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Cloning and expression profile of *PID1* in Yak

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Abstract

To elucidate the role of phosphotyrosine interaction domain containing 1 (*PID1*) gene in yak (*Bos grunniens*), the *PID1* gene of Jiulong yak was cloned by RT-PCR and analyzed by bioinformatics method. The tissue and temporal expression profile of *PID1* gene of Jiulong yak were detected using semi-quantitative RT-PCR and fluorescence quantitative PCR, respectively. The results showed that the length of Jiulong yak *PID1* gene cDNA was 776 bp (GenBank accession number: KC184121) with an ORF of 612 bp which encodes 203 amino acids. The deduced *PID1*amino acid sequence of Jiulong yak shares a high homology with that of cattle, goat, pig, human, pygmy chimpanzee, mouse, rat, chicken and african clawed frog (89%-100%), while a lower homology with that of zebrafish (71%). Semi-quantitative RT-PCR analysis showed that the *PID1* gene expression was observed in heart, liver, spleen, kidney, longissimus muscle and fat of Jiulong yaks. The mRNA level of *PID1* in longissimus muscle, heart and liver was significantly higher than that in other studied tissues ($P<0.05$). The expression levels of *PID1* gene in longissium muscle of 0.5 and 9-year Jiulong yaks were significantly higher than that in those yaks at the age of 3.5 - 5.5 years old ($P<0.05$). These results suggest that PID1 may play an important role in the regulation of meat quality of yak.

Keywords

PID1, Jiulong Yak, Gene Clone, Tissue Distribution, Temporal Expression

1. Introduction

Yak is one of the most remarkable livestock distributed in the area of Central Asia highlands at altitudes ranging from 2500 to 5500m where few other domestic animals can survive [1]. Yak is important for the herdsmen living in the cold high altitude area because it provides meat, milk, wool, fur and other products [2]. Among these products, yak meat is of good quality with a fine texture, high protein, rich in amino acids, and low fat content. Also, it lacks anabolic steroids and other drugs [3]. Therefore, there has been an increasing focus on yak meat in recent years. The meat quality is reflected in the ratio of its protein to fat content [4]. Studies on the mechanism of fat deposition may help better breeding strategies to improve yak meat quality.

Phosphotyrosine interaction domain containing 1 *(PID1)*, also known as NYGGF4, was firstly cloned from abdominal subcutaneous fat of obese subjects using suppression subtractive hybridization (SSH) and found that it was expressed primarily in adipose tissue, heart, and skeletal muscle[5]. Amino acid sequence analysis revealed a phosphotyrosine-binding (PTB) domain in PID1 [6]. This domain can serve as an adaptor or scaffold for the signaling complexes involved in a wide range physiological processes including neural development, tissue homeostasis, cell growth [7] and adiposeness [8]. In addition, the protein was located in a trimeric complex with two other membrane proteins: cubilin and low-density lipoprotein (LDL) receptor-related protein 1 (LRP1), also suggesting that PID1 might be involved in lipid transport and cellular signal transduction [9]. Further studies indicated that PID1 may be related to fat deposition and may regulate adipocyte growth and development as evidenced by dramatically increasing the proliferation of 3T3-L1 pre-adipocytes and that knockdown of PID1 improved

mitochondrial function in 3T3-L1 adipocytes [10, 11, 12]. Whereas PID1 hasn't been characterized in yak and the role of it still hasn't been defined too. Therefore, we cloned *PID1* gene and established its expression profile in yak.

2. Materials and Methods

2.1. Animals and Sample Collection

Healthy Jiulong yaks were slaughtered at 0.5 years, 3.5-5.5 years and 9 years old (n=5). Heart, liver, spleen, kidney, longissimus muscle and fat samples were harvested and frozen in liquid nitrogen jars for total RNA extraction. Animal studies were approved by the Southwest University for Nationalities Institutional Committee for the Care and Use of Animals.

