miR-188 promotes liver steatosis and insulin resistance via the autophagy pathway

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is becoming the most prevalent liver disease worldwide, characterized by liver steatosis and is often accompanied with other pathological features such as insulin resistance. However, the underlying mechanisms are not fully understood, and specific pharmacological agents need to be developed. Here, we investigated the role of microRNA-188 (miR-188) as a negative regulator in hepatic glucose and lipid metabolism. miR-188 was upregulated in the liver of obese mice. Loss of miR-188 alleviated diet-induced hepatosteatosis and insulin resistance. In contrast, liver-specific overexpression of miR-188 aggravated hepatic steatosis and insulin resistance during high-fat diet feeding. Mechanistically, we found that the negative effects of miR-188 on lipid and glucose metabolism were mediated by the autophagy pathway via targeting autophagy-related gene 12 (Atg12). Furthermore, suppressing miR-188 in the liver of obese mice improved liver steatosis and insulin resistance. Taken together, our findings reveal a new regulatory role of miR-188 in glucose and lipid metabolism through the autophagy pathway, and provide a therapeutic insight for NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD), characterized mainly by hepatic steatosis, is the most common liver disease worldwide (Birkenfeld & Shulman 2014, Meex & Watt 2017). It is reported that NAFLD is closely associated with type 2 diabetes mellitus (T2DM), as more than 70% of patients with T2DM have NAFLD (Tilg et al. 2017, Younossi et al. 2019). Both diseases share some common pathological features including hypertriglyceridaemia and insulin resistance (Newgard 2012, Baffy 2015). However, there are no pharmacological agents specifically designed to treat NAFLD (Saponaro et al. 2015, Hazlehurst et al. 2016). Thus, further exploring the underlying mechanisms and developing potential drugs for NAFLD is of great importance.

miRNA, a class of noncoding single-strand RNA with 22-24 nucleotides, exert their function by inhibiting or degrading target mRNAs (Bartel 2004). miRNAs have been reported to play important roles in many biological processes including cell growth, cell differentiation and cell apoptosis (Esau et al. 2006, Li et al. 2009). Several miRNAs have been proven to participate in the regulation of glucose and lipid metabolism, such as miR-130a, miR-34a, miR-133 and miR-21 (Min et al. 2012, Mori et al. 2012, Yin et al. 2013, Xiao et al. 2014, Loyer et al. 2016). Among these, miR-188 has been identified as a crucial regulator in various kinds of cancers, including breast cancer, lung cancer, prostate carcinoma and glioma
(Li et al. 2018b, Zhao et al. 2018, Zhang et al. 2019, Zhu et al. 2020), and in other diseases, such as Alzheimer’s disease, myocardial infarction and atherosclerosis (Lee et al. 2012, 2016, Wang et al. 2015, Zhang et al. 2018). In our previous studies, we have found that miR-188 promotes the adipogenic differentiation of bone marrow mesenchymal stem cells and fat accumulation in bone (Li et al. 2015), followed with the finding that adipose miR-188 increases fat mass accumulation and energy expenditure (Huang et al. 2020). Interestingly, we also noted that in aged mice, miR-188 knockout affected the expression of lipid metabolic genes in the liver (Huang et al. 2020). However, little is known regarding whether miR-188 plays a direct role in regulating hepatic lipid or glucose metabolism.

In the present study, we found that expression of miR-188 was increased in the liver of high-fat diet induced obese mice and genetically obese db/db mice, leading us to hypothesize that miR-188 plays an important role in glucose and lipid homeostasis. After 3 months of high-fat diet feeding, the level of liver steatosis and hepatic insulin resistance was ameliorated in miR-188-null mice, while mice with liver-specific miR-188 overexpression had the opposite phenotype. Further investigation demonstrated that miR-188 exerted its effects via the autophagy pathway and Atg12 was the potential downstream mediator. Inhibition of miR-188 expression in the liver of obese mice can alleviate liver steatosis and insulin resistance. Taken together, our findings revealed a novel role of miR-188 in regulating glucose and lipid metabolism and suggested that miR-188 may be a potential therapeutic target in NAFLD.

