Intravenous injection of microvesicle-delivery miR-130b alleviates high-fat diet-induced obesity in C57BL/6 mice through translational repression of PPAR-γ

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Abstract

Background: We have shown previously that microvesicle (MV)-delivered miR-130b (miR-130b-MV) is able to target PPAR-γ and subsequently reduce the lipid accumulation in vitro. However, the in vivo effect of miR-130b on fat deposition and glucose homeostasis remains unknown.

Results: Three-week-old C57BL/6 mice were fed a high-fat diet for 8 weeks and then intravenously injected with MV-packaged scrambled control microRNA (miRNA) or miR-130b every other day for 10 days. Glucose tolerance test was performed and body weight, epididymal fat weight, as well as the expression of lipid metabolic genes were determined. We showed that mice fed on high-fat diet for 8 weeks demonstrated significantly higher body weight, elevated blood glucose and impaired glucose tolerance. miR-130b-MV injection significantly reduced body weight and epididymal fat weight and partly restored glucose tolerance. miR-130b expression was significantly increased in the epididymal fat after miR-130b-MV injection while the protein content of its target gene PPAR-γ was significantly suppressed, together with a significant up-regulation of the lipolysis genes, hormone sensitive lipase, monoglyceride lipase and leptin. Moreover, miR-130b-MV injection increased the expression of miR-378a and miR-378-3p that are reported to participate in the regulation of fat deposition.

Conclusion: Our results indicate that miR-130b-MV is able to reduce the epididymal fat deposition and partly restore glucose tolerance, through translational repression of PPAR-γ in a high-fat diet-induced obese mouse model.

Keywords: MV-delivery miR-130b, PPAR-γ, Lipolysis, Epididymal fat deposition, High-fat diet induced obese mice

Background

Obesity is a major risk factor for the development of type II diabetes (T2DM), cardiovascular diseases, and some types of cancer [1–5]. Searching for more efficient and safe approaches to prevent and treat obesity has been the focus of intensive research. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a ligand-activated transcription factor which is regarded as a master regulator of fat deposition [6–8]. Previous studies have shown that activation or over-expression of PPAR-γ can stimulate lipogenesis and adipogenesis [1], while down-regulation of PPAR-γ decreases fat mass in mice [9–13]. Therefore, PPAR-γ is considered as a therapeutic target for the treatment of obesity.

MicroRNAs (miRNAs) are small non-coding RNAs which regulate gene expression through mRNA degradation and/or translational repression [14]. Numerous studies have demonstrated that some miRNAs can inhibit PPAR-γ expression and suppress adipogenesis and lipogenesis. Both miR-302a and miR-27a are reported to inhibit adipogenic differentiation and lipid accumulation in 3T3-L1 mouse adipocytes [15] and human multipotent adipose-derived stem cells [16] by down-regulating PPAR-γ expression. miR-130 has been shown to strongly reduce adipogenesis by repressing PPAR-γ biosynthesis in human primary preadipocytes and 3T3-L1 mouse adipocytes.
These findings suggest that miRNAs may be used as an effective therapy for the treatment of obesity. However, exogenous miRNAs without appropriate protection or modifications can be quickly degraded by RNases that are abundant in the blood [18]. Therefore, the stability of exogenous miRNAs is one of the primary concerns with respect to clinical application [19].

Microvesicles (MVs) are a heterogeneous population of membrane-covered vesicles ranging from 100 nm to 1 μm in diameter, being secreted by almost all types of cells in vivo and in vitro under both normal and pathological conditions [20–23]. MVs are able to protect, transport and deliver bioactive contents, including miRNAs [24], from parent cells to cells of other origins [25, 26]. Recently, we demonstrated that miR-130b can be packaged into MVs and delivered to the recipient primary cultured porcine adipocytes to reduce lipid accumulation in vitro by inhibiting PPAR-γ expression [27]. Nevertheless, it remains unknown whether MV-shuttled miR-130b can modulate fat deposition through targeting PPAR-γ in vivo.

