Deep Sequencing of Small RNA Repertoires in Mice Reveals Metabolic Disorders-Associated Hepatic miRNAs

Tingming Liang, Chang Liu, Zhenchao Ye

Jiangsu Key Laboratory for Molecular and Medical Biotechnology, College of Life Science, Nanjing Normal University, Nanjing, Jiangsu, China

Abstract

Obesity and associated metabolic disorders contribute importantly to the metabolic syndrome. On the other hand, microRNAs (miRNAs) are a class of small non-coding RNAs that repress target gene expression by inducing mRNA degradation and/or translation repression. Dysregulation of specific miRNAs in obesity may influence energy metabolism and cause insulin resistance, which leads to dyslipidemia, steatosis hepatis and type 2 diabetes. In the present study, we comprehensively analyzed and validated dysregulated miRNAs in ob/ob mouse liver, as well as miRNA groups based on miRNA gene cluster and gene family by using deep sequencing miRNA datasets. We found that over 13.8% of the total analyzed miRNAs were dysregulated, of which 37 miRNA species showed significantly differential expression. Further RT-qPCR analysis in some selected miRNAs validated the similar expression patterns observed in deep sequencing. Interestingly, we found that miRNA gene cluster and family always showed consistent dysregulation patterns in ob/ob mouse liver, although they had various enrichment levels. Functional enrichment analysis revealed the versatile physiological roles (over six signal pathways and five human diseases) of these miRNAs. Biological studies indicated that overexpression of miR-126 or inhibition of miR-24 in AML-12 cells attenuated free fatty acids-induced fat accumulation. Taken together, our data strongly suggest that obesity and metabolic disturbance are tightly associated with functional miRNAs. We also identified hepatic miRNA candidates serving as potential biomarkers for the diagnose of the metabolic syndrome.

Citation: Liang T, Liu C, Ye Z (2013) Deep Sequencing of Small RNA Repertoires in Mice Reveals Metabolic Disorders-Associated Hepatic miRNAs. PLoS ONE 8(11): e80774. doi:10.1371/journal.pone.0080774

Editor: Yu Xue, Huazhong University of Science and Technology, China

Received July 19, 2013; Accepted October 5, 2013; Published November 15, 2013

Copyright: © 2013 Liang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by grants from the National Basic Research Program of China (973 Program) (2012CB947600, 2013CB911600), the Program for New Century Excellent Talents in University by the Chinese Ministry of Education (NCET-11-0990), the National Natural Science Foundation of China (31171137,31271261), the Key Project of Chinese Ministry of Education (211062), the Research Fund for the Doctoral Program of Higher Education of China (20103207110007),the Fok Ying Tong Education Foundation (121022), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (12KJB360001), Natural Science Foundation of China for Talents Training in Basic Science (J1103507), the Priority Academic Program Development of Jiangsu Higher Education Institutions (164320H106) and Collaborative Innovation Center For Cardiovascular Disease Translational Medicine of Jiangsu Higher Education Institutions. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail: tmliang@njnu.edu.cn

Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs that repress target gene expression by a combination of mRNA degradation and/or translation repression. Dysregulation of specific miRNAs in obesity may influence energy metabolism and cause insulin resistance, which leads to dyslipidemia, steatosis hepatis and type 2 diabetes. In the present study, we comprehensively analyzed and validated dysregulated miRNAs in ob/ob mouse liver, as well as miRNA groups based on miRNA gene cluster and gene family by using deep sequencing miRNA datasets. We found that over 13.8% of the total analyzed miRNAs were dysregulated, of which 37 miRNA species showed significantly differential expression. Further RT-qPCR analysis in some selected miRNAs validated the similar expression patterns observed in deep sequencing. Interestingly, we found that miRNA gene cluster and family always showed consistent dysregulation patterns in ob/ob mouse liver, although they had various enrichment levels. Functional enrichment analysis revealed the versatile physiological roles (over six signal pathways and five human diseases) of these miRNAs. Biological studies indicated that overexpression of miR-126 or inhibition of miR-24 in AML-12 cells attenuated free fatty acids-induced fat accumulation. Taken together, our data strongly suggest that obesity and metabolic disturbance are tightly associated with functional miRNAs. We also identified hepatic miRNA candidates serving as potential biomarkers for the diagnose of the metabolic syndrome.
more-severe nonalcoholic steatohepatitis (NASH) [17]. Jordan et al. [18] reported that transgenic over-expression of miR-143 in mice impairs insulin-stimulated AKT activation and glucose homeostasis. Therefore, dysregulation of miRNAs may contribute importantly to the metabolic abnormalities.

