

Identification of a Differentially-expressed Gene in Fatty Liver of Overfeeding Geese

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Abstract In response to overfeeding, geese develop fatty liver. To understand the fattening mechanism, mRNA differential display reverse transcription PCR was used to study the gene expression differences between French Landes grey geese and Xupu white geese in conditions of overfeeding and normal feeding. One gene was found to be up-regulated in the fatty liver in both breeds, and it has a 1797 bp cDNA with 83% identity to chicken *SELENBP1*. The sequence analysis revealed that its open reading frame of 1413 bp encodes a protein of 471 amino acids, which contains a putative conserved domain of 56 kDa selenium binding protein with high homology to its homologues of chicken (95%), rat (86%), mouse (84%), human (86%), monkey (86%), dog (86%), and cattle (86%). The function of this protein has been briefly reviewed based on published information. In tissue expression analysis, the expression of geese *SELENBP1* mRNA was found to be higher in liver or kidney than in other tested tissues. The results showed that overfeeding could increase the mRNA expression level of geese *SELENBP1*.

Keywords fatty liver; mRNA differential display; *SELENBP1*; real-time quantitative PCR; Northern blotting

Geese have the ability to store energy in their liver through overfeeding to form fatty liver [1–5], which serves as a food product. Within 2–3 weeks, the goose liver accumulates large triglycerides in response to overfeeding, and its weight may increase up to 10-fold [4]. In the process of liver fattening, the metabolism of liver changes dramatically [1]. This change is different among different goose breeds, so their fatty liver performances are also different [5]. Landes, a well-known goose breed, can produce even large fatty liver (approximately 800 g). Other goose breeds show a relatively weak fatty liver performance. The weight of Xupu white geese is just approximately 400 g [6]. So far, the mechanism of fatty liver metabolism is poorly understood. Previous studies mainly focused on biochemical levels and confirmed that different levels of many biochemical indexes which were due to overfeeding, such as malic enzyme and isocitric dehydrogenase, had a

close relationship to fatty liver weight [1–5].

Because there are few reports on the molecular mechanism of goose fatty liver formation, we applied mRNA differential display reverse transcription PCR (DDRT-PCR) to study the expression profile between two goose breeds in different conditions of overfeeding and normal feeding. This technique has been proven to be an efficient method for isolating and characterizing the gene expression in different cell types [7–10].

A differentially-expressed gene identified as goose *SELENBP1* between the fatty liver and normal liver was discovered. Its complete cDNA sequence was isolated and its mRNA expression profile analyzed.

Materials and Methods

Animal feeding and sample preparation

Twenty French Landes grey geese and 20 Xupu white geese were allocated to two groups, respectively (5 males

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and 5 females in each group). All of the geese were hatched on the same day and bred under the same natural conditions. All of the geese had free access to food and water. At 100 d of age, one group of each breed began the period of overfeeding with a boiled, maize-based diet (5 meals per day, 400 g per goose per day, 16 d in total), and the other was still bred under the previous condition. After overfeeding, all of the geese were deprived of food for 8 h, and then killed by exsanguination. Liver samples (approximately 50 g) were immediately taken from the ventromedial portion of the main lobe (right lobe) of each goose. Tissue samples (approximately 30 g) were taken from the liver, spleen, ovary, uterus, muscular stomach, abdominal fat, and kidney from non-overfed Landes. These samples were immediately frozen in liquid nitrogen and stored at -80°C for later use.

Total RNA extraction, reverse transcription, and mRNA differential display

RNA was extracted according to Trizol reagent instructions (Invitrogen, Grand Island, USA). Total RNA samples were treated with RNase-free DNase I (Invitrogen) to digest the remaining genomic DNA. The quality and quantity of RNA was checked by formaldehyde-agarose gel electrophoresis and a spectrophotometer, respectively. Two micrograms of total RNA was used for reverse transcription with M-MLV (Invitrogen) according to the manufacturer's instruction.

The mRNA differential display was performed using liver RNA samples with a Delta differential display kit (Clontech, Palo Alto, USA). The primers were 5'-ATTAACCCTC ACTAAATGGAGCTGG-3' (arbitrary) and 5'-CATTATGCTGAGTGATATCTTTTTTTTTTAC-3' (anchored). The PCR was run as follows: 94°C for 5 min, 40°C for 5 min, 72°C for 5 min, 3 cycles, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, then 72°C extension for 10 min, and finally 4°C to terminate the reaction. The PCR products were separated on 8% denatured PAGE gel and the bands were visualized by silver staining.

