Resistin up-regulates LPL expression through the PPARγ-dependent PI3K/AKT signaling pathway impacting lipid accumulation in RAW264.7 macrophages

Bi Li[a,c,1], Jing Fang[a,1], Tingting He[a,1], Sirui Yin[a], Mingxian Yang[a,c], Hengmin Cui[a], Xiaoping Ma[a], Junliang Deng[a], Zhihua Ren[a], Yanchun Hu[a], Gang Ye[a], Ming Zhang[b], Yi Geng[a], Liping Gou[a], Zhicai Zuo[a,*]

[a] Key Laboratory of Animal Disease and Human Health of Sichuan Province, College of Veterinary Medicine, Sichuan Agricultural University, Huimin road 211, 611130, China
[b] College of Animal Science & Technology, Sichuan Agricultural University, Huimin road 211, 611130, China
[c] Sichuan Key Laboratory of Conservation Biology for Endangered Wildlife, Chengdu Research Base of Giant Panda Breeding, Chengdu, Sichuan 610081, China

ABSTRACT

Resistin is a cysteine-rich cytokine, which has been indicated as a mediator of insulin resistance and inflammation. Previous studies demonstrated that lipoprotein lipase (LPL) was an important enzyme that could mediate lipid accumulation in macrophages. Additionally, the intracellular molecules phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase (AKT)/peroxisome proliferator-activated receptor (PPARγ) were supposed to be involved in the lipid accumulation process in cells. However, it remains unclear whether resistin was correlated with the dysregulation of lipid metabolism in macrophages. The present study investigated that resistin could up-regulate the expression of LPL and increase the contents of intracellular triglyceride (TG) and total cholesterol (TC) in RAW264.7 macrophages. In addition, intracellular molecules PI3K, AKT and PPARγ were significantly up-regulated and activated in resitin-stimulated RAW264.7 macrophages ($P < 0.05$). In contrast, the effects of resistin on RAW264.7 macrophages could be abrogated by specific inhibitors for LPL (LPL-siRNA) and PI3K/AKT signaling pathway (LY294002). All together, this study demonstrated that resistin could up-regulate the expression of LPL and induce lipid accumulation in RAW264.7 macrophages. More importantly, the PPARγ-dependent PI3K/AKT signaling pathway was relevant to the lipid accumulation process in resistin-stimulated macrophages.

1. Introduction

Resistin is a cysterine-rich cytokine, a unique signaling hormone that potentially links obesity, insulin resistance and inflammation [1,2]. Resistin is mainly secreted from adipocytes and macrophages that might reduce the sensitivity of insulin and relate to the disorder of inflammatory lesions [3–5]. Increasing evidences proved that the serum level of resistin was positively correlated with lipid metabolic disorders, like obesity, type 2 diabetes mellitus and atherosclerosis [6–10]. Macrophages are players in these chronic diseases that orchestrate lipid metabolism and inflammation [11–14]. Some studies even implied that resistin might induce excessive lipid accumulation in macrophages [15–17]. However, the cellular mechanisms of resistin on the

dysregulation of lipid metabolism in macrophages are not well understood.

Lipoprotein lipase (LPL) is a primary enzyme that required for the metabolism of lipoprotein triglycerides [18]. In artery, LPL can further hydrolyze remnant-like lipoprotein particles (RLPs) and facilitate the uptake of RLPs, like oxidized low-density lipoprotein (ox-LDL), which may result in the augment of lipid deposition in monocytes/macrophages [19–21]. Meanwhile, resistin was supposed to be a mediator that could up-regulate the expression of LPL in adipose cells 3T3-L1 [22,23]. However, whether resistin could up-regulate the expression of LPL in macrophages was unclear.

Phosphatidylinositol 3-kinase (PI3K) and serine-threonine protein kinase (AKT), regarded as signal transduction molecules in cells, are

associated with varieties of biological processes, including apoptosis, insulin resistance and adipogenesis [24–27]. Previous studies elucidated that the PI3K/AKT mediated signaling pathway was participated in lipid accumulation process via phosphorylating or activating substrates, like peroxisome proliferator-activated receptor (PPARγ) [28–30]. PPARγ, a downstream signal molecule of AKT, is well-known as a transcription factor in lipid metabolism [31–33]. In macrophages, PPARγ is verified as a mediator to the expression of LPL [34,35]. Reciprocally, resistin could activate insulin receptor substrate-1 (IRS-1) to regulate PI3K/AKT signaling pathway [25]. However, it remained uncertain whether resistin could induce the uptake of lipid in macrophages, and if this process was relevant to intracellular signaling molecules PI3K, AKT and PPARγ.

Hence, the aims of this study were to testify the role of resistin in lipid accumulation process in macrophages and investigate the cellular mechanism. The present findings showed that PPARγ-dependent PI3K/AKT signaling pathway was correlated with the expression of LPL in resistin-stimulated RAW264.7 macrophages, and LPL was a factor that would facilitate the cellular uptake of ox-LDL, thus enhancing lipid accumulation in macrophages.

2. Materials and methods

2.1. Cell culture

RAW264.7 macrophages were purchased from China Center for Type Culture Collection (CCTCC, China). Cells were cultured in DMEM (High glucose) with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (Gibco, Life Technologies). RAW264.7 macrophages were incubated in a humidified incubator (Thermo, Life Technologies) at 37 °C, 95% air and 5% CO_2. RAW264.7 macrophages were used within 20 passages in this study. Endotoxin-free recombinant mouse resistin was purchased from Lifespan Biosciences. ox-LDL was purchased from Solarbio (Beijing, China).

2.2. Real-time quantitative PCR analysis (qRT-PCR)

Total RNA of RAW264.7 macrophages was extracted by using TRIzol reagent (Invitrogen, Life Technologies) in accordance with the instruction manual. β-actin gene was used as an internal control. Primer sets were as follows: murine β-actin [36], forward: 5′-CTGAGAGGGAAATCGTGCGT-3′, and reverse: 5′-CCACAGGATTCCATACCCAAGA-3′; murine LPL [20], forward: 5′-GGGAGTTTGGCTCCAGAGTTT-3′, and reverse: 5′-TGTGTCTTCAGGGGTCCTTAG-3′. qRT-PCR reactions were performed on QuantStudio Real-Time PCR system (Applied Biosystems) using SYBR Green detection chemistry. Melting curve analysis verified the reliability of each qRT-PCR reaction. Quantitative measurements were determined by using the ΔΔ Ct method, and the mRNA expression of β-actin gene was used as the internal control.

