

RESEARCH PAPER

Activation of PPAR- δ induces microRNA-100 and decreases the uptake of very low-density lipoprotein in endothelial cells

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BACKGROUND AND PURPOSE

Increased level of very low-density lipoprotein (VLDL) is a key feature of the metabolic syndrome and is associated with cardiovascular diseases. PPAR- δ agonists play a protective role in lipid metabolism and vascular function. In this study, we aimed to investigate the role of PPAR- δ in the uptake of VLDL in endothelial cells and its underlying mechanism(s).

EXPERIMENTAL APPROACH

Uptake of VLDL in HUVECs was assessed by Dil-fluorescent labelling of VLDL. Levels of VLDL receptor mRNA and microRNA (miR-100) were detected by quantitative PCR. The target genes of miR-100 were predicted using bioinformatics analysis. 3'-Untranslated region (3'-UTR) luciferase reporter and Argonaute 1 pull-down assays were used to validate the target of miR-100.

KEY RESULTS

PPAR- δ agonist GW501516 decreased uptake of VLDL and expression of VLDL receptor at mRNA and protein levels. GW501516 inhibited the luciferase reporter activity of the 3'-UTR of VLDL receptor. VLDL receptor was a direct target of miR-100. miR-100 was significantly increased by GW501516 in HUVECs. Transfection of a miR-100 mimic decreased the mRNA and protein levels of VLDL receptor and uptake of VLDL. Furthermore, a miR-100 inhibitor abolished the inhibitory effect of PPAR- δ on VLDL receptor expression and VLDL uptake.

CONCLUSIONS AND IMPLICATIONS

In endothelial cells, activation of PPAR- δ decreased VLDL receptor expression and VLDL uptake via the induction of miR-100. These results provided a novel mechanism for the vascular protective effect of PPAR- δ agonists.

Abbreviations

3'-UTR, 3'-untranslated region; Ago1, argonaute 1; ECs, endothelial cells; miRISC, miRNA-mediated silencing complex; miRNAs, microRNAs; VLDL, very low-density lipoprotein

Tables of Links

TARGETS
Nuclear hormone receptors^a
PPAR- δ (NR1C2)
Transporters^b
ABCA1, cholesterol efflux regulatory protein

LIGANDS
GSK0660
GW501516
GW0742

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (^{a,b}Alexander *et al.*, 2013a,b).

Introduction

Hypertriglyceridaemia is widely recognized as a risk factor for cardiovascular diseases, especially coronary artery diseases (Carroll *et al.*, 2005; Miller *et al.*, 2011). Very low-density lipoprotein (VLDL) is a major carrier of plasma triglyceride. Epidemiological studies showed that a high level of plasma VLDL predicts coronary events and contributes to atherogenesis (Hodis, 1999; Kugiyama *et al.*, 1999; Nordestgaard *et al.*, 2007). Also, VLDL increased endothelial permeability and apoptosis and enhanced inflammatory response, suggesting a direct impact of VLDL on atherogenesis through endothelial cell (EC) uptake (Eiselein *et al.*, 2007; Libby, 2007). The VLDL receptor, a member of the LDL receptor superfamily, binds and internalizes VLDL and other apoE-containing lipoproteins (Takahashi *et al.*, 1992). VLDL receptor is expressed ubiquitously in peripheral tissues including vascular tissues and particularly increased in atherosclerotic lesions (Karls *et al.*, 1992; Wyne *et al.*, 1996; Hiltunen *et al.*, 1998), which suggests the involvement of VLDL receptor in the development of atherosclerosis.

PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three isoforms of PPAR (α , β/δ and γ) play important roles in controlling lipid metabolism and energy homeostasis. PPAR- δ has many endogenous ligands, including saturated and polyunsaturated fatty acid. Synthetic ligands include GW501516, GW0742 and L0165041 (Wang, 2008). These synthetic ligands have been used in mouse models of obesity and diabetes. GW501516, which is 1000-fold selective for PPAR- δ over the other PPAR subtypes, reduces weight gain and circulating triglyceride level, while increasing HDL in high-fat diet-induced obese mice, *db/db* and *ob/ob* mice, through stimulating peripheral fatty acid catabolism (Oliver *et al.*, 2001; Tanaka *et al.*, 2003). GW501516 and GW0742 both improve insulin sensitivity and rescue hepatic steatosis in diabetic models (Lee *et al.*, 2006; Qin *et al.*, 2008). Moreover, PPAR- δ agonists significantly reduce atherosclerotic lesions in LDL receptor^{-/-} mice and restore endothelial function in diabetic mice. The potential mechanism for the vascular protective effects is that PPAR- δ regulates endothelial survival, activation and inflammation (Li *et al.*, 2004; Graham *et al.*, 2005; Barish *et al.*, 2008; Tian *et al.*, 2012). In the context of lipoprotein transport, GW501516 up-regulates expression of the cholesterol efflux regulatory protein ABCA1 and increases apolipoprotein A1-specific cholesterol efflux in human THP-1

monocytes. PPAR- δ is a VLDL sensor in macrophages (Chawla, 2003) and VLDL directly regulates gene expression via the activation of PPAR- δ . However, the role of PPAR- δ in the regulation of VLDL uptake has not been assessed.