Therefore, in the present study, we tested anti-obesity efficacy of MV-packaged miR-130b on a high-fat diet-induced C57BL/6 mouse model. miR-130b was packaged into MV by HeLa-229 cells transfected to over-express exogenous miR-130b. MV-packaged miR-130b was isolated from the culture media through ultracentrifugation and was injected intravenously to the obese mice. We show that miR-130b was delivered to the epididymal fat tissue and significantly decreased the fat deposition, which was associated with a significant down-regulation of PPAR-γ protein content and an activation of lipolytic genes. Our results provide the preliminary evidences that MV-mediated delivery of miR-130b is able to reduce fat deposition in a high-fat diet-induced obese model.

Methods
Reagents, cells, and antibodies
Dulbecco’s modified Eagle’s medium: Nutrient Mixture F-12 (DMEM/F-12) was supplied by Life Technologies Inc. (Carlsbad, CA, USA). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). The human cervix cancer cell line HeLa-229 was purchased from the Cell Resource Center of Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Anti-PPAR-γ (BS4444, 1: 500 dilution) and anti-GAPDH antibodies (AP0066, 1: 10,000 dilution) were purchased from Bioworld Technology (Minneapolis, MN, USA). Synthetic RNA molecules and scrambled negative control oligonucleotides were purchased from Life Technologies Inc.

Plasmid construction
The precursors of miR-130b (89 bp) and the negative control miRNA (Scrambled control, miR-SC) were synthesized by Life Technologies Inc., based on the sequence information from miRNA precursors (www.mirbase.org) and the requirements for pSilencer 3.1-H1 siRNA expression vector (Ambion, Austin, TX, USA). Precursors of miR-130b and miR-SC were produced by annealing the upstream and downstream (50 μmol/L each) miRNA precursor sequences (Table 1). The 50 μL reaction mix was incubated in 96-well plates at 95 °C for 2 min, and subjected to touchdown PCR. During this procedure the temperature was decreased 0.1 °C every 8 s until it reached 25 °C. The PCR products were subcloned into pSilencer 3.1-H1 siRNA expression vector using BamHI and HindIII restriction endonucleases (Life Technologies Inc.).

Cell culture, miR-130b transfection and microvesicle isolation
Approximately 3 × 10^5/cm^3 HeLa-229 cells were seeded in 150 mm cell culture dish and grown in DMEM/F-12 media supplemented with 15 mmol/L NaHCO_3, 100 IU/mL penicillin, 100 IU/mL streptomycin, and 10 % FBS at 37 °C in a 5 % CO_2 water-saturated incubator. When the cells reached 90-95 % confluence, plasmids of 50 μg miR-130b and 50 μg miR-SC were transfected separately with Lipofectamine 2000 (Life Technologies Inc.), according to the manufacturer’s instructions. The transfected cells were incubated at 5 % CO_2 and 37 °C. Four hours later, the transfection medium was changed to DMEM/F-12 containing 10 % MVs-free FBS prepared by ultracentrifugation and filtration [28]. Cells were harvested 24 h after transfection and the medium was collected.

MVs were isolated from the medium by differential centrifugation according to previously published methods [29]. Briefly, 18 mL media mixture from six dishes was subjected to serial centrifugation. Initial centrifugation was undertaken at 300 g for 10 min followed by 1200 g for 10 min and 10,000 g for 20 min, by this process dead cells and other debris were removed. Then the resulting supernatant was filtered through 0.22 μm filters (Millipore, Billerica, MA, USA) into Beckman Quick seal tubes. Ultracentrifugation was performed at 110,000 g for 2 h using a 70Ti rotor (Beckman Coulter, Brea, CA, USA). All steps were performed at 4 °C. MVs were collected from the pellets and re-suspended in FBS-free media for subsequent assay. The Bicinchoninic acid (BCA) method was used to quantify the total protein concentration in MVs preparations.