5'- and 3'-rapid amplification of cDNA ends (RACE)

The significant band was cut down from the PAGE gel and put into a 0.5 ml tube. After adding 40 μl sterile water, the tube was put in a thermal cycler and boiled at 98°C for 15 min. The solution was used as template for re-amplification PCR using the original primer pair. The band was then recovered from the agarose gel using a UNI-Q-10 column DNA collection kit (Sangon, Shanghai, China). The purified PCR product was ligated into a pMD 18-T vector (TaKaRa, Tokyo, Japan) and transformed into

competent *Escherichia coli* electro-cells (DH5 α). Eight colonies were random selected for each transformation. The inserts were sequenced using the pMD 18-T vector universal primers.

The sequences obtained were used to search available chicken-expressed sequence tags using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>). The sequences sharing more than 80% identity were assembled to produce a contig, according to which one pair of primers was designed to perform PCR to obtain a longer sequence. The primers were 5'-CCCAACCTGAAAGACGAG-3' (SeF-1) and 5'-GGAGCTTCTGTGGCTCAC-3' (SeR-2).

The 5'- and 3'- RACE were performed using the SMART RACE cDNA amplification kit (Clontech). The corresponding RACE-ready first-strand cDNA was prepared from 1 μg of total RNA. The nest PCR amplification reactions were performed using the universal primer mix provided by the kit and gene-specific primers (GSP). The GSP primers were as follows: 5'-GTCACCACTGCCCAGACAGTGAGA-3' (5'-RACE GSP1), 5'-CGAGTCCGTTCCACATCCACCACA-3' (5'-RACE GSP2), 5'-CCTGATGGCCTGTTCTCGCTCA-3' (3'-RACE GSP1), and 5'-GGTGAATCAGTTCAGCCAGGACT-3' (3'-RACE GSP2).

Nucleotide sequence analysis

The sequence analysis and protein prediction were carried out using Lasergene 6 software and the National Center for Biotechnology Information tools (NCBI).

Real-time quantitative PCR

Real-time quantitative PCR was carried out to confirm the differential expression of DNA isolated from DDRT-PCR using the iQ5 apparatus (BioRad, Hercules, USA) with the following primers: 5'-ATCTCCAACACTCGCAAAC-3' (sense), and 5'-ATCTGGGTAGAACTGCTTGT-3' (antisense). The cDNA was prepared from individual livers of three geese in each group, and each cDNA sample had four repetitions. The PCR was conducted using SYBR green real-time PCR master mix kit (Toyobo, Tokyo, Japan). PCR was carried out according to the following conditions: 95°C for 30 s, 55°C for 30 s, and 72°C for 15 s, 40 cycles. Accurate amplification of the target amplicon was checked by performing a melting curve. Parallel amplification of the β -actin transcript was carried out as an endogenous control to normalize the expression data of the target gene transcript. The β -actin gene primers (according to M26111.1) were 5'-CCAGGCAAATCTATACACCTC-3' (forward) and 5'-CGACACGAAATCTGGAATG-3' (reverse). The results were calculated by an

optimized comparative Ct ($\Delta\Delta C_t$) value method. To ensure that no false positive PCR fragments were generated from pseudogenes in the contaminating genomic DNA, the primers were derived from different exons.

Northern blotting analysis

Northern blotting was prepared using the DIG-high prime DNA labeling and detection starter kit (Roche, Mannheim, Germany). Total RNA was isolated from liver samples of four groups with Trizol reagent (Invitrogen). The denatured total RNA (10 μ g) was separated by electrophoresis on 1.5% formaldehyde-agarose gel and transferred to a positively-charged nylon membrane (Hybond-N⁺; Amersham, Buckingham, UK) using electric blotting. A cDNA fragment (505 bp) was generated as a probe via reverse transcriptase PCR using forward (5'-GGATGTGCTCTGAGTGT-3') and reverse (5'-CACCTCTTCA-GTATCCA-3') primers. The β -actin probe (394 bp) was used as an endogenous control. After the RNA was UV-crosslinked (120 mJ/cm², 30s) and prehybridized (68 °C, 2 h), the filters were hybridized with probes at 68 °C for 20 h. Then nylon membranes were washed at the appropriate stringency to remove non-specific bindings. After that, the hybridized probes were immunodetected with anti-digoxigenin-AP for 1.5 h, and visualized with the colorimetric substrates nitroblue tetrazolium/BCIP for 16–24 h. Finally, the membrane was washed with 50 ml of sterile double distills water for 5 min to terminate the reaction.

Quantitative relative reverse transcriptase polymerase chain reaction

Quantitative relative reverse transcription PCR (RT-PCR) was performed to measure the expression pattern of the differentially-expressed DNA isolated from differential display PCR in other tissues. The β -actin gene was used as an endogenous control to regulate the tissue cDNA concentrations in order to eliminate the effects of the cDNA concentration. The number of PCR cycles was optimized to ensure the reaction was terminated before the platform phase. The gene specific primers were 5'-ATCTCC-AACACTCGCAAAC-3' (sense) and 5'-ATCTGGTGA-GAAGTCTTGT-3' (antisense), and the β -actin gene primers were described earlier. PCR was performed as follows: 94 °C for 3 min, followed by 24 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 15 s, then 72 °C extension for 10 min, and finally 4 °C to terminate the reaction.

