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SIRT3 protects cardiomyocytes from oxidative stress-mediated cell death by activating NF-κB

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ABSTRACT

Oxidative stress-mediated cell death in cardiomyocytes reportedly plays an important role in many cardiac pathologies. Our previous report demonstrated that mitochondrial SIRT3 plays an essential role in mediating cell survival in cardiac myocytes, and that resveratrol protects cardiomyocytes from oxidative stress-induced apoptosis by activating SIRT3. However, the exact mechanism by which SIRT3 prevents oxidative stress remains unknown. Here, we show that exposure of H9c2 cells to 50 μM H_2O_2 for 6 h caused a significant increase in cell death and the down-regulation of SIRT3. Reactive oxygen species (ROS)-mediated NF-κB activation was involved in this SIRT3 down-regulation. The SIRT3 activator, resveratrol, which is considered an important antioxidant, protected against H_2O_2-induced cell death, whereas the SIRT inhibitor, nicotinamide, enhanced cell death. Moreover, resveratrol negatively regulated H_2O_2-induced NF-κB activation, whereas nicotinamide enhanced H_2O_2-induced NF-κB activation. We also found that SOD2, Bcl-2 and Bax, the downstream genes of NF-κB, were involved in this pathological process. These results suggest that SIRT3 protects cardiomyocytes exposed to oxidative stress from apoptosis via a mechanism that may involve the NF-κB pathway.

1. Introduction

Emerging evidence suggests that progressive cardiomyocyte death due to apoptosis is responsible for the anatomic remodeling of myocardium in pathological processes. Oxidative stress is a well-known factor that promotes apoptosis; it has been implicated in the pathogenesis of a variety of diseases, including cardiovascular disease [1,2]. Despite increasing experimental and clinical evidence, the mechanisms underlying oxidative stress-induced cardiomyocyte apoptosis are largely unexplored. Therefore, an understanding of the mechanisms regulating cardiomyocyte cell survival and/or death pathways remains an important goal in cardiac cell biology.

SIRT3 is a class III histone deacetylase (HDAC); the HDACs are also called sirtuins (SIRTs). SIRTs have been demonstrated to play important roles in many physiological and pathophysiological conditions, including metabolism, cell survival, cancer, aging and calorie restriction-mediated longevity of organisms ranging from yeasts to humans [3–5]. In addition to histones, SIRT3 also deacetylates other proteins, including acetyl-CoA synthetase 2 (AceCS2) [6,7], glutamate dehydrogenase (GDH) [8], FoxO3a [9,10], Ku70 [11], p53 [12], SOD2 [13], and PGC-1α [14]. Thus, SIRT3 can regulate cellular metabolism via its effects on gene expression. Several studies have shown that SIRT3 is a key regulator of cell defense and survival in response to stress [10–14]. Our previous report also demonstrated that mitochondrial SIRT3 plays an essential role in mediating cell survival in cardiac myocytes, and that resveratrol protects cardiomyocytes from oxidative stress-induced apoptosis by activating SIRT3 [15]. However, the exact mechanism by which SIRT3 prevents oxidative stress remains unknown.

NF-κB is a transcription factor that regulates genes involved in cell survival, cell adhesion, inflammation, differentiation and growth; it is activated by a variety of stimuli including carcinogenesis, inflammatory agents such as TNF-α and H_2O_2, and tumor promoters [16]. Extensive research during the last few years has shown that H_2O_2 may directly activate a unique intracellular signaling pathway responsible for NF-κB activation [17]. Whether the protective effect of SIRT3 on oxidative stress-mediated cell death in cardiomyocytes depends on an NF-κB pathway has not been demonstrated.

This study was designed to examine the role of the SIRT3–NF-κB pathway in cardiomyocytes. We show that exposure of H9c2 cells to 50 μM H_2O_2 for 6 h caused a significant increase in cell death and the down-regulation of SIRT3. Overexpression of SIRT3 protected cells against stress-mediated cell death. We also identified NF-κB as a new target of SIRT3. Activation of NF-κB by SIRT3

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altered the expression of SOD2, Bcl-2 and Bax, the downstream genes of NF-κB, thus making cells resistant to oxidative stress-mediated cell damage. These results demonstrate that SIRT3 is a survival factor for cardiomyocytes under stress conditions.

2. Materials and methods

2.1. Cell line and materials

The H9c2 embryonal rat heart-derived cell line was obtained from American Type Culture Collection. Resveratrol, nicotinamide and 3-(4,5-dimetrylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). Antibodies against SIRT3 and NF-κB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The In Situ Cell Apoptosis Detection Kit was purchased from Promega.