MicroRNAs (miRNAs) are a family of highly conserved, small non-coding RNA molecules, that regulate gene expression at the post-transcriptional level via the degradation or translational repression of their target mRNAs (Filipowicz *et al.*, 2008). As a transcription factor, PPAR- δ regulates both protein-coding and non-coding gene expressions (Yin *et al.*, 2010). In this study, we have identified miR-100 as a potential target of PPAR- δ and that this miRNA consequently regulates the expression of VLDL receptor and VLDL uptake.

Methods

Cells

HUVECs were cultured as described previously (Qin *et al.*, 2007). The investigation conformed to the principles outlined in the Declaration of Helsinki. Informed consent was documented and the protocol was approved by Institutional Ethic Committee. HEK293 cells (ATCC CRL-1573) were cultured in DMEM with 10% FBS.

Quantitative RT-PCR

Total RNA was isolated with TRIzol reagent. Quantitative RT-PCR (qRT-PCR) for microRNA was performed using the Taqman miR assay kits and TaqMan real-time PCR Master Mixes (Applied Biosystems, Foster City, CA, USA). Levels of miRs were normalized to the internal control U6 snRNA. Levels of mRNA for the VLDL receptor were measured using specific primers (forward: 5'-ACC TGA ATG ATG CCC AAG AC; reverse: 5'- CTC GGC CAT TTT CCT CTA CA) and normalized to the housekeeping gene GAPDH using SYBR Green real-time PCR Master Mixes (Applied Biosystems). The relative levels of gene expression were expressed as fold changes in relation to the control treatment.

Immunoblotting

Cellular proteins were extracted with lysis buffer [50 mmol·L⁻¹ Tris-HCl, pH 7.5, 15 mmol·L⁻¹ EGTA, 100 mmol·L⁻¹ NaCl, 0.1% (wt/vol) Triton X-100] supplemented with the protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). The samples were resolved on a

10% SDS-PAGE and blotted to nitrocellulose membranes. Immunoblots were reacted with primary antibodies at 1:1000 dilution, detected with HRP-conjugated secondary antibodies and visualized by enhanced chemoluminescence system.

Transfection, plasmid and reporter assay

The double-stranded miRNA mimic was designed to supplement endogenous miRNA activity, while the single-stranded inhibitor was designed to specifically bind to and inhibit endogenous miRNA activity. The miRNA mimic and inhibitor were transfected into HUVECs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at a final concentration of 5 nM or 40 nM respectively. Cells were harvested after 24 h of transfection.

The VLDL receptor 3'-untranslated region (3'-UTR) was cloned by PCR from the genomic DNA with specific primers (forward: 5'-AGC TTT GGA TGT GGT TAC CG; reverse: 5'-CTC AGT CTT TGC AAA CCT CCA). The amplified product was cloned into Hind III and Spe I of the pMir-Report luciferase vector (Ambion, Austin, TX, USA) to generate VLDL receptor 3'-UTR reporter construct (wt-VLDL receptor 3'-UTR-luc). The VLDL receptor 3'-UTR mutation vector (mu-VLDLR 3'-UTR) was generated using the method of site-directed mutagenesis as previous described (Laible and Boonrod, 2009). The parental plasmid (wt-VLDL receptor 3'-UTR-luc) was amplified using the primer-contained mutated sequence (GGT TGG GAC AAT GGC AAT AGG ACA AAG AAT TCT ACT AAG ATG AAA TTG CCA AAA AAA) and KOD DNA polymerase (Millipore, Billerica, MA, USA). After DpnI digestion, the plasmid was subjected to the transformation. The mutant construct was confirmed by sequencing. pcDNA3.1-PPAR- δ and miR-100 mimic were co-transfected into HEK293 cells using Lipofectamine 2000. For luciferase assay, the pRSV- β -galactosidase plasmid was co-transfected with the luciferase reporter vector to normalize the transfection efficiency. Luciferase and β -galactosidase activities were measured as described previously (Wang *et al.*, 2013).

Immunoprecipitation of argonaute (AGO)-miRNA-mediated silencing complex (miRISC)

HUVECs were harvested and lysed with a buffer containing 50 mM Tris-HCl at pH 7.4, 150 mM NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 100 U- μ L⁻¹ RNase inhibitor and 1 mM PMSF. The lysates were incubated with anti-Ago1 antibody. After incubation at 4°C overnight, the immune complexes were pulled down with protein A/G sepharose beads for 4 h and washed with the lysis buffer. Following the last wash, the beads were then centrifuged and the immunoprecipitated RNAs were extracted with TRIzol and analysed by qRT-PCR (Chen *et al.*, 2013). The level of VLDL mRNA indicated the association of VLDL receptor mRNA with AGO-containing miRISC.