Results

Identification of differentially-expressed transcript in geese liver in response to overfeeding and full-length cDNA cloning

The mRNA differential display was carried out to study the response of geese liver to overfeeding. Ninety primer pair combinations were used and approximately 800 bp cDNA fragments were analyzed after silver straining. The band pattern of the cDNA fragments amplified by one primer pair is shown in **Fig. 1**. To decrease the “false positive” rate, each primer pair was used three times to amplify three different cDNA samples reversed from the total RNA of each identical goose liver. The PCR products were separated on an 8% denatured PAGE gel. Only the reproducible and larger than 250 bp bands were selected. One hundred and forty putative, differentially-expressed DNA fragments were cloned. Eight clones were examined for each band obtained from the gel. These colonies were randomly selected from each transformation. Each of the colonies was sequenced and analyzed. One gene was confirmed as an up-regulated gene in the Landes fatty liver (LFL) and Xupu fatty liver (XpFL). In addition, its expression level was higher in Landes liver (LL) than that in Xupu geese (XpL). This gene was named *Gene 8216*.

Gene 8216 was cloned and sequenced as described above, and a 353-bp fragment was obtained [**Fig. 2(A)**]. By blasting in NCBI, this fragment showed 92% identities to chicken selenium binding protein 1 gene (*SELENBP1*; XM 423397.2). Then, the fragments of *Gene 8216* and

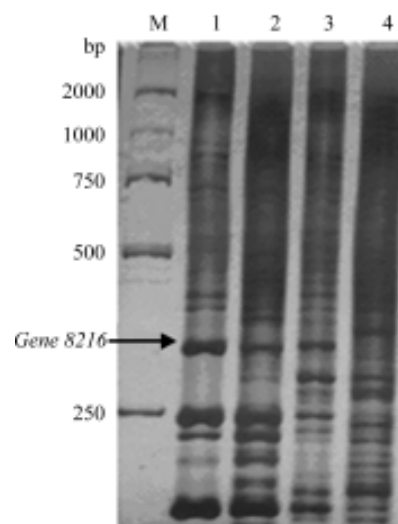


Fig. 1 Differential expression analysis of *Gene 8216* in different geese

M, DL2000 marker; 1, Landes fatty liver; 2, Landes liver; 3, Xupu goose fatty liver; 4, Xupu goose liver.

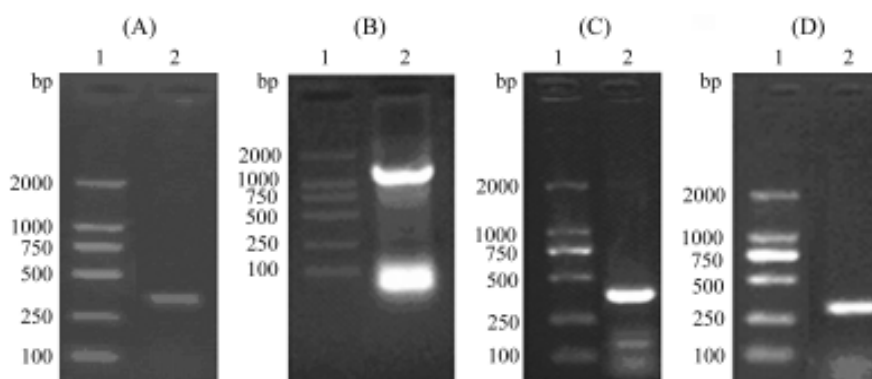


Fig. 2 cDNA cloning and rapid amplification of cDNA ends (RACE) for *Gene 8216*

(A) Re-amplification of the differential expression band in differential display reverse transcription PCR. (B) cDNA cloning PCR results. (C) 5'-RACE product. (D) 3'-RACE product. 1, DL2000 marker; 2, amplification product.

chicken *SELENBP1* were used as templates to perform PCR using specific primers (SeF-1 and SeR-1). A 1359-bp fragment was then obtained [Fig. 2(B)]. The first step of the nest PCR was carried out using the 5'-RACE GSP1 and 3'-RACE GSP1 primers, respectively. The PCR product was used as a template to perform the second step of nest PCR with the 5'-RACE GSP2 and 3'-RACE GSP2 primers. One fragment of 344 bp was obtained for 5'-RACE [Fig. 2(C)] and another fragment of 281 bp for 3'-RACE [Fig. 2(D)]. After assembling these sequences together, a 1797 bp complete cDNA sequence was finally obtained (Fig. 3).

