Identification and characterization of circular RNAs in *Longissimus dorsi* muscle tissue from two goat breeds using RNA-Seq

Jiyuan Shen · Huimin Zhen · Lu Li · Yuting Zhang · Jiqing Wang · Jiang Hu · Xiu Liu · Shaobin Li · Zhiyun Hao · Mingna Li · Zhidong Zhao · Yuzhu Luo

Received: 29 November 2021 / Accepted: 17 March 2022 / Published online: 16 April 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Circular RNAs (circRNAs) are a class of non-coding RNA that play crucial roles in the growth and development of skeletal muscle. However, little is known about the role of circRNAs in caprine skeletal muscle. In this study, the size of muscle fiber and the expression profiles of circRNAs were compared in *Longissimus dorsi* muscle of Liaoning cashmere (LC) goats and Ziwuling black (ZB) goats with significant phenotypic differences in meat production performance, using hematoxylin and eosin staining and RNA-Seq, respectively. The size of muscle fiber in LC goats was larger than those in ZB goats (*P* < 0.05). A total of 10,875 circRNAs were identified and 214 of these were differentially expressed between the two caprine breeds. The parent genes of differentially expressed circRNAs were mainly enriched in connective tissue development, Rap1, cGMP-PKG, cAMP and Ras signaling pathway. In conclusion, circRNAs may play important roles in skeletal mass, meat production performance and meat quality traits in goats. The results provide an improved understanding of the functions of circRNAs in skeletal muscle development of goats.

Keywords Circular RNAs · *Longissimus dorsi* muscle · Goats · RNA-Seq

Abbreviations

| Abbreviations | Description |
|---------------|-------------|
| circRNAs      | Circular RNAs |
| miRNA         | MicroRNA    |
| LC            | Liaoning cashmere |
| ZB            | Ziwuling black |
| RT-PCR        | Reverse transcriptase-polymerase chain reaction |
| STAT1         | Signal transducer and activator of transcription 1 |
| MYH4          | Myosin-4    |
| LMO7          | LIM domain 7 |
| GO            | Gene Ontology |
| KEGG          | Kyoto Encyclopedia of Genes and Genomes |

Introduction

Skeletal muscle is the largest organ of animals and its growth and development directly determines skeletal muscle mass, meat production performance and meat quality in domestic animals. It is now found that many functional genes and non-coding RNAs are involved in the growth and development of skeletal muscle, so the identification of RNAs that regulate skeletal muscle activity can provide a chance to improve meat production. However, compared with our knowledge of skeletal muscle mRNAs (Hernández-Hernández et al. 2017; Taylor and Hughes 2017; Zammit 2017) and miRNAs (Tao et al. 2018; Cheng et al. 2020; Cai et al. 2021), the reports on the roles of circular RNA (circRNAs) in the skeletal muscle are very limited.

CircRNAs are a novel class of non-coding RNA and they are result from the covalently linage of 5’ and 3’ ends of linear RNA (Lasda and Parker 2014). Due to the closed loop structure, circRNAs are not susceptible to be affected by RNA exonucleases. It is, therefore, known that circRNAs are more stable and evolutionarily conserved than the linear mRNAs (Jeck and Sharpless 2014). In recent years, the function of circRNAs is gradually uncovered. CircRNAs mainly function as microRNA (miRNA) sponges. They...
thereby relieve the inhibition of target mRNAs by miRNAs, eventually resulting in an increase in expression level of target mRNAs (Hansen et al. 2013; Ouyang et al. 2018; Chen et al. 2019). In addition, exon–intron circRNAs predominantly localized in the nucleus can regulate the transcription of their parent genes in a cis-regulatory way (Li et al. 2015). Finally, other functions of circRNAs have also been reported, including regulating alternative splicing (Ashwal-Fluss et al. 2014), interacting with RNA-binding proteins (Hentze and Preiss 2014), and even being translated into protein (Wang and Wang 2015).

To date, the studies of circRNAs in skeletal muscle tissues have mainly been focused on pigs (Hong et al. 2019; Shen et al. 2019a; Wang et al. 2019a; Li et al. 2020), chickens (Ouyang et al. 2018; Chen et al. 2019; Shen et al. 2019b) and cattle (Wei et al. 2017; Liu et al. 2020; Yan et al. 2020). These studies reported the expression profiles and characterization of circRNAs in skeletal muscle either from different breeds, or from different development stages, and also analyzed the function of circRNAs more by investigating the functions of their parent genes using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. For example, 13,377 circRNAs were identified in leg muscles of chickens between 11 embryo age, 16 embryo age and 1 day post hatch periods. Subsequently, one (circRBFOX2s) of the differentially expressed circRNAs identified was found to promote the proliferation of chicken myoblast by binding with miR-206 (Ouyang et al. 2018). In addition, a total of 655 circRNAs were found to be differentially expressed in muscle tissues between Shandong black cattle and Luxi cattle with significant differences in the diameter, length and weight of muscle fibers, and their parent genes were mainly involved in muscle fiber development process, and MAPK and mTOR signaling pathways (Liu et al. 2020).

However, there are only two reports about the activity of circRNAs in muscle tissues in goats. Ling et al. (2020) identified 9090 circRNAs in skeletal muscle of Anhui white goats across seven development stages, and the parent genes of the differentially expressed circRNAs were involved in the regulation of myoblast differentiation, skeletal muscle maturation and hypertrophy. In addition, a circRNA CDR1as has been found to activate the differentiation of caprine skeletal muscle satellite cells by relieving the inhibition of IGF1R targeted by miR-7 (Li et al. 2019a).

Liaoning cashmere (LC) goats and Ziwuling black (ZB) goats are famous local breeds in China and of economic importance for goat farmers. Compared with ZB goats, LC goats have higher carcass weight, content of intramuscular fat, and proportion of intramuscular collagen fibers, but poorer meat tenderness ($P < 0.05$) (Shen et al. 2021; Wang et al. 2021). In this study, the circRNAs expression profiles of Longissimus dorsi muscle tissue were compared between LC and ZB goats using RNA-Seq. The GO enrichment and KEGG pathway were also analyzed for the parent genes of differentially expressed circRNAs between the two caprine breeds. The results will provide an improved understanding of the function of circRNAs in skeletal muscle growth and development processes in goats.

**Materials and methods**

**Ethics statement**

All experimental procedures were approved by Faculty of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China. The investigation for experimental animals was also coincide with the rules for animal care and use published by the Ministry of Science and Technology of the People’s Republic of China (Approval number 2006–398).

**Experimental animals and sampling**

The same Longissimus dorsi muscle samples as described by Shen et al. (2021) were used in this study. Briefly, five 9-month-old LC rams and five 9-month-old ZB rams were selected from the Yongfeng Goat Breeding Company (Huan County, China) and then slaughtered. These goats were raised under the same feeding and management conditions. The carcass weight, muscle components and meat quality of these goats investigated in the study are presented in Table 1 (Shen et al. 2021; Wang et al. 2021).

The Longissimus dorsi muscle samples from the area between 12 and 13th ribs on the left carcass were collected and then used for RNA isolation and hematoxylin and eosin staining. The samples for RNA isolation were frozen in liquid nitrogen, whereas the samples for hematoxylin and eosin staining was fixed with 4% paraformaldehyde.

