Biochemical and Biophysical Research Communications 450 (2014) 1363–1369

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SIRT2 ameliorates lipopolysaccharide-induced inflammation in macrophages

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ARTICLE INFO

Article history:
Received 22 June 2014
Available online 5 July 2014

Keywords:
SIRT2
Macrophage
Inducible nitric oxide synthase
Nitric oxide
Reactive oxygen species

ABSTRACT

Introduction: SIRT2 is a NAD(+)-dependent deacetylases and associated with numerous processes such as infection, carcinogenesis, DNA damage and cell cycle regulation. However, the role of SIRT2 in inflammatory process in macrophage remains unclear.

Materials and methods: In the present study, we have evaluated the regulatory effects of SIRT2 in lipopolysaccharide (LPS)-stimulated macrophages isolated from SIRT2 knockout (KO) and wild type (WT) mice or Raw264.7 macrophage cells. As inflammatory parameters, expression of inducible nitric oxide synthase (iNOS), the productions of nitric oxide, reactive oxygen species (ROS) and M1-macrophage-related factors were evaluated. We also examined the effects of SIRT2 on activation of nuclear factor-kappaB (NFκB) signaling.

Results: SIRT2 deficiency inhibits LPS-induced iNOS mRNA and protein expression in bone marrow derived macrophages. SIRT2-siRNA transfection also suppressed LPS-induced iNOS expression in Raw264.7 macrophage cells. Bone marrow derived macrophages isolated from SIRT2 KO mice produced lower nitric oxide and expressed lower levels of M1-macrophage related markers including iNOS and CD86 in response to LPS than WT mice. Decrease of SIRT2 reduced the LPS-induced reactive oxygen species production. Deficiency of SIRT2 resulted in inhibition of NFκB activation through reducing the phosphorylation and degradation of IκBα. The phosphorylation and nuclear translocation of p65 was significantly decreased in SIRT2-deficient macrophages after LPS stimulation.

Discussion: Our data suggested that deficiency of SIRT2 ameliorates iNOS, NO expression and reactive oxygen species production with suppressing LPS-induced activation of NFκB in macrophages.

1. Introduction

Inflammatory process leads to an increase in nitric oxide synthases (NOSs). Expression of inducible NOS (iNOS) is up-regulated in response to inflammatory stimuli and thus, production of nitric oxide (NO) is highly increased [1]. The inflammatory responses are also well characterized by the secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) [2]. Oxygen free radicals are suggested as mediators in lipopolysaccharide (LPS)-mediated inflammatory responses [3]. Reactive oxygen species (ROS) production is increased by inflammatory cytokines and NO in macrophages. Thus, macrophages play critical roles in inflammation. Macrophages have been classified into classically activated macrophages (M1), which is activated by interferon gamma (IFN-γ) and alternatively activated macrophages (M2), which is induced by interleukin (IL)-4 [4]. M1-macrophages express iNOS, TNF-α and CD86, and M2-macrophages express arginase-1, CD206 and FIZZ1 [5].

LPS is one of the potent pro-inflammatory stimulants and exerts its effects by activation of nuclear factor-κB (NF-κB), which is related with expression of many genes related to inflammatory reactions [6]. In inflammatory conditions, p65 also known as RelA, is involved in heterodimer formation, nuclear translocation and activation of NF-κB. Especially, phosphorylation of p65 is critical for NF-κB activity in synthesis of genes. LPS activates upstream mediators of NF-κB activation that requires phosphorylation of p65 in macrophages [7]. Thus, regulation of pro-inflammatory mediators and cytokines including iNOS, NO, phosphorylation of

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http://dx.doi.org/10.1016/j.bbrc.2014.06.135
0006-291X/

p65 in macrophage has been proposed to be a good avenue for the treatment of various inflammatory injury.

SIRT2, a NAD-dependent class I histone deacetylase, is linked to a variety of physiological and pathological conditions such as genomic instability [8], carcinogenesis [9], and cell cycle progression [10]. Recently, it has been demonstrated that SIRT2 is involved in bacterial infection [11], brain microglial cell activation [12] and arthritis [13]. In addition, it has been well known that macrophage has a critical role in host defense process in innate immunity. However, the role of SIRT2 in macrophages during LPS-induced inflammatory process remains to be clarified.

On the basis of above considerations, we have evaluated roles of SIRT2 in LPS-induced inflammatory responses and the molecular basis in macrophages. Our results showed that SIRT2 modulates LPS-mediated inflammatory responses through inhibiting NF-κB activation.

