

Original Article

MicroRNA profile changes in liraglutide treated steatotic HepG2 cells

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Abstract: The dysregulation and modulation of microRNAs (miRNAs) expression has been reported in non-alcoholic fatty liver disease (NAFLD). Glucagon-like peptide-1 receptor agonists (GLP-1RAs) can reduce hepatic lipid content. The effects of GLP-1RAs on the miRNA profile of hepatic steatosis are poorly defined. In this study, we constructed a cell model of simple hepatic steatosis with HepG2 cell lines and treated the cells with or without liraglutide (LG), a GLP-1RA. miRNA Next-Generation Sequencing was then conducted. A total of 242 and 239 differentially expressed miRNAs (DEMs) were identified between steatotic and control HepG2 cells, and between LG-treated and non-treated steatotic HepG2 cells, respectively. Moreover, 36 miRNAs were increased in steatotic cells and reduced by additional LG treatment, and 87 miRNAs have the opposite changing tendency. These 123 miRNAs, including miR-34a, miR-21, miR-451 and miR-183, may be involved in the regulation of cell apoptosis, cell growth and cell cycle, inflammatory, lipid metabolism and oxidative stress response, thus may be more important to the protection effects of LG on hepatic steatosis. Our study is useful to advance understanding of the development of hepatic steatosis and how GLP-1RA improved hepatic steatosis, thus provided novel insights into NAFLD pathogenesis and treatment.

Keywords: miRNAs, NAFLD, GLP-1RAs, hepatic steatosis

Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a clinicopathologic condition characterized by excessive fat deposition in the liver caused by factors other than excessive alcohol intake [1, 2]. NAFLD has close association with insulin resistance and genetic susceptibility [3]. NAFLD comprises a histological spectrum ranging from simple hepatic steatosis (SHS) to non-alcoholic steatohepatitis (NASH) [4]. Approximately 30% of NAFLD patients subsequently progressed to NASH and have high risk for hepatic fibrosis, cirrhosis and hepatocellular carcinoma [5]. With the rising incidence of obesity and obesity-related metabolic comprehensive syndrome, NAFLD has become the major cause of chronic liver disease in the developed countries and areas [6]. Nowadays, NAFLD is becoming a new and major public health problem. Besides dietary and lifestyle interventions, there are currently no other effective therapeutic options for NAFLD.

MicroRNAs (miRNAs) are 21-23nt regulatory RNA molecules. Over 1,000 distinct miRNAs have been identified in mammalian cells [7] since the first miRNA was discovered in *Caenorhabditis elegans* in 1993 [8, 9]. miRNAs can post-transcriptionally repress gene expression [7, 10], thus playing a critical role in lipid metabolism, inflammation, cell apoptosis and tissue development [11-15]. Several studies have shown the dysregulation and modulation of microRNA expression in NAFLD, NASH and HCC [16-19].

Glucagon-like peptide-1 receptor agonists (GLP-1RAs), which are modified forms of GLP-1 and can lower blood glucose levels and stimulate weight loss, is an effective treatment option for type 2 diabetes mellitus (T2DM) [20]. Emerging evidences indicate that GLP-1RAs can markedly reduce hepatic lipid content by inhibiting the expression of fatty acid synthesis-related genes and enhancing the expression of fatty acid oxidation-related genes [21, 22]. However, little is

known about the effects of GLP-1RAs on the miRNA profile of hepatic steatosis.

In the present study, we constructed a cell model of simple hepatic steatosis with HepG2 cell lines [23] and then used liraglutide (LG) [24], a GLP-1RA, to treat the cell model. We tried to elucidate miRNAs involved in the protective effects of LG on hepatic steatosis by miRNA Next-Generation Sequencing. The differentially expressed miRNAs were identified and the results were then validated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Materials and methods

Cell culture

The human hepatoma cell line HepG2 was purchased from the Cell Bank of the Shanghai Branch of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) and 100 U/mL penicillin/streptomycin (Invitrogen).

Free fatty acid (FFA)-induced steatosis

To induce steatosis, HepG2 cells at 70% confluence were exposed to 0.5 mM free fatty acid (FFA) mixture (oleic acid/palmitic acid, 2:1, Sigma-Aldrich, St. Louis, MO, USA) for 24 h as described previously [23].

Experimental grouping

HepG2 cells were seeded onto 6-well plates and divided into three groups: 1) control group; 2) FFA-treated group; 3) FFA and liraglutide (LG, Novo Nordisk, Bagsvaerd, Denmark)-treated group. For Group 2 and 3, HepG2 cells were firstly exposed to 0.5 mM FFA for 24 h and then exposed to saline and 100 nM LG, respectively. For Group 1, HepG2 cells were cultured for 24 h and then exposed to saline. After another 24 h, cells were collected for oil red O staining and RNA extraction.

Oil Red O Staining

After washed with PBS, HepG2 cells were fixed in 4% paraformaldehyde for 30 min. Cells were incubated in 60% isopropanol briefly and then

with 0.5% Oil red O solution (w/v in 60% isopropanol, Sigma-Aldrich) for 15 min at room temperature. Then, cells were counterstained with hematoxylin (Sigma-Aldrich). Cells with Oil Red O Staining were observed under a microscope (Nikon, Tokyo, Japan).

Total RNA extraction and miRNA Next-Generation Sequencing

Total RNA was extracted from cells with Direct-zol RNA MiniPrep Kit (Zymo Research Corporation, Irvine, CA, USA) as per manufacturer's instructions, and then incubated with DNase (Promega, Madison, WI, USA) to remove any contaminating genomic DNA. The RNA integrity was checked by denaturing agarose gel electrophoresis. The quantity and quality of isolated RNA were assessed by using Nanodrop 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

Libraries for sequencing for Illumina HiSeq-2000 platform (Illumina Inc. San Diego, CA, USA) were prepared according to IlluminaTruSeq small RNA sample preparation protocol. Sequencing data were converted to FASTQ files using Illumina CASAVA (v.1.8.2). The raw sequences were quality controlled with FastQC v. 0.11.2 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The raw reads were cleaned by trimming of adaptor sequences, empty reads, low quality Sequences, shorter and longer reads. Cleaned sequence reads were then aligned against microRNA database (miRBASE release 21 at <http://www.mirbase.org/>) with miRDeep2 v2.0.0.7 [25]. Differential miRNA expression was then analyzed using R package DESeq (<http://www.bioconductor.org/packages/release/bioc/html/DESeq.html>). Heat map was generated using the heatmap.2.

Stem-loop real-time RT-PCR

Stem-loop real-time RT-PCR with SYBR Green was performed to analyze miRNA expression. The stem-loop RT primers were as follows: hsa-miR-34a-3p, 5'-CTCAACTGGTGTCG-TGGAGTCGGCAATTCAGTTGAGAGGGCA-3'; hsa-miR-21-3p, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACAGCC-3'; hsa-miR-21-5p, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACA-3'; hsa-miR-451a, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAC-

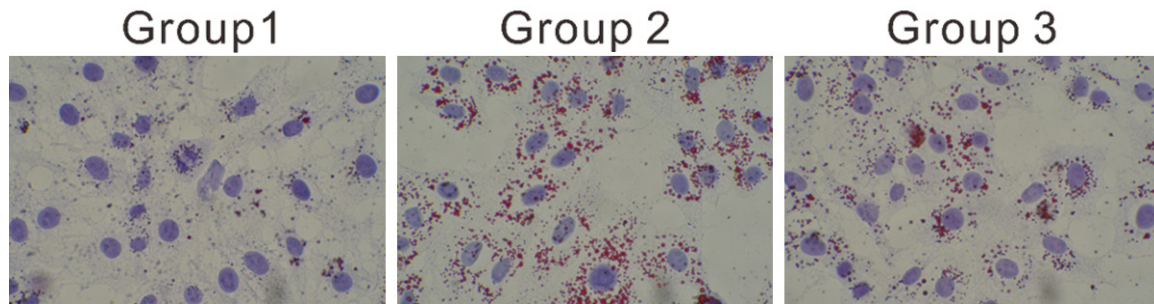


Figure 1. Lipid accumulation in HepG2 cells was evaluated by Oil Red O staining. Lipid droplets in HepG2 cells was observed by microscope (200×). Group 1, control HepG2 cells; Group 2, 0.5 mM FFA-treated-HepG2 cells; and Group 3, 0.5 mM FFA and 100 nM LG-treated HepG2 cells.