**Hematoxylin and eosin staining of Longissimus dorsi muscle**

The Longissimus dorsi muscle samples fixed with 4% paraformaldehyde were treated using graded ethanol (75, 85, 95, and 100%) to remove moisture. Subsequently, the dehydrated specimens were embedded in paraffin and then cut into about 5 µm of thickness using Rotary cutting machine (Leica, Wetzlar, Germany). The paraffin sections were used for hematoxylin and eosin staining as suggested by Cao et al. (2014).

Micrographs (400×) of hematoxylin and eosin staining from three different fields of view for each sample were taken by BA200 Digital microscope (MOTIC, Xiamen, China). The diameter and cross-sectional area of muscle...
fibers were then measured using Motic Images Advanced v3.2. The difference in these measurements between LC and ZB goats was analyzed using Student’s t test in SPSS v24.0.

RNA samples preparation and sequencing

Total RNA was isolated from ten caprine Longissimus dorsi muscle samples using Trizol reagent (Invitrogen, Carlsbad, CA, United States). The integrity and concentration of these RNA samples were measured using Agilent 2100 Bioanalyzer (Agilent, CA, United States) and Nanodrop 2000 (Thermo Scientific, MA, United States), respectively. High-quality RNA samples were screened with a parameter of RNA integrity number > 7 being fitted as the threshold. The ribosomal RNA (rRNA) was removed from these high quality RNA samples using a Ribo-Zero Gold rRNA Removal Kit (Illumina, CA, United States). The remaining RNA was fragmented into 200–300 bp in length and then used for constructing cDNA libraries using a NEBNext Ultra RNA Library Prep Kit (New England Biolabs, MA, United States). The cDNA libraries were paired-end sequenced with a sequencing depth of 12 G clean data on a HiSeq™ 4000 sequencer (Illumina, CA, United States).

Analysis of RNA-Seq data

The clean reads were obtained by removing the reads with quality scores < Q20, reads containing sequencing adapters and reads with > 10% unknown nucleotides from raw reads produced from sequencer, using fastp v0.18.0 (Chen et al. 2018). These clean reads were then mapped to Caprine Genome Assembly ARS1 (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/704/415/GCF_001704415.1_AR51) using HISAT2 v2.1.0 (Kim et al. 2015). For the reads that were unmapped against Caprine Genome Assembly ARS1, 20-mers from both ends were defined as anchor reads. The anchor reads were mapped to the reference genome again using bowtie2 v2.2.8 (Langmead and Salzberg, 2012) and circRNAs were identified using software Find_circ (Memczak et al. 2013). The identified circRNAs were characterized by counting their chromosomal distribution, type and length. The type of differentially expressed circRNAs was also counted. The expression level of each annotated circRNA was normalized by calculating the Reads Per Million mapped reads (RPM). CircRNAs with RPM > 0 were considered to be expressed. DESeq v2.0 (Love et al. 2014) was used to screen differentially expressed circRNAs in Longissimus dorsi muscle tissues between LC and ZB goats, with fold change > 2.0 and P value < 0.05. It is notable that the correction for multiple testing was not performed in this study as the calculation method of P value in DESeq2 is very strict and sufficient as the screening criterion for differentially expressed circRNAs. In addition, if the corrected P value was used, the number of differentially expressed circRNAs will sharply decrease, which was not conducive to analyze the function of the parent genes of differentially expressed circRNAs by GO and KEGG analysis.

Validation of the authenticity of circRNAs using reverse transcriptase–PCR and DNA sequencing

Based on the characteristic that circRNA has a unique head-to-tail junction, its authenticity was validated using reverse transcriptase–PCR (RT–PCR) and DNA sequencing. Briefly, 20 differentially expressed circRNAs between the two caprine breeds were randomly selected. The ten RNA samples that were used for RNA-Seq were also used to produce cDNA using an RT–PCR kit (Takara, Dalian, China). The cDNA products were then amplified using divergent primers (Table 2) in a 20-µL reaction, containing 0.8-µL of the cDNA, 0.5 U of Taq DNA polymerase (Takara, Dalian, China), 150 µM of each dNTP (Takara, Dalian, China), 2.5 mM Mg²⁺, 0.25 µM of each primer and 1× PCR buffer supplied with DNA polymerase enzyme. RT–PCR amplicons were checked in 1% agarose gels electrophoresis and then sequenced using Sanger sequencing. To confirm the authenticity of these circRNAs, the sequences from Sanger sequencing were aligned to the goat reference genome and RNA-Seq data to validate the location of the junction sites of these circRNAs.

| Table 1 Carcass weight, muscle components and meat quality of the two goat breeds |
|---------------------------------|---------------------------------|---------------------------------|------------------|
| Traits                          | Liaoning cashmere goats (n = 5) | Ziwuling black goats (n = 5)    | P value          |
| Carcass weight (kg)             | 14.10 ± 1.17                    | 7.45 ± 1.28                     | 2.600E–05        |
| Lion-eye area (cm²)             | 13.44 ± 2.26                    | 5.86 ± 1.97                     | 4.836E–04        |
| Muscle shear force value (N)    | 22.71 ± 2.63                    | 18.11 ± 1.27                    | 0.027            |
| The proportion of intramuscular collagen fibers (%) | 17.78 ± 1.21                    | 12.31 ± 1.25                    | 0.017            |
| Intramuscular fat content (%)   | 3.23 ± 0.23                     | 1.88 ± 0.40                     | 0.004            |

*The results are derived from independent t-test and shown as mean±SD (n = 5)*
Validation of the reliability of RNA-Seq results using RT-quantitative PCR

The same 20 differentially expressed circRNAs as those used for RT–PCR, were selected to confirm the reliability of RNA-Seq results using RT–quantitative PCR (RT–qPCR). The ten RNA samples that were used for RNA-Seq were also used to produce cDNA using an RT–PCR kit (Takara, Dalian, China). The RT–qPCR was performed in triplicate using a 2× ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) on an Applied Biosystems QuantStudio® 6 Flex (Thermo Lifetech, MA, USA). Caprine GAPDH and β-actin were used as internal control for normalizing the expression levels of these circRNAs as suggested by Ling et al. (2020) and Wang et al. (2019a). A 2−ΔΔCt method was finally used to calculate the relative expression levels.

Functional enrichment analysis of the parent genes of differentially expressed circRNAs and construction of circRNA-miRNA-mRNA network

The parent genes of circRNAs were annotated by aligning their sequence to the RefSeq non-redundant proteins (nr) database using BLASTX (E value < 10−5). GO (Ashburner et al. 2000) analysis was used to analyze the main function of the parent genes of the differentially expressed circRNAs using DAVID (Dennis et al. 2003). The KOBAS 3.0 (Buet al. 2021) was used to identify the pathway that included the parent genes of the differentially expressed circRNAs using KEGG database. The GO terms and KEGG pathways with P value < 0.05 were considered to be significantly enriched based on hypergeometric test. Meanwhile, five differentially expressed circRNAs were used to predict their miRNA binding sites using Mireap v0.2 (Liu et al. 2015), Miranda v3.3a (Turner 1985) and TargetScan v7.0 (Lewis et al. 2005). The three software were also used to predict the target genes of the miRNAs sponged by the five circRNAs and predicted results from the three kinds of software were compared. The Pearson's coefficients in expression levels between the five differentially expressed circRNAs and their corresponding mRNAs was calculated using SPSS v24.0. An interaction network among these circRNAs and their sponged miRNAs as well as corresponding mRNAs was constructed using StarBase v3.0 (Li et al. 2014) and then drawn using Cytoscape v3.5.1 (Shannon et al. 2003).

