SOLEXA SEQUENCING AND BIOINFORMATICS ANALYSIS ON MICRO-RNA FROM THE RABBIT MUSCLE

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ABSTRACT

As a new regulatory factor, miRNA is involved in the growth and development of skeletal muscle and the differentiation of muscle cells through specific binding with target genes. In this study, miRNA-sequencing was performed on the longissimus dorsi muscle of three 30-day-old male rabbits using Solexa platform, aiming to evaluate the data quality of high-throughput sequencing in the systematic identification of miRNA in the muscles of meat rabbits, and analyze the expression profile of miRNA in the muscle tissues of meat rabbits. Only 2.6% of exon and intron sequences were aligned, which fully indicated that the constructed miRNA libraries were of high quality. miR-1, miR-133 and miR-206 were highly expressed in the muscles of meat rabbits. High expression of miRNA might play an important role in myogenic differentiation, muscle repair and energy metabolism of skeletal muscle. The specific highly expressed miRNA in muscle tissues identified in this study can be used as an important reference for further functional studies.

Key words: rabbit; muscle; microRNA; sequencing; expression;

INTRODUCTION

Meat rabbit is not only an important source of meat, but also an ideal animal model for many human diseases research. Rabbit meat is characterized by high protein, high lecithin, high amino acid and low cholesterol, which makes it an ideal source of meat protein for many patients with chronic diseases (obesity, cardio-cerebrovascular diseases, diabetes mellitus). Therefore, rabbit has an irreplaceable position in animal husbandry and scientific research (Piles et al., 2000). The growth and development of muscle not only affect the meat production, but also affect the meat quality, energy metabolism and blood circulation system (Horak et al., 2015). Current studies have shown that multiple genes participate in the growth and development of rabbit muscle and affect the function of muscle, thus affecting the important economic traits of livestock and poultry (Callis et al., 2007). Li used SOLiD and Solexa high-throughput sequencing platforms to detect miRNAs in the brains and hearts of rabbits (Li et al., 2011). A total of 464 miRNA precursors and 886 mature miRNAs were detected. There are differences in the expression of some miRNAs and their isomers in brain and heart. Zhao et al. conducted non-coding RNAs analysis on the three different development stages of hair follicles in Angorarabbits, and found 97 miRNAs, of which miR-320-3p was involved in the hair follicle development process. There are few studies on the systematic discovery and identification of miRNA in rabbit, especially the expression profile analysis in muscle tissue. In this study, Solexa technology was used to evaluate the accuracy and reliability of high-throughput sequencing technology in the systematic identification of miRNA in rabbit muscle tissue, and specific miRNA related to muscle development was screened out, which laid a foundation for further revealing the mechanism of miRNA in the process of rabbit muscle development.

MATERIALS AND METHODS

Sample collection and preparation

The rabbits were 3 New Zealand white rabbits (male rabbits) born on the same day, which were raised in the rabbit farm of Sichuan Academy of Science Animal. Under the same feeding and management conditions, the animals were raised to 30 days old and slaughtered. After slaughtering, the longissimus dorsi muscle was quickly collected and put into a centrifuge tube without RNasee, which was immediately put into liquid nitrogen for preservation for the extraction of total RNA.

Extraction of total RNA

The total RNA was extracted by one-step method with Trizol ® reagent (Invitrogen, San Diego, CA, USA) kit, and the operation steps were carried out according to the operation instructions. The degradation and integrity of total RNA was monitored on 1% agarose gels. Three muscle total RNA qualified for integrity test were mixed into one RNA pool according to the same concentration, and the mixed total RNA was sent to Beijing Novogene company for sequencing.

Statistical analysis of sequencing data

After the low-quality reads, reads with adapter contamination or without inserted fragments, and reads containing poly A / T / G / C was removed in the raw sequencing data by Perl and python scripts, the filtered clean reads are obtained. The clean sequences in the range of 18-35nt were screened for subsequent statistical analysis. Using bowtie to map small RNA screened by the length on the reference sequence, and analyze the distribution of small RNA on the reference. By comparing the mapped reads on the reference sequence with the miRNA precursor sequence of the specified rabbit and mouse in the miRBase database (version 20.0), and the details of the miRNA on each matching sample were obtained, including the secondary structure of the known matching miRNA, the sequence, length, occurrence times and other information of miRNA in each sample. All small RNA could be classified and annotated by comparing with all kinds of RNAs. In order to make each annotated small RNA have a unique annotation, small RNA tags were mapped to RepeatMasker and Rfam database

