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Keratin 26, a novel member of the goat type I keratin gene family

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ABSTRACT

Keratins and keratin-associated proteins make up the main structural components of the cashmere fiber. In this study, we constructed a skin cDNA library of Liaoning new-breeding cashmere goat during follicle anagen. A total of 1986 ESTs were sequenced from the skin library and the 1126 unisequences were homologous to functionally characterized or hypothetical proteins, including 14 unisequences that encoded structural proteins of cashmere fiber. We also isolated a full-length cDNA clone, which showed high homology with another known sequences, termed keratin 26 (*K26*). The *K26* protein consisted of 468 amino acids, exhibited a high amount of histidine, lysine and methionine, but low content of arginine, isoleucine and cysteine, and possessed a glycine-rich region. Additionally, *K26* contained a relatively high proportion of α-helix structures and showed a close relationship with *K25* and *K27*. RT-PCR analysis showed that *K26* were only present in goat skin. The above results point to *K26* being a novel cashmere goat keratin family member.

1. Introduction

The wool follicle is a complex mini-organ existing in goat skin and can be divided into primary and secondary follicle. After the wool follicles become mature and active, they vary with periods of growth (anagen), regression (catagen) and rest (telogen). In anagen, the outer root sheath grows downward, while the inner root sheath and hair shaft grow upward (Porter et al., 2004). Additionally, there are a number of genes that involved in the growth of wool follicle or the regulation of cashmere quality also highly expressed during anagen, such as keratin and keratin-associated protein genes. To date, however, the signal pathway related to cyclic changes in wool follicles is still unclear.

Keratin, which is a member of the intermediate filament proteins family, can be divided into type I (acidic) and type II (basic) keratin (Tanaka et al., 2007). Based on new consensus nomenclature for mammalian keratin, in addition to keratin pseudogene, type I keratin can be further classified into human type I epithelial keratin (*K9-K28*), human type I hair keratin (*K31-K40*), nonhuman type I epithelial and hair keratin (*K41-K70*). Type II keratin can also be further divided into human type II epithelial keratin (*K1-K8*, *K71-K80*), human type II hair keratin (*K81-K86*), nonhuman type II epithelial and hair keratin (*K87-K120*) (Schweizer et al., 2006). Recently, significant advances have been made toward the investigation of keratin family. Novel human type I keratin genes *K25irs1-K25irs4*, *Ka35*, *Ka36* and type II keratin genes *K1b*, *K6l*, *Kb20* were identified by Rogers et al. (2004, 2005). Langbein et al. (2003, 2006) found type I (*K25-K28*) and type II (*K6irs1-K6irs4*) keratin genes specifically expressed in inner root sheath of hair follicle. The location of the *K27*, *K31*, *K35*, *K38* and *K85* genes in Wiltshire sheep follicles were analyzed by Yu et al. (2009). However, only few of cashmere goat keratin genes were identified.

2. Materials and methods

2.1. Sample collection

Four adult cashmere goats (*Capra hircus*), including two bucks and two nannies were selected randomly from

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new-breeding cashmere goat farm in Liaoning province, China. During anagen, skin, heart, liver, spleen, lung and kidney were collected from the cashmere goats and snap frozen in liquid nitrogen. All experiments that involved in animals were approved by the animal care and use committee at the Life Science Department of Liaoning Normal University and also had approval and authorization from the Chinese Ministry of Agriculture.

2.2. Library construction and DNA sequencing

The cDNA synthesis and library construction were performed using total RNA isolated from skin during follicle anagen, with the SMART™ cDNA library construction kit (Clontech, USA) following the instruction provided by the manufacturer. The isolated cDNA was ligated into the pDNR-LIB vector, and used to transform DH10B *Escherichia coli* cells via electroporation, overnight at 37 °C. Randomly selected cDNA were sequenced with M13 primer (5′-GTAAAACGACGGCCAGT-3′) from the 5′-end on an ABI 3730-XL DNA sequencer.