Table 2 Information of primers used for RT-PCR and RT-qPCR

| CircRNA/Gene | Forward (5′ → 3′) | Reverse (5′ → 3′) |
|--------------|-------------------|------------------|
| circ_009262  | ACCAACTGCTTTTCCGAAAGTG | GCTTCATTAAACGCCCACCACA |
| circ_003757  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_008447  | TGCAGGAGCTCTTGTTGGA | TGTTCATTAAACGCCCACCACA |
| circ_002956  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_007137  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_001875  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_006718  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_008092  | TCAAGGAGCTAGGATTTGCA | CATGAAACAGGATGTGGGCA |
| circ_008770  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_009387  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_006172  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_005348  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_004394  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_003976  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_002473  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_008047  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_010303  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_007151  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_008117  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| circ_009217  | CAGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| GAPDH        | AGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |
| β-actin      | AGCAGCAGATGCCAACACTAA | GCAACTCAATGTGCCATCAC |

Results

Comparison of the size of muscle fiber between LC and ZB goats

Muscle fibers are the main structural components of skeletal muscle tissues, accounting for about 70% of the total
weight. The diameter and cross-sectional area are important morphology characteristics of muscle fibers and they play key roles in determining meat production performance of animals (Tuma et al. 1962; Larzul et al. 1997; Rehfeldt et al. 2000; Choi and Oh 2016). The comparison results of the diameter and cross-sectional area of Longissimus dorsi muscle fiber between LC and ZB goats are shown in Fig. 1. The diameter and cross-sectional area of muscle fibers from LC goats were 38.52 ± 2.20 μm and 1902.91 ± 156.92 μm², respectively, which was larger than ZB goats with a diameter of 29.09 ± 3.81 μm and a cross-sectional area of 1117.72 ± 210.10 μm² (Fig. 1).

The identification of circRNAs in the caprine Longissimus dorsi muscle tissues

The raw reads obtained in the study from ten Longissimus dorsi muscle samples have been submitted to GenBank database (https://www.ncbi.nlm.nih.gov/sra) with accession numbers SRR13008213-SRR13008222. The RNA-Seq reads and mapped results to the goat reference genome have also been described in our previous study (Shen et al. 2021). Concisely, an average of 88,773,430 and 87,190,490 clean reads were obtained from LC and ZB goats, respectively. After removing the rRNA mapped reads, an average of 77,996,504 and 82,000,854 high quality clean reads could be mapped to Caprine Genome Assembly ARS1 from LC and ZB goats, respectively. A total of 8781 and 8872 circRNAs were identified in Longissimus dorsi muscle tissues of LC and ZB goats, respectively, with 6778 circRNAs being commonly expressed across the two breeds. Of these circRNAs identified, 2003 circRNAs were only expressed in LC goats, while 2094 were only expressed in ZB goats. Most notably, circ_001086 derived from LIM domain 7 (LMO7) was the most highly expressed circRNA in muscle tissues of both LC and ZB goats.

Authentication of caprine circRNAs in the Longissimus dorsi muscle tissue

A total of 20 circRNAs were selected to validate the presence of their specific head-to-tail junctions using RT–PCR and Sanger sequencing. Agarose gel electrophoresis results showed that all 20 circRNAs were expressed and they produced a band with an expected size (Fig. 2A). Sanger sequencing further affirmed the presence of head-to-tail splice junctions and the size for these caprine circRNAs,
which was consistent with those provided by RNA-seq (Fig. 2B).

**Characterization of circRNAs identified in the caprine Longissimus dorsi muscle tissue**

Of the six types of circRNAs, annot_exons were the most common sequences with an average proportion of 78.03%, followed by exon_intron, one_exon and antisense sequence types with an average proportion of 7.69%, 5.25%, and 3.97%, respectively. Intronic and intergenic sequences were the least common types with an average proportion of 3.14 and 1.91%, respectively (Fig. 3A).

The circRNAs identified from *Longissimus dorsi* muscles were widely distributed across all the caprine chromosomes, with the exception of Y chromosome. The most circRNAs were distributed on chromosomes 2 and 1, while the least circRNAs were distributed on chromosome 27 (Fig. 3B). The length of most circRNAs was less than 1 kb (Fig. 3C).
Identification and validation of differentially expressed circRNAs

Of all the 10,875 circRNAs identified in caprine *Longissimus dorsi* muscle tissues, 214 circRNAs were found to be differentially expressed between LC and ZB goats, including 85 up-regulated circRNAs and 129 down-regulated circRNAs in *Longissimus dorsi* muscle of LC goats when compared to ZB goats (Supplementary File 1). The most up-regulated circRNA in LC goats was circ_008092 derived from signal transducer and activator of transcription 1 (*STAT1*) with a 19.4-fold increase in expression, while circ_003628 derived from myosin-4 (*MYH4*) was the most down-regulated circRNA in LC goats with a 55.6-fold decrease in expression. Most notably, of 214 differentially expressed circRNAs identified, 25 circRNAs were only expressed in LC goats, while 39 circRNAs were only expressed in ZB goats. The type distribution of differentially expressed circRNAs was similar to the overall distribution of all circRNAs identified. Briefly, annot_exons were the most common sequences with an average proportion of 81.31%, followed by exon_intron, one_exon and intergenic sequences with an average proportion of 9.35%, 5.14%, and 2.34%, respectively. Antisense and intronic sequences were the least common types with an average proportion of 0.93% and 0.93%, respectively.

To validate the repeatability of RNA-seq data, 20 differentially expressed circRNAs that were used for the validation of the presence of circRNAs, were also subjected to RT-qPCR analysis. Because circ_006172 and circ_009217 were only expressed in LC and ZB goats, respectively, the log2 fold-change for LC goats relative to ZB goats was infinity for these two circRNAs. Their relative expression levels are therefore not presented in Fig. 4. As shown in Fig. 4, the expression levels of circ_009262, circ_003757, circ_008447, circ_002956, circ_008770, circ_009387, and circ_002473 in LC goats were higher than those in ZB goats. In contrast, compared to ZB goats, the expression levels of circ_005348, circ_004394, circ_003976, circ_008047, circ_010303, circ_007151, and circ_008117 were lower in LC goats. The results suggest that RT-qPCR results for these circRNAs in the *Longissimus dorsi* muscle were in accordance with those obtained from RNA-Seq (Fig. 4), and that our RNA-Seq results are repeatable and reliable.

