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SIRT1 inactivation induces inflammation through the dysregulation of autophagy in human THP-1 cells

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ABSTRACT

Inflammation plays a crucial role in atherosclerosis. Monocytes/macrophages are some of the cells involved in the inflammatory process in atherogenesis. Autophagy exerts a protective effect against cellular stresses like inflammation, and it is regulated by nutrient-sensing pathways. The nutrient-sensing pathway includes SIRT1, a NAD^+-dependent histone deacetylase, which is implicated in the regulation of a variety of cellular processes including inflammation and autophagy. The mechanism through which the dysfunction of SIRT1 contributes to the regulation of inflammation in relation to autophagy in monocytes/macrophages is unclear. In the present study, we demonstrate that treatment with 2-[(2-Hydroxynaphthalen-1-ylmethylene)amino]-*N*-(1-phenethyl)benzamide (Sirtinol), a chemical inhibitor of SIRT1, induces the overexpression of inflammation-related genes such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 through nuclear factor (NF)-κB signaling activation, which is associated with autophagy dysfunction, as shown through p62/Sqstm1 accumulation and decreased expression of light chain (LC) 3 II in THP-1 cells. The autophagy inhibitor, 3-methyladenine, also induces inflammation-related NF-κB activation. In p62/Sqstm1 knockdown cells, Sirtinol-induced inflammation through NF-κB activation is blocked. In addition, inhibition of SIRT1 is involved in the activation of the mammalian target of rapamycin (mTOR) pathway and is implicated in decreased 5′-AMP activated kinase (AMPK) activation, leading to the impairment of autophagy. The mTOR inhibitor, rapamycin, abolishes Sirtinol-induced inflammation and NF-κB activation associated with p62/Sqstm1 accumulation. In summary, SIRT1 inactivation induces inflammation through NF-κB activation and dysregulates autophagy via nutrient-sensing pathways such as the mTOR and AMPK pathways, in THP-1 cells.

1. Introduction

Cardiovascular disease (CVD) is the most frequent cause of death in patients with diabetes mellitus [1] and metabolic syndrome [2], and atherosclerosis is a major cause of CVD. Atherosclerosis has been identified as an inflammatory disease, and the underlying pathology is characterized by persistent inflammation of the vascular tissue [3]. Systemic and vascular micro-inflammation is related to insulin resistance in diabetes or metabolic syndrome, and monocytes/macrophages are key players in the inflammatory pathogenesis of atherosclerosis. Activation of monocytes in the circulation and infiltrating macrophages in the atherosclerotic lesion releases various inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 [4]. Therefore, studying the regulatory mechanisms of inflammation in monocytes/macrophages is important for preventing cardiovascular diseases.

Autophagy, a lysosomal degradation pathway, plays a crucial role in removing protein aggregates as well as damaged organelles for the maintenance of intracellular homeostasis during various stress conditions [5]. Suppression of basal homeostatic autophagy may cause age-related diseases including atherosclerosis. Recent reports show that autophagy in macrophages may produce protective effects against advanced atherosclerosis [6], and the impairment of autophagy in macrophages leads to the inflammation, resulting in the progression of atherosclerosis [7]. Autophagy is regulated by nutrient-sensing pathways including SIRT1, mammalian target of rapamycin (mTOR) and 5′-AMP activated kinase (AMPK) [5]. The dysregulation of nutrient-sensing pathways and the subsequent impairment of cellular functions are involved in the pathogenesis of insulin resistance and vascular dysfunction. Among the nutrient-sensing pathways, SIRT1 dysfunction is observed in various tissues and cell types in animals models of insulin resistance [8–11]. In addition, de Kreutzenberg et al. reported that insulin resistance negatively affects both SIRT1 gene and protein expression in the peripheral blood monocytes of humans with

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metabolic syndrome [12]. SIRT1, a NAD^+-dependent deacetylase, is a homologue of silent information regulator (Sir2), which was initially identified from studies in aging yeast [13]. Homologues of Sir2 in higher eukaryotes are known as sirtuins, and there are seven sirtuins in mammals. Numerous reports show that SIRT1 activation may improve or retard age-related diseases such as diabetes and cardiovascular disease by regulating a variety of cellular processes including decreasing inflammation and inducing autophagy [14]. Therefore, we hypothesize that the dysregulation of SIRT1 plays a role in impairing autophagy, resulting in the dysregulation of the maintenance of accumulated molecules, with subsequent effects on inflammation in monocytes/macrophage. In addition, we hypothesize that decreased autophagy may lead to insulin resistance and atherosclerosis.

In the present study, we demonstrate that SIRT1 inactivation impairs starvation-induced autophagy leading to the accumulation of p62/Sqstm1 and resulting in inflammation through NF-κB activation. In addition, inhibition of SIRT1 contributes to the regulation of nutrient-sensing pathways by the activation of mTOR and the inhibiting AMPK activation, thereby causing the suppression of autophagy.

