Global Landscape of m6A Methylation of Differently Expressed Genes in Muscle Tissue of Liaoyu White Cattle and Simmental Cattle

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Liaoyu white cattle (LYWC) is a local breed in Liaoning Province, China. It has the advantages of grow quickly, high slaughter rate, high meat quality and strong anti-stress ability. N6 methyladenosine (m6A) is a methylation modification of N6 position of RNA adenine, which is an important modification mechanism affecting physiological phenomena. In this study, we used the longissimus dorsi muscle of LYWC and SIMC for m6A-seq and RNA-seq high-throughput sequencing, and identified the key genes involved in muscle growth and m6A modification development by bioinformatics analysis. There were 31532 m6A peaks in the whole genome of LYWC and 47217 m6A peaks in the whole genome of SIMC. Compared with Simmental cattle group, LYWC group had 17,351 differentially expressed genes: 10,697 genes were up-regulated, 6,654 genes were down regulated, 620 differentially expressed genes were significant, while 16,731 differentially expressed genes were not significant. Among the 620 significantly differentially expressed genes, 295 genes were up-regulated and 325 genes were down regulated. In order to explore the relationship between m6A and mRNA expression in the muscles of LYWC and SIMC, the combined analysis of MeRIP-seq and RNA-seq revealed that 316 genes were m6A modified with mRNA expression. To identify differentially methylated genes related to muscle growth, four related genes were selected for quantitative verification in LYWC and SIMC. GO enrichment and KEGG analysis showed that the differentially expressed genes modified by m6A are mainly involved in skeletal muscle contraction, steroid biosynthesis process, redox process, PPAR pathway and fatty acid metabolism, and galactose metabolism. These results provide a theoretical basis for further research on the role of m6A in muscle growth and development.

Keywords: m6A methylation, RNA-seq, muscle growth and development, genetic modification, species

INTRODUCTION

To date, more than 150 types of posttranscriptional modifications have been identified in the RNA of all living organisms (Boccaletto et al., 2018). The N6-methyladenosine (m6A) modification was discovered in the 1970s and was originally considered to be an abundant nucleotide modification of mRNA in eukaryotic cells (Jia et al., 2013; Yue et al., 2015). Biological functions of m6A modification are mediated...
by special binding proteins, including methyltransferases, demethylases, and effectors (Zhang et al., 2019). It is involved in various biological processes, such as lipid production and energy metabolism (Zhu et al., 2014; Wang et al., 2015a). In addition, m6A methylation regulates the splicing, expression, decay and translation of mRNA (Wang et al., 2014; Wang et al., 2015b; Xiao et al., 2016). Until recently, little was known about the specific function and mechanism of m6A. Similar to DNA and histone methylation, m6A methylation is also dynamic and reversible in mammals (Wang et al., 2019). It is modulated by several genes, including methyltransferases (METTL3, METTL4 and WTAP) (Liu et al., 2014; Ping et al., 2014), demethylases (ALKBH5 and FTO) (Jia et al., 2011; Zheng et al., 2013) and reading proteins (YTHDF, elf3 and HNRNPC) (Cao et al., 2016). m6A modification is co-installed by a variety of protein complexes (Roundtree et al., 2017). For example, YTHDF2 binds to m6A in mRNA to degrade target genes, while YTHDF1, YTHDF3 and elf3 promote the translation of m6A containing transcripts (Wang et al., 2014; Wang et al., 2015a; Meyer et al., 2015). As the transferase of writers, METTL3 is composed of catalytic subunit and many other auxiliary subunits. This protein is very important for embryonic growth and development. Embryos lacking METTL3 show pluripotent degradation and damage (Guelu et al., 2015). The distribution of mettl3 varies with the type of cell line. In some cases, the change of cell state will lead to the change of its distribution (Knuckles et al., 2017; Xiang et al., 2017). In the cytoplasm, METTL3 itself recognizes the 3′UTR m6A site on mRNA and promotes the formation of translation loop through the interaction with elf3β, so as to promote the translation of transcripts (Shi et al., 2019). METTL3 can functionally be regulated by PTM or its protein interaction. It is reported that METTL14 in human cells is phosphorylated at the residue serine399 site on the protein interface with METTL3, indicating that mettl3 is a regulatory function (Wang et al., 2016; Shi et al., 2019). m6A readers protein contains two kinds: one is a stable and direct protein containing YTH domain (YTH) domain, and the other is the common RNA binding domain (RBD) (Shi et al., 2019). Both the YTH domain family 1–3 (YTHDF1-3) and the YTH domain containing 1–2 (YTHDC1-2) in humans are stable and directly exercise the reading function of m6A.YTHDF1 and YTHDF3 translation initiation factors promote the translation of target transcripts in cells, and YTHDC2 mediates mRNA stability and translation and regulates cell development (Hsu et al., 2017). The other uses the common RNA binding domain (RBD), such as K homology (KH) domain, RNA recognition motif (RRM) domain and arginine/glycine rich (RGG) domain to preferentially bind the m6A containing region in RNA and exercise the function of m6A reader by regulating the surrounding RNA protein interaction (Shi et al., 2019). Most studies on m6A modification have focused on humans and mice (Dominiessi et al., 2012). The m6A methylation is related to obesity (Wang et al., 2020). FTO was the first m6A mRNA demethylase that was discovered. It mediates DNA and RNA demethylation (Jia et al., 2011). The m6A demethylase FTO plays a key role in regulating postpartum growth and energy consumption. A study reported that AMPK regulates lipid accumulation in skeletal muscle cells by regulating FTO expression and FTO-dependent demethylation of m6A (Wu et al., 2017). Research reports on mouse animal models have shown that FTO plays an important role in the regulation of fat mass, adipogenesis, and body weight (Goh et al., 2009; Fischer et al., 2009; Church et al., 2010; Gao et al., 2010; McMurray et al., 2013; Merkentse et al., 2015; Ronkainen et al., 2016).