Functional annotation of the parent genes of differentially expressed circRNAs

A total of 4112 parent genes were annotated for 10,875 circRNAs identified. To further understand how these differentially expressed circRNAs regulate phenotypic differences in muscle mass, meat production performance and meat quality between the two goat breeds, GO enrichment and KEGG pathway analysis were performed for the parent genes of the differentially expressed circRNAs. A total of 195 parent genes were annotated for 214 differentially expressed
circRNAs. The most significant GO term with the lowest \( P \) value was connective tissue development \( (P = 6.43 \times 10^{-04}) \), which was enriched by the parent genes of five differentially expressed circRNAs, including LOC102187872 circRNA (circ_002339), SOX6 circRNA (circ_006718), ZBTB16 circRNA (circ_008022), ADAMTS12 circRNA (circ_004981), and CREB5 circRNA (circ_000980). This was followed by syncytium formation by plasma membrane fusion \( (P = 0.001, 3 \text{ parent genes}) \) and syncytium formation \( (P = 0.001, 3 \text{ parent genes}) \). In addition, several important GO terms related to skeletal muscle hypertrophy were also found, including regulation of GTPase activity \( (P = 0.004, 9 \text{ parent genes}) \), Rho GTPase binding \( (P = 0.005, 3 \text{ parent genes}) \), Ras GTPase binding \( (P = 0.006, 6 \text{ parent genes}) \), small GTPase binding \( (P = 0.011, 6 \text{ parent genes}) \), and GTPase binding \( (P = 0.012, 6 \text{ parent genes}) \) (Fig. 5A).

Several significant KEGG pathways \( (P < 0.05) \) associated with the growth and development of skeletal muscle and the deposition of intramuscular fat were also found in this study, including Rap1 signaling pathway \( (P = 0.002) \), cGMP-PKG signaling pathway \( (P = 0.009) \), cAMP signaling pathway \( (P = 0.043) \), Ras signaling pathway \( (P = 0.045) \), and adipocytokine signaling pathway \( (P = 0.047) \) (Fig. 5B). It was worth noting that nine differentially expressed circRNAs that their parent genes were closely associated with growth and development of skeletal muscle and adipose tissues, were involved in these pathways. These consisted of MAPK1 circRNA (circ_001875), AKT3 circRNA (circ_001709), MET circRNA (circ_009387), MEF2A circRNA (circ_006172), NFKB1 circRNA (circ_002300), PLCE1 circRNA (circ_008117), AFDN circRNA (circ_007151), PLCB4 circRNA (circ_001835), and CREB5 circRNA (circ_000980).

**Functional analysis of circRNAs as miRNA sponges**

For the 214 differentially expressed circRNAs identified, a total of 431 miRNA binding sites were predicted. For clearly presenting the interaction effect of circRNAs and miRNAs, 5 circRNAs with the type of annot_exons were further selected, including four up-regulated circRNAs (circ_002300, circ_006172, circ_008092, and circ_001875) and one down-regulated circRNA (circ_010839) in LC goats compared to ZB goats, given that annot_exons are mainly localized in the cytoplasm and they may, therefore, play biological function by acting miRNA sponges. There were 55 miRNA binding sites in total for these 5 circRNAs.

---

**Fig. 4** RT-qPCR validation of differentially expressed circRNAs identified using RNA-Seq. These included 11 up-regulated circRNAs **A** and 7 down-regulated circRNAs **B** in LC goats compared to ZB goats.

---

\( \text{circRNAs} \)
Of these miRNAs, a total of eight sponged miRNAs were selected, including miR-34b-5p, miR-27b-3p, miR-34c-5p, miR-424-5p, miR-15b-5p, miR-148a-3p, miR-96 and miR-330-5p, based on the findings that miR-330-5p were related with adipose development (Shi et al. 2018), while other miRNAs have been reported to play important roles in the growth and development of skeletal muscle (Wang et al. 2019b; Ling et al. 2018; Hou et al. 2017; Connolly et al. 2018; Li et al. 2019b; Yin et al. 2020; Nguyen et al. 2020). In addition, the target genes of these miRNAs had positive correlations in expression with their corresponding circRNAs ($r > 0.6$, $P < 0.05$) (Table 3 and Supplementary File 2). A total of 11 circRNA–miRNA–mRNA pairs were screened and then used to construct a circRNA–miRNA–mRNA network (Fig. 6).

**Discussion**

In this study, the diameter and cross-sectional area of *L. dorsi* muscle fiber from LC goats were larger than those from ZB goats. It has been reported that the
diameter or cross-sectional area reflects the size of muscle fiber, which directly determines skeletal muscle mass during postnatal period of animals (Glass 2005; Schiaffino et al. 2013). This may partly explain why in our findings LC goats had higher carcass weight than ZB goats. Choi and Oh (2016) also found that pigs with greater cross-sectional area of muscle fibers had higher carcass weight ($P < 0.001$).

Besides carcass weight, cross-sectional area of muscle fiber has been reported to positively correlated with intramuscular fat content ($r = 0.68$) of pork (Larzul et al. 1997), and muscle fiber diameter was also positively correlated with shear force value ($r = 0.63$) and loin eye area ($r = 0.56$) of beef (Tuma et al. 1962). These studies further supported our observation that meat from LC goats with higher diameter and cross-sectional area of Longissimus dorsi muscle fiber had higher muscle shear force value, intramuscular fat content and loin eye area than meat from ZB goats.

The number of circRNAs (10,875) identified in the study was higher than what was investigated by Ling et al. (2020), who described 9090 circRNAs in caprine Longissimus dorsi muscle tissues of Anhui white goats. In addition, 14,640 and 6988 circRNAs were found in muscle tissues from cattle (Liu et al. 2020) and pigs (Wang et al. 2019a), respectively. This likely revealed species-specific expression pattern of circRNAs. Our observation that most of circRNAs identified were the type of annot_exons, was in accordance with the

### Table 3

Pearson correlation of selected circRNAs in expression level with the target mRNA of corresponding miRNA

| CircRNA     | Corresponding miRNA | Target mRNA | Pearson correlation of circRNA in expression with the target mRNA of corresponding miRNA | $P$ value* |
|-------------|----------------------|-------------|--------------------------------------------------------------------------------------|------------|
| circ_002300 | miR-34b-5p           | IGFBP2      | 0.721*                                                                               | 0.019      |
| circ_002300 | miR-34b-5p           | CCDC80      | 0.949**                                                                              | 0.000      |
| circ_002300 | miR-27b-3p           | PAX3        | 0.792**                                                                              | 0.006      |
| circ_002300 | miR-27b-3p           | FGF1        | 0.902**                                                                              | 0.000      |
| circ_002300 | miR-34c-5p           | COL1A1      | 0.861**                                                                              | 0.001      |
| circ_006172 | miR-27b-3p, miR-96   | FGF1        | 0.746*                                                                               | 0.013      |
| circ_001875 | miR-15b-5p           | HOMER1      | 0.637*                                                                               | 0.048      |
| circ_001875 | miR-424-5p           | FGF1        | 0.799**                                                                              | 0.006      |
| circ_010839 | miR-148a-3p          | SIK1        | 0.941**                                                                              | 0.000      |
| circ_008092 | miR-330-5p           | CTRP6       | 0.850**                                                                              | 0.002      |

*The results are derived from two-tailed $t$ test. *$P < 0.05$; **$P < 0.01$
findings in muscle tissues in Anhui white goats (Ling et al. 2020), pigs (Liang et al. 2017), cattle (Wei et al. 2017), and chicken (Ouyang et al. 2018). Other types of circRNAs that have been identified in Longissimus dorsi muscle of Anhui white goats (Ling et al. 2020), chicken (Ouyang et al. 2018) and pigs (Li et al. 2020), were also found in the study. Most of circRNAs identified in this study were less than 1 kb, which was also consistent with the length distribution of circRNAs reported in skeletal muscle of cattle (Wei et al. 2017) and pigs (Cao et al. 2022). It is noteworthy that of multiple circRNAs produced by a single gene, there were only 1–2 circRNAs with a high expression level. This phenomenon was also observed in bovine muscle circRNAs (Wei et al. 2017). The uneven distribution of circRNAs in caprine chromosome observed in the study is perhaps unsurprising as caprine chromosomes 1 and 2 are the largest in size, while chromosome 27 is relatively small in the goat genome. Ling et al. (2020) found similar chromosome distribution of circRNAs in caprine skeletal muscle tissues, and studies in cattle, chicken and pigs also confirmed that the numbers of circRNAs found was proportional to chromosome size (Wei et al. 2017; Shen et al. 2019b; Cao et al. 2022).