2. Material and methods

2.1. Materials

The anti-phospho-AMPK (Thr172), anti-light chain (LC) 3, anti-phospho-mTOR, anti-mTOR, anti-phospho-p70 S6 kinase (Thr421/Ser424), anti-p70 S6 kinase, anti-phospho-NF-κB (p65), and anti-NF-kB (p65) antibodies were obtained from Cell Signaling (Beverly, MA, USA). The p62/Sqstm1 antibodies were purchased from Medical & Biological Laboratories (Nagoya, Japan). 2-[(2-Hydroxynaphthalen-1-ylmethylene)amino]-*N*-(1-phenethyl)benzamide (Sirtinol) was from Calbiochem (San Diego, CA, USA). Horseradish peroxidase-linked anti-rabbit, anti-mouse secondary antibodies and RIPA buffer were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). RPMI1640 medium and fetal bovine serum were obtained from American Type Culture Collection (Rockville, MD, USA). 6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide (Ex-527), Rapamycin, 3-methyladenine (3-MA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. THP-1 cell culture

THP-1 cells (American Type Culture Collection, Rockville, MD) were cultured in Falcon flasks in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, 10 mM HEPES and 1500 mg/L sodium bicarbonate, and they were maintained at 37 °C in a 5% CO_2 incubator. After serum starvation for 24 h, cells were incubated with 10 μM Sirtinol, 10 μM EX-527, 5 mM or 10 mM 3-MA for 5, 10 or 20 h at 37 °C. In some experiments, cells were pre-incubated with 100 ng/ml of rapamycin for 3 h, and then incubated with 10 μM Sirtinol. Prior to the assays, the culture medium was centrifuged at 1000*g* for 10 min, the supernatant was discarded, and the cells were used for the assays described below.

2.3. Western blotting

The samples were homogenized in ice-cold RIPA buffer. Total protein samples from the cells were used for Western blot analysis. These samples were separated on 5–20% gradient SDS-PAGE gels and transferred to polyvinylidene difluoride fmembranes (Immobilon; Millipore, Bedford, MA, USA). After blocking with 5% milk, the membranes were incubated overnight with the previously mentioned antibodies at 4 °C. The membranes were then incubated with the appropriate HRP-conjugated secondary antibodies, and the bands were detected by enhanced chemiluminescence (Amersham, Buckinghamshire, UK).

2.4. Quantitative RT-PCR

The isolation of total RNA from the cells, cDNA synthesis by reverse transcription, and quantitative real-time PCR were performed as described previously [15]. Analytical data were normalized by using 18S as an internal control. The PCR primer sets are listed below. TNF-α: sense (5′–3′) CCCAGGCAGTCAGATCATCTTC, anti-sense (5′–3′) AGCTGCCCCTCAGCTTGA. IL-6: sense (5′–3′) AAGCCAGAGCTGTG CAGATGAGTA, anti-sense (5′–3′) TGTCCTGCAGCCACTGGTTC, 18S: sense (5′–3′) ACTCAACACGGGAAACCTCA, anti-sense (5′–3′) AACCA GACAAATCGCTCCAC.

2.5. RNA interference

The siRNA for human p62/Sqstm1 (Accell SMARTpool) and a negative control (Accell non-targeting siRNA) were purchased from Thermo Scientific (Fremont, CA). THP-1 cells were seeded onto a forty-eight-well plate and were transfected with p62/Sqstm1 siRNA using Accell delivery media. After 72 h, the cells were exposed to Sirtinol for 10 or 20 h, and the levels of TNF-α mRNA expression or p62/Sqstm1 and phospho-NF-kB (p65) expression were determined by quantitative RT-PCT or Western blotting.

2.6. Statistics

The data are expressed as the mean ± standard deviation (S.D.). The Tukey multiple-comparison test was used to determine the significance of pairwise differences among three or more groups. Statistical significance between two groups was determined using the unpaired *t*-test. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Inhibition of SIRT1 decreases autophagy in THP-1 cells

We first investigated the effects of SIRT1 inhibition on starvation-induced autophagy by immunoblotting for p62/Sqstm1 and LC3 II. Treatment with Sirtinol, a chemical inhibitor of sirtuins such as SIRT1, significantly increased the p62/Sqstm1 levels and decreased the LC3 II levels when compared to the control (Fig. 1A). Moreover, treatment with EX-527, a different SIRT1 chemical inhibitor, also decreased the starvation-induced autophagy (Fig. 1B). These data indicate that inhibition of SIRT1 decreases starvation-induced autophagy in THP-1 cells.

3.2. Inhibition of SIRT1 increases inflammation in THP-1 cells

We examined whether inhibition of SIRT1 induces inflammation in THP-1 cells. The activation of transcription factor NF-κB (p65) plays a crucial role in inflammation-related gene expression in the pathogenesis of atherosclerosis [16]. Therefore, NF-κB activation was assessed by immunoblotting for the phosphorylated species of NF-κB (p65). Treatment with Sirtinol significantly increased NF-κB (p65)-phosphorylation in THP-1 cells (Fig. 1C) and induced the overexpression of inflammation-related genes such as TNF-α and IL-6 when compared to the control cells (Fig. 1D).

