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MAPK–PPARα/γ signal transduction pathways are involved in *Chlamydia pneumoniae*-induced macrophage-derived foam cell formation

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ABSTRACT

Chlamydia pneumoniae (*C. pneumoniae*) is now widely accepted as an independent risk of atherosclerosis development. In this paper, our results showed that *C. pneumoniae* infection significantly increased the number of foam cells in LDL-treated THP-1 macrophages. C-Jun NH2 terminal kinase (JNK1/2) inhibitor SP600125 and extracellular signal-regulated kinase (ERK1/2) inhibitor PD98059 strongly inhibited *C. pneumoniae*-induced accumulation of lipid droplet, whereas p38 inhibitor SB203580 had no obvious effect on lipid accumulation. Furthermore, we found that *C. pneumoniae* not only stimulated the phosphorylation of Mitogen-activated protein kinase (MAPK) including JNK1/2, ERK1/2 and p38 but also down-regulated the expression of peroxisome proliferator-activated receptors (PPARγ and PPARα) at mRNA and protein levels. However, the phosphorylation of JNK1/2, ERK1/2 and p38 MAPK by *C. pneumoniae* was substantially reversed after PPARγ agonist (rosiglitazone) or PPARα agonist (fenofibrate) treatment while PPARγ inhibitor (GW9662) and PPARα antagonist (MK886) enhanced *C. pneumoniae*-induced phosphorylation of JNK1/2, ERK1/2 and p38. In addition, we demonstrated that *C. pneumoniae*-induced PPARγ and PPARα down-regulation were significantly suppressed by JNK1/2 inhibitor (SP600125) and ERK1/2 inhibitor (PD98059), but not p38 inhibitor (SB203580). These results first declare that MAPK–PPARα/γ reciprocal signal pathways are involved in *C. pneumoniae*, which induces foam cell formation, thus facilitating atherogenesis.

1. Introduction

In recent decades, there is extensive evidence indicating that atherosclerosis is a chronic inflammatory disorder and *Chlamydia pneumoniae* (*C. pneumoniae*) plays a key role in the development of atherosclerosis and coronary artery disease [1]. *C. pneumoniae* is an obligate intracellular bacterial pathogen that has a biphasic elementary body and reticulate body life cycle and causes pneumonia, bronchitis and other respiratory tract disease as a widespread respiratory pathogen [2]. Saikku et al. [3] first mentioned the role for *C. pneumoniae* in the pathogenesis of atherosclerosis in 1988. Subsequently large amounts of epidemiological and clinical studies suggested that *C. pneumoniae* infection aggravated the progression of atherosclerotic lesions [4]. *C. pneumoniae* is able to disseminate via the circulation throughout the body within monocytes/macrophages and promote inflammatory atherogenous process [5]. Furthermore, in vitro study suggested that *C. pneumoniae* induced macrophage foam cell formation when cultured in the presence of low density lipoprotein (LDL), implicating that this organism is intimately linked with atherosclerosis progression [6]. However, the underlying mechanisms and pathways involved in *C. pneumoniae* induced foam cell formation have not yet been fully understood.

Peroxisome proliferator-activated receptors (PPARs) are a ligand-activated nuclear hormone receptor superfamily existing in

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three isoforms: PPARα, PPARβ/δ and PPARγ. As members of transcription factors, PPARγ and PPARα can regulate key genes involved in all stages of atherosclerosis, such as monocyte recruitments, macrophage activation, foam cell formation, vascular inflammation and thrombosis [7]. Recent studies revealed that activated PPARγ and PPARα significantly suppressed the development of atherosclerosis in LDL receptor-deficient mice [8,9]. In addition, our earlier study has demonstrated that ligand-activated PPARγ inhibited the expression of Acylcoenzyme A: cholesterol acyltransferase 1 (ACAT1) in macrophages, which is a key and exclusive microsomal enzyme that esterifies free cholesterol with fatty acids to form cholesteryl ester [10]. Subsequently, we further confirmed the involvement of PPARγ dependent pathway in the foam cell formation induced by *C. pneumoniae*, while *C. pneumoniae* up-regulated ACAT1 mRNA and protein levels via PPARγ dependent pathway [11,12]. These studies indicate that PPARγ expression by macrophages has antiatherogenic effects via modulation of cell cholesterol trafficking.

Mitogen-activated protein kinase (MAPK) is a key component of the signaling pathways that transduct extracellular stimuli into cells and regulate expression of numerous genes [13]. It was reported that *C. pneumoniae* infection induced activation of MAPK [14,15]. Kitazawa et al. have demonstrated that chlamydophilal antigens-induced macrophage foam cell formation was mediated by JNK [16]. Moreover, our previous works have demonstrated that *C. pneumoniae* induced disequilibrium of intracellular cholesterol homeostasis by up-regulating ACAT1 expression and down-regulating ATP binding cassette transporters (ABCA1/G1) expression via JNK-PPARγ signal transduction pathway [17].