2. Materials and methods

2.1. Chemicals and reagents

LPS was purchased from Sigma–Aldrich (St Louis, MO), AGK2 from Tocris (Bristol, UK), AK-1 from Calbiochem (San Diego, CA). Antibodies against SIRT2 (Abcam, Cambridge, MA), iNOS (BD Transduction Laboratories, San Jose, CA), phospho-p65 (Cell Signaling Technology, Danvers, MA), p65 (EMD Millipore Corporation, Billerica, MA) and β-actin (Sigma–Aldrich) were obtained. HRP-conjugated secondary antibodies were purchased from Enzo Life Science Inc. (Farmingdale, NY). All other reagents were purchased from Sigma–Aldrich (St Louis, MO) unless otherwise indicated.

2.2. Preparation of BMDM cells

Experimental procedures were in agreement with our institutional guidelines on animal care. For the isolation of bone marrow derived macrophage (BMDM) cells, *Sirt2*$^{+/+}$ and *Sirt2*$^{-/-}$ male mice (aged 6 weeks, Jackson Laboratory, Bar Harbor, ME) were killed by cervical dislocation and lower limbs were removed [14]. BMDM cells were flushed from the medullary cavities of tibias and femurs with phosphate buffered saline (PBS) using a 23 G needle. The cell suspension was filtered through a cell strainer (40 μm) to remove debris, followed by centrifugation at 1500 rpm for 5 min. After centrifugation, the supernatant was removed and the cells were suspended in culture medium to give 1.0×10^6 cells/mL.

2.3. Cell culture and treatment

BMDM cells were seeded at a density of 1×10^6 cells/mL as described previously [15,16]. At 18 h after seeding, floating cells were transferred to a new dish with the complete culture media. After incubation for 2 days, cultured BMDM cells that had attached to the dish were removed using cold PBS.

Raw 264.7 macrophage cells (RAW cells; American Type Culture Collection, Manassas, VA) were grown in DMEM-high glucose supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified incubator with 5% CO_2. To evaluate the effects of SIRT2 on iNOS mRNA expression, RAW cells were plated in 6 cm dish (5×10^5 cells/dish) and allowed to grow overnight. siRNA (100 pmoL/L, Dharmacon ON-TARGET plus SMART pool, Dharmacon Inc., CO) and 20 μL Lipofectamine™ 2000 (Invitrogen) were diluted in Opti-MEM (Invitrogen) to a total volume of 2 mL. The diluted siRNA and Lipofectamine™ 2000 were mixed and incubated to generate the transfection mixture at room temperature for 5 min. The cells were washed with Opti-MEM medium, and then the transfection mixture was added to the dish and incubated for 24 h.

2.4. MTT cell viability

The measurement of cell viability of AGK2 or AK-1 was performed using the MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [17]. Briefly, RAW cells were plated at a density of 2×10^3/well in a 96-well plate and incubated at 37 °C for 24 h. The cells were treated with AGK2 (10 and 20 μmol/L), AK-1 (10 and 20 μmol/L) or vehicle alone. After 8 h of incubation at 37 °C, 50 μL of the MTT solution was added to each well and incubated under the same conditions for another 4 h. The absorbance was measured at 490 nm using a spectrofluorometer (VersaMax, Molecular Devices, Sunnyvale, CA).

2.5. Western blotting

For the detection of target proteins, Western blotting was performed as described previously [18,19]. In brief, cells were washed with PBS and lysed with Radio-Immunoprecipitation Assay (RIPA) buffer supplemented with protease and phosphatase inhibitors (Sigma–Aldrich). The cells were sonicated for 5 min for complete cell lysis and microcentrifuged for 15 min 16,000*g*. Samples (20 μg/lane) were loaded onto 10% SDS–polyacrylamide gel and after electrophoresis, separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA). The PVDF membrane was incubated with 5% non-fat dry milk in TBS (20 mM Tris–HCl (pH 7.6) and 150 mM NaCl) containing 0.1% Tween 20 (v/v) (TBS/Tween) for 1 h before incubation with antibodies against phospho-p65 (dilution 1/500) or iNOS in 5% non-fat milk overnight at 4 °C. Cytoplasmic cell extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Inc., Waltham, MA) and blotted with primary antibody against phosphor-IκBα or IκBα. Anti-β-actin or p65 antibody was used as a loading control.

2.6. Immunocytochemistry of p65

Immunocytochemistry was performed as previously described [20]. BMDM cells from SIRT2 wild and knockout mice were stimulated for 6 h with LPS. After incubation, cells were fixed and further analyzed for nuclear translocation of p65 immune reactions using immune cytochemistry with a primary antibody against NFκB p65 (EMD Millipore Corporation). Stained cells were washed and examined using a laser scanning confocal microscope (Zeiss LSM 510 confocal microscope, Carl Zeiss, Gottingen, Germany). The grading system was used as previously described [20]. In brief, 0 = no immune reaction in cell nucleus; 1 = 1/3 of the cell nucleus demonstrated immune reactions; 2 = 2/3 of the cell nucleus demonstrated immune reactions; 3 = immune reaction all over the cell nucleus and the cytoplasm were visible; 4 = immune reactivity was only located in cell nucleus.