LYWC are excellent beef cattle based on Charolais, breeding the fourth-generation hybrid herd with Liaoning local cattle as the female parent in Liaoning Province, China. A stable population with 93.75% Charolais pedigree and 6.25% local cattle pedigree was formed. LYWC grow quickly, and the slaughter rate was also higher than that of other beef cattle breeds. Due to the large market demand for beef in China, most farms choose LYWC for its excellent production performance. LYWC has wide adaptability, strong stress resistance, and outstanding cold resistance ability and can withstand a low-temperature environment of −30°C. Although LYWC has a better growth rate and slaughter rate, its rough myofiber always influences beef quality directly compared to Simmental and other beef cattle (Jing et al., 2011; Shuangyong et al., 2011). A large number studies have shown that m6A modification plays an important role in regulating lipid production and energy metabolism, inflammatory mechanisms and tumor formation. Based on the necessary functions of m6A modification in regulating gene expression and involving various biological processes, we speculate that m6A modification is involved in beef cattle muscle growth and development. In this study, we aimed to explore the global landscape of differentially expressed m6A methylation genes in muscle tissue between LYWC and SIMC and provide a theoretical basis for further research on the specific regulatory mechanism of unique meat quality and the optimization and selection of LYWC breeds. However, the effect, mechanism, and function of m6A modification on muscle growth and development still needs further research in the future.

**MATERIAL AND METHOD**

**Sample Collection and Ethics Statement**

Three healthy male Liaoyu white cattle and three Simmental cattle were selected for this study and provided the same feed and drinking water during the breeding period. The breeding environment conditions were identical. The average birth