Animals and diets
All procedures involving laboratory animal use were approved by the Animal Ethics Committee of Nanjing Agricultural University, with the project number 2012CB124703. The slaughter and sampling procedures complied with the “Guidelines on Ethical Treatment of Experimental Animals”
Three-week-old male specific pathogen-free (SPF) C57BL/6 mice weighing 9 ~ 10 g were obtained from the Comparative Medicine Center of Yangzhou University (Yangzhou, China, certificate of quality is SCXK (Su) 2012-0004) and fed in the Laboratory Animal Center of Jiangsu Province Integrative Medicine Hospital. The mice were housed in standard cages (33 × 23 × 12 cm, five mice/cage), maintained under controlled conditions (22 ± 0.5 °C, 50 ± 5 % relative humidity, 12-h/12-h dark/light cycle) with free access to both food and water.

After 7-day adaptation, thirty-six mice were randomly divided into two groups as follows: (1) the control group (MD10% fat group, n = 12) fed with normal fat diet (MD12031, 10 % fat); (2) the high fat group (MD45% fat group, n = 24) fed with high-fat diet (MD12032, 45 % fat). Both control and high fat diets were purchased from Medicience Ltd. (Yangzhou, China). The diets were replaced every 2 days to prevent oxidization of the fats in diets. After 8 weeks, the body weight was recorded and the glucose tolerance test was performed to confirm the successful establishment of the obese mouse model.

Then, we divided the obese mice randomly into two groups: (1) the control group injected with miR-SC-MV (HF-SC-MV); (2) the treated group injected with miR-130b-MV (HF-130b-MV). The mice were injected every other day for 10 days. During the 10 days of treatment, the mice in both groups were still fed high-fat diet.

**Oral glucose tolerance test (OGTT)**

After 10 h fasting (from 8:30 in the morning till 18:30 in the afternoon), the mice were given glucose at 2.5 g/kg body weight by intraperitoneal injection. The blood glucose levels before glucose injection (0 min) and 15, 30, 60, 90, 120 min after glucose injection were determined.

**Preparation of blood and epididymal fat tissue**

After ten days of treatment, the mice were fasted for 10 h and the body weight was recorded. Then the blood...
was drawn from the abdominal aorta using a syringe. The plasma was separated by centrifugation at 3000 rpm for 15 min at 4 °C and stored at -20 °C. The epididymal fat, gastrocnemius muscle and liver samples were removed, weighed and snap-frozen in liquid nitrogen and then stored at -70 °C.

**Analyses of plasma biochemical parameters, hormones and cytokines**

Plasma concentrations of biochemical metabolites, including alanine transaminase (ALT, no.C009-2), aspartate transaminase (AST, no.C010-1), glucose (GLU, no.F006), triglycerides (TG, no.F001-1), total cholesterol (TCh, no.F002-1), high-density lipoprotein cholesterol (HDLc, no.A112-2), low-density lipoprotein cholesterol (LDLc, no.A113-1) and nonesterified fatty acid (NEFA, no.A042-1) were detected by automatic biochemical analyzer (Beckman coulter, AU2700) using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). The plasma concentrations of hormones and cytokines, such as interleukin-6 (IL-6, no.96-407), insulin (no.96-416), leptin (no.96-421) and tumor necrosis factor (TNF-α, no.96-422), were measured by China Biomarker Service, Luminex 200 (no.CNBMSLX200) using Magnetic Bead MAPmate (Merck&Millipore, Darmstadt, Germany) according to the instructions provided by the manufacturer.