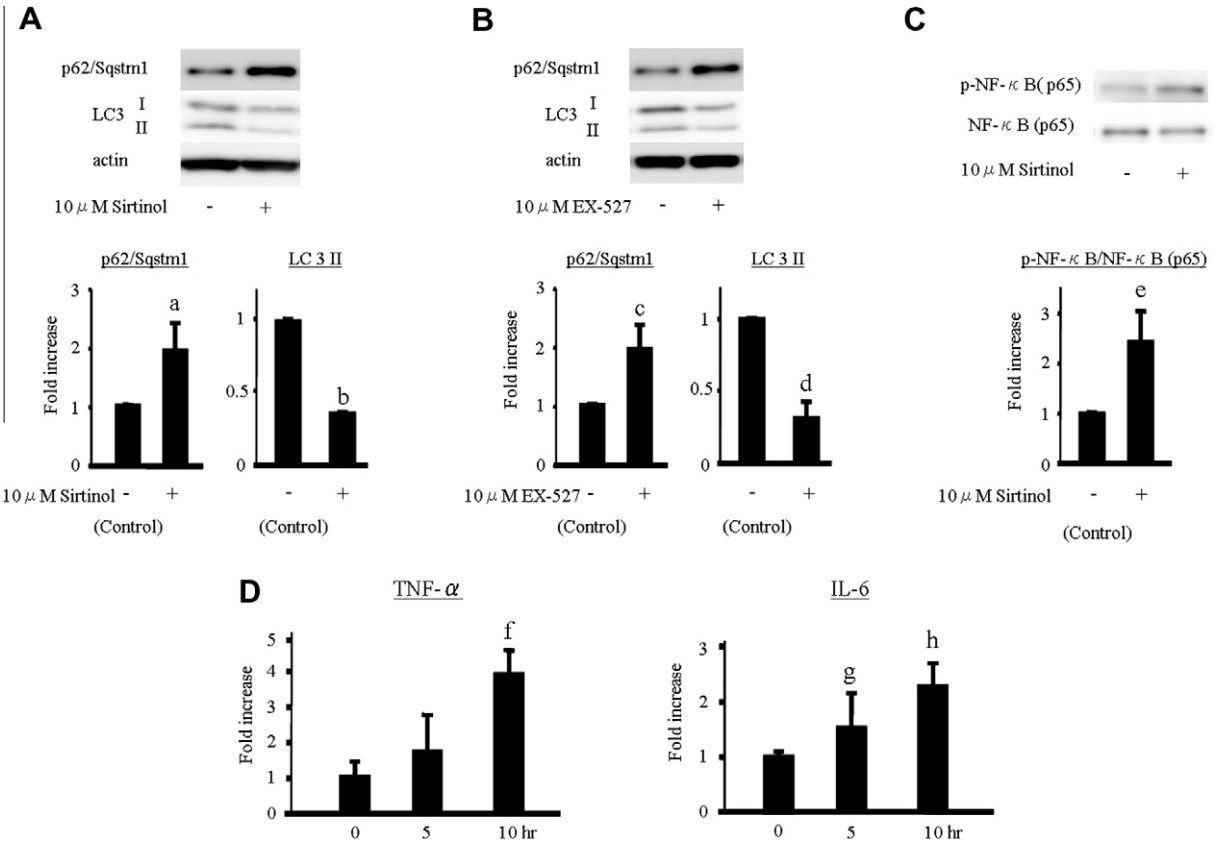

Fig. 1. (A) Treatment with 10 μM Sirtinol for 20 h significantly increases p62/Sqstm1 and decreases light chain (LC) 3 II expression when compared to the control. The data are presented as the mean ± S.D. ($N = 4$, a and b: $p < 0.01$ vs. Control). (B) Treatment with 10 μM EX-527 for 20 h significantly increases p62/Sqstm1 and decreases light chain (LC) 3 II expression when compared to the control. The data are presented as the mean ± S.D. ($N = 4$, c and d: $p < 0.01$ vs. Control). (C) Treatment with 10 μM Sirtinol for 20 h significantly increases phospho-nuclear factor (NF)-κB (p65) expression when compared to the control. The data are presented as the mean ± S.D. ($N = 3$, e: $p < 0.05$ vs. Control). (D) Treatment with 10 μM Sirtinol significantly increases the mRNA expression of tumor necrosis factor (TNF)-α for 10 h and interleukin (IL)-6 for 5 and 10 h when compared to the control. The data are presented as the mean ± S.D. ($N = 6$, f: $p < 0.01$ vs. other groups, g: $p < 0.05$ vs. 10 h, h: $p < 0.01$ vs. 0 h).

3.3. Suppression of autophagy increases inflammatory responses

To define the potential role of autophagy in inflammation, we examined whether suppression of autophagy induces inflammation in THP-1 cells, using a chemical inhibitor of autophagy, 3-MA. Treatment with 3-MA significantly increased the mRNA expression of TNF-α and IL-6 (Fig. 2B), which was accompanied by the activation of NF-κB, compared to the control (Fig. 2A).