Materials and methods

Materials

The following antibodies were purchased from Cell Signaling Technology: anti-AKT (#4691), anti-Phospho-AKT (Ser473)(#9271), anti-GSK3β(#9315), anti-Phospho-GSK3β (Ser9) (#9336), anti-Insulin Receptor β (IR) (#3025), anti-Phospho-IR β (Tyr1135/1136) (#3024), anti-P62 (#5114), and anti-LC3B (#2775).

Animals and treatment

Male C57BL/6j mice and leptin receptor-mutated (db/db) mice were purchased from Hunan SJA Laboratory Animal Company. The miR-188 null mice were generated by transcription activator-like effector nuclease (TALEN) technique as reported previously (Li et al. 2015). All mice were kept in Laboratory Animal Research Center with specific pathogen-free standard at Central South University, and maintained on a 12-h light/dark cycle at 20–24°C with free access to rodent chow diet (CD). Mice were fed high-fat diet (HFD) (60% fat, D12492; Research Diets) for 3 months at 8–10 weeks. All animal care protocols and experiments were reviewed and approved by the Animal Care and Use Committees of the Laboratory Animal Research Center at Xiangya Medical School of Central South University, and this study was compliant with all relevant ethical regulations regarding animal research.

Intravenous administration of adeno-associated virus

Adeno-associated viral vector 8 (AAV8) for miR-188 overexpression (AAV-miR-188) was purchased from Hanbio Biotechnology Co (Shanghai, China). AAV8 for miR-188 knockdown (AAV-anti-miR-188) was purchased from OBIO Technology Corporation (Shanghai, China). Virus were diluted in sterilized PBS and administrated at a dose of 10^{11}–10^{12} vg/mL per mice via tail-vein injection. Functional validation was verified by qPCR (Ising et al. 2017, Xiao et al. 2020).

Blood glucose, serum insulin and homeostasis model assessment of insulin resistance index (HOMA-IR)

The levels of blood glucose were measured by a glucometer monitor (Roche, ACCU-CHEK Active) and the levels of serum insulin were detected by an ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using following formula: fasting glucose levels (mmol/L) × fasting serum insulin (mIU/L)/22.5.

Measurement of blood and liver TC, TG, FFA and serum ALT, AST

Hepatic lipids were extracted with chloroform-methanol (2:1). Triglyceride (TG), total cholesterol (TC), free fatty acid (FFA) and alanine transaminase (ALT), aspartate aminotransferase (AST) were measured with a TG kit, TC kit, FFA kit, ALT kit or AST kit respectively, according to the manufacturers’ instructions. All these kits were purchased from Shanghai Shensuo UNF Medical Diagnostic Articles Company (Shanghai, China).
In vivo insulin signaling assay

Mice were euthanized after 6-h fasting. Liver, muscle and white adipose tissue were excised and snap frozen for the untreated group and the remaining liver, muscle and white adipose tissue were excised at 3, 4 and 5 min after injection of insulin (2 U/kg for mice with chow diet, 5 U/kg for mice with high-fat diet) via portal vein. The frozen tissues were used for further Western blot analysis.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

GTT and ITT were performed by intraperitoneal injection of 1 g/kg glucose after overnight fasting and 1 U/kg insulin after 4-h fasting respectively (Su et al. 2019). Blood glucose levels were examined at 0 min, 15 min, 30 min, 60 min, 90 min and 120 min after injection.

Primary hepatocyte isolation and adenovirus infection

Isolation and culture of primary hepatocytes from male C57BL/6j mice were performed as previously reported (Li et al. 2010). Briefly, mice were anesthetized and perfused with perfusion buffer containing collagenase through the vena cava. After fully digesting, the liver was excised and dispersed, followed by washing three times. The supernatant was discarded and cells were plated at 5 x 10^6 cells/well in a 12-well culture dish. Following culturing for 8 h, cells were infected for 2 or 3 days with adenoviruses at a MOI of 7. Infected hepatocytes were then collected for further qRT-PCR and Western blot analysis. To inhibit the autophagy process, primary hepatocytes were treated with 50 μm chloroquine (C6628-25G, Sigma) for 6 h. Ad-miR-188 and Ad-anti-miR-188 were constructed from OBiO Technology Corporation (Shanghai, China). miR-188 mimics and siRNA-atg12 were purchased from RiboBio Co (Guangzhou, China).

