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Retinoid acid receptor-related orphan receptor alpha (RORα) regulates macrophage M2 polarization via activation of AMPKα

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ABSTRACT

Macrophages are able to polarize to pro-inflammatory M1 or anti-inflammatory M2 states with distinct phenotypes and physiological functions. RORα is a member of the nuclear receptor super family and plays important roles in lipid, glucose metabolism, as well as the inflammatory response. In this study, we examined the potential function of RORα in the regulation of macrophage polarization. Treatment of RAW264.7 macrophages with RORα agonist cholesterol sulfate (CH-S) and overexpression of RORα increased M2 macrophage markers expressions (Arg1, Ym1 and Fizz1) both on mRNA and protein levels. Conversely, selective antagonism (SR1001) abrogated the induction of M2 macrophage markers which induced by CH-S. In addition, CH-S induced phosphorylation of Adenosine monophosphate (AMP)-activated protein kinase α (AMPKα), which was accompanied by the activation of acetyl-CoA carboxylase 1 (ACC). However, SR1001 abolished the activation of AMPKα and ACC induced by CH-S. Meanwhile, inactivation of AMPKα by its inhibitor Compound C (Comp C) abrogated the mRNA and protein levels of CH-S-induced M2 macrophage markers expressions. Together these findings reveal that RORα regulates macrophage M2 polarization via activation of AMPKα, which may provide a novel beneficial effect of RORα against inflammation.

1. Introduction

Inflammation is a body protective response to various endogenous and exogenous stimuli, which can be triggered by infection (acute inflammation) and tissue damage (chronic inflammation) (Medzhitov 2008; Takeuchi and Akira, 2010). Macrophages are an essential component of innate immunity and play a central role in both acute and chronic inflammation (Gordon and Martinez, 2010). During the inflammatory process, macrophages may undergo classical M1 activation [stimulated by Toll like receptor (TLR) ligands and interferon-γ (IFN-γ)] or alternative M2 activation [stimulated by interleukin 4/13 (IL-4/IL-13)] (Biswas and Mantovani, 2010). Act as switches of the immune system, macrophages maintain the balance between pro- (M1) and anti-inflammatory (M2) activities. M1 macrophage produces high levels of reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS) and pro-inflammatory cytokines including tumor necrosis factor α (TNF-α), IL-1β, IL-6, IL-12, IL-23, as well as the cell membrane molecule CD86 (Devaraj and Jialal 2011). On the other hand, IL-4, IL-13 and colony-stimulating factor (CSF) polarize macrophages to M2 subset, which produces anti-inflammatory cytokines, such as vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), IL-10 which characterized by increased levels of chitinase-like protein 3 (Ym1), arginase1 (Arg1), resistin-like molecule RELMα (Fizz1), as well as scavenger and the mannose receptor (CD206) (Devaraj and Jialal, 2011; Fernando et al., 2014). Macrophage phenotypic plasticity is regulated on transcriptional level through gene expression cascade induced by inflammatory stimuli, such as signal transducers and activators of transcription (STATs) family members, transcrip-

Abbreviations: RORα, retinoic acid receptor related orphan receptor α; CH-S, cholesterol sulfate; LPS, lipopolysaccharides; AMPKα, adenosine monophosphate (AMP)-activated protein kinase α; ACC, acetyl-CoA carboxylase 1; Comp C, compound C; TLR, toll like receptor; IFN-γ, interferon-γ; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor α; CSF, colony-stimulating factor; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor β; Arg1, arginase1; Ym1, chitinase-like protein 3; Fizz1, resistin-like molecule RELMα; STATs, signal transducers and activators of transcription; SMCs, smooth muscle cells; COX-2, cyclooxygenase 2; PPARs, peroxisome proliferator–activated receptors; Bmal1, brain and muscle ARNT-like; ApoC3, apolipoprotein C III; KLF, Krüppel-like factors; HIFs, hypoxia inducible factors; IRFs, interferon regulatory factors; JNK, C-Jun N-terminal kinase; PI3K, phosphatidylinositol-3-kinase; MFI, mean fluorescence intensity.