VLDL uptake assay

The uptake of VLDL was measured by Dil-VLDL. HUVECs were cultured on glass-bottom culture dishes (MatTek Corp., Ashland, MA, USA) and treated with GW501516 for 36 h. The assay was performed in a serum-free medium containing 1% BSA. Cells were incubated with Dil-VLDL (10 mg·mL⁻¹ in pre-incubation medium) for 2 h at 37°C, then washed three times

with probe-free medium (Takahashi *et al.*, 1992). Fluorescence confocal microscopy was performed using 514 nm excitation and 550 nm emission filters to visualize Dil-VLDL. Hoechst dye was used to counterstain nuclei. The fluorescence intensity was determined from 200 cells normalized to the areas of the cells.

Data analysis

Data were expressed as means \pm SEM from at least three independent experiments. Student's *t*-test or two-way ANOVA were used for statistical analyses. *P* < 0.05 was considered significant.

Materials

GW501516 ((4-[(4-methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-5-yl)methyl]sulfanyl]-2-methylphenoxy)acetic acid), a selective agonist of PPAR- δ , was synthesized by WuXi Pharmatech Inc. (Shanghai, China), as described previously (Oliver *et al.*, 2001; Fan *et al.*, 2007). The specific PPAR- δ antagonist GSK0660 was obtained from Tocris Bioscience (Bristol, UK). Dil-labelled VLDL (Dil-VLDL) (BT-922) was from Biomedical Technologies Inc. (Stoughton, MA, USA). The VLDL receptor (6A6) mouse monoclonal antibody (sc-18824) was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and the rabbit polyclonal argonaute 1 (Ago1) antibody (#9338) was from Cell Signaling Inc. (Danvers, MA, USA). The miRNA mimic and inhibitors were from Genepharma (Shanghai, China).

Results

Activation of PPAR- δ decreases VLDL receptor expression and VLDL uptake in vascular ECs

To examine the effect of PPAR- δ on endothelial VLDL uptake, HUVECs were pretreated with the PPAR- δ agonist GW501516 for 24 h before incubation with Dil-VLDL. By tracing Dil fluorescence, we found that GW501516 decreased Dil-VLDL uptake into HUVECs compared with DMSO control (Figure 1A and B).

To determine if the effect of GW501516 was mediated by modulation of VLDL receptor we measured the levels of VLDL receptor protein and mRNA by Western blotting and qRT-PCR. Both the mRNA and protein levels of the VLDL receptor were decreased by GW501516 (Figure 1C-E).

To assess the involvement of miRNAs in the regulation of VLDL receptor expression by PPAR- δ , we co-transfected wild-type VLDL receptor 3'-UTR (wt-VLDL receptor 3'-UTR-luc) with either pcDNA 3.1-PPAR- δ or pcDNA 3.1 control vector in HEK293 cells. As shown in Figure 1F, the luciferase activity was decreased with GW501516 treatment in control cells and was further decreased in cells with PPAR- δ overexpression, indicating that the 3'-UTR of the VLDL receptor gene was a target of the PPAR- δ action.

VLDL receptor is a target of miR-100

By using Pictar (<http://pictar.mdc-berlin.de/>) to predict microRNA target sites in the 3'-UTR of VLDL receptor, we found that