**RNA isolation and mRNA quantification**

Total RNA was isolated from liver using Trizol reagent (Life Technologies Inc.), according to the manufacturer's instructions. Concentration of the extracted RNA was measured using a NanoDrop-1000 spectrophotometer. RNA integrity was confirmed by denaturing agarose electrophoresis, and DNA contamination was evaluated by PCR using isolated RNA as template with the primers of 18s. M-MLV (Promega, Madison, WI, USA) and dN6 random primer (Takara, Kyoto, Japan) were used to synthesize cDNA from 2 μg of total RNA from each sample according to manufacturer's instructions. Three reference genes (PPIA, GAPDH and 18s) were tested and the mRNA abundances showed no difference between the two groups, and at last PPIA was chosen as a reference gene. Real-time PCR was performed in Mx3000P (Stratagene, Palo Alto, CA, USA). All primers used for this experiment were listed in Table 1.

**miRNAs real-time PCR quantification**

Total RNA was treated with RNase-free, DNase I (TaKaRa). The total RNA (4 μg) was polyadenylated by poly (A) polymerase at 37 °C for 1 h in a 20 μL reaction mixture using a Poly (A) tailing kit (AM1350, Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer’s instructions. The polyadenylated RNA was then dissolved and reverse transcribed using the poly (T) adapter.

Real-time PCR was performed, in triplicate, using the SYBR green qPCR master mix reagent (Takara) with a miRNA-specific forward primer and a universal reverse primer that is complementary to part of the poly (T) adapter sequence. Since no validated reference gene was available for pig miRNAs, a random DNA oligonucleotide was added to RNase-free DNase I-treated total RNA samples before polyadenylation, as an exogenous reference, to normalize the expression of miRNAs. The sequences of all the mature miRNAs, the poly (T) adapter and the exogenous reference gene used in the present study are listed in Table 2.

**Table 2: primers used for miRNA detection**

| Name   | Primer sequence (5′ to 3′) | miBase Acc No. |
|--------|---------------------------|----------------|
| miR-130b | CAGUGCAAUAGUAAGGCAU         | MIMAT001922     |
| miR-130a | CAGUGCAAUAGUAAAGGCAU        | MIMAT0007758    |
| miR-27a  | UUCAGUGCGUAGUCUCCGC         | MIMAT002148     |
| miR-27b  | UUCAGUGCGUAGUCUCCGC         | MIMAT003890     |
| miR-103  | AGCAGCGAAGACAGCGCAUGA        | MIMAT002154     |
| miR-143-3P | UGAGAUGAGCGACUGAGCUC         | MIMAT0013879    |
| miR-143-5P | GUGUGCAGUGCCGACUCUGG         | MIMAT0017374    |
| miR-378  | ACUGGACUUGAGUGUGAGGAGGC     | MIMAT0013868    |
| miR-378b-3p | ACUGGACUUGAGUGUGAGGAGGAG   | MIMAT0037082    |
| miR-455  | UAUGUGCCUUGAGGACUCACUGC     | MIMAT002957     |
| miR-320  | AAGCGUUGGCGAGAGGCGAAGA      | MIMAT0013878    |
| miR-106a | AAAAGUGCUUAGAGCAGCGUGGAGG   | MIMAT002118     |
| poly(T) adapter | TAGAGTGATTGAGGACGAGCAAGA | N/A             |
| Universal primer | TAGAGTGATTGAGGACGAGCAAGA | N/A             |
| Exogenous reference | GTGCCCCACGATGTGATTCCGC | N/A             |
(ANOVA). All statistical analyses were undertaken using Statistical Program for Social Sciences (SPSS) version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The level of significance was set at \( P < 0.05 \).

**Results**

**Establishment of high-fat diet-induced obese mouse model**

C57BL/6 obese mouse model was successfully established after feeding high-fat diet ad libitum for 8 weeks (Fig. 1). The experimental design for establishing the obese mice model is depicted in a flow chart (Fig. 1a). The body weight was significantly higher \( (P < 0.01) \) in high-fat diet group (Fig. 1b-c) with significantly increased \( (P < 0.05) \) body weight gain (Fig. 1d). The oral glucose tolerance test (OGTT) demonstrated impaired glucose tolerance in high-fat diet group as compared with the control group (Fig. 1e), which was indicated by significantly increased \( (P < 0.001) \) area under the blood glucose-time curve (AUC) (Fig. 1f).