3.4. The accumulation of p62/Sqstm1 is associated with the dysfunction of autophagy and is required to promote inflammation

Previous reports show that the accumulation of p62/Sqstm1, by inhibiting autophagy, may lead to inflammation through the activation of the NF-κB signaling pathway [17]. Therefore, to elucidate the mechanism by which inhibition of autophagy induces inflammation in THP-1 cells, we determined whether p62/Sqstm1 levels contribute to the overexpression of inflammation-related genes and cause NF-κB activation by using siRNA to knockdown p62/Sqstm1. In p62/Sqstm1 knockdown cells, the phosphorylation of NF-κB (p65) and overexpression of TNF-α, induced by treatment with Sirtinol, were blocked (Fig. 3A and B).

3.5. Inhibition of SIRT1 induces inflammation by suppressing autophagy via mTOR activation

Autophagy is regulated by nutrient-sensing pathways including SIRT1, mTOR and AMPK. The imbalance of these pathways in the cell may lead to the dysregulation of autophagy. Therefore, we determined whether the mTOR pathway and AMPK activation were altered after treatment with Sirtinol. The phosphorylation of mTOR and the phosphorylation of p70 S6 kinase, a down-stream target of the mTOR pathway, were significantly increased after treatment with Sirtinol when compared to control cells (Fig. 4A). However, phosphorylation of AMPK decreased after Sirtinol treatment (Fig. 4B). In addition, rapamycin, an mTOR inhibitor, restored the Sirtinol-induced suppression of autophagy with increased levels of p62/Sqstm1, the activation of NF-κB (Fig. 4C) and the overexpression of TNF-α mRNA (Fig. 4D).

4. Discussion

Inflammation plays a crucial role in atherosclerosis, and monocytes/macrophages are involved in the systemic and vascular inflammatory process [3]. Therefore, understanding the regulatory mechanisms of inflammation in monocytes/macrophages is important for preventing CVD. Autophagy is the only mechanism for degrading large molecules, organelles, proteins, and end-products. Therefore, homeostatic autophagy eliminates damaged components and serves an important housekeeping function [5]. Recent reports show that autophagy may exert protective effects for age-related diseases including atherosclerosis [6,7]. Therefore, the dysregulation of autophagy may be implicated in the pathogenesis of vascular dysfunction in insulin resistant, diabetes or metabolic syndrome. Autophagy may regulate inflammation [18], and conversely, inflammation may regulate autophagy for the maintenance of

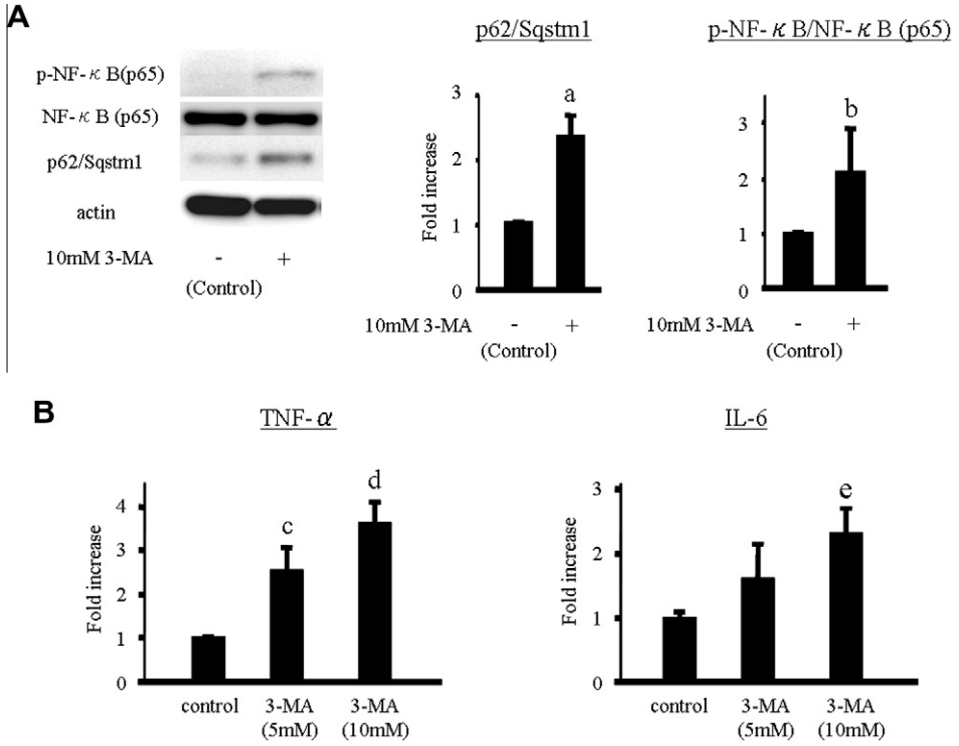

Fig. 2. (A) 10 mM 3-methyladenine (3-MA) treatment, an inhibitor of autophagy, for 20 h significantly increases phopsho-NF-κB (p65) expression in THP-1 cells when compared to control cells. The data are presented as the mean ± S.D. ($N = 3$, a: $p < 0.01$ vs. Control, b: $p < 0.05$ vs. Control). (B) mRNA expression of TNF-α is significantly increased after 5 mM or 10 mM 3-MA treatment for 10 h. mRNA expression of IL-6 is significantly increased by 10 mM 3-MA. The data are presented as the mean ± S.D. ($N = 3$, c: $p < 0.05$ vs. 10 mM 3-MA, d: $p < 0.01$ vs. Control, e: $p < 0.05$ vs. Control).

