Research article

Transcriptome profiling reveals differential expression of genes potentially involved in muscle and adipose tissue development of cattle

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ABSTRACT

Background: To identify differentially expressed genes (DEGs) between muscle and adipose in cattle, we analyzed the data from the RNA sequencing of three Angus × Qinhuang crossbred cattle.

Results: Searched the Gene Expression Omnibus (GEO) for a microarray dataset of Yan yellow cattle, GSE49992. After the DEGs were identified, we used STRING and Cytoescape to construct a protein–protein interaction (PPI) network, subsequently analyzing the major modules of key genes. In total, 340 DEGs were discovered, including 21 hub genes, which were mainly enriched in muscle contraction, skeletal muscle contraction, troponin complex, lipid particle, Z disc, tropomyosin binding, and actin filament binding. Conclusions: In summary, these genes can be regarded as candidate biomarkers for the regulation of muscle and adipose development.


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1. Introduction

The quality of meat is affected by a higher fat content [1], which has an important impact on the biological composition and affects the juiciness and flavor. Currently, consumers are paying more attention to their health and demanding better tasting and healthier meat. Fat is made up of large clusters of adipocytes and primarily functions in animals as energy storage and as an endocrine organ. Fat is of importance in the regulation of metabolism and fat deposition, and it can improve animal health and meat quality [2,3].

Adipocytes mainly gather under the subcutaneous, intermuscular, visceral, and mesentery connective tissue, and some are scattered between and inside the muscle bundle; while on one hand, intramuscular or marbling fat plays a vital role in improving flavor and palatability of meat, on the other, subcutaneous and visceral fat pads are considered to be of no worth [4]. When subjected to texture profile analysis (TPA), compared with the high-fat control, the chewability of the low-fat sample increased, while the hardness and elasticity remained unchanged [5]. In many cases, muscle and fat selection are product-driven. Previous research has shown that after adjusting for intramuscular fat, Wagyu had more intense flavor and higher tenderness and juiciness compared to Angus [6]; for example, at the level of gene regulation, KLF3 gene [7] and SIRT1 gene [8] alter intramuscular fat content in cattle. Muscle and adipose functionality are affected by a comprehensive genetic regulatory network. Biological explanations, especially for DEGs, remain a challenge with RNA-seq. Further research is required to discover the genes and their mechanisms which make a difference in bovine muscle and fat function, particularly through mining hub genes (biomarkers). In this study, integration with functional enrichment, pathway analysis, and a protein–protein interaction (PPI) network was used to analyze DEGs between the longissimus dorsi muscle and adipose tissues.

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2. Materials and methods

2.1. Experimental cattle

The cattle for this experiment came from the breeding ground of the National Beef Cattle Improvement Center (Yangling, China), with the same feeding conditions as supported by the ethics committee of the Institutional Animal Care and Use Committee (Northwest A&F University, China). Three 18-month-old Angus × Qinhuann crossbred cattle were slaughtered, and the longissimus dorsi and subcutaneous adipose tissues over the 12th to 13th rib over the midline of the carcass were sampled and stored at −80°C. The experiment was conducted on the longissimus dorsi muscle tissue and subcutaneous adipose tissue group, with three replicate animals for each tissue identified using the following code: RAN-Q-M102, RAN-Q-M12, RAN-Q-M89, and RAN-Q-F102, RAN-Q-F12, RAN-Q-F89.

2.2. RNA extraction, sequencing, and mapping

For the procedure of extraction of total RNA and sequencing, we referred to Mei et al. [9]. Then, after using the FASTX-Toolkit [10] to control the quality of raw reads and removing the reads containing the adapter, reads containing over 10% poly-Ns, and reads of low quality (>50% of bases with Phred scores <10), we mapped the extracted sequence to the reference genome (UMD3.1) of cattle with TopHat v2.0.9 [11] and Bowtie v2.0.6 [12] using the default parameters.

2.3. Microarray data

One dataset [GSE49992] (GPL2112 platform, Affymetrix Bovine Genome Array) was downloaded from the Gene Expression Omnibus (GEO) [13], where a total of nine bulls of the same breed (Yan yellow cattle) were included, and the dataset contained three longissimus dorsi muscle tissues samples, three subcutaneous adipose tissues samples, and three abdominal adipose tissues samples.

2.4. Identification of DEGs

The DESeq (1.18.0) [14] was used for analyzing DEGs from the transcriptome data of crossbred cattle. Meanwhile, the DEGs between muscle tissue and adipose tissue samples downloaded from GEO datasets were analyzed using GEO2R, an interactive web tool, with |logFC| > 2 and adj. P-value < 0.001 as the criterion of significance. Downloaded data from GEO were divided into three groups: the LodAba group, for the longissimus dorsi muscle and abdominal adipose tissues; the LodSua group, for the longissimus dorsi muscle and subcutaneous adipose tissues; and the LodAdi group, for the longissimus dorsi muscle and adipose tissues.

2.5. GO enrichment analyses and KEGG of DEGs

We used the DAVID online database (version 6.8; http://david.ncifcrf.gov) [15] to analyze the function, biological processes, and KEGG of DEGs to provide comprehensive information regarding the gene and protein functions. To analyze the DEGs, FDR <0.05 was considered as the criterion of significance.