2.2. Cell culture and treatment

H9c2 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C for 48 h in a 5% CO_2 atmosphere. The myocytes were then divided into the following groups: (1) control (Control), in which myocytes were incubated in normal medium for an additional 6 h; (2) oxidative stress (H_2O_2), in which myocytes were incubated in DMEM containing 50 μM H_2O_2 for 6 h; (3) resveratrol (RES), in which 20 μM resveratrol was added 30 min before exposure to H_2O_2; and (4) nicotinamide (NAM), in which 20 mM nicotinamide was added 30 min before exposure to H_2O_2.

2.3. Cell viability measurement by MTT assay

H9c2 cells were dispensed in flat-bottomed 96-well microtiter plates at a density of 1×10^4 cells/well. MTT assays were performed to determine cell viability according to the manufacturer's protocols. Briefly, after treatment with H_2O_2, the MTT labeling reagent (5 mg/ml MTT in PBS) was added to the cells at a final concentration of 0.5 mg/ml, and the cells were incubated for 4 h. The medium was then removed and 150 μl of dimethyl sulfoxide (DMSO) were added to each well, and the plates were agitated for 10 min on a shaker to dissolve the formazan product. Finally, a well with DMSO but without cells was used as a blank and the OD value of each well at 490 nm was determined on a microtiter plate reader (KHB labsystems Wellscan K3, Finland).

2.4. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining

We detected TUNEL-positive cells using the *In Situ* Cell Apoptosis Detection kit, according to the manufacturer's instructions. Cells were fixed with 4% paraformaldehyde and stained with the reagents in the TUNEL assay kit. The number of stained cells was assessed using the computer software program, Simple PCI/BX51 Image.

2.5. Immunofluorescence microscopy

For immunofluorescence analysis, H9c2 cells were fixed with 4% paraformaldehyde in PBS for 20 min and permeabilized by treatment with 0.5% Triton X-100 in PBS for 10 min. After blocking with 2% BSA in PBS for 30 min, the cells were incubated with a rabbit polyclonal antibody against NF-κB p65, followed by staining with an Alexa Fluor 555 anti-rabbit secondary antibody (Molecular Probes). The nuclei of the cells were stained with 10 mM Tris–HCl (pH 7.4), 10 mM EDTA, 100 mM NaCl, and 500 ng/ml 4′,6-diamidino-2-phenyl-indol dihydrochloride (DAPI, Sigma) for 15 min at room temperature. The stained cells were visualized on a fluorescence microscope (Olympus BX51, Japan).

2.6. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated from H9c2 cells using the RNA simple Total RNA Kit (Tiangen, Beijing, China). cDNA was synthesized from 1 μg of total RNA using a cDNA Synthesis Kit (Takara Biotechnology Co., Ltd., China). Primer sequences (Genecore, Shanghai, China) were as follows: 18S rRNA (forward: 5′-ATTCCGATAACGAACGAG AC-3′, reverse: 5′-GGCATCACAGACCTGTTA TTG-3′); SIRT3 (forward: 5′-TACTTCCTTCGGCTGCTTCA-3′, reverse: 5′-AAG GCGAA ATCAGCCACA-3′); SOD2 (forward: 5′-TTGCCACCCGTGTTGCTA-3′, reverse: 5′-AGCTTGTTGGGCCGCTAA-3′); Bcl-2 (forward: 5′-TCCACCCTCC TTCGGTTT-3′, reverse: 5′-ACTGTGCCAATGATCCCG-3′); Bax (forward: 5′-TACCCTTGAGCTGCACTCC-3′, reverse: 5′-AGGGCACAAGACACACACA T-3′). After cDNA synthesis, the PCR reaction consisted of 32 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a further 6 min at 72 °C in the last cycle. PCR products were separated by electrophoresis on a 2% agarose gel and visualized by staining with ethidium bromide. The relative expression was quantified densitometrically using the Gel Image Ver. 3.74 System (Tianon, Shanghai, China).