High-throughput sequencing of miRNAs provides a highly quantitative evaluation of known individual miRNA species [19]. Since the liver plays a central role in glucose and lipid metabolism, we attempted to identify dysregulated miRNAs by sequencing small RNAs in ob/ob mouse liver using Illumina/Solexa sequencing platform. The hepatic miRNA candidates under metabolic disorder conditions were validated using RT-qPCR method. We then analyzed the expression of miRNA groups at the miRNA gene cluster and gene family levels. The functional enrichment analysis of the differential miRNAs was also performed to understand their potential physiological roles.

Results

Overview of miRNA Expression Profiles in ob/ob Mouse Liver

To examine the difference of miRNA expression between ob/ob and WT mouse liver, we used the Illumina miRNA expression profiling assay. According to small RNAs that could be mapped to mouse miRNA precursors, the most abundant length was 22 nt, as expected (Figure 1). 510 miRNAs were detected, and over 13.8% of which showed dysregulated expression. Using a 2-fold expression difference as a cutoff, 37 miRNAs showed significantly differential expression in ob/ob mouse liver. Among which, 12 were up-regulated and 25 were down-regulated (Figure 2). For those up-regulated, the top ten were miR-122, 24, 195a, 106b, 15b, 802, 185, 214, 378, and let-7c. miR-122 expression was most robustly dysregulated (> 6-fold) in ob/ob mouse liver (Figure 2A). In contrast, for those down-regulated, the top ten were miR-224, 126, 7a, 128, 455, 452, 135b, 145, 18a and 196a (Figure 2B). miR-224 expression decreased most (< -5-fold). Furthermore, multiple isomiRs (miRNA variants) have been shown to be detected from the miRNA locus due to imprecise and alternative cleavage of Drosha and Dicer. Herein, assessed fold change (log2) based on the different selection of isomiRs (the most abundant isomiR and sum of all isomiRs) showed various values (Table S1).

Differentially Expressed miRNA Gene Cluster and Gene Family

Next, we performed a comprehensive analysis of isomiR expression patterns in miRNA gene clusters and families [20]. Four abundantly expressed miRNA gene clusters and four gene families were selected. Various enrichment levels could be found among members in miRNA gene cluster and gene family based on the original sequence counts (Figure 3). Clustered miRNAs and homologous miRNAs had various expression levels (even involved in larger expression divergence), but they always showed consistent dysregulation patterns (For example, mir-15b cluster and mir-193b cluster; mir-15 family) although the fold change may differ.

Functional Enrichment Analysis

Functional enrichment analysis of dysregulated miRNAs suggested that the versatile biological roles (Table 1) of these miRNAs were included. These miRNAs contributed to many essential biological processes, such as TGF-beta signaling pathway, cell cycle, Wnt signaling pathway, MAPK signaling pathway, ErbB signaling pathway, Jak-STAT signaling pathway, and etc. Furthermore, these aberrantly expressed miRNA species were involved in occurrence and development of some human diseases, including chronic myeloid leukemia, pancreatic cancer, colorectal cancer, glioma, prostate cancer, and etc.