**miR-130b-MV partly restored glucose tolerance**

To determine the effects of miR-130b-MV injection on the glucose tolerance, OGTT was performed (Fig. 2). Blood glucose concentrations in miR-130b-MV-injected mice were significantly reduced \( (P = 0.003) \) after the intravenous injection of glucose (2.5 g/kg body weight) when compared with miR-SC-MV-injected mice (Fig. 2a). In addition, miR-130b-MV injection tended to reduce \( (P = 0.057) \) the AUC when compared with miR-SC-MV-injected mice (Fig. 2b). The above results suggest that intravenous injection of miR-130b-MV partly restored the glucose tolerance caused by high-fat diet.

**miR-130b-MV reduced body weight and epididymal fat weight**

The protocol of MV injection via tail vein is depicted in the flow chart (Fig. 3a). We delivered miR-130b-MV to serve as a carrier host for the over-expression of exogenous miR-130b [27], so it was chosen to serve as a carrier host for the over-expression of exogenous miR-130b. HeLa-229 cells were transfected with the miR-130b over-expression plasmid, while plasmid over-expressing miR-SC was also transfected to serve as a negative control. MVs containing miR-130b and miR-SC were purified from the supernatant of transfected cells and RT-PCR verified the package of GR, TNF-\( \alpha \), UCP-3, SCD-1, LDLR, STAT3, and PPAR-\( \gamma \) in miR-130b-MV preparations (Fig. 3b). miR-130b-MV and miR-SC-MV preparations were injected via tail vein into the obese mice of treatment and control groups respectively every other day for 10 days. The phenotypic changes in body size and fat deposition are shown in Fig. 3c-e, which indicate obviously reduced body weight and the epididymal fat mass in mice treated with miR-130b-MV.

Quantitatively, miR-130b-MV significantly reduced the body weight \( (P < 0.01) \), the epididymal fat weight \( (P < 0.05) \) (Fig. 3f) and the muscle weight \( (P < 0.05) \), but not the liver weight. Furthermore, the epididymal fat weight relative to the body weight tended to be lower \( (P = 0.063) \) (Fig. 3g) in miR-130b-MV group when compared with miR-SC-MV group. The relative muscle weight was not different, yet the liver weight relative to the body weight was significantly higher \( (P < 0.05) \) in miR-130b-MV group compared with miR-SC-MV counterparts (Table 3).

**miR-130b-MV treatment did not affect the plasma concentrations of ALT, AST, Tch, HDLc, LDLc, IL-6, insulin, leptin or TNF-\( \alpha \), while TG \( (P = 0.109) \) and glucose \( (P = 0.074) \) concentrations tended to be lower in miR-130b-MV group (Table 3).**

**miR-130b-MV increased miR-130b expression and suppressed PPAR-\( \gamma \) protein content in epididymal fat**

The abundance of miR-130b in the epididymal fat tissue was significantly higher \( (P < 0.05) \) in HF-130b-MV group (Fig. 3h). The mRNA expression of the predicted target genes of miR-130b, including GR, TNF-\( \alpha \), UCP-3, SCD-1, LDLR, STAT3, and PPAR-\( \gamma \) (Fig. 3i), was detected and only PPAR-\( \gamma \) tended to be increased \( (P = 0.07, \) Fig. 3j) in miR-130b-MV group. However, the protein content of PPAR-\( \gamma \) was significantly reduced \( (P < 0.05) \) in the epididymal fat of mice injected with miR-130b-MV (Fig. 3k).