TCA-3'; hsa-miR-183-5p, 5'-CTCAACTGGTGTC-GTGGAGTCGGCAATTCAGTTGAGAGTGAA-3'. Real-time PCR was done with miRNA-specific forward primers and universal reverse primer (5'-TGGTGTCTGGAGTCG-3') on an Applied Biosystems 7300 detection system (Applied Biosystem, Foster City, CA, USA) with U6 as internal control. miRNA-specific forward primers were as follows: hsa-miR-34a-3p, 5'-AC-CTCCAGCTGGGCAATCAGCAAGTATAC-3'; hsa-miR-21-3p, 5'-ACACTCCAGCTGGGCAACACCAGTCGATG-3'; hsa-miR-21-5p, 5'-ACACTCCAGCTGGTAGCTTATCAGACTGA-3'; hsa-miR-451a, 5'-ACACTCCAGCTGGGAAACCGTTACCATTAC-3'; hsa-miR-183-5p, 5'-ACACTCCAGCTGGGTATGGCACTGGTAGAA-3'. For the internal control, U6, 1 µl of cDNAs synthesized by using oligodT) were used as template. The U6 primers were: forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Data analysis

The differences among three groups were analyzed with GraphPadPrism 6 software (GraphPad Software, La Jolla, CA, USA) with ANOVA and Sidak's multiple comparisons test. Bars showed the means $\pm$ SD. The differences between the means were considered statistically significant when $P < 0.05$.

Results

LG reduced lipid accumulation in steatotic HepG2 cells

Firstly, we established an *in vitro* NAFLD model by treating HepG2 cells with 0.5 mM FFA mixture for 24 h as describe previously [23]. To determine the effect of LG on lipid accumu-

lation in steatotic HepG2 cells, FFA-treated HepG2 cells were exposed to 100 nM LG and the lipid accumulation was evaluated by oil red O staining (**Figure 1**). After 24 h incubation with FFA, lipid droplets were accumulated in the cytoplasm of HepG2 cells (Group 2), whereas no evident lipid droplet was observed in control group (Group 1). LG treatment significantly reduced the number of lipid droplets (Group 3).

Identification of differentially expressed miRNAs (DEMs)

To investigate the effects of LG on the miRNA profile of hepatic steatosis, RNA was extracted from Group 1-3 HepG2 cells and subjected to miRNA Next-Generation Sequencing on the Illumina Hiseq-2000 platform. After removing the low-quality, adaptor, less than 12nt sequences and longer than 30nt sequences, we obtained 861369, 156569 and 190945 unique reads in Group 1, Group 2 and Group 3, respectively.

miRNA that exhibited more than 2-fold differentially expressed were then defined as DEMs (**Figure 2**). Here, we identified a total of 242 significantly DEMs with 113 up-regulations ([Table S1](#)) and 129 down-regulations ([Table S2](#)) in Group 2 (steatotic HepG2 cells), when compared with Group 1. These 242 DEMs may be associated with the lipid accumulation of HepG2 cells. A total of 239 significantly DEMs with 116 up-regulations ([Table S3](#)) and 123 down-regulations ([Table S4](#)) were identified in Group 3 (LG-treated steatotic HepG2 cells), when compared with Group 2. These 239 DEMs may be related with the effects of LG on the lipid accumulation of HepG2. More importantly,

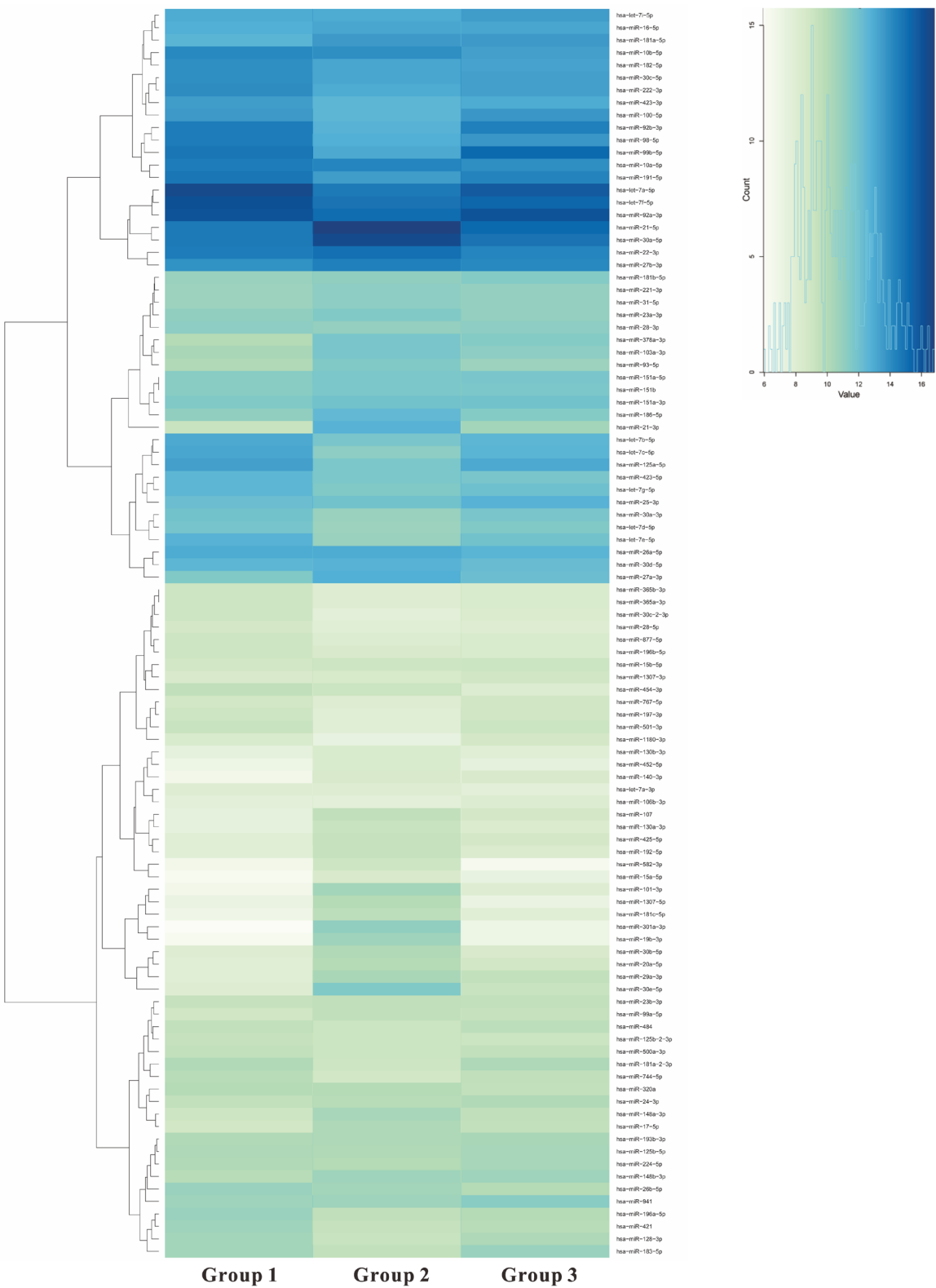


Figure 2. Global miRNA expression profile. A heat map shows the results of differentially expressed miRNAs. Group 1, control HepG2 cells; Group 2, FFA-treated-HepG2 cells; and Group 3, FFA and LG-treated HepG2 cells.