Nucleotide sequence analysis

The complete cDNA sequence finally obtained was not homologous to any of the known geese genes and it was then submitted to Genbank (accession No. EF488993). By blasting in NCBI, this sequence shows high homology with *SELENBP1* mRNA of other species, such as chicken (83%), human (71%), rat (71%), and mouse (70%). The sequence prediction showed that the CDS length of this gene was 1416 bp (from 9 to 1424), encoding 471 amino acids. The coding region score was 1147, the probability of exon was 0.999, and the ploy(A) signal was from 1771 to 1776. The complete sequence of this gene and the encoded amino acids are shown in Fig. 3. The putative domain was identified as a 56-kDa selenium binding protein (SBP56) (Fig. 4), which is a conserved domain of *SELENBP1* among species. Further blasting in NCBI revealed that this protein shared high homology with *SELENBP1* of seven species: chicken (95%), rat (86%), human (86%), monkey (86%), dog (86%), cattle (86%), and mouse (84%) as shown in Fig. 5. Taking these results together, it is suggested that the *Gene 8216* is the goose

SELENBP1. The phylogenetic tree of goose *SELENBP1* and its homologues was also constructed as shown in Fig. 6. Geese and chicken were clustered into one category.

Confirmation of the differential expression of *SELENBP1*

The result of the real-time quantitative PCR is shown in Fig. 7. The expression levels were not considered detectable when the Ct value of *SELENBP1* exceeded 35 in the liver sample. The expression level was calculated as $2^{-\Delta\Delta Ct}$ to compare the relative expression. It revealed that the gene expression level was higher in the fatty liver (LFL and XpFL) than in the normal liver (LL and XpL), and Landes (LL and LFL) showed a higher expression than Xupu geese (XpL and XpFL). These outcomes confirmed the results obtained from the DDRT-PCR.

Northern blotting with an *SELENBP1* specific cDNA probe detected a single transcript approximately 1800 bp in size in each liver RNA (Fig. 8), which is consistent with the complete cDNA length of 1797 bp isolated using PCR-based approaches. Higher expression levels of *SELENBP1* were detected in the fatty liver (LFL and XpFL), and Landes (LL and LFL) showed higher expression than Xupu geese (XpL and XpFL). These coincided with the results of the real-time quantitative PCR and DDRT-PCR.

SELENBP1 expression profile in other tissues

The tissue expression pattern was examined in seven tissues of non-overfed Landes by RT-PCR. The results indicated that *SELENBP1* was differentially expressed in these tested tissues (Fig. 9). The mRNA expression level of *SELENBP1* is high in the liver and kidney, moderate in the spleen, and low in the ovary, uterus, muscular stomach, and abdominal fat.