2.3. Western-blotting analysis

Monoclonal antibodies against LPL (bs-1973r; 1:1000), PI3K (bs-10657r; 1:800), AKT (bs-0115r; 1:1000), PPARγ (bs-0530r; 1:1000), p-PI3K (bs-6417r; 1:1000), p-AKT (bs-5194r; 1:1000), p-PPARγ (bs-4888r; 1:1000) and β-actin (BA2305; 1:800) were purchased from Bioss Antibodies, Inc. (Beijing, China), and the horseradish peroxidase (HRP)-conjugated secondary antibody (BA2305; 1:3000) was purchased from Wuhan Boster Biological Technology, Ltd. (Wuhan, China). Proteins in cells were extracted by using a Cytoplasmic and Nuclear Protein Extraction kit (BestBio, Inc., Shanghai, China), and the protein concentrations were calculated by using bicinchoninic acid assay kits (Pierce, Life technologies). Protein lysates were kept at −20 °C until detected by western-blotting. The protein lysates were fractionated via 12% SDS-PAGE and transferred to PVDF membranes (EMD Millipore, Billerica, MA, USA). Then the PVDF membranes were blocked with 5%

fat-free milk powder at room temperature for 1 h and immunoblotted overnight at 4 °C with primary antibodies. Next, the PVDF membranes were incubated with HRP-conjugated secondary antibody for 1 h at room temperature. After that, the PVDF membranes were washed 5 times with Tween-20 mixed PBS for 5 min. Finally, the blots in PVDF membranes were developed by using the enhanced chemiluminescence (ECL) system (GE Healthcare Life Sciences). The integrated optical density (IOD) value of each blot was detected and analyzed by Image-Pro Plus 6.0 software (Media Cybernetics, Inc.).

2.4. Detection of triglycerides (TG) and total cholesterol (TC) contents

RAW264.7 macrophages were starved for 12 h and then treated with 50 μg/ml ox-LDL and LPL-siRNA for 24 h; or incubated with resistin (200 ng/ml) for 24 h. The contents of TG and TC were detected by using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Transfection for LPL knock-down

Small-interfering RNA specific for LPL (LPL-siRNA) was designed and synthesized by Invitrogen (Life Technologies). After RAW264.7 macrophages were grown to 70–80%, the cells were then transfected with 150 nM LPL-siRNA or 150 nM control siRNA (ctrl-siRNA) by using Lipofectamine® 3000 (Invitrogen, Life technologies). siRNAs were as follows: LPL-siRNA, forward: 5′-CAGCUGAGGACACUUGUCAUCUCAU-3′, and reverse: 5′-AUGAGAUGACAAGUGUCCUCAGCUG-3′; ctrl-siRNA, forward: 5′-CAGAGGGCACAUUUGACCUUUCCAU-3′, and reverse: 5′-AUGGAAAGGUCAAAUGUGCCCUCUG-3′.

2.6. Statistical analysis

Data were presented as Mean ± SD. Statistical analyses and graphs plot were performed by using GraphPad Prism software (Version 4.03; GraphPad Software Inc., San Diego, CA, USA). All data were evaluated by using one-way analysis of variance (ANOVA) or Student's t test to compare quantitative data among the experimental groups. Differences with $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Effects of resistin on the expression of LPL in RAW264.7 macrophages

RAW264.7 macrophages were exposed to resistin in a diverse set of concentrations and for the designated times (Fig. 1), and the expression of LPL in cells was tested by qRT-PCR and western-blotting. Data in Fig. 1A illustrated that the mRNA expression level of LPL in RAW264.7 was increased with the dosage increase of resistin. Data in Fig. 1B further illustrated that the mRNA expression level of LPL was significantly up-regulated with the designated times and to the peak at 24 h ($P < 0.05$). Similar protein expression patterns of LPL were detected by western-blotting (Fig. 1C–F). Taken together, these data supported that resistin could up-regulate the expression of LPL in RAW264.7 macrophages with time and dose.