Luciferase assay

HEK 293T cells were co-transfected with miR-188-mimics or miR-188-NC, using pRL-Renilla as internal control. After 48 h transfection, the firefly and Renilla luciferase activities were assayed using a Dual-Luciferase Reporter Assay System (Promega) (Neudecker et al. 2017).

Immunofluorescence assay

After infection with adenoviruses, primary hepatocytes were transfected with green fluorescent protein (GFP)-LC3 plasmids (Xiao et al. 2016). Forty-eight hours later, primary hepatocytes were fixed with 4% paraformaldehyde and examined via an LSM 510 META confocal laser microscope (Carl Zeiss Imaging). The number of green punctates were counted using ImageJ software.

In vitro cell model of lipid accumulation

Primary hepatocytes were cultured with 0.5 mM palmitate acid (P0500, Sigma) and 1 mM oleic acid (O1008, Sigma) for 24 h before harvest. Intracellular triglyceride levels were measured by TG kit according to the manufacturer’s protocol.

Quantitative real-time PCR and Western blot analysis

qRT-PCR analysis and Western blot analysis were conducted as previously described (Yang et al. 2017b, 2019, Li et al. 2018a). The primer pairs used for qRT-PCR were listed (Table 1).

Histological analysis of tissues

Frozen sections of liver were stained with Oil Red O. Paraformaldehyde-fixed, paraffin-embedded sections of liver were stained with hematoxylin and eosin (H&E) for histology.

Statistical analysis

All data are expressed as means±s.e.m. The statistical significance of the differences between various treatments or groups was measured by either Student’s t test or ANOVA followed by Bonferroni post-test. Data analyses were performed using GraphPad Prism 7.0. P<0.05 was considered statistically significant.

Results

miR-188 deficiency alleviates HFD-induced hepatic steatosis and insulin resistance

To study the potential link between miR-188 and NAFLD, we measured miR-188 expression in the liver of obese mice. Notably, miR-188 expression was elevated in the liver of both HFD-induced obese mice and db/db
genetically obese mice (Fig. 1A). To understand the role of miR-188 in the progression of obesity-induced glucose and lipid dysregulation, we generated miR-188-null mice (Supplementary Fig. 1A, see section on supplementary materials given at the end of this article). A normal liver structure and function were found in miR-188 null mice, comparable with that found in wild-type mice (Supplementary Fig. 1B and C). No significant differences were shown in the levels of serum insulin and fasting blood glucose between the miR-188 null mice and their WT littermates under normal chow feeding conditions (Fig. 1B, C and D). miR-188 null mice had a slightly decreased glucose level on glucose and insulin exposure (Fig. 1E and F). After 3 months of HFD feeding, the increased level of serum insulin and HOMA-IR index were selectively blunted by miR-188 loss (Fig. 1C and D). This was further evidenced by an improved glucose tolerance and clearance ability (Fig. 1E and F). Furthermore, the insulin-stimulated phosphorylation of IR, AKT and GSK3β were all upregulated in the liver of HFD fed miR-188-null mice but not in muscle and white adipose tissue (Fig. 1G, H and I), suggesting an improvement in hepatic insulin signaling by miR-188 loss.

As hepatic steatosis and insulin resistance are closely associated in obesity, we next tested whether miR-188 was involved in the regulation of HFD-induced hepatic steatosis. miR-188-null mice and wild-type mice were fed with HFD for 3 months. The ratio of liver weight to body weight (LW/BW) was lower in miR-188-null mice without significant differences in body weight and food intake between these two groups (Fig. 1J, K and L). Hepatic lipid accumulation was decelerated by miR-188 knockout (Fig. 1M). This was further evidenced by decreased liver and serum triglyceride (TG) contents (Fig. 1N and O). There was no significant difference in the level of total cholesterol (TC) and free fatty acids (Fig. 1N and O). Serum ALT and AST, markers of liver injury, were also lower in miR-188-null mice than in WT mice (Fig. 1P).