**miR-130b-MV affected mRNA expression of lipid metabolic genes in epididymal fat**

Injection of miR-130b-MV affected the expression of lipid metabolic genes in the epididymal fat of high-fat diet-induced obese mice (Fig. 4). Moreover, miR-130b-MV did not affect the mRNA expression of CCTTA enhancer binding protein-\( \beta \) (C/EBP-\( \beta \)), peroxisome proliferators-activated receptor-\( \alpha \) (PPAR-\( \alpha \)), sterol regulatory element-binding protein 1 (SREBP1), or the lipogenic enzymes, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). However, 11\( \beta \)-hydroxysteroid dehydrogenase type 1 (11\( \beta \)-HSD1) mRNA expression was significantly up-regulated \( (P < 0.01) \) in miR-130b-MV-injected mice when compared with miR-SC-MV mice (Fig. 4).
**Fig. 1** (See legend on next page.)
miR-130b-MV affected expression of other miRNAs involved in lipid metabolism in epididymal fat

The abundance of 11 other miRNAs in the epididymal fat that are closely related to fat deposition was also determined. The expression of miR-378a and miR-378b-3P was significantly increased ($P < 0.05$) in the epididymal fat of miR-130b-MV-injected mice when compared with miR-SC-MV-injected mice (Fig. 5).

Discussion

High-fat induced obese C57BL/6 mice have been widely used as a model for studying human visceral obesity because they represent the human simple obesity both metabolically and pathophysiologically [30–32]. In this study, feeding C57BL/6 mice with high-fat diet for 8 weeks significantly increased the body weight by over 20%, indicating that the obese mice model was successfully established [33]. Furthermore, blood glucose concentration was also significantly increased, suggesting impaired glucose tolerance which is closely associated with obesity [34].

In our previous study, we demonstrated that miR-130b-MV was able to reduce the lipid deposition in porcine primary adipocytes in vitro by targeting PPAR-γ gene [27]. Here we provide the evidence that miR-130b-MV injection was effective to decrease body weight and reduce epididymal fat deposition in high-fat diet-induced obese mice in vivo, at least partly through the translational repression of PPAR-γ. This is in agreement with a previous observation that miR-150-MV injection suppressed its target c-Myb expression and enhanced cell migration in mice [35]. Similarly, it was reported that miR-143-MV injection via tail vein was able to suppress tumor growth in mice [36]. Although there have been several attempts to administer MV-shuttled miRNAs by intravenous injection, this study provides, to our knowledge, the first evidence that miRNA packaged in MVs has an anti-obesity efficacy in an in vivo animal model.

However, the safety of miR-130b-MV-mediated therapy for obesity has to be considered. In the present study, we inspected histologically the possible side effects of miR-130b-MV on other tissues, including liver, kidney, heart and spleen. No obvious pathological changes were observed (H&E results not shown). Based on the consideration that each miRNA may target multiple target genes and function through different pathways [37], we detected numerous biochemical and hormonal parameters in the plasma including ALT, AST, Tch, TG, glucose, HDLc, LDLc, IL-6, insulin, leptin, and TNF-α. Interestingly, none of these blood parameters showed significant change, except TG and glucose displayed a tendency of decrease. This indicates that the general metabolic homeostasis of the body was not disturbed by miR-130b-MV treatment. Moreover, GR, TNF-α, UCP-3, SCD-1, LDLR, and STAT3, in addition to PPAR-γ, are also predicted to be the target of miR-130b, yet the...
Fig. 3 (See legend on next page.)
mRNA expression of these genes in the epididymal fat tissue was not affected by miR-130b-MV treatment. Nevertheless, it remains to be determined whether these genes are affected at the level of protein.

