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In silico identification of conserved microRNAs and their targets in bovine fat tissue

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ABSTRACT

MicroRNAs (miRNAs) represent a newly identified class of non-protein-coding ~22 nt small RNA which plays important roles in multiple biological processes by degrading targeted mRNA or repressing mRNA translation. Here we present EST (expressed sequence tags)-based and GSS (Genomic Survey Sequences)-based combined approach for the detection of conserved miRNAs of cattle. A total of 20 conserved miRNAs that belong to 18 miRNA families were detected following a range of filtering criteria; their functions were further predicted and analyzed. To confirm our prediction, a miRNA-detecting microarray was designed with probes complementary to previously known mature miRNA sequences from 131 organisms. After hybridizing with small RNAs extracted from beef cattle subcutaneous fat tissue, 219 (32.30%) miRNAs were detected in the 679 known *Bos taurus* miRNAs and all the miRNAs predicted above were also detected. Conformation of 22 most abundant miRNA expression by qRT-PCR indicated that they were highly accumulated not only in subcutaneous fat tissue but also in intramuscular fat tissue. Bioinformatics of KEGG pathway analysis suggested that 4 differential expression miRNAs (miR-143, miR-145, miR-2325c and miR-2361) involved in different pathways and target genes may regulate the fat deposition differently. Taken together, our results expand the number of known bovine miRNAs and provide a thorough account of the miRNA transcriptome in bovine adipose tissue.

1. Introduction

miRNAs are a class of regulatory small RNAs (18–24 nucleotides in length) important in a variety of developmental and physiological processes (Flynt and Lai, 2008). These small RNAs are broadly present in eukaryotic organisms and repress gene expression by destabilizing target mRNA as well as inhibiting their translation (Wang and Blelloch, 2009). All miRNAs have similar secondary hairpin structures, and many of these are evolutionarily conserved (Ambros et al., 2003). This suggests a powerful approach for predicting the existence of new miRNA orthologs or homologues in other species. Computational approaches have been widely used to identify miRNAs in plants and animals (Brown and Sanseau, 2005; Lai et al., 2003). However, a majority of computational approaches are based on whole genome sequences, which makes it a little difficult to identify miRNAs in a wide range of species (Brown and Sanseau, 2005). Thus, currently a majority of identified miRNAs and their targets are limited to a few species, especially in the model species, such as *Arabidopsis thaliana* and rice in plants, *Caenorhabditis elegans*, human and mouse in animals (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006; Weber, 2005).

Previous research has used the publicly available expressed sequence tag (EST) and genomic survey sequences (GSS) to search new plant miRNA genes (Yang et al., 2012; Zhang et al., 2008). In the present study, we also used known animal miRNA sequences of previously identified to search the bovine homologues of miRNAs in the publicly available EST and GSS database (National Center for Biotechnology Information, NCBI, http://www.ncbi.nlm.nih.gov/). Currently 15,644 mature miRNAs have been discovered from 131 species and deposited in the publicly available miRNA database miRBase (Release 15.0, http://www.mirbase.org). Specifically, the number of miRNAs from bovine species is limited with only 679 mature and 765 precursors reported, compared with 1105 mature from human and 722 mature from mouse. In order to identify more miRNAs in bovine and to increase our understanding of the gene regulatory networks, we predicted miRNA candidates in bovine by homology searching, starting from the data set involving various known animal miRNAs, and the prediction was validated verified by miRNA microarray.

Abbreviations: miRNA, microRNA; 3′-UTR, 3′-untranslated region; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; EST, expressed sequence tags; GSS, genomic survey sequences; DAVID, database for annotation, visualization and integrated discovery; nt, nucleotide(s); BLAST, basic local alignment search tool; MFEs, minimal folding free energies; MFEIs, minimal folding free energy indices; NCBI, National Centre for Biotechnology Information; HMGA2, high mobility group A2; ERK5, extracellular signal-regulated protein kinase 5; LM, length of mature miRNAs; NM, number of mismatch; LP, length of precursor

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The role of miRNA in lipid metabolism was first reported in *Drosophila*, where the deletion of miR-14 increased the levels of triacylglycerol and diacylglycerol (Xu et al., 2003). Thereafter, several miRNAs were shown to promote preadipocyte differentiation in the human and mouse (Esau et al., 2004; Wang et al., 2008; Xie et al., 2009). Analysis of the 3′ UTR from 395 ESTs expressed in 3T3-L1 preadipocytes during conversion into lipid-assimilating adipocytes showed that >70% of the differentially expressed genes may be potentially regulated by miRNAs (Hackl et al., 2005). A recent study on the expression of 155 miRNAs in human omental and subcutaneous adipose tissues found that the expression of miRNAs was adipose depot specific and that some miRNAs were correlated with the morphology of adipose tissue and adipocyte size (Kloting et al., 2009). Although the roles of some miRNAs in lipid metabolism (Xu et al., 2003) and adipocyte formation (Esau et al., 2004; Hackl et al., 2005; Wang et al., 2008; Xie et al., 2009) have been demonstrated, no miRNA has been reported to regulate lipid metabolism in beef cattle. Lipid deposition, especially in subcutaneous adipose tissue, is directly associated with the yield and the quality of meat (Adams et al., 1982). To gain insight into the association between subcutaneous adipose tissue and miRNA expression, the expression profiles of 679 miRNAs (miRBase, http://microrna.sanger.ac.uk) were determined in beef cattle subcutaneous adipose tissues. In order to understand the molecular function of 20 newly identified miRNAs and 22 most abundant miRNAs in bovine subcutaneous fat tissue, we also used the TargetScan to predict the target genes.