3.2. Effects of resistin on lipid accumulation in ox-LDL incubated RAW264.7 macrophages

The ox-LDL (50 μg/ml, 24 h) and resistin (200 ng/ml, 24 h) were sequentially added into RAW264.7 macrophages (Fig. 2), and the storage levels of intracellular triglyceride (TG) and total cholesterol (TC) were measured by kits. Results in Fig. 2A and B illustrated that resistin obviously enhanced the contents of TG and TC in ox-LDL incubated RAW264.7 macrophages. These results indicated that resistin could promote the cellular lipid accumulation in ox-LDL-incubated RAW264.7 macropahages.
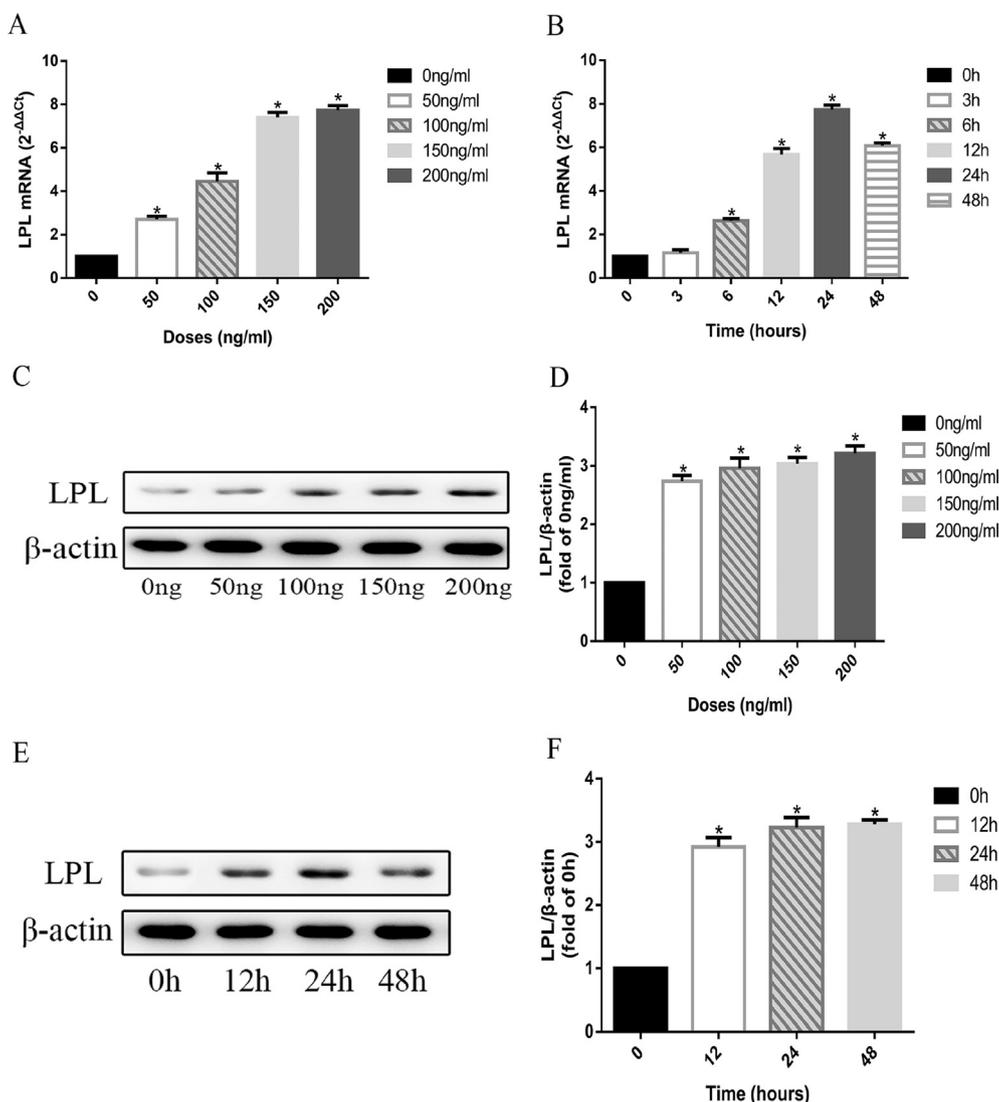
Fig. 1. Effects of resistin on the expression of LPL in RAW264.7 macrophages. RAW264.7 macrophages were exposed with 0 ng/ml, 50 ng/ml, 100 ng/ml and 200 ng/ml resistin for 24 h. (A) The mRNA expression level of LPL was analyzed by qRT-PCR and (C, D) the protein expression level of LPL was detected by western-blotting. $^*P < 0.05$ compared with 0 ng/ml resistin treatment group. RAW264.7 macrophages were treated with 200 ng/ml resistin for 0 h, 3 h, 6 h, 12 h, 24 h and 48 h. (B) The mRNA expression of LPL was analyzed by qRT-PCR and (E, F) the protein expression of LPL were detected by western-blotting. $^*P < 0.05$ compared with 0 h group.

3.3. Inhibition of LPL abrogates lipid accumulation in resistin-stimulated RAW264.7 macrophages

To clarify the role of LPL in resistin-mediated lipid accumulation process, RAW267.4 cells were transiently transfected with LPL-siRNA for 48 h prior to the treatment of resistin. In Fig. 3A, the knocking down efficiency of LPL-siRNA was approximately 86%, when compared with the control. Thereafter, the transfected RAW264.7 cells were pretreated with ox-LDL for 1 h prior to the stimulation of resistin (200 ng/ml) for 24 h. In Fig. 3, stimulating the cells with resistin significantly enhanced the mRNA and protein expression levels of LPL, and the contents of TG and TC ($P < 0.05$); whereas lack of LPL remarkably impaired the
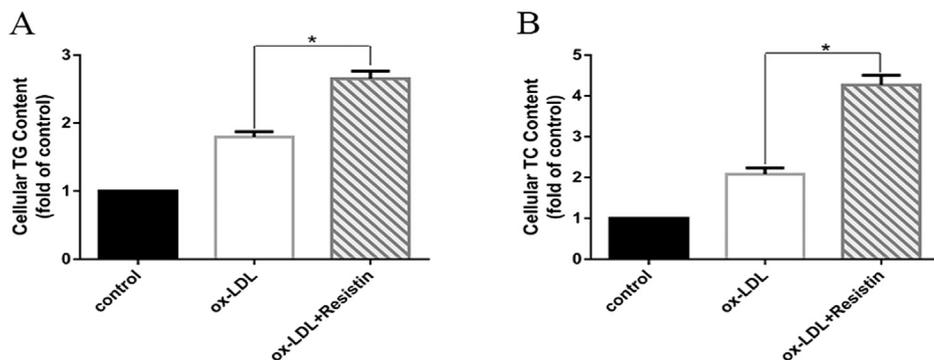