Validation of the Differentially Expressed miRNAs by RT-qPCR

To further validate the abnormal expression of the collected miRNA species, we selected several typical miRNAs and performed RT-qPCR for double-check. As expected, the result showed similar expression patterns as observed in deep sequencing. For example, miR-122, 24, 106b, 696 and 15b were up-regulated, but miR-126, 145 and 103 were down-regulated (Figure 4).

miR-126 or miR-24 Regulates Lipid Accumulation in AML12 Hepatocytes Exposed to FFAs

To determine whether the disordered miR-126 or miR-24 levels induced by free fat acid (FFA) affect cellular triglyceride (TG) accumulation, we examined the TG levels in AML-12 cells transfected with mimic (Negative control) NC, miR-126 mimic or inhibitor NC, miR-24 inhibitor using Nile red staining. FFA significantly increased the TG accumulation in AML-12 cells compared with controls (Figure 5). More important, overexpression of miR-126 or inhibition of miR-24 markedly improved fat accumulation in AML-12 cells expose to FFA (Figure 5).

Discussion

Dysregulation of miRNA expression has been shown to influence glucose homeostasis, cholesterol metabolism and cause insulin resistance, which thus plays an important role in the pathogenesis of metabolic disorders such as T2D, atherosclerosis, fatty liver and Alzheimer's disease [21,22]. In the present study, deep sequencing of small RNA populations in ob/ob mice reveals metabolic disorders-associated hepatic miRNAs.

Here, we observed that 37 metabolic disorder-associated hepatic miRNAs were differentially expressed in ob/ob mouse liver, and they regulated the expression of several important downstream target genes. Overview of the miRNA expression profiles in ob/ob mouse liver, miR-122 is the most abundant miRNA (Figure 2). It has been demonstrated that miR-370 induces the accumulation of hepatic triglycerides through interacting with miR-122 and Cpt 1a [23]. Another study showed that hepatocytes from anti-miR-122-treated mice showed increased fatty acid oxidation rates and reduced fatty acid synthesis. Accordingly, antagonist of miR-122 used in diet-induced obese mice significantly improved hepatic steatosis.
Figure 1. Length distributions of reads. A: The data of *ob/ob* mouse liver samples. B: The data of wild-type mouse liver samples.
doi: 10.1371/journal.pone.0080774.g001
and reduced levels of triglyceride accumulation. Taken these previous observations and our data, miR-122 is a risk factor to induce obesity and hepatic metabolic dysfunction. On the other hand, miR-126 is known as an endothelium-specific miRNA, and has been reported to promote angiogenesis by targeting SPRED1 to inhibit VEGF signaling [24]. It also acts as an oncogene by targeting SOX2 in gastric cancer cells [25]. Our results validated that miR-126 was down-regulated in ob/ob mouse liver. Similarly, miR-33 was another down-regulated miRNA in ob/ob mouse liver. Silencing of miR-33 in vivo increases hepatic expression of ABCA1 and SREBP-2 [26,27], leading to dysregulation of cholesterol homeostasis. Thus, miR-33 is critical to maintain normal cholesterol metabolism. A recent study suggested that miR-27b was responsive to lipid levels and regulated several key lipid-metabolism genes during dyslipidemia [28]. This miRNA was also detected to be decreased in ob/ob mouse liver, suggesting the impaired responsiveness to fatty acids under this pathophysiological condition. Last but not least, miR-106b was very interesting and it decreased ABCA1 levels and impairs cellular cholesterol homeostasis in neuronal cells [29]. Its function may extend to the liver system according to our data. Collectively, the abnormally expressed miRNAs in ob/ob livers were involved in lipid synthesis, fatty acid oxidation and cholesterol homeostasis through their target genes.
Most miRNA studies focus on finding targets of individual miRNAs, yet half of the total miRNAs are co-expressed as clusters [30]. The expression pattern of miRNA gene clusters and gene families in ob/ob mouse liver showed that these miRNAs were coordinately regulated (Figure 3). The miR-15b/16-2 cluster generates miR-15b/15b* and 16-2/16*-2. Both miR-15b and miR-16-2 were over-expressed in ob/ob mouse liver, however, they were down-regulated in SGC790/VR cells [31]. On the other hand, miR-103 family (including miR-103 and miR-107) was dysregulated in ob/ob mouse liver. These results demonstrate that the miRNA gene family coordinate interaction with each other and function together in the pathogenesis of liver metabolic disorders with consistent dysregulation patterns.