![Table 1 | The primer list.](http://example.com/table1)

| Gene name | Primer |
|-----------|--------|
| MYH6-F    | 5'-AGCAGCTAGCTACGCGCTTC-3' |
| MYH6-R    | 5'-GTCAGCTTGTGACACGCCGCG-3' |
| MYOM2-F   | 5'-CGTGCTTGCAGCCGCTTTAT-3' |
| MYOM2-R   | 5'-GCTTGCGAAGGTTGTAGAACCCG-3' |
| ACTB-F    | 5'-CTCTCCAGGCTCCCCCTTTGCT-3' |
| ACTB-R    | 5'-GGGCGAGTCCTCTCTTCTGCG-3' |
| XIRP1-F   | 5'-GAAAACAAGAGGAAACGCAGACA-3' |
| XIRP1-R   | 5'-GGCCAGGGCCATCTTCCT-3' |
| TNNT1-F   | 5'-AGAAGTTTCGGGAAGGCGG-3' |
| TNNT1-R   | 5'-ACAGGGCAAGGACCTCCA-3' |
The longissimus dorsi muscle samples of two breeds of beef cattle were collected after slaughter. A 1 cm³ muscle sample was taken from the inner side of the spine near the shoulder area. After that, muscle samples were immediately stored in liquid nitrogen. All experimental procedures were approved and performed according to the guidelines of the Laboratory Animal Management Committee of Shenyang Agricultural University.

**Experimental Procedure**

Total RNA was extracted using TRIzol reagent (Invitrogen, CA, United States). The quality and quantity of total RNA were analyzed by Bioanalyzer 2100 and RNA 6000 Nano Labchip kits (Angelon, CA, United States) with value of RIN >7.0. Oligo-dT magnetic beads were used to enrich total RNA with poly(A) mRNA. Approximately 200 µg of total RNA was subjected to isolation of poly(A) mRNA with poly-T oligo-attached magnetic beads (Invitrogen). The lysed RNA fragments were then incubated with m6A-specific antibodies (No. 202003, Synaptic Systems, Germany) in IP buffer (50 mM Tris-HCl, 750 mM NaCl and 0.5% Igepal CA-630) at 4°C for 2 h with BSA (0.5 µg/µl) (1 ml). The mixture was then incubated with protein A beads and eluted with elution buffer (1×IP buffer and 6.7 mM m6A). The eluted RNA was precipitated with 75% ethanol. According to the chain-specific library prepared by the dUTP method, the eluted m6A fragment (IP) and the unprocessed input control fragment were converted into the final cDNA library. The average insert size of the paired-end library was 100 ± 50 bp. We performed paired-end 2 × 150 bp sequencing on the Illumina NovaSeq™ 6000 platform of LC-BIO Biotech Ltd. (Hangzhou, China) according to the protocol recommended by the supplier.

**Bioinformatics Analysis Process**

First, Cutadapt and local Perl scripts were used to process the data obtained from sequencing to remove low-quality sequences, contaminated sequences, and linker sequences generated by the sequencer to obtain CleanData (Martin, 2011). Fastp (v0.12.6, data quality control doi: 10.1093/bioinformatics/btp616) was used to verify sequence quality, and HISAT2(v2.0.4, alignment reference sequence: doi: 10.1038/nmeth.3317)) was used to map the read data to the *Bos taurus* genome of cattle with default parameters (Bos taurus_NCBI genome version NA) (USDA ARS) (Kim et al., 2015). The threshold settings of differential peak and differential expression are generally | log2Fc | ≥ 1 and P-val < 0.05. At the same time, qval/fdr is corrected for P-val. Exome Peak

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**TABLE 2 | Summary of reads quality control.**

| Sample     | Raw_Reads       | Valid_Reads     | Valid%  | Q20%  | Q30%  | GC%   |
|------------|-----------------|-----------------|---------|-------|-------|-------|
| LYWC1_IP   | 75018648        | 69503364        | 71.85   | 98.71 | 96.25 | 58.98 |
| LYWC2_IP   | 63985000        | 58996716        | 71.45   | 98.64 | 96.03 | 59.02 |
| LYWC3_IP   | 73355244        | 69624344        | 73.96   | 98.81 | 96.37 | 57.88 |
| SIM1_IP    | 34991688        | 32396532        | 90.24   | 98.30 | 95.29 | 57.62 |
| SIM2_IP    | 42841030        | 40364782        | 91.73   | 97.87 | 94.15 | 58.51 |
| SIM3_IP    | 50385720        | 49785152        | 96.68   | 98.20 | 94.82 | 57.03 |
| LYWC1_input| 53535846        | 52583702        | 96.52   | 97.43 | 92.96 | 57.89 |
| LYWC2_input| 73285174        | 71862022        | 96.42   | 97.35 | 92.81 | 57.35 |
| LYWC3_input| 42315990        | 41541788        | 96.24   | 97.40 | 92.95 | 56.52 |
| SIM1_input | 70936986        | 68696324        | 90.09   | 98.18 | 94.97 | 58.41 |
| SIM2_input | 96166358        | 95102418        | 98.52   | 98.11 | 94.57 | 56.15 |
| SIM3_input | 66577584        | 64093630        | 90.23   | 97.98 | 94.45 | 57.91 |

