Apolipoprotein CIII regulates lipoprotein-associated phospholipase A2 expression via the MAPK and NFκB pathways

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ABSTRACT

Apolipoprotein CIII (apo CIII), a small glycoprotein that binds to the surfaces of certain lipoproteins, is associated with inflammatory and atherogenic responses in vascular cells. Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been proposed as an inflammatory biomarker and potential therapeutic target for cardiovascular disease (CVD). Here, we report that apo CIII increases Lp-PLA2 mRNA and protein levels in dose- and time-dependent manner in human monocyteic THP-1 cells, and the increase can be abolished by MAPK and NFκB pathway inhibitors. Lp-PLA2 inhibitor, 1-stearoyl glycerol attenuates the inflammation induced by apo CIII. In turn, exogenous Lp-PLA2 expression upregulates apo CIII and the upregulation can be inhibited by 1-stearoyl glycerol in HepG2 cells. Moreover, plasma Lp-PLA2 level is correlated with apo CIII expression in pig liver. In vivo, Lp-PLA2 expression in monocytes and its activity in serum were significantly increased in human apo CIII transgenic porcine models compared with wild-type pigs. Our results suggest that Lp-PLA2 and apo CIII expression level is correlated with each other in vitro and in vivo.

KEY WORDS: Apolipoprotein CIII, Lipoprotein-associated phospholipase A2, MAPK pathway, NFκB pathway, Inflammation

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease that is associated with hypertriglyceridemia, hypercholesterolemia and vascular cell dysfunction. Apolipoprotein CIII (apo CIII), a small glycoprotein, is synthesized by the liver and to a lesser extent by the intestines, is one of the major components of triglyceride-rich lipoproteins (TRL) (Aalto-Setälä et al., 1992). The overexpression of human apo CIII in mice, rabbits and pigs has been shown to reduce lipoprotein lipase (LPL) activity and lead to hypertriglyceridemia, apo CIII in mice, rabbits and pigs has been shown to reduce circulating lipoproteins and catalyzes the hydrolysis of oxidized LDL with a truncated sn-2 acyl chain to release inflammatory products, oxidized fatty acids and lysophosphatidylcholine (LysoPC) (Rosenson and Stafforini, 2012; Stafforini et al., 1987; Tew et al., 1996; Zalewski and Macphee, 2005). These products have been shown to cause pro-inflammatory and pro-apoptotic effects in vitro studies (Matsumoto et al., 2007; Wang et al., 2010). In addition, increased Lp-PLA2 expression has been observed in necrotic cores and in macrophages of vulnerable and ruptured plaques from human and rabbit atherosclerotic lesions (Häkkinen et al., 1999; Lavi et al., 2007). The WOSCOPS (West of Scotland Coronary Prevention Study) was the first report that demonstrated the plasma concentration of Lp-PLA2 has a strong and positive association with the risk of coronary events, even when age, systolic blood pressure, and lipoprotein levels were put into consideration (Packard et al., 2000); these results were confirmed in subsequent investigations (Hatoum et al., 2011; Sabatine et al., 2007). The importance of Lp-PLA2 as an independent biomarker of CVD remains controversial (Mallat et al., 2010; Thompson et al., 2010) due to recent clinical investigations (White et al., 2014) that contradict the positive association reported by the WOSCOPS. However, Darapladib, an Lp-PLA2 inhibitor, decreased the levels of interleukin (IL)-6 and high-sensitivity C-reactive protein (hs-CRP) by 12.3% and 13%, respectively, after the oral administration of 160 mg daily for 12 weeks (Mohler et al., 2008) and significantly halted the necrotic core volume increase compared to a placebo at 12 months (Serruys et al., 2008). Therefore, investigation of the factors that regulate Lp-PLA2 levels is needed.

Here, we investigated the effects of apo CIII on Lp-PLA2 expression. In addition, we previously developed a genetically modified, human apo CIII overexpression porcine model, which has a longer triglyceride absorbance and clearance time than the wild types and exhibits a 2.5-fold and 2.3-fold increase in plasma triglycerides in the postprandial and fasting states, respectively (Wei et al., 2012). In these models, plasma Lp-PLA2 activity and expression are also investigated. These data may benefit to understanding the regulation of Lp-PLA2 and the relationship with apo CIII in vitro and in vivo.