2.7. Nitrite analysis

NO synthesis was spectrophotometrically determined by assaying the culture supernatants for nitrite using Total Nitric oxide and Nitrate/Nitrite Parameter Assay Kit (R&D systems, Minneapolis, MN). Absorbance was measured at 540 nm using a spectrofluorometer (VersaMax, Molecular Devices, Sunnyvale, CA).

2.8. Detection of ROS production

RAW cells were incubated with or without AK-1 for 30 min, followed by stimulation with or without LPS for 4 h. Cells were then

washed and incubated with 2′,7′-dichlorodihydrofluorescein-diacetate (DCF-DA, Sigma–Aldrich, 10 μmol/L) at 37 °C for 20 min and then washed three times with serum free medium. The number of fluorescence-positive cells per unit area was counted under a Zeiss Z1 microscope (Carl Zeiss, Göttingen, Germany). The fluorescence was measured using a spectrofluorometer (SpectraMax Gemini EM, Molecular Devices, Sunnyvale, CA) (excitation at 488 nm and emission at 535 nm).

2.9. Quantitative real time polymerase chain reaction

Total RNA was extracted from cells using TRI Reagent (MRC, Cincinnati, OH). The same amount of RNA was loaded in triplicates for each assay and quantitative real time polymerase chain reaction (PCR) was performed using a SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, CA) on a Rotor-Gene Q (Qiagen, Hilden, Germany) to measure iNOS, TNF-α, CD86, arginase-1, CD206 and FIZZ1. Gene expression values were calculated based on the comparative threshold cycle method, normalized to the expression values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as described previously [14]. Supplementary Table S1 summarizes the primer sequences of mouse iNOS, TNF-α, CD86, arginase-1, CD206, FIZZ1 and GAPDH.

2.10. Statistical analysis

Statistical analysis was performed using the SPSS soft-ware version 19.0 (IBM SPSS Statistics, IBM corporation, Armonk, NY). Data with normal distribution were analyzed with the Student's *t* parametric test to assess statistical significance between the means of two independent groups. Data without normally distribution were analyzed using Mann–Whitney *U* test. All values are presented as mean ± standard deviation (SD).

3. Results

3.1. Knockout of SIRT2 attenuates LPS-induced iNOS expression

To assess the role of SIRT2 in LPS-induced iNOS expression, we evaluated expression of iNOS in BMDM cells isolated from SIRT2 knockout (KO) mice. As expected, Western blot analysis revealed that SIRT2 protein was absent in BMDM cells from SIRT2 KO mice (SIRT2 KO BMDM cells) whereas expression of SIRT2 was evident in BMDM cells from wild type (WT) mice (Fig. 1A). Treatment with LPS increased expression level of iNOS in both SIRT2 WT and KO BMDM cells (Fig. 1B). However, the increased level of iNOS was significantly higher in SIRT2 WT BMDM cells than the level in SIRT2 KO BMDM cells. The iNOS mRNA expression in the BMDM cells after LPS exposure was significantly lower in SIRT2 KO than SIRT2 WT cells (Fig. 1C). To ensure the observations, RAW cells were transfected with SIRT2 siRNA or control siRNA. iNOS protein and mRNA expression in SIRT2 siRNA-transfected RAW cells was significantly decreased compared with that in control siRNA-transfected cells (Fig. 1D and E). We also found that SIRT2 inhibition with AGK2 or AK-1 ameliorated LPS-induced iNOS protein and mRNA expression in RAW cells (Supplementary Fig. 1A–C). These results suggest that LPS-induced iNOS expression is regulated by SIRT2 signaling. In addition, determination of cytotoxic effect of AGK2 or AK-1 on viability of RAW cells showed that both inhibitors had no cytotoxic activity up to 20 μmol/L (Supplementary Fig. 1C). Therefore, the non-toxic concentrations (10 and 20 μmol/L) were used throughout the experiments.

3.2. LPS-induced NO production is decreased in SIRT2 KO BMDM cells

To investigate the potential effect of SIRT2 deficiency on NO production, we measure LPS-induced NO production in the BMDM cells by measuring the accumulated nitrite in the culture medium. Results demonstrated that LPS challenge significantly increased the production of NO in BMDM cells compared with vehicle-treated group from WT mice. SIRT2 deficiency of BMDM cells have decreased NO production relative to SIRT2 WT mice (Fig. 1F).