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**TABLE 3 | Summary of reads mapped to the cattle reference genome.**

| Sample     | Valid reads       | Mapped reads     | Unique mapped reads | Multi mapped reads |
|------------|-------------------|------------------|---------------------|-------------------|
| LYWC1_IP   | 69503364          | 65423613 (94.13%)| 53565538 (77.07%)   | 11858075 (17.06%) |
| LYWC2_IP   | 58996716          | 55868831 (94.87%)| 46290788 (78.46%)   | 9678043 (16.40%)  |
| LYWC3_IP   | 69624344          | 66284434 (95.20%)| 53985255 (76.96%)   | 12899179 (18.24%) |
| LYWC1_input| 32396532          | 30103051 (92.92%)| 24045688 (74.22%)   | 6057393 (18.70%)  |
| LYWC2_input| 40364782          | 36721102 (90.97%)| 29120560 (72.14%)   | 7600542 (18.83%)  |
| LYWC3_input| 49785152          | 45949312 (92.30%)| 34638134 (69.58%)   | 11311178 (22.72%) |
| SIM1_IP    | 66998264          | 62525662 (94.95%)| 53655224 (78.11%)   | 11570438 (16.84%) |
| SIM2_IP    | 95102418          | 93001052 (97.79%)| 63883053 (69.96%)   | 29518000 (30.83%) |
| SIM3_IP    | 64093630          | 60045990 (93.68%)| 47919015 (74.76%)   | 12126975 (18.92%) |
This program uses bed or bam format files to identify the m6A peak and visualize it in the UCSC genome browser or with IGV software (http://www.igv.org). MEME and HOMER were used to discover known motifs and locate the peak of the obtained motifs using a Perl script (diff_peak | log2FC|≥1, pval<0.05; call peak log2FC ≥ 1, pval<0.05) (Bailey et al., 2009; Heinz et al., 2010). ChIPseeker analyzes the scanned peak calling and annotates the peak genes (Yu et al., 2015). Then, StringTie (v1.3.4d, assembly quantity: doi: 10.1038/NBT.3122) was used to perform expression operations on all mRNA in the input database to calculate FPKM (FPKM = [total exon fragments/mapped exon readings (million) × exon length (kB)]) (Pertea et al., 2015). Using the R language package edgeR (v3.20.9, difference analysis: doi: 10.1093/bioinformatics/btp616), differentially expressed mRNAs with log2 (fold change) > 1 or log2 (fold change)<−1 and p-value<0.05 were selected, the reference genome was ARS-UCD1.2 (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/002/263/795/GCF_002263795.1_ARS -UCD1.2/) (Robinson et al., 2010). The edger input file was raw counts, and we used edger to analyze the PVAL and qval of the results, calculate fpkm values to measure the expression levels of genes, and compare the fold difference obtained by means of fpkm expression compared to the fold change. Functional enrichment we mapped differential gene functional annotations into different GO term/KEGG pathways by writing our own script, embodying the difference test as a hypergeometric test. The integration of MeRIP-seq and RNA-seq data is related through the annotation of peak, and the qualitative correlation is determined through the up/down of the two parts of regulation. Because exomepeak cannot output the quantification of peak level, it cannot calculate the correlation with the expression.