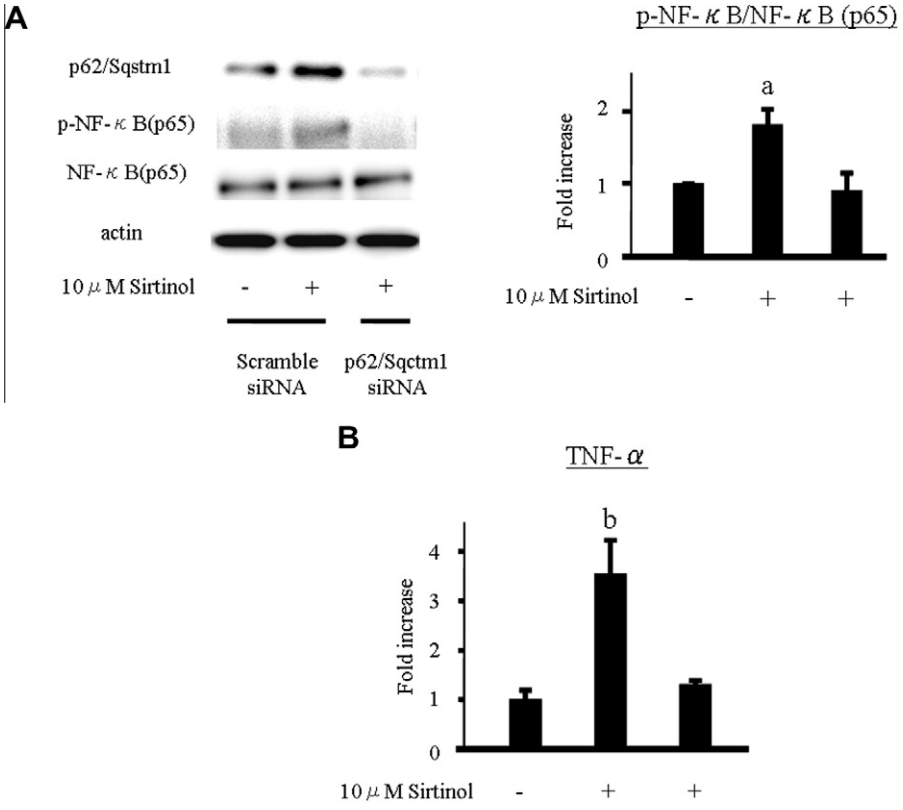

Fig. 3. (A) p62/Sqstm1 knockdown in THP-1 cells blocks NF-κB (p65) phosphorylation after 10 μM Sirtinol treatment. The data are presented as the mean ± S.D. ($N = 3$, a: $p < 0.01$ vs. other groups). (B) 10 μM Sirtinol-induced overexpression of TNF-α mRNA is abolished in p62/Sqstm1 knockdown THP-1 cells. The data are presented as the mean ± S.D. ($N = 4$, b: $p < 0.01$ vs. other groups).