2.2. Cloning of *PID1* Gene

About 100 mg liver sample was collected and triturated in liquid nitrogen. Total RNA was extracted with Trizol reagents according to the manufacturer's instructions (TaKaRa, Dalian, China). First strand cDNA was synthesized from 2 μg of purified total RNA in a reverse transcription (RT-PCR) system (TaKaRa), as described in the product protocol. A pair of homologous primers (Table 1) was designed with Primer Premier 5.0 software based on cattle *PID1* gene sequence (GenBank: NM_001079584.2).With the primers, *PID1* cDNA was amplified by PCR using the first strand cDNA as template. The PCR started with pre-denaturation at 94 °C for 3 min, followed by 38 cycles (94 °C for 30s; 58.4 °C for 45s; 72 °C for 45s), and ended with a final extension at 72 °C for 10min. The PCR products were detected by 1 % agarose gel electrophoresis, and recovered with an agarose gel DNA extraction Kit (Shanghai Biotechnology Co., China).The DNA fragment were cloned by pMD19-T vector (TaKaRa), transformed into E.coli DH5α and sequenced (Shanghai Sangon Biological Engineering Technology Co. Ltd., Shanghai, China).

2.3. Bioinformatics Sequence Analysis

ORF of Jiulong yak gene was determined by the ORF finder on NCBI website (http://www.ncbi.nlm.nih.gov). The isoelectric point and molecular weight of the protein deduced from the nucleotide sequence were analyzed by ExPASy (http://www.expasy.org/tools). The conservative domain was predicted by NCBI tools (http://www.ncbi.nlm.nih.gov/Structure/cdd /wrpsb.cgi). Signal peptide was identified by Signal P4.0 (http://www.cbs.dtu.dk/services/SignalP/)[13] (Petersen et al.2011), and amino acid sequence homology of PID1 protein sequences from related species was established with the BioEdit 5.0.6 software version[14](Hall,2001), phylogenic tree was constructed by MEGA 6.0 (Tamura et al.,2013)[15].

2.4. Semi-Quantitative RT-PCR

Total RNA was extracted from the heart, liver, spleen, kidney, longissimus muscle and fat of 3.5-5.5-aged Jiulong yaks as above, respectively. The primers (table 1) were designed according to the characterized yak *PID1* gene sequence and PCR amplification procedure was as followed: pre-denatured at 94°C for 2min, then 33 cycles of amplification at 94°C for 30s, 57.5C for 30s and 72°C for 30s, followed by a final extension at 72°C for 10 min. The PCR fragments were separated by 1% agarose gel electrophoresis and analyzed with Quantity One software (Bio-Rad, Hercules, CA, USA). Yak β-actin (primers in table 1) was used as loading control.

Table 1. *Primers for cloning and detection*

Names	Sequences	Annealing temperature(℃)	Utilizations
PID1-F1 *PID1*- R1	5'- GCCCATCGACAGAGTCTTGC-3' 5'-TCGCTTATCCGTCTATCATCTTG -3'	58.2	For cloning of *PID1* cDNA
PID1-F2 *PID1*- R2	5'- GAAATCCGACCATTCCAAGT -3' 5'- TCTGGTAGGACAGGTCATCATT-3'	57.5	For SQ RT-PCR and qPCR
β-actin-F *β-actin*-R	5'- CCCATCTA TGAGGG GTACGC-3' 5'- CCTTGATGTCACGGACGATTT -3'	54	loading control

2.5. Real Time Quantitative RT-PCR

Total RNA was prepared from longissimus muscle of 0.5, 3.5-5.5 and 9-aged Jiulong yaks as above, respectively. The real time quantitative PCR primers (table 1) were designed also based on the characterized yak *PID1* gene sequence. The amplification mixture contained 10 μl SYBR® Premix Ex TaqTM (2×) (TaKaRa Biotechnology (Dalian) Co., Ltd.), 1 μl of RT reaction mix, 0.5 μl of 10 mmol/L each of primers and add ddH2O to 20 μl. The amplification was carried out as follows: pre-denatured at 94°C for 1min, then 45 cycles of amplification at 95 °C for 30 s, 57.5/54 °C for 30 s and 72 °C for 30 s.

2.6. Statistical Analysis

Data were analyzed using SPSS13.0 and showed as mean±SEM. The developmental pattern difference of *PID1* was assayed by one-way ANOVA, and significance level was set at $P<0.05$. The threshold cycle was analyzed using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001)[16].