3.3. LPS-induced ROS production is suppressed in SIRT2 KO cells

We further investigated whether SIRT2 is involved in ROS production in *in vitro*. LPS-treated BMDM cells were incubated with a fluorescent probe DCF-DA and the number of DCF-DA-positive cells was evaluated by fluorescent microscopy. Treatment of SIRT2 KO BMDM cells with LPS substantially decreased the number of DCF-DA-positive cells per unit area compared to SIRT2 WT BMDM cells (Fig. 2A and B). Intracellular ROS levels also were monitored by a spectrofluorometer. The level of ROS in SIRT2 KO BMDM cells was significantly lower than the level in SIRT2 WT BMDM cells (Fig. 2C). In addition, treatment of RAW cells with a SIRT2 inhibitor, AK-1 significantly lowered the LPS-induced ROS production (Supplementary Fig. 2A–C). These data suggest that SIRT2 is involved in LPS-induced ROS production.

3.4. M1-macrophage related factors are decreased in SIRT2 deficient cells

We evaluated the expression of M1- and M2-macrophage related factors after treatment with INF-γ and IL-4, respectively. iNOS, TNF-α and CD86 were examined as M1-macrophage related factors while arginase-1, CD206 and FIZZ1 were examined as M2-macrophage related factors using quantitative real time PCR. mRNA expression of M1-macrophage related factors, iNOS and CD86, was significantly decreased in SIRT2 KO BMDM cells compared to SIRT2 WT cells (Fig. 3A–C). TNF-α expression had a tendency to decreased, but did not reach to statistical significance. However, mRNA expression of M2-macrophage related factors, arginase-1, CD206 and FIZZ1, was not significantly changed in SIRT2 KO BMDM cells compared to SIRT2 WT (Fig. 3D–F). mRNA expression of M1-macrophage related factors, iNOS, TNF-α and CD86, was also significantly lower in AGK2-treated RAW cells (Supplementary Fig. 2D and E).

3.5. SIRT2 deficiency decreases LPS-induced NFκB activation

NF-κB is activated in response to LPS [7]. In activated macrophages by LPS, the IκBα is phosphorylated and finally degraded by ubiquitination. The activated NF-κB is translocated from cytoplasm to nucleus [21,22]. Therefore, Western blot analysis was performed with cytoplasmic extracts to assess the phosphorylation and degradation of IκBα. We observed that deficiency of SIRT2 ameliorated LPS-induced phosphorylation and degradation of IκBα in BMDM cells (Fig. 4A and B). In next experiment, to determine the blocking effect of SIRT2 on activation of NF-κB, phosphorylation of p65 subunit was determined using BMDM cells from SIRT2 KO and WT mice. Our Western blot analysis showed that LPS-induced phosphorylation of p65 in SIRT2 KO BMDM cells significantly lower than that of SIRT2 WT BMDM cells (Fig. 4C). We also examined p65 translocation to the nucleus by immunocytochemistry. After treatment with LPS, a pronounced accumulation of p65 protein in the cell nucleus was observed in SIRT2 WT BMDM cells (Fig. 4D). We found that LPS-induced nuclear translocation of p65 was significantly suppressed in SIRT2 KO BMDM cells compared with SIRT2 WT BMDM cells (Fig. 4D).

