Biochemical and Biophysical Research Communications xxx (2015) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep

Xiangyang Miao*, Qingmiao Luo, Xiaoyu Qin, Yuntao Guo, Huijing Zhao

Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100193, China

ARTICLE INFO

Article history:
Received 17 September 2015
Accepted 23 September 2015
Available online xxx

Keywords:
Sheep
Adipose
RNA-seq
Gene ontology
Metabolism

ABSTRACT

Small Tail Han and Dorset sheep are two different sheep with distinguished morphologies in fat depositions. In order to characterize their gene expression profiles, our present study took the advantages of RNA sequencing technology with the aims to identify important genes regulating the metabolisms in adipose tissues of two different sheep. In obtained high quality sequencing reads, 85.9 (Han) and 86.1% (Dorset) were uniquely aligned to Oar v3.1 sheep reference genome, and over 76% of bases in mapped reads corresponded to mRNA. Using R package EBSeq, we identified 602 differentially expressed genes. Using the 602 genes, GO analysis showed that 30 out of 56 significantly enriched biological processes were metabolism related, of which the most significant one was triglyceride biosynthetic process. The KEGG pathway analysis indicated the down-regulation of several fat metabolic pathways. The predominant down-regulation of massive metabolic processes, particularly the lipid metabolism, in adipose tissues of Han sheep could explain, at least in part, the distinguished fat deposition between two different sheep, and our data constitute a basic picture of transcriptomes in these sheep for better understanding of underline biological mechanism in their lipid metabolisms.

1. Introduction

Meat is an important protein source in the world, and fat is an important component in meat quality and impacts animal productivity [7,12]. Adipose tissue is one of the principal tissues involved in the regulation of fat deposition and lipid metabolism in domestic animals, such as pigs and sheep. Therefore an understanding of the molecular regulation of lipid metabolic processes is pivotal in the development of strategies to manipulate adiposity and improve sheep quality. Being a great part of the global agricultural economy, sheep is one of the major meat sources for human health.

A variety of sheep breeds around the world provide a great resource for meat market [5,32]. Dorset is a sheep bred in America, with medium size of body having good muscle conformation to produce a desirable carcass. The Small Tail Han sheep (Han) are excellent local breeds in China, with good flavor of meats and plentiful fat concentrated in the tail area. Contrast to Dorset sheep being of rapid growth, Han sheep is of high prolificacy with the mean litter size of 2.61 [28], compared to 1.45 for Dorset [3]. Significant differences in fat deposition between these two breeds have lured increasing interest in the characterization of genetic profiles in these sheep [29,30,32]. However, the factors and mechanisms responsible for the differences in fat deposition have not been elucidated completely.

Global gene expression profiling has been for over a decade mainly using microarray analysis [8,9]. In recent years, however, deep sequencing of transcriptomes is increasingly being utilized with promises of being higher sensitivity in identification of differential expression as well as holding opportunities to discover novel transcripts ([13,20–22,33]). Recently, the next-generation sequencing (NGS) technology has been employed to explore differentially expressed genes in the adipose tissues in pigs [2], cattle [14,18]. There have been no transcriptome studies of adipose tissues except for fat tails in sheep. Furthermore, understanding of sheep adipose regulation at the molecular level is still limited. Therefore, the regulation of fat deposition in sheep at a genome-wide level remain to be identified.

* Corresponding author.
E-mail address: mxy32@sohu.com (X. Miao).

http://dx.doi.org/10.1016/j.bbrc.2015.09.129
0006-291X/

In order to characterize the gene expression profiles in these sheep breeds, our present study took the advantages of RNA sequencing technology. With gene ontology and pathway analyses, a comprehensive genome wide gene expression profiling was conducted with the aims to identify important genes regulating the metabolisms in adipose tissues of the two different breeds of livestock sheep. As studies on sheep fat transcriptomes that employed RNA sequencing technology are relatively rare as far, results from our current study will constitute a basic picture of the sheep gene expression profiles.

2. Materials and methods

2.1. Animals

All procedures involving animals were approved by the animal care and use committee at the respective institution where the experiment was conducted. All experiments were performed in accordance with relevant guidelines and regulations set by Ministry of Agriculture of the People's Republic of China. Dorset and Small Tail Han sheep at 2 years old were obtained from Qingdao Aote Sheep Farm (Shandong, China). All animals were raised in the same way under the condition of free access to water and food in natural lighting. Adipose tissue samples from the backfat of the sheep were sampled, immediately frozen in liquid nitrogen, and stored at −80 °C until required for RNA isolation. For gene expression studies, six sheep within each breed (three were males and three were females) were randomly selected, which were as close as possible to the median average daily weight and carcass weight of their group.