2.7. Immunoblotting

Immunoblotting was used to analyze SIRT3 expression relative to beta-actin. After treatment, H9c2 cells were washed three times with cold PBS, and then lysed with RIPA lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1% NP-40, 1 mM phenylmethyl-sulfonyl fluoride (PMSF) and 1 mM EDTA. Samples were separated on 12% SDS–polyacrylamide gels (BioRad, Hercules, CA, USA) and transferred to polyvinylidene fluoride membranes. Membranes were blocked for 1 h at room temperature with 5% dry milk in TPBS (PBS containing 0.1% Tween 20), and then incubated with the appropriate primary antibodies (SIRT3 antibody was diluted 1:200, and beta-actin 1:1000) overnight at 4 °C. After washing with TPBS, membranes were incubated with horseradish peroxidase (HRP)-linked secondary antibodies (diluted 1:2000 in TPBS containing 5% dry milk) at room temperature for 1 h. The immune complexes were visualized by enhanced chemiluminescence (ECL) methods, and the band intensities were measured and quantitated. The resulting images were analyzed with Quantity One software (BioRad, Hercules, CA, USA).

2.8. Statistical analysis

Dates are expressed as means ± SD for three or more independent experiments. Statistical significance was estimated by one-way ANOVA followed by Student–Newman–Keuls test for comparison of several groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. H_2O_2-induced cytotoxicity in H9c2 cells

H9c2 cells treated with 50 μM H_2O_2 for 6 h exhibited morphological alterations such as cell shrinkage, and detached from the wells of the culture plates (Fig. 1(A)). Resveratrol pre-treatment prevented these changes, whereas nicotinamide exacerbated these effects. We also assessed cell viability using the MTT assay. H_2O_2

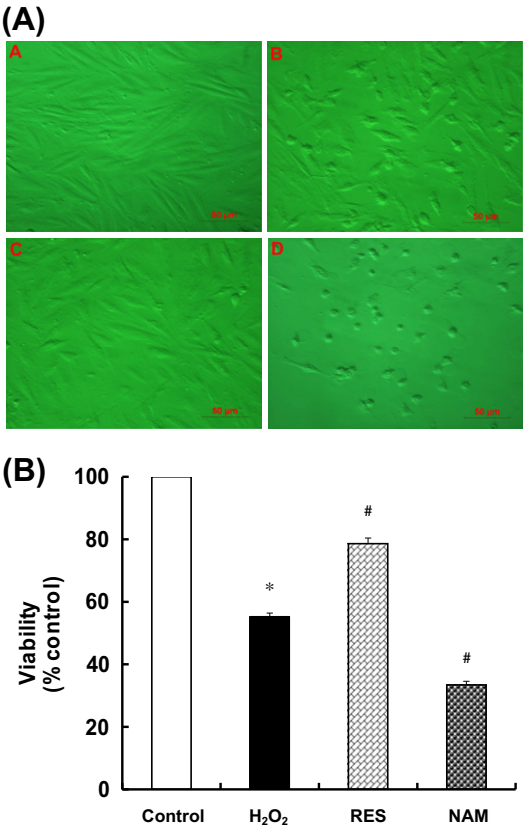

Fig. 1. (A) Morphological characteristics of H9c2 cells after H_2O_2 treatment in the absence or presence of resveratrol or nicotinamide: A. no treatment; B. H9c2 cells exposed to 50 μM H_2O_2 for 6 h; C. H9c2 cells treated with 20 μM resveratrol for 30 min before exposure to 50 μM H_2O_2 for 6 h; D. H9c2 cells treated with 20 mM nicotinamide for 30 min before exposure to 50 μM H_2O_2 for 6 h. (B) Effects of resveratrol or nicotinamide on H_2O_2-induced cytotoxicity in H9c2 cells. H9c2 cells were pretreated with resveratrol (20 μM) or nicotinamide (20 mM) for 30 min, and the indicated concentrations of H_2O_2 were then added to the medium. After incubation for 6 h at 37 °C, cell viability was determined by the MTT reduction assay as described in Section 2. Data are the mean ± SD values for three independent experiments. ($^*P < 0.05$ compared with Control; $^\#P < 0.05$ compared with H_2O_2).