Table 1. 36 DEMs up-regulated by FFA exposure was down-regulated by additional LG treatment

ID	G2 VS G1	G3 VS G2
hsa-miR-4454	47	0.04937
hsa-miR-2467-5p	39	0.1730827
hsa-miR-301a-3p	37.095238	0.0701343
hsa-miR-19a-3p	29.357143	0.0585102
hsa-miR-106b-5p	21.166667	0.1577932
hsa-miR-19b-3p	20.714286	0.0920155
hsa-miR-148b-5p	17.1	0.3651438
hsa-miR-101-3p	15.069444	0.2033622
hsa-miR-210-3p	13.888889	0.2986975
hsa-miR-33b-5p	13	0.2109446
hsa-miR-29b-3p	11.5	0.2201161
hsa-miR-30e-5p	11.053191	0.2558134
hsa-miR-301b	10.583333	0.16942
hsa-miR-21-3p	10.366667	0.270407
hsa-miR-193a-3p	9.8333333	0.2717252
hsa-miR-18a-5p	9.5	0.4329915
hsa-miR-100-3p	8	0.1450244
hsa-miR-1307-5p	7.18	0.177452
hsa-miR-374a-3p	7.1666667	0.0752205
hsa-miR-143-3p	7	0.1119298
hsa-miR-32-5p	6.5	0.129812
hsa-miR-374a-5p	6.125	0.2798244
hsa-miR-4517	6	0.0351574
hsa-miR-31-3p	5.5	0.2812594
hsa-miR-190a-5p	5.3333333	0.1713925
hsa-miR-335-5p	5	0.0632834
hsa-miR-340-5p	4.5517241	0.1534142
hsa-miR-21-5p	4.4175202	0.4152659
hsa-miR-24-2-5p	4.2391304	0.4197256
hsa-miR-20a-3p	4	0.1054723
hsa-miR-30b-5p	3.9438776	0.3946
hsa-miR-30a-5p	3.0513761	0.46874
hsa-let-7i-3p	2.6666667	0.3955211
hsa-miR-181c-5p	2.6	0.3273278
hsa-miR-34a-3p	2.5	0.3375113
hsa-miR-32-3p	2.25	0.2812594
hsa-miR-451a	6.75	0.2031318

36 DEMs up-regulated by FFA exposure was down-regulated by additional LG treatment, while 87 DEMs down-regulated by FFA exposure was increased by additional LG treatment. These 123 DEMs were functionally related with cell apoptosis (miR-106b-5p [26] and miR-143-3p [27]), cell growth and cell cycle (miR-101-3p

Table 2. 87 DEMs down-regulated by FFA exposure was increased by additional LG treatment

ID	G2 VS G1	G3 VS G2
hsa-miR-1275	0.125	2.5313347
hsa-miR-222-5p	0.14	3.375113
hsa-miR-1249	0.1428571	4.2892061
hsa-miR-1292-5p	0.1666667	4.6407803
hsa-miR-1468-5p	0.1666667	3.7970021
hsa-miR-26a-2-3p	0.1666667	2.390705
hsa-miR-3605-3p	0.1666667	6.7502259
hsa-let-7c-3p	0.18	2.9063473
hsa-miR-629-5p	0.18	3.5157427
hsa-miR-1247-3p	0.1875	3.5157427
hsa-miR-1260a	0.1956522	7.4533745
hsa-miR-4741	0.2	3.375113
hsa-miR-23a-5p	0.2025316	3.2960088
hsa-miR-1306-5p	0.2142857	4.6407803
hsa-miR-454-5p	0.2142857	4.3419422
hsa-let-7e-3p	0.2222222	6.3283368
hsa-miR-1908-5p	0.2222222	4.2188912
hsa-miR-130b-5p	0.241573	3.6302087
hsa-miR-1268a	0.25	2.1094456
hsa-miR-1268b	0.25	2.1094456
hsa-miR-197-5p	0.25	3.375113
hsa-miR-23b-5p	0.25	2.5313347
hsa-miR-3143	0.25	2.5313347
hsa-miR-3173-5p	0.25	5.0626695
hsa-miR-338-5p	0.25	3.7970021
hsa-miR-4510	0.25	3.1114323
hsa-miR-767-3p	0.25	2.3203902
hsa-let-7c-5p	0.2522922	3.1362238
hsa-miR-92b-3p	0.2654369	3.9507116
hsa-let-7b-3p	0.265625	3.1467965
hsa-miR-1301-3p	0.2826087	5.4196526
hsa-let-7e-5p	0.2870332	2.5421287
hsa-miR-92b-5p	0.2916667	3.3148431
hsa-miR-98-5p	0.2956958	2.2783677
hsa-miR-125a-5p	0.2977941	3.104091
hsa-miR-99b-5p	0.2996466	5.4230094
hsa-miR-1343-3p	0.3	4.9220397
hsa-miR-200c-3p	0.3	3.0938536
hsa-let-7a-5p	0.3007397	2.6079707
hsa-miR-574-5p	0.3035714	2.7298708
hsa-miR-1304-3p	0.326087	3.7407502
hsa-miR-628-3p	0.3269231	2.2335306
hsa-let-7d-3p	0.3285714	2.7147648
hsa-miR-4449	0.3333333	2.1094456
hsa-miR-6511a-3p	0.3333333	3.1641684

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hsa-miR-7976	0.3333333	4.6407803
hsa-miR-3615	0.3548387	3.0203426
hsa-miR-219a-1-3p	0.3571429	2.0250678
hsa-miR-146b-5p	0.36	5.3439289
hsa-miR-501-3p	0.3733032	2.8739598
hsa-miR-346	0.375	2.5313347
hsa-miR-760	0.375	2.390705
hsa-miR-1247-5p	0.3793103	2.7806328
hsa-let-7b-5p	0.388675	2.0873326
hsa-miR-128-3p	0.3927911	2.359601
hsa-miR-1260b	0.4032258	2.868846
hsa-miR-191-5p	0.4107287	2.2359188
hsa-miR-128-1-5p	0.4117647	3.4655178
hsa-miR-191-3p	0.4130435	2.442516
hsa-miR-365a-5p	0.4166667	2.0250678
hsa-miR-18a-3p	0.4375	2.3143632
hsa-miR-197-3p	0.4391534	2.4652557
hsa-miR-1910-5p	0.4411765	2.7000904
hsa-let-7d-5p	0.4480568	2.0651103
hsa-miR-181a-2-3p	0.4544304	2.7663704
hsa-miR-744-5p	0.4545455	2.0606647
hsa-miR-30a-3p	0.4567439	2.2658057
hsa-miR-1180-3p	0.4568966	3.3790931
hsa-miR-100-5p	0.4688773	2.7875435
hsa-miR-183-5p	0.4748603	3.1170161
hsa-miR-505-3p	0.4893617	2.1277886
hsa-miR-1908-3p	0.5	5.3158029
hsa-miR-2682-3p	0.5	2.5313347
hsa-miR-3126-5p	0.5	8.0158933
hsa-miR-3188	0.5	4.2188912
hsa-miR-320c	0.5	2.6217395
hsa-miR-320d	0.5	4.0079467
hsa-miR-3935	0.5	3.1641684
hsa-miR-3960	0.5	6.3283368
hsa-miR-4435	0.5	2.3203902
hsa-miR-4473	0.5	2.9532238
hsa-miR-4671-3p	0.5	2.9532238
hsa-miR-4781-3p	0.5	2.1094456
hsa-miR-505-5p	0.5	2.390705
hsa-miR-576-5p	0.5	4.2188912
hsa-miR-636	0.5	5.4845586
hsa-miR-6716-3p	0.5	7.5940042

[28] and miR-29b-3p [29]), inflammatory (miR-23b-5p [30] and miR-21 [31]), lipid metabolism (miR-33b-5p [32, 33] and miR-335-5p [34]) and oxidative stress response (miR-200c-3p [35]), and may be more critical to the protection effects of LG on FFA-induced steatosis (Tables 1, 2 and Figure 3).

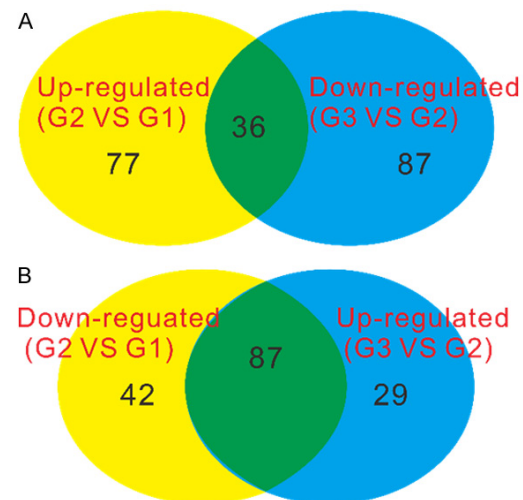


Figure 3. Venn diagram.