Although a number of studies indicated the vital roles of MAPK and PPARs in the modulation of foam cell formation, the effect of MAPK—PPARα/γ crosstalk on *C. pneumoniae*-induced foam cell formation has not been fully investigated. Therefore, we examined the role of MAPK—PPARα/γ crosstalk in THP-1 macrophage-derived foam cell formation induced by *C. pneumoniae* infection.

2. Materials and methods

2.1. Ethics statement

This experiment involving fresh plasma was approved by normolipidemic volunteers and Wuhan Blood Centre (authorizations: 2010-8) and conformed to the Declaration of Helsinki.

2.2. Cell lines and other reagents

Human monocytic THP-1 cells were purchased from American Type Culture Collection (ATCC, Rockville, MA, USA). Epithelial HEp-2 cells were obtained from Wuhan University, China. *C. pneumoniae* strain AR-39 was purchased from ATCC. RPMI1640 powder and fetal bovine serum (FBS) were purchased from Hyclone (Thermo Scientific, Waltham, MA, USA). Fluorescein isothiocyanate (FITC)-conjugated specific anti-chlamydial monoclonal antibody was obtained from Dako (Copenhagen, Denmark). SB203580, PD98059, SP60125, fenofibrate, rosiglitazone, MK886, GW9662, Phorbol 12-myristate 13-acetate (PMA) and cycloheximide were from Sigma (St. Louis, MO, USA). PrimeScript RT Reagent Kit and qRT-PCR using SYBR® Premix Ex Taq™ were from TAKARA Bio INC. PPARα antibody was from Abcam (Cambridge, UK). The following protein antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA): phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), phospho-SAPK/JNK (Thr183/Tyr185), Phospho-p38 MAPK (Thr180/Tyr182), p44/42 MAPK (Erk1/2), SAPK/JNK, p38 MAPK and PPARγ.

2.3. Low density lipoprotein (LDL) isolation and assessment

Amounts of plasma LDL ($d = 1.006–1.063$ g/ml) was isolated by density-gradient ultracentrifugation as described [18]. The isolated LDL was dialyzed in phosphate-buffered saline (PBS) for 24 h (h), and then was concentrated with polyoxyl 20,000. LDL was stored for no longer than 2 weeks in the dark at 4 °C before use.

2.4. Propagation of C. pneumoniae and purification

C. pneumoniae strain AR39 was propagated in HEp-2 cells by centrifugation-driven infection at 750 × g and 25 °C for 1 h followed by incubation at 37 °C and 5% CO_2 for 1 h as described [19]. Fresh RPMI1640 medium containing 2 mg/ml cycloheximide and 10% heat-inactivated FBS was added. Infected HEp-2 cells were harvested after 72 h incubation, and disrupted by freezing, thawing and ultrasonication. After centrifugation at 500 × g and 4 °C for 30 min (min) in order to remove cell debris, the supernatants containing elementary bodies were harvested and stored at 70 °C. Chlamydial inclusion forming units (IFU) were determined by counting chlamydial inclusions formed in HEp-2 cells with FITC-conjugated specific anti-chlamydial monoclonal antibody.

2.5. Cell culture and infection of THP-1-derived macrophages

THP-1 cells were cultured in RPMI1640 medium containing 10% FBS at 37 °C in 5% CO_2 and differentiated into macrophage by 160 nmol/l PMA for 48 h. THP-1-derived macrophages were pretreated with 50 μM fenofibrate, 20 μM rosiglitazone, 20 μM MK886, 20 μM GW9662 for 2 h or 20 μM SP600125, 50 μM PD98059, 10 μM SB203580 for 1 h. Then macrophages were infected or uninfected with *C. pneumoniae* (1×10^6 IFU) in the presence of LDL (50 mg/l).

2.6. Oil red O staining

Cells were counterstained with hematoxylin and oil red O. The intracellular lipid droplets were stained red, and cell nuclei were stained blue. Cells with a lipid droplet area no less than the width of the nucleus were designated oil red O positive. Foam cells of morphological criterion were defined as cells that have ten Oil red O-positive lipid droplets or more (≥10) under a microscope [20].

2.7. Real-time quantitative RT-PCR

Total cellular RNA was isolated from treated cells using Trizol (TAKARA Bio INC). Real-time quantitative RT-PCR assay was performed using the two-step method following the manufacturer's procedure. Transcript levels of selected genes were measured by the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA). The gene specific primers and probes were purchased from Invitrogen life technologies (Shanghai, China). β-actin was used as an endogenous control. The sequences of the PCR primers were as follows:

PPARα	5′-GCCACCGATTTCATACAACA-3′ (forward) 5′-TCTGGGATCTGCCTCATTCT-3′ (reverse)
PPARγ	5′-CGTGGCCGCAGATTTGAA-3′ (forward) 5′-CTTCCATTACGGAGAGATCCAC-3′ (reverse)
β-actin	5′-GTCCACCTTCCAGCAGATGT-3′ (forward) 5′-CACCTTCACCGTTCCAGTTT-3′ (reverse)