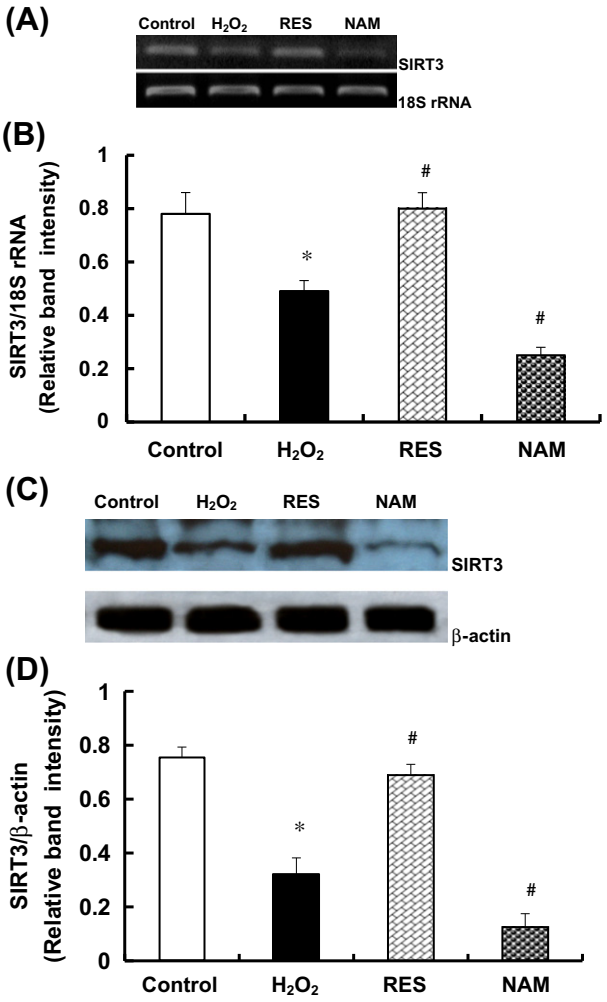

Fig. 2. SIRT3 mRNA and Protein expression in H9c2 cells measured by RT-PCR and Western blot. Cells were incubated in normal medium (Control) or in the presence of 50 μM H_2O_2 (H_2O_2), 20 μM resveratrol + H_2O_2 (RES), or 20 mM nicotinamide + H_2O_2 (NAM) for 6 h. (A) Representative ethidium bromide stained gels showing SIRT3 and 18S rRNA in the same samples. (B) The abundance of SIRT3 mRNA is shown as a ratio to 18S rRNA. (C) Representative western blots showing SIRT3 protein and β-actin in the same samples. (D) The abundance of SIRT3 protein is shown as a ratio to β-actin. The results represent the mean ± SD of three independent determinations. ($^*P < 0.05$ compared with Control; $^\#P < 0.05$ compared with H_2O_2).

decreased cell viability and its cytotoxic effects were attenuated in the presence of resveratrol (20 uM), a SIRT3 activator, which is considered an important antioxidant (Fig. 1(B)). However, a significant increase in cell death was observed when the SIRT inhibitor (20 mM nicotinamide) was added before H_2O_2 pretreatment.

3.2. SIRT3 plays an essential role in H_2O_2-induced cell death

H9c2 cells were treated with H_2O_2 (50 μM, 6 h) and SIRT3 mRNA and protein levels were determined by RT-PCR and western blot analysis, respectively. H9c2 cells treated with H_2O_2 exhibited significantly decreased SIRT3 mRNA and protein expression (Fig. 2). Moreover, pretreatment with resveratrol significantly increased SIRT3 mRNA and protein expression (Fig. 2). However, SIRT3 mRNA and protein expression were significantly decreased when the cells were pretreated with nicotinamide (Fig. 2).

3.3. Overexpression of SIRT3 attenuated H_2O_2-induced apoptotic cell death

Apoptosis in H9c2 cells was detected by TUNEL assay. As shown in Supplementary Fig. 1, the proportion of TUNEL-positive cells was significantly greater in the H_2O_2 group than in the normal control group (Control: 1.23 ± 0.34% vs. H_2O_2: 21.45 ± 3.08%, $P < 0.05$). However, pretreatment with resveratrol significantly inhibited the induction of apoptosis by H_2O_2 (RES: 8.04 ± 1.46% vs. H_2O_2, $P < 0.05$). Meanwhile, the number of apoptotic cells was significantly increased following addition of 20 mM nicotinamide, a SIRT inhibitor (NAM: 35.66 ± 4.24% vs. H_2O_2; $P < 0.05$).

3.4. SIRT3 protects cardiomyocytes from H_2O_2-mediated cell death by activating NF-κB

To explore the molecular mechanisms responsible for the protective effect of SIRT3 against H_2O_2-induced oxidative cell death, we examined NF-κB intranuclear translocation by immunofluorescence microscopy. H9c2 cells treated with H_2O_2 exhibited more pronounced intranuclear NF-κB p65 (Fig. 3B, images at 60 min are shown). Moreover, while exposure of cells to 20 μM resveratrol caused NF-κB p65 to exit the nucleus (Fig. 3C), this effect was reversed by 20 mM nicotinamide (Fig. 3D).

