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AMPK activates LXRα and ABCA1 expression in human macrophages

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ABSTRACT

ATP-binding cassette transporter A1 (ABCA1) is a key modulator of macrophage cholesterol homeostasis. We studied the impact of AMP-activated protein kinase (AMPK) on ABCA1 expression in primary human and THP-1 macrophages. Pharmacological or genetic activation of AMPK increased mRNA and protein expression of ABCA1 and its transcriptional activator liver X receptor (LXR) α, resulting in increased cholesterol efflux to apolipoprotein AI-containing medium. On the other side, an AMPK knockdown decreased ABCA1 and LXRα mRNA and protein. Silencing LXRα, but not LXRβ, attenuated ABCA1 expression after AMPK activation, and luciferase reporter as well as chromatin immunoprecipitation analyses showed the binding of LXRα to the LXR responsive element in the ABCA1 promoter. Inhibition of extracellular-signal regulated kinase and mechanistic target of rapamycin signalling increased ABCA1 expression, at the same time making it unresponsive to AMPK activation. Considering other potential regulators of ABCA1 expression, we excluded histone deacetylase HDAC9 and FOXO3 involvement in mediating AMPK effects on ABCA1. Our data link AMPK activation to an increased cholesterol efflux capacity of macrophages, suggesting an atheroprotective effect of macrophage AMPK.

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1. Introduction

Atherosclerosis, a major cause of mortality in the Western world, is propagated by disturbed cholesterol metabolism and inflammation. Atherosclerosis progression is accompanied by recruitment of monocytes and their differentiation to macrophages, which take up lipoproteins to become foam cells (Moore and Tabas, 2011). Foam cell formation is governed by balancing rates of lipid uptake and efflux. The latter is mainly controlled by transporters belonging to a superfamily of ATP-binding cassette (ABC) transporters. ABCA1, an integral membrane protein mediating cholesterol and phospholipid efflux to lipid-poor apolipoprotein AI (apoAI), is a key determinant of the cellular cholesterol efflux capacity (Westerterp et al., 2014).

Molecular details of ABCA1 mRNA expression are described to involve transcriptional activation by peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs). PPARs and LXRs are transcription factors, which regulate inflammation and lipid metabolism (Nagy et al., 2012). Early studies in human macrophages showed expression regulation of ABCA1 by PPARα and PPARγ (Chinetti et al., 2001), and that PPARγ increases ABCA1 expression and cholesterol efflux via LXRα activation (Chawla et al., 2001). There are two isoforms of LXRs, LXRα (NR1H3 gene) and LXRβ (NR1H2 gene), which can be activated by oxidized derivatives of cholesterol (Calkin and Tontonoz, 2012). LXRs regulate cholesterol homeostasis by activating genes that govern transport and catabolism of cholesterol through binding to LXR response elements (LXREs) within their promoters. Activation of LXRs reduces atherosclerosis in mice by promoting cholesterol efflux via ABCA1, particularly in macrophages (Joseph et al., 2002; Levin et al., 2005). LXR transcriptional activity is regulated by ligand availability (Spann et al., 2012) or by post-translational modifications, such as phosphorylation (Chen et al., 2006; Torra et al., 2008), acetylation (Li et al., 2007), or SUMOylation (Ghisletti et al., 2007). Human LXRα, but not LXRβ, also activates its own expression due to the

Abbreviations: ABC, ATP-binding cassette; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; ATF1, activating transcription factor-1; ChIP, chromatin immunoprecipitation; CV, control virus; ERK, extracellular-signal regulated kinase; HDAC, histone deacetylase; KD, knockdown; LXR, liver X receptor; LXRE, LXR response element; mTOR, mechanistic target of rapamycin; NCOR1, nuclear receptor corepressor 1; PPAR, peroxisome proliferator-activated receptor; PTM, post-translational modification.

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presence of a LXRE in the human, but not murine, LXRα promoter (Laffitte et al., 2001; Whitney et al., 2001).

AMP-activated protein kinase (AMPK) is a key cellular energy sensor (Hardie et al., 2012). It is a heterotrimetic complex consisting of α, β, and γ subunits, which can be allosterically activated by AMP binding to the γ subunit, underlying AMPK activation by metabolic stress. Binding of AMP facilitates phosphorylation of AMPK at a conserved Thr residue (Thr172 in the rat sequence) by upstream kinases, which greatly increases AMPK activity. AMPK activates ATP generating processes, such as fatty acid oxidation and diminishes ATP-consuming processes like fatty acid and protein synthesis thus, regulating energy homeostasis (Hardie et al., 2012). Several synthetic AMPK activators are known. Most of them, like the widely used antidiabetic drug metformin, change the AMP/ATP ratio (Hawley et al., 2010). In contrast, A-769662 (A-769) has been shown to activate AMPK directly by an allosteric mechanism involving the β1– subunit (Göransson et al., 2007; Sanders et al., 2007).

AMPK is anti-inflammatory in cells of the innate and adaptive immune systems through several independent mechanisms (O'Neill et al., 2013). AMPK is also reported to be atheroprotective. Mechanisms may involve reduced endoplasmic reticulum stress in the endothelium (Dong et al., 2010), or inducing uncoupling protein 2 expression (Wang et al., 2011). Recently, AMPK has been linked to cholesterol metabolism in immune cells. In human macrophages, AMPK influences cholesterol efflux through phosphorylation and activation of the activating transcription factor-1 (ATF1), which in turn induced a LXRβ-LXRα regulatory cascade, culminating in activation of ABCA1 expression and increased cholesterol efflux (Wan et al., 2013). Salicylate and A-76962 increased LXRα and ABCA1 expression and promoted cholesterol efflux in murine bone marrow-derived macrophages from wild type, but not from AMPKβ1-deficient mice (Fullerton et al., 2015). Furthermore, AMPK activation in mouse macrophages stabilized the mRNA of the cholesterol transporter ABCG1 in a LXR-independent manner (Li et al., 2010). However, the exact mechanisms governing expression regulation of LXRα/ABCA1 by AMPK is still incompletely resolved.