Fig. 2. Effects of resistin on the lipid accumulation in ox-LDL incubated RAW264.7 macrophages. RAW264.7 macrophages were pre-treated with 50 µg/ml ox-LDL for 24 h and then treated with the resistin (200 ng/ml) for 24 h. The storage levels of intracellular TG (A) and TC (B) were measured by kits. PBS was used as blank control; $^*P < 0.05$ compared with ox-LDL treatment group.
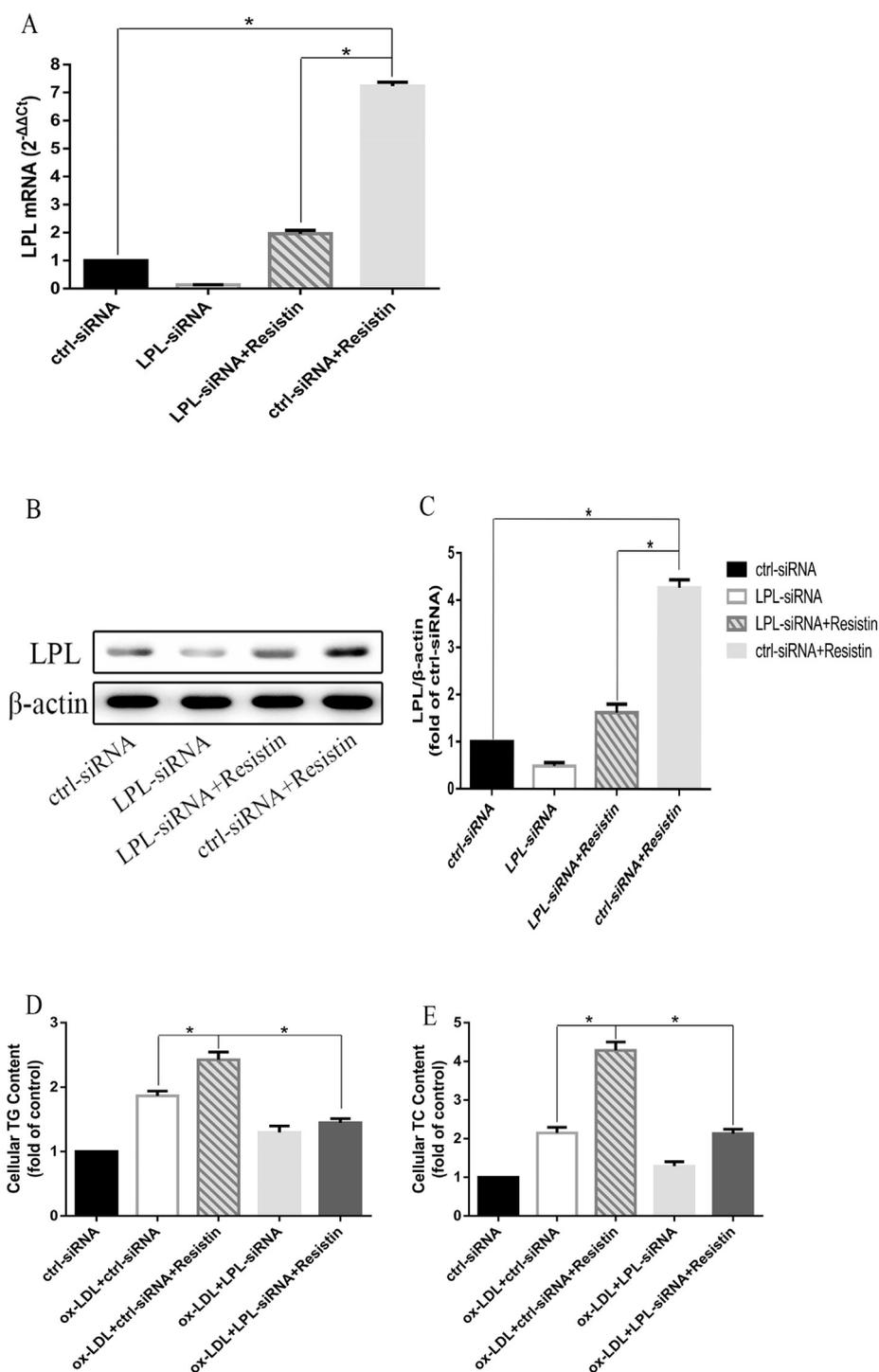
Fig. 3. Inhibition of LPL abrogates lipid accumulation in resistin-stimulated RAW264.7 macrophages. RAW264.7 macrophages were transfected with LPL-siRNA for 48 h, and then cells were pretreated with 50 μg/ml ox-LDL for 24 h and then treated with 200 ng/ml resistin for 24 h. (A) The mRNA expression level of LPL was analyzed by qRT-PCR and (B, C) the protein expression level of LPL was detected by western-blotting. *P < 0.05 compared with 0 ng/ml ox-LDL treatment group. The storage levels of intracellular TG (D) and TC (E) were also measured by kits. ctrl-siRNA was used as blank control; *P < 0.05 compared with ctrl-siRNA treatment group.

resistin-induced effects in RAW264.7 ($P < 0.05$), thus indicating that the lipid accumulation effects exerted by resistin in ox-LDL incubated RAW264.7 macrophages were associated with LPL.

3.4. Resistin up-regulates the protein expression and phosphorylation levels of PI3K, AKT, and PPARγ in RAW264.7 macrophages

To investigate whether signaling molecules PI3K, AKT and PPARγ were involved in resistin-stimulated lipid accumulation process in macrophages, RAW264.7 macrophages were exposed to 200 ng/ml resistin for 24 h (Fig. 3), and the protein expression and phosphorylation levels of PI3K, AKT and PPARγ in cells were tested by western-blotting.

In Fig. 3A, incubation with resistin significantly increased the protein expression and phosphorylation levels of PI3K, AKT and PPARγ in RAW264.7 ($P < 0.05$), indicating that the resistin-induced lipid accumulation process might be relevant to the activation/phosphorylation of PI3K, AKT and PPARγ.