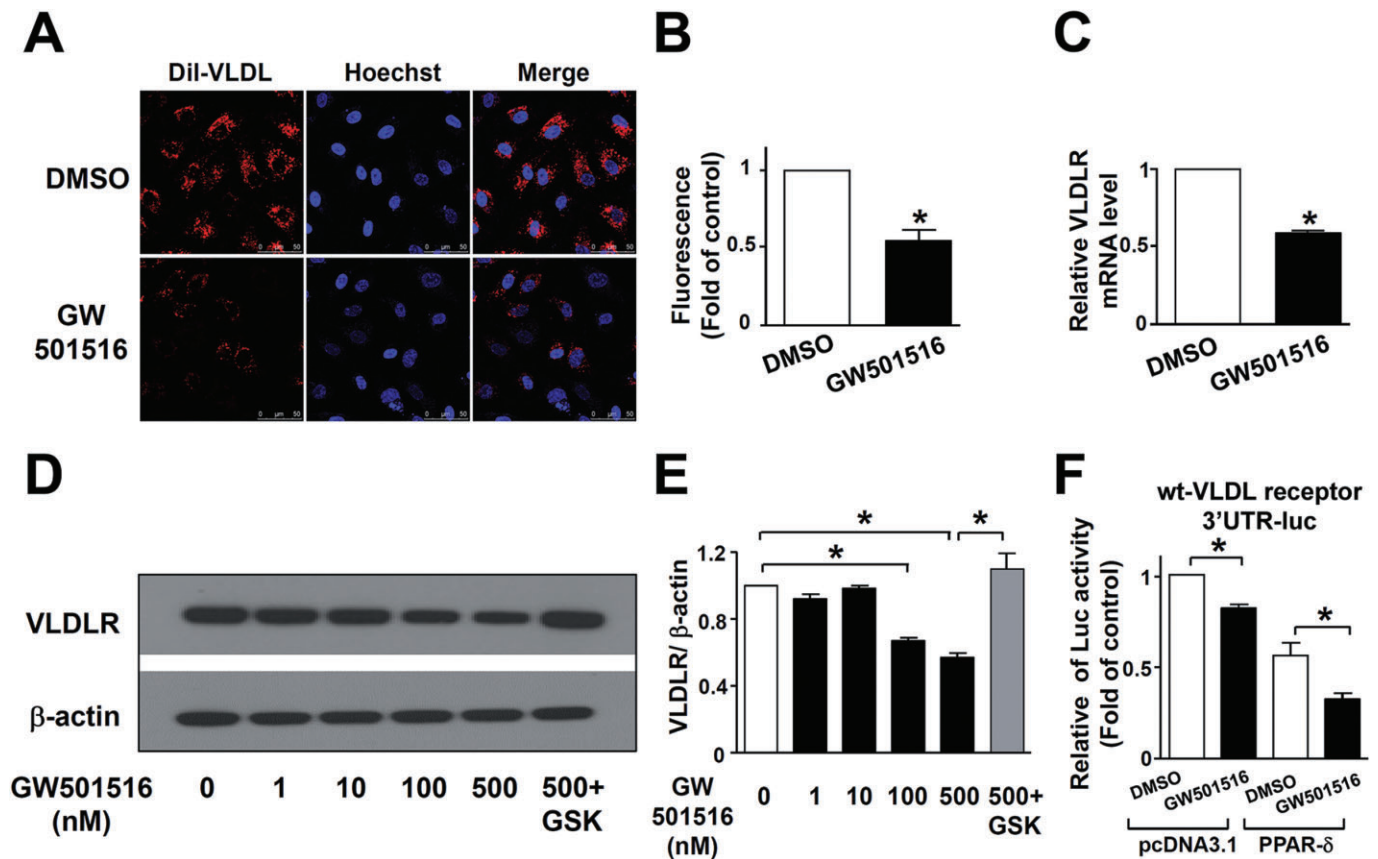


Figure 1

GW501516 reduces VLDL uptake through decreased expression of VLDL receptor (VLDLR). (A) Representative fluorescence micrographs showing the fluorescently labelled VLDL (Dil-VLDL) uptake into HUVECs after 36 h treatment with GW501516 (500 nM) or DMSO control. Dil-VLDL is shown in red and nuclei in blue; $n = 4$. (B) Quantification of Dil-VLDL uptake into HUVECs. (C) VLDL receptor mRNA level after 36 h GW501516 (500 nM) or DMSO control treatment in HUVECs; $n = 4$. (D) Representative immunoblots for VLDL receptor in HUVECs after 36 h GW501516 in different concentrations or in the presence of GSK0660. DMSO treatment was used as a control; $n = 6$. (E) Quantification of VLDL receptor immunoblotting results. (F) The effect of GW501516 (500 nM) or DMSO control treatment on the luciferase activity of the wt-VLDL receptor 3'-UTR-luc in HEK293 cells co-transfected with pcDNA3.1-PPAR- δ or pcDNA3.1 control vector; $n = 3$. Data are presented as mean \pm SEM. * $P < 0.05$, significantly different from DMSO control.

miR-100 might conservatively target this 3'-UTR mRNA in several species (human, chimpanzee, mouse, rat, dog and chicken; Supporting Information Fig. S1). This analysis led us to focus on the role of miR-100 in our subsequent experiments. We first transfected a miR-100 mimic in HUVECs and, as shown in Figure 2A, found this transfection increased the miR-100 level ~20-fold, compared with the control RNA. Immunoblotting and qRT-PCR analyses revealed that this transfection also decreased both VLDL receptor protein and mRNA levels (Figure 2B–D). The functional consequence of the transfection of miR-100 mimic was a significantly decreased Dil-VLDL uptake (Figure 2E and F).