Validation of miRNA differential expression by qRT-PCR

To validate the results observed in our miRNA sequencing data, we performed qRT-PCR analysis on several human miRNAs. Among the 123 DEMs, miR-34a [16], miR-21 and miR-451 [36] have been reported increased in patients with NAFLD, while miR-183 [37] was decreased in a rat model with steatosis. **Figure 4** showed that the differential expression of all five miRNAs tested by qRT-PCR was significant and consistent with our sequencing data.

Discussion

miRNAs are involved in lipid metabolism, inflammation, cell apoptosis and tissue development [11-15]. The dysregulation and modulation of microRNA expression has been reported in NAFLD, NASH and HCC [16-19]. A recent study showed that GLP-1RAs can improve metabolic dysfunction, insulin resistance and lipotoxicity in patients with NASH [38]. Studies on the effects of GLP-1RAs on the miRNA profile of hepatic steatosis are rare. In this study, we conducted miRNA Next-Generation Sequencing for a cell model of hepatic steatosis with or without liraglutide (LG) treatment. We analyzed the miRNA expression difference between steatotic and control HepG2 cells, and identified 242 differentially expressed miRNAs (DEMs), many of which were identified as DEMs in previous studies on patients with NAFLD and rat model with steatosis, such as miR-34a [16], miR-21 [36], miR-451 [36] and miR-183 [37]. Moreover,

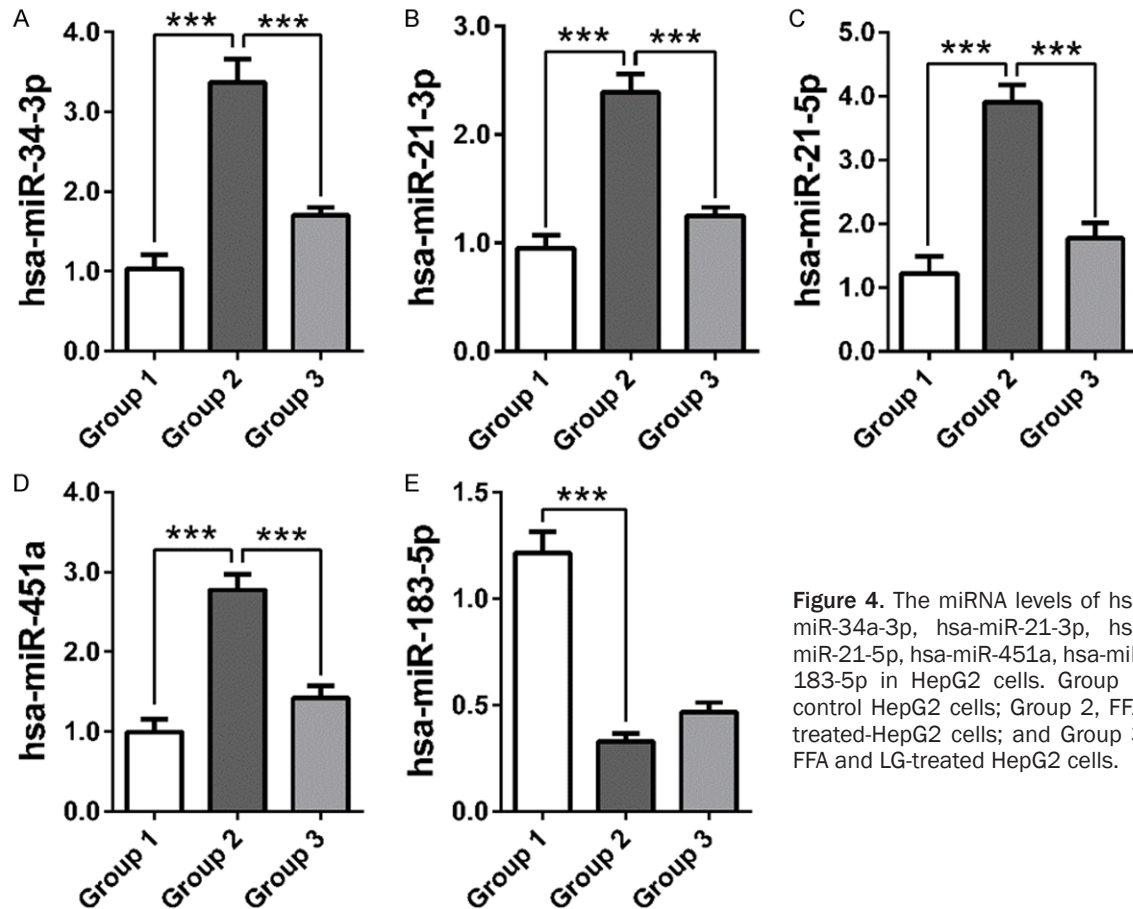


Figure 4. The miRNA levels of hsa-miR-34a-3p, hsa-miR-21-3p, hsa-miR-21-5p, hsa-miR-451a, hsa-miR-183-5p in HepG2 cells. Group 1, control HepG2 cells; Group 2, FFA-treated-HepG2 cells; and Group 3, FFA and LG-treated HepG2 cells.

we elucidated miRNAs involved in the protective effects of LG on hepatic steatosis. A total of 239 DEMs were identified in LG-treated steatotic HepG2 cells when compared with steatotic HepG2 cells. Our data suggested that miRNA Next-Generation Sequencing is a powerful approach to study miRNA expression profiling.

More importantly, among the 239 DEMs, 36 miRNAs were increased in steatotic cells and reduced by additional LG treatment, and 87 miRNAs have the opposite changing tendency. These 123 miRNAs may be more important to the protection effects of LG on hepatic steatosis. The targets of 123 DEMs may be involved in the regulation of cell apoptosis, cell growth and cell cycle, inflammatory, lipid metabolism and oxidative stress response.

In conclusion, our study showed the differentially expressed miRNAs between steatotic and control HepG2 cells, and between LG-treated and non-treated steatotic cells. Investigating DEMs is useful to advance understanding of

mechanisms important to the development of hepatic steatosis and how GLP-1RA improved hepatic steatosis, thus provided novel insights into NAFLD pathogenesis and treatment. However, there are some limitations of our study including lacking of experimental data confirming the targets of DEMs, and shortage of in vivo experiments and clinical data. Further investigations are needed.

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Disclosure of conflict of interest

None.

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Table S1. Upregulated miRNAs (Group 2 VS Group 1)

ID	Group 1	Group 2	Fold change
hsa-miR-4454	1.414213562	66.46803743	47
hsa-miR-2467-5p	1.414213562	55.15432893	39
hsa-miR-301a-3p	29.69848481	1101.672365	37.0952381
hsa-miR-19a-3p	9.899494937	290.6208871	29.35714286
hsa-miR-106b-5p	8.485281374	179.6051224	21.16666667
hsa-miR-19b-3p	39.59797975	820.2438662	20.71428571
hsa-miR-6087	1.414213562	27.57716447	19.5
hsa-miR-148b-5p	7.071067812	120.9152596	17.1
hsa-miR-3065-3p	1.414213562	22.627417	16
hsa-miR-101-3p	50.91168825	767.2108576	15.06944444
hsa-miR-210-3p	12.72792206	176.7766953	13.88888889
hsa-miR-33b-5p	1.414213562	18.38477631	13
hsa-miR-29b-3p	4.242640687	48.7903679	11.5
hsa-miR-338-3p	2.828427125	32.52691193	11.5
hsa-miR-34a-5p	5.656854249	62.93250353	11.125
hsa-miR-30e-5p	132.9360749	1469.367891	11.05319149
hsa-miR-301b	8.485281374	89.80256121	10.58333333
hsa-miR-21-3p	318.1980515	3298.653134	10.36666667
hsa-miR-193a-3p	8.485281374	83.43860018	9.833333333
hsa-miR-18a-5p	8.485281374	80.61017306	9.5
hsa-miR-194-5p	1.414213562	12.72792206	9
hsa-miR-100-3p	5.656854249	45.254834	8
hsa-miR-590-3p	2.828427125	22.627417	8
hsa-miR-152-5p	1.414213562	10.60660172	7.5
hsa-miR-4485	1.414213562	10.60660172	7.5
hsa-miR-1307-5p	70.71067812	507.7026689	7.18
hsa-miR-374a-3p	25.45584412	182.4335495	7.166666667
hsa-miR-181c-5p	57.98275606	410.1219331	7.073170732
hsa-miR-143-3p	9.899494937	69.29646456	7
hsa-miR-96-5p	4.242640687	28.99137803	6.833333333
hsa-miR-451a	2.828427125	19.09188309	6.75
hsa-miR-32-5p	1.414213562	9.192388155	6.5
hsa-miR-374a-5p	11.3137085	69.29646456	6.125
hsa-miR-378a-5p	1.414213562	8.485281374	6
hsa-miR-4517	1.414213562	8.485281374	6
hsa-miR-17-3p	12.72792206	70.00357134	5.5
hsa-miR-31-3p	4.242640687	23.33452378	5.5
hsa-miR-378c	5.656854249	31.11269837	5.5
hsa-miR-378i	1.414213562	7.778174593	5.5
hsa-miR-29a-3p	120.2081528	650.5382387	5.411764706
hsa-miR-190a-5p	4.242640687	22.627417	5.333333333
hsa-miR-582-3p	50.91168825	271.529004	5.333333333
hsa-miR-582-5p	16.97056275	89.09545443	5.25
hsa-miR-769-5p	26.87005769	135.0573952	5.026315789
hsa-miR-335-5p	2.828427125	14.14213562	5
hsa-miR-516a-5p	2.828427125	14.14213562	5
hsa-miR-3176	2.828427125	13.43502884	4.75