2. Materials and methods

2.1. Sequences of miRNAs, EST, GSS and mRNA

To search for potential conserved miRNAs, we firstly downloaded the known animal miRNA sequences of previously identified mature miRNAs and their pre-miRNAs from various species of metazoan from the miRNA Registry Database (http://microrna.sanger.ac.uk). To avoid the overlap of miRNAs, the repeated sequences of miRNAs within the above species were removed and the remaining sequences were used as a reference of miRNA. The bovine EST, GSS, and mRNA databases were obtained from the NCBI (http://www.ncbi.nlm.nih.gov/).

2.2. Potential miRNAs and their precursors

Fig. 1 shows the procedure of search for potential miRNAs in bovine. Since only mature miRNAs, rather than miRNA precursor sequences, were conserved, all previously known mature miRNA sequences were compared with all sequences available in the bovine EST and GSS databases using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/).

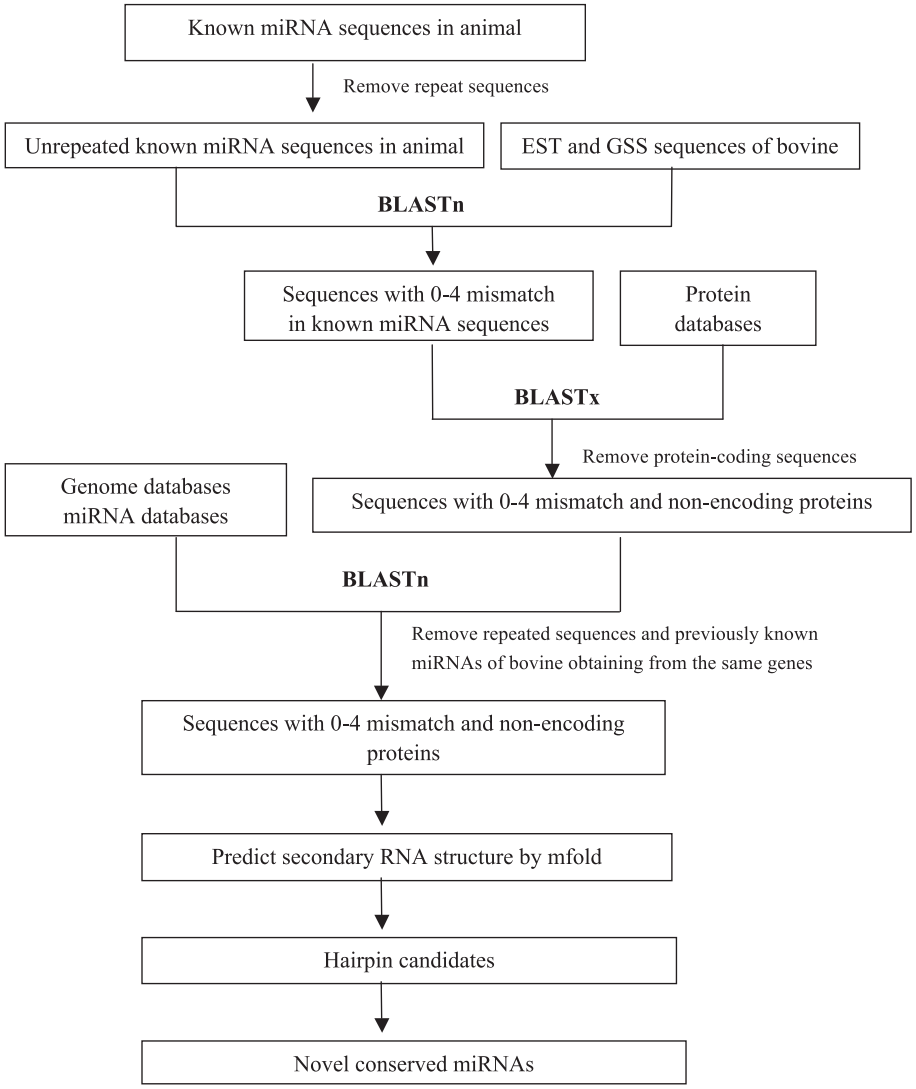

Fig. 1. The procedures to identify cattle miRNA candidate genes by homology search method.