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Growth differentiation factor-15 induces expression of ATP-binding cassette transporter A1 through PI3-K/PKCζ/SP1 pathway in THP-1 macrophages

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ABSTRACT

Objective: The aim of this study was to determine whether ATP-binding cassette transporter A1 (ABCA1) was up-regulated by growth differentiation factor-15 (GDF-15) via the phosphoinositide 3-kinase (PI3K)/protein kinase Cζ (PKCζ)/specificity protein 1 (SP1) pathway in THP-1 macrophages.
Methods and results: We investigated the effects of different concentrations of GDF-15 on ABCA1 expression in THP-1 macrophages. The results showed that GDF-15 dramatically increased cholesterol efflux and decreased cellular cholesterol levels. In addition, GDF15 increased ABCA1 mRNA and protein levels. The effects of GDF-15 on ABCA1 protein expression and cellular cholesterol efflux were abolished by wither inhibition or depletion of PI3K, PKCζ and SP1, respectively, suggesting the potential roles of PI3K, PKCζ and SP1 in ABCA1 expression. Taken together, GDF-15 appears to activate PI3K, PKCζ and SP1 cascade, and then increase ABCA1 expression, thereby promoting cholesterol efflux and reducing foam cell formation.
Conclusion: Our results suggest that GDF-15 has an overall protective effect on the progression of atherosclerosis, likely through inducing ABCA1 expression via the PI3K/PKCζ/SP1 signaling pathway and enhancing cholesterol efflux.

1. Introduction

Atherosclerosis is the basic pathology of coronary heart disease. The accumulation of lipids in the arterial wall leads to the formation of foam cells, which are thought to play a key role in the development of atherosclerosis. It is known that the reverse cholesterol transport (RCT) [1] plays an important role in inversing the process of atherosclerotic lesion formation, and is a primary atheroprotective property of high-density lipoproteins (HDL) and its major protein, apolipoprotein (Apo) A-I. HDL and ApoA-I are shown to promote efflux of excess cholesterol from macrophages through the cholesterol transporters, such as ATP-binding cassette transporter A1 (ABCA1), ABCG1 and scavenger receptor class B type I (SR-BI). A recent report showed that transcription factor specificity protein 1 (SP1) is a potential regulator in oxysterol-induced ABCA1 expression [2]. It is known that phosphorylation of SP1 increases its DNA binding and transcription activity, and inhibition of SP1 DNA binding attenuates ABCA1 expression [3]. Several protein kinases, including DNA-dependent protein kinase, protein kinase Cζ (PKCζ), casein kinase II and cyclin-dependent kinase 2 (Cdk2) can mediate SP1 phosphorylation [3]. Low-density lipoprotein (LDL)-induced ABCA1 expression is also related to an increased phosphorylation of SP1 and PKCζ, whereas inhibition of PKCζ activity attenuates LDL-induced SP1 phosphorylation and ABCA1

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expression in macrophages [2]. It has also been reported that both reelin and apoE3 significantly increased phosphorylated disabled-1 (Dab1), phosphoinositide 3-kinase (PI3K), PKCζ and SP1, a sequential activation of PI3K, PKCζ and SP1, and then up-regulate the expression of ABCA1 [4].

Here more attention has been paid to cytokine growth differentiation factor-15 (GDF-15), a 12-kDa secreted protein, also named macrophage inhibitory cytokine-1. GDF-15, a distant member of the transforming growth factor (TGF)-β superfamily, is produced and secreted in many cells, such as activated macrophages [5], endothelial cells [6] and vascular smooth muscle cells [7]. GDF-15 serum levels are related to future ischemic events following acute myocardial events [8]. Recently, GDF-15 has been identified to participate in cardiovascular pathology in mouse models. The results indicate that transgenic mice overexpressing GDF-15 are protected from the development of atherosclerosis [9,10], but further studies will be required to define the underlying mechanism. It has also been reported that GDF-15 protects cultured cardiomyocytes via PI3K/Akt-dependent mechanisms [11]. In the present research, we conducted the experiments to investigate the relationship between GDF-15 and ABCA1 expression in THP-1 macrophages. We found that GDF-15 could increase ABCA1 expression via the PI3K/PKCζ/SP1 pathway. These findings indicate that GDF-15 might be a potential target to prevent foam cell formation and progression of atherosclerosis.

2. Materials and methods

2.1. Reagents and antibodies

RPMI medium 1640 was obtained from Solarbio (Beijing, China). Fetal bovine serum (FBS) was purchased from Invitrogen (Carlsbad, CA). Recombinant GDF-15 was from PeproTech Inc. (Jiangsu, China). Phosphoinositide 3-kinase (PI3K) inhibitor 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), PKCζ inhibitor bisindolylmaleimide I hydrochloride (109203X) and antibodies for phosphorylated proteins were obtained from Cell Signaling Technology (Boston, MA). SP1 inhibitor mithramycin A, cholesterol and phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma–Aldrich (St Louis, MO). Ox-LDL was prepared as described previously [12]. Mouse monoclonal antibody to ABCA1 was from Abcam (Cambridge, United Kingdom).

2.2. Cell culture and treatment

Human THP-1 cells were cultured as previously described [13,14]. After 3–4 d, cells were treated with PMA (160 nmol/L) for 24 h to be differentiated into macrophages. The medium was then replaced with serum-free medium containing ox-LDL (50 μg/mL) for 48 h so as to let cells become fully differentiated macrophages for the subsequent experiments. The cells were then fixed with 4% paraformaldehyde and, followed by 0.5% Oil red O dye. The cells were counterstained with hematoxylin and photographed at ×400 magnification.