To confirm that miR-100 directly targeted VLDL receptor, we co-transfected HEK293 cells with wt-VLDL receptor 3'-UTR-luc reporter plasmid and the miR-100 mimic. As shown in Figure 3A, the miR-100 mimic significantly decreased the luciferase activity of wt-VLDL receptor 3'-UTR-luc. However, after mutating the seed sequence of miR-100 in the 3'-UTR of VLDL receptor, we found that the luciferase activity was not decreased by miR-100 mimic (Figure 3B).

As a post-transcriptional regulation, miRNAs control gene expression by guiding AGO-containing miRISC complexes to target mRNA. To investigate the association of VLDL receptor mRNA with Ago1, we transfected HUVECs with the miR-100 mimic or control RNA, then quantitated mRNA level after Ago1 immunoprecipitation. The equal input of cell lysate as well as the equal amount of Ago1 pulled down were shown in Supporting Information Fig. S2. As expected, the miR-100 mimic transfection increased the level of VLDL receptor mRNA complexed to Ago1 compared with the IgG control (Figure 3C), indicating a direct targeting action of miR-100.

miR-100 mediates the effect of PPAR- δ on VLDL uptake

To investigate whether PPAR- δ activation induced miR-100 expression, HUVECs were treated with the PPAR- δ agonist GW501516 and the level of miR-100 was quantified. As shown in Figure 4, GW501516 increased the expression of miR-100, both time- and dose-dependently. This effect was blocked by GSK0660, a selective PPAR- δ antagonist. Further-

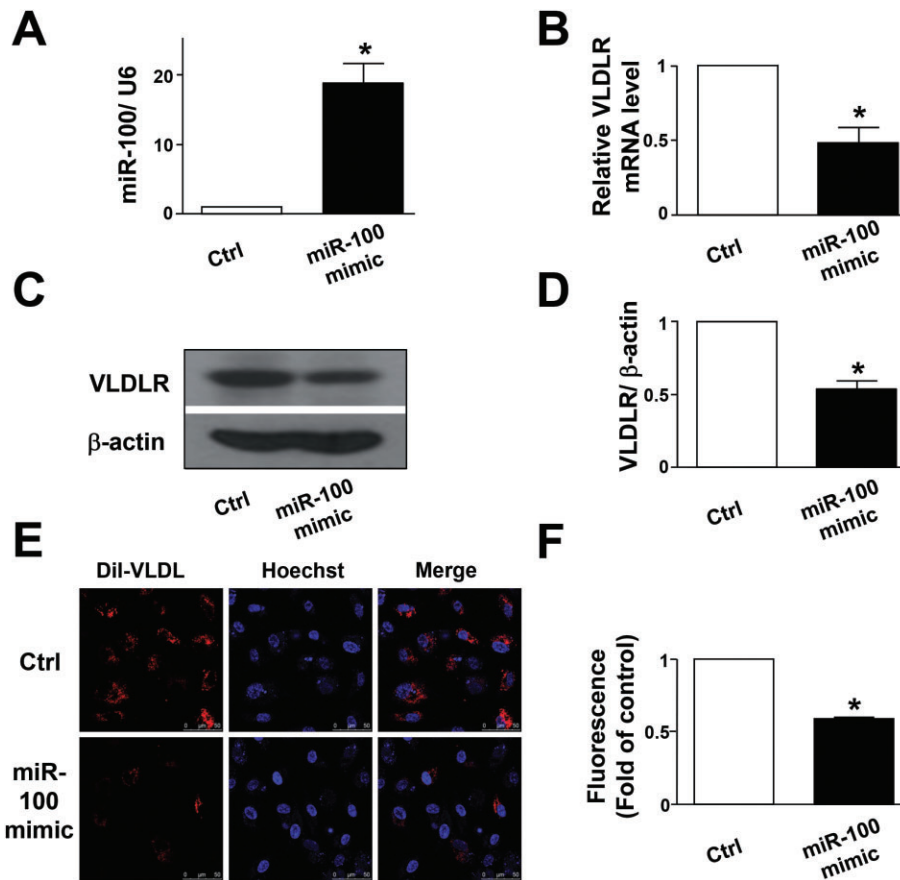


Figure 2

miR-100 suppresses VLDL receptor (VLDLR) expression and VLDL uptake. (A) Quantitative PCR for validation of the efficiency of HUVECs transfected with miR-100 mimic (5 nM). (B) Quantitative PCR analysing VLDL receptor mRNA level after 24 h transfection with miR-100 mimic (5 nM) or control RNA (Ctrl). (C) Representative immunoblot for VLDL receptor in HUVECs after 24 h miR-100 mimic (5 nM) or control RNA (Ctrl) transfection. (D) Quantification of VLDL receptor immunoblotting results. (E) Representative fluorescence micrographs showing the Dil-VLDL uptake into HUVECs following 24 h transfection with miR-100 mimic (5 nM) or control RNA (Ctrl). Dil-VLDL is shown in red and nuclei in blue. (F) Quantification of Dil-VLDL uptake into HUVECs. Data are presented as mean \pm SEM of three independent experiments. * $P < 0.05$, significantly different from control.

more, a miR-100 inhibitor blocked the inhibitory effects of GW501516 on Dil-VLDL uptake (Figure 5A and B) and expression of the VLDL receptors (Figure 5C and D).

