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Short communication

ABC-cassette transporter 1 (ABCA1) expression in epithelial cells in *Chlamydia pneumoniae* infection

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ABSTRACT

ATP-binding cassette transporter A1 (ABCA1) mediates reverse cholesterol transport and innate immunity response in different cell types. We have investigated the regulation of ABCA1 expression in response to intracellular *Chlamydia pneumoniae* infection in A549 epithelial lung carcinoma cells. *C. pneumoniae* infection decreased ABCA1 expression in A549 cells, and the activity of the ABCA1 promoter was decreased. The decreased promoter activity was dependent on its E-box and GnT-box elements of the promoter. Chlamydial growth was decreased in ABCA1-silenced epithelial lung carcinoma cells. These data indicate an important role for ABCA1 in intracellular bacterial infection.

1. Introduction

ATP-binding cassette transporter A1 (ABCA1) is abundant protein in macrophages [1]. It exports cellular free cholesterol and phospholipids from the Golgi apparatus to apolipoprotein A-I (apoA-I) on plasma membrane, resulting in formation of high-density lipoprotein (HDL) particles. The transcription of ABCA1 is induced by oxysterols and 9-cis-retinoic acid (RA) through liver and retinoic X receptors (LXR and RXR). In addition, the expression of ABCA1 is responsive to many cytokines and microbial stimuli [1]. In macrophages, ABCA1 deficiency results in foam cell formation, an early hallmark of the development of atherosclerotic lesions. In addition to the function on lipid transport, ABCA1 has also an important anti-inflammatory function. ABCA1-deficient mice secrete high concentrations of pro-inflammatory cytokines, including TNF-α, interleukin-1β, IL-6, and IFN-γ in lipopolysaccharide (LPS) induced sepsis [2], and the loss of ABCA1 function in macrophages may lead to low-grade chronic inflammation in vivo [3]. The anti-inflammatory function of ABCA1 seems to be dependent on its interaction with apoA-I and thus to its function in lipid efflux [4].

While the function of ABCA1 in the lipid efflux is well documented in macrophages, it seems to have a less well characterized but equally important role in lung epithelium. McNeish and co-workers observed that alveolar type II epithelial cells of ABCA1−/− knockout mice accumulate lipids and aberrant lamellar bodies [5]. Experimental data suggest that ABCA1 mediates basolateral surfactant efflux in these cells [6].

Chlamydia pneumoniae, a Gram-negative obligate intracellular bacterium, is a common cause of community-acquired pneumonia [7]. Like all *Chlamydia* species, it replicates only inside the host within a membrane-bound intracellular vesicle, termed inclusion [7]. *C. pneumoniae* infection has a tendency to persist, and the persistent infection has been suggested to contribute to chronic inflammatory states, such as atherosclerosis (for a review see Ref. [8]).

C. pneumoniae infection disrupts the intracellular transport of surfactant lipids in type II pneumocytes [9]. Taking into account the central role of ABCA1 in surfactant metabolism, we investigated ABCA1 expression in *C. pneumoniae*-infected A549 epithelial lung carcinoma cells. In addition, we studied the activity of ABCA1 promoter in the infected cells. Finally, we examined chlamydial growth in ABCA1-silenced epithelial lung carcinoma cells.

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2. Materials and methods

2.1. Cell culture

A549 epithelial lung carcinoma cells (CCL-185) cells were obtained from American Type Culture Collection (LGC Standards AB, Sweden) and maintained as in Ref. [10].

2.2. Preparation of C. pneumoniae elementary bodies

C. pneumoniae elementary bodies (EBs) were prepared as previously described [10]. In some instances, *C. pneumoniae* was heat-inactivated by incubating at 56 °C for 30 min, as indicated in results.

2.3. Antibodies and reagents

The following primary antibodies were used for immunoblotting and microscopy: monoclonal mouse anti-ABCA1 (Abcam), rabbit polyclonal antibody against *C. pneumoniae* IncA [11], monoclonal anti-β-actin-peroxidase (Sigma–Aldrich), and goat anti-mouse IgG HRP-conjugated (SantaCruz Biotechnology). Fluorophore-conjugated secondary antibodies (Invitrogen) were used for confocal imaging. 9-cis-retinoic acid (RA), 22-hydroxy-R-cholesterol (22-OH), and GW3965 were purchased from Sigma.

2.4. Plasmids

A fragment of the human ABCA1 promoter (from −928 to +101 bp) linked to firefly luciferase reporter [13], and its versions with the DR4 element, E-box, or GnT-box mutagenized have been previously described [14].