It was noteworthy that the most highly expressed circRNA in both LC and ZB goats was circ_001086 derived from LMO7. LMO7 is essential to skeletal muscle development as it maintains proper myoblast differentiation (Dedeic et al. 2011). Knockdown of LMO7 inhibited myogenesis by preventing myotube formation and decreasing the number of myoblast in chicken (Possidonio et al. 2016). In addition, circLMO7 produced from LMO7 promoted the proliferation of myoblast, but inhibited the differentiation and apoptosis of myoblasts by sponging miR-378a-3p in cattle (Wei et al. 2017). These suggest that circ_001086 play key roles for skeletal muscle development in both LC and ZB goats and it is worthy of further investigation.

Compared to ZB goats, the most up-regulated and down-regulated circRNAs in LC goats were circ_008092 and circ_003628, respectively, which originated from STAT1 and MYH4, respectively. STAT1 is the parent gene of circ_003976, and involved in the accumulation of crucial muscle proteins, such as myosin heavy chain and troponins (Reyes et al. 2015). CYFIP1 producing circ_007919 promoted the remodeling of actin, which is one of the most important muscle proteins (De Rubeis et al. 2013). It was, therefore, inferred that differential expression of these circRNAs in Longissimus dorsi muscle tissues between LC and ZB goats may be involved in significant phenotypic differences in muscle mass and carcass weight originated from muscle fiber hypertrophy.

The cAMP signaling pathway, Ras signaling pathway and cGMP-PKG signaling pathway play important roles in growth and development of skeletal muscle and
adipose tissue. The cAMP signaling and cGMP-PKG signaling pathways are crucial for skeletal muscle development (Berdeaux and Stewart 2012; Kuo and Ehrlich 2015) and also associated with adipocyte differentiation and lipolysis (Madsen and Kristiansen 2010; Nishikimi et al. 2009). Ras signaling was involved in inhibition of myoblast differentiation and skeletal myogenesis (Olson et al. 1987; Mitin et al. 2001), and regulation of adipocyte differentiation during brown adipogenesis (Murholm et al. 2010). As might be expected, the parent genes of nine differentially expressed circRNAs enriched in the pathways were also related with muscle hypertrophy and atrophy. For example, the proteins MAPK1 and AKT3 promoted the hypertrophy of postnatal skeletal muscle (Fadia and Adams 2004), and also participated in terminal differentiation and proliferation of myoblast (Li and Johnson 2006; Wei et al. 2013). The protein MET has been found to promote muscle hypertrophy by preventing apoptosis of myogenic progenitors (Ronzoni et al. 2017; Cassano et al. 2008). The significant role of MEF2A has well been established in controlling embryonic myogenesis, adult skeletal muscle growth, hypertrophy and regeneration (Schiaffino et al. 2018). The knockout of NFkB1 inhibited the unloading-induced muscle atrophy by increasing cross-sectional areas of muscle fiber (Hunter and Kandarian 2004). Meanwhile, the regulation effects of parent genes MAPK1, AKT3 and MEF2A on adipogenesis have also been described (Zhao et al. 2011; Ding et al. 2017; Wu et al. 2018). These indicate that the parent genes of these differentially expressed circRNAs detected contributed to the differences in carcass weight and content of intramuscular fat between LC and ZB goats.

Other differentially expressed circRNAs of interest, which their parent genes are crucial for skeletal muscle growth and development, included WWPI circRNA (circ_008374), RNF13 circRNA (circ_007619), STAU2 circRNA (circ_00404), and STAU2 circRNA (circ_000993). WWPI has been reported to regulate skeletal muscle hypertrophy and atrophy (Hirata et al., 2019), while RNF13 and STAU2 were involved in the regulation of myoblast proliferation and differentiation (Bélanger et al. 2003; Zhang et al. 2010).

It was notable that most differentially expressed circRNAs screened in the study were the type of annot_exons (81.31%), suggesting that they may mainly be localized in the cytoplasm and can function as miRNA sponges to positively regulate the expression levels of the target genes. For the sponge mechanism of circRNA-miRNA-mRNA, there is a positive correlation in expression levels between circRNA and corresponding mRNA (Quan et al. 2021). The positive correlation relationships were also observed in the 11 circRNA-miRNA-mRNA pairs constructed in the study, indicating possible sponge mechanism existed. In this context, the roles of circRNAs in various cell activities can be reflected by the functions of their binding miRNAs. In the study, some predicted binding miRNAs have previously been reported to be associated with skeletal muscle development and intramuscular fat deposition. For example, the miR-424-5p and miR-15b-5p would target with circ_001875 (Fig. 6), which was up-regulated in *Longissimus dorsi* muscle of LC goats with higher carcass weight. Previous studies found that miR-424-5p inhibited human skeletal muscle mass by reducing protein synthesis (Connolly et al. 2018), and miR-15b-5p suppress myoblast proliferation and differentiation by targeting IRS1 (Li et al. 2019b). It was therefore inferred that the higher expression level of circ_001875 may contribute to higher muscle mass of LC goats by sequencing the negative effect of miR-424-5p and miR-15b-5p on skeletal development. Besides these miRNAs described above, miR-27b-3p that would be sponged by circ_002300 and circ_006172, also played important roles in proliferation and differentiation of myoblast by targeting PAX3 in goats (Ling et al. 2018). These results indicate that these differentially expressed circRNAs identified in the study may play key miRNAs sponge roles in regulating the differences in meat production performance between LC and ZB goats.

In this study, a total of 214 circRNAs were differentially expressed between the two goat breeds with significant phenotype difference in meat production performance. The parent genes and sponged miRNAs of differentially expressed circRNAs were associated with muscle development and intramuscular fat deposition. This study provides an improved understanding of the roles of circRNAs in skeletal muscle development of goats. Moreover, this study lays the foundation for further research into the function of individual circRNAs in the development of muscle and adipose tissues.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00438-022-01887-1.

**Acknowledgements** This research was funded by the fund for Basic Research Creative Groups of Gansu Province (18JR3RA190), the Fuxi Young Talents Fund of Gansu Agricultural University (Gaufx-02Y02) and the Projects of Gansu Agricultural University (GSAU-ZL-2015-033).