Furthermore, loss of miR-188 decreased expression of lipogenesis genes including *Fas* (fatty acid synthase), *Srebp1c* (sterol regulatory element binding protein 1c) and *Gpat* (glycerol-3-phosphate acyltransferase), and increased expression of genes related to fatty acid uptake and β-oxidation such as *Cd36* (cluster of differentiation 36), *Fabp4* (fatty acid binding protein), *Ppara* (peroxisome proliferator-activated receptor α) and *Cpt1a* (carnitine palmitoyltransferase 1α) was found in the liver, which might be partly attributed to alleviated hepatic steatosis in miR-188-null mice (Fig. 1Q and R). Together, these observations demonstrated that miR-188 knockout alleviates HFD-induced hepatosteatosis and insulin resistance.

Liver-specific overexpression of miR-188 promotes HFD-induced hepatosteatosis and hepatic insulin resistance

To further understand the unique role of miR-188 in promoting NAFLD progression, male C57BL/6J mice were infected with AAV-miR-188 and control AAV-GFP via tail vein injection, followed by HFD for 3 months. As a result, miR-188 was overexpressed exclusively in the liver (Fig. 2A). Mice with AAV tail vein injection had normal AST and ALT levels, and normal liver structure (Supplementary Fig. 1D). In contrast to the protective effect of miR-188 deficiency, hepatic miR-188 overexpression increased serum insulin level and HOMA-IR index, with no significant effect on fasting blood glucose level (Fig. 2B, C and D). Consistently, a deterioration in glucose tolerance and clearance was detected in AAV-miR-188-injected mice compared with their control group (Fig. 2E and F). Furthermore, the insulin-stimulated phosphorylation of IR, AKT and GSK3β were lower in the liver of AAV-miR-188 mice, indicating worse hepatic insulin resistance (Fig. 2G). Further investigation revealed an increased LW/BW in AAV-miR-188 mice with little difference in body weight or food intake (Fig. 2H, I and J). In line with this, more severe
Figure 1
miR-188 knockout alleviates hepatosteatosis and insulin resistance during HFD feeding. Male 8-week-old miR-188-null mice and their wildtype littermates were fed with chow diet and HFD for 3 months. (A) The expression of miR-188 in the liver of male lean mice, HFD-induced obese mice and db/db mice. (B, C and D) Fasting blood glucose level, fasting serum insulin level and HOMA-IR index of lean and obese miR-188-null mice and their controls. (E and F) GTT and ITT assay of lean and obese miR-188-null mice and their controls. (G, H and I) Insulin signal assay in the liver, muscle and fat of obese miR-188-null mice and their controls. (J, K and L) LW/BW, body weight and food intake of lean and obese miR-188-null mice and their controls. (M) Liver H&E and Oil Red O staining of obese miR-188-null mice and their controls, scale bar: 300 μm. (N and O) TG, TC and FFA level in serum and liver of obese miR-188-null mice and their controls. (P) Serum ALT and AST level of obese miR-188-null mice and their controls. (Q and R) The mRNA levels of genes related to lipid metabolism in the liver of obese miR-188-null mice and their controls. Scd1 (stearoyl-CoA desaturase), Chrebp (carbohydrate-responsive element-binding protein), Ppary (peroxisome proliferator-activated receptor γ). All the data are shown as means ± s.e.m. (n = 5–6). Statistical significance was calculated by Student’s t test or two-way ANOVA, *P < 0.05; **P < 0.01; ***P < 0.001.
Liver steatosis was found in miR-188 overexpressing mice (Fig. 2K), as well as higher TG in both liver and serum (Fig. 2L and M). Serum ALT and AST were also higher in obese mice with liver-specific miR-188 overexpression compared to their controls (Fig. 2N). Aggravated hepatosteatosis can be explained by an increased trend of lipogenesis-related gene expression and a decreased level of genes related to β-oxidation and fatty acid uptake in the liver of AAV-miR-188 mice (Fig. 2O and P). Together, overexpressing miR-188 in the liver exacerbated liver steatosis and hepatic insulin resistance during obesity.

