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Negative regulation of NLRP3 inflammasome by SIRT1 in vascular endothelial cells

Yanxiang Li[a,d], Xiaofeng Yang[a], Yanhao He[a], Weirong Wang[b], Jiye Zhang[c], Wei Zhang[a], Ting Jing[a], Bo Wang[a], Rong Lin[a,d,*]

[a] Department of Pharmacology, Xi'an Jiaotong University, Health Science Center, Xi'an, China
[b] Laboratory Animal Center, Xi'an Jiaotong University, Health Science Center, Xi'an, China
[c] School of Pharmacy, Xi'an Jiaotong University, Health Science Center, Xi'an, China
[d] Cardiovascular Research Center, Xi'an Jiaotong University, Health Science Center, Xi'an, China

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ABSTRACT

NLRP3 inflammasome not only functions as a critical effector in innate immunity, but also triggers the production of proinflammatory cytokines involved in inflammation-associated diseases. Sirtuin 1 (SIRT1) plays an important role in the regulation of cellular inflammation. However, whether the activation of NLRP3 inflammasome is regulated by SIRT1 remains unknown. In this study, we investigated the regulatory effect of SIRT1 on NLRP3 inflammasome and the underlying mechanisms. We found that lipopolysaccharide (LPS) and adenosine triphosphate (ATP)-induced the activation of NLRP3 inflammasome in human umbilical vein endothelial cells (HUVECs). Activation of SIRT1 inhibited NLRP3 inflammasome activation and subsequent caspase-1 cleavage as well as interleukin (IL)-1β secretion, whereas SIRT1 knockdown obviously enhanced the activation of NLRP3 inflammasome in HUVECs. Importantly, gene silencing of SIRT1 abrogated the inhibitory effect of SIRT1 activator on NLRP3 inflammasome formation and IL-1β production in HUVECs stimulated with LPS plus ATP. Further study indicated that cluster of differentiation 40 (CD40) may be involved in the regulation of NLRP3 inflammasome by SIRT1. *In vivo* studies indicated that implantation of the periarterial carotid collar increased the arterial expression levels of CD40 and CD40 Ligand (CD40L), but inhibited arterial SIRT1 expression in the rabbits. Moreover, treatment with SIRT1 activator decreased CD40 and CD40L levels in collared arteries. Meanwhile, serum IL-1β level, the marker of inflammasome activation, was also inhibited by SIRT1 activation. Taken together, these findings revealed a novel regulatory mechanism of NLRP3 inflammasome by SIRT1, which may be related to suppression of CD40.

1. Introduction

The NLRP3 (NOD-like receptor family, pyrin domain-containing 3) inflammasome is a multi-protein complex that consists of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and caspase-1 (Lu et al., 2016). The aberrant activation of NLRP3 inflammasome has been implicated in a wide range of diseases, including complex diseases and inherited disorders (Coll et al., 2015). Recent evidences indicate that NLRP3 inflammasome initiates inflammatory response, which plays a crucial role in the progression of cardiovascular disease (Chen et al., 2016; Xi et al., 2016; Janoudi et al., 2015). A report showed that the activation of NLRP3 inflammasome by oscillatory flow augmented the production of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) in vascular endothelial cells (ECs) (Xiao et al., 2013). An *in vivo* study has also revealed that tumor necrosis factor (TNF)-α expression in cardiac tissue was reduced in a NLRP3$^{-/-}$ mice ischemic heart disease model (Jong et al., 2014). It is well accepted that sirtuin 1 (SIRT1), a NAD$^+$-dependent deacetylase, has been proven to be a key regulator in inflammatory response during cardiovascular disease (Winnik et al., 2015; Hubbard et al., 2013; Gorenne et al., 2013). In the pre-

Abbreviations: SIRT1, sirtuin 1; NLRP3, NOD-like receptor family pyrin domain-containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; CD40, cluster of differentiation 40; HUVECs, human umbilical vein endothelial cells; ECs, endothelial cells; LPS, lipopolysaccharide; ATP, adenosine triphosphate; IL, interleukin; RSV, resveratrol; NZW rabbits, New Zealand white rabbits; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1; TNF, tumor necrosis factor.