**Author contributions** JS, JW, and YL conceived and designed the experiments. JS, HZ, LL, and YZ performed the experiments. JS analyzed the data. JW, YL, JH, XL, SL, ZH, ML, and ZZ contributed reagents, materials and tools and collected the samples. JS and JW wrote the manuscript and revised the manuscript. All authors read and approved the final manuscript.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**Ethics approval** Ethical approval by the Ethics Committee of Gansu Agricultural University, was obtained (GAU-LC-2020-27).
References

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25:25–29. https://doi.org/10.1038/75556

Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N, Kadener S (2014) CircRNA biogenesis competes with pre-mRNA splicing. Mol Cell 56:55–66. https://doi.org/10.1016/j.molcel.2014.08.019

Bar DZ, Charar C, Gruenbaum Y (2018) Small GTPases in C. elegans metabolism. Small GTPases 9:415–419. https://doi.org/10.1080/21541248.2016.1247940

Begue G, Douillard A, Galbes O, Rossano B, Vernus B, Candau R, Begue G, Douillard A, Galbes O, Rossano B, Vernus B, Candau R, Bélanger G, Stocksley MA, Vromme M, Schaeffer L, Furic L, Des-groseillers L, Jasmin BJ (2003) Localization of the RNA-binding proteins Staufen1 and Staufen2 at the mammalian neuromuscular junction. J Neurochem 86:669–677. https://doi.org/10.1046/j.1471-4159.2003.01883.x

Berdeaux R, Stewart R (2012) CAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. Am J Physiol Endocrinol Metab 303:1–17. https://doi.org/10.1152/ajpendo.00555.2011

Bu D, Luo H, Huo P, Wang Z, Zhang S, He Z, Wu Y, Zhao L, Liu J, Guo J, Fang S, Cao W, Yi L, Zhao Y, Kong L (2021) KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. Nucleic Acids Res 49:W317–W325. https://doi.org/10.1093/nar/gkab447

Cai R, Zhang Q, Wang Y, Yong W, Pang W (2021) Lnc-ORA interacts with microRNA-532-3p and IGF2BP2 to inhibit skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. Am J Physiol Endocrinol Metab 303:1–17. https://doi.org/10.1152/ajpendo.00555.2011

Cao H, Liu J, Du T, Liu Y, Zhang X, Guo Y, Wang J, Zhou X, Li X, Yang G, Shi X (2022) Circular RNA screening identifies circ-ORSAH1 3p as a regulator of fast/slow myofibers in porcine skeletal muscles. Mol Genet Genom 297:87–99. https://doi.org/10.1007/s00439-021-01835-5

Cassano M, Biressi S, Finan A, Benedetti L, Omes C, Boratto R, Martin F, Allegretti M, Broccoli V, Cusella De Angelis G, Comoglio PM, Basilico C, Torrente Y, Michieli P, Sampaolesi M (2008) Magic-factor 1, a partial agonist of Met, induces muscle hypertrophy. J Biol Chem 296:100376. https://doi.org/10.1016/j.jbc.2021.100376

Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:1884–1890. https://doi.org/10.1093/bioinformatics/bty560

Chen B, Yu J, Guo L, Byers M, Wang Z, Chen X, Xu H, Nie Q (2019) Circular RNA circhmp3 promotes the proliferation and differentiation of chicken myoblast cells by sponging miR-30a-3p. Cells 8:177. https://doi.org/10.3390/cells8020177

Cheng X, Li L, Shi G, Chen L, Li C (2020) MEG3 promotes differentiation of porcine satellite cells by sponging miR-423-5p to relieve inhibiting effect on SRF. Cells 9:449. https://doi.org/10.3390/cells9020449

Choi YM, Oh HK (2016) Carcass performance, muscle fiber, meat quality, and sensory quality characteristics of crossbred pigs with different live weights. Korean J Food Sci An 36:389–396. https://doi.org/10.5851/kosfa.2016.36.3.389

Connolly M, Paul R, Farre-Garrós R, Natenak SA, Bloch S, Lee J, Lorenzo JP, Patel H, Cooper C, Sayer AA, Wort SJ, Griffiths M, Polkey MI, Kemp PR (2018) miR-424-5p reduces ribosomal RNA and protein synthesis in muscle wasting. J Cachexia Sarcopenia 9:400–416. https://doi.org/10.1007/s13279-017-01226

De Rubeis S, Pascuito E, Li KW, Fernández E, Di Marino D, Buzzi A, Ostroff LE, Klann E, Zwartkruis FJ, Komiyama NH, Grant SG, Poujol C, Chequet D, Aichsel T, Posthuma D, Smit AB, Bagni C (2013) CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron 79:1169–1182. https://doi.org/10.1016/j.neuron.2013.06.039

Dedeic Z, Cetera M, Cohen TV, Holaska JM (2011) Emerin inhibits Lmo7 binding to the Pax3 and MyoD promoters and expression of myoblast proliferation genes. J Cell Sci 124:1691–1702. https://doi.org/10.1242/jcs.080259

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA (2003) DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 4:P3

Ding L, Zhang L, Biswas S, Schugar RC, Brown JM, Byzova T, Podrez E (2017) Akt3 inhibits adipogenesis and protects from diet-induced obesity via WNK1/SKG1 signaling. JCI Insight 2:e95687. https://doi.org/10.1172/jci.insight.95687

Fadia H, Adams GR (2004) Inhibition of MAPK/ERK kinase prevents IGF-I-induced hypertrophy in rat muscles. J Appl Physiol 96:203. https://doi.org/10.1152/japplphysiol.00856.2004

Glass DJ (2005) Skeletal muscle hypertrophy and atrophy signaling pathways. Int J Biochem Cell B 37:1974–1984. https://doi.org/10.1016/j.biocell.2005.04.018

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495:384–388. https://doi.org/10.1038/nature11993

Hentze MW, Preiss T (2014) Circular RNAs: splicing’s enigma variations. EMBO J 32:923–925. https://doi.org/10.1002/embj.2013.53

Hernández-Hernández JM, Garcia-González EG, Bru CE, Rudnichi MA (2017) The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Semin Cell Dev Biol 72:10–18. https://doi.org/10.1016/j.sedev.2017.11.010

Hirata Y, Nomura K, Senga Y, Okada Y, Kobayashi K, Okamoto S, Minokoshi Y, Imamura M, Takeda S, Hosooka T, Ogawa W (2019) Hyperglycemia induces skeletal muscle atrophy via a WWP1/KLFL15 axis. JCI Insight 4:e124952. https://doi.org/10.1172/jci.insight.124952

Hong L, Gu T, He Y, Zhou C, Hu Q, Wang X, Zheng E, Huang S, Xu Z, Yang J, Yang H, Li Z, Liu D, Cai G, Wu Z (2019) Genome-Wide analysis of circular RNAs mediated ceRNA regulation in porcine embryonic muscle development. Front Cell Dev Biol 7:289. https://doi.org/10.3389/fcell.2019.00289

Hou L, Xu J, Li H, Ou J, Jiao Y, Hu C, Wang C (2017) MiR-34c represses muscle development by forming a regulatory loop with Notch1. Sci Rep 7:9346. https://doi.org/10.1038/s41598-017-09688-y

Hunter RB, Kandarian SC (2004) Disruption of either the Nfkb1 or the Bcl3 gene inhibits skeletal muscle atrophy. J Clin Invest 114:1504–1511. https://doi.org/10.1172/JCI21696

Jeck WR, Sharpless NE (2014) Detecting and characterizing circular RNAs. Nat Biotechnol 32:453–461. https://doi.org/10.1038/nbt.2890