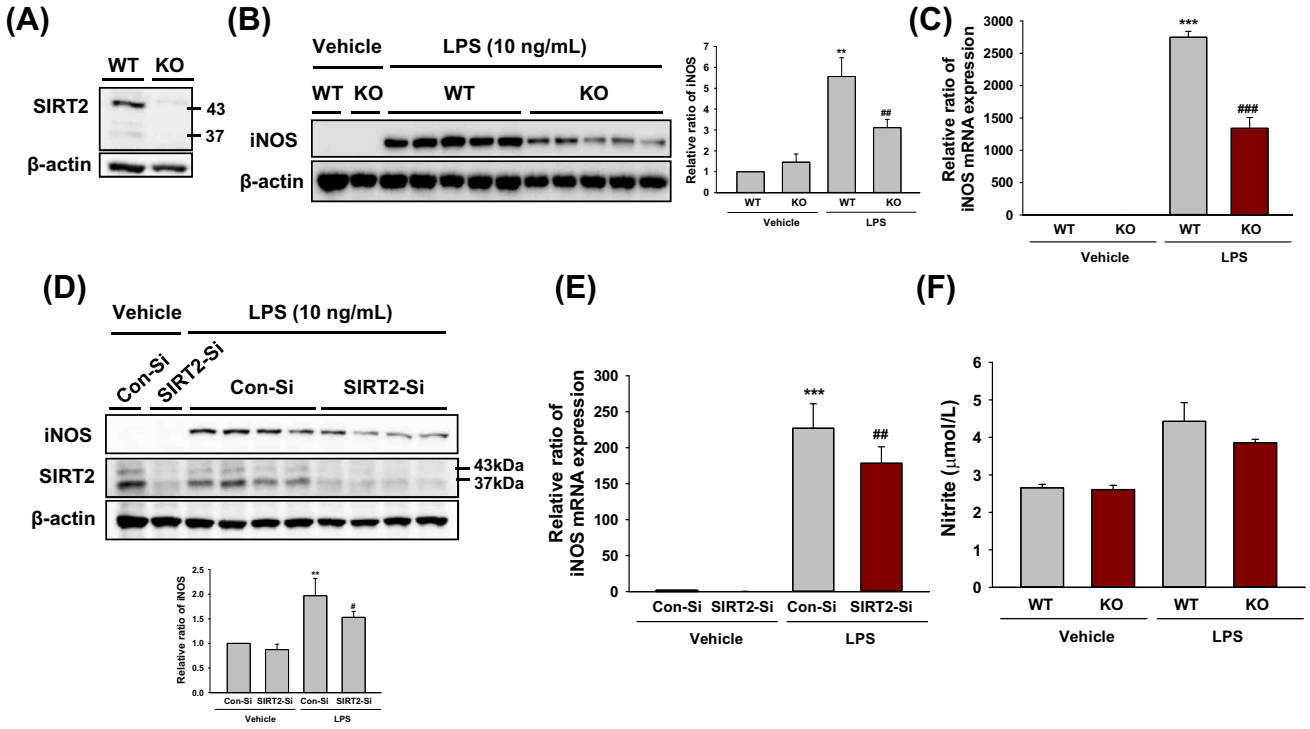

Fig. 1. (A) Expression of SIRT2 protein in BMDM cells from SIRT2 wild (WT) and knockout (KO) mice. (B, C) iNOS protein and mRNA levels in BMDM cells from SIRT2 WT and KO mice. (D, E) Expression of iNOS at protein and mRNA level in RAW cells after transfection with SIRT2-siRNA (SIRT2-Si) or control-siRNA (Con-Si). (F) NO levels in the culture medium in BMDM cells from SIRT2 WT and KO mice. Each bar represents the mean ± SD of three independent experiments. **, $p < 0.01$ *vs* vehicle + WT or vehicle + Con-Si; ***, $p < 0.001$ *vs* vehicle + WT or vehicle + Con-Si; $^{\#\#}$, $p < 0.01$ *vs* LPS + WT or LPS + Con-Si; $^{\#\#\#}$, $p < 0.001$ *vs* LPS + WT or LPS + Con-Si.

