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RESEARCH ARTICLES

Resveratrol up-regulates SIRT1 and inhibits cellular oxidative stress in the diabetic milieu: mechanistic insights☆,☆☆

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Abstract

Several lines of evidence support a role for oxidative stress in diabetic complications. Diabetic patients have increased O_2^- production in monocytes. Loss of SIRT1 activity may be associated with metabolic diseases such as diabetes. Several studies have shown that SIRT1 can regulate mammalian FOXO transcription factors through direct binding and/or deacetylation. However, interactions between SIRT1 and FOXO under diabetic conditions are unclear. The phytochemical resveratrol has recently gained attention for its protection against metabolic disease. Resveratrol has been shown to increase mitochondrial function by activating SIRT1.

In this study, we tested the protective effect of resveratrol on cellular oxidative stress through the SIRT1–FOXO pathway under high-glucose conditions. Human monocytic (THP-1) cells were cultured in the presence of mannitol (osmolar control) or normoglycemic (NG, 5.5 mmol/l glucose) or hyperglycemic (HG, 25 mmol/l glucose) conditions in absence or presence of resveratrol (3 and 6 μmol/l) for 48 h. We first examined SIRT1 activity and oxidative stress in monocytes of Type 1 diabetes mellitus (T1DM) patients compared with healthy controls. In T1DM patients, monocytic SIRT1 expression was significantly decreased and p47phox expression was increased compared with controls. Under HG *in vitro*, SIRT1 and FOXO3a were significantly decreased compared with NG, and this was reversed by resveratrol treatment, concomitant with reduction in HG-induced superoxide production and p47phox. Under HG, SIRT1 small interfering RNA (siRNA) inhibited FOXO3a, and there was no beneficial effect of resveratrol in siRNA-treated HG-induced cells. Thus, resveratrol decreases HG-induced superoxide production via up-regulation of SIRT1, induction of FOXO3a and inhibition of p47phox in monocytes.

Keywords: SIRT1; FOXO3a; p47phox; Oxidative stress; Resveratrol and diabetes

1. Introduction

Hyperglycemia contributes to vascular complications of diabetes. High glucose has been shown to induce inflammatory cytokines, chemokines, p38 mitogen-activated protein kinase, reactive oxygen species (ROS), protein kinase C (PKC) and nuclear factor-κB (NF-κB) activity in both clinical and experimental systems [1–6]. Several lines of evidence support a role for oxidative stress in the development of diabetes complications [7,8]. Diabetic patients have increased O_2^- production in monocytes and neutrophils [2,7,9]. Excess accumulation of ROS can result from defects in ROS scavenging and is believed to have an impact on cellular aging and the senescence process [10]. nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is accepted as the most important mechanism for ROS generation in phagocytic cells. Previously, we have shown that p47phox, an essential component of monocyte NADPH oxidase, is required for ROS generation under high-glucose conditions [11].

Recently, SIRT1, the mammalian homologue of yeast Sir2, was identified as a key mediator that links calorie restriction and longevity in mammals. Sirtuins are a conserved family of NAD-dependent deacetylases (class III histone deacetylases) [12–16]. To date, seven members of sirtuin proteins (*i.e.*, SIRT1–SIRT7) have been identified in humans. Recent studies have demonstrated that SIRT1 plays an important role in the regulation of cell death/survival and stress response in mammals. SIRT1 promotes cell survival by inhibiting apoptosis or cellular senescence induced by stresses, including DNA damage and oxidative stress [12–17]. An increasing number of proteins have been identified as substrates of SIRT1, including p53 [18–21], Forkhead box O (FOXO) transcription factors [22–28]. Improper regulation of sirtuin proteins has been reported in a number of diseases, including Bowen's disease [29], type I diabetic nephropathy [30], Alzheimer's disease and amyotrophic lateral sclerosis [31], and nonalcoholic fatty liver disease [32]. It has been suggested that loss of Sirt1 activity may be associated with metabolic diseases such as T2DM and atherosclerosis [33–35].

