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Novel splice isoforms of dairy goat *DBC1* and their diverse mRNA expression profiles

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ABSTRACT

As a critical regulator of nuclear factors affecting adipogenesis, deleted in breast cancer 1 (DBC1) leads to the hyperacetylation of p53, a target of the NAD^+-dependent histone deacetylase Sirtuin 1, and activation of the apoptotic pathway. To date, the transcript variants of *DBC1* and their functions have never been reported in dairy goats. Herein, we identified two novel transcripts of dairy goat *DBC1*, namely, *DBC1* and *DBC1a*. *DBC1* was 42 base pairs longer than *DBC1a*, and the splice site of the 42- nucleotide deletion complied with the classic "GT-AG" rule. The goat *DBC1a* in this study had a high degree of homology with other predicted goat *DBC1* variants as well as the only known bovine *DBC1* variant, *DBC1-X4*. *DBC1* and *DBC1a* were widely expressed in different tissues and highly expressed in the pancreas, spleen, lung, liver, kidney, and mammary gland. Moreover, *DBC1* has a significantly higher expression than *DBC1a* in the pancreas, kidney, liver, heart, muscle, brain, and testis. Interestingly, in mammary gland, *DBC1* and *DBC1a* demonstrated similar mRNA expression levels, implying that *DBC1a* variant plays an important role in milk production. This is the first report on the alternative splicing of goat *DBC1* and its mRNA expression profile, which will provide a foundation for further study the function of *DBC1* in dairy goat industry.

1. Introduction

Deleted in breast cancer 1 (*DBC1*), also known as *KIAA1967* and *CCAR2* (the cell cycle and apoptosis regulator 2), located on human chromosome 8pq21, was originally described as a homozygous deleted gene in breast cancer (Chini et al., 2013; Joshi et al., 2013). DBC1 regulates several nuclear factors involved in adipogenesis, including Sirtuin 1 (SIRT1), estrogen receptor (ER)-α, and ERβ (Trauernicht et al., 2007; Koyama et al., 2010; Yu et al., 2011). The NAD^+-dependent histone deacetylase SIRT1 affects gene silencing, stress resistance, apoptosis, senescence, and fat and glucose metabolism (Cohen et al., 2004; Motta et al., 2004). DBC1 is a specific inhibitor of SIRT1 that regulates adipose tissue metabolism (Van der Horst et al., 2004; Vaquero et al., 2004; Yeung et al., 2004). SIRT1 is critical for tumorigenesis owing to its ability to silence the expression of several tumor suppressor genes (Pruitt et al., 2006). For example, in colorectal cancer, high levels of SIRT1 expression enhance tumorigenesis and associate with a poor prognosis of colorectal carcinoma patients (Chen et al., 2014). Therefore, it is conceivable that DBC1 acts as tumor suppressor by promoting cell apoptosis. Additionally, DBC1 not only interacts with the estrogen receptors ERα and ERβ directly (Trauernicht et al., 2007; Koyama et al., 2010; Yu et al., 2011; Hagrass et al., 2014; Pei et al., 2015), but also concomitantly binds to cell cycle and apoptosis regulator 1 (CCAR1), the activator of ERα. Together, DBC1 and CCAR1

Abbreviations: DBC1, deleted in breast cancer 1; DBC1a, deleted in breast cancer 1a; SIRT1, sirtuin 1; ERα, estrogen receptors α; ERβ, estrogen receptors β; NAD^+, nicotinamide adenine dinucleotide; RT-PCR, reverse transcript polymerase chain reaction; qRT-PCR, quantitative real-time polymerase chain reaction; mRNA, messenger RNA; CCAR2, cell cycle and apoptosis regulator 2; CCAR1, cell cycle and apoptosis regulator 1; AR, androgen receptor; AS, alternative splicing; RNA-Seq, RNA sequencing; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGC-1a, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PEPCK, phosphoenolpyruvate carboxykinase; CRCC, cell renal cell carcinoma; SNP, single nucleotide polymorphism; NCBI, the National Center for Biotechnology Information; CDS, coding DNA sequence; cDNA, complementary DNA; aa, amino acid; nt, nucleotide; bp, base pair.

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can stabilize ERα, promoting estrogen-dependent proliferation in breast cancer cells (Garapaty et al., 2009; Yu et al., 2011).

Numerous studies have shown that more than 90% of mammalian genes have tremendous transcriptional complexity through alternative splicing (AS) (Pan et al., 2008; Wang et al., 2008; Alam et al., 2014). Along with the rapid development of sequencing and bioinformatics, deep RNA sequencing (RNA-Seq) has become a powerful and useful tool for genome-wide analysis of AS (Shen et al., 2014; Sohail et al., 2014), uncovering novel AS data from animal transcriptome databases. AS is a common mode of gene regulation in diverse biological processes, such as cell differentiation (Kianianmomeni et al., 2014; Cotter et al., 2015), cell signaling (Wright and Dyson, 2014; Li et al., 2015), and disease development (Wang and Cooper, 2007; Cooper et al., 2009; Sohail et al., 2014). Moreover, differential alternative splicing is regulated through the variable expression or interaction of splicing proteins and their regulatory factors. AS is important in processes, such as spermatogenesis (Guo et al., 2013; Li et al., 2014), adipogenesis (Li et al., 2013; Zhou et al., 2014), and gluconeogenesis (Anemaet et al., 2008). Recently, it was discovered that bovine *DBC1* has two transcript variants involved in adipogenesis (Li et al., 2013), but alternative splice variants of *DBC1* have not been reported in goat. Because transcript variants are extremely variable due to alternative splicing patterns, whether alternative splicing of goat *DBC1* exists or not is unknown.