RESULTS AND DISCUSSION

Sequencing data quality and classification annotation

The total reads of the raw sequencing reached 8600494, and the low quality sequence only accounted for 0.17% of the raw sequences, which indicated that the quality of the constructed sequencing library was good. After removing some contaminated sequences from the sequencing library, the total reads of clean reads reached 8409454, accounting for 97.78% of the total sequence reads. Unique reads accounted for 3.25% of the total sequences. In the sequencing library, 7683439 (95.18%) reads (mappable reads) can be aligned to the rabbit genome. In the sequencing library, the total sequencing reads from the exons and introns of the gene encoding the protein accounted for about

2.60% of the total clean reads. The non-coding RNA such as rRNA, tRNA, snRNA, snoRNA and repeat RNA, which accounted for 2.94% of the total clean reads, of which the proportion of rRNA was slightly higher than 0.20% compared with the reference miRBase database of rabbits. At present, RNA-seq technology has been widely used in the analysis of miRNA expression profiles in different tissues, development stages and physiological states of pigs, cattle, sheep and poultry, and a large number of miRNAs with different expressions have been successfully detected (Wold et al., 2007). However, the accuracy of miRNA expression profile depends on the quality of sequencing data. The results of genome comparison show that the small RNA species with low expression amount occupy the vast majority of sequencing copies, and have high detection efficiency and coverage for the small RNA with low expression abundance in muscle tissue. In addition, only 0.06% of tRNA was sequenced and 2.60% of the sequences were compared with the exon and intron of mRNA, which fully shows that the constructed miRNA library has high quality and high reliability. The sequencing results of this study accord with the characteristics of miRNA length distribution and basically cover the miRNA in muscle tissue, which lays a foundation for our systematic and comprehensive analysis of miRNA expression in muscle tissue.

Known miRNA comparison and expression profile Analysis

By comparing with the mature sequence or precursor sequence of rabbit and mouse known miRNA in miRNA, the known miRNA was obtained. The top 10 of expression level was shown in Table 1, and the expression level was above 50000.

Table 1 The expression level of known miRNA by Solexa sequencing (top 10)

precursor_id	precursor_sequence	Expression level
mmu-mir-1a-2	UCAGAGCACAUACUUCUUUA	3603501
mmu-mir-1b	UUCCUUUAGCUUCUUCUUGG	3595652
mmu-mir-206	CCAGGCCACAUGCUUCUUUA	418853
mmu-mir-378a	AGGGCUCCUGACUCCAGGU	215076
mmu-mir-133a-2	AGAAGCCAAAUGCUUUGCUG	158750
mmu-mir-27b	AGGUGCAGAGCUUAGCUGA	108722
mmu-mir-26a-2	GGCUGCGGCUGGAUUCAAG	87130
mmu-let-7i	CUGGCUGAGGUAGUAGUUU	68289
mmu-mir-127	CCAGCCUGCUGAAGCUCAG	66401
mmu-let-7f-2	UGUGGGAUGAGGUAGUAG	64849

The study of miRNA in pig, cattle, sheep and other livestock muscle showed that the expression of miRNA was tissue-specific. Zhou analyzed the miRNA expression profiles in 90 day gestation and longissimus dorsi of 120-day-old pig (Zhou et al., 2010). The results showed that miRNA-206 and let-7 were abundant in 90 day gestation, while miR-1a, miR-133a, miR-26a and miR-1826 were higher in 120 day gestation. Guo sequenced miRNAs from the longissimus dorsi of different embryonic stages of Capra hircus, and found that miR-1, miR-206, mir-136, miR-125b and let-7 families were highly expressed in multiple periods (Guo et al., 2016). miR-206 and miR-133 are expressed abundantly in skeletal muscles of different domestic animals after birth, which is basically

consistent with the results of this study. It is speculated that there may be similar functions in skeletal muscle development.The sequences of miR-1 and miR-206 have high homology and belong to the same family, which can target Notch 3 and Pax 7 genes to inhibit the proliferation of skeletal muscle satellite cells and promote the myogenic differentiation of skeletal muscle cells (Chen et al., 2010). miR-133 regulates the proliferation and differentiation of myocytes by targeting Srf, igf1R, CycD2, FgfR and Sp1 genes (Feng et al., 2013). The mitochondrion activity of miR-133 knockout mice was significantly lower than that of control mice (Zhang et al., 2012). After birth, the number of muscle fibers is no longer increased, but more importantly, the repair of damaged muscles and the maintenance of normal physiological functions. Therefore, miR-1, miR-206 and miR-133, which play an important role in myogenic differentiation, muscle repair and energy metabolism, are abundant in postnatal skeletal muscle.

CONCLUSIONS

The yield and quality of rabbit meat has always been the focus of genetic improvement in rabbits, which is mainly affected by muscle growth and development.The expression levels of miR-133, miR-206 and miR-1 were expressed abundantly in skeletal muscles of rabbit.The specific highly expressed miRNA in muscle tissues identified in this study can be used as an important reference for further functional studies.

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