**Real-Time Fluorescence Quantitative PCR**

We tested four different genes with m6A methylation modification for qRT-PCR analysis, they are related to muscle growth and development (Kee and Hardeman, 2008; Otten et al., 2012; Flix et al., 2013; Siddique et al., 2016). We validated the methylation-modified differential genes and used
a qRT-PCR kit (Takara, Dalian, China) to reverse-transcribe the total RNA extracted from the muscle into cDNA. SYBR Green (Vazyme-Q711, China) was used to perform real-time fluorescent quantitative PCR according to the instructions. The ACTB gene was used as an internal reference gene to standardize the expression level of genes. Three trials were performed on three LYWC and three SIM muscle samples. Primer 5 was used to design four pairs of primers, the primer list is shown in Table 1. All primers span the end of the gene. The relative expression of differentially expressed genes was calculated by the \( 2^{-\Delta \Delta Ct} \) method. The data are expressed as the mean ± standard error (sample number \( n = 3 \)). The \( t \)-test in SPSS statistical software (version 22.0, Chicago, IL, United States) was used to perform the statistical analyses in the two groups, and the difference was significant when \( p < 0.05 \).

RESULTS AND ANALYSIS

Sequence Statistics and Quality Control

First, the raw data generated by sequencing needed to be preprocessed. Cutadapt filtered out unqualified sequences and removed reads with an adaptor, low quality, and unsure base information. The original sequencing volume, effective sequencing volume, Q20, Q30, and GC content were counted, and appropriate evaluation was conducted. Effective data (Clean Data) was prepared for analysis. In the MeRIP-seq library, we obtained two sets of muscle sample data reads. Three biological replicates were performed in each group, and the effective reading data were as follows: LYWC group: 75018648, 63985000 and 73353244; SIM group: 34991588, 42841030 and 50385720. The percentages of valid data (Clean Data) in the two groups are shown in Figure 2. The heat map of the enrichment of reads near the TSS and the peak distribution near the TSS at the start site of gene transcription. The distribution of peaks of original differences in gene function. Distribution of differential peak on gene functional elements.
groups of data were 71.85, 71.45, and 73.96% in the LYWC group and 90.24, 91.73, and 96.68% in the SIM group, respectively. In the RNA-seq library, we obtained two sets of muscle sample data reads, each of which was subjected to three biological replicates: the effective read data were as follows: LYWC group: 53535846, 73285174, and 42315990; SIM group: 71853823, 80695947, and 57733109. In the RNA-seq library, we obtained two sets of data with sequencing error rates less than 0.01 and the proportion of Q20% bases with a quality value ≥20 (sequencing error rate is less than 0.01) and the proportion of Q30% bases with a quality value ≥30 (the sequencing error rate is less than 0.001) are shown.

Map Data to Genome
We used HISAT2 for reference genome comparison of the preprocessed valid data and mapped reads to the Bos taurus (Bos taurus_NCBI, version NA) genome with default parameters. By comparing the obtained reads with the reference genome sequence, we can perform detailed statistics on the data obtained by sequencing and its distribution in the genome. In the m6A-seq library, the IP samples of the longissimus dorsi muscle of beef cattle were analyzed with the reference genome. In general, the percentage of the sequence alignment to the exonic region should be the highest. The results of this experiment showed that the IP samples of Liaoyu white cattle accounted for 97.54, 97.24, and 96.96% in the exonic region; the input samples were 98.08, 98.10, and 97.79%, respectively. The IP samples of Simmental cattle accounted for 96.81, 97.21, and 96.68% in the exonic region; the input samples were 96.86, 97.71, and 96.94%, and the results are shown in Figure 1.

Identification of m6A Modification Sites and Analysis of Differentially Methylated Genes
Use peak-calling software, the R language toolkit exomePeak, was used to scan the m6A peak in the entire genomic dataset. Based on the identification of the IP and input libraries, we performed further filtering to obtain the differential peaks and perform GO and KEGG enrichment analyses. In general, the default p < 0.05 was the filter condition of the peak. Compared with the Simmental cattle group, we screened 5631 difference peaks in Liaoyu white cattle, of which 4,059 m6A peaks were significantly up-regulated and 66 m6A peaks were significantly down regulated, as shown in Figure 2B. The distribution of m6A peaks is shown in Table 2.
peak in transcripts was analyzed. Analysis of the distribution of m6A peak in the transcript was performed. We divided the transcript into four parts: 5'-UTR, 3'-UTR, first exon and other exons. It was used to analyze the distribution of different peaks in the original gene function, as shown in Figure 2C. As shown in Table 4, the m6A modification was mainly enriched in the 3'-UTR, and we report the top 20 differences m6A peak. Difference factor <1 indicates hypomethylation, and difference factor >1 indicates hypermethylation.