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360. https://doi.org/10.1038/nmeth.3317
Kimball SR, Jefferson LS (2006) Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr 136:227S-S231. https://doi.org/10.1093/jn/136.1.227S

Kuo IY, Ehrlich BE (2015) Signaling in muscle contraction. CSH Perspect Biol 7:e006023. https://doi.org/10.1101/cshperspect.a006023

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923

Larzul C, Lefaucheur L, Ecolan P, Gogué J, Talmant A, Sellier P, Le Roy P, Monin G (1997) Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. J Anim Sci 75:3126–3137. https://doi.org/10.2527/1997.75123126x

Lasda E, Parker R (2014) Circular RNAs: diversity of form and function. RNA 20:1829–1842. https://doi.org/10.1261/rna.047126.114

Lefebvre V, Li P, de Crombrugghe B (1998) A new long form of Sox5 (L-Sox5). Sox5 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J 17:5718–5733. https://doi.org/10.1093/embryo/17.19.5718

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20. https://doi.org/10.1016/j.cell.2004.12.035

Li J, Johnson SE (2006) ERK2 is required for efficient terminal differentiation of skeletal myoblasts. Biochem Bioph Res Co 345:1425–1430. https://doi.org/10.1016/j.bbrc.2006.05.051

Li JH, Liu S, Zhou H, Qu LH, Yang JH (2014) starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 42:D92–D97. https://doi.org/10.1093/nar/gkt1248

Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G (2015) Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol 22:256–264. https://doi.org/10.1038/nsmb.2959

Li L, Chen Y, Nie L, Ding X, Zhang X, Zhao W, Xu X, Kyei B, Dai D, Zhan S, Guo J, Zhong T, Wang L, Zhang H (2019a) MyoD-induced circular RNA CDR1as promotes myogenic differentiation of skeletal muscle satellite cells. Beta-Gene Regul Mech 1862:807–821. https://doi.org/10.1016/j.bbgrm.2019.07.001

Li Z, Cai B, Abdalla BA, Zhu X, Zheng M, Han P, Nie Q, Zhang X (2019b) LncIRS1 controls muscle atrophy via sponging miR-15 family to activate IGF1-PI3K/akt pathway. J Cachexia Sarcopeni 10:391–410. https://doi.org/10.1002/jcsm.12374

Li B, Yin D, Li P, Zhang Z, Zhang X, Li H, Li R, Hou L, Liu H, Wu W (2020) Profiling and functional analysis of circular RNAs in porcine fast and slow muscles. Front Cell Dev Biol 8:322. https://doi.org/10.3389/fcell.2020.00322

Liang G, Yang Y, Niu G, Tang Z, Li K (2017) Genome-wide profiling of Sus scrofa circular RNAs across nine organs and three developmental stages. DNA Res 24:523–535. https://doi.org/10.1093/dnares/dsx022

Ling YH, Sui MH, Zheng Q, Wang KY, Wu H, Li WY, Liu Y, Chu MX, Fang FG, Xu LN (2018) miR-27b regulates myogenic proliferation and differentiation by targeting PAX3 in goat. Sci Rep 8:3909. https://doi.org/10.1038/s41598-018-22262-4

Ling Y, Zheng Q, Zhu L, Xu L, Sui M, Zhang Y, Liu Y, Fang F, Chu M, Ma Y, Zhang X (2020) Trend analysis of the role of circular RNA in goat skeletal muscle development. BMC Genom 21:220. https://doi.org/10.1186/s12864-020-06649-2

Liu W, Xu L, Wang Y, Shen H, Zhu X, Zhang K, Chen Y, Yu R, Limera C, Liu L (2015) Transcriptome-wide analysis of chromatin-stress responsive microRNAs to explore miRNA-mediated regulatory networks in radish (Raphanus sativus L.). Sci Rep 5:14024. https://doi.org/10.1038/srep14024

Liu R, Liu X, Bai X, Xiao C, Dong Y (2020) Identification and characterization of circRNA in Longissimus dorsi of different breeds of cattle. Front Genet 11:565085. https://doi.org/10.3389/fgene.2020.5650851

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8

Madsen L, Kristiansen K (2010) The importance of dietary modulation of cAMP and insulin signaling in adipose tissue and the development of obesity. Ann NY Acad Sci 1190:1–14. https://doi.org/10.1111/j.1749-6632.2009.05262.x

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gersgenes LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. https://doi.org/10.1038/nature11928

Mtin N, Kudla AJ, Konieczny SF, Taparovsky EJ (2001) Differential effects of Ras signaling through NFkappB on skeletal myogenesis. Oncogene 20:1276–1286. https://doi.org/10.1038/sj.onc.1204223

Murholm M, Dixen K, Hansen JB (2010) Ras signalling regulates differentiation and UCPI expression in models of brown adipogenesis. Biochim Biophys Acta 1800:619–627. https://doi.org/10.1016/j.bbagen.2010.03.008

Nguyen MT, Min KH, Lee W (2020) MiR-96-5p induced by palmitic acid suppresses the myogenic differentiation of C2C12 myoblasts by targeting FHL1. Int J Mol Sci 21:9445. https://doi.org/10.3390/ijms21244945

Nishikimi T, Iemura-Inaba C, Akimoto K, Ishikawa K, Koshikawa S, Matsuoka H (2009) Stimulatory and Inhibitory regulation of lipolysis by the NPR-A/C/GMP/PKG and NPR-C/G(i) pathways in rat cultured adipocytes. Regul Pept 153:53–63. https://doi.org/10.1016/j.regpep.2008.10.010

Olson EN, Spizz G, Tainsky MA (1987) The oncogenic forms of N-ras or H-ras prevent skeletal myoblast differentiation. Mol Cell Biol 7:2104–2111. https://doi.org/10.1128/mcb.7.6.2104

Ouyang H, Chen X, Wang Z, Yu J, Jia X, Li Z, Luo L, Abdalla BA, Jebesa E, Nie Q, Zhang X (2018) Circular RNAs are abundant and dynamically expressed during embryonic muscle development in chickens. DNA Res 25:71–86. https://doi.org/10.1093/dnares/dsx039

Possidonio AC, Soares CP, Fontenele M, Morris ER, Mody V, Costa ML, Mermelstein C (2016) Knockdown of Lmo7 inhibits chick myogenesis. FEBS Lett 590:317–329. https://doi.org/10.1002/febs.23375

Ronzoni F, Ceccarelli G, Perini I, Benedetti L, Galli D, Mulas F, Balli M, Magenes G, Bellazzi R, De Angelis GC, Sampaolesi M (2017) Met-activating genetically improved chimeric factor-I promotes angiogenesis and hypertrophy in adult myogenesis. Curr Pharm