FOXO transcription factors FOXO1 (FKHR) [36], FOXO3a (FKHRL1) [37], FOXO4 (ARX) [38] and FOXO6 [39] are emerging as an

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important family of proteins that modulate the expression of genes involved in apoptosis, the cell cycle, DNA damage repair, oxidative stress, cell differentiation, glucose metabolism and other cellular functions [22–24,26,40]. Several studies have shown that SIRT1 can control the cellular response stress by regulate mammalian FOXO transcription factors through direct binding and/or deacetylation [12,22,24,26,28,41]. However, the mechanism of the interactions between SIRT1 and FOXO under hyperglycemic conditions is not well understood. FOXO transcription factors regulate antioxidant expression and DNA damage repair. Among all FOXO members, FOXO3a appears to have an important role under oxidative stress. Foxo3 plays an important role in the *in vivo* regulation of oxidative stress in mammals. FOXO3a up-regulates transcription of the ROS scavenging enzymes superoxide dismutase 2 [SOD2, also known as manganese superoxide dismutase (MnSOD)] and catalase [42–44]. Thus, FOXO3 appears to be a critical physiological regulator of oxidative stress in mammalian cells.

One important sirtuin-activating compounds is the natural product resveratrol (3,4,5-trihydroxystilbene), a polyphenol that is synthesized by plants and is present in grapes and red wine [45]. Recently, resveratrol has been shown to improve energy balance and increase mitochondrial function in mice by activating SIRT1 [46]. Resveratrol has previously gained considerable attention because of its beneficial effects as a cardioprotective, cancer chemopreventive, and chemotherapeutic agent [47–51].

Previously, we have shown increased monocytic superoxide in Types 1 and 2 diabetes mellitus (T1DM and T2DM, respectively) and subsequently showed under HG condition that this is mediated via up-regulation of PKCα and p47phox [11]. However, the role of SIRT1 in regulating monocytic superoxide under HG condition is not well understood. Moreover, the effect of resveratrol, a potent SIRT1 inducer, on monocyte superoxide under HG conditions is not elucidated and is the focus of the present report. Thus, we hypothesize that resveratrol can suppress ROS production via regulatory mechanism involving FOXO3a, SIRT1 and p47phox under high-glucose conditions in human monocytes.

2. Materials and methods

2.1. Materials

Anti-FoxO family antibodies were procured from Cell Signaling Technology (Danvers, MA) and anti-SIRT1 was procured from Abcam (Cambridge, MA). Dihydroethidium (DHE) was purchased from Molecular Probes (Eugene, OR). Class III histone/protein deacetylase (HDAC) assay reagents (colorimetric) were purchased from Biovision (Mountain View, CA). The BCA protein assay kit was purchased from Pierce. Novex pre-cast Tris–glycine gels were obtained from Invitrogen (Carlsbad, CA). All other chemicals, unless otherwise stated, were obtained from Sigma (St. Louis, MO).

2.2. Cell culture and treatment with resveratrol

Human monocytes were obtained from T1DM patients and healthy controls as detailed previously [52]. Human monocytic THP-1 cell line was obtained from American Type Culture Collection (Manassas, VA). THP-1 cells were cultured in RPMI medium containing 10% fetal bovine serum and 1% antibiotics at 37 °C and 5% CO_2. resveratrol (dissolved in DMSO) was used for the treatment of cells. The final concentration of DMSO used was 0.1% (v/v) for each treatment. Human monocytic (THP-1) cells (1×10^5 cells/ml) were cultured in the presence of osmolar control (19.5 mmol/l mannitol), normoglycemic (NG, 5.5 mmol/l glucose) or hyperglycemic (HG, 25 mmol/l) conditions in absence or presence of resveratrol (3 and 6 μmol/l) for 48 h. Cells were washed with phosphate-buffered saline (PBS) and then harvested.

2.3. Trypan blue exclusion assay

Following treatments, cells were trypsinized and collected in a 1.5-ml Eppendorf tube. The cells were pelleted by centrifugation and resuspended in PBS (120 μl). Trypan blue (0.4% in PBS; 10 μl) was added to a smaller aliquot (10 μl) of cell suspension, and the number of cells (viable unstained and nonviable blue) were counted.