* Corresponding author at: Department of Pharmacology, Xi'an Jiaotong University, Health Science Center, Xi'an, Shaanxi 710061, China.
E-mail address: linrong@mail.xjtu.edu.cn (R. Lin).

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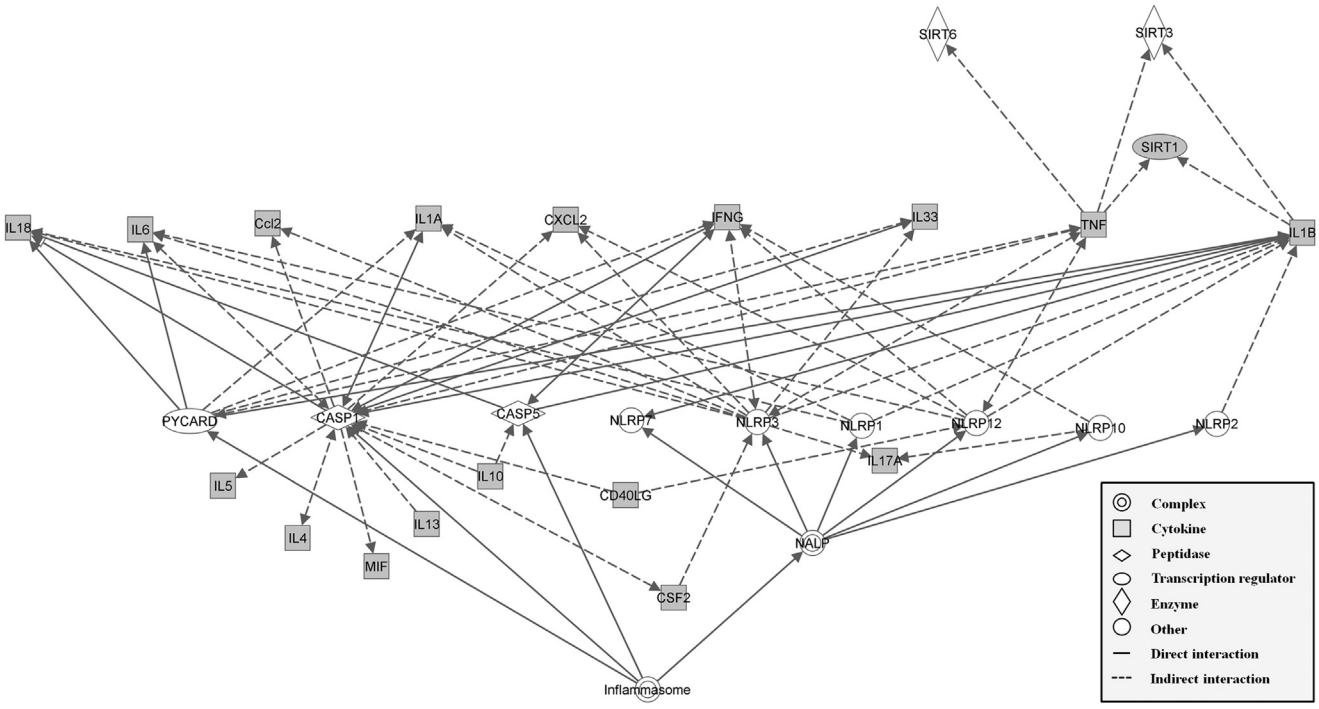

Fig. 1. Ingenuity pathway analysis-based interactions network of SIRT1 and inflammasome.