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hsa-miR-340-5p	41.01219331	186.6761902	4.551724138
hsa-miR-15a-5p	38.18376618	173.2411614	4.537037037
hsa-miR-99a-3p	2.828427125	12.72792206	4.5
hsa-miR-21-5p	12979.65208	57337.87467	4.417520157
hsa-miR-107	89.09545443	388.2016229	4.357142857
hsa-miR-20a-5p	123.0365799	530.3300859	4.310344828
hsa-miR-424-5p	5.656854249	24.04163056	4.25
hsa-miR-24-2-5p	32.52691193	137.8858223	4.239130435
hsa-let-7f-2-3p	5.656854249	22.627417	4
hsa-miR-20a-3p	1.414213562	5.656854249	4
hsa-miR-374b-3p	1.414213562	5.656854249	4
hsa-miR-378d	1.414213562	5.656854249	4
hsa-miR-550a-3p	2.828427125	11.3137085	4
hsa-miR-30b-5p	138.5929291	546.5935419	3.943877551
hsa-miR-873-5p	4.242640687	15.55634919	3.666666667
hsa-miR-130a-3p	86.2670273	316.0767312	3.663934426
hsa-miR-140-3p	49.49747468	177.4838021	3.585714286
hsa-miR-660-5p	26.87005769	95.45941546	3.552631579
hsa-miR-181a-3p	14.14213562	49.49747468	3.5
hsa-miR-3690	1.414213562	4.949747468	3.5
hsa-miR-378a-3p	519.0163774	1771.302487	3.41280654
hsa-miR-339-3p	7.071067812	23.33452378	3.3
hsa-miR-361-3p	16.97056275	55.86143571	3.291666667
hsa-miR-127-3p	2.828427125	9.192388155	3.25
hsa-miR-30a-5p	13102.68866	39981.23162	3.051376147
hsa-miR-30d-3p	15.55634919	47.37615434	3.045454545
hsa-miR-2682-5p	4.242640687	12.72792206	3
hsa-miR-3614-5p	1.414213562	4.242640687	3
hsa-miR-874-3p	1.414213562	4.242640687	3
hsa-miR-93-3p	7.071067812	21.21320344	3
hsa-miR-192-5p	117.3797257	334.4615075	2.84939759
hsa-miR-132-5p	2.828427125	7.778174593	2.75
hsa-miR-93-5p	551.5432893	1504.72323	2.728205128
hsa-let-7i-3p	4.242640687	11.3137085	2.666666667
hsa-miR-17-5p	223.4457429	590.4341623	2.642405063
hsa-miR-103a-3p	678.8225099	1777.666448	2.61875
hsa-miR-181c-3p	21.21320344	55.15432893	2.6
hsa-miR-584-5p	15.55634919	40.30508653	2.590909091
hsa-miR-452-5p	63.63961031	162.6345597	2.555555556
hsa-miR-148a-3p	265.8721497	677.4082964	2.54787234
hsa-miR-425-5p	128.6934342	325.2691193	2.527472527
hsa-miR-186-5p	1098.843938	2771.151475	2.521879022
hsa-miR-27a-3p	1484.92424	3715.139028	2.501904762
hsa-miR-185-3p	1.414213562	3.535533906	2.5
hsa-miR-324-5p	9.899494937	24.74873734	2.5
hsa-miR-33b-3p	2.828427125	7.071067812	2.5
hsa-miR-34a-3p	1.414213562	3.535533906	2.5
hsa-miR-450a-5p	1.414213562	3.535533906	2.5
hsa-miR-4664-3p	1.414213562	3.535533906	2.5

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hsa-miR-4677-3p	4.242640687	10.60660172	2.5
hsa-miR-9-5p	1.414213562	3.535533906	2.5
hsa-miR-7704	8.485281374	20.50609665	2.416666667
hsa-miR-181d-5p	55.15432893	127.2792206	2.307692308
hsa-miR-148a-5p	9.899494937	22.627417	2.285714286
hsa-miR-183-3p	2.828427125	6.363961031	2.25
hsa-miR-32-3p	2.828427125	6.363961031	2.25
hsa-miR-548av-5p	19.79898987	44.54772721	2.25
hsa-miR-548k	19.79898987	44.54772721	2.25
hsa-miR-532-5p	74.95331881	164.0487732	2.188679245
hsa-miR-145-5p	1.414213562	2.828427125	2
hsa-miR-342-5p	1.414213562	2.828427125	2
hsa-miR-3934-5p	1.414213562	2.828427125	2
hsa-miR-4768-5p	1.414213562	2.828427125	2
hsa-miR-502-5p	1.414213562	2.828427125	2
hsa-miR-550a-3-5p	8.485281374	16.97056275	2
hsa-miR-550a-5p	8.485281374	16.97056275	2

Table S2. Downregulated miRNAs (Group 2 VS Group 1)

ID	Group 1	Group 2	Fold change
hsa-miR-1275	5.656854249	0.707106781	0.125
hsa-miR-576-5p	25.45584412	3.535533906	0.138888889
hsa-miR-222-5p	70.71067812	9.899494937	0.14
hsa-miR-1249	29.69848481	4.242640687	0.142857143
hsa-miR-1292-5p	4.242640687	0.707106781	0.166666667
hsa-miR-1468-5p	4.242640687	0.707106781	0.166666667
hsa-miR-26a-2-3p	12.72792206	2.121320344	0.166666667
hsa-miR-3605-3p	4.242640687	0.707106781	0.166666667
hsa-let-7c-3p	35.35533906	6.363961031	0.18
hsa-miR-629-5p	35.35533906	6.363961031	0.18
hsa-miR-1247-3p	11.3137085	2.121320344	0.1875
hsa-miR-3197	25.45584412	4.949747468	0.194444444
hsa-miR-1260a	32.52691193	6.363961031	0.195652174
hsa-miR-4741	7.071067812	1.414213562	0.2
hsa-miR-23a-5p	111.7228714	22.627417	0.202531646
hsa-miR-1306-5p	9.899494937	2.121320344	0.214285714
hsa-miR-454-5p	79.19595949	16.97056275	0.214285714
hsa-let-7e-3p	12.72792206	2.828427125	0.222222222
hsa-miR-1908-5p	12.72792206	2.828427125	0.222222222
hsa-miR-130b-5p	125.8650071	30.40559159	0.241573034
hsa-miR-1268a	2.828427125	0.707106781	0.25
hsa-miR-1268b	2.828427125	0.707106781	0.25
hsa-miR-1915-5p	2.828427125	0.707106781	0.25
hsa-miR-195-5p	2.828427125	0.707106781	0.25
hsa-miR-197-5p	2.828427125	0.707106781	0.25
hsa-miR-2116-3p	33.9411255	8.485281374	0.25
hsa-miR-23b-5p	2.828427125	0.707106781	0.25
hsa-miR-3143	2.828427125	0.707106781	0.25