2.3. Cellular cholesterol efflux determination

Cells were cultured as indicated above, followed by labeling with 0.2 μCi/mL of [^{3}H]cholesterol. After 72 h, cells were washed with PBS and incubated with RPMI 1640 medium containing 0.1% (wt/vol) bovine serum albumin (BSA) overnight to allow equilibration of [^{3}H]cholesterol in all cellular pools. The cells were then washed with PBS and incubated in 2 mL of efflux medium containing RPMI 1640 medium and 0.1% BSA. Medium- and cell-associated [^{3}H]cholesterol was then measured by liquid scintillation counting. Percent efflux was calculated by the following equation: [total media counts /(total cellular counts + total media counts)] × 100%.

2.4. RNA isolation and real-time quantitative polymerase chain reaction (RT-PCR) analysis

Total RNA from cells was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RT-PCR was performed on a Mx3000 Multiplex quantitative PCR system (Stratagene, La Jolla, CA) using SYBR Green detection chemistry. Melt curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were determined using the ΔΔCt method, and the expression of β-actin was used as the internal control.

2.5. Transfection of PI3-K, PKCζ and SP1 short interfering RNA (siRNA)

Specific siRNAs for human PI3K, PKCζ and SP1 (Santa Cruz Biotechnology) and non-silencing control siRNA were synthesized by the Biology Engineering Corporation in Shanghai, China. THP-1 macrophages (2×10^6 cells per well) were transfected with the siRNA of PI3K, PKCζ and SP1 or control siRNA, respectively, by using Lipofectamine 2000 (Invitrogen).

2.6. Western blot analysis

Western blot analysis was performed as described before [13]. Proteins (20 μg each) were loaded on 8% SDS–polyacrylamide electrophoresis gel and electrophoresed for 2 h at 100 V in the buffer containing 25 mM Tris base, 250 mM glycine, and 0.1% SDS. The protein samples were visualized using a chemiluminescence method (ECL Plus Western Blotting Detection System; Amersham Biosciences).

2.7. High performance liquid chromatography (HPLC) assay

HPLC analysis was conducted as previously described [15,16]. Data were analyzed with Total Chrom software from PerkinElmer.

2.8. Statistical analysis

All data are presented as means ± S.D. Results were analyzed by one-way analysis of variance and Student's *t* test. Statistical analyses were conducted with SPSS 13.0. *P* value <0.05 was considered as significance.

3. Results

3.1. GDF-15 promotes cholesterol efflux in THP-1 macrophages

In this study, we investigated the effect of recombinant GDF-15 on cholesterol efflux in THP-1 macrophages. We found that at a concentration of 1.5 ng/mL significantly reduced Oil red O dye in ox-LDL-treated THP-1 macrophages (Fig. 1A and B). Then we assessed the cholesterol efflux activity in the presence or absence of GDF-15. We observed that GDF-15 enhanced cellular cholesterol efflux from THP-1 macrophages in a dose- and time- dependent manner (Fig. 1C and D). GDF-15 started to promote cellular cholesterol efflux when its concentration reached to 1 ng/mL under a 24 h incubation time point. The enhanced effect of GDF-15 on cholesterol efflux was also observed when the cells were incubated with 1.5 ng/mL GDF-15 for 12 h. To further confirm these findings, HPLC was then conducted to determine cellular cholesterol contents. The concentrations of total cellular cholesterol, free cholesterol and cholesterol ester in GDF-15-treated cells were