2.4. Immunostaining

For detection of SIRT1 by immunofluorescence, after treatment of cells with resveratrol, the cells were centrifuged and medium was aspirated. Cells were washed twice in PBS (10 mmol/l, pH 7.4) and placed on L-lysine coasted slides; the slides were air-dried, fixed with 4% formaldehyde for 30 min at 4 °C and stained overnight at 4 °C with SIRT1 and FOXO3a antibodies (1:1000 dilution). After being air-dried, slides were incubated with appropriate secondary antibody for 60 min. The slides were washed as described above, air-dried, mounted with mounting medium and then examined with a fluorescence microscope at ×400 magnification. To measure immunostaining intensity of Foxo3a and Sirt1, images were captured with a Nikon eclipse TE200 camera (Japan). The signal intensity was measured using ImageJ software.

2.5. Preparation of nuclear fraction

After treatment of cells with resveratrol the medium was aspirated and the cells washed twice in PBS (10 mmol/l, pH 7.4). Nuclear lysates were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce). Nuclear lysates were collected and cleared by centrifugation, and the supernatant aliquoted and stored at −80°C. The protein content in the lysates was measured by BCA protein assay (Pierce) per manufacturer's protocol.

2.6. Measurement of HDAC activity using ELISA

Following treatment of cells with various concentrations of resveratrol for 48 h, cells were harvested, nuclear lysates were prepared and 10 μg of nuclear lysate protein from each group were taken for determination of HDACs activity, respectively. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 405 and 440 nm.

2.7. Western blot analysis

For Western blot analysis, 12 μg of protein resolved over 10% Tris–glycine polyacrylamide gels (Novex), transferred onto nitrocellulose membranes and subsequently incubated in blocking buffer [5% nonfat dry milk/1% Tween 20; in 20 mmol/l TBS (pH 7.6)] for 2 h. The blots were incubated with appropriate primary antibody (SIRT1, FOXO1a, FOXO3a and p47phox), washed and incubated with appropriate secondary horseradish peroxidase-conjugated antibody (Amersham Biosciences). The blots were detected with chemiluminescence (ECL kit; Amersham Biosciences) and autoradiography using XAR-5 film (Eastman Kodak). Equal loading of protein was confirmed by stripping the blots and reprobing with Lamin (Sigma).

2.8. Measurement of intracellular superoxide production

To assess the production of superoxide, NG, HG and HG–resveratrol-treated cells were incubated with 5 μmol/l DHE (Molecular Probes), which is oxidized to the fluorescent intercalator, ethidium by cellular oxidants, particularly superoxide radicals. Cells (1×10^5) were stained with 5 μmol/l DHE for 30 min at 37°C and were washed and resuspended in PBS. The oxidative conversion of DHE to ethidium was measured by flow cytometry.

2.9. Small interfering RNA transfection assays

Prevalidated small interfering RNAs (siRNAs) were obtained from Santa Cruz Biotechnology, and transfection assays were performed as described previously [53,54] following the manufacturer's instructions, with suitable vehicle and scrambled siRNA controls. Transfection efficiency was around 70%. Subsequently, human monocytic cells (1×10^5 cells/ml) were cultured in the presence of osmolar control (9.5 mmol/l mannitol) or euglycemic (EG, 5.5 mmol/l glucose) or hyperglycemic (HG, 25 mmol/l) conditions in absence or presence of resveratrol (3 and 6 μmol/l) for 48 h. Then, cells were washed with PBS and harvested.

2.10. Statistical analysis

Each experiment was performed at least three times. Results are expressed as the means±S.D. Statistical analysis was performed using Student's *t* test and statistical significance is expressed as $^*P<.05$ and $^{**}P<.01$.

3. Results

SIRT1 expression and O_2^- production are altered in T1DM patients compared with normal healthy controls.