2.6. PPI network and module analyses

For the DEGs, we used the STRING online database [16] to predict the PPI network, with an interaction score > 0.4. Then, we used Cytoscape (version 3.7.1) [17] and Molecular Complex Detection (MCODE) (version 1.5.1) [18] to cluster key modules from the PPI network by the default parameters. Subsequently, the functional major module genes were analyzed using DAVID. Meanwhile, the R visualization package GOPlot [19] was used to obtain a visualization of the relationships between genes and the functional categories.

3. Results

3.1. RNA-Seq data analyses

We obtained a total of 260.9 million clean reads, with approximately 88.71% of these reads aligned to the reference genome, of which 70.07% were unique (Table 1).

3.2. Discovered DEGs in muscle tissue and adipose tissue

Through standardization of the results, the DEGs (889 in LodAba group, 912 in LodSua group, 957 LodAdi group from Yan yellow cattle, and 1832 in AnQin group from Angus × Qinhuann crossbred cattle) were confirmed, along with the overlap of four groups, including 340 genes displayed in a Venn diagram (Fig. 1A).

3.3. Enrichment and KEGG analyses of DEGs

For the gene ontology (GO) term analyses of biological processes (BP), DEGs were significantly clustered in skeletal muscle contraction (GO:0000309), myofibril assembly (GO:0030239), gluconeogenesis (GO:0006094), and muscle contraction (GO:0006936) (Table 2). Cell components (CC) of DEGs were significantly clustered in the Z disc (GO:00030018), extracellular exosome (GO:0070062), troponin complex (GO:0005861), and blood microparticle (GO:0072562) (Table 2). Molecular functions (MF) were clustered and enriched in actin filament binding (GO:0051015), actin binding (GO:0003779), calcium ion binding (GO:0005509), and FATZ binding (GO:0051373) (Table 2). KEGG pathway analyses showed that DEGs were greatly enriched in adrenergic signaling in cardiomyocytes (bta04261), pertussis (bta05133), cardiac muscle contraction (bta04260), and carbon metabolism (bta01200) (Table 2).

3.4. PPI network and module analyses

The PPI network of DEGs was produced using STRING (Fig. 1B), and core modules were generated using Cytoscape (Fig. 1C–E). Functional enrichment analyses of these modules’ genes were mainly enriched in skeletal muscle contraction (GO:0006936), muscle contraction (GO:0000309), troponin complex (GO:0005861), and lipid particle (GO:0005811), Z disc (GO:0030018), troponymosin binding (GO:0055253), and actin filament binding (GO:0051015) (Table 3, Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clean reads</th>
<th>Total mapped (%)</th>
<th>Uniquely mapped (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAN-Q-M102</td>
<td>42,087,136</td>
<td>87.7%</td>
<td>67.0%</td>
</tr>
<tr>
<td>RAN-Q-M12</td>
<td>27,164,290</td>
<td>86.4%</td>
<td>67.0%</td>
</tr>
<tr>
<td>RAN-Q-M89</td>
<td>49,965,854</td>
<td>87.5%</td>
<td>66.9%</td>
</tr>
<tr>
<td>RAN-Q-F102</td>
<td>47,120,010</td>
<td>91.5%</td>
<td>73.7%</td>
</tr>
<tr>
<td>RAN-Q-F12</td>
<td>47,828,122</td>
<td>89.6%</td>
<td>72.9%</td>
</tr>
<tr>
<td>RAN-Q-F89</td>
<td>46,766,436</td>
<td>89.8%</td>
<td>72.7%</td>
</tr>
<tr>
<td>Total/Average</td>
<td>260,931,848</td>
<td>88.7%</td>
<td>70.0%</td>
</tr>
</tbody>
</table>
Fig. 1. Venn diagram of the protein–protein interaction (PPI) network and the most significant module of differentially expressed genes (DEGs). (A) The DEGs were selected with a fold change >2 and adj. \( P \)-value < 0.001 among the four groups: the LodAba group, for the longissimus dorsi muscle and abdominal adipose tissues; the LodSua group, for the longissimus dorsi muscle and subcutaneous adipose tissues; the LodAdi group, for the longissimus dorsi muscle and adipose tissues; and the AnQin group, for the Angus × Qinhuai crossbred cattle longissimus dorsi muscle and subcutaneous adipose tissues. (B) The PPI network of DEGs was constructed by STRING. (C-E) Molecular Complex Detection (MCODE) modules from DEG screening.
In conclusion, this study showed that the following results: (1) a total of 340 DEGs, which included 21 hub genes, were identified, and the hub genes can be regarded as candidate biomarkers for muscle and adipose functionality; and (2) these hub genes revealed their functional roles in lipid metabolism which may be useful for modifying sensory attributes which are associated with fat in the meat such as the flavor and texture. Due to the lack of other studies in this area, the mechanisms responsible for these effects remain unknown and require further research. These results should help to better understand the genetic and physiological mechanisms that regulate muscle tissue and subcutaneous fat expression and might be useful for cattle breeding.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

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References