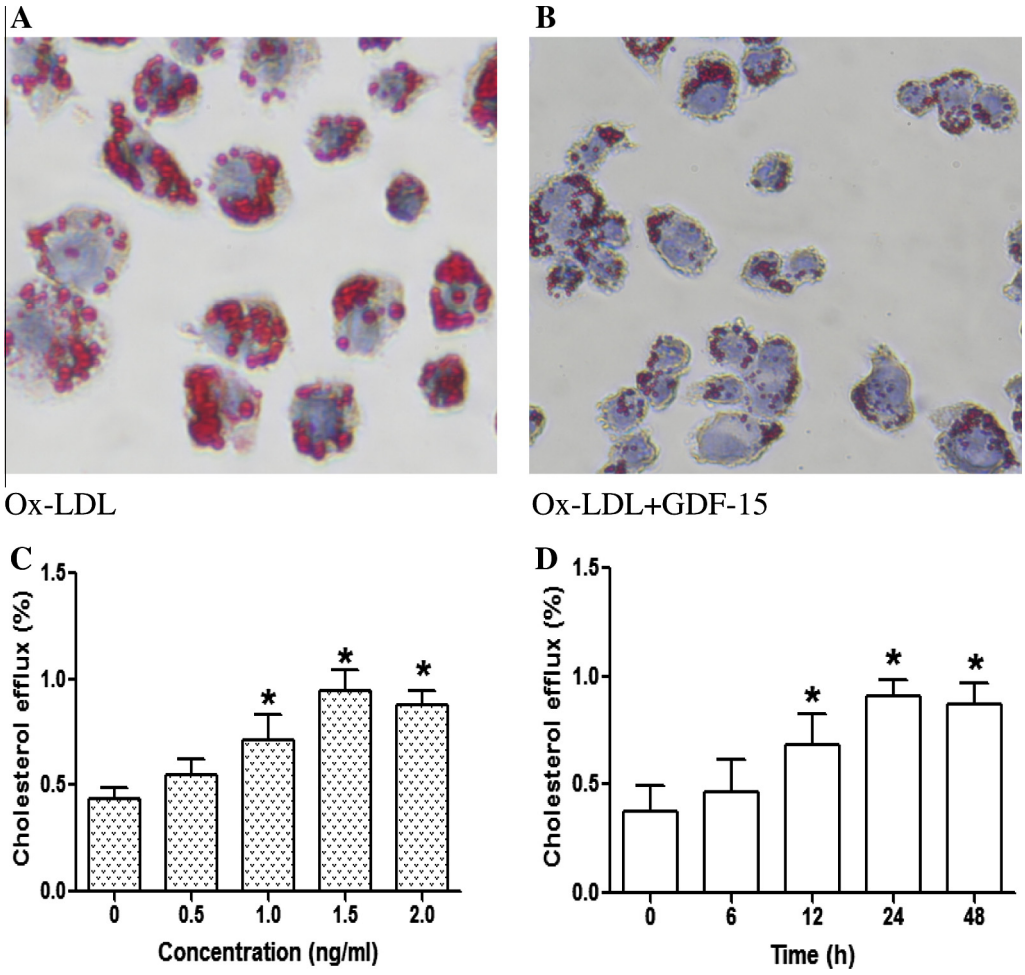

Fig. 1. GDF-15 promotes cholesterol efflux in THP-1 macrophages. (A and B) Effect of GDF-15 on cholesterol efflux in THP-1 cells. THP-1 macrophages incubated with recombinant GDF-15 (1.5 ng/mL) for 24 h were fixed and stained with Oil red O dye. The magnification of each panel is ×400. (C and D) Dose- and time-dependent effects of GDF-15 on cholesterol efflux in THP-1 macrophages. THP-1 macrophages were incubated with indicated concentrations of GDF-15 for 24 h (C) or 1.5 ng/mL (D) of GDF-15 for indicated times. Then cholesterol efflux assay was analyzed by liquid scintillation counting assays as described. Values represent the mean ± SD from three independent experiments. Ox-LDL, oxidized low-density lipoprotein. $^{*}P < 0.05$ vs. control.

significantly lower than that in the control cells, especially when the cells were incubated with 1.5 ng/mL of GDF-15 for 24 h (Table 1). These results suggest that GDF-15 is able to activate the cholesterol efflux system to export excess cholesterol from macrophages.

3.2. GDF-15 induces ABCA1 protein and mRNA expression

Given the important role of ABCA1 in cholesterol efflux from macrophages [17], we next investigated whether GDF-15 altered the levels of ABCA1. THP-1 macrophages were treated with various concentrations of GDF-15 (0–2.0 ng/mL) or with 1.5 ng/mL of GDF-15 for different time points. Quantitative real-time PCR and Western blot were performed to measure ABCA1 mRNA abundance and protein levels, respectively. As shown in Fig. 2, GDF-15 starting from a concentration of 1.0 ng/mL increased the levels of ABCA1 protein (Fig. 2A) and mRNA (Fig. 2C) compared with the untreated cells. ABCA1 protein and mRNA levels were also increased when the cells were incubated with 1.5 ng/mL of GDF-15 for 12 h (Fig. 2B and D). Taken together, these results suggest that GDF-15 activate the ABCA1-dependent cholesterol efflux system to reduce cellular cholesterol levels.

Table 1
Effects of GDF-15 on cholesterol content in THP-1 macrophages.

Time (h)	0	6	12	24	48
TC (mg/g)	520 ± 32	519 ± 28	509 ± 27*	488 ± 30*	475 ± 24*
FC (mg/g)	262 ± 29	176 ± 26	193 ± 30*	200 ± 27*	199 ± 40*
CE (mg/g)	349 ± 38	343 ± 37	316 ± 29*	288 ± 31*	276 ± 33*
CE/TC (%)	0.67	0.66	0.62	0.59	0.58

THP-1 macrophages were divided into 5 groups and cultured in medium at 37 °C containing 1.5 ng/mL GDF-15 for the indicated times. HPLC was then performed to determine the levels of cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). The results are expressed as mean ± SD from three independent experiments, each was performed in triplicate.
* $P < 0.05$ vs. control.

3.3. PI3-K, PKCζ and SP1 are involved in GDF-15-induced up-regulation of ABCA1 expression

Subsequently, we studied the mechanism account for GDF-15-induced increase in ABCA1 levels. The cardioprotective effect of GDF-15 is dependent on PI3K-Akt signaling pathway [11]. Activation of PI3K can increase the expression of ABCA1 [4]. Thus, we investigated if PI3K is involved in GDF-15-induced expression of ABCA1. THP-1 macrophages were treated with LY294002, an inhibitor of PI3K [18], or specific PI3K siRNA to inhibit PI3K signaling pathway. PI3K siRNA reduced PI3K levels (Fig. 3A). PI3K can