Springer
Biotechnol 18:309–317. https://doi.org/10.2174/138920101866617021124602
Schiaffino S, Dyar KA, Ciciliot S, Blauw B, Sandri M (2013) Mechanisms regulating skeletal muscle growth and atrophy. FEBS J 280:4294–4314. https://doi.org/10.1111/febs.12253
Schiaffino S, Dyar KA, Calabria E (2018) Skeletal muscle mass is controlled by the MRF4-MEF2 axis. Curr Opin Clin Nutr 21:164–167. https://doi.org/10.1097/MCO.0000000000000456
Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504. https://doi.org/10.1101/gr.1239303
Shen L, Gan M, Tang Q, Tang G, Jiang Y, Li M, Chen L, Bai L, Shuai S, Wang J, Li X, Liao K, Zhang S, Zhu L (2019a) Comprehensive analysis of lncRNAs and circRNAs reveals the metabolic specialization in oxidative and glycolytic skeletal muscles. Int J Mol Sci 20:2855. https://doi.org/10.3390/ijms20122855
Shen X, Liu Z, Cao X, Hu H, Han S, Chen Y, Cui C, Zhao J, Li D, Wang Y, Zhu Q, Yin H (2019b) Circular RNA profiling identified an abundant circular RNA circTMTC1 that inhibits chicken skeletal muscle satellite cell differentiation by sponging miR-128-3p. Int J Biol Sci 15:2265–2281. https://doi.org/10.7150/ijbss.36412
Shen J, Hao Z, Wang J, Hu J, Liu X, Li S, Ke N, Song Y, Lu Y, Hu L, Qiao L, Wu X, Luo Y (2021) Comparative transcriptome profile analysis of Longissimus dorsi muscle tissues from two goat breeds with different meat production performance using RNA-Seq. Front Genet 11:619399. https://doi.org/10.3389/fgene.2020.619399
Shi T, Yan X, Qiao L, Li B, Cheng L, Pan Y, Jing J, Cao N, Liu W (2018) MiR-330-5p negatively regulates ovine prediploidy differentiation by targeting branched-chain aminotransferase 2. Anim Sci J 89:858–867. https://doi.org/10.1111/asj.12995
Sun L, Ma K, Wang H, Xiao F, Gao Y, Zhang W, Wang K, Gao X, Ip N, Wu Z (2007) JAK1-STAT1-STAT3, a key pathway promoting proliferation and preventing premature differentiation of myoblasts. J Cell Biol 179:129–138. https://doi.org/10.1083/jcb.200703184
Tao S, Yan X, Qiao L, Li B, Liu W (2018) MiR-330-5p negatively regulates ovine prediploidy differentiation by targeting branched-chain aminotransferase 2. Anim Sci J 89:858–867. https://doi.org/10.1111/asj.129995
Taylor MV, Hughes SM (2017) Met2 and the skeletal muscle differentiation program. Semin Cell Dev Biol 72:33–44. https://doi.org/10.1016/j.semcdb.2017.11.020
Tuma HJ, Venable JH, Wuthier PR, Henriksson RL (1962) Relationship of fiber diameter to tenderness and meatiness as influenced by bovine age. J Animalience. https://doi.org/10.2307/1235645
Turner DA (1985) Miranda: a non-strict functional language with polymorphic types. In: Conference on functional programming languages and computer architecture. Springer, Berlin, Heidelberg, p 1–16
Varendi K, Kumar A, Harma MA, Andressoo JO (2014) MiR-1, miR-10b, miR-155, and miR-191 are novel regulators of BDNF. Cell Mol Life Sci 71:4443–4456. https://doi.org/10.1007/s00018-014-1628-x
Wang Y, Wang Z (2015) Efficient backsplicing produces translatable circular miRNAs. RNA 21:172–179. https://doi.org/10.1261/rna.048272
Wang J, Ren Q, Hua L, Chen J, Zhang J, Bai H, Li H, Xu B, Shi Z, Cao H, Xing B, Bai X (2019a) Comprehensive analysis of differentially expressed mRNA, lncRNA and circRNA and their ceRNA networks in the Longissimus dorsi muscle of two different pig breeds. Int J Mol Sci 20:1107. https://doi.org/10.3390/ijms20110011
Wang Z, Zhang X, Li Z, Abdalla BA, Chen Y, Nie Q (2019b) MiR-34b-5p mediates the proliferation and differentiation of myoblasts by targeting IGFBP2. Cells 8:360. https://doi.org/10.3390/cells8040360
Wang J, Song C, Cao X, Li H, Cai H, Ma Y, Huang Y, Lan X, Lei C, Ma Y, Bai Y, Lin F, Chen H (2019c) MiR-208b regulates cell cycle and promotes skeletal muscle cell proliferation by targeting CDKN1A. J Cell Physiol 234:3720–3729. https://doi.org/10.1002/jcp.27146
Wang J, Shen J, Liu X, Li S, Luo Y, Zhao M, Hao Z, Ke N, Song Y, Qiao L (2021) Comparative analysis of meat production traits, meat quality, and muscle nutrient and fatty acid contents between Ziwuling black goats and Liaoning cashmere goats. Acta Pratacult Sin 30:166–177. https://doi.org/10.11686/cyxh2020199
Wei W, He HB, Zhang WY, Zhang PX, Bai JB, Liu HZ, Cao JH, Chang KC, Li XY, Zhao SH (2013) miR-29 targets Akt3 to reduce proliferation and facilitate differentiation of myoblasts in skeletal muscle development. Cell Death Dis 4:e668. https://doi.org/10.1038/cddis.2013.184
Wei X, Li H, Yang J, Hao D, Dong D, Huang Y, Lan X, Plath M, Lei C, Lin F, Bai Y, Chen H (2017) Circular RNA profiling reveals an abundant circLMO7 that regulates myoblasts differentiation and survival by sponging miR-378a-3p. Cell Death Dis 8:e3153. https://doi.org/10.1038/cddis.2017.541
Wu W, Wang S, Xu Z, Wang X, Feng J, Shan T, Wang Y (2018) Betaine promotes lipid accumulation in adipogenic-differentiated skeletal muscle cells through ERK/PPARγ signalling pathway. Mol Cell Biochem 447:137–149. https://doi.org/10.1007/s11010-018-3299-7
Yan XM, Zhang Z, Meng Y, Li HB, Gao L, Luo D, Jiang H, Gao Y, Yuan B, Zhang JB (2020) Genome-wide identification and analysis of circular RNAs differentially expressed in the longissimus dorsi of two different breeds with different meat production performance using RNA-Seq. Front Genet 11:619399. https://doi.org/10.3389/fgene.2020.619399
Yin H, He H, Cao X, Shen X, Han S, Cui Z, Zhao J, Wei Y, Chen Y, Xia L, Wang Y, Li D, Zhu Q (2020) MiR-148a-3p regulates skeletal muscle satellite cell differentiation and apoptosis via the PI3K/AKT signaling pathway by targeting Meox2. Front Genet 11:512. https://doi.org/10.3389/fgene.2020.00512
Zammit PS (2017) Function of the myogenic regulatory factors Myf5, MyoD, myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Semin Cell Dev Biol 72:19–32. https://doi.org/10.1016/j.semcdb.2017.11.011
Zhang Q, Wang K, Yong Z, Meng J, Yu F, Yan C, Zhu D (2010) The myostatin-induced E3 ubiquitin ligase RNF13 negatively regulates the proliferation of chicken myoblasts. FEBS J 277:466–476. https://doi.org/10.1111/j.1742-4658.2009.07498.x
Zhao X, Mo D, Li A, Gong W, Xiao S, Zhang Y, Qin L, Niu Y, Guo Y, Liu X, Cong P, He Z, Wang C, Li J, Chen Y (2011) Comparative analyses by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness. PLoS ONE 6:e19774. https://doi.org/10.1371/journal.pone.0019774

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.