3. Results

3.1. Molecular Characteristics of *PID1* Gene in Jiulong Yak

A 776 bp fragment of yak *PID1* gene was obtained by

RT-PCR (GenBank No. KC184121, Fig. 1). This fragment covers an ORF of 612bp from positions 97 to 708 in the nucleotide sequence encoding a protein containing 217 amino acids with a PTB motif (Fig. 2 and Fig.3). The predicted molecular weight of yak PID1 protein was 23.09 kDa, the iso-electric point was 6.25, and no signal peptide was found with Signal P 4.0 analysis. The deduced amino acid sequence of yak PID1 was 100 %, 99 %, 98 %, 98 %, 98 %, 97 %, 97 %, 92 %, 89 % and 71 % identical to cattle, goat, pig, human, pygmy chimpanzee, mouse, rat, chicken, African clawed frog and zebrafish, respectively. The highest homology was with cattle and the lowest homology was with bony fishes. The phylogenetic tree was constructed according to the deduced yak PID1 and the PID1 sequences from other species (Fig. 4).

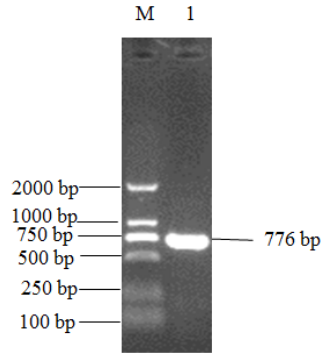

Fig 1. *Yak PID1 cDNA in agarose gel electrophoresis. RT-PCR was used to amplify PID1 gene from liver of Jiulong yak. The target fragment was 776 bp.1.PCR amplification product; 2.M.DNA marker DL 2000.*

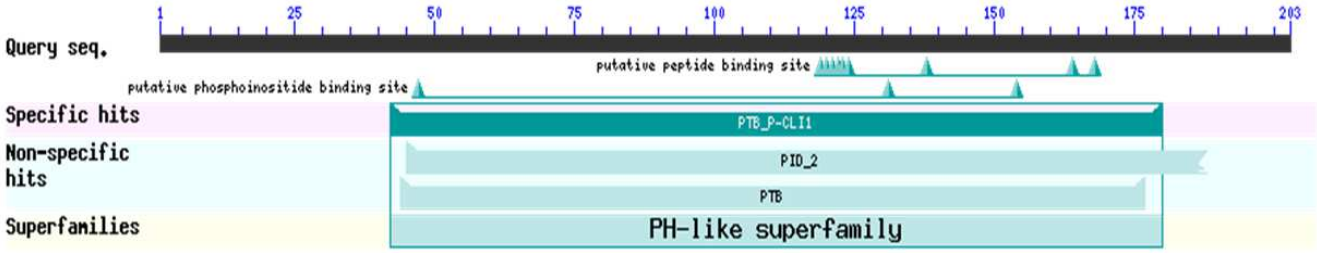

Fig 2. *Prediction of biological function of the deduced amino acid sequence of Jiulong yak PID1.*