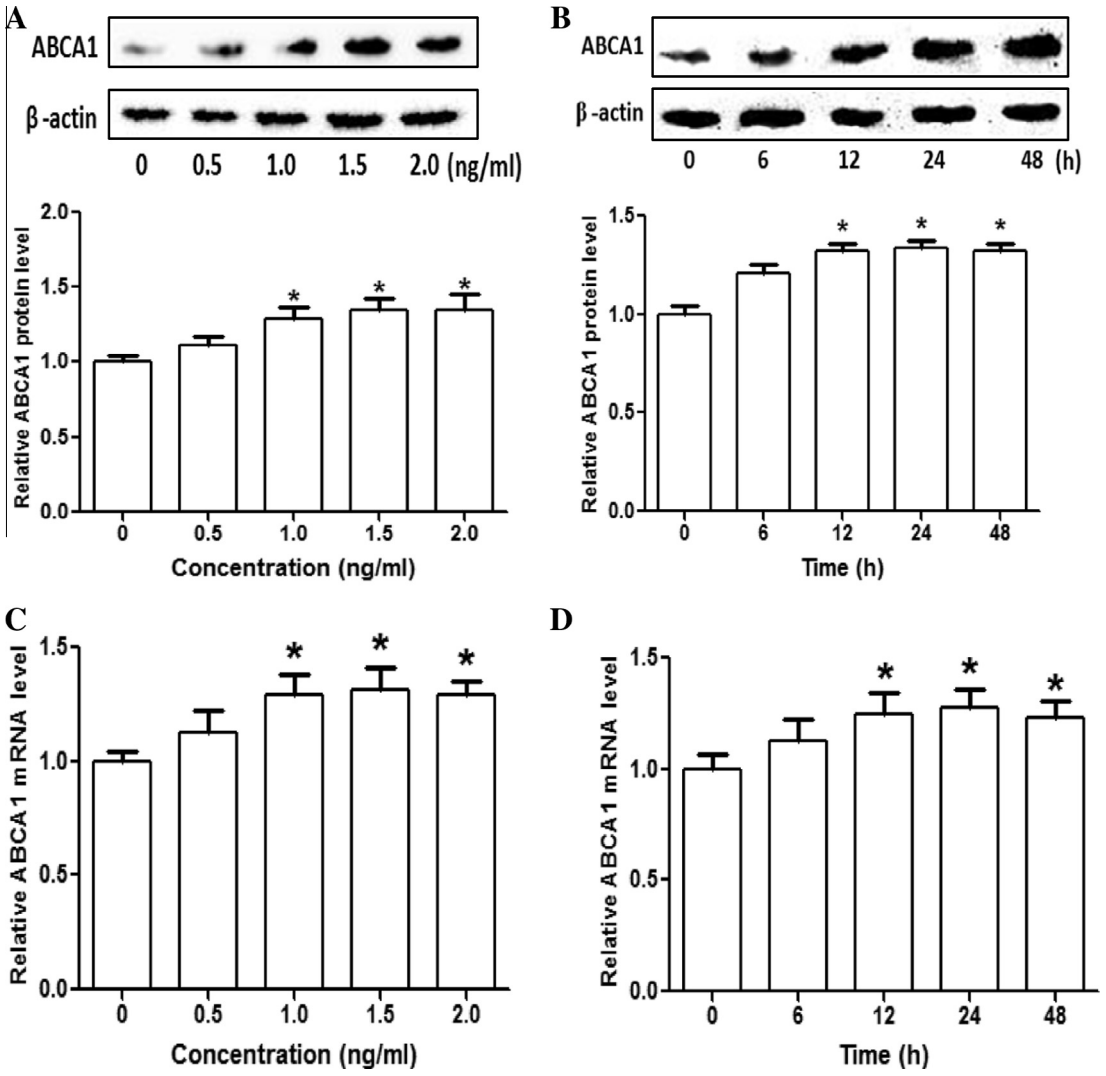

Fig. 2. Effects of GDF-15-induced ABCA1 expression in THP-1 macrophages. Cells were treated with various concentrations of GDF-15 as indicated for 24 h (A and C) or incubated with 1.5 ng/mL GDF-15 for various time periods (B and D). Effects of GDF-15 on ABCA1 protein expression (A and B). Total proteins were extracted. The protein levels of ABCA1 or β-actin were measured by Western blot assays. Effects of GDF-15 on ABCA1 mRNA levels (C and D). Total RNA was extracted and RT-PCR was performed as described. All results are expressed as mean ± SD from three independent experiments, each was performed in triplicate. $^{*}P < 0.05$ vs. control.

mediate PKCζ phosphorylation [19]. We observed that inhibition of PI3K by LY294002 or PI3K siRNA suppressed GDF-15-induced increase in PKCζ phosphorylation and in the levels of ABCA1 protein and mRNA expression (Fig. 3B and C). These results suggest the involvement of PI3K and PKCζ in ABCA1 expression induced by GDF-15.

To further confirm the regulatory roles of PKCζ in GDF-15-induced ABCA1 expression. THP-1 macrophages were treated with 109203X, a chemical inhibitor of PKCζ, and PKCζ siRNA to inhibit PKCζ activity. As shown in Fig. 3D, PKCζ siRNA significantly reduced the level of PKCζ protein. We observed that the enhanced effect of GDF-15 on ABCA1 protein and mRNA expression was abolished by 109203X and PKCζ siRNA (Fig. 3E and F). As expected, inhibition of PKCζ also reduced the phosphorylation of SP1, a downstream target of PKCζ (Fig. 3E).

Next, we investigated the potential role of SP1 in GDF-15-induced ABCA1 expression. We transfected THP-1 macrophages with siRNA specific to SP1 to reduce endogenous SP1 expression. SP1 siRNA significantly decreased SP1 levels (Fig. 3G). Meanwhile, we also treated the cells with mithramycin A, a chemotherapeutic drug that binds to GC-rich DNA sequences and blocks binding of transcription factors such as SP1 to GC-specific regions of DNA. As shown in Fig. 3H, addition of 100 nM mithramycin A or SP1 siRNA treatment abolished GDF-15-induced increase in ABCA1 protein and mRNA levels (Fig. 3H and I). Taken together, these data indicate that GDF-15 appears to induce a sequential activation of PI3K and PKCζ, which phosphorylates SP1 and then increases its binding to the ABCA1 promoter to enhance ABCA1 transcription.

