed frog       FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERI
Caligus rogercresseyi     FNVETVEYRNICFTVWDVGGQDKIRPLWRHYFQNTQGIIFVVDSNDRERI
                          ********:**************:*****:********:************:

Atlantic salmon           AESAEELSKMLQEDELREAVLLVFANKQDLPNAMAVSDLTDKLGLQSLRS
Rainbow trout             AESAEELSKMLQEDELREAVLLVFANKQDLPNAMAVSDLTDKLGLQSLRS
Zebrafish                 AESAEELSKMLQEDELRDAVLLVFANKQDLPNAMAVSELTDKLGLQSLRS
Sheep                     QEGAEELQKMLQEDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Cattle                    QEGAEELQKMLQEDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Platypus                  QEGAEELQKMLQEDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQALRN
Pig                       QEGADELQKMLQEDELQDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Mouse                     QEGAAVLQKMLLEDELQDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Rabbit                    QEGAEELRKILQEDELQDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Red jungle fowl           EEAADELQKMLQEDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Human                     QEVADELQKMLLVDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
Green_anole               QEAAEELQKMLQEDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRN
African clawed frog       QEAAEELQKMLQEDELRDAVLLVFANKQDLPNAMAISEMTDKLTLQTLRN
Caligus rogercresseyi     FEAREELQKMLQEDELREAHLLVFANKQDLPEAMNAAELTDKMGLNDLRS
                           *    * *:*  ***::* **:*********:**  :::***: *: **.

Atlantic_salmon           RVWHVQATCATQGTGLYEGLDWLSNELSKR
Rainbow trout             RVWHVQATCATQGTGLYEGLDWLSNELPKR
Zebrafish                 RTWYVQATCATQGTGLYEGLDWLSNELSKR
Sheep                     RTWYVQATCATQGTGLYEGLDWLSNELSKR
Cattle                    RTWYVQATCATQGTGLYEGLDWLSNELSKR
Platypus                  RTWYVQATCATQGTGLYEGLDWLSNELSKR
Pig                       RTWYVQATCATQGTGLYEGLDWLSNELSKR
Mouse                     RTWYVQATCATQGTGLYEGLDWLSNELSKR
Rabbit                    RTWYVQATCATQGTGLYEGLDWLSNELSKR
Red jungle fowl           RTWYVQATCATQGTGLYEGLDWLSNELSKR
Human                     RTWYVQATCATQGTGLYEGLDWLSNELSKR
Green anole               RTWYVQATCATQGTGLYEGLDWLSNELSKR
African clawed frog       RTWYVQATCATQGTGLYEGLDWLSNELSKR
Caligus rogercresseyi     RKWYIQATCATQGNGLYEGLDWLSNELSKS
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Fig. 3: The alignment of the protein encoded by sheep *ARF4* gene and third other kinds of ARF4 proteins

No.: NP-990656; 98%), channel catfish (Accession No.: NP-001188198; 93%) and zebrafish (Accession No.: NP-954969; 93%) (Fig. 4). Based on the results of the alignment of ARF3, ARF4 and ARF5 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software (http://align.genome.jp/) as shown in Fig. 5-7. The phylogenetic analysis revealed that the sheep *ARF3* gene has a closer genetic relationship with the *ARF3* gene of human.

The sheep *ARF4* gene has a closer genetic relationship with the *ARF4* gene of cattle. The sheep *ARF5* gene has a closer genetic relationship with the *ARF5* genes of human, mouse and rat.

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Sheep_Human_Mouse_Rat          MGLTVSALFSRIFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Gray short-tailed opossum      MGLTVSALFSRIFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Chicken                        MGLTVSAIFSRIFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Channel catfish                MGLTISSIFGRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
Zebrafish                      MGLTISSLFGRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIG
                               ****:*::*.*:**************************************

Sheep_Human_Mouse_Rat          FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Gray short-tailed opossum      FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Chicken                        FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Channel catfish                FNVETVEYRNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
Zebrafish                      FNVETVEYKNICFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERV
                               ********:*****************************************

Sheep_Human_Mouse_Rat          QESADELQKMLQEDELRDAVLLVFANKQDMPNAMPVSELTDKLGLQHLRS
Gray short-tailed opossum      QESADELQKMLQEDELREAVLLVFANKQDMPNAMPVSELTDKLGLQNLRS
Chicken                        QESAEELQKMLQEDELRDAVLLVFANKQDMPNAMVVSELTDKLGLQALRS
Channel catfish                AESADELSKMLQEDELRDTVLLVFANKQDLPNAMPVSELTDKLGLQSLRS
Zebrafish                      AESAEELSKMLQEDELRDAVLLVFANKQDLPNAMAVSELTDKLGLQSLRS
                                ***:**.*********::**********:**** ********** ***

Sheep_Human_Mouse_Rat          RTWYVQATCATQGTGLYDGLDWLSHELSKR
Gray short-tailed opossum      RTWYVQATCATQGTGLYDGLDWLSHELSKR
Chicken                        RTWYVQATCATQGTGLYDGLDWLSHELSKR
Channel catfish                RTWYVQATCATQGTGLYEGLDWLSNELSKR
Zebrafish                      RTWYVQATCATQGTGLYEGLDWLSNELSKR
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Fig. 4: The alignment of the protein encoded by sheep *ARF5* gene and seven other kinds of ARF5 proteins