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hsa-miR-3173-5p	2.828427125	0.707106781	0.25
hsa-miR-338-5p	5.656854249	1.414213562	0.25
hsa-miR-3605-5p	5.656854249	1.414213562	0.25
hsa-miR-4510	22.627417	5.656854249	0.25
hsa-miR-627-5p	2.828427125	0.707106781	0.25
hsa-miR-6511b-5p	2.828427125	0.707106781	0.25
hsa-miR-767-3p	5.656854249	1.414213562	0.25
hsa-let-7c-5p	4781.456054	1206.324169	0.252292221
hsa-miR-92b-3p	12802.87538	3398.35519	0.265436872
hsa-let-7b-3p	226.27417	60.1040764	0.265625
hsa-miR-1301-3p	32.52691193	9.192388155	0.282608696
hsa-let-7e-5p	3370.070919	967.3220767	0.287033151
hsa-miR-125a-5p	5577.65829	1660.993829	0.297794118
hsa-miR-1229-3p	7.071067812	2.121320344	0.3
hsa-miR-1343-3p	7.071067812	2.121320344	0.3
hsa-miR-200c-3p	7.071067812	2.121320344	0.3
hsa-miR-4775	7.071067812	2.121320344	0.3
hsa-let-7a-5p	44358.2226	13340.27653	0.300739654
hsa-miR-574-5p	39.59797975	12.02081528	0.303571429
hsa-miR-424-3p	28.28427125	9.192388155	0.325
hsa-miR-1304-3p	32.52691193	10.60660172	0.326086957
hsa-miR-628-3p	36.76955262	12.02081528	0.326923077
hsa-let-7d-3p	49.49747468	16.26345597	0.328571429
hsa-miR-125a-3p	38.18376618	12.72792206	0.333333333
hsa-miR-4421	4.242640687	1.414213562	0.333333333
hsa-miR-4449	4.242640687	1.414213562	0.333333333
hsa-miR-6511a-3p	4.242640687	1.414213562	0.333333333
hsa-miR-7976	4.242640687	1.414213562	0.333333333
hsa-miR-3615	87.68124087	31.11269837	0.35483871
hsa-miR-219a-1-3p	9.899494937	3.535533906	0.357142857
hsa-miR-146b-5p	35.35533906	12.72792206	0.36
hsa-miR-501-3p	312.5411973	116.6726189	0.373303167
hsa-miR-1226-3p	5.656854249	2.121320344	0.375
hsa-miR-346	5.656854249	2.121320344	0.375
hsa-miR-760	11.3137085	4.242640687	0.375
hsa-miR-30c-2-3p	271.529004	102.5304833	0.377604167
hsa-miR-1247-5p	41.01219331	15.55634919	0.379310345
hsa-let-7b-5p	4408.103674	1713.319731	0.388675008
hsa-miR-128-3p	765.0895372	300.520382	0.392791128
hsa-miR-421	818.8296526	326.6833329	0.398963731
hsa-miR-1260b	43.84062043	17.67766953	0.403225806
hsa-let-7f-5p	36618.23177	14850.65662	0.405553625
hsa-miR-196a-5p	944.6946597	384.666089	0.407185629
hsa-miR-191-5p	13972.43	5738.878636	0.410728745
hsa-miR-128-1-5p	24.04163056	9.899494937	0.411764706
hsa-miR-191-3p	32.52691193	13.43502884	0.413043478
hsa-miR-215-5p	16.97056275	7.071067812	0.416666667
hsa-miR-365a-5p	8.485281374	3.535533906	0.416666667
hsa-miR-25-5p	65.05382387	28.28427125	0.434782609

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hsa-miR-18a-3p	56.56854249	24.74873734	0.4375
hsa-miR-197-3p	267.2863633	117.3797257	0.439153439
hsa-miR-1910-5p	24.04163056	10.60660172	0.441176471
hsa-let-7d-5p	1892.217746	847.8210306	0.448056801
hsa-miR-2110	14.14213562	6.363961031	0.45
hsa-miR-30c-1-3p	14.14213562	6.363961031	0.45
hsa-miR-181a-2-3p	558.6143571	253.8513344	0.45443038
hsa-miR-744-5p	497.803174	226.27417	0.454545455
hsa-miR-30a-3p	2076.06551	948.2301936	0.456743869
hsa-miR-1180-3p	164.0487732	74.95331881	0.456896552
hsa-miR-4326	33.9411255	15.55634919	0.458333333
hsa-miR-222-3p	8432.955472	3914.543141	0.464195875
hsa-miR-100-5p	6247.995519	2929.543394	0.46887732
hsa-let-7f-1-3p	26.87005769	12.72792206	0.473684211
hsa-miR-628-5p	53.74011537	25.45584412	0.473684211
hsa-miR-183-5p	759.432683	360.6244584	0.474860335
hsa-miR-675-5p	91.92388155	43.84062043	0.476923077
hsa-miR-505-3p	66.46803743	32.52691193	0.489361702
hsa-miR-1908-3p	7.071067812	3.535533906	0.5
hsa-miR-2116-5p	2.828427125	1.414213562	0.5
hsa-miR-2682-3p	1.414213562	0.707106781	0.5
hsa-miR-29c-5p	4.242640687	2.121320344	0.5
hsa-miR-3074-5p	4.242640687	2.121320344	0.5
hsa-miR-3126-5p	1.414213562	0.707106781	0.5
hsa-miR-3188	1.414213562	0.707106781	0.5
hsa-miR-3200-5p	1.414213562	0.707106781	0.5
hsa-miR-320c	19.79898987	9.899494937	0.5
hsa-miR-320d	5.656854249	2.828427125	0.5
hsa-miR-335-3p	32.52691193	16.26345597	0.5
hsa-miR-3691-5p	9.899494937	4.949747468	0.5
hsa-miR-3935	2.828427125	1.414213562	0.5
hsa-miR-3960	1.414213562	0.707106781	0.5
hsa-miR-4435	11.3137085	5.656854249	0.5
hsa-miR-4473	1.414213562	0.707106781	0.5
hsa-miR-4660	1.414213562	0.707106781	0.5
hsa-miR-4671-3p	1.414213562	0.707106781	0.5
hsa-miR-4781-3p	1.414213562	0.707106781	0.5
hsa-miR-5003-3p	1.414213562	0.707106781	0.5
hsa-miR-5010-3p	2.828427125	1.414213562	0.5
hsa-miR-501-5p	11.3137085	5.656854249	0.5
hsa-miR-505-5p	4.242640687	2.121320344	0.5
hsa-miR-576-3p	8.485281374	4.242640687	0.5
hsa-miR-636	1.414213562	0.707106781	0.5
hsa-miR-6505-5p	1.414213562	0.707106781	0.5
hsa-miR-6511a-5p	1.414213562	0.707106781	0.5
hsa-miR-6716-3p	1.414213562	0.707106781	0.5
hsa-miR-6724-5p	2.828427125	1.414213562	0.5
hsa-miR-6858-5p	1.414213562	0.707106781	0.5
hsa-miR-7110-3p	1.414213562	0.707106781	0.5

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Table S3. Upregulated miRNAs (Group 3 VS Group 2)