We further investigated the downstream molecular mechanisms underlying the miR-130b-MV-mediated inhibition in fat deposition. FAS and ACC are key adipogenic enzymes [38] that play pivotal roles in fat deposition, while HSL, ATGL and MGL are important lipases responsible for TG hydrolysis [39, 40]. Leptin, a cytokine secreted predominantly from the fat tissue, plays an important role in regulating energy balance, and increased leptin can stimulate lipolysis, by up-regulating HSL, ATGL and MGL expression [41]. In the present study, miR-130b-MV increased HSL, MGL and leptin mRNA expression, but did not influence FAS and ACC mRNA expression. Therefore, it is presumed that miR-130b-MV decreases fat deposition predominantly by enhanced lipolysis but not lipogenesis.

It is noted that miR-130b-MV also altered the expression of other miRNAs related to fat deposition. For instance, miR-378a and miR-378b-3p were up-regulated significantly in the epididymal fat tissue of miR-130b-MV-injected mice. miR-378 is highly induced during adipogenesis and has been reported to be positively regulated in adipogenesis. The role of miR-378 family in fat deposition has been

### Table 3 Effect of miR-130b-MV injection for 10 days on the apparent and blood biochemical parameters in mice

| Parameters                        | HF-SC-MV         | HF-130b-MV       | P Value |
|-----------------------------------|------------------|------------------|---------|
| **Sampling parameters**           |                  |                  |         |
| Body weight (g)                   | 29.75 ± 0.53     | 26.30 ± 0.90     | 0.000   |
| Epididymal fat weight (mg)        | 1.02 ± 0.09      | 0.72 ± 0.09      | 0.034   |
| Liver weight (mg)                 | 1.18 ± 0.05      | 1.17 ± 0.06      | 0.822   |
| Muscle weight (mg)                | 0.34 ± 0.02      | 0.29 ± 0.01      | 0.025   |
| Epididymal fat weight/Body weight | 0.034 ± 0.003    | 0.027 ± 0.003    | 0.063   |
| Liver weight/Body weight          | 0.039 ± 0.002    | 0.045 ± 0.002    | 0.004   |
| Muscle weight/Body weight         | 0.011 ± 0.005    | 0.011 ± 0.005    | 0.825   |
| **Biochemical parameters**        |                  |                  |         |
| ALT (U/L)                         | 10.83 ± 1.72     | 10.45 ± 1.84     | 0.882   |
| AST (U/L)                         | 53.64 ± 5.68     | 52.50 ± 2.00     | 0.858   |
| GLU (mmol/L)                      | 11.92 ± 0.60     | 10.13 ± 0.74     | 0.074   |
| TG (mmol/L)                       | 0.55 ± 0.04      | 0.44 ± 0.04      | 0.109   |
| Tch (mmol/L)                      | 2.59 ± 0.13      | 2.41 ± 0.14      | 0.334   |
| HDLc (mmol/L)                     | 1.46 ± 0.08      | 1.33 ± 0.05      | 0.167   |
| LDLc (mmol/L)                     | 0.15 ± 0.02      | 0.20 ± 0.03      | 0.066   |
| NEFA (μmol/L)                     | 857.67 ± 41.17   | 879.82 ± 56.14   | 0.751   |
| **Hormones and cytokines**        |                  |                  |         |
| IL-6 (pg/mL)                      | 69.94 ± 14.17    | 216.98 ± 96.37   | 0.163   |
| Insulin (pg/mL)                   | 1668.54 ± 243.60 | 1618.35 ± 223.44 | 0.881   |
| Leptin (pg/mL)                    | 2566.43 ± 421.64 | 2289.77 ± 550.37 | 0.698   |
| TNF-α (pg/mL)                     | 6.66 ± 0.48      | 7.81 ± 0.69      | 0.194   |