Discussion

In this study, we have demonstrated that activation of PPAR- δ induced miR-100 expression and subsequently decreased the expression of VLDL receptor, resulting in the reduction of VLDL uptake in ECs. PPAR- δ , a ligand-dependent transcription factor, plays an important role on lipid metabolism and vascular protection. For instance, PPAR- δ activation inhibited inflammation, oxidative stress and apoptosis (Liou *et al.*, 2006; Fan *et al.*, 2007) in ECs. *In vivo* evidence showed that a PPAR- δ agonist restored endothelial function in diabetic and atherosclerosis models (Graham *et al.*, 2005; Tian *et al.*, 2012). Importantly, PPAR- δ acts as a sensor for VLDL, regulating triglyceride homeostasis locally in peripheral tissues such as macrophages and vessel walls (Chawla, 2003). In this study,

we found that the PPAR- δ agonist triggered translational repression of VLDL receptor and the decrease of VLDL internalization, effects that would provide endothelial protection. Chawla had shown that the expression of VLDL receptors was dramatically increased in PPAR- δ null macrophages, suggesting that VLDL receptor was either directly or indirectly decreased by activation of PPAR- δ (Chawla, 2003). Our finding provides a novel mechanism for the inhibiting effect of PPAR- δ on the expression of VLDL receptors, via miRNAs.

The major finding of this study was that miR-100, which was induced by PPAR- δ , directly targeted VLDL receptor expression. This was validated by the reporter assay and Western blotting analysis. AGO pull-down assay demonstrated the direct binding of miR-100 and miRISC to VLDL receptor mRNA. Functionally, we showed that miR-100 decreased VLDL uptake in ECs, via this target gene. As shown in Figure 4, the PPAR- δ agonist GW501516 increased miR-100 expression, while the specific antagonist GSK0660 attenuated this induction. A genome-wide analysis by chromatin immunoprecipitation sequencing revealed a PPAR β/δ -enrichment

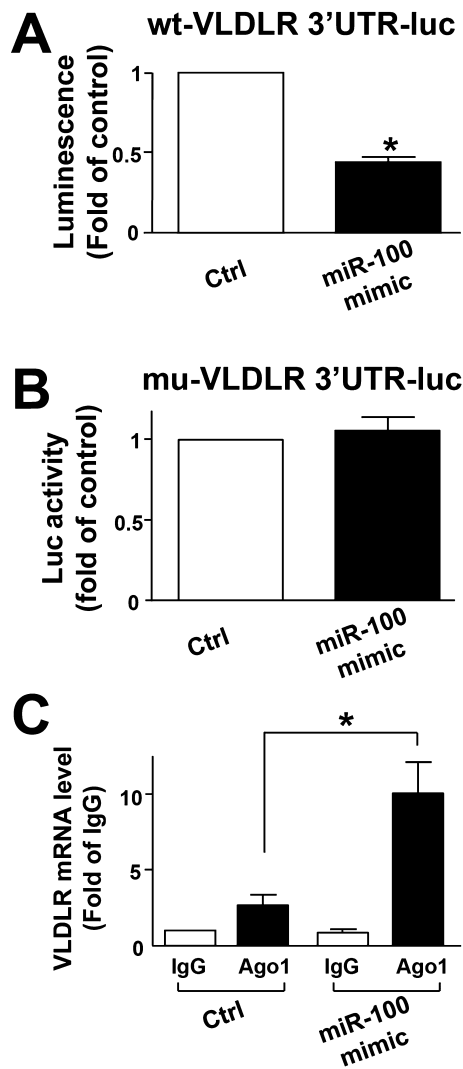


Figure 3

MicroRNA-100 targets VLDL receptor 3'UTR. (A and B) Luciferase activity of the reporter of wt-VLDL receptor 3'-UTR-luc plasmid (A), but not mutated VLDL receptor 3'-UTR-luc plasmid (B), was decreased in miR-100 mimic transfected cells. (C) Quantitative PCR analysing the Ago1 immunocomplexes after 48 h of miR-100 mimic or control RNA transfection. Data are presented as the mean \pm SEM from three independent experiments. * $P < 0.05$, significantly different from control.

peak in the promoter of miR-100 (–4016 bp from the putative transcription start site) (Tora *et al.*, 2011), which indicates that PPAR- δ may transcriptionally regulate the expression of miR-100. The specific role of miR-100 in the inhibition of VLDL expression by the PPAR- δ agonist GW501516 was addressed by using a miR-100 inhibitor. GW501516 was unable to decrease the protein level of VLDL receptor in the presence of anti-miR-100 (Figure 5C and D), which confirmed that GW501516 suppressed the expression of VLDL receptor mainly through the action of miR-100. Treatment with GW501516 and transfection of miR-100 had a similar efficacy in terms of the inhibition of VLDL receptors, although very different levels of miR-100 were achieved. The reasons for the