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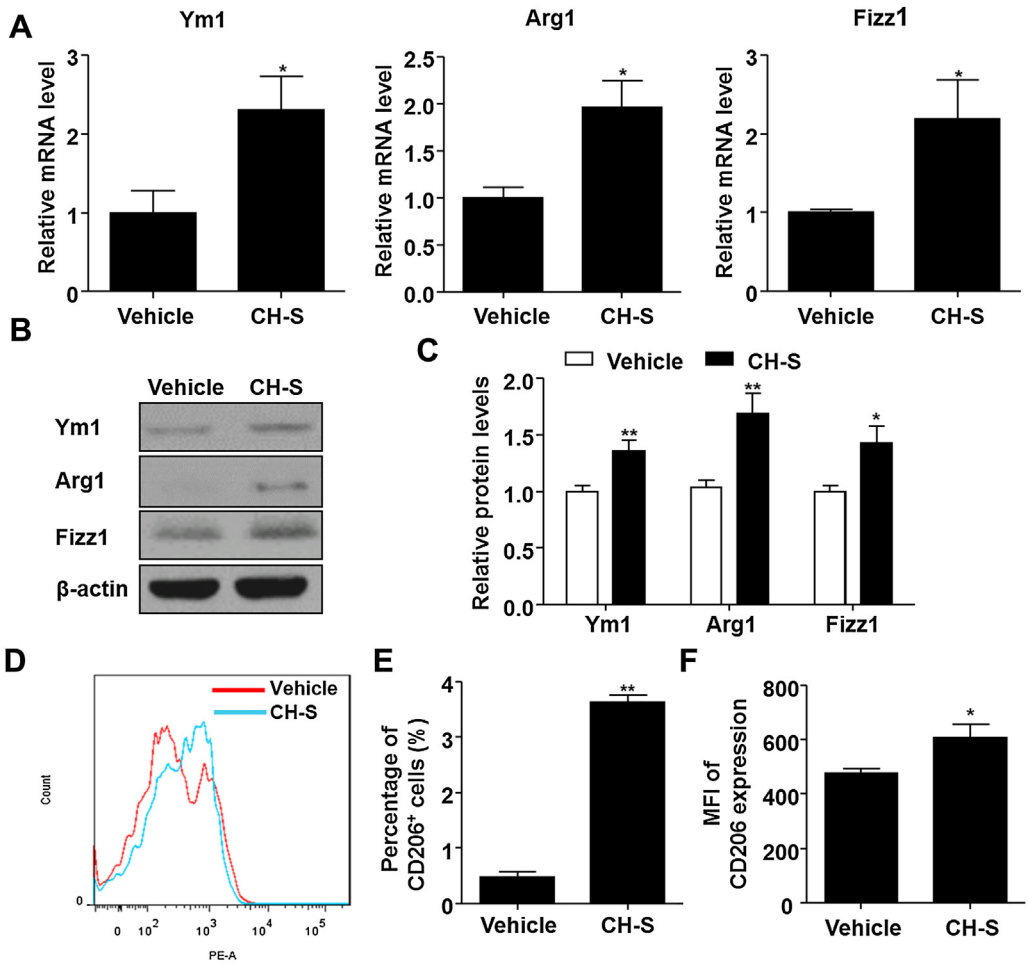

Fig. 1. RORα agonist CH-S promotes macrophage M2 polarization. RAW264.7 cells were incubated with 10 μM CH-S for 24 h and DMSO as vehicle control. (A) Total RNA was extracted and subjected to qRT-PCR for the assessment of Ym1, Arg1 and Fizz1 levels. (B) Cells lysates were analyzed for the levels of Ym1, Arg1 and Fizz1 by using western blotting. (C) Quantification of Ym1, Arg1 and Fizz1 protein levels, which was normalized to the amount of β-actin. The expression of CD206 was evaluated by flow cytometry analysis. Representative histogram obtained by flow cytometry analysis (D). Percentages of CD206+ macrophages (E). Mean fluorescence intensity (MFI) of CD206 expression on macrophages (F). Data represented the mean ± SD, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. Vehicle.

Table 1
List of primer pairs used for qRT-PCR.

Primer	Forward (5′-3′)	Reverse (5′-3′)
Arg1	ATGCTCACACTGACATCAACACTC	CTCTTCCATCACCTTGCCAATCC
Ym1	AGAAGGGAGTTTCAAACCTGGT	GTCTTGCTCATGTGTGTAAGTGA
Fizz1	TCCAGCTAACTATCCCTCCACTGT	GGCCCATCTGTTCATAGTCTTGA
ApoC3	GCATCTGCCCGAGCTGAAGAG	CTGAAGTGATTGTCCATCCAGC
Bmal1	GTCGGGACAAAATGAACAGTTT	TCCTGGACATTGCATTGCAT
RORα	ACGCCCACCTACAACATCTC	TCACATATGGGTTCGGGTTT
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

tional repressors, nuclear receptors and a series signaling pathways (Tugal et al., 2013).

RORα (NR1F1) is a ligand-activated transcription factor, belongs to the nuclear receptor superfamily. RORα is activated by a large number of endogenous ligands including cholesterol derivatives in which cholesterol sulfate (CH-S) is the most active form, oxygenated sterols such as 7-oxygenated sterols, and melatonin; as well as synthetic compounds like certain thiazolidinediones, SR1078, and CGP52608 (Laitinen and Staels, 2003; Solt and Burris, 2012; Kojetin and Burris, 2014). RORα involves in many metabolic pathways, particularly in lipid and glucose metabolism, cerebellar ataxia, angiogenesis, circadian clock regulation (Raichur et al., 2010; Wang et al., 2012b). Importantly, RORα also takes part in immune response regulation. RORα negatively regulates inflammatory response through inbition of NF-κB signalling pathway in smooth muscle cells (SMCs) (Delerive et al., 2001). Overexpression of RORα in human SMCs inhibits TNF-α-induced expression of pro-inflammatory cytokines such as IL-6, IL-8, and cyclooxygenase 2 (COX-2) (Delerive et al., 2001; Stapleton et al., 2005). In addition, stimulated of peritoneal macrophages from RORα$^{sg/sg}$ mice with lipopolysaccharides (LPS) results in hyper-induction of IL-1β and TNF-α (Kopmels et al., 1992; Dzhagalov et al., 2004). These all indicates that RORα negatively regulates inflammation response. More recently, nuclear receptors or their specific coactivators and some other transcriptional factors are proven to be involved in macrophage polarization regulation, such as peroxisome proliferator–activated receptors (PPARs) and Nr4A2 (Bouhlel et al., 2007; Charo, 2007; Kang et al., 2008; Mahajan et al., 2015). However, whether nuclear receptor RORα regulates M2 macrophage polarization is still unclear.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a heterotrimeric complex comprised of a catalytic α-subunit and two regulatory β- and γ-subunits (Hardie, 2007), maintains fatty acid, cholesterol, and glucose homeostasis, regulates ROS/redox balance, autophagy, cell proliferation, cell apoptosis, cellular polarity, mitochondria function and genotoxic response (Wang et al., 2012a; Weng and Schuppan, 2013). The α-subunit contains the catalytic domain of the serine/threonine protein kinase in the N-terminus. The phosphory-