2.5. ABCA1 protein expression analysis

A549 cells were plated on 24-well plates in culture media a day before inoculation. The cells were inoculated with viable or heat-inactivated *C. pneumoniae* K6 at a multiplication of infection (MOI) of 0.01. The cells were then centrifuged at 900 g at 37 °C for 1 h, and incubated in culture media at 37 °C for 1 h. The cells were washed to remove any unbound *Chlamydia* particles, and incubated in culture media with or without 1 μM RA and 1 μM 22-OH or 1 μM GW3965 at 37 °C for different time periods, as indicated in results. Subsequently, the cells were harvested and immunoblotted as in Ref. [10].

2.6. Quantitative RT-PCR

For ABCA1 mRNA detection total RNA was isolated with RNAeasy Kit (Qiagen) according to the manufacturer's protocol. Universal ProbeLibrary (Roche) probe No. 11 was added to a final concentration of 1 μM. The primers (5′-TCAGGATCAGGAAGATAAATAGAGG and 5′-ACCTCACTTTCAGAAGAAGACAAAC) were added to a final concentration of 3 μM. RT-PCR amplifications were performed as previously described [10]. The fold change (FD) was estimated from the threshold cycle by $\Delta\Delta C_T$ method [12], and the ABCA1 transcript levels were normalized against those of hypoxanthine-guanine phosphoribosyltransferase-2.

2.7. Luciferase reporter assay

A549 cells, plated on 24-well plates, were inoculated with viable or heat-inactivated *C. pneumoniae*. As a control some cells were not inoculated. Then, the cells were centrifuged at 900 g at 37 °C for 1 h, and incubated at 37 °C for 24 h, followed by co-transfection with luciferase reporter constructs and Renilla internal control plasmids (Promega) using Lipofectamine (Invitrogen) as in Ref. [15]. Transfected cells were incubated with 1 μM GW3965 at 37 °C for 24 h, after which the cells were harvested. Luminescence-measurements were carried out with Dual Luciferase Assay Reporter System according to the manufacturer's protocol (Promega).

2.8. RNA interference assays

The following siRNA duplex with dTdT-overhangs were synthesized (Sigma) to target ABCA1 transcripts: (5′-CUGUAUGGGUGGGUCAAUCA and 5′-UGAUUGACCACCCAUACAG). A non-targeting siRNA duplex (5′-CCUACAUCCCGAUCGAUGAUG and 5′-CAUCAUCGAUCGGGAUGUAGG) was used as a negative control. The cells were transfected with siRNA duplexes as in Ref. [10]. Then, the cells were incubated with 1 μM GW3965 for 24 h to induce ABCA1 expression.

2.9. C. pneumoniae growth assay

ABCA1-silenced cells were inoculated with *C. pneumoniae* K6 at a MOI of 0.01. Then the cells were centrifuged at 900 g at 37 °C for 1 h, and incubated at 37 °C for 1 h. After washing, the cells were incubated in culture media supplemented with 0.5 μg ml^{-1} cycloheximide at 37 °C for 48 h. The cells were fixed, stained, and examined as in Ref. [10].

2.10. Confocal microscopy

To examine localization of ABCA1 in *C. pneumoniae*-infected cells, the cells were fixed, stained with monoclonal ABCA1 antibody, and examined as in Ref. [10] with LSM510 Meta Confocal Microscope (Zeiss).

3. Results

3.1. The expression of ABCA1 is decreased in A549 epithelial lung carcinoma cells infected with C. pneumoniae

To study ABCA1 expression in *C. pneumoniae* infection, A549 cells were infected with or without the RXR and LXR activators, RA and 22-OH. The ABCA1 expression was analyzed at 48 h post infection (hpi). The basal expression of ABCA1 was low without RA and 22-OH (Fig. 1). In the infected cells, the expression of ABCA1 mRNA was significantly decreased compared with non-infected cells (Fig. 1A). In agreement with this, the expression of ABCA1 protein was decreased in the infected cells (Fig. 1B).

3.2. The activity of ABCA1 promoter is suppressed upon C. pneumoniae infection, and the effect is mediated by E-box and GnT-box elements

To investigate the regulation of ABCA1 expression at a promoter level, A549 cells were inoculated with viable *C. pneumoniae*, heat-inactivated *C. pneumoniae*, or mock-inoculated (control). The heat-inactivated bacteria were not able to grow inside the host (data not shown). Then, the cells were transfected with luciferase reporter plasmids linked to human wild-type ABCA1 promoter or the ABCA1 promoter encompassing mutated DR4 element, E-box, or GnT-box. The activity of wild-type ABCA1 promoter was decreased to 62% and 66% in the cells that were inoculated with viable or heat-inactivated bacteria, respectively, compared with the control (Fig. 2A). As expected, the activity of DR4-mutated promoter was low irrespective of inoculation status. The activities of the E-box or GnT-box mutants were comparable between the cells inoculated with heat-inactivated bacteria and control (Fig. 2A), suggesting that inhibition of the promoter activity by