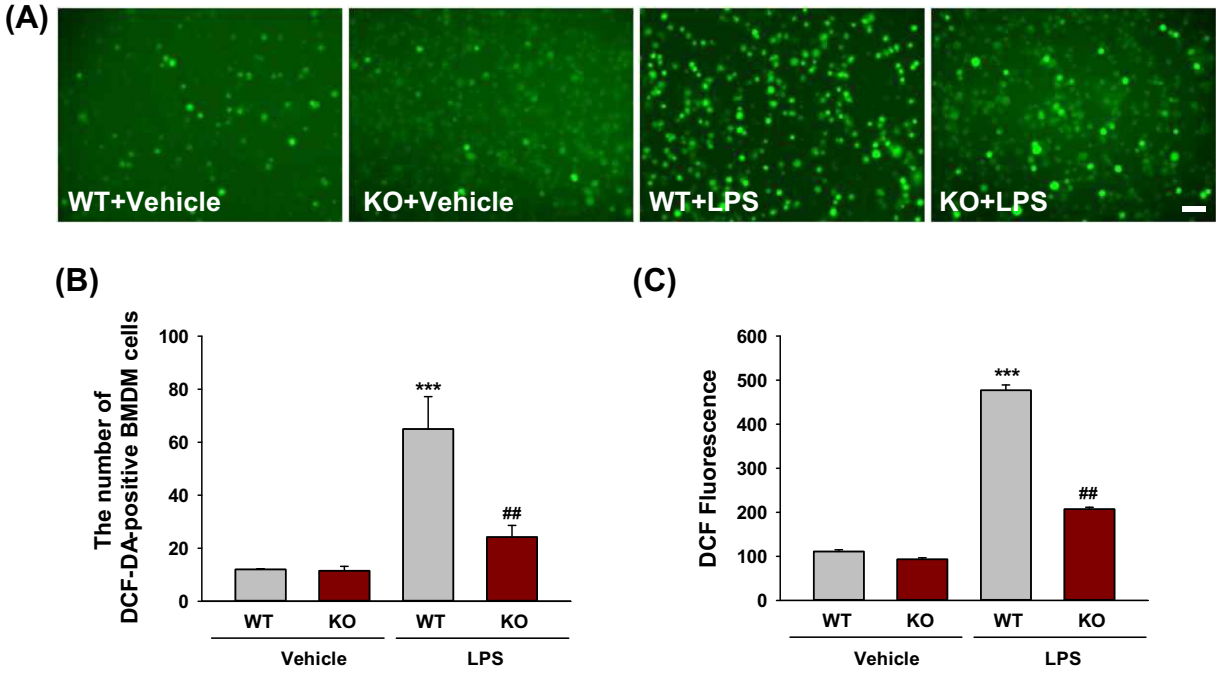

Fig. 2. Deficiency of SIRT2 attenuated LPS-induced ROS accumulation. (A, B) Intracellular ROS of BMDM cells were visualized using DCF-DA fluorescent staining. BMDM cells stimulated with LPS (10 ng/mL) for 4 h. BMDM cells were then washed with serum free medium and 10 μmol/L of DCF-DA was added for 20 min. The number of DCF-DA-positive cells was counted under a fluorescent microscope. Bar = 50 μm. Each bar represents the mean ± SD of three independent experiments. (C) Fluorescence DCF-DA-treated BMDM cells was measured by a spectrofluorometer after excitation at 488 nm and emission at 535 nm. ***, $p < 0.001$ *vs* vehicle + WT; $^{\#\#}$, $p < 0.01$ *vs* LPS + WT.

4. Discussion

Macrophages play a critical role in the innate immunity in early inflammatory process and are main source inflammatory mediators [23]. The inflammatory mediators are regulated by various pathogens. Recognition of pathogens is primarily mediated by pathogen-associated molecular patterns (PAMPs) including LPS [24]. In RAW cells, NF-κB, a transcriptional factor, is implicated in the