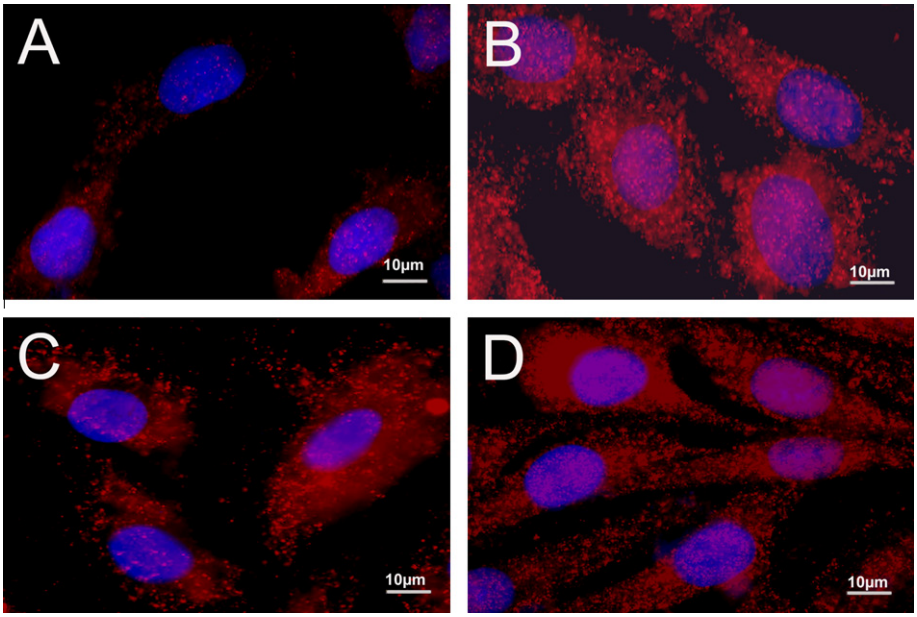

Fig. 3. Analysis of the effect of SIRT3 on H_2O_2-induced NF-κB activation by immunofluorescence microscopy. A. Control group, B. H_2O_2 group, C. RES group, D. NAM group. H9c2 cells treated with H_2O_2 for 60 min exhibited more pronounced intranuclear NF-κB p65 (B). Exposure of cells to resveratrol (20 μM) induced the translocation of NF-κB p65 from the nucleus to the cytoplasm (C), but this effect could be reversed by 20 mM nicotinamide (D). Images were acquired at a 1000× magnification. The data are representative of three independent experiments that provided similar results.

3.5. Activation of NF-κB by SIRT3 altered the expression of mitochondrial-related genes

NF-κB is a transcription factor that regulates genes involved in cell survival, cell adhesion, inflammation, differentiation and growth [16]. To investigate the role of SIRT3 in response to oxidative stress, we analyzed the expression of SOD2, Bcl-2 and Bax, the downstream genes of NF-κB, by PT-PCR. The RT-PCR results showed that oxidative stress caused the down-regulation of SOD2 and Bcl-2, but the up-regulation of Bax. Exposure of cells to 20 uM resveratrol caused rapid activation of SOD2 and Bcl-2, but inhibited the expression of Bax. This effect could be also reversed by 20 mM nicotinamide (Fig. 4).

4. Discussion

Oxidative stress in cardiomyocytes plays an important role in the pathogenesis of both heart failure and ischemic-reperfusion injury [2]. Our previous report demonstrated that mitochondrial SIRT3 plays an essential role in mediating cell survival, and that resveratrol protects cardiomyocytes from oxidative stress-induced apoptosis by activating SIRT3 [15]. However, the pro-survival effects of SIRT3 on oxidative damage in cardiac myocytes have not been elucidated. In this study, we have identified NF-κB as a new target of SIRT3. Activation of NF-κB by SIRT3 alters the expression of SOD2, Bcl-2 and Bax, the downstream genes of NF-κB, and confers resistance to oxidative stress-mediated cell damage. These studies demonstrate that SIRT3 protects cardiomyocytes from oxidative stress-induced apoptosis by activating the NF-κB pathway.