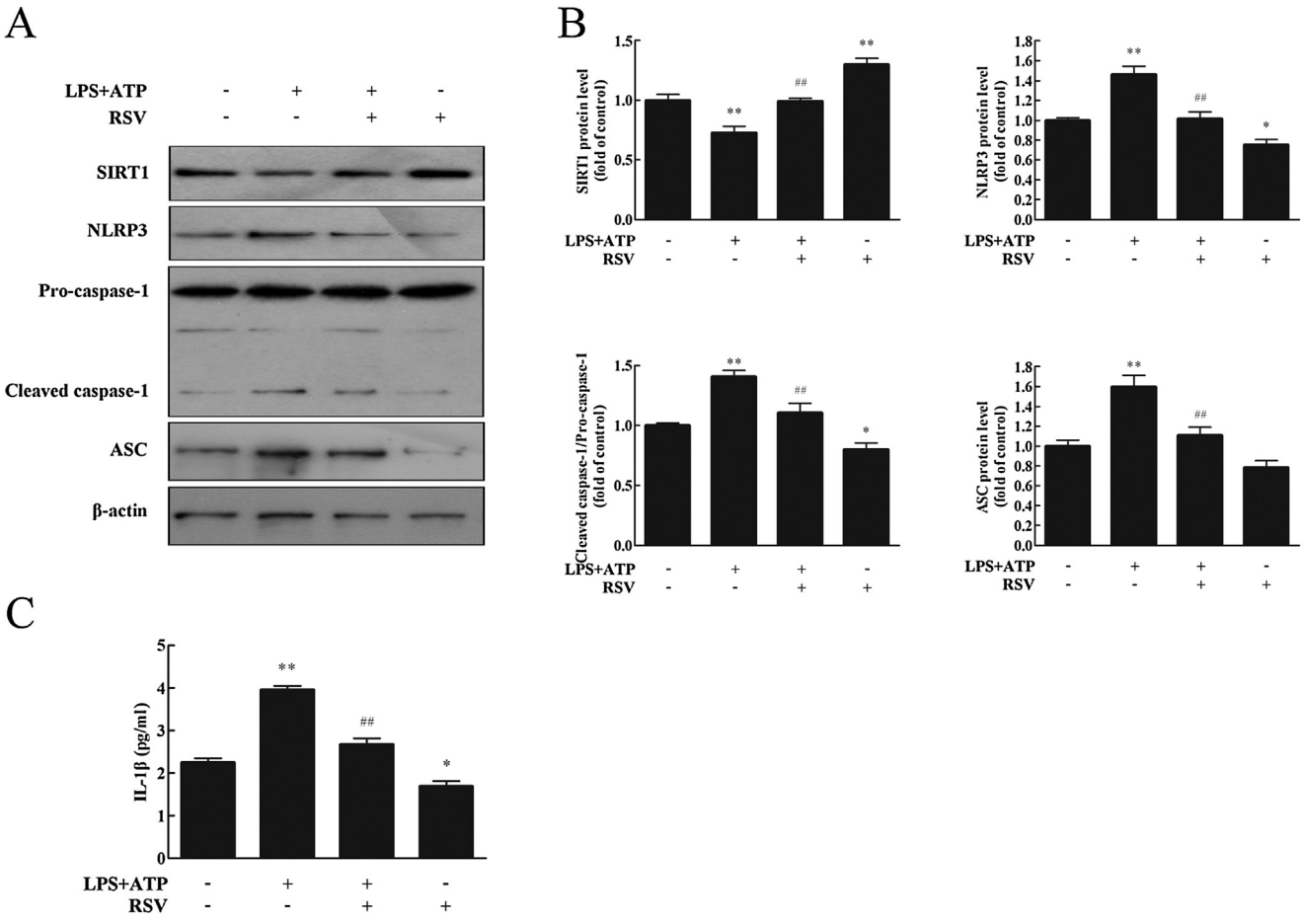

Fig. 2. SIRT1 activator attenuates LPS and ATP-induced NLRP3 inflammasome activation in HUVECs. The cells were pre-incubated with SIRT1 activator resveratrol (RSV) (20 μM for 1 h) and then stimulated with LPS (0.5 μg/ml for 3.5 h) and ATP (5 mM for 30 min). (A) The expression of SIRT1, NLRP3, pro-caspase-1, cleaved caspase-1 and ASC was determined by western blotting. (B) The bar graphs show the quantified results of western blotting. (C) The secretion of IL-1β in cell supernatants was detected by ELISA. Data are expressed as mean ± SEM, n = 3. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* control; $^{\#\#}P < 0.01$ *vs.* LPS + ATP group.