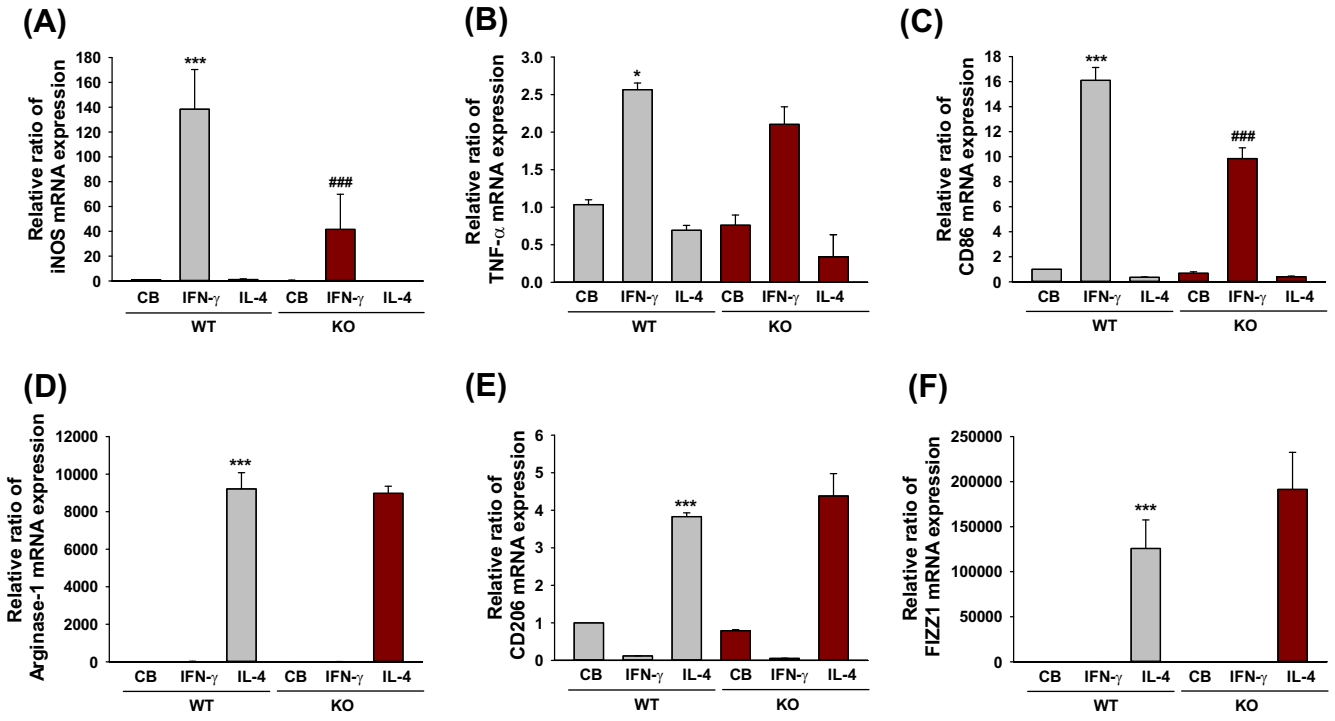

Fig. 3. mRNA expression of M1-macrophage related factors: iNOS (A), TNF-α (B), CD86 (C); M2-related factors: arginase-1 (D), CD206 (E) and FIZZ1 (F) in the BMDM cells isolated from SIRT2 WT and KO mice. BMDM cells from SIRT2 WT and KO mice cells were stimulated with INF-γ (100 U/mL) or IL-4 (20 ng/mL) for 48 h. The expression levels of iNOS, TNF-α, CD86, arginase-1, CD206 and FIZZ1 were measured by quantitative real time PCR. The expression levels were normalized to GAPDH level. Bar represents the mean ± SD of three independent experiments. *, $p < 0.05$ *vs* WT + CB; ***, $p < 0.001$ *vs* WT + CB; $^{\#\#\#}$, $p < 0.001$ *vs* WT + INF-γ.

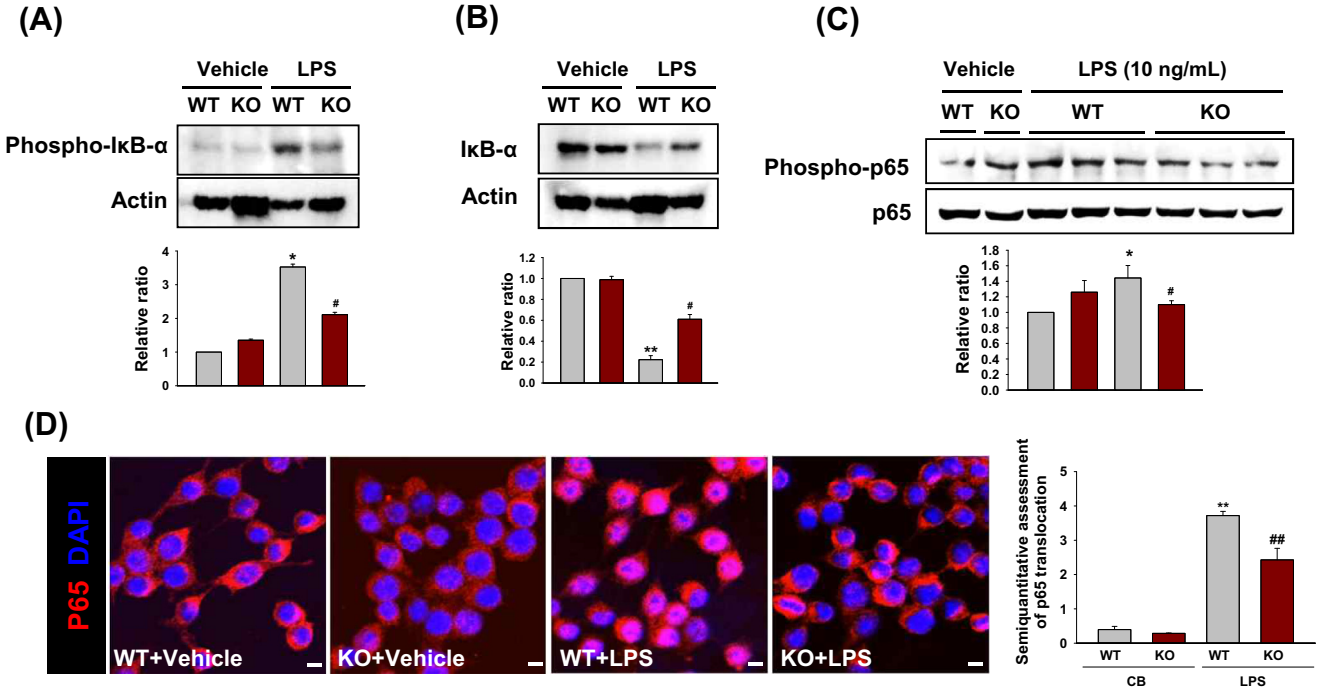

Fig. 4. (A and B) The effects of SIRT2 deficiency on LPS-induced phosphorylation and degradation of α in cytoplasmic extracts of BMDM cells. Phosphorylation and degradation of IκBα protein from SIRT2 WT and KO BMDM cells was assessed by Western blotting after incubation for 0.5 and 1 h with LPS, respectively. (C) Effects of SIRT2 deficiency on LPS-induced p65 phosphorylation in BMDM cells from SIRT2 WT and KO mice. BMDM cells were treated with LPS (10 ng/mL) for 8 h. Lysates were prepared and Western blotting was performed using a specific phospho-p65 antibody. p65 was used as an internal control. (D) Immunofluorescence subcellular localization of p65 protein. SIRT2 WT and KO BMDM cells were treated with LPS for 1 h, and p65 protein localization was detected using anti-p65 antibody. Data are presented as the mean ± SD of three independent experiments. Bar = 10 μm. *, $p < 0.05$ *vs* vehicle + WT; $^{\#}$, $p < 0.05$ *vs* LPS + WT.

regulation of many inflammation related genes including iNOS gene by LPS [25]. iNOS is not expressed under normal physiological conditions, but can be increased by pro-inflammatory stimulators or oxidative stress. NO has a dual biologic role in inflammatory process. It may protect the cells from inflammation and atherosclerosis. However, overproduction of NO produced by iNOS is cytotoxic.