Functional enrichment analysis showed that over six signal pathways and five human diseases were involved. In addition, miRNAs may serve as nodes cross-linking various signaling pathways by the integration of transcriptional inputs or by their functional regulatory outputs, thus highlighting the potential important roles in the epigenetic regulation of lipid metabolic abnormalities. The small non-coding regulatory molecules have opened new avenues for the treatment of metabolic diseases.

The functional analysis in AML-12 liver cells showed that dysregulation of miR-126 and miR-24 is correlated with fat accumulation (Figure 5). In recent years, several miRNAs, such as miR-155 [32], and miR-217 [33], related fat liver disease have been identified. Although our present study supports the
concept that miRNAs contribute to the fat accumulation in mice cell, further work is warranted to identify more specific hepatic miRNAs and their functions involved in lipid metabolic disorders. Whether up-regulation of reduced hepatic miR-126 using miR-126 mimic (or down-regulation of elevated hepatic miR-24 using antagomiR-24) approaches would alleviate liver steatosis in high fat diet-fed mice is currently under investigation in our laboratory.

In conclusion, the present study demonstrated that various miRNAs were differentially expressed in ob/ob mouse liver (especially for miR-126 and miR-24), suggesting that they were tightly linked to obesity and other metabolic disorders. The candidate miRNAs we identified may be potential biomarkers for the diagnose of the metabolic syndrome.

Materials and Methods

Animal Studies

Male obese ob/ob mice on a C57BL/6J background (12 weeks old) and their male WT littermates were purchased from

Table 1. Enrichment pathway analysis based on experimentally validated target mRNAs.

| Pathway                        | Gene Numbers | EnrichmentP-value | Target Genes                                                                 |
|--------------------------------|--------------|-------------------|------------------------------------------------------------------------------|
| Chronic myeloid leukemia       | 11           | 2.81E-23          | Acvr1b;Ccdn1;Cdk6;Cdkn1b;E2f3;Myc;Pik3r2;Runx1;Smad3;Smad4;Tgfbr1            |
| Pancreatic cancer              | 9            | 1.00E-18          | Acvr1b;Ccdn1;Cdk6;E2f3;Pik3r2;Smad3;Smad4;Stat3;Tgfbr1                      |
| Colorectal cancer              | 8            | 1.54E-15          | Acvr1b;Ccdn1;Myc;Pik3r2;Smad3;Smad4;Tcf7f2;Tgfbr1                           |
| Acute myeloid leukemia         | 7            | 8.53E-15          | Ccdn1;Kit;Myc;Pik3r2;Runx1;Stat3;Tcf7f2                                     |
| TGF-beta signaling pathway     | 7            | 3.09E-13          | Acvr1b;Myc;Rb2;Smad3;Smad4;Smad5;Tgfbr1                                    |
| Cell cycle                     | 7            | 1.95E-12          | Ccdn1;Cdk6;Cdkn1b;E2f3;Rb2;Smad3;Smad4                                     |
| Wnt signaling pathway          | 7            | 8.77E-12          | Cank2d;Canc2b;Ccdn1;Myc;Smad3;Smad4;Tcf7f2                                 |
| Glioma                         | 6            | 5.55E-12          | Cank2d;Canc2b;Ccdn1;Cdk6;E2f3;Pik3r2                                      |
| Prostate cancer                | 6            | 3.58E-11          | Ccdn1;Cdkn1b;E2f3;Foxo1;Pik3r2;Tcf7f2                                     |
| Small cell lung cancer         | 6            | 4.08E-11          | Ccdn1;Cdk6;Cdkn1b;E2f3;Myc;Pik3r2                                         |
| MAPK signaling pathway         | 6            | 2.01E-08          | Acvr1b;Bdnf;Mapk14;Mef2c;Myc;Tgfbr1                                       |
| Adherens junction              | 5            | 1.63E-09          | Acvr1b;Smad3;Smad4;Tcf7f2;Tgfbr1                                          |
| ErbB signaling pathway         | 5            | 3.44E-09          | Cank2d;Canc2b;Ccdn1;Myc;Pik3r2                                            |
| Jak-STAT signaling pathway     | 5            | 5.94E-08          | Ccdn1;Myc;Pik3r2;Spre1;Stat3                                               |