4. Discussion

The development of atherosclerosis is a complex process involving multiple pathways. Many studies have been carried out to investigate pro- or anti-atherogenic factors, but it is usually unclear whether a certain factor has promoting or inhibitory effects on atherosclerosis [20,21]. Here is the case with GDF-15, which is a divergent member of the transforming growth factor-β cytokine superfamily. Previous community-based studies have shown that higher levels of GDF-15 are associated with prevalent cardiovascular disease [22,23]. However, overexpression of GDF-15 in apolipoprotein E deficient (apoE−/−) mice reduces atherosclerosis, which

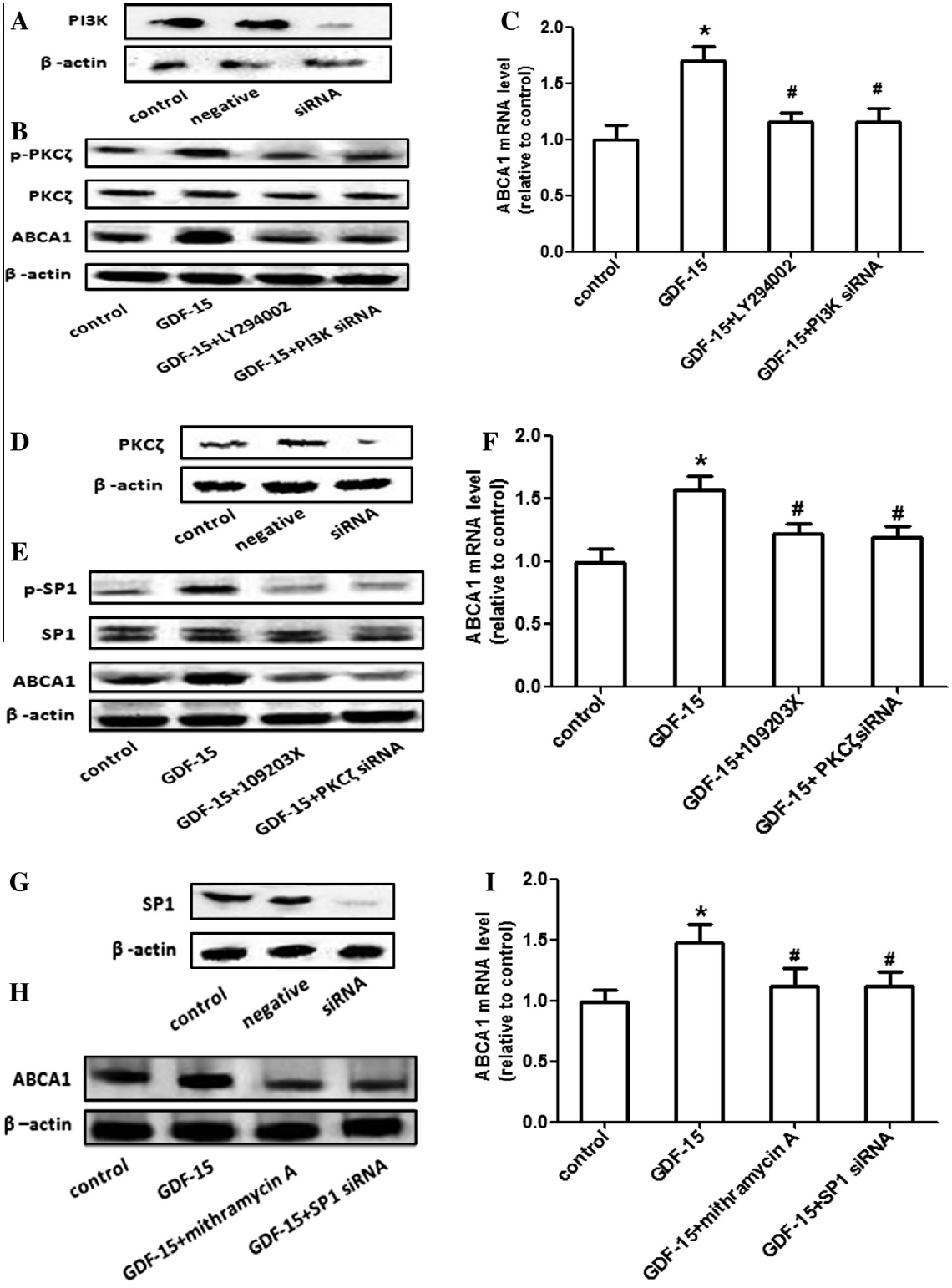

Fig. 3. The roles of PI3-K, PKCζ and SP1 in GDF-15-induced up-regulation of ABCA1 expression in THP-1 macrophages. Cells were treated with siRNA for PI3K, PKCζ or SP1 or control siRNA for 24 h (A, D, G). PI3-K, PKCζ or SP1 protein levels were subjected to SDS–PAGE and Western blot analysis. (B) Cells were treated with LY294002 (50 μmol/L), siRNA of PI3K or culture medium alone for 30 min, followed by treatment with 1.5 ng/mL of GDF-15 for the indicated time periods. ABCA1 and PKCζ (p-PKCζ) protein levels were determined by Western blot analysis. (E) Cells were treated with 8 μmol/L of 109203X or siRNA of PKCζ or culture medium alone for 30 min, and then treated with 1.5 ng/mL of GDF-15 for the indicated time periods. ABCA1 or SP1 (p-SP1) protein levels were determined by Western blot analysis. (H) Cells were treated with mithramycin A (100 nmol/L) or siRNA of Sp1 or culture medium alone for 30 min, and then treated with GDF-15 (1.5 ng/mL) for the indicated time periods. ABCA1 protein levels were determined by Western blot analysis and quantitative real-time RT-PCR and normalized to β-actin. Total ABCA1 mRNA (C, F, I) levels were determined by RT-PCR and normalized to β-actin. Values represent the mean ± SD from three independent experiments, each was performed in triplicate. $^{*}P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. GDF-15 group.