The present study demonstrated that LPS markedly increased iNOS expression and NO production, which were significantly attenuated by SIRT2 deficiency in BMDM cells. We also found that SIRT2 knockdown or inhibition significantly decreased LPS-induced iNOS expression and NO production in RAW cells. To our best knowledge, there is no report on the effect of SIRT2 on iNOS expression in macrophages.

Our data demonstrated that LPS-induced iNOS protein or mRNA expression in SIRT2 KO mice was not completely blocked as compared with WT mice (Fig. 1B) and the inhibitory effect of SIRT2 siRNA-transfected RAW cells was very weak (Fig. 1D). Thus, it seems that the participation of SIRT2 against LPS signaling is restrictive. SIRT1, another class I sirtuins, is involved in regulation of LPS-induced iNOS and NO expression [26]. Zong et al. [26] have reported that resveratrol, an activator of SIRT1, inhibits LPS-induced NO production and iNOS expression in murine RAW cells. However, our data showed that decrease of SIRT2 mitigated the LPS-induced NO production and iNOS expression in macrophages. Although SIRT2 has the deacetylation activity on histone protein like SIRT1, our data are not consistent with Zong's findings. The exact mechanism regarding the different effect on iNOS expression and NO production between SIRT2 and SIRT1 should be clarified in the future.

Polarization of M1 and M2 macrophages may have a critical role in regulation of inflammatory processes. In the study, we evaluated whether SIRT2 is involved in polarization of M1 and M2 macrophage after LPS treatment. mRNA expression of M1-macrophage related factors (iNOS, TNF-α, CD86) and of M2-macrophage related factors (arginase-1, CD 206 and FIZZ1) in the BMDM cells was measured using quantitative real time PCR. Our results showed that SIRT2 deficiency exerted an apparently inhibitory effect on expression of M1-macrophage related factors while SIRT2 deficiency did not show any significant inhibitory effect on M2-macrophage related factors (Fig. 3). Yang et al. [27] have demonstrated that SIRT1 specific deletion in myeloid cells increases infiltration of M1 macrophages and decreases M2 macrophages in adipose tissue. These findings suggest that SIRT2 may be involved in polarization of macrophages.

Activation of Toll-like receptor-4 (TLR-4) by LPS leads to the activation of NF-κB by phosphorylation of p65 Ser (536) [6]. Phosphorylation of p65 increases the translocation of NF-κB into the nucleus and regulates the transcription of many inflammatory genes. In our present study, SIRT2 deficiency significantly inhibited LPS-induced phosphorylation of p65 as well as iNOS expression and NO production in BMDM cells. It has been demonstrated that SIRT2 regulates NF-κB by deacetylation of p65 Lys (310) [28]. As posttranslational modifications of the p65 subunit by acetylation or phosphorylation are a major aspect of the regulation of NF-κB activity, the precise molecular basis on the relation between phosphorylation and acetylation of p65 after LPS treatment remained to be clarified.

Activated macrophages increase ROS production during inflammatory process and ROS is critical for LPS-induced inflammation [29,30]. We showed that SIRT2-deficient in BMDM cells decreases ROS after LPS stimulation. Thus, inhibition of ROS generation by decrease of SIRT2 might also contribute to inhibit expression of inflammatory mediators. Thus, our data indicated that inhibition of LPS-induced might be one of the mechanisms responsible for its anti-inflammatory property.

In conclusion, our results have demonstrated that in macrophages, SIRT2 modulates LPS-induced iNOS expression, NO production and M1-related inflammatory gene expression through the regulation of p65 phosphorylation. Although further exploration of SIRT2 *in vivo* is required, our findings may provide a molecular basis for the ability of SIRT2 serving as a promising candidate for treating LPS-induced inflammatory diseases.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of (NRF) funded by the Ministry of Education, Science and Technology (NRF-2011-0016712, K.W.), a grant (CUHBRI-2012- 02-003, K.W.) of the CNUH-BRI (K.W.) and research funds of Chonbuk National University in 2012 (J.Y.J.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.06.135.

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