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GCCCATCGACAGAGTCTTGCCAGCGTCTCCAGTGCCCAACCCCGCGGCTGGAAGATGTGG 60
CAGCCGGCCACGGAGCGCCTGCAGCACTTTCAGACCATGCTGAAGTCTAAGTTGAATGTC 120
                                      M  L  K  S  K  L  N  V
CTAACACTGAAAAAGGAACCTCTGCCAGCAGTCATCTTCCACGAGCCAGAGGCCATCGAG 180
 L  T  L  K  K  E  P  L  P  A  V  I  F  H  E  P  E  A  I  E
CTGTGCACGACCACGCCCCTGATGAAGACCAGGACTCAGAGTGGCTGCAAGGTGACCTAT 240
 L  C  T  T  T  P  L  M  K  T  R  T  Q  S  G  C  K  V  T  Y
CTGGGTAAGGTGCCCACCACAGGCATGCAGTTTTTGTCAGGCTGCACAGAAAAGCCAGTC 300
 L  G  K  V  P  T  T  G  M  Q  F  L  S  G  C  T  E  K  P  V
ATCGAGCTCTGGAAGAAGCACACACTGGCCCGAGAAGACGTCTTTCCGGCTAATGCCCTC 360
 I  E  L  W  K  K  H  T  L  A  R  E  D  V  F  P  A  N  A  L
CTGGAAATCCGACCATTCCAAGTGTGGCTCCATCACCTCGACCACAAAGGGGAGGCCACG 420
 L  E  I  R  P  F  Q  V  W  L  H  H  L  D  H  K  G  E  A  T
GTACACATGGATACCTTCCAGGTGGCCCGCATCGCCTACTGCACCGCCGACCACAACGTG 480
 V  H  M  D  T  F  Q  V  A  R  I  A  Y  C  T  A  D  H  N  V
AGCCCCAACATCTTCGCCTGGGTTTACAGGGAGATTAATGATGACCTGTCCTACCAGATG 540
 S  P  N  I  F  A  W  V  Y  R  E  I  N  D  D  L  S  Y  Q  M
GACTGCCATGCTGTCGAGTGCGAGAGCAAGCTGGAGGCCAAGAAGCTGGCCCACGCCATG 600
 D  C  H  A  V  E  C  E  S  K  L  E  A  K  K  L  A  H  A  M
ATGGAGGCCTTCAAGAAGACTTTCCACAGTATGAAGAGCGATGGCCGGATCCACAGGAAC 660
 M  E  A  F  K  K  T  F  H  S  M  K  S  D  G  R  I  H  R  N
AGCTCCTCAGAAGAAGCATCCCAGGAATTAGAATCTGATGATGGCTGAGTGAACTGAGAG 720
 S  S  S  E  E  A  S  Q  E  L  E  S  D  D  G  *
AGCTTCAGCAAAGGCAGCATTGGTCAAGGAATTCAAGATGATAGACGGATAAGCGA     776
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Fig 3. *The nucleotide sequence and the deduced amino acid sequence of Jiulong yak PID1. The asterisk represents the stop codon. The PTB domain was underlined.*

3.2. Expression Profile of *PID1* mRNA in Jiulong Yak

Semi-quantitative RT-PCR was employed to analyze the relatively transcription level of *PID1* mRNA in different tissues of Jiulong yak. The *PID1* mRNA was observed in all the tissues tested. In particular, high expression levels of *PID1* were detected in heart, liver and abdominal muscle ($P<0.05$), low expression levels were found in spleen and kidney and fat (Fig. 5). Furthermore, real-time quantitative RT-PCR showed higher *PID1* expression level in longissimus muscle of Jiulong yaks at 0.5 and 9 years than at 3.5-5.5 years old ($P<0.05$, Fig.6).

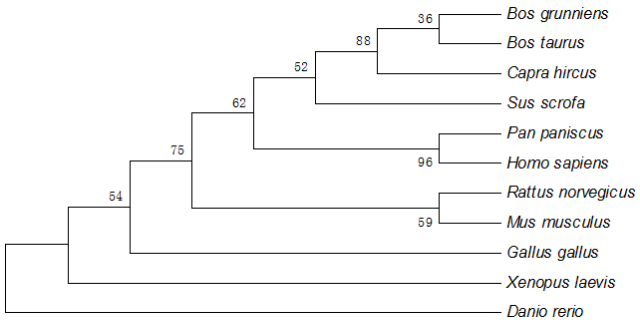

Fig 4. *Phylogenetic analysis of PID1 amino acid sequences. The phylogenetic tree was constructed with neighbour-joining (NJ) methods (Kimura two-parameter model, 10,000 replicates, bootstrap phylogeny test) based on PID1 amino acid sequences using MEGA software version 3.1. Bootstrap values and genetic distance are also indicated. GeneBank accession numbers used in this analysis are as follows: domestic yak AGF90519, cattle NP_001073052, goat AEV66316, pig AGK63078, human NP_001094288, pygmy chimpanzee XP_003821924, mouse NP_001003948, rat NP_001103963, chicken NM_001178144.1, African clawed frog NP_001090300, zebrafish NP_001013522.*