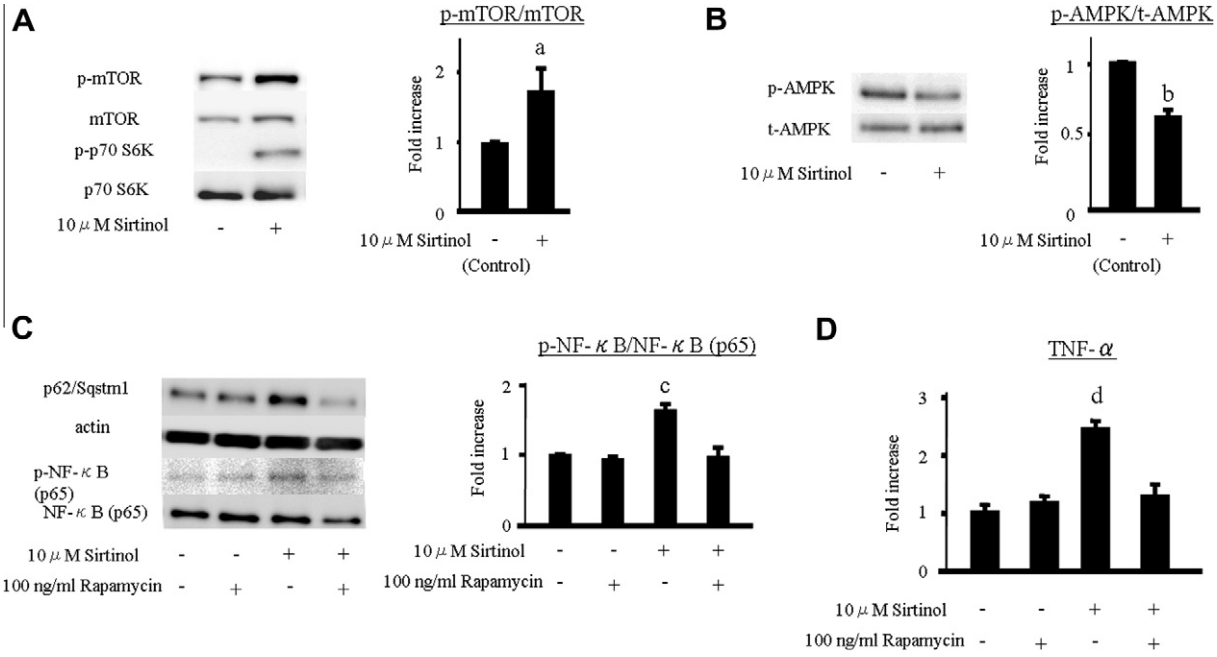

Fig. 4. (A) Treatment with 10 μM Sirtinol induces the phosphorylation of mammalian target of rapamycin (mTOR) and p70 S6 kinase, a down-stream target of the TOR pathway. The data are presented as the mean ± S.D. ($N = 4$, a: $p < 0.01$ vs. Control). (B) Treatment with 10 μM Sirtinol significantly decreases the phosphorylation of AMP activated kinase (AMPK) when compared to control cells. The data are presented as the mean ± S.D. ($N = 4$, b: $p < 0.01$ vs. Control). (C) Treatment with 100 ng/ml rapamycin inhibits the Sirtinol-induced activation of NF-κB and the accumulation of p62/Sqstm1. The data are presented as the mean ± S.D. ($N = 4$, c: $p < 0.01$ vs. other groups). (D) Treatment with 100 ng/ml rapamycin inhibits the Sirtinol-induced overexpression of TNF-α mRNA. The data are presented as the mean ± S.D. ($N = 4$, a: $p < 0.01$ vs. other groups).

cellular functions [5]. SIRT1 is one of the nutrient-sensing pathways and its activation may exert beneficial effects by regulating many cellular functions including inflammation and autophagy [14]. Therefore, we hypothesize that the dysfunction of SIRT1 leads to the impairment of autophagy and the dysregulation of inflammation in monocytes. To test our hypothesis, we mimicked SIRT1 inactivation using Sirtinol, a chemical inhibitor of SIRT1, and determined its effects on autophagy and inflammation in THP-1 cells. We found that inhibition of SIRT1 induces the overexpression of inflammation-related genes and increased phosphorylation of NF-κB (p65). This was accompanied by the inhibition of starvation-induced autophagy. In addition, we showed that inhibition of autophagy induced NF-κB dependent inflammation using a chemical inhibitor, 3-MA. These data indicate that the impairment of autophagy induced by the inhibition of SIRT1 may increase the expression of inflammation-related genes and activate NF-κB signaling. One of the mechanisms through which the impairment of autophagy can activate NF-κB signaling is p62/Sqstm1 accumulation. p62/Sqstm1 accumulation has been associated with the dysregulation of autophagy and is involved in increasing inflammation or tumorigenesis via alteration to NF-κB signaling in several cell lines [17,19–22]. Our results support the previous data and indicate that Sirtinol-induced overexpression of inflammation-related genes and NF-κB activation is blocked in p62/Sqstm1 knockdown THP-1 cells. Therefore, the dysregulation of autophagy and the accumulation of p62/Sqstm1 induced by SIRT1 inactivation may contribute to increased inflammation through the activation of the NF-κB pathway in monocytes/macrophages. In addition to the regulation of inflammation by autophagy, it has been reported that autophagy is induced by the activation of NF-κB signaling [23,24]. Thus, the regulation of autophagy and inflammation may be dependent on cell type, cellular stresses and conditions.

We also found that inhibition of SIRT1 leads to activation of the mTOR pathway and decreases AMPK activation. Nutrient starvation induces autophagy through the inhibition of mTOR [25]. mTOR consist of mTOR complex (C) 1 and mTORC2, and mTORC1 is rapamycin sensitive [25]. mTORC1 plays a key role at the interface of pathways that coordinate the balance between cell growth and autophagy. This is in response to nutritional status [25]. The mTORC1 pathway is regulated by other nutrient-sensing pathways including SIRT1 and AMPK [25]. Ghosh et al. demonstrated that SIRT1 negatively regulates mTORC1 signaling through an interaction with tuberous sclerosis complex 2 (TSC2) [26]. In addition, AMPK negatively regulates mTORC1 through the phosphorylation of TSC2 [27] and the regulatory associated protein of mTOR (Raptor) [28]. Furthermore, SIRT1 activates AMPK by the deacetylation of liver kinase B (LKB) 1 [29]. Thus, inhibition of SIRT1 may activate the mTORC1 pathway through direct or indirect mechanisms and result in the dysfunction of autophagy.