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GAGCCAACATGGCAAAATGTGGAGCATGTGGTCCAGGATATGCGACTCCTCTGGATGCCATGAAAGGTCCCGTGAG
    M A K C G A C G P G Y A T P L D A M K G P R E
GAGATCTTGTACCTGCCATGTCATCTACAGAAACACTGGGATAGAGAAGCCTGACTACCTGGCCACTGTGGATGTTGAC
    E I L Y L P C I Y R N T G I E K P D Y L A T V D V D
CCCAGATCTCCDCACTACTGTCAGGTTCATCCATOGCCTGCCCATGCCAATCTTAAAGADGAGCTCCATCATTAGGG
    P R S P H Y C Q V I H R L P M P N L K D E L H H S G
TGGAAATGCTGTAGCAGCTGCTTTGGGGATGCTACAAAGAGAAGAAATCGTCTCATTCTACCAAGTCTGATCTCGTCT
    W N A C S S C F G D A T K R R N R L I L P S L I S S
CGCAITTTATGTGGTGGATGTGGGAACGGACTCGAAGCTCCTAGAATOCATAAGATTGTGGAGCCAGTGGAGCTGTC
    R I Y V V D V G T D S K A P R I H K I V E P V E L F
TGGAAAGGTAAATGTGGCTAATCCTCACACCTCTCACTGTCTGGGCAGTGGTGACATCTTAATCAATTGTTTGGGAGAT
    W K G N V A N P H T S H C L G S G D I L I N C L G D
CCTTCTGGCAATGGAAAAGGTGGCTTTATTTGCTCGATGGAGAGACCTTTGAAAGTAAAAGGAACTGGGAGAAAAGGG
    P S G N G K G G F I L L D G E T F E V K G N W E K G
GAGAAGGTCCCACCCCTTGGTTATGACTTCTGGTATCAGCCACGGCATAATGTCTGATGAGCACTGAGTGGGAAACC
    E K V P P L G Y D F W Y Q P R H N V L M S T E W G T
CCAAAAGTCTTGGCAGATGGGTTTAAACCAGCTGATGTAGAGAAAAGGCATTATGGCCGTCATATTAACGTGTGGGAC
    E K V P P L G Y D F W Y Q P R H N V L M S T E W G T
TGGAACTCTCATACTTACATTGAGAAAATGACTTAGGGAAGGACTCCATACCTTTGAAATCOGATTCCTCCACAAT
    W N S H T Y I Q K I D L G K D S I P L E I R F L H N
CCGGATGCTGCAGAAGGGTTCGTTGGATGTGCTCTGAGTAGTACAGTACATCGGTTTTACAAGACAGAGAAAGGAAAC
    P D A A E G F V G C A L S S T V H R F Y K T E K G N
TGGGCACAGAGAAAAGTGAATGAAGTACCCAGCAAGAAGGTGCAAGGATGGCTTCTCCCTGACATGCCTGGTCTTATT
    W A A E K V I E V P S K K V Q G W L L P D M P G L I
ACTGATATCCTCATCTCACTGGATGACAGGTTCCCTGTATTTGAGCAACTGGCTGCATGGAGACATCCGTCAGTATGAT
    T D I L I S L D D R F L Y F S N W L H G D I R Q Y D
ATCTCCAACACTCGCAAADCCAAAGCTOGTGGGTCAGGTGTTTCTGGGAGGCAGTATCATAAAAGGTGGACCTGTAAC
    I S N T R K P K L V G Q V F L G G S I I K G G P V T
GTAGTGAAGACAAAGGAGCTGCAGTGTACCCAGAGCCATTGTGATCAAAGGGAAGAGAGTGCAGGTGGACCTCAG
    V V E D K E L Q C Q P E P F V I K G K R V P G G P Q
ATGATTCAGCTTGTGGATGGGAAGAGATTGTATGTACCAGCTCTCTACAGTGGGTGGGACAAGCAGTCTTAC
    M I Q L S L D G K R L Y V T S S L Y S G W D K Q F Y
CCAGATCTTGTCAAAGAAGGCTCTGTTATGCTGCAGATTGATGTGGATACTGAAAGAGGTGGACTGAAAGTAAAAGAA
    P D L V K E G S V M L Q I D V D T E R G G L K V N E
AACTTCTAGTGGATTTGGGAAGGAACCTGATGGCCTGTCTCGCTCATGAGATTGTTATCCTGGGGAGACAGT
    N F L V D F G K E P D G P V L A H E I R Y P G G D S
ACCTCGGATATCTGGATTAAATGTTGCATGGAGCTGGTGTITTTTTTCATGTTGTTTGTITGTTTGTITTCCTC
    T S D I W I *
TCTCTCCTCCCTACCCCACTTCTGATTTTATACAGGTGAATCAGTTCAGCCAGGACTTGTCCGCTGCTAAATTA
    GAATTCATTAGGGTGAAGCACAGAAGCTCCATTTCTTCTCATTCTCTGAGGCAGCCAAAGTGAATCTTCTGAAACA
    TGATATACATCTCATTATTTCTTAATCTTTGCTTAAAGTCTGTGTAATCACTATTTACTGTAGGTTTTGAGGCCA
    AGCGCATCAATTTTTAAGGATTGTTGCCACGTTGTGGAGGAGAAGCTATCCCGAAAAA
    AAAAAA
    
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Fig. 3 cDNA sequence of *Gene 8216* goose *SELENBP1* and the predicted amino acid sequence
* indicates the stop codon.



Fig. 4 Putative domain of the protein encoded by *Gene 8216* goose *SELENBP1*
SBP56, 56 kDa selenium binding protein.