3.5. LY294002 abrogates resistin function by PI3K/AKT/PPARγ signaling pathway

To further confirm the role of PI3K, AKT and PPARγ in resistin-induced lipid accumulation process, RAW264.7 macrophages were pretreated with a specific PI3K/AKT signaling pathway inhibitor
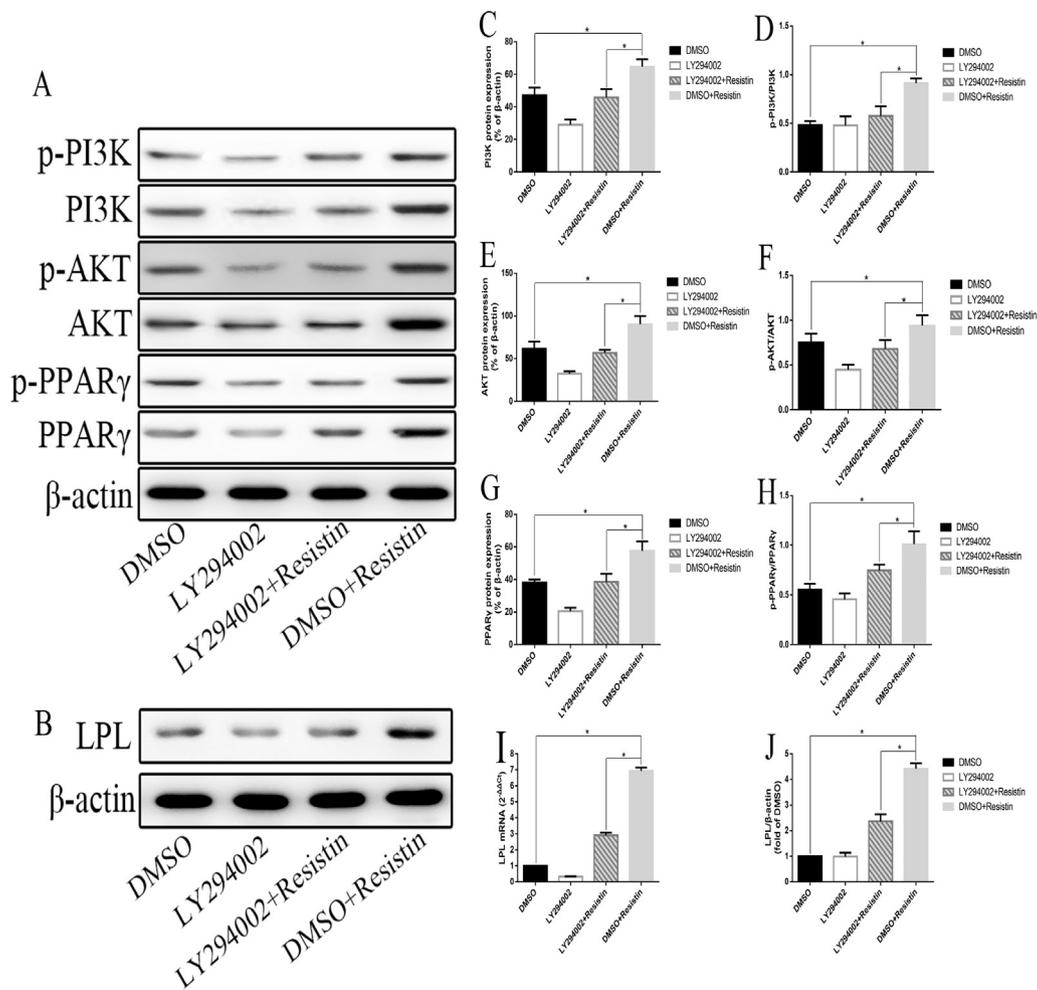
Fig. 4. The expression of LPL in resistin-stimulated RAW264.7 macrophages was mediated by the PPARγ-dependent PI3K/AKT signaling pathway. RAW264.7 macrophages were pretreated with LY294002 for 2 h before resistin treatment (200 ng/ml, 24 h). (A, C ~ H) The protein expression and phosphorylation levels of PI3K, AKT, and PPARγ were detected by western-blotting. (B, J) The protein expression level of LPL was detected by western-blotting. (I) The mRNA expression level of LPL was analyzed by qRT-PCR. $^{*}P < 0.05$ compared with DMSO treatment group.

LY294002 (40 µmol/l) for 2 h and then treated with resistin for 24 h, the protein expression and phosphorylation levels of PI3K, AKT and PPARγ were analyzed by western-blotting (Fig. 4A). Results showed that LY294002 significantly reduced the protein expression and phosphorylation levels of PI3K, AKT and PPARγ in resistin-treated RAW264.7 ($P < 0.05$). Besides, the mRNA expression and protein expression levels of LPL were also decreased significantly ($P < 0.05$), along with the down-regulation of PI3K, AKT and PPARγ (Fig. 4B). The abovementioned results indicated that the expression of LPL in resistin-stimulated RAW264.7 was dependent on the PI3K/AKT/PPARγ signaling pathway.

4. Discussion

Resistin is a unique cytokine, also known as FIZZ3 (found in inflammatory zone 3), belongs to the cysteine-rich secretory proteins family [5]. Resistin is postulated as a molecule link, collecting obesity, insulin resistance and inflammation in humans and rodents. Recently, obesity and atherosclerosis have been viewed as pathological states of chronic inflammation [37,38]. Besides, it has been approved that resistin could acutely regulate lipid metabolism in obesity and might play a certain role in the pathogenesis of atherosclerosis [15,39,40]. The connection between lipid metabolism and macrophages has two faces: on one hand, lipid metabolism fulfils the energetic needs for macrophages and supports membrane fluidity necessary for the function of

phagocytosis; on the other hand, the excessive uptake of oxidized lipids in macrophages may accelerate the formation of foam cells, leading to dysfunction of the arteries [41–43]. In this study, we found that resistin significantly enhanced the uptake of ox-LDL in RAW264.7 macrophages ($P < 0.05$), and the internalization process of lipid in macrophages was thought to play a central role in the promotion of atherosclerosis.

LPL is a 55-kDa glycoprotein that regulates multi aspects of lipid metabolism. In adipose cells 3T3-L1, it has been proved that resistin could activate LPL through stimulating glucose-dependent insulinotropic polypeptide (GIP) [23,44]. To examine the hypothesis that resistin could induce the activity of LPL in macrophages, we firstly used qRT-PCR and western blot analysis to assess the expression changes of LPL in RAW264.7 exposed to different concentrations of resistin and indicated times (Fig. 1). Results showed that the expression level of LPL in RAW264.7 macrophages was significantly increased after the treatment of resistin ($P < 0.05$). Broadly, LPL is supposed to bind with heparan sulfate proteoglycans (HSPG) on cell surface that will facilitate the adhesion of macrophages to the endothelium and promote the retention of RLPs in the arterial intima [18,45–47]. Additionally, LPL is viewed as a molecular bridge on cell surface to bind ox-LDL and LDL receptor (LDLR) that will accelerate the uptake of extracellular ox-LDL into macrophages [18,48–50]. Generally, the up-regulated expression of LPL will not only facilitate the hydrolysis of lipoprotein triglycerides in cells, but also contribute to the lipid accumulation in macrophages,
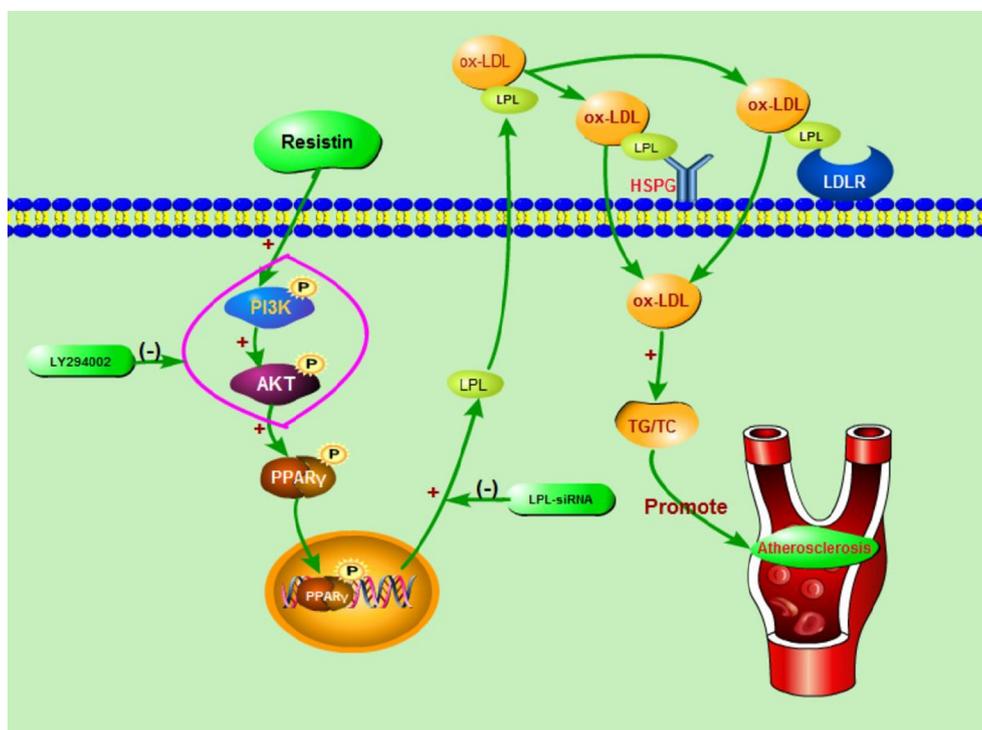
Fig. 5. Resistin modulates LPL expression via the PPARγ-dependent PI3K/AKT signaling pathway promoting lipid accumulation in RAW264.7 macrophages. These observations might provide intracellular targets for the prevention and control of lipid metabolic disorders, like the atherosclerosis.