Oxidative stress influences multiple anti- and pro-apoptotic signaling pathways. Here, we focused particularly on the role of the Sir2 family in apoptosis pathways. Sir2, a class III histone deacetylase (HDAC), mediates lifespan extension in model organisms and prevents apoptosis in mammalian cells [18]. SIRT3 is a class III HDAC. AceCS2, a mitochondrial enzyme that converts acetate into acetyl-CoA, was the first identified mitochondrial substrate of SIRT3 [6,7]. GDH, a mitochondrial protein involved in energy production, is deacetylated by SIRT3 [8]. Ku70 and the forkhead transcription factor, FoxO3a, are also substrates of SIRT3 [9–11]. SIRT3 deficiency caused increased mitochondrial ROS production and subsequent developmental arrest attributed to p53 activation in pre-implantation embryos [12]. Overexpression of SIRT3 in brown adipocytes activates CREB phosphorylation and results in stimulation of PGC-1α gene expression, which consequently decreases the cellular ROS level [14]. Another recent study reported that SIRT3 deacetylates and activates SOD2 [13]. Thus, SIRT3 can regulate cellular metabolism and gene expression. Several studies have shown that SIRT3 is a key regulator of cell defense and survival responses to stress [10–14].

In this study, we demonstrated that H_2O_2 treatment induces apoptosis in H9c2 cells. A sustained oxidative insult, mediated by exposure of H9c2 cells to 50 μM H_2O_2 for up to 6 h, triggered an intracellular death cascade leading to remarkable cell death. The H_2O_2-induced cell death was typical of apoptosis, as demonstrated by TUNEL assay. The expression of endogenous SIRT3 was decreased under conditions of oxidative stress, suggesting that endogenous SIRT3 plays an essential role in mediating cell survival in cardiac myocytes. Resveratrol, a natural SIRT3 activator, protected against H_2O_2-induced cell death, whereas the SIRT inhibitor, nicotinamide, enhanced cell death.

The nuclear transcription factor NF-κB, is recognized as an important target for analyzing the physiological roles of early response genes in the cellular response to changes in intracellular redox status and subsequent oxidative stress [17]. Accumulating evidence has demonstrated that certain antioxidants and antioxidant enzymes can lower the intracellular accumulation of ROS by inhibiting the activation of NF-κB [19,20]. The present study revealed that H_2O_2 induced NF-κB activation in H9c2 cells. Resveratrol negatively regulated H_2O_2-induced NF-κB activation, whereas nicotinamide enhanced H_2O_2-induced NF-κB activation.

NF-κB induces the transcription of a variety of genes, including genes involved in the oxidative stress response (SODs), cell adhesion (ICAM-1 and VCAM-1), inflammation (IL-6 and IL-8), and apoptosis (Bcl-2 and Bax) [21]. Superoxide dismutases (SODs) are a class of enzymes that catalyze the detoxification of superoxide