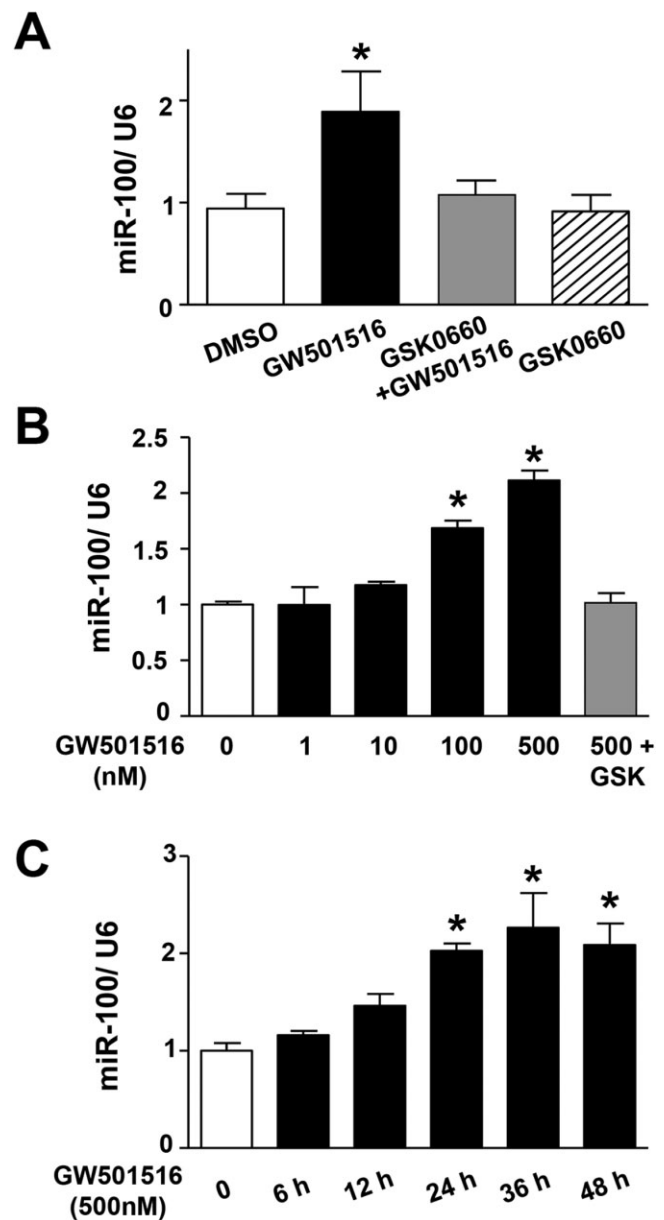


Figure 4

Specific ligand for PPAR- δ induces miR-100 expression. (A) Quantitative PCR analysing miR-100 level in HUVECs after treatment with DMSO control, GSK0660 (2.5 μ M) or GW501516 (500 nM) in the presence or absence of GSK0660 (2.5 μ M); $n = 7$. (B) Quantitative PCR analysing miR-100 level in HUVECs after 24 h treatment with GW501516 at different concentrations or in the presence of GSK0660. DMSO treatment was used as a control; $n = 4$. (C) Quantitative PCR analysing miR-100 level in HUVECs after treatment with GW501516 (500 nM) for different time; $n = 5$. Data are presented as the mean \pm SEM. * $P < 0.05$, significantly different from control.

disproportionate dose–effect relationship are unclear although it has been a common observation that an exogenously transfected gene and an endogenously induced gene are similarly effective. One interpretation may be that over-expressed miRNAs potentially saturated RISC complexes and consequently reached a plateau state for the silencing effect