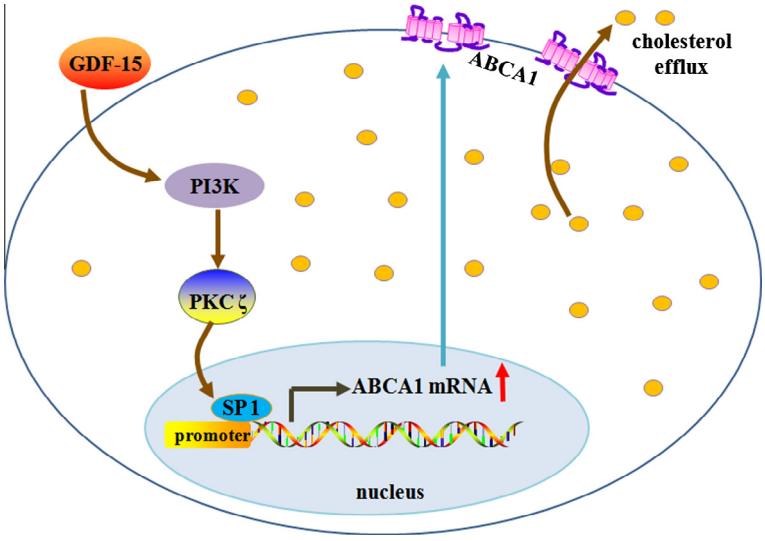

Fig. 4. Model of the mechanism of GDF-15-induced ABCA1 expression and cellular cholesterol efflux. Treatment of THP-1 macrophages with recombinant GDF-15 activates the PI3-K/PKCζ/SP1 signaling cascade, which consequently increases ABCA1 expression and promotes cellular cholesterol efflux. GDF-15, Growth differentiation factor 15; PI3K, phosphoinositide 3-kinase; PKCζ, protein kinase Cζ; SP1, specificity protein 1; ABCA1, ATP-binding cassette transporter A1.

suggests that the increased serum GDF-15 levels observed in atherosclerotic disease may represent an attempt to reduce disease progression [9]. However, the mechanisms underlying this protection have not been identified. Elimination of excess cholesterol from macrophages in the artery vascular wall reduces foam cell formation and protects against the progress of atherosclerosis [24]. ABCA1 mediates the efflux of cellular phospholipids and cholesterol onto lipid-free ApoA-I to form nascent high density lipoprotein (HDL) [25,26], a critical step in RCT that transports cholesterol from peripheral tissues back to the liver for excretion into bile and eventually the feces [24]. Studies have shown that enhancing ABCA1 expression leads to increased cellular cholesterol efflux and reduced lipid accumulation in macrophages, thereby reducing total cholesterol levels and foam cell formation [27,28]. It suggests that regulating expression of ABCA1 could, at least partially, inhibit the progression of atherosclerosis. Our findings clearly indicate that recombinant GDF-15 up-regulates ABCA1 expression, enhances ApoA-I-mediated cholesterol efflux and reduces cellular lipid accumulation in THP-1 macrophage.

The cellular ABCA1 protein levels are regulated at both transcriptional and post-translational levels [29,30]. Liver X receptors (LXRs), cholesterol-sensing nuclear receptors, are key transcriptional regulators of lipid metabolism. LXR activation in macrophages is an emerging therapeutic strategy against the development of atherosclerotic diseases. We have demonstrated that synthetic LXR agonists attenuate coronary heart disease in apoE−/− mouse model of atherosclerosis through increasing ABCA1 protein levels and promoting cholesterol efflux from peritoneal macrophages [31]. In THP-1 macrophage-derived foam cells, TGF-β1 up-regulates the expression of ABCA1 through LXR-α signaling pathway [12]. SP1 also regulates ABCA1 transcription. In apoE−/− mice, enhanced SP1 phosphorylation and the subsequent binding to the ABCA1 promoter increase ABCA1 promoter activity and induce ABCA1 expression [32]. PI3K is a protein kinase that mediates PKCζ phosphorylation [19]. Our data showed that inhibition of PI3K with LY294002 reduced GDF-15-induced PKCζ phosphorylation and ABCA1 expression. Specifically, treatment of THP-1 macrophages with GDF-15 activates PI3K, increases PKCζ phosphorylation and induces physical interaction of PKCζ and SP1. Additionally, transfection of THP-1 macrophages with siRNA of PI3K, PKCζ and SP1 or treatment of the cells with inhibitors, respectively, attenuated GDF-15-induced ABCA1 expression. These findings suggest that the regulatory role of GDF-15 in ABCA1 expression in THP-1 macrophages is at least partially dependent on PI3K/PKCζ/SP1 signaling pathway (Fig. 4).

In summary, results from this report clearly indicate that recombinant GDF-15 can induce ABCA1 expression and cholesterol efflux, and that sequential activation of PI3K, PKCζ and SP1 is responsible for GDF-15-induced ABCA1 expression. These data reveal a novel pathway in regulating ABCA1 expression in macrophage and indicate that GDF-15 might be a potential target to prevent foam cell formation and atherosclerosis progression. However, there are several limitations in our study. Future studies are needed to explore which membrane receptor(s) is involved in GDF-15-mediated signal pathway and whether other proteins such as SR-BI that also mediates cholesterol efflux are the targets of GDF-15.