**Motif Analysis**
As a dynamic modification phenomenon, RNA methylation is mainly accomplished by the combined action of multiple methylases and methylation binding site motifs. A motif is a nucleotide sequence pattern with biological significance, and the sequence has a high degree of conservation. The methylases involved in the process of RNA methylation recognize the motifs in the gene to generate methylation and regulate gene expression. The motif software MEME was used to search for more credible motifs in the peak area and obtain information about the width, E-value, and location of each motif. We performed motif prediction on each set of samples, and the results are shown in Figure 3. A motif structure that is reported commonly in RNA modifications are RRACH (where R = A or G, H = A, C or U).

**Whole Gene Analysis and Differential Gene Analysis**
The expression level of genes is mainly measured by RPKM (reads per kilobase of exon model per million mapped reads) or FPKM (fragments per kilobase of exon model per million mapped reads) to measure the abundance value of gene expression. In our research, we chose FPKM to report the expression abundance values of different samples of known genes. Compared with Simmental cattle, Liao yu white cattle detected 17,351 differentially expressed genes, 620 genes were significantly different and 16,731 genes were not significantly different (|log2fc| ≥ 1 and p < 0.05). Among the differentially expressed genes, 10,697 genes were upregulated and 6,654 genes were downregulated. Among the 620 significantly differentially expressed genes, 295 were up-regulated and 325 were downregulated. Table 5 shows the top 20 differentially expressed genes we screened. Among the top 20 differentially expressed genes, there are 10 up-regulated genes and 10 down-regulated genes. We used Figures 4A,B to show the gene expression and expression density. We plotted the overall distribution statistics of differentially expressed genes, as shown in Figure 4C. Figure 4D shows the gene heat map between LYWC and SIM samples.

**Joint Analysis of Differentially Expressed Genes and Differentially Expressed Genes**
In the entire transcriptome sequencing, we found that there were upregulated and downregulated genes. In the MeRIP-seq sequencing results, according to the changes in abundance, we found that the methylation of the gene itself was upregulated and downregulated. Therefore, we combined the correlation analysis of the two sequencing results to compare and analyze the transcription level and methylation level. In the
samples of the LYWC group, 13,624 genes have been modified by m6A, and in the samples of the SIMC group, 24,522 genes have been modified by m6A. We found that among the differentially expressed genes, 620 genes were significantly expressed. Based on this, we screened 316 genes with significant differential expression and m6A methylation modification. The result is shown in Figure 5. Since this experiment mainly explored the regulation of muscle growth and development, we screened four candidate genes related to muscle cell growth and development, as shown in Table 6. The m6A regulation of these genes was upregulated, while gene regulation was downregulated. At the same time, the difference between the two sets of samples was significant.

**GO Analysis and KEGG Pathway Analysis of Differentially Expressed m6A Methylation Genes**

To deeply study the significance of m6A modification in physiological and biochemical processes, we conducted GO (http://www.geneontology.org/) and KEGG (http://www.kegg.jp/) analyses of the different peaks of m6A. The peaks selected were enriched with 898 GO items and 162 pathways. Figure 6A shows the top 25 items in biological processes, the top 15 items in cell components, and the top 10 items in molecular functions. GO analysis (Figure 6B) showed that differentially methylated genes significantly enriched fibers in fat granule tissue, skeletal muscle contraction, and muscle contraction. KEGG pathway analysis (Figure 6C) showed that differentially methylated genes were related to the p53 signaling pathway and PPAR pathway. At the same time, they are also involved in biological processes such as galactose metabolism, fatty acid metabolism, adipocytokine pathway, nitrogen metabolism, arginine synthesis, etc.