which may be proatherogenic. Therefore, the contents of intracellular triglyceride (TG) and total cholesterol (TC) of RAW264.7 macrophages were subsequently tested in this study, and the results showed that resistin significantly induced lipid accumulation in ox-LDL incubated RAW264.7 ($P < 0.05$) (Fig. 2). Given the aforementioned results that the expression of LPL was up-regulated by resistin, we thought the lipid accumulation process in RAW264.7 macrophages was positively correlated with the elevated LPL expression induced by resistin. Therefore, a LPL specific inhibitor (LPL-siRNA) was transiently transfected into RAW267.4 prior to the treatment of resistin for further disclosing the involvement of LPL in resistin-stimulated RAW264.7 macrophages (Fig. 3). Results showed that the expression level of LPL and the contents of TG and TC were significantly decreased ($P < 0.05$), indicating that the lipid accumulation process in resistin-stimulated RAW264.7 macrophages was correlated with the expression of LPL.

PI3K is a heterodimeric phosphatidylinositol kinase that will activate the downstream messenger molecules [51]. AKT, also known as protein kinase B (PKB), is a downstream messenger molecule of PI3K that is associated with pathological processes, like cancer, inflammation, insulin resistance and cardiovascular disease [28]. PI3K/AKT signaling pathway was reported to be participated in a diverse set of intracellular signal transaction pathways, many of which would propose adipogenesis [28]. In addition, some biological effects exerted by resistin were found to be closely related to PI3K/AKT signaling pathway [25]. In this study, the protein expression levels of PI3K/AKT and their phosphorylation (activation) were significantly up-regulated ($P < 0.05$) after RAW264.7 macrophages were treated with resistin (Fig. 4A). PPARγ, a member of nuclear hormone receptors super-family, binds retinoic acid receptor (RXR) to form a structure named PPAR-RXR [52–55]. The PPAR-RXR can be activated by fatty acids and LPL lipolytic products to trigger the lipid accumulation process [56]. Besides, a peroxisome proliferator activated receptor responsive element (PPRE) was discovered in the promoter site of LPL gene that could regulate the expression of LPL in cells [57]. The LPL-PPRE is a specific sequence element that was capable to bind with different types of PPAR-RXR, like haPPARγ-mRXRα. What's more, PPARγ has been denoted as a

downstream signal molecule of AKT that could be activated by PI3K/AKT signaling pathway [58]. As illustrated in Fig. 4A, G and H, treatment of resistin significantly increased the expression level of PPARγ and its phosphorylation in RAW264.7 ($P < 0.05$). However, the PI3K/AKT pathway blocker (LY294002) has effectively reversed the resistin-induced changes of PI3K/AKT and caused a significant reduction of PPARγ ($P < 0.05$), indicating that PI3K/AKT signaling pathway was closely related to PPARγ in RAW264.7 macrophages (Fig. 4). Besides, the mRNA expression and protein expression levels of LPL were decreased after the PPARγ-dependent PI3K/AKT signaling pathway was blocked by LY294002 (Fig. 4B). Collectively, these findings manifested that the up-regulation of LPL and the accumulation of lipid in resistin-stimulated RAW264.7 macrophages were correlated with PI3K/AKT signaling pathway, and PPARγ might play a vital role in this process.

5. Conclusions

The present study found that resistin could up-regulate the expression of LPL to accelerate lipid accumulation process in RAW264.7 macrophages, indicating resistin as a causative agent that would facilitate the transformation of macrophages into lipid-laden foam cells. Moreover, we found that the function of resistin on lipid accumulation in RAW264.7 macrophages was correlated to the PPARγ-dependent PI3K/AKT pathway, which might provide intracellular targets for the prevention and control of lipid metabolic disorders, like the atherosclerosis (Fig. 5).

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

References

[1] C.M. Steppan, S.T. Bailey, S. Bhat, E.J. Brown, R.R. Banerjee, et al., The hormone resistin links obesity to diabetes, Nature (2001) 307–312.

[2] B. Li, J. Fang, Z. Zuo, S. Yin, T. He, et al., Activation of the porcine alveolar macrophages via toll-like receptor 4/NF-κB mediated pathway provides a mechanism of resistin leading to inflammation, Cytokine (2018).

[3] L. Patel, A.C. Buckels, I.J. Kinghorn, P.R. Murdock, J.D. Holbrook, et al., Resistin is expressed in human macrophages and directly regulated by PPARγ activators, Biochem. Biophys. Res. Commun. 300 (2) (2003) 472–476.

[4] F.A. Hannan, K.G. Culligan, Human resistin and the RELM of inflammation in diabesity, Diabetol. Metab. Syndr. 7 (1) (2015) 54–64.

[5] F. Molica, S. Morel, B.R. Kwak, F. Rohnerjeanrenaud, S. Steffens, Adipokines at the crossroad between obesity and cardiovascular disease, Thromb. Haemostasis 113 (03) (2015) 553–566.