2.2. Preparation of fat tissues

Samples of subcutaneous adipose tissue were obtained within 30 min after slaughter, and cut into pieces of 2 mm × 2 mm × 2 mm. These small pieces were quickly placed into RNA preservation solution (RNA-later, Life Technologies, Carlsbad, CA) for 24 h at room temperature, and then the tissue blocks were transferred to −80 °C freezer for longer preservation.

2.3. Library preparation and RNA sequencing

The fat tissues were disrupted with liquid nitrogen and total RNA was extracted with Trizol reagents (Cat.#15596026, Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. The quality and quantity of the RNA samples were assessed on a Bioanalyzer 2100 system using an RNA 6000 Nano kit (Agilent Technologies, Palo Alto, CA). Approximately 1 μg of RNA from each sample was used to generate RNA-Seq cDNA libraries for sequencing using the TruSeq RNA Sample Prep Kit v2 (Illumina Inc., San Diego, CA). Sample preparation followed the manufacturer's protocol with a workflow that included isolation of polyadenylated RNA molecules using poly-T oligo-attached magnetic beads, enzymatic RNA fragmentation, cDNA synthesis, ligation of bar-coded adapters, and PCR amplification. DNA size and purity of the cDNA library were checked using a high sensitivity DNA 1000 kit on a Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA), and quantification of the cDNA libraries was performed with Qubit™ dsDNA HS kit on Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA). The cDNA libraries were then diluted to 4 nM, and a 120 μl aliquot was used to generate clusters on a paired-end flow cell using the cBOT (Illumina) and sequenced on the Illumina Genome Analyzer IIx (GAIIx) using the SBS 36-cycle Sequencing Kit (v5) at Shanghai Biotechnology Corporation (Shanghai, China), according to manufacturer-recommended cycling parameters. One lane for samples from each species was sequenced as 100-bp reads, and image analysis and base calling were performed with SCS2.8/RTA1.8 (Illumina). FASTQ file generation and the removal of failed reads were performed by CASAVA ver.1.8.2 (Illumina).

2.4. Reads mapping

Illumina Genome Analyzer outputs base-calling files that were converted to FASTQ files with quality scores for reads mapping. Adaptor sequences and low quality sequences were removed from the original reads by fastx_toolkit (v0.0.13.2, downloadable at http://hannonlab.cshl.edu/fastx_toolkit/), and then raw reads for each sample were mapped to the sheep reference genome Oar_v3.1 with TopHat (v.2.0.4) using Bowtie (v2.1.0) mapping algorithm [27].

2.5. Determination of differential expression

Differentially expressed genes were identified by an R package "EBSeq" using raw gene counts as input and quantile normalization was applied for variable library sizes [19]. EBSeq is an empirical Bayesian approach that models a number of features observed in RNA-seq data. A list of differentially expressed genes were generated at a 2-fold difference and controlled with false discovery rate (FDR) at 0.05 in an experiment comparing two biological conditions without replicates. When replicates are not available, EBSeq estimates the variance by pooling similar genes into a certain number of bins, and this approach works well when there are no more than 50% differentially expressed genes in the data set [19].

2.6. Annotations of differentially expressed genes

In order to extract biological meanings from list of differentially expressed genes, we conducted gene enrichment analysis with Gene Ontology (GO) [10] and Kyoto Encyclopedia of Genes and Genomes databases (KEGG, http://www.genome.jp/kegg) [16,17].

2.7. Quantitative PCR (qPCR)

Firstly, reverse transcription was carried out from 0.5 μg of the same RNA samples using PrimerScript RT reagent Kit (Cat. # DRR037A, TaKaRa, Japan), and then the synthesized cDNAs were used as template in qPCR. SYBR Green chemistry was utilized to measure the gene expression level, and the qPCR reactions were performed in Roche LightCycler 480 II system, with a 10-μl reaction consisting of 5 ng of cDNA, 200 uM of forward and reverse primers each, and 2 μl of 5X SuperReal PreMix (SYBR Green) (Cat# FP205, TIANGEN Biotech, Beijing, China). The qPCR program was 95 °C for 15 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, and a final stage of dissociation analysis. The comparative Ct method and internal control gene GAPDH were used in calculation of relative expression level of genes. All qPCR reactions yielded a single peak on the dissociation curve, indicating specific amplifications achieved with those primers designed by Generay Biotech (Shanghai, China).

3. Results

3.1. Summary of the raw sequence reads

In the present study, two cDNA sequencing libraries were constructed using fat tissues from Han and Dorset sheep. With quality filter at Q-score >30, we obtained 55.17 and 63.65 million raw sequence reads per sample from Han and Dorset libraries, respectively (Table 1), of which, 85.9% (47.39/55.17 million reads, Han) and

Please cite this article in press as: X. Miao, et al., Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.09.129