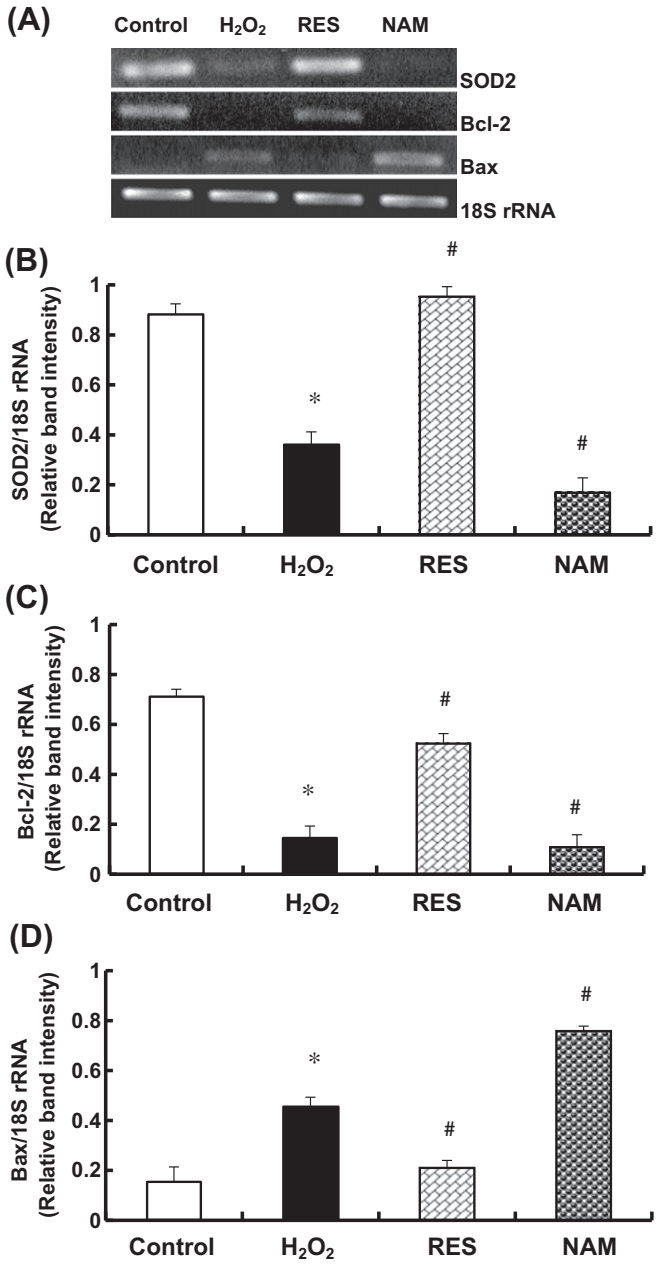

Fig. 4. Analysis of the expression of SOD2, Bcl-2 and Bax mRNAs, the downstream genes of NF-κB, by PT-PCR. H9c2 cells were incubated in normal medium (Control) or in the presence of 50 μM H_2O_2 (H_2O_2), 20 μM resveratrol + H_2O_2 (RES), or 20 mM nicotinamide + H_2O_2 (NAM) for 6 h. (A) Representative ethidium bromide stained gels showing the target genes and 18S rRNA in the same sample. (B) The abundance of SOD2 mRNA is shown as a ratio to 18S rRNA. (C) The abundance of Bcl-2 mRNA is shown as a ratio to 18S rRNA. (D) The abundance of Bax mRNA is shown as a ratio to 18S rRNA. The results represent the mean ± SD of three independent determinations. ($^{*}P < 0.05$ compared with Control; $^{\#}P < 0.05$ compared with H_2O_2).

into oxygen and hydrogen peroxide, the latter of which is then converted to oxygen and water by catalase. SODs are believed to be present in all oxygen-metabolizing organisms, and the physiological role of SODs is to balance the level of intracellular reactive oxygen species (ROS), products of aerobic metabolism normally produced in the mitochondria. Although low levels of ROS contribute to cell signaling and cell proliferation, excess ROS, because of their highly reactive nature, cause damage to a range of cellular constituents, including proteins, lipids and, in particular, DNA.

Mammalian cells express three forms of SOD that, although catalyzing the same reaction, differ in their metal co-factor binding and subcellular localization. SOD1 binds to copper and zinc and localizes in the cytosol; SOD2 binds to manganese and localizes in the mitochondria; and SOD3 binds to copper and zinc and is secreted into extracellular fluid. Of these three forms, mitochondrial SOD2 is thought to play a crucial role in controlling the level of oxygen and producing a large flux of ROS. Mutations in SOD2 are associated with ageing and various human diseases including idiopathic cardiomyopathy, sporadic motor neuron disease and cancer [22].

Oxidative stress-induced SOD2 expression is believed to be an important cellular defense mechanism [22,23]. Recently, Chen et al. [13] demonstrated that the mitochondrial deacetylase, SIRT3, binds to, deacetylates, and activates SOD2. Increased reactive oxygen species (ROS) levels stimulate SIRT3 transcription, leading to SOD2 deacetylation and activation [13]. In this study, we found that overexpression of SIRT3 in H9c2 cells restrained NF-κB intranuclear translocation, which stimulated SOD2 gene expression and consequently conferred cells with resistance against oxidative insults.

Furthermore, we observed the expression of apoptosis-related genes, including the anti-apoptotic protein, Bcl-2, and the pro-apoptotic protein, Bax. Resveratrol promoted the expression of Bcl-2, reduced the expression of Bax and inhibited apoptosis. By contrast, treatment with 20 mM nicotinamide promoted the expression of Bax, but reduced Bcl-2, and increased the rate of apoptosis. A study by Sundaresan et al. [11] demonstrated that deacetylation of Ku70 by SIRT3 promotes the interaction between Ku70 and Bax, and confers resistance to Bax-mediated cell damage. However, our results showed that overexpression of SIRT3 can activate NF-κB, enhance the ratio of Bcl-2 to Bax, and thereby protect cells against stress-mediated cell death.

5. Conclusion

In summary, the results presented here show that: (1) endogenous SIRT3 plays an essential role in mediating cell survival in cardiomyocytes; (2) overexpression of SIRT3 protects cells against oxidative stress-mediated cell death; and (3) the molecular mechanisms responsible for the protective effect of SIRT3 may involve the activity of the NF-κB pathway. Because oxidative stress plays an important role in the development of cardiovascular disease, our findings suggest that the stress-responsive regulatory enzyme, SIRT3, may be a potential therapeutic target for ROS-related diseases.

Acknowledgments

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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.2012.11.066.