RESULTS AND DISCUSSION

Effects of apo CIII on Lp-PLA2 gene expression

Lp-PLA2, as an independent biomarker and regulator of atherosclerosis, the level may be regulated by lipid associated

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factors. Apo CIII level is closely related with hypertriglyceridemia. To investigate the effect of apo CIII on Lp-PLA2 expression level, we treated human monocytic THP-1 cells with apo CIII. As shown in Fig. 1, the levels of Lp-PLA2 mRNA and protein expression were increased in THP-1 cells incubated with apo CIII in serum-free medium in a dose- and time-dependent manner (Fig. 1A–E). Furthermore, apo CIII-transfected THP-1 cells also exhibited increased Lp-PLA2 expression and activity (Fig. 1F,G). These data suggest that apo CIII can induce Lp-PLA2 expression in vitro.

**Lp-PLA2 involves apo CIII-induced inflammation**

Apo CIII treatment significantly increases TNF-α, IL-6 and MCP-1 release from monocytic THP-1 cells (Fig. 2A–C). In circulation, Lp-PLA2 can act on oxidized LDL and produce oxidized fatty acids and lysophosphatidylcholine (LysoPC) which are proinflammatory factors. To address the question that if Lp-PLA2 mediates apo CIII’s proinflammatory effect, the Lp-PLA2 inhibitor 1-linoleoyl glycerol was used to pretreat the cells before apo CIII treatment. As shown in Fig. 2D–F, the pro-inflammatory effects of apo CIII were attenuated by 1-linoleoyl glycerol, and no inhibitory effect was observed in the cells that lack apo CIII. These results suggest that Lp-PLA2 at least partially mediates the effects of apo CIII on TNF-α, IL-6 and MCP-1 release in monocytic THP-1 cells.

**Apo CIII stimulates Lp-PLA2 expression is regulated by MAPK and NFκB pathways**

The nuclear factor (NF)-κ B and mitogen-activated protein kinase (MAPK)-dependent pathways were closely related to the inflammatory response in cells. Other researcher and our previous report demonstrated that NF-κB and MAPK pathway mediate the regulation of Lp-PLA2 gene expression. Therefore, here we assessed the possibility that both pathways involve in regulating Lp-PLA2 expression by apo CIII in THP-1 cells. Apo CIII stimulated the phosphorylation of p65 NFκB and p42/44 MAPK and upregulated Lp-PLA2 expression (Fig. 3A–D). The MAPK kinase inhibitor PD98059 and NFκB inhibitor PDTC can completely block the increase in Lp-PLA2 mRNA and protein expression induced by apo CIII (Fig. 3D–G). These results indicate that the p65 NFκB and p42/44 MAPK pathways mediate the upregulation of Lp-PLA2 expression induced by apo CIII.
The relationship between apo CIII and Lp-PLA2 expression in vivo and Lp-PLA2 expression and activity in Apo CIII transgenic pigs

Twenty pigs samples of blood and liver tissues were collected to observe the expression of apo CIII and Lp-PLA2, the results show that the mRNA level of apo CIII and Lp-PLA2 are correlated (Fig. 4E). The previously developed apo CIII transgenic pigs specifically overexpress human apo CIII in the liver and intestines (Wei et al., 2012). The model was used to confirm the correlation of apo CIII and Lp-PLA2 expression. When fed a normal chow diet, the apo CIII transgenic pigs exhibited a 4-fold increase in plasma Lp-PLA2 activity and a 10-fold increase of Lp-PLA2 mRNA in macrophage compared to the wild-type controls (Fig. 4A,B). There was no change in the plasma Lp-PLA2 activity in wild-type pigs in the fasting or fed (olive oil) states (Fig. 4C). Interestingly, the Lp-PLA2 activity can be increased in the apo CIII transgenic pigs after ingesting olive oil for 2 h (Fig. 4C). These findings demonstrate that apo CIII may be responsible for the changes in Lp-PLA2 expression at the transcriptional level.

In addition, we observed that exogenous Lp-PLA2 stable expressed in HepG2 cells could increase apo CIII mRNA level in liver could upregulate Lp-PLA2 expression via p65 NFκB and p42/44 MAPK pathways in macrophage in circulation. The increased Lp-PLA2 could stimulate macrophage inflammation independent with lipoproteins and the inflammatory factors in turn stimulate apo CIII expression in liver (Fig. 4F). The detailed mechanisms in the circle need to be further investigated.