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References

[1] G.J. Zhao, K. Yin, Y.C. Fu, C.K. Tang, The interaction of ApoA-I and ABCA1 triggers signal transduction pathways to mediate efflux of cellular lipids, Mol. Med. 18 (2012) 149–158.

[2] X. Chen, Y. Zhao, Z. Guo, L. Zhou, E.U. Okoro, H. Yang, Transcriptional regulation of ATP-binding cassette transporter A1 expression by a novel signaling pathway, J. Biol. Chem. 286 (2011) 8917–8923.

[3] E. Thymiakou, V.I. Zannis, D. Kardassis, Physical and functional interactions between liver X receptor/retinoid X receptor and Sp1 modulate the transcriptional induction of the human ATP binding cassette transporter A1 gene by oxysterols and retinoids, Biochemistry 46 (2007) 11473–11483.

[4] X. Chen, Z. Guo, E.U. Okoro, H. Zhang, L. Zhou, X. Lin, A.T. Rollins, H. Yang, Up-regulation of ATP binding cassette transporter A1 expression by very low density lipoprotein receptor and apolipoprotein E receptor 2, J. Biol. Chem. 287 (2012) 3751–3759.

[5] M.R. Bootcov, A.R. Bauskin, S.M. Valenzuela, A.G. Moore, M. Bansal, X.Y. He, H.P. Zhang, M. Donnellan, S. Mahler, K. Pryor, B.J. Walsh, R.C. Nicholson, W.D. Fairlie, S.B. Por, J.M. Robbins, S.N. Breit, MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 11514–11519.

[6] P. Secchiero, F. Corallini, A. Gonelli, R. Dell'Eva, M. Vitale, S. Capitani, A. Albini, G. Zauli, Antiangiogenic activity of the MDM2 antagonist nutlin-3, Circ. Res. 100 (2007) 61–69.

[7] B. Bermudez, S. Lopez, Y.M. Pacheco, J. Villar, F.J. Muriana, J.D. Hoheisel, A. Bauer, R. Abia, Influence of postprandial triglyceride-rich lipoproteins on lipid-mediated gene expression in smooth muscle cells of the human coronary artery, Cardiovasc. Res. 79 (2008) 294–303.

[8] K.C. Wollert, T. Kempf, T. Peter, S. Olofsson, S. James, N. Johnston, B. Lindahl, R. Horn-Wichmann, G. Brabant, M.L. Simoons, P.W. Armstrong, R.M. Califf, H. Drexler, L. Wallentin, Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome, Circulation 115 (2007) 962–971.

[9] H. Johnen, T. Kuffner, D.A. Brown, B.J. Wu, R. Stocker, S.N. Breit, Increased expression of the TGF-b superfamily cytokine MIC-1/GDF15 protects ApoE(−/−) mice from the development of atherosclerosis, Cardiovasc. Pathol. 21 (2012) 499–505.

[10] M.R. Preusch, M. Baeuerle, C. Albrecht, E. Blessing, M. Bischof, H.A. Katus, F. Bea, GDF-15 protects from macrophage accumulation in a mousemodel of advanced atherosclerosis, Eur. J. Med. Res. 18 (2013) 19.

[11] T. Kempf, M. Eden, J. Strelau, M. Naguib, C. Willenbockel, J. Tongers, J. Heineke, D. Kotlarz, J. Xu, J.D. Molkentin, H.W. Niessen, H. Drexler, K.C. Wollert, The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury, Circ. Res. 98 (2006) 351–360.

[12] Y.W. Hu, Q. Wang, X. Ma, X.X. Li, X.H. Liu, J. Xiao, D.F. Liao, J. Xiang, C.K. Tang, TGF-beta1 up-regulates expression of ABCA1, ABCG1 and SR-BI through liver X receptor alpha signaling pathway in THP-1 macrophage-derived foam cells, J. Atheroscler. Thromb. 17 (2010) 493–502.

[13] X.R. Hao, D.L. Cao, Y.W. Hu, X.X. Li, X.H. Liu, J. Xiao, D.F. Liao, J. Xiang, C.K. Tang, IFN-gamma down-regulates ABCA1 expression by inhibiting LXRalpha in a JAK/STAT signaling pathway-dependent manner, Atherosclerosis 203 (2009) 417–428.

[14] C.K. Tang, G.H. Yi, J.H. Yang, L.S. Liu, Z. Wang, C.G. Ruan, Y.Z. Yang, Oxidized LDL upregulated ATP binding cassette transporter-1 in THP-1 macrophages, Acta Pharmacol. Sin. 25 (2004) 581–586.

[15] C.K. Tang, G.H. Tang, G.H. Yi, Z. Wang, L.S. Liu, S. Wan, Z.H. Yuan, X.S. He, J.H. Yang, C.G. Ruan, Y.Z. Yang, Effect of apolipoprotein A-I on ATP binding cassette transporter A1 degradation and cholesterol efflux in THP-1 macrophage-derived foam cells, Acta Biochim. Biophys. Sin. 36 (2004) 218–226.

[16] C.K. Tang, J.H. Yang, G.H. Yi, Z. Wang, L.S. Liu, Z.Y. Wan, Z.H. Yuan, C.G. Ruan, Y.Z. Yang, Effects of oleate on ATP binding cassette transporter A1 expression and cholesterol efflux in THP-1 macrophage-derived foam cells, Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao 35 (2003) 1077–1082.