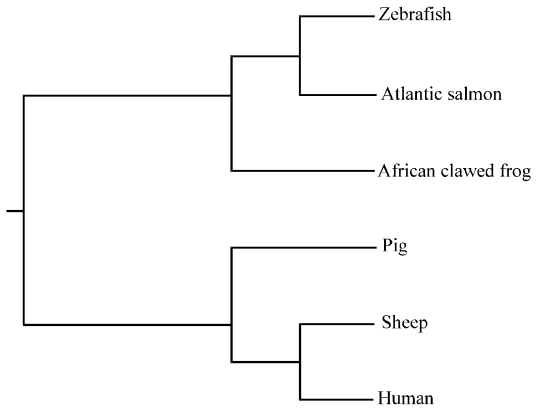

Fig. 5: The phylogenetic analysis for six kinds of *ARF3* genes

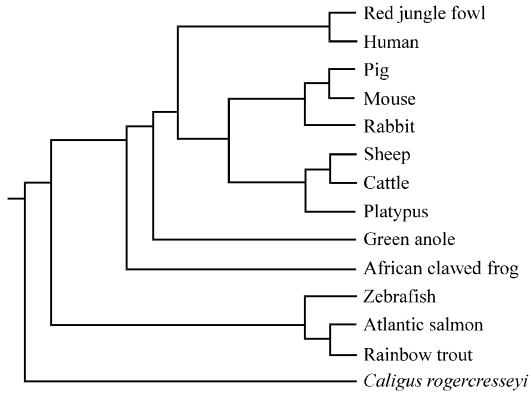

Fig. 6: The phylogenetic analysis for fourteen kinds of *ARF4* genes

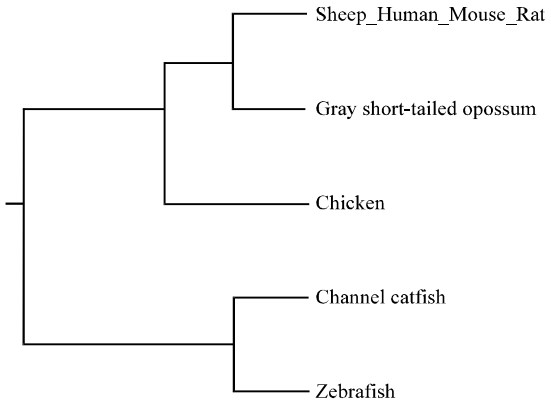

Fig. 7: The phylogenetic analysis for eight kinds of *ARF5* genes

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *ARF3* gene was highly expressed in liver, moderately expressed in skin, lung, muscle, spleen, heart and fat and weakly expressed in kidney and ovary. The sheep *ARF4* gene and *ARF5* gene displayed similar tissue expression distribution. They were both highly expressed in spleen, lung, muscle, kidney and ovary, moderately in skin, liver and heart and hardly expressed in fat (Fig. 8).

In the current study, we firstly get the coding sequences of sheep *ARF3*, *ARF4* and *ARF5* genes by RT-PCR. With the development of modern bioinformatics and specific sheep NCBI EST database established along with different convenient analysis tools, researchers can easily find the useful ESTs which were highly

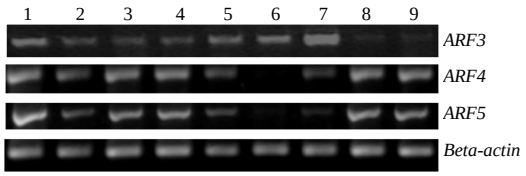

Fig. 8: Tissue expression distribution of sheep *ARF3*, *ARF4* and *ARF5* genes. The β-actin expression is the internal control.1: Spleen; 2: Skin; 3: Lung; 4: Muscle; 5: Heart; 6: Fat; 7: Liver; 8: Kidney and 9: Ovary

homologous to the coding sequences of human genes. Based on these sheep EST sequences, we 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 *ARF3*, *ARF4* and *ARF5* genes, it could be seen that this is an effective method to isolate some novel sheep genes.

Through sequence analysis, we found that the encoding protein of the sheep *ARF3*, *ARF4* and *ARF5* genes are highly homologous with ARF3, ARF4 and ARF5 proteins of human and some other animals. This implied that the *ARF3*, *ARF4* and *ARF5* genes were highly conserved in some species and the sheep *ARF3*, *ARF4* and *ARF5* genes will have similar functions as the *ARF3*, *ARF4* and *ARF5* genes of human and other animals. We also found that the sheep ARF3, ARF4 and ARF5 proteins do not show complete identity to human or other animals. This implied that the sheep *ARF3*, *ARF4* and *ARF5* genes will have some differences in functions to those of human or other mammals.

The phylogenetic analysis revealed that the sheep *ARF3* gene has a closer genetic relationship with the *ARF3* gene of human. This implied that we can use sheep as a model organism to study the human *ARF3* gene or use human as a model organism to study the sheep *ARF3* gene. Similarly, the sheep *ARF4* gene has a closer genetic relationship with the *ARF3* gene of cattle and the sheep *ARF5* gene has a closer genetic relationship with the *ARF5* genes of human, mouse and rat so that we can use cattle as a model organism to study the sheep *ARF3* gene and use human, mouse and rat as model organisms to study the sheep *ARF5* gene. From the tissue distribution analysis in the experiment, it can be seen that the sheep *ARF3*, *ARF4* and *ARF5* genes were 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 *ARF3*, *ARF4* and *ARF5* 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 *ARF3*, *ARF4* and *ARF5* genes were presented diversely in different tissues.

CONCLUSION

In this study, we 1st isolated the sheep *ARF3*, *ARF4* and *ARF5* genes and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into these novel sheep genes.

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