MATERIALS AND METHODS

Animals and cells
The apo CIII transgenic pigs were developed using Chinese experimental miniature pig fibroblasts according to a previously described method, and they were fed a chow diet (Wei et al., 2012). For the experiments, wild-type and apo CIII transgenic pigs were administered olive oil orally (fat 23%) for fat load at 10 ml/kg body weight after a 16-h fast, and blood was collected for analysis. Human mononcytic THP-1 cells were donated by Dr. Yang’s lab (Jilin University) and maintained in RPMI-1640 medium with 10% fetal bovine serum (PAA, Austria). Pig monocytes were isolated from apo CIII transgenic or wild-type pigs using Histopaque-1077 (Sigma-Aldrich, USA). All of the animal experiments were conducted according to Jilin University Animal Care and Use Committee protocol no. 2008-11.

Reagents
Apolipoprotein C-III was purchased from Sigma-Aldrich. The antibodies p42/44, p-p42/44, p65 and p-p65 were purchased from Cell Signaling.
schematic model of apolipoprotein CIII regulates Lp-PLA2 expression.

obtained from Cayman. Human MCP-1, TNF- and PDTC were purchased from Beyotime, and 1-linoleoyl glycerol was obtained from Boster.

The protein concentrations were determined using the Enhanced BCA (Beyotime, China) containing 1 mM PMSF and protein phosphatase for the expression analysis, the cells were lysed in cell lysis buffer (Beyotime, China) containing 1 mM PMSF and protein phosphatase inhibitor (Applyingen, China) for 30 min on ice and were then centrifuged. The protein concentrations were determined using the Enhanced BCA protein assay kit (Beyotime, China). Equal amounts of protein were used for 12% SDS-PAGE and then transferred to nitrocellulose membranes. Immunoblots were performed with the indicated primary antibodies and the corresponding secondary antibodies. The signal was detected using BeyoECL Plus (Beyotime, China).

Lp-PLA2 activity

Blood was collected from pigs using anticoagulant EDTA tubes and centrifuged at 1000 g for 10 min. The plasma was analyzed using a PAF Acetylhydrolase kit (Cayman, USA) according to the manufacturer’s instructions.

Quantitative real-time PCR

Total RNAs from the pig monocytes, THP-1 cells, HepG2 cells and liver tissues were extracted using the TRNzolA+ reagent according to the manufacturer’s instructions (Tiangen, China), and 1 µg of RNA was used for reverse transcription (Invitrogen, China). The obtained cDNA was used for real-time PCR using the BioEasy SYBR Green I kit (Bioer, China) in an qRT system (Bio-Rad, USA) according to the manufacturer’s recommendations. The expression of β-actin mRNA was used as an internal control to normalize target gene expression. Pig Lp-PLA2, primers, forward: 5'-CACTGAACGTGCACTTACTAC'-3', reverse: 5'-TACTCTGCTCTGTGGTAG-3'; Human Lp-PLA2: primers, forward: 5'-TTAGTGTCCCTGACACACC-3', reverse: 5'-TACAGCAGGACTTACCATACACC-3'. Human apo CIII primers, forward: 5'-GCCACCAAGACGCACAAGGAT-3', reverse: 5'-GCCGGACACCCAGAGTGCA-3'. Pig apo CIII primers, forward: 5'-AACCAAGCTGAAGGAGCTCAG-3', reverse: 5'-GTGAACTTGGCCTGAACGTCG-3'.

Quantification of proinflammatory cytokines

The levels of proinflammatory cytokines which stimulated by apo CIII and released in the culture media were evaluated by Human IL-6 ELISA Kit, Human TNFα ELISA Kit and Human MCP-1 ELISA Kit (Boster Inc., China), following the manufacturer’s instructions.

Statistical analyses

The data are expressed as the mean±SEM and were analyzed using a two-tailed unpaired t test in the GraphPad Prism software. P<0.05 was considered to be statistically significant.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

X.C.T. and H.S.O.Y. designed the experiments. X.L.H., T.D.W., X.X.L., Z.L., and G.Q.W. and Q.S. performed the experiments. J.F.Z. and D.X.P. analysed all data. We thank Xuming Deng at Jilin University, Yuhui Wang and George Liu at the Peking University Health Science Center for their collaborative development of the apo CIII transgenic pigs and discussion for the paper.

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