[6] X.H. Niu, L. Li, J.Y. Li, Q. Song, M.M. Jin, J.X. Liu, Serum resistin positively correlates with serum lipids, but not with insulin resistance, in first-degree relatives of type-2 diabetes patients: an observational study in China, Medicine 96 (16) (2017) e6622.

[7] S. Younis, M. Blumenberg, Q. Javed, Resistin gene polymorphisms are associated with acne and serum lipid levels, providing a potential nexus between lipid metabolism and inflammation, Arch. Dermatol. Res. 308 (4) (2016) 229–237.

[8] N. Zayani, H. Hamdouni, I. Boumaiza, O. Achour, F. Neffati, A. Omezzine, M.F. Najjar, A. Bouslama, Resistin polymorphims, plasma resistin levels and obesity in Tunisian volunteers, J. Clin. Lab. Anal. (2017) e22227.

[9] F. Santilli, R. Liani, F.P. Di, G. Formoso, P. Simeone, R. Tripaldi, T. Ueland, P. Aukrust, G. Davì, Increased circulating resistin is associated with insulin resistance, oxidative stress and platelet activation in type 2 diabetes mellitus, Thromb. Haemostasis 115 (06) (2016) 1089–1099.

[10] Y. He, X.J. Bai, F.X. Li, L.H. Fan, J. Ren, Q. Liang, H.B. Li, L. Bai, H.Y. Tian, F.L. Fan, Resistin may be an independent predictor of subclinical atherosclerosis formale smokers, Biomarkers 22 (3–4) (2016) 291–295.

[11] A. Remmerie, C.L. Scott, Macrophages and lipid metabolism, Cell. Immunol. 330 (2018) 27–42.

[12] J.I. Odegaard, A. Chawla, Alternative macrophage activation and metabolism, Annu. Rev. Pathol. 6 (1) (2010) 275–297.

[13] X. Geeraerts, E. Bolli, S.M. Fendt, J.A.V. Ginderachter, Macrophage metabolism as therapeutic target for cancer, atherosclerosis, and obesity, Front. Immuno. 8 (2017) 1–13.

[14] S. Saha, I.N. Shalova, S.K. Biswas, Metabolic regulation of macrophage phenotype and function, Immunol. Rev. 280 (1) (2017) 102–111.

[15] W. Xu, L. Yu, W. Zhou, M. Luo, Resistin increases lipid accumulation and CD36 expression in human macrophages, Biochem. Biophys. Res. Commun. 351 (2) (2006) 376–382.

[16] T.S. Lee, C.Y. Lin, J.Y. Tsai, Y.L. Wu, K.H. Su, et al., Resistin increases lipid accumulation by affecting class A scavenger receptor, CD36 and ATP-binding cassette transporter-A1 in macrophages, Life Sci. 84 (3) (2009) 97–104.

[17] C. Rae, A. Graham, Human resistin promotes macrophage lipid accumulation, Diabetologia 49 (5) (2006) 1112–1114.

[18] Y. Li, P.P. He, D.W. Zhang, X.L. Zheng, F.S. Cayabyab, et al., Lipoprotein lipase: from gene to atherosclerosis, Atherosclerosis 237 (2) (2014) 597–608.

[19] Y.C. Lv, Y.Y. Tang, J. Peng, G.J. Zhao, J. Yang, et al., MicroRNA-19b promotes macrophage cholesterol accumulation and aortic atherosclerosis by targeting ATP-binding cassette transporter A1, Atherosclerosis 236 (1) (2014) 215–226.

[20] J. Peng, Y.C. Lv, P.P. He, Y.Y. Tang, W. Xie, et al., Betulinic acid downregulates expression of oxidative stress-Induced lipoprotein lipase via the PKC/ERK/c-Fos pathway in RAW264.7 macrophages, Biochimie 119 (2015) 192–203.

[21] G.P. Tian, W.J. Chen, P.P. He, S.L. Tang, G.J. Zhao, et al., MicroRNA-467b targets LPL gene in RAW 264.7 macrophages and attenuates lipid accumulation and proinflammatory cytokine secretion, Biochimie 94 (12) (2012) 2749–2755.

[22] H. Gong, Y. Ni, X. Guo, L. Fei, X. Pan, et al., Resistin promotes 3T3-L1 preadipocyte differentiation, Eur. J. Endocrinol. 150 (6) (2004) 885–892.

[23] S.J. Kim, C. Nian, C.H. Mcintosh, Resistin knockout mice exhibit impaired adipocyte glucose-dependent insulinotropic polypeptide receptor (GIPR) expression, Diabetes 62 (2) (2013) 471–477.

[24] F. Maingrette, G. Renier, Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C, Diabetes 52 (8) (2003) 2121–2128.

[25] Y. Zhang, J. Hai, M. Cao, S. Pei, J. Wang, et al., Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway, Int. Immunopharmacol. 17 (3) (2013) 714–720.

[26] M.B. Jiménez-Castro, A. Casillas-Ramírez, M. Mendes-Braz, M. Massip-Salcedo, J. Gracia-Sancho, et al., Adiponectin and resistin protect steatotic livers undergoing transplantation, J. Hepatol. 59 (6) (2013) 1208–1214.

[27] L. Pang, Y. Zhang, Y. Yu, S. Zhang, Resistin promotes the expression of vascular endothelial growth factor in ovary carcinoma cells, Int. J. Mol. Sci. 14 (5) (2013) 9751–9766.

[28] B.D. Manning, A. Toker, AKT/PKB signaling: navigating the network, Cell 169 (3) (2017) 381–405.

[29] X. Peng, R. Chen, Y. Wu, B. Huang, C. Tang, et al., PPARγ-PI3K/AKT-NO signal pathway is involved in cardiomyocyte hypertrophy induced by high glucose and insulin, J. Diabetes. Complicat. 29 (6) (2015) 755–760.

[30] D. Zhu, J. Wang, X. Sun, J. Chen, Y. Duan, et al., Septin4_i1 regulates apoptosis in

[31] H.J. Park, J. Yun, S.H. Jang, S.N. Kang, B.S. Jeon, et al., Coprinus comatus cap inhibits adipocyte differentiation via regulation of PPARγ and Akt signaling pathway, Plos One 9 (9) (2014) e105809.