References

[1] M.T. Crow, K. Mani, Y.J. Nam, R.N. Kitsis, The mitochondrial death pathway and cardiac myocyte apoptosis, Circ. Res. 95 (2004) 957–970.
[2] C.E. Murdoch, M. Zhang, A.C. Cave, A.M. Shah, NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure, Cardiovasc. Res. 71 (2006) 208–215.
[3] S. Michan, D. Sinclair, Sirtuins in mammals: insights into their biological function, Biochem. J. 404 (2007) 1–13.
[4] L.R. Saunders, E. Verdin, Sirtuins: critical regulators at the crossroads between cancer and aging, Oncogene 26 (2007) 5489–5504.

[5] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, Annu. Rev. Pathol. 5 (2010) 253–295.
[6] W.C. Hallows, S. Lee, J.M. Denu, Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases, Proc. Natl. Acad. Sci. USA 103 (2006) 10230–10235.
[7] B. Schwer, J. Bunkenborg, R.O. Verdin, J.S. Andersen, E. Verdin, Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2, Proc. Natl. Acad. Sci. USA 103 (2006) 10224–10229.
[8] D.B. Lombard, F.W. Alt, H.L. Cheng, J. Bunkenborg, R.S. Streeper, R. Mostoslavsky, et al., Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation, Mol. Cell Biol. 27 (2007) 8807–8814.
[9] K.M. Jacobs, J.D. Pennington, K.S. Bisht, N. Aykin-Burns, H.S. Kim, M. Mishra, et al., SIRT3 interacts with the daf-16 homolog FOXO3a in the mitochondria, as well as increases FOXO3a dependent gene expression, Int. J. Biol. Sci. 4 (2008) 291–299.
[10] N.R. Sundaresan, M. Gupta, G. Kim, S.B. Rajamohan, A. Isbatan, M.P. Gupta, Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice, J. Clin. Invest. 119 (2009) 2758–2771.
[11] N.R. Sundaresan, S.A. Samant, V.B. Pillai, S.B. Rajamohan, M.P. Gupta, SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70, Mol. Cell Biol. 28 (2008) 6384–6401.
[12] Y. Kawamura, Y. Uchijima, N. Horike, K. Tonami, K. Nishiyama, T. Amano, et al., Sirt3 protects in vitro-fertilized mouse preimplantation embryos against oxidative stress-induced p53-mediated developmental arrest, J. Clin. Invest. 120 (2010) 2817–2828.
[13] Y. Chen, J. Zhang, Y. Lin, Q. Lei, K.L. Guan, S. Zhao, et al., Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS, EMBO Rep. 12 (2011) 534–541.
[14] X. Kong, R. Wang, Y. Xue, X. Liu, H. Zhang, Y. Chen, et al., Sirtuin 3, a new target of PGC-1α, plays an important role in the suppression of ROS and mitochondrial biogenesis, PLoS One 5 (2010) e11707.
[15] W. Yu, Y.C. Fu, X.H. Zhou, C.J. Chen, X. Wang, R.B. Lin, et al., Effects of resveratrol on H_2O_2-induced apoptosis and expression of SIRTs in H9c2 cells, J. Cell Biochem. 107 (2009) 741–747.
[16] C.J. Chen, W. Yu, W. Wang, Red wine may be used in the therapy of myocarditis, J. Cell Biochem. 111 (2010) 808–810.
[17] J.H. Jang, Y.J. Surh, Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells, Mutat. Res. 496 (2001) 181–190.
[18] R.R. Alcendor, L.A. Kirshenbaum, S. Imai, S.F. Vatner, J. Sadoshima, Silent information regulator 2alpha, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes, Circ. Res. 95 (2004) 971–980.
[19] M. Meyer, R. Schreck, P.A. Baeuerle, H_2O_2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor, EMBO J. 12 (1993) 2005–2015.
[20] K.N. Schmidt, P. Amstad, P. Cerutti, P.A. Baeuerle, The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B, Chem. Biol. 2 (1995) 13–22.
[21] J.W. Gordon, J.A. Shaw, L.A. Kirshenbaum, Multiple facets of NF-κB in the heart: to be or not to NF-κB, Circ. Res. 108 (2011) 1122–1132.
[22] L. Miao, D.K. St Clai, Regulation of superoxide dismutase genes: implications in disease, Free Radic. Biol. Med. 47 (2009) 344–356.
[23] X. Qiu, K. Brown, M.D. Hirschey, E. Verdin, D. Chen, Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation, Cell Metab. 12 (2010) 662–667.