Here we used the human THP-1 cell line and primary human macrophages to investigate mechanistic details how AMPK affects ABCA1 expression and activity. We show that AMPK activation stimulates LXRα, but not LXRβ signalling. AMPK-dependent ABCA1 expression also involves inhibition of extracellular-signal regulated kinase (ERK) and mechanistic target of rapamycin (mTOR) pathways. As a consequence, ABCA1 expression by AMPK causes cholesterol efflux from human macrophages.

2. Materials and methods

2.1. Cell culture and treatments

THP-1 human monocytic cells (ATCC) were maintained in RPMI 1640 medium (GE Healthcare) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin at 37 °C, 5% CO2. Differentiation of THP-1 cells into macrophages was induced by treatment with phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) at a final concentration of 100 nM for 48 h. All subsequent treatments were done in RPMI 1640 medium without PMA. Hepatoma cell line HepG2 was cultured in MEM medium (Sigma-Aldrich) containing 10% FCS, 2 mM L-glutamine, 1x non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C, 5% CO2. Human peripheral blood monocytes were isolated from buffy coats (DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt, Germany) using Ficoll gradient (LSM 1077, GE Healthcare) centrifugation according to the manufacturer's protocol and CD14 microbead selection (Miltenyi Biotec). Monocytes were seeded for differentiation in serum-free medium (Macrophage-SFM, Life Technologies) supplemented with 50 ng/ml human recombinant macrophage colony-stimulating factor (M-CSF, Immunotools) and maintained for 6 days, before cells were switched to RPMI 1640 medium with 10% FCS. Cells were treated for indicated times with A-769662 (Tocris, 250 μM), AICAR (EMD biosciences, 1 mM), phenformin (Sigma-Aldrich, 250 μM), salicylate (Sigma-Aldrich, 3 mM), T0901317 (Enzo Life Sciences, 1 μM), PD98059 (Enzo Life Sciences, 25 μM), U0126 (Enzo Life Sciences, 10 μM), rapamycin (Sigma-Aldrich, 100 nM), LY294002 (Enzo Life Sciences, 30 μM) or cycloheximide (Sigma-Aldrich, 10 μg/ml)

2.2. Plasmid constructs and transfection

Cloning of human truncated AMPKα1 subunit and production of AMPKα1 lentiviruses was performed as described (Kemmerer et al., 2015). Adenovirus coding for a mutated R70Q regulatory γ1 subunit (AdAMPKγ1 RQ) was kindly provided by Dr Jason Dyck, University of Alberta, Canada. Firefly luciferase plasmids ABCA1 luc and mutABCA1 luc were kindly provided by Dr Andrew Brown, University of New South Wales, Australia. HDAC9 overexpression plasmid (pSG5-fHDAC9) (Kaluza et al., 2013) was kindly provided by Dr Stefanie Dimmeler, Goethe-University Frankfurt, Germany. The LXRα overexpression construct (TrueORF, Cat. No. RC223767) was purchased from OriGene. Transfection of THP-1 cells was achieved by nucleofection using Amaxa Human Monocyte Nucleofector Kit (Lonza) in a Nucleofector device according to manufacturer's instructions.

2.3. Quantitative PCR

Total RNA was isolated using PeqGold RNAPure kit (PeqLab) and transcribed using cDNA Synthesis kit (Fermentas). Quantitative PCR was performed with iQ SYBR green Supermix (Bio-Rad) using CFX96 system from Bio-Rad. Primer sequences for quantitative PCR can be obtained upon request. Expression was normalized to GAPDH

2.4. Western analysis

Cell pellets were harvested in lysis buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 1 mM Na3VO4, 0.5% NP-40, 1 μM PMSF, protease inhibitor cocktail (Roche)) Protein extracts were separated on polyacrylamide gels, transferred to nitrocellulose membrane, blocked in 5% nonfat milk or 5% BSA in TBS-Tween buffer (0,1% Tween 20, pH 7.4) and incubated with a desired primary antibody overnight at 4 °C. Primary antibodies directed against ABCA1 (Novus Biologicals, NB400-105), phospho-ACC (Ser79) (#3661), AMPK (#2532), phospho-HDAC (#3443), phospho-ERK (#9101), ERK (#4696) (all Cell Signaling Technology), LXRα (Abcam, ab41902), HDAC9 (Biovision, 3609-100), and Nucleolin (Santa Cruz Biotechnology, sc-13057) were used, followed by IRDye 680 and IRDye 800-coupled secondary antibodies (LICOR Biosciences). Blots were visualized and quantified using the Odyssey imaging system (LICOR Biosciences)

2.5. RNA interference

siRNAs targeting human LXRα and LXRβ (Qiagen), human PPARs, AMPKα1, HDAC4, HDAC9, ATF1 (siGENOME SMARTpool, Thermo Fisher Scientific) or scrambled control RNA oligonucleotides were transfected into primary macrophages at a final concentration of 50 nM for 72 h using Hiperfect transfection

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