[32] D. Yoshiga, N. Sato, T. Torisu, H. Mori, R. Yoshida, et al., Adaptor protein SH2-B linking receptor-tyrosine kinase and Akt promotes adipocyte differentiation by regulating peroxisome proliferator-activated receptor gamma messenger ribonucleic acid levels, Mol. Endocrinol. 21 (5) (2007) 1120–1131.

[33] B. Bermudez, T.B. Dahl, I. Medina, S. Groeneweg, S. Holm, et al., Leukocyte overexpression of intracellular NAMPT attenuates atherosclerosis by regulating PPARγ-dependent monocyte differentiation and function, Arterioscl. Throm. Vas. 37 (6) (2017) 1157–1167.

[34] L.A. Stechschulte, H.T. Jr, S.S. Ghanem, W. Shou, S.M. Najjar, et al., FKBP51 reciprocally regulates GRα and PPARγ activation via the Akt-p38 pathway, Mol. Endocrinol. 28 (8) (2014) 1254–1265.

[35] J. Zhong, W. Gong, L. Lu, J. Chen, Z. Lu, et al., Irbesartan ameliorates hyperlipidemia and liver steatosis in type 2 diabetic db/db mice via stimulating PPAR-γ, AMPK/Akt/mTOR signaling and autophagy, Int. Immunopharmacol. 42 (2017) 176–184.

[36] H. Luan, Q. Zhang, L. Wang, C. Wang, M. Zhang, et al., OM85-BV induced the productions of IL-1β, IL-6, and TNF-α via TLR4- and TLR2-mediated ERK1/2/NF-κB pathway in RAW264.7 cells, J. Interf. Cytok. Res. 34 (7) (2014) 526–536.

[37] A.D. Mooradian, Obesity: a rational target for managing diabetes mellitus, Growth Horm. Igf Res. 11 (11 Suppl A) (2001) S79–S83.

[38] S.D. Patel, M.W. Rajala, L. Rossetti, P.E. Scherer, L. Shapiro, Disulfide-dependent multimeric assembly of resistin family hormones, Science 304 (5674) (2004) 1154–1158.

[39] O.A. Mojiminiyi, N.A. Abdella, Associations of resistin with inflammation and insulin resistance in patients with type 2 diabetes mellitus, Scand. J. Clin. Lab. Inv. 67 (2) (2007) 215–225.

[40] C. Rae, A. Graham, Fatty acid synthase inhibitor, C75, blocks resistin-induced increases in lipid accumulation by human macrophages, Diabetes Obes. Metab. 10 (12) (2010) 1271–1274.

[41] S. Biswas, A. Mantovani, Orchestration of metabolism by macrophages, Cell Metab. 15 (4) (2012) 432–437.

[42] J. Mcnelis, J. Olefsky, Macrophages, immunity, and metabolic disease, Immunity 41 (1) (2014) 36–48.

[43] P.K. Langston, M. Shibata, T. Horng, Metabolism supports macrophage activation, Front. Immuno. 8 (9) (2017) 1–7.

[44] V.R. Babaev, S. Fazio, L.A. Gleaves, K.J. Carter, et al., Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in vivo, J. Clin. Invest. 103 (12) (1999) 1697–1705.

[45] J.C. Mamputu, A.C. Desfaits, G. Renier, Lipoprotein lipase enhances human monocyte adhesion to aortic endothelial cells, J. Lipid Res. 38 (9) (1997) 1722–1729.

[46] J.C. Obunike, S. Paka, S. Pillarisetti, I.J. Goldberg, Lipoprotein lipase can function as a monocyte adhesion protein, Arterioscl. Throm. Vas. 17 (7) (1997) 1414–1420.

[47] L. Eiselein, D.W. Wilson, M.W. Lamé, J.C. Rutledge, Lipolysis products from triglyceride-rich lipoproteins increase endothelial permeability, perturb zonula occludens-1 and F-actin, and induce apoptosis, Am. J. Physiol. Heart Circ. Physiol. 292 (6) (2007) H2745–H2753.

[48] U. Beisiegel, W. Weber, G. Bengtsson-Olivecrona, Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein, Proc. Nati. Acad. Sci. USA 88 (19) (1991) 8342–8346.

[49] B. Loeffler, J. Heeren, M. Blaeser, H. Radner, D. Kayser, et al., Lipoprotein lipase-facilitated uptake of LDL is mediated by the LDL receptor, J. Lipid Res. 48 (2) (2007) 288–298.

[50] S. Eisenberg, E. Sehayek, T. Olivecrona, I. Vlodavsky, Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix, J. Clin. Invest. 90 (5) (1992) 2013–2021.

[51] M.S. Jamaluddin, S.M. Weakley, Q. Yao, C. Chen, Resistin: functional roles and therapeutic considerations for cardiovascular disease, Brit. J. Pharmacol. 165 (3) (2012) 622–632.

[52] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, et al., PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation, Nat. Med. 7 (1) (2001) 48–52.

[53] S.M. Watkins, P.R. Reifsnyder, H.J. Pan, J.B. German, E.H. Leiter, Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone, J. Lipid Res. 43 (11) (2002) 1809–1817.

[54] J. Cao, J. Lockwood, P. Burn, Y. Shi, Cloning and functional characterization of a mouse intestinal acyl-CoA: monoacylglycerol acyltransferase, MGAT2, J. Biol. Chem. 278 (16) (2003) 13860.

[55] Y.J. Lee, E.H. Ko, E.K. Ji, E. Kim, H. Lee, et al., Nuclear receptor PPARγ-regulated monoacylglycerol O-acyltransferase 1 (MGAT1) expression is responsible for the lipid accumulation in diet-induced hepatic steatosis, Proc. Nati. Acad. Sci. USA 109 (34) (2012) 13656–13661.

[56] D. Prusty, B.H. Park, K.E. Davis, S.R. Farmer, Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes, J. Biol. Chem. 277 (48) (2002) 46226–46232.

[57] K. Schoonjans, J. Peinadoonsurbe, A.M. Lefebvre, R.A. Heyman, M. Briggs, et al., PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene, Embo J. 15 (19) (1996) 5336–5348.

[58] L. Liu, Z. Yang, Y. Xu, J. Li, D. Xu, et al., Inhibition of oxidative stress-elicited AKT activation facilitates PPARγ agonist-mediated inhibition of stem cell character and tumor growth of liver cancer cells, Plos One 8 (8) (2013) e73038.