Molecular Cloning, Sequence Identification and Tissue Expression Profile of
Three Novel Sheep (Ovis aries) Genes-ZFAND5, ZGPAT and ZDHHC7

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Abstract: The complete coding sequences of three sheep genes ZFAND5, ZGPAT and ZDHHC7 were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The sheep ZFAND5 gene encodes a protein of 213 amino acids that shares high homology with the Zinc finger, AN1-type Domain 5 (ZFAND5) proteins of eight species; cattle (100%), pig (99%), human (99%), rabbit (99%), mouse (98%), giant panda (98%), gray short-tailed opossum (97%) and Northern white-cheeked gibbon (97%). The sheep ZGPAT gene encodes a protein of 513 amino acids that shares high homology with the Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT) proteins of seven species; cattle (97%), giant panda (84%), rabbit (99%), human (79%), rat (76%), mouse (77%) and chimpanzee (78%). The sheep ZDHHC7 gene encodes a protein of 308 amino acids that shares high homology with the Zinc finger, DHHC-type containing 7 (ZDHHC7) proteins of nine species; cattle (99%), dog (93%), pig (93%), human (93%), giant panda (93%), Northern white-cheeked gibbon (92%), mouse (92%), white-tufted-ear marmoset (92%) and rat (92%). Finally, these three novel sheep genes were assigned to GenelDs; 100302558, 100302028 and 100302557. The phylogenetic analysis revealed that the sheep ZFAND5, ZGPAT and ZDHHC7 genes all have a closer genetic relationship with the ZFAND5, ZGPAT and ZDHHC7 genes of cattle. Tissue expression profile analysis was also carried out and results demonstrated that sheep ZFAND5, ZGPAT and ZDHHC7 genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, ZFAND5, ZGPAT, ZDHHC7, tissue expression, China

INTRODUCTION

Zinc finger, AN1-type Domain 5 (ZFAND5) contains an A2O-like Zinc finger domain (ZnF-A2O) at its N terminus and an AN1-like domain (Znf-AN1) at its C terminus. Similar to A2O, ZFAND5 interacted with IKKgama, RIP and TRAF6 in co-immunoprecipitation experiments (Huang et al., 2004). Latest research demonstrated that ZFAND5 is a potent inhibitory factor for osteoclast differentiation and that the mechanism is unlikely due to direct attenuation of the NF-kappa B pathway (Hishiya et al., 2005, 2006). Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT), a Zinc finger and G-patch domain-containing protein, acts as a transcriptional repressor through the recruitment of the nucleosome remodelling and deacetylase complexes. Transcriptional target analysis revealed that ZGPAT regulates several cellular signalling pathways including EGFR pathways that are critically involved in cell proliferation, survival and migration. This gene inhibits cell proliferation and suppresses breast carcinogenesis and that ZGPAT depletion leads to a drastic tumour growth in vivo. ZGPAT is downregulated in breast carcinomas and that its level of expression is negatively correlated with that of EGFR. These indicate that ZGPAT is a novel transcription repressor and a potential tumour suppressor (Li et al., 2009).

Zinc finger, DHHC-type containing 7 (ZDHHC7) also, a Zinc finger domain-containing protein, appears to have a role in maintaining spermatid cell differentiated functions and mediating FSH actions (Chaudhary and Skinner, 2002).

As mentioned, ZFAND5, ZGPAT and ZDHHC7 genes are three genes which have important functions. Until today, ZFAND5, ZGPAT and ZDHHC7 genes had been reported in human and other animals but the sheep ZFAND5, ZGPAT and ZDHHC7 genes have not been reported yet.

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In present experiment, researchers will isolate the coding sequences of sheep ZFAND5, ZG PAT and ZDHHC7 genes based on the coding sequence information of ZFAND5, ZGPAT and ZDHHC7 genes from human or other mammals and their highly homologous sheep EST's 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 1st-strand cDNA samples were used to perform RT-PCR for the isolation of sheep ZFAND5, ZG PAT and ZDHHC7 genes and for the tissue expression profile analysis.

Isolation of the sheep ZFAND5, ZG PAT and ZDHHC7 genes: The primers for sheep ZFAND5 gene isolation were designed based on the coding sequence information of human ZFAND5 gene and its highly homologous sheep EST sequences; EE756605 and EE814109. Similarly, the primers for sheep ZGPAT gene isolation were designed based on the coding sequence information from human ZGPAT gene and its highly homologous sheep EST sequences; EE809332 and EE802245. The primers for sheep ZDHHC7 gene isolation were designed based on the coding sequence information from human and mouse ZDHHC7 genes and their highly homologous sheep EST sequences; EE832186 and EE809375. 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 MgCl₂, 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⁻¹) and 9.5 µL sterile water. 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: Primers for sheep ZFAND5, ZG PAT, ZDHHC7 and beta-actin genes and their annealing temperatures

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequences</th>
<th>Ta°C</th>
</tr>
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<tbody>
<tr>
<td>ZFAND5</td>
<td>Forward:5'-ATGGGTCACACGAGACTAAC-3',</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Reverse:5'-TTATATTCTCTGAAATTTTTCT-3'</td>
<td></td>
</tr>
<tr>
<td>ZG PAT</td>
<td>Forward:5'-ATGGGACCGAAGGAGACGGCTG-3',</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Reverse:5'-CTAGAACCTCAGTCATCGTT-3'</td>
<td></td>
</tr>
<tr>
<td>ZDHHC7</td>
<td>Forward:5'-AGGCCATCCTCAGAGAC-3',</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Reverse:5'-CCTACACTGGAAACCTCGGGG-3'</td>
<td></td>
</tr>
<tr>
<td>Beta-actin</td>
<td>Forward:5'-CTTGAATTCCACGAGATT-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Reverse:5'-CAAGGCTATTGCAGAACC-3'</td>
<td></td>
</tr>
</tbody>
</table>

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 ZFAND5, ZG PAT and ZDHHC7 genes which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR. 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 MgCl₂, 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.