2.3. EST clustering and sequence analysis

After quality and vector trimming, sequences containing more than 100 bp were assembled by CAP3 software, and viewed by Editplus. All unisequences were searched against the non-redundant nucleotide/protein database in GenBank and Swiss-Prot by BLASTN/BLASTX to identify DNA/protein homologues and possible functions. Open reading frame was identified with help of ORF finder in NCBI. Multiple alignment analysis of keratins was carried out using CLUSTALX program. Evolutionary tree was constructed using the MEGA program. Amino acid composition of keratins was shown using ProParam. Protein domain prediction was performed using the Pfam and PROSITE program. Secondary/tertiary structures of keratins were predicted using PredictProtein (Rost et al., 2004)/SWISS-MODEL program.

2.4. Semi-quantitative RT-PCR

RT-PCR was performed using total RNA isolated from skin, heart, liver, spleen, lung and kidney, following the manufacturer's instructions (TIANGEN, Peking, China). The following *K26* and *β-actin* specific primers were designed according to their sequence (GenBank accession no. GU084153, AF481159): *K26* (forward primer: 5′-CACTGGTCGGCTAACTGG-3′; GTGCTGTTGCCATGTCTT; reverse primer: 5′-CACGCCCTTCTGATTTGT-3′), *β-actin* (forward primer: 5′-CCAAAGCCAACCGTGAGAA-3′; reverse primer: 5′-AGAGGCGTACAGGGACAGCA-3′).

3. Results

3.1. Generating ESTs of goat skin cDNA library

A cDNA library was constructed using RNA isolated from the skin of Liaoning new-breeding cashmere goat. On average, the insert length of the cDNA clones was about 1 kb and the titer of the cDNA library was about 2×10^5 cfu/ml. A total of 2770 random cDNA clones were sequenced from 5′-end. Trimming of vector sequences, low quality, and polyA/T tails provided a data set of 1986 high quality ESTs.

Table 1
Keratin/keratin-associated protein genes and its number of EST sequences included in skin cDNA library.

Gene	Number of EST	Gene	Number of EST
Keratin 1	1	KAP6	19
Keratin 2	3	KAP8	7
Keratin 5	2	KAP9	7
Keratin 26	2	KAP11	35
Keratin 27	5	KAP13	2
Keratin 31	5	KAP16	17
KAP3	5	KAP26	3

3.2. Data analysis and functional classification

The remaining 1986 ESTs were aligned and assembled into 243 contigs and 883 singletons. About 64% of the resulting 1126 unisequences shared homology to functionally characterized or hypothetical proteins, including 14 unisequences that encoded structural proteins of cashmere fiber. Among them, *KAP6*, *KAP11* and *KAP16* had a relatively high amount of EST numbers (Table 1). The rest 36% unisequences had no homologous sequences in GenBank.

3.3. Characterization of K26

In this study, screening of the skin library led to the isolation of a full-length cDNA clone, 1822 bp in size, which possessed an intact open reading frame of 1407 bp encoding a protein of 468 amino acids. In addition to the first methionine codon, there were also 12 methionine codons occurred 587 bp, 35 bp, 59 bp, 8 bp, 80 bp, 113 bp, 20 bp, 56 bp, 86 bp, 26 bp, 74 bp, 218 bp downstream of the former one respectively and could represent an alternative site for the initiation of translation. Additionally, the cDNA clone contained a conventional polyadenylation site ca. 334 bp downstream of the coding region (Fig. 1). Homologous analysis showed that the nucleotide/protein sequence for this cDNA had high homology with keratin 26 of cattle, human, mouse and rabbit submitted to the GenBank database (Nos. NM001099096, BC132951, BC116672, and NM001008823). As the corresponding gene was designated *KRT26* (cDNA/protein termed *K26*), we have named our cDNA accordingly.

Amino acid analysis revealed that *K26* of Liaoning new-breeding cashmere goat contained a conservative domain of intermediate filaments which was homologous to that of cattle, human, mouse and rabbit (black boxes in Fig. 2). *K26* also possessed a glycine-rich region which had greatest homology with that of cattle and human *K26* (92% and 67%, respectively, underlined in Fig. 2). However, in contrast to most keratins of goat or sheep, the *K26* protein exhibited a high amount of histidine, lysine and methionine (2.8, 6.0 and 2.8 mol%, respectively), but a relatively low content of arginine, isoleucine and cysteine (4.7, 3.4 and 2.6 mol%, respectively, Table 2). In addition, the values of molecular weight and isoelectric point (51.749 kDa and 5.16, respectively) were relatively higher than that of