Figure 4. Validation of the differential expression pattern of miRNA species by RT-qPCR analysis. RT-qPCR analysis was performed to quantify the expression levels of miR-122, 126, 145, 24, 106b, 103, 696 and 15b.
the Model Animal Research Center of Nanjing University (Nanjing, China), and were housed and maintained in a 12-h light/12-h dark cycle (light/dark, 12:12) in a temperature- and humidity-controlled environment. All protocols complied with, and all animals received humane care according to the criteria outlined in the NIH ‘Guide for the Care and Use of Laboratory Animals’ and the approved regulations set by the Laboratory Animal Care Committee at Nanjing Normal University.

RNA Isolation, Construction of Small RNA Library and High-Throughput Small RNA Sequencing

Five liver samples from ob/ob or WT mice (20 mg each in weight) were equally pooled together and kept in RNAfixer (BioTeke Co. Ltd, Beijing, China) at -70 °C until use.

RNA with low molecular weight from liver was extracted using a mirVana™ miRNA isolation kit (Ambion, Austin, TX, USA) following the manufacturer’s protocol. The purity and concentration of RNA samples were determined with NanoDrop ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA) at 260/280 nm (ratio > 2.0). 60 μg of total RNA from the pooled samples was used for library preparation and sequencing. The libraries were used for the Illumina/Solexa miRNA deep sequencing which included the following steps: total RNA of each sample was sequentially ligated to 3’ and 5’ small RNA adapters. cDNA was then synthesized and amplified using Illumina’s proprietary RT primers and amplification primers, respectively. Subsequently, PCR amplified fragments within 110-130 bp were extracted and purified from the PAGE gel. When the completed libraries were quantified with Agilent 2100 Bioanalyzer, the DNA fragments in the libraries were denatured with 0.1M NaOH to generate single-stranded DNA molecules, which were captured on Illumina flow cells, amplified in situ and finally sequenced for 36 cycles on Illumina’s Genome Analyzer IIX according to the vendor’s recommended protocol (Illumina, San Diego, CA, USA) [34-36].

Data deposition: The data reported in this paper have been deposited in the Sequence Read Archive (SRA) database, www.ncbi.nlm.nih.gov/sra (accession no. SRR987316; SRR987317).

Bioinformatic Analysis of Solexa Sequencing Data

Low-quality sequence reads failed to pass the quality filter were removed according to the criteria of Solexa/Illumina. 3’ adapter sequences were trimmed from clean reads and the tags shorter than 15nt were discarded. Then reads were aligned to the latest known mouse reference miRNA precursor set (Sanger miRBase 19.0, http://www.mirbase.org/) [37-39] using Novoaalign software (v2.07.11). In this aspect, reads (counts < 2) were discarded when calculating the miRNA expression. In order to characterize the isomiR variability, sequences matching the miRNA precursors in the mature miRNAs region ± 4 nt (no more than 1 mismatch) were accepted as mature miRNA isomiRs, which were grouped according to the 5-prime (5p) or 3-prime (3p) arm of the precursor hairpin [40].

The relative abundance of miRNAs was estimated by the number of reads for each miRNA sequenced, and the read amount of each unique sequence was normalized to reads per million according to the total read count of the mapped reads data set [41]. To compare the differential expression between ob/ob and WT mice, the log2 ratio of miRNA (ob/ob)/miRNA
was extracted with TRIzol Reagent (Invitrogen Corp., Carlsbad, CA). The target mRNAs of these miRNAs were determined based on known experimentally validated target mRNAs from the miRTarBase database [42], the miRecords database [43] and the TarBase database [44]. If no validated target was identified in these databases, the prediction software of TargetScan [45] was used to predict target mRNAs.