Notes: HF, high fat; HF-SC-MV, high-fat diet-induced obese mice injected with miR-SC-MV; HF-130b-MV, high-fat diet-induced obese mice injected with miR-130b-MV; The values shown represent the means ± SEM; *P < 0.05 vs. HF-SC-MV group; **P < 0.01 vs. HF-SC-MV group.
Over-expression of miR-378 was shown to increase fat accumulation [42], yet the opposite result was also reported [43]. Over-expression of miR-378 increased lipolysis genes expression, while inhibition of miR-378 expression attenuated stimulated lipolysis and reduced the expression of lipolytic regulators [44]. MVs have been utilized for the delivery of therapeutic RNAi and are considered as a more effective, advantageous method than other options. Compared with the delivery strategies of viruses, lipid nanoparticles and polymeric nanoparticles, MVs present some major advantages. MVs are natural carriers and are not subjected to the attacks by antibodies, complements or opsonins in circulation. Other methods are prone to be cleared or trigger unwanted immune responses [45]. In the present study, MVs delivered miR-130b into the epididymal fat tissue efficiently and repressed the fat deposition, further suggesting that MVs are advantageous carriers for transferring therapeutic small RNAs compared to other methods. However, miR-130b-MV injection was conducted every other day for 10 days due to limited quantity of the miR-130b-MV preparations. The effects and the side-effects, if any, of prolonged treatment of miR-130b-MV remain unclear. It was reported that 7 days after miR-150b-MV injection, miR-150 still maintained at a low level in plasma, and its target gene VEGF was repressed and the tumor development was also suppressed [46]. Future studies are required to test the half-life of MV-protected miR-130b.

Conclusion
In summary, this is the first in vivo study demonstrating that miR-130b-MV can be shuttled into the epididymal fat tissue to down-regulate PPAR-γ expression and to stimulate the expression of lipolysis genes. Further studies may be directed to assess the cytotoxicity and the half-life of miR-130b-MV in the blood, so as to further contribute to the development of anti-obesity drugs for clinical application.

Abbreviations
ACC: Acetyl-CoA carboxylase; ALT: Alanine transaminase; AST: Aspartate transaminase; ATGL: Adipose tissue triglyceride lipase; ANOVA: Analysis of variance; BCA: Bicinchoninic acid; C/EBP-β: CCTTA enhancer binding protein-β; DMEM/F-12: Dulbecco’s modified eagle’s medium: nutrient mixture F-12; FAS: Fatty acid synthase; PBS: Fetal bovine serum; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GLUT: Glucose; GR: Glucocorticoid receptor; HDLc: High-density lipoprotein cholesterol; HF: High fat; HF-SC-MV: High fat diet-induced obese mice injected with miR-SC-MV; HF-130b-MV: High fat diet-induced obese mice injected with miR-130b-MV; HMEC-1: Human microvascular endothelial cell line-1; HSL: Hormone sensitive lipase; IBMX: 3-isobutyl-1-methylxanthine; IL-6: Interleukin-6; LDLc: Low-density lipoprotein cholesterol; MD: Mediciene diet; MGL: Monoglyceride lipase; miRNAs: microRNAs; MV: Microvesicle; NEFA: Non esterified fatty acid; OGTT: Oral glucose tolerance test; PPAR-α: Peroxisome proliferators-activated receptor-α; PPAR-γ: Peroxisome proliferators-activated receptor-γ; PPIA: Peptidylprolyl isomerase A; qRT-PCR: quantitative reverse transcription polymerase chain reaction; SCD-1: Stearoyl-coenzyme A desaturase Type-1; SPSS: Statistical program for social sciences; SREBP1: Sterol regulatory element-binding protein 1; TCH: Total cholesterol; T2DM: Type II diabetes; THP-1: Human acute monocytic leukemia cell line-1; TNF-α: Tumor necrosis factor-α; WB: Western blotting; 11β-HSD1: 11β-hydroxysteroid dehydrogenase type 1.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
RZ designed the experiment, supervised the laboratory work and critically revised the manuscript; SF mainly preformed the experiment and analyzed the data; YJ and XY assisted with discussion of results and writing the
manuscript; YL, MW, RC provided samples and carried out the detection of the serum parameters, and all authors read the manuscript and approved its final version.

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