ID	Group 2	Group3	Fold change
hsa-miR-3126-5p	1.539574951	12.34106854	8.015893304
hsa-miR-6716-3p	1.539574951	11.69153862	7.594004182
hsa-miR-1260a	13.85617456	103.2752578	7.453374475
hsa-miR-3605-3p	1.539574951	10.39247877	6.75022594
hsa-let-7e-3p	6.158299805	38.9717954	6.328336819
hsa-miR-3960	1.539574951	9.742948849	6.328336819
hsa-miR-636	1.539574951	8.443889003	5.484558576
hsa-miR-99b-5p	9138.916911	49560.43221	5.423009388
hsa-miR-1301-3p	20.01447437	108.4714972	5.419652557
hsa-miR-146b-5p	27.71234912	148.0928225	5.343928869
hsa-miR-1908-3p	7.697874756	40.92038517	5.315802928
hsa-miR-3200-3p	7.697874756	40.27085524	5.231425103
hsa-miR-3173-5p	1.539574951	7.794359079	5.062669455
hsa-miR-1343-3p	4.618724854	22.73354731	4.922039748
hsa-miR-1292-5p	1.539574951	7.144829156	4.640780334
hsa-miR-1306-5p	4.618724854	21.43448747	4.640780334
hsa-miR-7976	3.079149903	14.28965831	4.640780334
hsa-miR-425-3p	15.39574951	69.49970179	4.514213597
hsa-miR-454-5p	36.94979883	160.4338911	4.341942206
hsa-miR-1249	9.237449708	39.62132532	4.289206066
hsa-miR-1908-5p	6.158299805	25.98119693	4.218891212
hsa-miR-3188	1.539574951	6.495299233	4.218891212
hsa-miR-328-3p	1.539574951	6.495299233	4.218891212
hsa-miR-576-5p	7.697874756	32.47649616	4.218891212
hsa-miR-320d	6.158299805	24.68213708	4.007946652
hsa-miR-92b-3p	7399.197216	29232.0942	3.95071159
hsa-miR-1468-5p	1.539574951	5.84576931	3.797002091
hsa-miR-2277-3p	1.539574951	5.84576931	3.797002091
hsa-miR-338-5p	3.079149903	11.69153862	3.797002091
hsa-miR-3609	1.539574951	5.84576931	3.797002091
hsa-miR-4742-3p	1.539574951	5.84576931	3.797002091
hsa-miR-1304-3p	23.09362427	86.3874798	3.740750208
hsa-miR-130b-5p	66.20172291	240.3260716	3.630208718
hsa-miR-1247-3p	4.618724854	16.23824808	3.515742677
hsa-miR-629-5p	13.85617456	48.71474425	3.515742677
hsa-miR-128-1-5p	21.55404932	74.69594118	3.465517782
hsa-miR-1180-3p	163.1949448	551.4509049	3.379093056
hsa-miR-197-5p	1.539574951	5.196239386	3.37511297
hsa-miR-222-5p	21.55404932	72.74735141	3.37511297
hsa-miR-4741	3.079149903	10.39247877	3.37511297
hsa-miR-92b-5p	10.77702466	35.72414578	3.314843095
hsa-miR-23a-5p	49.26639844	162.3824808	3.29600876
hsa-miR-3935	3.079149903	9.742948849	3.164168409
hsa-miR-6511a-3p	3.079149903	9.742948849	3.164168409
hsa-miR-937-3p	3.079149903	9.742948849	3.164168409
hsa-let-7b-3p	130.8638709	411.8019714	3.146796504
hsa-let-7c-5p	2626.514867	8237.338487	3.136223819

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hsa-miR-183-5p	785.1832252	2447.428751	3.117016096
hsa-miR-4510	12.31659961	38.32226547	3.111432269
hsa-miR-125a-5p	3616.461561	11225.82566	3.104090967
hsa-miR-200c-3p	4.618724854	14.28965831	3.093853556
hsa-miR-3615	67.74129786	204.6019258	3.020342573
hsa-miR-99b-3p	55.42469825	166.2796604	3.000100418
hsa-miR-142-5p	6.158299805	18.18683785	2.953223849
hsa-miR-181b-3p	1.539574951	4.546709463	2.953223849
hsa-miR-4473	1.539574951	4.546709463	2.953223849
hsa-miR-4671-3p	1.539574951	4.546709463	2.953223849
hsa-miR-503-5p	1.539574951	4.546709463	2.953223849
hsa-miR-6858-3p	1.539574951	4.546709463	2.953223849
hsa-let-7c-3p	13.85617456	40.27085524	2.90634728
hsa-miR-501-3p	254.029867	730.0716338	2.873959832
hsa-miR-574-3p	129.3242959	371.5311161	2.872864016
hsa-miR-1260b	38.48937378	110.420087	2.868846024
hsa-miR-615-5p	4.618724854	12.99059847	2.812594142
hsa-miR-100-5p	6378.459023	17780.23212	2.78754352
hsa-miR-1247-5p	33.87064893	94.18183888	2.780632845
hsa-miR-181a-2-3p	552.7074075	1528.993439	2.76637045
hsa-miR-574-5p	26.17277417	71.44829156	2.729870785
hsa-let-7d-3p	35.41022388	96.13042865	2.71476478
hsa-miR-1910-5p	23.09362427	62.35487264	2.700090376
hsa-miR-671-3p	115.4681213	308.5267136	2.671964435
hsa-miR-486-5p	163.1949448	435.1850486	2.666657653
hsa-miR-320c	21.55404932	56.50910333	2.621739539
hsa-let-7a-5p	29045.62103	75750.12824	2.607970687
hsa-let-7e-5p	2106.138533	5354.075158	2.542128674
hsa-miR-1275	1.539574951	3.89717954	2.531334727
hsa-miR-142-3p	3.079149903	7.794359079	2.531334727
hsa-miR-23b-5p	1.539574951	3.89717954	2.531334727
hsa-miR-2682-3p	1.539574951	3.89717954	2.531334727
hsa-miR-3143	1.539574951	3.89717954	2.531334727
hsa-miR-346	4.618724854	11.69153862	2.531334727
hsa-miR-197-3p	255.5694419	630.0440256	2.465255708
hsa-miR-191-3p	29.25192407	71.44829156	2.442515965
hsa-miR-193a-5p	78.51832252	187.7141478	2.39070502
hsa-miR-26a-2-3p	4.618724854	11.0420087	2.39070502
hsa-miR-505-5p	4.618724854	11.0420087	2.39070502
hsa-miR-760	9.237449708	22.08401739	2.39070502
hsa-miR-128-3p	654.3193543	1543.932628	2.359601038
hsa-miR-4435	12.31659961	28.57931662	2.320390167
hsa-miR-6511b-3p	3.079149903	7.144829156	2.320390167
hsa-miR-6840-5p	3.079149903	7.144829156	2.320390167
hsa-miR-767-3p	3.079149903	7.144829156	2.320390167
hsa-miR-615-3p	135.4825957	313.7229529	2.315595972
hsa-miR-18a-3p	53.8851233	124.7097453	2.314363179
hsa-miR-98-5p	7805.645003	17784.1293	2.27836768
hsa-miR-30a-3p	2064.57001	4677.914507	2.265805706

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hsa-miR-105-5p	52.34554834	118.214446	2.258347649
hsa-miR-191-5p	12495.1903	27938.23059	2.235918774
hsa-miR-628-3p	26.17277417	58.4576931	2.233530642
hsa-miR-767-5p	295.5983906	654.0766327	2.212720547
hsa-miR-301a-5p	10.77702466	23.38307724	2.169715481
hsa-miR-505-3p	70.82044776	150.6909422	2.127788611
hsa-miR-1266-5p	6.158299805	12.99059847	2.109445606
hsa-miR-1268a	1.539574951	3.247649616	2.109445606
hsa-miR-1268b	1.539574951	3.247649616	2.109445606
hsa-miR-3940-3p	4.618724854	9.742948849	2.109445606
hsa-miR-4449	3.079149903	6.495299233	2.109445606
hsa-miR-4781-3p	1.539574951	3.247649616	2.109445606
hsa-miR-522-3p	10.77702466	22.73354731	2.109445606
hsa-miR-92a-3p	40024.33001	84211.55455	2.1040091
hsa-miR-25-3p	4300.032839	9016.774395	2.096908264
hsa-let-7b-5p	3730.390107	7786.56472	2.087332557
hsa-let-7d-5p	1845.950367	3812.09112	2.065110302
hsa-miR-744-5p	492.6639844	1015.21527	2.060664677
hsa-miR-219a-1-3p	7.697874756	15.58871816	2.025067782
hsa-miR-365a-5p	7.697874756	15.58871816	2.025067782

Table S4. Downregulated miRNAs (Group 3 VS Group 2)