Dog	MNSAKAKKQPSEATPCRTAVPTGLTAARLADGAMQSLPRITTAQLL3LLTPGSPVRIKAYO	60
Cattle	-----	
Human	-----	
Monkey	-----	
Rat	-----	
Mouse	-----	
Goose	-----	
Chicken	-----	
Dog	EAAWTFSSAIQVGLASSPRSESKGRKTRSVPLGVEISSAVAKQVVPVQAQAAVQAHWLPPGK	120
Cattle	-----	
Human	-----	
Monkey	-----	
Rat	-----	
Mouse	-----	
Goose	-----	
Chicken	-----	
Dog	HGAAVLIAPARGLLPGAHSTQCCKGCGPGYSPLEAHKGPREEIVYLPFCIYRMTGTEADPY	180
Cattle	-----HATKCGKCGPGYSPLEAHKGPREEIVYLPFCIYRMTGTEADPY	43
Human	-----HATKCGKCGPGYSPLEAHKGPREEIVYLPFCIYRMTGTEADPY	43
Monkey	-----HATKCGKCGPGYSPLEAHKGPREEIVYLPFCIYRMTGTEADPY	43
Rat	-----HATKCTKCGPGYATPLEAHKGPREEIVYLPFCIYRMTGTEADPY	43
Mouse	-----HATKCTKCGPGYATPLEAHKGPREEIVYLPFCIYRMTGTEADPY	43
Goose	-----HAKCGACGPGYATPLDAHKGPREEIVYLPFCIYRMTGTEADPY	42
Chicken	-----HAKCGACGPGYATPLDAHKGPREEIVYLPFCIYRMTGTEADPY	42
Dog	LATVDIDPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	240
Cattle	LATVDVMPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	103
Human	LATVDVDPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	103
Monkey	LATVDVDPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	103
Rat	LATVDVDPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	103
Mouse	LATVDVDPKSPQYCVIHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	103
Goose	LATVDVDPKSPHYCQVHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	102
Chicken	LATVDVDPKSPHYCQVHRLLPFPNLKDELHHSGMNACSSCFGDSIKSRTKLLPLSLISSR	102
Dog	IYVVDVGSDFRAPKLIHKVIEAEDIHAKCGLGVVHTSHCLASGEVMI STLGDPSKGNKGGF	300
Cattle	YVVVDVATEPRAPKLIHKVIEAEDIHAKCGLGVVHTSHCLASGEVMI STLGDPSKGNKGGF	163
Human	IYVVDVGSDFRAPKLIHKVIEPKDIHAKCELAFLHTSHCLASGEVMI STLGDPSKGNKGGF	163
Monkey	IYVVDVGSDFRAPKLIHKVIEPKDIHAKCELAFLHTSHCLASGEVMI STLGDPSKGNKGGF	163
Rat	IYVVDVGSDFRAPKLIHKVIEPNEIHAACNLGNLHTSHCLASGEVMI STLGDPSKGNKGGF	163
Mouse	IYVVDVGSDFRAPKLIHKVIEASEIQAACNVSSLHTSHCLASGEVMI STLGDPSKGNKGGF	163
Goose	IYVVDVGTDSKAPRIHKVIEPVELFVKGNVANPHTSHCLASGSDILINCLGDPSPGNKGGF	162
Chicken	IYVVDVGTDSKAPRIHKVIEPVELFVKGNVANPHTSHCLASGSDILINCLGDPSPGNKGGF	162
Dog	VLLDGETFEVKGTWEQPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	360
Cattle	VLLDGETFEVKGTWEQPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	223
Human	VLLDGETFEVKGTWEQPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	223
Monkey	VLLDGETFEVKGTWEQPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	223
Rat	VLLDGETFEVKGTWEKPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	223
Mouse	VLLDGETFEVKGTWEKPGGAAPHGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	223
Goose	ILLDGETFEVKGNMEKGGKPPFGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	222
Chicken	VLLDGETFEVKGNMEKGGKPPFGYDFWYQPRHNVMI STEWAAPNVFRDGFNPADVEAGL	222
Dog	YGSHLHVVDWQRHEIIQTLQDGLIPLERFLHNPDAAGPFVGCALSSTIQRFYKNGGG	420
Cattle	YGQHLVYVDWQRHERVOTLTLQDGLIPLERFLHNPDAAGPFVGCALSSNIQRFYKNGGG	283
Human	YGSHLVYVDWQRHEIVOTLTLQDGLIPLERFLHNPDAAGPFVGCALSSTIQRFYKNGGG	283
Monkey	YGSHLVYVDWQRHEIVOTLTLQDGLIPLERFLHNPDAAGPFVGCALSSTIQRFYKNGGG	283
Rat	YGSHLVYVDWQRHEIIQTLQMDGLIPLERFLHNPDAAGPFVGCALSSNIQRFYKNGGG	283
Mouse	YGSRIYVVDWQRHEIIQTLQMTDGLIPLERFLHNPDAAGPFVGCALSSNIQRFYKNGGG	283
Goose	YGRHNVVDWNSHTYIQKIDLGKDSIPLERFLHNPDAAGPFVGCALSSVHRFYKTEKG	282
Chicken	YGRHNVVDWNSHTYIQAIDLGKDSIPLERFLHNPDAAGPFVGCALSSVHRFYKTEKG	282
Dog	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	480
Cattle	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	343
Human	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	343
Monkey	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	343
Rat	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	343
Mouse	TWSVEKVIQVFPKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISDPORP	343
Goose	NWAAEKVIEVPSKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISNTRKP	342
Chicken	DWAAEKVIEVPSKVKVGMWLLPEMPLGITDILSLDDRFLYFSSNWLHGDLRQYDISNTRKP	342
Dog	RLTGQIFLGGSIIVKGGFPVOVLEDEELKSOPEPLVVKGKRVVAGGPOHIQLSLDGKRLVYTT	540
Cattle	RLVGOIFLGGSIIVKGGFPVOVLEDEELKSOPEPLVVKGKRVVAGGPOHIQLSLDGTRLYVTT	403
Human	RLTGQIFLGGSIIVKGGFPVOVLEDEELKSOPEPLVVKGKRVVAGGPOHIQLSLDGKRLVYTT	403
Monkey	RLTGQIFLGGSIIVKGGFPVOVLEDEELKSOPEPLVVKGKRVVAGGPOHIQLSLDGKRLVYTT	403
Rat	RLAGQIFLGGSIIVKGGFPVOVLEDEELKSOPEPLVVKGKRVVAGGPOHIQLSLDGKRLVYTT	403
Mouse	KLVGQVFLGGSIIVKGGFPVTVVDEKELQCOPEPFVVKGKRVVAGGPOHIQLSLDGKRLVYTS	402
Goose	KLVGQVFLGGSIIVKGGFPVTVVDEKELQCOPEPFVVKGKRVVAGGPOHIQLSLDGKRLVYTS	402
Chicken	KLVGQVFLGGSIIVKGGFPVTVVDEKELQCOPEPFVVKGKRVVAGGPOHIQLSLDGKRLVYTS	402
Dog	SLYSANDKQFFPDLIREGSMVLQIDVDTVRGGLKLNPNFLVDFGKEPLGPAALAEHLYRYPG	600
Cattle	SLYSANDKQFFPDLIREGSMVLQIDVDTVRGGLKLNPNFLVDFGKEPLGPAALAEHLYRYPG	463
Human	SLYSANDKQFFPDLIREGSMVLQVVDTVRGGLKLNPNFLVDFGKEPLGPAALAEHLYRYPG	463
Monkey	SLYSANDKQFFPDLIR-WPCLNGPEACVSSVYALY--LVQEKAKEASGQVNVTSSSHQ--	458
Rat	SLYSANDKQFFPDLIREGSMVLQIDVDTVRGGLKLNPNFLVDFGKEPLGPAALAEHLYRYPG	463
Mouse	SLYSANDKQFFPDLIREGSMVLQIDVDTVRGGLKLNPNFLVDFGKEPLGPAALAEHLYRYPG	463
Goose	SLYSGNDKQFFPDLVKEGSMVLQIDVDTVRGGLKVNPNFLVDFGKEPDGPAALAEHLYRYPG	462
Chicken	SLYSGNDKQFFPDLVKEGSMVLQIDVDTVRGGLKVNPNFLVDFGKEPDGPAALAEHLYRYPG	462
Dog	GDSSDIWI 609	
Cattle	GDSSDIWI 472	
Human	GDSSDIWI 472	
Monkey	-----	
Rat	GDSSDIWI 472	
Mouse	GDSSDIWI 472	
Goose	GDSTSDIWI 471	
Chicken	GDSTSDIWI 471	