**miR-188 regulates insulin sensitivity and lipid accumulation in vitro**

We further verified the regulatory function of miR-188 in insulin sensitivity and lipid accumulation through directly targeting hepatocytes. The insulin-stimulated phosphorylation of IR, AKT and GSK3β was elevated in primary hepatocytes with adenovirus-mediated miR-188 knockdown (Fig. 3A), which was then reversed in primary hepatocytes with adenovirus-mediated miR-188 overexpression (Fig. 3B). Consistent with this, primary hepatocytes with miR-188 knockdown had improved glucose output compared to the control group after insulin stimulation (Fig. 3C), while primary hepatocytes with miR-188 overexpression had opposite results (Fig. 3D). We further detected the intracellular TG level of primary hepatocytes with palmitate acid and oleic acid stimulation (PO), which is a cell model of lipid accumulation. After PO exposure for 24 h, primary hepatocytes with miR-188 inhibition had lower TG (Fig. 3E), whereas primary hepatocytes with miR-188 overexpression had higher TG (Fig. 3F). These results suggested that miR-188 regulates
hepatic insulin signaling and lipid accumulation in a cell autonomous way.

**miR-188 regulates insulin sensitivity and lipid accumulation via autophagy**

Autophagy has been demonstrated to play an important role in glucose and lipid metabolism (Codogno & Meijer 2010, Altshuler-Keylin et al. 2016). Inhibiting the autophagy process increases liver triglyceride storage and decelerates fatty acid β-oxidation (Yang et al. 2010), and also leads to deteriorated hepatic insulin sensitivity and glucose homeostasis (Singh et al. 2009). We further hypothesized that autophagy is involved in miR-188 regulated glucose and lipid metabolism. Knockdown of miR-188 elevated LC-3II and reduced SQSTM1/p62 expression, two autophagy markers, in primary hepatocytes, while miR-188 overexpression displayed opposite results (Fig. 4A and B). Similar results were also observed in the liver of mice with miR-188 knockout and liver-specific overexpression (Supplementary Fig. 1F and G), suggesting that miR-188 negatively regulates autophagy level in hepatocytes. Since autophagy is a dynamic process, we next detected autophagy flux by overexpressing the GFP-LC3 fusion protein in primary hepatocytes. As a result, miR-188-deficient and -overexpressing hepatocytes had higher and lower autophagy level, respectively (Fig. 4C). The cells were further treated with chloroquine, an autophagy inhibitor which can alter the pH of lysosomes and block autophagosome degradation, leading to LC3-II accumulation (Pasquier 2016). We observed a higher number of GFP-LC3 puncta in primary hepatocytes with miR-188 deficiency, and fewer GFP-LC3 puncta in primary hepatocytes with miR-188 overexpression (Fig. 4C). These results indicated that miR-188 inhibits autophagic flux in primary hepatocytes.

We next investigated whether autophagy was involved in miR-188 regulated insulin sensitivity and lipid accumulation. Primary hepatocytes were infected with Ad-anti-miR-188 and treated with chloroquine to suppress the autophagy process. We found that autophagy inhibition abolished miR-188 deficiency enhanced insulin signaling in primary hepatocytes (Fig. 4D). Similarly, the improved glucose output achieved by miR-188 knockdown was also reversed by autophagy suppression (Fig. 4E). Inhibition of autophagy in primary hepatocytes with less miR-188 also led to more intracellular TG (Fig. 4F). Taken together, miR-188 promotes insulin resistance and lipid accumulation via negatively regulating the autophagy pathway.

**Atg12 as a miR-188 target to regulate insulin sensitivity and lipid accumulation**

To further investigate the downstream of miR-188, we predicted potential mRNA targets using online bioinformatics tools, including TargetScan (Garcia et al. 2011, Tao et al. 2013), miRanda (Betel et al. 2010, Bezman et al. 2011) and miRDB (Huang et al. 2020). The top 2000 candidate genes from each software program were compared, and a total of 175 common genes were found (Supplementary Table 1). Among these, Atg12 was the most likely candidate gene to be involved in both the autophagy process and the regulation of metabolism. ATG12, an autophagy-related protein, is essential for the initiation and elongation of phagophores during...
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It has been reported to regulate energy expenditure and beige adipocyte maintenance (Malhotra et al. 2015, Altshuler-Keylin et al. 2016). Deleting Atg12 in UCP1-specific adipose tissue protected mice against diet-induced obesity and insulin resistance (Altshuler-Keylin et al. 2016). We found that hepatic ATG12 expression decreased in obese mice, increased in miR-188 null mice, and decreased in mice with liver-specific miR-188 overexpression (Fig. 5A, B and C). Similarly, ATG12 expression was induced by miR-188 knockdown but reduced by miR-188 overexpression in primary hepatocytes (Fig. 5D and E). To further verify that Atg12 is the target gene of miR-188, we generated luciferase reporter plasmids containing the WT 3′ UTR of Atg12 (Atg12 WT 3′ UTR) and a mutant 3′ UTR of Atg12 (Atg12 MT 3′ UTR) (Fig. 5F). Overexpressing miR-188 suppressed the luciferase activity of WT Atg12 3′ UTR, and this suppressive effect was abolished by mutant 3′ UTR (Fig. 5G). These results demonstrated that miR-188 targets Atg12 and inhibits its expression in hepatocytes.