Previous reports show that SIRT1 expression is decreased in human monocytes with insulin resistance [12], and the myeloid cell-specific SIRT1 knockout mice challenged with a high-fat diet display high levels of activated macrophages in liver and adipose tissue, predisposing the animals to development of systemic insulin resistance and metabolic derangement [30]. Moreover, Timmers et al. demonstrates that treatment with resveratrol, an activator of SIRT1, in obese humans decreases circulating inflammation makers and improves insulin resistance [31]. These data supports our hypothesis that the role of SIRT1 in monocytes/macrophages is important for the regulating of inflammation in relation to insulin resistance. SIRT1 may suppress inflammation by directly deacetylating NF-κB (p65) [30,32,33] however, there was no difference in acetylated- NF-κB (p65) levels between the control and Sirtinol-treated cells after serum starvation (data not shown). In the present study, we demonstrate that SIRT1 inactivation is implicated in inflammation through the phosphorylation of NF-κB (p65) which is associated with dysregulation of autophagy. Autophagy in macrophages may produce protective effects against

advanced atherosclerosis [6], and the impairment of autophagy in macrophages leads to the inflammation, resulting in the progression of atherosclerosis [7]. Further studies using animal models are needed to elucidate a detailed mechanism by which SIRT1 dysfunction-induced inflammation through dysregulation of autophagy in monocytes/macrophages causes insulin resistance and atherosclerosis.

In conclusion, inhibition of SIRT1 induces inflammation through NF-κB activation and the accumulation of p62/Sqstm1. SIRT1 inactivation contributes to the dysregulation of other nutrient-sensing pathways including mTOR and AMPK, thereby leading to the impairment of autophagy. Therefore, our data suggest that improving the imbalance of nutrient-sensing pathways associated with SIRT1 dysfunction may suppress inflammation in monocytes during insulin resistance.