Fig. 5 Alignment of the protein encoded by gene 8216 for goose SELENBP1 and seven other kinds of SBP56 homologues

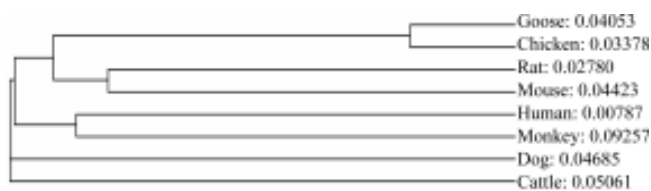


Fig. 6 Phylogenetic tree of eight kinds of 56 kDa selenium binding protein SELENBP1

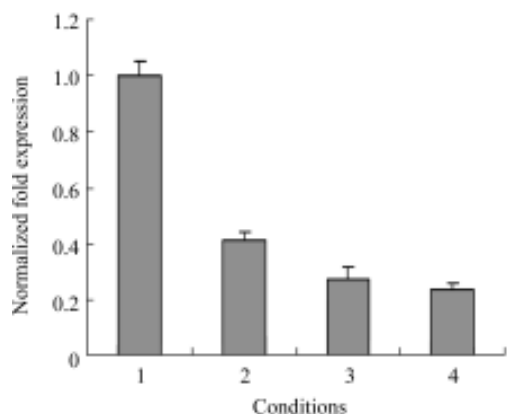


Fig. 7 Relative expression abundance of *Gene 8216* goose *SELENBP1* in Landes and Xupu geese between liver and fatty liver by real-time quantitative PCR

Each condition was normalized by β -actin. Values are shown as the mean \pm SE. Data were calculated by an optimized comparative Ct ($\Delta\Delta$ Ct) value method. 1, Landes fatty liver; 2, Landes liver; 3, Xupu goose fatty liver; 4, Xupu goose liver.

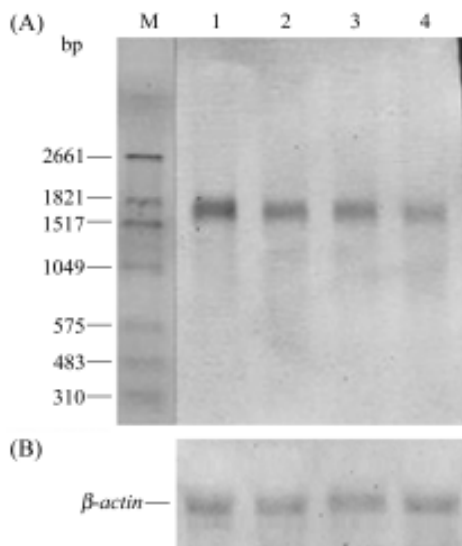


Fig. 8 Length of goose *SELENBP1* mRNA and expression abundance of goose *SELENBP1* mRNA in Landes and Xupu geese between liver and fatty liver determined by Northern blotting

(A) Northern blotting analysis of *SELENBP1*. M, RNA size marker, 1, Landes fatty liver; 2, Landes liver; 3, Xupu goose fatty liver; 4, Xupu goose liver. (B) Northern blotting analysis of β -actin gene.