Previous studies of *DBC1* have demonstrated that *DBC1* is a candidate tumor suppressor gene; these studies have focused on the specific regulatory functions of nuclear receptors related to adipogenesis. There are few reports about DBC1 on domestic animals, especially about its AS in dairy goats. As the most common dairy breed in China, the Xinong Saanen dairy goat is bred at Northwest A&F University and distributed throughout China. However, its milk production has decreased and cannot adapt to the increasing demands of the Chinese dairy goat industry. The study of AS in adipogenesis genes may provide insight into which genes are key regulators for increasing milk production in this breed. The aim of this study was to identify the alternatively spliced transcript variants of the dairy goat DBC1 and measure their mRNA expression profiles to provide a foundation for further study the function of dairy goat *DBC1* in the dairy goat industry.

2. Materials and methods

Use of animals and the animal experimental procedures were approved by International Animal Care and Use Committee of Northwest A&F University (NWSUAF). Furthermore, the care and use of experimental animals completely adhered to the local animal welfare laws, guidelines, and policies.

2.1. Animal and tissue collection

In this study, 22 goats (18 rams and 4 ewes) from the Xinong Saanen dairy goat breeding farm of Northwest A&F University, Yangling, Shaanxi province and the goat breeding farm in Lingyou county, Shaanxi province, PR China were used. Heart, liver, spleen, lung, kidney, pancreas, adipose, small intestine, muscle, brain, and testis tissues were collected from male dairy goats. Heart, spleen, lung, kidney, muscle, and mammary tissue were collected from female dairy goats. All samples were immediately placed in liquid nitrogen and stored at −80 °C (Zhou et al., 2014).

2.2. Total RNA extraction and cDNA synthesis

Total RNA was isolated using the RNAiso Plus reagent (TaKaRa, Dalian, China). RNase-free DNase I (TaKaRa Biotechnology, China) was used to remove the genomic DNA from RNA samples. RNA concentrations were measured by $OD_{260/280}$ value with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE) (Lan et al., 2013). RNA degradation and contamination were examined by ethidium bromide electrophoresis in 1.0% agarose gels. Reverse transcription PCR was performed to synthesize cDNA using the PrimeSript™ RT reagent Kit (TaKaRa, Dalian, China) using 0.5 μg total RNA according to the manufacturer's recommended procedure. The reaction was as follows: 0.5 μg of total RNA was combined with 5 × PrimeScript™ buffer, 0.5 μL of Random 6 mers primer (100 μM), 0.5 μL of PrimeScript™ RT Enzyme Mix I, and RNase-free water to total volume 10 μL. The mixture was incubated at 37 °C for 15 min, and the reaction was stopped by heating at 85 °C for 5 s.

2.3. PCR amplification and identification of DBC1 splice variants in dairy goats

Since there is high nucleotide sequence identity in many genes between goat and cows, the two reported transcript sequences of the bovine *DBC1* (Li et al., 2013) were used to discover unknown goat DBC1 transcripts. Four pairs of primers were designed to amplify the two distinctive variants using the known primers (Li et al., 2013) (Table 1). To prevent genomic DNA contamination and verify the effective amplification of different primers, genomic DNA was also used as the template for amplification for the P3 and P4 primer sets. PCR reactions were performed in a 25 μL reaction volume containing 50 ng cDNA (or genomic DNA) as the template, 0.5 μM of each primer, 2 × buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs, and 0.625 units of *Taq* DNA polymerase (MBI, Vilnius, Lithuania). Touch-Down PCR reaction conditions were as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 68–51 °C for 30 s, and 72 °C for 30 s, with a final exten-

Table 1
Primers used for real-time quantitative PCR (qPCR) of goat *DBC1* gene.

Names	Primer sequences (5′ > 3′)	Product sizes (bp)	Annealing (Tm)	Notes
P1	F1: CTGTTTCTGGAGATGCTGC R1: AGCTGTGCCCTCACTCTG	180	Touch-down protocol	qRT-PCR for DBC1
P2	F1: CTGTTTCTGGAGATGCTGC R2: CCATCTTCCTTCTCATCCT	170	Touch-down protocol	qRT-PCR for DBC1a
P3	F3: TCCAAGGATGAGAAGGAAGAT R3: GTCTCTCAGGAGCAGCAGTTC	133 (if cDNA as the template)/no products (if gDNA as the template)	Touch-down protocol	qRT-PCR for DBC1a (spanning the intron)
P4	F4: GTACAGAGTGAGGGCACAGC R3: GTCTCTCAGGAGCAGCAGTTC	168 (if cDNA as the template)/802 (if gDNA as the template)	Touch-down protocol	qRT-PCR for DBC1 (spanning partial intron)
GAPDH	F: AATGAAAGGGCCATCACCATC R: GTGGTTCACGCCCATCACA	204	Touch-Down	qRT-PCR for GAPDH

Note: cDNA: complementary DNA; gDNA: genomic DNA.