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References

[1] S.M. Haffner, S. Lehto, T. Ronnemaa, K. Pyorala, M. Laakso, Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction, N. Engl. J. Med. 339 (1998) 229–234.
[2] R.H. Eckel, S.M. Grundy, P.Z. Zimmet, The metabolic syndrome, Lancet 365 (2005) 1415–1428.
[3] R. Ross, Atherosclerosis – an inflammatory disease, N. Engl. J. Med. 340 (1999) 115–126.
[4] T.A. Pearson, G.A. Mensah, R.W. Alexander, J.L. Anderson, R.O. Cannon 3rd, M. Criqui, Y.Y. Fadl, S.P. Fortmann, Y. Hong, G.L. Myers, N. Rifai, S.C. Smith Jr., K. Taubert, R.P. Tracy, F. Vinicor, Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association, Circulation 107 (2003) 499–511.
[5] G. Kroemer, G. Marino, B. Levine, Autophagy and the integrated stress response, Mol. Cell 40 (2010) 280–293.
[6] X. Liao, J.C. Sluimer, Y. Wang, M. Subramanian, K. Brown, J.S. Pattison, J. Robbins, J. Martinez, I. Tabas, Macrophage autophagy plays a protective role in advanced atherosclerosis, Cell Metab. 15 (2012) 545–553.
[7] B. Razani, C. Feng, T. Coleman, R. Emanuel, H. Wen, S. Hwang, J.P. Ting, H.W. Virgin, M.B. Kastan, C.F. Semenkovich, Autophagy links inflammasomes to atherosclerotic progression, Cell Metab. 15 (2012) 534–544.
[8] T. Yoshizaki, S. Schenk, T. Imamura, J.L. Babendure, N. Sonoda, E.J. Bae, D.Y. Oh, M. Lu, J.C. Milne, C. Westphal, G. Bandyopadhyay, J.M. Olefsky, SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E419–E428.
[9] T. Yoshizaki, J.C. Milne, T. Imamura, S. Schenk, N. Sonoda, J.L. Babendure, J.C. Lu, J.J. Smith, M.R. Jirousek, J.M. Olefsky, SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes, Mol. Cell. Biol. 29 (2009) 1363–1374.
[10] M. Kitada, A. Takeda, T. Nagai, H. Ito, K. Kanasaki, D. Koya, Dietary restriction ameliorates diabetic nephropathy through anti-inflammatory effects and regulation of the autophagy via restoration of Sirt1 in diabetic Wistar fatty (fa/fa) rats: a model of type 2 diabetes, Exp. Diabetes Res. 2011 (2011) 908185.
[11] F. Liang, S. Kume, D. Koya, SIRT1 and insulin resistance, Nat. Rev. Endocrinol. 5 (2009) 367–373.
[12] S.V. de Kreutzenberg, G. Ceolotto, I. Papparella, A. Bortoluzzi, A. Semplicini, C. Dalla Man, C. Cobelli, G.P. Fadini, A. Avogaro, Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms, Diabetes 59 (2010) 1006–1015.
[13] S. Imai, L. Guarente, Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases, Trends Pharmacol. Sci. 31 (2010) 212–220.
[14] L. Guarente, Franklin H. Epstein lecture: sirtuins, aging, and medicine, N. Engl. J. Med. 364 (2011) 2235–2244.
[15] M. Kitada, S. Kume, N. Imaizumi, D. Koya, Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway, Diabetes 60 (2011) 634–643.
[16] K. Brand, S. Page, G. Rogler, A. Bartsch, R. Brandl, R. Knuechel, M. Page, C. Kaltschmidt, P.A. Baeuerle, D. Neumeier, Activated transcription factor nuclear factor-kappaB is present in the atherosclerotic lesion, J. Clin. Invest. 97 (1996) 1715–1722.
[17] H.M. Lee, D.M. Shin, J.M. Yuk, G. Shi, D.K. Choi, S.H. Lee, S.M. Huang, J.M. Kim, C.D. Kim, J.H. Lee, E.K. Jo, Autophagy negatively regulates keratinocyte inflammatory responses via scaffolding protein p62/SQSTM1, J. Immunol. 186 (2011) 1248–1258.
[18] T. Yoshizaki, C. Kusunoki, M. Kondo, M. Yasuda, S. Kume, K. Morino, O. Sekine, S. Ugi, T. Uzu, Y. Nishio, A. Kashiwagi, H. Maegawa, Autophagy regulates inflammation in adipocytes, Biochem. Biophys. Res. Commun. 417 (2012) 352–357.
[19] J. Moscat, M.T. Diaz-Meco, p62 at the crossroads of autophagy, apoptosis, and cancer, Cell 137 (2009) 1001–1004.
[20] G. Bjorkoy, T. Lamark, A. Brech, H. Outzen, M. Perander, A. Overvatn, H. Stenmark, T. Johansen, p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death, J. Cell Biol. 171 (2005) 603–614.
[21] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, T. Johansen, p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J. Biol. Chem. 282 (2007) 24131–24145.
[22] R. Mathew, C.M. Karp, B. Beaudoin, N. Vuong, G. Chen, H.Y. Chen, K. Bray, A. Reddy, G. Bhanot, C. Gelinas, R.S. Dipaola, V. Karantza-Wadsworth, E. White, Autophagy suppresses tumorigenesis through elimination of p62, Cell 137 (2009) 1062–1075.
[23] A. Criollo, L. Senovilla, H. Authier, M.C. Maiuri, E. Morselli, I. Vitale, O. Kepp, E. Tasdemir, L. Galluzzi, S. Shen, M. Tailler, N. Delahaye, A. Tesniere, D. De Stefano, A.B. Younes, F. Harper, G. Pierron, S. Lavandero, L. Zitvogel, A. Israel, V. Baud, G. Kroemer, The IKK complex contributes to the induction of autophagy, EMBO J. 29 (2010) 619–631.
[24] A. Criollo, F. Chereau, S.A. Malik, M. Niso-Santano, G. Marino, L. Galluzzi, M.C. Maiuri, V. Baud, G. Kroemer, Autophagy is required for the activation of NFkappaB, Cell Cycle 11 (2012) 194–199.
[25] P. Kapahi, D. Chen, A.N. Rogers, S.D. Katewa, P.W. Li, E.L. Thomas, L. Kockel, With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging, Cell Metab. 11 (2010) 453–465.
[26] H.S. Ghosh, M. McBurney, P.D. Robbins, SIRT1 negatively regulates the mammalian target of rapamycin, PLoS One 5 (2010) e9199.
[27] K. Inoki, T. Zhu, K.L. Guan, TSC2 mediates cellular energy response to control cell growth and survival, Cell 115 (2003) 577–590.
[28] D.M. Gwinn, D.B. Shackelford, D.F. Egan, M.M. Mihaylova, A. Mery, D.S. Vasquez, B.E. Turk, R.J. Shaw, AMPK phosphorylation of raptor mediates a metabolic checkpoint, Mol. Cell 30 (2008) 214–226.
[29] F. Lan, J.M. Cacicedo, N. Ruderman, Y. Ido, SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation, J. Biol. Chem. 283 (2008) 27628–27635.
[30] T.T. Schug, Q. Xu, H. Gao, A. Peres-da-Silva, D.W. Draper, M.B. Fessler, A. Purushotham, X. Li, Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress, Mol. Cell Biol. 30 (2010) 4712–4721.
[31] S. Timmers, E. Konings, L. Bilet, R.H. Houtkooper, T. van de Weijer, G.H. Goossens, J. Hoeks, S. van der Krieken, D. Ryu, S. Kersten, E. Moonen-Kornips, M.K. Hesselink, I. Kunz, V.B. Schrauwen-Hinderling, E.E. Blaak, J. Auwerx, P. Schrauwen, Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans, Cell Metab. 14 (2011) 612–622.
[32] L. Chen, W. Fischle, E. Verdin, W.C. Greene, Duration of nuclear NF-kappaB action regulated by reversible acetylation, Science 293 (2001) 1653–1657.
[33] R. Kiernan, V. Bres, R.W. Ng, M.P. Coudart, S. El Messaoudi, C. Sardet, D.Y. Jin, S. Emiliani, M. Benkirane, Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65, J. Biol. Chem. 278 (2003) 2758–2766.