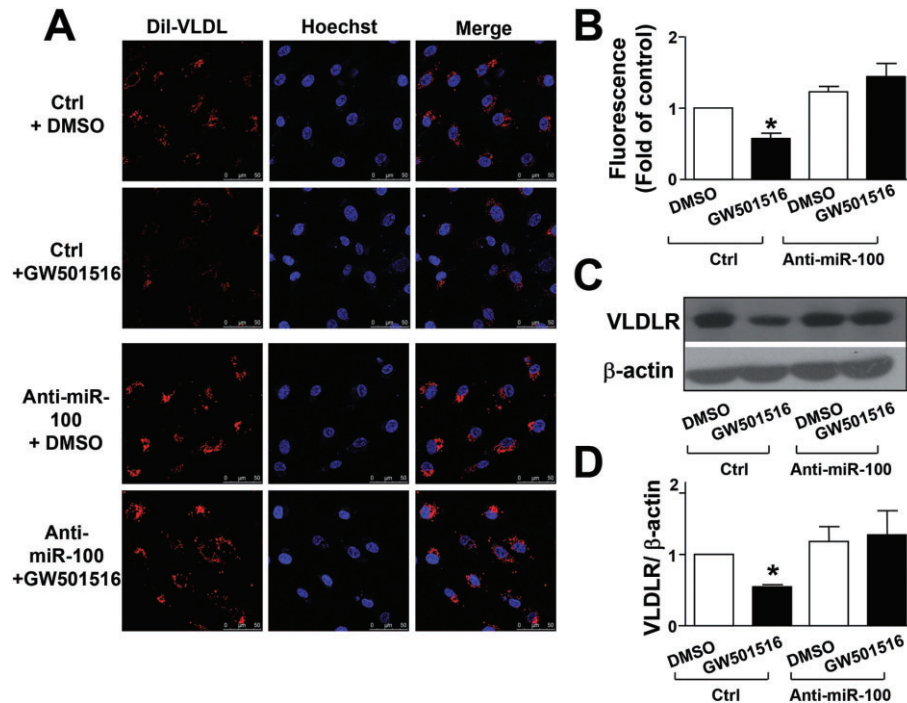


Figure 5

miR-100 mediates the suppressive effect of GW501516 on VLDL receptor (VLDLR) in HUVECs. (A) Representative fluorescence micrographs showing the Dil-VLDL uptake into HUVECs following 36 h treatment with GW501516 (500 nM) or DMSO control after 24 h of 40 nM miR-100 antagonist (anti-miR100) or control RNA (Ctrl) transfection. Dil-VLDL is shown in red and nuclei in blue. (B) Quantification of Dil-VLDL uptake into HUVECs. (C) Representative immunoblot for VLDL receptor in HUVECs following 36 h treatment with GW501516 (500 nM) or DMSO control after 24 h 40 nM miR-100 inhibitor (anti-miR100) or control RNA (Ctrl) transfection. (D) Quantification of VLDL receptor immunoblotting results. Data are presented as the mean \pm SEM from three independent experiments. * $P < 0.05$, significantly different from control.

(Khan *et al.*, 2009). This miRNA, miR-100, is highly expressed in ECs. Recently, Grundmann *et al.* showed that miRNA-100 was significantly down-regulated after induction of hind-limb ischaemia in mice and modulated ECs proliferation, tube formation and sprouting activity, which established an anti-angiogenic role of miR-100 (Grundmann *et al.*, 2011). In this study, we have identified miR-100 as a mediator regulating VLDL internalization through the direct inhibition of the expression of VLDL receptor in ECs.

Being a member of the LDL receptor superfamily, the VLDL receptor plays a major role in VLDL internalization and metabolism. Although VLDL receptor knockout mice had no lipoprotein abnormalities, reconstitution of VLDL receptor expression in VLDL receptor knockout mice greatly increased atherosclerotic lesion development, indicating a pro-atherogenic role of this receptor (Eck *et al.*, 2005). Moreover, the increased expression of VLDL receptor in atherosclerotic lesions suggests an important contribution to the endothelial dysfunction and the development of atherosclerosis (Hiltunen *et al.*, 1998). Our results in this study showed that PPAR- δ decreased VLDL receptor expression and VLDL uptake in ECs, via miR-100. This evidence has provided a new mechanism for the endothelial protective effect of PPAR- δ . This regulation could be a tissue-/organ-specific effect. The decreased uptake by ECs would restore the endothelial function in hyperlipidaemia. However, these results are limited to

in vitro experiments. Understanding the physiological significance of PPAR- δ -induced miR-100 needs further investigation in animal models, using EC-specific miR-100 overexpression or knockout.

In summary, we have demonstrated that PPAR- δ activation induced the expression of miR-100 and decreased the expression of VLDL receptor and, in turn, attenuated VLDL internalization in ECs. Our finding provides evidence that PPAR- δ regulates VLDL in ECs via a miRNA mechanism.

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Author contributions

X. F. and N. W. designed the research. X. F., L. F., A. L., X. W. and B. Z. performed the research. X. F. analysed the data. X. F. and N. W. wrote the paper.

Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

<http://dx.doi.org/10.1111/bph.13160>

Figure S1 Comparison of the seed region of miR-100 with putative target sequences of the 3'UTR conserved regions at human, chimpanzee, mouse, rat, dog and chicken VLDL receptor mRNA.

Figure S2 Western blot analysing the Ago1 immunocomplexes after 48 h of miR-100 mimic or control RNA transfection in input of cell lysate as well as IgG and Ago1 pulled-down sample. Data are representative of three independent experiments.