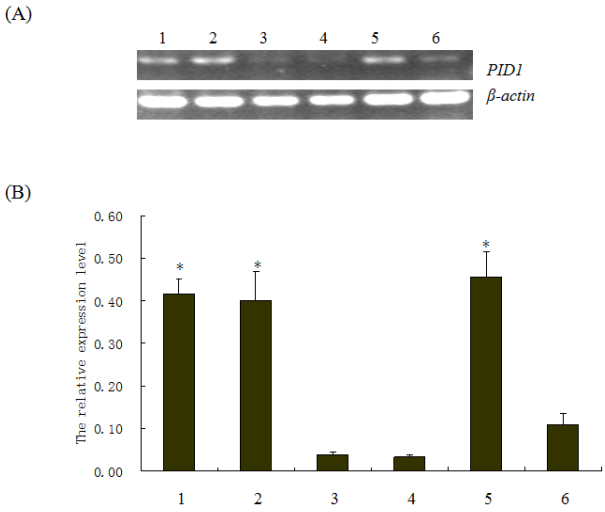

Fig 5. *Tissue distribution of PID1 in Jiulong yaks (n=5). (A) PID1 cDNA in six tissues. 1 Heart, 2 liver, 3 spleen, 4 kidney, 5 longissimus muscle, 6 fat. The PID1 cDNA were obtained by semi-quantitative RT-PCR. (B) The relative levels of PID1 mRNA in tissues from Jiulong yaks (the average values of five yaks). Yak β-actin was used as a loading control to normalize PID1 mRNA expression.* P< 0. 05.*

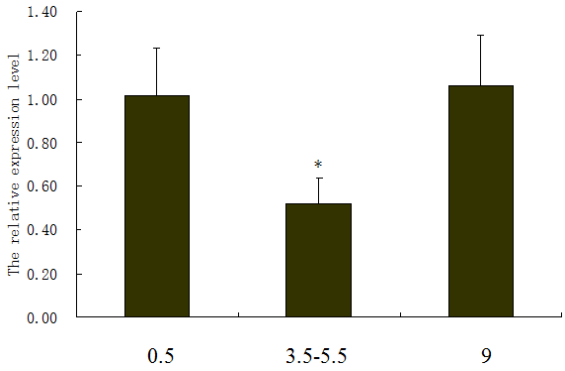

Fig 6. *The relatively levels of PID1 mRNA in longissimus muscle of Jiulong yaks at different ages. The mRNA was detected by fluorescence quantitative PCR. Expression levels of Jiulong yak PID1 gene in longissimus muscle at 0.5, 3.5-5.5 and 9 years old, respectively. * P< 0. 05.*

4. Discussion

Meat quality is one of the important economic traits for farm animals. Intramuscular fat content, an intrinsic factor contributing to meat palatability, thus is used as an indicator for beef quality grading (USDA, 1997; CMA, 2003). PID1 is highly expressed in obese people [17], which suggests that it may be related to the presence of intramuscular fat deposits. In the present study, we cloned yak *PID1* cDNA and found it covered an ORF of 612bp encoding a protein containing 217 amino acids with a PTB motif, which suggested that PID1 could be associated with fat development by interacting with growth factor receptors in signal transduction. The sequence alignments and phylogeny analysis showed that the amino acid sequences of PID1 are higher homologous among mammals (99%-89%) and yak sequences have highest homology with the bovine sequences.

Furthermore, the expression profile of *PID1* in Jiulong yaks was analyzed. The results showed that yak *PID1* mRNA were found in all of the tested tissues; with high levels in heart, liver, longissium muscle, which is consistent with the reports that PID1 were primarily expressed in the skeletal muscles and in the heart of chicken [18], human [5] and mice [19]. Xu et al indicated that PID1 was highest expressed in the liver of the goats[20].The tissue distribution of PID1 supported the hypothesis that this gene may be involved in lipidmetabolism, including the metabolism of intramuscle fat and differentiation of its function [10]. In this study, meanwhile, real time quantitative PCR also showed that the *PID1* expression levels in longissium muscle of 0.5 and 9-year Jiulong yaks were significantly higher than that in those yaks at the age of 3.5-5.5 years old ($P<0.05$). This result is inconsistency with previous studies on other animals. Xu et al., showed that the PID1 expression levels in longissimus muscle of goats increased at the age of 6-24 months old[20].

5. Conclusion

In conclusion, we cloned Jiulong yak *PID1* gene and used bioinformatics tools to analyze the gene and deduced protein sequences. Further, we also analyzed the temporal and spatial expression patterns of yak *PID1*, the results shows that PIDI may be a candidate gene for meat quality.

Acknowledgments

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