Furthermore, we examined whether Atg12 is required for the regulation of miR-188 in insulin sensitivity and lipid accumulation. We transfected primary hepatocytes from miR-188-null mice with siRNA-Atg12 and siRNA-NC. Atg12 silencing decreased insulin-stimulated insulin signaling, enhanced glucose output, and elevated PO-induced cellular TG (Fig. 5H, I and J). Thus, Atg12 is a potential target of miR-188 in the regulation of glucose and lipid metabolism.

Knockdown of hepatic miR-188 ameliorates insulin resistance and liver steatosis in obese mice

To further verify the potential of miR-188 as a therapeutic target in reversing obesity-induced hepatosteatosis and insulin resistance, we used AAV-anti-miR-188 to knockdown miR-188 in the liver of diet-induced obesity (DIO) mice (Fig. 6A). Hepatic miR-188 suppression improved a systemic glucose and insulin response in DIO mice, as evidenced by lower fasting blood glucose tendency, decreased HOMA-IR index, and increased glucose tolerance and insulin sensitivity (Fig. 6B, C, D, E and F). This was further proven by restored hepatic insulin signaling (Fig. 6G). Furthermore, mice with lower hepatic miR-188 had a decreased LV/BW ratio without changes in body weight and food intake when compared with their...
controls (Fig. 6H, I and J). In line with this, reduced lipid accumulation and TG contents were observed in the liver of DIO mice injected with AAV-anti-miR-188 (Fig. 6K and L), though other lipid contents in the liver and serum were not changed between these two group (Fig. 6L and M). Meanwhile, lower miR-188 also improved the liver function of DIO mice, as evidenced by the elevated levels of serum AST and ALT (Fig. 6N). Hepatic miR-188 knockdown decreased expression of lipogenesis genes such as Fas, Srebp1c, Pparγ and Gpat, and elevated expression of genes related to fatty acid uptake and oxidation including Cd36, Fabp4, Pparα and Cpt1a (Fig. 6O and P). These data showed that miR-188 knockdown alleviated hepatic steatosis and insulin resistance in obese mice, which could be mediated by increased autophagy level (Supplementary Fig. 1H). Together, these findings suggested that suppressing miR-188 could be a strategy to improve hepatic insulin resistance and steatosis in obese mice.

Discussion

In this study, we firstly uncovered the regulatory role of miR-188 in glucose and lipid metabolism, which was mediated by the autophagy pathway. Briefly speaking, miR-188-null mice fed with high-fat diet ameliorated liver steatosis and hepatic insulin resistance compared with their controls, meanwhile mice with miR-188 overexpression in the liver had the opposite phenotype. Further investigation demonstrated that the regulatory effects of miR-188 were mediated by the autophagy process and Atg12 could be the potential target gene of miR-188. Administration of AAV-anti-miR-188 improved the high-fat diet induced hepatosteatosis and insulin resistance. Our findings revealed a novel function of miR-188 and provided a new therapeutic candidate in NAFLD (Fig. 7).