**RT-qPCR Validation**

To confirm the expression of miRNAs identified by the deep sequencing approach, RT-qPCR analysis was performed using the total RNA samples from five ob/ob or WT mouse livers. For miRNA quantification, Bulge-loop™ miRNA RT-qPCR Primer Sets (one RT primer and a pair of qPCR primers for each set) specific for miR-106b, 24, 122, 126, 103, 696, 15b and 145 were designed by RiboBio (Guangzhou, China). The total RNA was extracted with TRIzol Reagent (Invitrogen Corp., Carlsbad, CA). miRNA bulge-loop was then reverse transcribed with the PrimeScript RT reagent kit (Takara Bio, Tokyo, Japan) and quantified by qPCR using SYBR Premix Ex Taq™ II (Takara). The data were normalized to snRNA U6 and analyzed using the 2^(-ΔΔCt) method. The differentially expressed miRNAs were selected to query against the miRNA bulge-loop in the miRTarBase database [42]. The validated target mRNAs from the miRTarBase database [42] were considered differentially expressed.

**Cell Culture and Treatments**

The mouse AML12 hepatocyte cells were obtained from American Type Culture Collection and were cultured in DMEM/F12 (Wisent, Nanjing) containing 10% heat-inactivated fetal bovine serum (Wisent, Nanjing) and 1% penicillin-streptomycin (Invitrogen) according to the indicated manufacturer’s instructions. The cells were used at passage 3. DMEM/F12 media were changed every 2 to 3 days. The culture medium was replaced by media containing 10% bovine serum albumin (BSA) for 18 hr to stimulate the FFAs.

**Nile Red Staining**

Cell were fixed with 4% formaldehyde and stained with Nile Red solution (1µg/ml) for 10 min at 37 °C. Lipid-bound Nile Red fluorescence was observed with a fluorescence microscope (Ti-S, Nikon).

**Statistical Analysis**

Data are presented as the mean ± S.E. Unless otherwise indicated, the statistics was performed using Student’s t-test when only two groups were compared. Results with a P < 0.05 were considered statistically significant.

**Supporting Information**

Table S1. Various fold change values (log2) based on different selection of miRNA sequences.

**Author Contributions**

Conceived and designed the experiments: TML CL. Performed the experiments: TML ZCY. Analyzed the data: TML ZCY. Contributed reagents/materials/analysis tools: TML ZCY. Wrote the manuscript: TML.

**References**

1. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136: 215-233. doi:10.1016/j.cell.2009.01.002. PubMed: 19167326.

2. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM (2003) Bantam Encodes a Developmentally Regulated microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene hid in Drosophila. Cell 113: 25-36. doi:10.1016/S0092-8674(03)00231-9. PubMed: 12679032.

3. Hayasita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K et al. (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 65: 9628-9632. doi:10.1158/0008-5472.CAN-05-2352. PubMed: 16269980.

4. Farazi TA, Horlings HM, Ten Hoeve JJ, Mihalovic A, Halfwerk H et al. (2011) MicroRNA sequence and expression analysis in breast tumors by deep sequencing. Cancer Res 71: 4443-4453. doi: 10.1158/0008-5472.CAN-11-0630. PubMed: 21586611.

5. Hwang HW, Mendell JT (2006) MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 94: 776-780. doi:10.1038/sj.bjc.6603023. PubMed: 16495913.

6. Krüttfeldt J, Stoffel M (2006) MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab 4: 9-12. doi:10.1016/j.cmet.2006.05.009. PubMed: 16814728.

7. Heneghan HM, Miller N, Kerin MJ (2010) Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 11: 354-361. doi:10.1111/j.1467-789X.2009.00659.x. PubMed: 19793375.

8. Rottiers V, Nálar ÁM (2012) MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 13: 239-250. doi:10.1038/nrm3313. PubMed: 22436747.

9. WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation 0512-3054 (Linking): 1-253.

10. Peleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D et al. (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540-543. doi:10.1126/science.7624776.

11. Cohen B, Novick D, Rubinstein M (1996) Modulation of insulin activities by leptin. Science 274: 1185-1188. doi:10.1126/science.274.5290.1185. PubMed: 8865466.

12. Xu P, Vermeoy SY, Guo M, Hay BA (2003) The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol 13: 790-795. doi:10.1016/S0960-9822(03)00250-1. PubMed: 12725740.