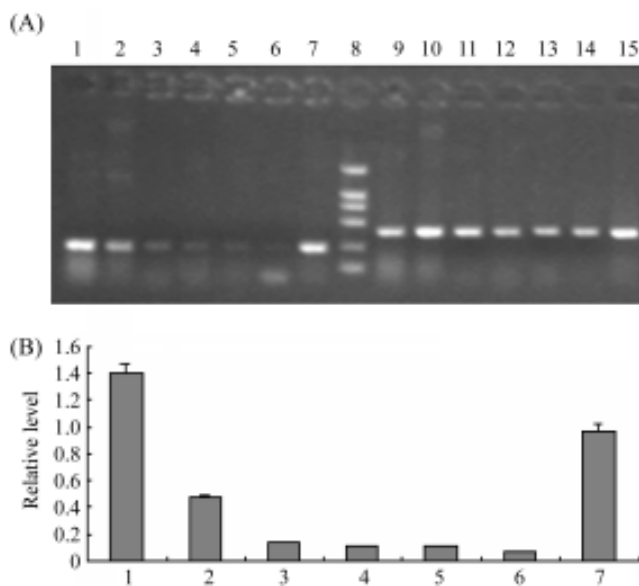


Fig. 9 Expression analysis of *SELENBP1* in multiple tissues by reverse transcription PCR (RT-PCR)

(A) Semiquantitative RT-PCR analysis of *selenbp1* in tested tissues. 1, liver; 2, spleen; 3, ovary; 4, uterus; 5, muscular stomach; 6, abdominal fat; 7, kidney; 8, DL2000 marker; 9–15, $\hat{\alpha}$ -actin expressed in 1–7 tissues, respectively. (B) Ratio of *SELENBP1* to $\hat{\alpha}$ -actin.

Discussion

One up-regulated gene, *Gene 8216*, in the fatty liver of geese due to overfeeding was identified. The sequence of *Gene 8216* is closely related to the chicken *SELENBP1* gene, which encodes SELENBP1 that contains a conserved domain of SBP56. SBP56 was originally discovered as a cytosolic protein that binds selenium [11], which has no Se-cysteine [12] and is highly conserved in many species [12,13]. This protein is highly expressed in the liver, lung, colon, and prostate, and its expression level is correlated with a low frequency of cancer in these tissues [14]. Up till now, the exact physiological function of this protein is still poorly known. Previous reports suggested that SBP56 might be a transport protein [15–17], and had confirmed that SBP56 participated in the late stages of intra-Golgi protein transport [18].

Goose *SELENBP1* was up-regulated in the fatty liver in the two breeds (LFL and XpFL), which may reveal that the expression level of this gene can be changed in response to overfeeding. *SELENBP1* is highly expressed in the rat liver after given a toxic coplanar polychlorinated biphenyl [19–22]. These results indicated that the *SELENBP1* gene could be regulated by offering some specific materials. In the process of overfeeding, the goose liver accumulated

large triglycerides and the liver cell showed hypertrophy [1–5]. This is a natural adaptability change of birds. In addition, with the liver fattening, the capability of antioxidation in the liver and other biological functions should be adjusted accordingly, and some mechanisms should exist to avoid the liver cell malignant denaturation. SBP56 has been proven to have these related biological functions in organisms as antioxidation [23,24] and depression malignant changes of cells [14,25]. Furthermore, we found that the expression profile of the goose *SELENBP1* gene differed between the two breeds. The expression level of this gene in the Landes liver was almost 2-fold higher than that in the Xupu geese liver. Moreover, the variation of this gene expression level between the fatty liver and normal liver in Landes was larger than that in Xupu geese, which revealed that the goose *SELENBP1* gene in the Landes liver was more susceptible to overfeeding. Previous reports had confirmed that fatty liver performance was different among goose breeds [5]. The different sensitivity of the goose *SELENBP1* gene to overfeeding may partially account for the difference of fatty liver performances among goose breeds.

In this study, we first obtained the complete cDNA sequence of the goose *SELENBP1* gene and discovered that it was involved in goose liver fattening events, but the exact function of *SELENBP1* in the fatty liver and the mechanism of *SELENBP1* regulation in response to overfeeding still remain unclear.

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