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 ZFAND5, ZG PAT and ZDHHC7 genes: Through RT-PCR with pooled tissue cDNAs for sheep ZFAND5, ZG PAT and ZDHHC7 genes, the resulting PCR products were 642, 1542 and 927 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.: FJ937959, FJ943995)
and FJ937952). The sequence prediction was carried out using the GenScan software and results showed that the 642, 1542 and 927 bp cDNA sequences represent three single genes which encoded 213, 513 and 308 amino acids, respectively. Finally, these three novel sheep genes were assigned to GenIDs: 100302558, 100302028 and 100302557.

Further BLAST analysis of these proteins revealed that the sheep ZFAND5 protein has high homology with the Zinc finger, AN1-type domain 5 (ZFAND5) proteins of eight species; cattle (Accession No.: NP_001094515; 100%), pig (Accession No.: NP_001090975; 99%), human (Accession No.: NP_005998; 99%), rabbit (Accession No.: XP_002708253; 99%), mouse (Accession No.: NP_033577; 98%), Giant panda (Accession No.: XP_002914790; 98%), gray short-tailed opossum (Accession No.: XP_001365007; 97%) and Northern white-cheeked gibbon (Accession No.: XP_003267457; 97%) (Fig. 2).

The sheep ZGPAT protein has high homology with the Zinc finger CCCH-type with G patch domain-containing protein (ZGPAT) proteins of seven species;
cattle (Accession No.: NP_001019685; 97%), Giant panda (Accession No.: XP_002925738; 84%), rabbit (Accession No.: XP_002708808; 99%), human (Accession No.: AAH32612; 79%), rat (Accession No.: NP_001009656; 76%), mouse (Accession No.: NP_659143; 77%) and chimpanzee (Accession No.: XP_003317102; 78%) (Fig. 3).

The sheep ZDHHC7 protein has high homology with the Zinc finger, DHHC-type containing 7 (ZDHHC7) proteins of nine species-cattle (Accession No.: XP_874326; 99%), dog (Accession No.: XP_546796; 93%), pig (Accession No.: XP_003126871; 93%), human (Accession No.: NP_006210; 93%), Giant panda (Accession No.: XP_002913343; 93%), Northern white-cheeked gibbon (Accession No.: XP_003272546; 92%), mouse (Accession No.: NP_598728; 92%), white-tufted-eared marmoset (Accession No.: XP_002761264; 92%) and rat (Accession No.: NP_596885; 92%) (Fig. 4).

Based on the results of the alignment of ZFAND5, ZGPAT and ZDHHC7 proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software as shown in Fig. 5-7.
Fig. 4: The alignment of the protein encoded by sheep ZDHHC7 gene and ten other kinds of ZDHHC7 proteins. White-tufted-ear represents white-tufted-ear marmoset; Giant represents giant panda; Northern represents Northern white-cheeked gibbon.

The phylogenetic analysis revealed that the sheep ZFAND5, ZGPAT and ZDHHC7 genes all have a closer genetic relationship with the ZFAND5, ZGPAT and ZDHHC7 genes of cattle.

**Tissue expression profile:** Tissue expression profile analysis was carried out and results revealed that the sheep ZFAND5, ZGPAT and ZDHHC7 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, we firstly get the coding sequences of sheep ZFAND5, ZGPAT and ZDHHC7 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 genes. Based on these sheep EST sequences, researchers
can obtain the complete coding sequences of some novel sheep genes through the same experimental methods such as RT-PCR. From the clone and sequence analysis of sheep ZFAND5, ZGPAT and ZDHHC7 genes, it could be seen that this is an effective method to isolate some novel sheep genes. Through sequence analysis, researchers found that the encoding protein of the sheep ZFAND5, ZGPAT and ZDHHC7 genes are highly homologous with ZFAND5, ZGPAT and ZDHHC7 proteins of human and some other animals. This implied that the ZFAND5, ZGPAT and ZDHHC7 genes were highly conserved in some species and the sheep ZFAND5, ZGPAT and ZDHHC7 genes will have similar functions as the ZFAND5, ZGPAT and ZDHHC7 genes of human and other animals.

The researchers also found that the sheep ZFAND5, ZGPAT and ZDHHC7 proteins do not show complete identity to some animals. This implied that the sheep ZFAND5, ZGPAT and ZDHHC7 genes will have some differences in functions to those of other animals.

The phylogenetic analysis revealed that the sheep ZFAND5, ZGPAT and ZDHHC7 genes all have a closer genetic relationship with the ZFAND5, ZGPAT and ZDHHC7 genes of cattle. This implied that we can use cattle as a model organism to study the sheep ZFAND5, ZGPAT and ZDHHC7 genes or use sheep as a model organism to study the cattle ZFAND5, ZGPAT and ZDHHC7 genes.

From the tissue distribution analysis in this experiment it can be seen that the sheep ZFAND5, ZGPAT and ZDHHC7 genes were obviously differentially expressed in some tissues.

As researchers did not study functions at protein levels yet, there might be many possible reasons for differential expression of sheep ZFAND5, ZGPAT and ZDHHC7 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 ZFAND5, ZGPAT and ZDHHC7 genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep ZFAND5, ZGPAT and ZDHHC7 genes and performed necessary sequence and tissue expression profile analysis. This established the primary foundation for further insight into these novel sheep genes.

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REFERENCES