[17] K. Yin, D.F. Liao, C.K. Tang, ATP-binding membrane cassette transporter A1 (ABCA1): a possible link between inflammation and reverse cholesterol transport, Mol. Med. 16 (2010) 438–449.

[18] M. Guo, A. Joiakim, J.J. Reiners Jr., Suppression of 2,3,7,8-tetrachlorodibenzo-p-dioxin(TCDD)-mediated aryl hydrocarbon receptor transformation and CYP1A1 induction by the phosphatidylinositol 3-kinase inhibitor 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), Biochem. Pharmacol. 60 (2000) 635–642.

[19] N.G. Shenoy, G.J. Gleich, L.L. Thomas, Eosinophil major basic protein stimulates neutrophil superoxide production by a class IA phosphoinositide 3-kinase and protein kinase C-zeta-dependent pathway, J. Immunol. 171 (2003) 3734–3741.

[20] X.H. Yu, H.L. Jiang, W.J. Chen, K. Yin, G.J. Zhao, Z.C. Mo, X.P. Ouyang, Y.C. Lv, Z.S. Jiang, D.W. Zhang, C.K. Tang, Interleukin-18 and interleukin-12 together downregulate ATP-binding cassette transporter A1 expression through the interleukin-18R/nuclear factor-kappaB signaling pathway in THP-1 macrophage-derived foam cells, Circ. J. 76 (2012) 1780–1791.

[21] K. Nakaya, M. Ayaori, H. Uto-Kondo, G.M. Sotherden, T. Nishida, H. Katamoto, Y. Miura, S. Takiguchi, E. Yakushiji, M. Iizuka, M. Ogura, M. Sasaki, M. Yogo, T. Komatsu, T. Adachi, C. Maruyama, K. Ikewaki, Overexpression of stearoyl-coenzyme A desaturase 1 in macrophages promotes reverse cholesterol transport, Biochim. Biophys. Acta 1831 (2013) 1402–1411.

[22] L. Lind, L. Wallentin, T. Kempf, H. Tapken, A. Quint, B. Lindahl, S. Olofsson, P. Venge, A. Larsson, J. Hulthe, A. Elmgren, K.C. Wollert, Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study, Eur. Heart J. 30 (2009) 2346–2353.

[23] A. Rohatgi, P. Patel, S.R. Das, C.R. Ayers, A. Khera, A. Martinez-Rumayor, J.D. Berry, D.K. McGuire, J.A. de Lemos, Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study, Clin. Chem. 58 (2012) 172–182.

[24] X. Zhou, Z. Yin, X. Guo, D.P. Hajjar, J. Han, Inhibition of ERK1/2 and activation of liver X receptor synergistically induce macrophage ABCA1 expression and cholesterol efflux, J. Biol. Chem. 285 (2010) 6316–6326.

[25] D. Duffy, D.J. Rader, Update on strategies to increase HDL quantity and function, Nat. Rev. Cardiol. 6 (2009) 455–463.

[26] Y.R. Su, D.E. Dove, A.S. Major, A.H. Hasty, B. Boone, M.F. Linton, S. Fazio, Reduced ABCA1-mediated cholesterol efflux and accelerated atherosclerosis in apolipoprotein E-deficient mice lacking macrophage-derived ACAT1, Circulation 111 (2005) 2373–2381.

[27] S.G. Chen, J. Xiao, X.H. Liu, M.M. Liu, Z.C. Mo, K. Yin, G.J. Zhao, J. Jiang, L.B. Cui, C.Z. Tan, W.D. Yin, C.K. Tang, Ibrolipim increases ABCA1/G1 expression by the LXRalpha signaling pathway in THP-1 macrophage-derived foam cells, Acta Pharmacol. Sin. 31 (2010) 1343–1349.

[28] J. Jiang, Z.C. Mo, K. Yin, G.J. Zhao, Y.C. Lv, X.P. Ouyang, Z.S. Jiang, Y. Fu, C.K. Tang, Epigallocatechin-3-gallate prevents TNF-alpha-induced NF-kappaB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells, Int. J. Mol. Med. 29 (2012) 946–956.

[29] N. Wang, A.R. Tall, Regulation and mechanisms of ATP-binding cassette transporter A1-mediated cellular cholesterol efflux, Arterioscler., Thromb., Vasc. Biol. 23 (2003) 1178–1184.

[30] G.J. Zhao, S.L. Tang, Y.C. Lv, X.P. Ouyang, P.P. He, F. Yao, Y.Y. Tang, M. Zhang, Y.L. Tang, D.P. Tang, F.S. Cayabyab, G.P. Tian, C.K. Tang, NF-kappaB suppresses the expression of ATP-binding cassette transporter A1/G1 by regulating SREBP-2 and miR-33a in mice, Int. J. Cardiol. 171 (2014) e93–e95.

[31] X.Y. Dai, X. Ou, X.R. Hao, D.L. Cao, Y.L. Tang, Y.W. Hu, X.X. Li, C.K. Tang, The effect of T0901317 on ATP-binding cassette transporter A1 and Niemann–Pick type C1 in apoE−/− mice, J. Cardiovasc. Pharmacol. 51 (2008) 467–475.

[32] Y. Zhao, X. Chen, H. Yang, L. Zhou, E.U. Okoro, Z. Guo, A novel function of apolipoprotein E: upregulation of ATP-binding cassette transporter A1 expression, PLoS One 6 (2011) e21453.