13. Roberts APE, Lewis AP, Jopling CL (2011) miR-122 activates hepatitis C virus translation by a specialized mechanism requiring RNA components. Nucleic Acids Res 39: 7716-7729. doi:10.1093/nar/gkr426. PubMed: 21653556.
14. Esau C, Davis S, Murray SF, Yu XX, Pandey SK et al. (2006) miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 3: 87-98. doi:10.1016/j.cmet.2006.01.005. PubMed: 16459310.

15. Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracini M et al. (2009) MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 69: 5761-5767. doi: 10.1158/0008-5472.CAN-08-4797. PubMed: 19584283.

16. Traković M, Hauser J, Soutschek J, Bhat B, Akin A et al. (2011) MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 474: 649-653. doi:10.1038/nature10112. PubMed: 21654750.

17. Castro RE, Ferreira D, Afonso MB, Borralho PM, Machado MV et al. (2012) miR-34a/SIRT1/p53 is Suppressed by Ursodeoxycholic Acid in Rat Liver and Activated by Disease Severity in Human Non-alcoholic Fatty Liver Disease. J Hepatol.

18. Jordan SD, Krüger M, Willmes DM, Redemann N, Wunderlich FT et al. (2011) Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat Cell Biol 13: 434-446. doi:10.1038/ncb2211. PubMed: 21441927.

19. Friedländer MR, Chen W, Adamidi C, Maasska M, Ainspanier R et al. (2008) Discovering microRNAs from deep sequencing data using miRDeep. Nat Biotechnol 26: 407-415. doi:10.1038/nbt1394. PubMed: 18390266.

20. Guo L, Lu Z (2010) Global expression analysis of miRNA gene cluster and family based on isomiRs from deep sequencing data. Comput Biol Chem 34: 165-171. doi:10.1016/j.compbiolchem.2010.06.001. PubMed: 20619743.

21. Maxfield FR, Tabas I (2005) Role of cholesterol and lipid organization in disease. Nature 438: 612-621. doi:10.1038/nature04399. PubMed: 16319881.

22. Foley P (2010) Lipids in Alzheimer’s disease: a century-old story. Biochim Biophys Acta 1801: 750-753. doi:10.1016/j.bbapap.2010.05.004. PubMed: 20471492.

23. Ioopoulos D, Drosatos K, Hiyama Y, Goldberg IJ, Zannis VI (2010) MicroRNA-370 controls the expression of microRNA-122 and Cpt1alpha and affects lipid metabolism. J Lipid Res 51: 1513-1523. doi:10.1194/jlr.M004812. PubMed: 21062749.

24. Wang S, Auranova AB, Johnson BA, Qi X, McAnally J et al. (2008) The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 15: 261-271. doi:10.1016/j.devcel.2008.07.002. PubMed: 18695456.

25. Tsubo T, Akiyama Y, Hashimoto Y, Shimada S, Goto K et al. (2011) MicroRNA-126 inhibits SOX2 expression and contributes to gastric carcinogenesis. PLOS ONE 6: e16617. doi:10.1371/journal.pone.0016617. PubMed: 21304604.

26. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM et al. (2011) Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature 478: 404-407. doi:10.1038/nature10481. PubMed: 21365488.

27. Vickers KC, Shoucri BM, Levin MG, Wu H, Pearson DS et al. (2012) MicroRNA-27b is a regulatory hub in lipid metabolism and is altered in dyslipidemia. Hepatology.

28. Kim J, Yoon H, Ramírez CM, Lee SM, Hoe HS et al. (2012) miR-106b impairs cholesterol efflux and increases AB levels by repressing ABCA1 expression. Exp Neurol, 235: 476-83. doi:10.1016/j.expneurol.2011.09.012. PubMed: 22119192.

29. Megraw M, Sethupathy P, Corda B, Hatzigeorgiou AG (2007) miRGen: a database for the study of animal microRNA genomic organization and function. Nucleic Acids Res 35: D149-D155. doi:10.1093/nar/gkm971. PubMed: 17105354.

30. Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003) Prediction of mammalian microRNA targets. Cell 115: 787-798. doi:10.1016/S0092-8674(03)00183-3. PubMed: 12835179.