**Verification of Differentially Expressed Genes**

To study the function of gene m6A modification and determine the key genes that regulate muscle growth and development in beef cattle muscle cells, We used qRT-PCR for experimental verification. RNA-seq results showed that among the screened differential genes, the expression of Liaoyu white cattle group was lower than that of Simmental cattle group. The qRT-PCR results also confirmed that the m6A-modified gene is indeed present in the Liaoyu white cattle muscle. The trends of these genes are consistent with the RNA-seq results (Figure 7).

**DISCUSSION**

The modification of m6A is involved in many physiological processes, such as: Mediates mRNA output and synthesis, affects cell maturation, lipogenesis, maintains embryonic development stability, affects cell circadian rhythm, regulates physiological and biochemical processes such as division. At the same time, the modification of RNA methylation is a dynamic change (Jia et al., 2011) (Zheng et al., 2013) (Feng et al., 2018). m6A RNA modification is dynamically regulated by methyltransferases (writers) and demethylases (erasers). Since m6A bases cannot be directly detected by sequencing, the dissection of the m6A landscape is hindered; they do not change the base pairing properties and cannot be distinguished from conventional bases by reverse transcription (Brocard et al., 2017). Recently, new methods based on m6A immunoprecipitation or modified selective RNA chemistry methods to isolate modified RNA fragments coupled with high-throughput sequencing, namely,
m6A-seq and MeRIP-seq, have identified thousands of hundred-nucleotide fragments containing modifications in the transcriptomes of mammalian cells (Dominissini et al., 2012; Meyer et al., 2012). Modification of m6A has been successively discovered in many animals, plants, bacteria and other microorganisms.
Based on numerous research reports, it is found that the majority of cows mammary gland and lymphocytes undergo m6A modification phenomenon (Burtseva et al., 1979; Horowitz et al., 1984; Wu et al., 2021). However, there are still few reports on beef cattle. We speculate that m6A is involved in the muscle growth and development process of beef cattle. Our data show that there are a large number of methylation modifications during muscle growth and development. It may have an important effect on the types of muscle fibers, the maturation of muscle cells, and the changes in muscle structure.

Through laser-induced microdamage of zebrafish muscles combined with cell repair, it was found that the XIRP1 gene is abundant in skeletal muscle and involved in cell repair, cells and new myofibrils, and the repair of damage does not involve cell proliferation (Otten et al., 2012). Troponin T (TNNT1) exists as a group of homologous proteins in the striated muscle of vertebrates and invertebrates. Mutations in the TNNT1 gene can cause rod-shaped myopathy. From animal model experiments, it was found that lack of TNNT1 reduced the content of slow fibers, accompanied by type II fiber hypertrophic growth and increased muscle fatigue (Feng et al., 2009; Wei et al., 2014). Myosin 2 (MYOM2) is the main component of the myofibril M-band of the sarcomere and the central gene in the interaction of sarcomere genes (Auxerre-Plantie et al., 2020). Research by Auxerre-Plantie found that loss of function and moderate knockdown of this gene can lead to myocardial expansion, and severe knockdown can lead to increased sarcomeric myosin (Auxerre-Plantie et al., 2020). Research by Andrei found that in hypothyroid rats, MYOM2 expression increased 3.4 times. Through small-molecule interference of RNA with MYOM2, it was found that the contraction speed of cardiomyocytes were severely reduced (Rozanski et al., 2013). Myosin heavy chain 6 (MYH6) is widely found mainly in the heart and smooth muscle. This gene is mainly expressed in type I fibers. The presence or absence of MYH6 and its family gene MYH7 determines the slow or fast-twitch phenotype of skeletal muscle (Stuart et al., 2016).