ID	Group 2	Group3	Fold change
hsa-miR-33a-5p	20.01447437	0.649529923	0.032453009
hsa-miR-6087	60.0434231	1.94858977	0.032453009
hsa-miR-4517	18.47489942	0.649529923	0.035157427
hsa-miR-4454	144.7200454	7.144829156	0.049370004
hsa-miR-2355-5p	12.31659961	0.649529923	0.05273614
hsa-miR-19a-3p	632.765305	37.02320563	0.05851017
hsa-miR-335-5p	30.79149903	1.94858977	0.063283368
hsa-miR-301a-3p	2398.657774	168.2282501	0.070134328
hsa-miR-374a-3p	397.2103374	29.87837647	0.075220541
hsa-miR-3677-3p	16.93532446	1.299059847	0.076707113
hsa-miR-1272	7.697874756	0.649529923	0.084377824
hsa-miR-3912-3p	7.697874756	0.649529923	0.084377824
hsa-miR-455-5p	21.55404932	1.94858977	0.090404812
hsa-miR-19b-3p	1785.906943	164.3310706	0.092015472
hsa-miR-20a-3p	12.31659961	1.299059847	0.10547228
hsa-miR-4523	6.158299805	0.649529923	0.10547228
hsa-miR-624-5p	6.158299805	0.649529923	0.10547228
hsa-miR-143-3p	150.8783452	16.88777801	0.111929767
hsa-miR-580-3p	10.77702466	1.299059847	0.120539749
hsa-miR-4792	96.99322193	12.34106854	0.127236402
hsa-miR-32-5p	20.01447437	2.598119693	0.129812037
hsa-miR-137	4.618724854	0.649529923	0.140629707
hsa-miR-548e-5p	4.618724854	0.649529923	0.140629707
hsa-miR-548h-5p	4.618724854	0.649529923	0.140629707
hsa-miR-548u	4.618724854	0.649529923	0.140629707

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hsa-miR-652-5p	4.618724854	0.649529923	0.140629707
hsa-miR-100-3p	98.53279688	14.28965831	0.145024385
hsa-miR-340-5p	406.4477871	62.35487264	0.153414226
hsa-miR-106b-5p	391.0520376	61.70534271	0.157793175
hsa-miR-590-3p	49.26639844	7.794359079	0.15820842
hsa-miR-301b	195.5260188	33.12602609	0.169420041
hsa-miR-190a-5p	49.26639844	8.443889003	0.171392456
hsa-miR-2467-5p	120.0868462	20.78495755	0.173082716
hsa-miR-1307-5p	1105.414815	196.1580368	0.17745197
hsa-miR-582-3p	591.1967813	113.0182067	0.191168508
hsa-miR-451a	41.56852368	8.443889003	0.203131799
hsa-miR-101-3p	1670.438822	339.7041499	0.203362222
hsa-miR-1284	3.079149903	0.649529923	0.210944561
hsa-miR-138-5p	6.158299805	1.299059847	0.210944561
hsa-miR-141-3p	12.31659961	2.598119693	0.210944561
hsa-miR-144-3p	3.079149903	0.649529923	0.210944561
hsa-miR-200b-3p	3.079149903	0.649529923	0.210944561
hsa-miR-216b-5p	3.079149903	0.649529923	0.210944561
hsa-miR-33b-5p	40.02894873	8.443889003	0.210944561
hsa-miR-4467	3.079149903	0.649529923	0.210944561
hsa-miR-4638-3p	3.079149903	0.649529923	0.210944561
hsa-miR-4728-3p	3.079149903	0.649529923	0.210944561
hsa-miR-548aq-3p	3.079149903	0.649529923	0.210944561
hsa-miR-548d-3p	3.079149903	0.649529923	0.210944561
hsa-miR-548w	3.079149903	0.649529923	0.210944561
hsa-miR-29b-3p	106.2306716	23.38307724	0.220116063
hsa-miR-106a-5p	7.697874756	1.94858977	0.253133473
hsa-miR-199a-5p	7.697874756	1.94858977	0.253133473
hsa-miR-30e-5p	3199.236749	818.4077033	0.255813423
hsa-miR-542-3p	12.31659961	3.247649616	0.263680701
hsa-miR-196a-3p	29.25192407	7.794359079	0.266456287
hsa-miR-4461	16.93532446	4.546709463	0.268474895
hsa-miR-561-5p	38.48937378	10.39247877	0.270009038
hsa-miR-21-3p	7182.117148	1942.094471	0.270406961
hsa-miR-193a-3p	181.6698443	49.36427417	0.271725197
hsa-miR-374a-5p	150.8783452	42.21944501	0.279824417
hsa-miR-31-3p	50.80597339	14.28965831	0.281259414
hsa-miR-32-3p	13.85617456	3.89717954	0.281259414
hsa-miR-551b-5p	4.618724854	1.299059847	0.281259414
hsa-miR-573	9.237449708	2.598119693	0.281259414
hsa-miR-210-3p	384.8937378	114.9667964	0.298697498
hsa-miR-548f-3p	10.77702466	3.247649616	0.301349372
hsa-miR-140-5p	18.47489942	5.84576931	0.316416841
hsa-miR-29a-5p	12.31659961	3.89717954	0.316416841
hsa-miR-181c-5p	892.9534717	292.2884655	0.327327766
hsa-miR-374b-5p	64.66214795	21.43448747	0.33148431
hsa-miR-34a-3p	7.697874756	2.598119693	0.337511297
hsa-miR-9-5p	7.697874756	2.598119693	0.337511297
hsa-miR-651-5p	9.237449708	3.247649616	0.351574268

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hsa-miR-148b-5p	263.2673167	96.13042865	0.365143801
hsa-miR-30b-5p	1190.091437	469.6101345	0.394600045
hsa-let-7i-3p	24.63319922	9.742948849	0.395521051
hsa-miR-21-5p	124841.0536	51842.23083	0.415265887
hsa-miR-24-2-5p	300.2171155	126.0088051	0.419725587
hsa-miR-1278	3.079149903	1.299059847	0.421889121
hsa-miR-1303	1.539574951	0.649529923	0.421889121
hsa-miR-141-5p	1.539574951	0.649529923	0.421889121
hsa-miR-147b	1.539574951	0.649529923	0.421889121
hsa-miR-19a-5p	1.539574951	0.649529923	0.421889121
hsa-miR-20b-5p	1.539574951	0.649529923	0.421889121
hsa-miR-3184-5p	1.539574951	0.649529923	0.421889121
hsa-miR-3662	3.079149903	1.299059847	0.421889121
hsa-miR-3929	3.079149903	1.299059847	0.421889121
hsa-miR-4508	1.539574951	0.649529923	0.421889121
hsa-miR-4512	1.539574951	0.649529923	0.421889121
hsa-miR-4645-3p	1.539574951	0.649529923	0.421889121
hsa-miR-4659a-3p	3.079149903	1.299059847	0.421889121
hsa-miR-4745-5p	3.079149903	1.299059847	0.421889121
hsa-miR-4753-5p	1.539574951	0.649529923	0.421889121
hsa-miR-5003-3p	1.539574951	0.649529923	0.421889121
hsa-miR-519a-3p	3.079149903	1.299059847	0.421889121
hsa-miR-545-5p	10.77702466	4.546709463	0.421889121
hsa-miR-548f-5p	1.539574951	0.649529923	0.421889121
hsa-miR-548s	1.539574951	0.649529923	0.421889121
hsa-miR-548y	3.079149903	1.299059847	0.421889121
hsa-miR-561-3p	1.539574951	0.649529923	0.421889121
hsa-miR-5706	1.539574951	0.649529923	0.421889121
hsa-miR-576-3p	9.237449708	3.89717954	0.421889121
hsa-miR-579-5p	1.539574951	0.649529923	0.421889121
hsa-miR-592	3.079149903	1.299059847	0.421889121
hsa-miR-642a-3p	1.539574951	0.649529923	0.421889121
hsa-miR-643	1.539574951	0.649529923	0.421889121
hsa-miR-6505-5p	1.539574951	0.649529923	0.421889121
hsa-miR-6737-3p	3.079149903	1.299059847	0.421889121
hsa-miR-6747-3p	1.539574951	0.649529923	0.421889121
hsa-miR-6767-5p	1.539574951	0.649529923	0.421889121
hsa-miR-6810-5p	1.539574951	0.649529923	0.421889121
hsa-miR-6894-3p	1.539574951	0.649529923	0.421889121
hsa-miR-7108-5p	1.539574951	0.649529923	0.421889121
hsa-miR-7110-3p	1.539574951	0.649529923	0.421889121
hsa-miR-874-5p	1.539574951	0.649529923	0.421889121
hsa-miR-933	1.539574951	0.649529923	0.421889121
hsa-miR-18a-5p	175.5115444	75.99500102	0.432991467
hsa-miR-671-5p	40.02894873	17.53730793	0.438115626
hsa-miR-30a-5p	87050.6469	40804.11931	0.468739989
hsa-miR-516a-5p	30.79149903	14.93918824	0.485172489
hsa-miR-101-5p	9.237449708	4.546709463	0.492203975
hsa-miR-10a-3p	16.93532446	8.443889003	0.498596234