In our previous study, we found that miR-188 exerted a function in the aging-related pathophysiological process, including aging-associated BMSC differentiation (Li et al. 2015) and aging-associated metabolic phenotype (Huang et al. 2020). There was no significant difference in the level of glucose and lipid metabolism between elderly miR-188-null mice and their WT littermates. But the slightly lower HOMA-IR index and serum TG level in elderly miR-188-null mice still gave us a hint for further detecting the phenotype in miR-188 null mice under high-fat diet conditions. As we expected, 3 months of...
Hepatic miR-188 suppression ameliorates insulin resistance and liver steatosis in obese mice. Male 8-week old C57BL/6J mice was fed with HFD for 3 months, then received AAV-anti-miR-188 and AAV-GFP by tail injection, functional validation was tested after 3 weeks. (A) The expression of miR-188 in the liver of obese mice injected with AAV-anti-miR-188 and their controls. (B, C and D) Fasting blood glucose level, fasting serum insulin level and HOMA-IR index of obese mice injected with AAV-anti-miR-188 and their controls. (E and F) GTT and ITT assay of obese mice injected with AAV-anti-miR-188 and their controls. (G, H, I and J) BW/BW, body weight and food intake of obese mice injected with AAV-anti-miR-188 and their controls. (K) Liver H&E and Oil Red O staining of obese mice injected with AAV-anti-miR-188 and their controls, scale bar: 300 μm. (L and M) TG, TC and FFA level in serum and liver of obese mice injected with AAV-anti-miR-188 and their controls. (N) Serum ALT and AST level of obese mice injected with AAV-miR-188 and their controls. (O and P) The mRNA levels of genes related to lipid metabolism. All the data are shown as means ± s.e.m. (n = 5–6). Statistical significance was calculated by Student’s t test or two-way ANOVA, *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 7
Illustration of miR-188 promoted hepatosteatosis and insulin resistance by suppressing autophagy in regard to targeting Atg12.
when feeding with HFD. Thus, the role of miR-188 in other tissues requires further investigation.

Autophagy is characterized as a degenerative system for maintaining homeostatic balance (Ebatto et al. 2008, Ueno & Komatsu 2017). Accumulating evidence has demonstrated that autophagy was impaired during NAFLD both in mouse models (Tanaka et al. 2016) and in human disease (Gonzalez-Rodriguez et al. 2014). Damaged autophagic ability in the liver can lead to cellular TG accumulation and fatty acid oxidation reduction, the promotion of ER stress and the deterioration of glucose tolerance and insulin sensitivity, forming a vicious circle (Liu et al. 2009, Lavallard & Gual 2014). We confirmed the involvement of autophagy in miR-188 regulation based on the findings that autophagy markers and autophagy flux were altered along with different miR-188 expression in vivo and in vitro. Moreover, the improved insulin sensitivity and reduced triglyceride accumulation achieved by miR-188 knockdown was abolished by autophagy inhibitor administration.

ATG12 is a key component of the ATG5-ATG12-ATG16 complex, which is essential for the initiation and elongation of phagophores during autophagosome formation (Haller et al. 2014). ATG12 can also exert its function via a ATG12-ATG3 or autophagy-independent manner (Yang et al. 2017a). Atg12 has been reported to be involved in the regulation of metabolism, such as beige adipocyte maintenance (Altshuler-Keylin et al. 2016), body weight gain (Malhotra et al. 2015), and liver disease, such as liver fibrosis (Kim et al. 2018) and hepatocellular carcinoma (Kunanopparat et al. 2016). Combining data from online bioinformatics tools, luciferase assays and Western blotting, we regard Atg12 as the potential target of miR-188 (Fig. 5A, B, C, D, E, F and G). Deletion of miR-188 increased autophagy by upregulating ATG12, and vice versa. Furthermore, Atg12 silencing reversed the improved insulin sensitivity induced by miR-188 deficiency, leading to higher glucose production and lipid content (Fig. 5H, I and J). These results suggest that the ATG12-autophagy pathway mediates the role of miR-188 in regulating hepatic glucose and lipid metabolism, which is also evidenced by hepatic miR-188 suppression-improved metabolic profile in obese mice via enhancement of ATG12-autophagy signaling (Supplementary Fig. 1H). Nonetheless, it is still not clear whether and how the miR-188-ATG12-autophagy pathway regulates hepatosteatosis by controlling the expression of metabolic genes such as Cd36, Ppara and Cpt1a. Manipulation of Atg12 in the livers or hepatocytes of miR-188 knockout and overexpressed mice are required to answer this question.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/JOE-20-0033.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Y L, X Q Z, and X H L designed the experiments and wrote the manuscript; Y L, X Q Z, Y X, C J L, Y H, Q G and T S performed most of the experiments; L F helped to design the project, wrote the response letter, did the manuscript correction and proof reading during revision with L L. L L supervised the experiments, analyzed the data and led the project.

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