Based on GO enrichment and KEGG pathway analysis, we speculate that m6A modification in genes has potentially important functions and may play a vital role in certain pathways are involved in cell growth and development. Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors activated by fatty acids and their derivatives. They are ligand-activated receptors in the nuclear hormone receptor family. Three subtypes have been found in different species, which control many intracellular metabolic processes. The subtypes include PPARα (also known as NR1C1). PPARα participates in the liver and skeletal muscle through regulation and expression of lipid metabolism genes. PPARβ/δ participates in lipid oxidation and cell proliferation. PPARγ promotes the differentiation of adipocytes to enhance blood glucose uptake. PPAR transcriptional activity can be regulated by nongene crosstalk with phosphatases and kinases, including ERK1/2, p38-MAPK, PKA, PKC, AMPK and GSK3. At the same time, nuclear receptor coactivator (coactivator) and PPAR-RXR act synergistically and complement and stabilize the active transcription complex, which can regulate lipid metabolism and fat formation, maintain metabolic homeostasis and the expression of inflammation genes, and induce anticancer effects in a variety of human tumors.

The m6A regulation level of the differentially expressed genes screened in this study was negatively correlated with the transcription level. The RNA-seq results showed that the differential gene expression in LYWC was lower than that in SIM. The results of qRT-PCR showed that the differentially expressed genes for m6A methylation were all present in the transcription level. The RNA-seq results showed that the differentially expressed genes for m6A methylation were all present in the muscle tissue of beef cattle. Therefore, this indicates that m6A modification not only participates in the process of muscle growth and development but may also regulate mRNA degradation.

Skeletal muscle development is a complex biological process. The regulatory role of myogenic regulatory factors and the study of apparent modifications, including DNA methylation and histone modification, in the regulation of skeletal muscle development have given us a preliminary understanding of the regulatory network of skeletal muscle development. Based on the involvement of m6A in the regulation of mouse brain development, fat formation, and other tissue development processes, we speculate that m6A is also involved in the regulation of skeletal muscle development (Zhao et al., 2014; Ma et al., 2018; Wang et al., 2018). Studies have shown that the regulation of METTL3 gene expression and regulation of m6A levels in myoblasts affect the differentiation process of myocytes and the expression of key regulatory genes (Kudou et al., 2017; Chen et al., 2019). This shows that m6A is involved in the regulation of muscle cell differentiation. During the development of animal embryos and the growth and development of brain tissue after birth, neural stem cells are required for differentiation and self-
### TABLE 6 | m6A-modified candidate genes related to muscle cell growth and development.

| Gene name | Gene ID | M6A regulation | Gene regulation | FPKM.LYWC_input | FPKM.SIM_input |
|-----------|---------|----------------|----------------|-----------------|----------------|
| TNNT1     | 282095  | up             | Down           | 6852.32         | 14067.95       |
| MYOM2     | 524077  | up             | Down           | 603.17          | 990.94         |
| XIRP1     | 509670  | up             | Down           | 235.30          | 1260.87        |
| MYH6      | 100296004 | up         | Down           | 156.23          | 508.30         |

### FIGURE 6 | m6A differential peak gene ontology enrichment analysis and KEGG pathway analysis. (A) Main enrichment 3 and meaningful GO entries of m6A peak. (B) The first 20 items have significantly enrichment GO terms. (C) The first 20 enriched pathways of the m6A peak.
In conclusion, this study analyzed m6A methylation modification in the muscle tissue of Liaoyu white cattle and Simmental cattle. Based on our experimental results, we speculate that m6A modification plays an important role in muscle growth and development. This study shows that TNNT1, XIRP1, MYOM2, and MYH6 are likely to play a key role in muscle growth and muscle differentiation. In addition, the data obtained through high-throughput sequencing provide a theoretical basis for further exploring the function of m6A modification on muscle growth and development. At the same time, the regulatory mechanism of m6A modification in muscle still needs to be studied in depth in the future.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI [accession: PRJNA778440].

ETHICS STATEMENT

The animal study was reviewed and approved by Laboratory Animal Management Committee of Shenyang Agricultural University.

AUTHOR CONTRIBUTIONS

PL and LL conceived and designed the study. YD and QD performed the main experiments. BW, JS, GC, and WX performed data analysis. SY, MZ, and BW performed sample collection. PL and LL conceived and designed the study. YD and QD performed the main experiments. BW, JS, GC, and WX performed data analysis. SY, MZ, and BW performed sample collection. BW, JS, GC, and WX drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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