We first examined SIRT1 expression in monocytes of T1DM patients compared with normal healthy controls (Fig. 1). In T1DM patients, SIRT1 expression was significantly decreased compared with healthy controls ($n=8$/group). Monocyte membrane p47phox expression was significantly increased in T1DM compared with healthy

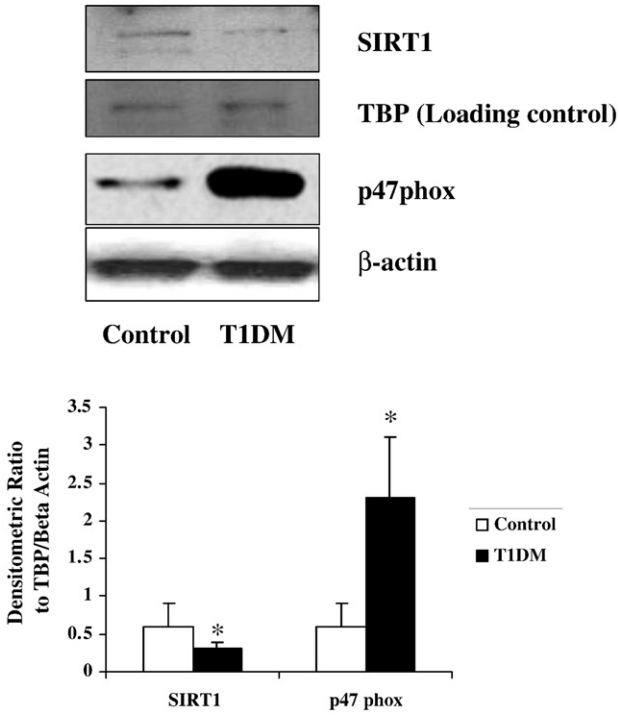

Fig. 1. Decreased SIRT1 expression and increased p47phox expression in T1DM subjects compared wirh normal subjects. Monocytes were isolated from fasting blood obtained from T1DM and normal subjects as described in Materials and Methods. For the Western blot analysis, protein was subjected to SDS-PAGE and used SIRT1 and p47phox antibodies (1:1000 dilutions) as detailed in Materials and Methods. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for TATA binding protein (TBP) and β-actin. The immunoblot shown here are representative of eight experiments with similar results. Densitometric ratios to TBP and β-actin are provided in the lower panel. *P<.01 compared with controls.

controls (P<.001). In addition, as shown previously [11], T1DM monocytes released significantly increased O_2^- as evidenced by increased DHE staining.

3.1 In vitro treatment with resveratrol significantly induced SIRT1 expression in HG conditions

To examine mechanisms, we tested pathways in human monocytic cells under HG conditions. Furthermore, since resveratrol upregulates SIRT1, we tested the effect of resveratrol on O_2^- production in monocytes under HG conditions. Next, we examined the effect of HG compared with NG on SIRT1 expression level by immunoblotting (Fig. 2A). Under HG, SIRT1 was significantly decreased compared with NG. Also, as shown in Fig. 2B, HDACs activity was decreased in HG conditions and this decrease was reversed by resveratrol (3 and 6 μmol/l) treatment.

3.2. In vitro treatment with resveratrol significantly induced FOXO3a expression in HG conditions

Next, we studied its specific regulation mechanisms. SIRT1 expression is associated with activation of FOXO family proteins. Under HG, FOXO3a expression was significantly decreased compared with NG (P<.001). As shown in Fig. 2C, down-regulation of FOXO3a under high glucose-induced conditions was significantly reversed by resveratrol (3 and 6 μmol/l) treatment. These findings were confirmed by immunofluorescence analysis (Fig. 3A and B). However, there was no effect on FOXO1a or p53 (data not shown).

3.3. In vitro treatment with resveratrol significantly suppressed O_2^- production in HG conditions

We examined the production of superoxide using DHE. Under HG, superoxide production was significantly increased compared with NG (P<.001). As shown in Fig. 4A, increased superoxide production under high glucose-induced monocytes was significantly down-regulated by resveratrol (3 and 6 μM) treatment. p47phox is an essential component of monocyte NADPH oxidase production and required for ROS generation under high-glucose conditions [11]. Therefore, we examined effect of resveratrol on NADPH oxidase (p47phox) under high glucose. Under HG, p47phox expression in the cell membrane was significantly increased compared with NG (P<.001). As shown in Fig. 4B, up-regulated p47phox expression in high glucose-induced monocytes was significantly down-regulated by resveratrol (3 and 6 μmol/l) treatment.

3.4. With SIRT1 knockdown, resveratrol fails to affect intracellular superoxide production

To determine if SIRT1 modulates FOXO3a and p47phox specifically in HG conditions in monocytes resulting in decreased superoxide, we

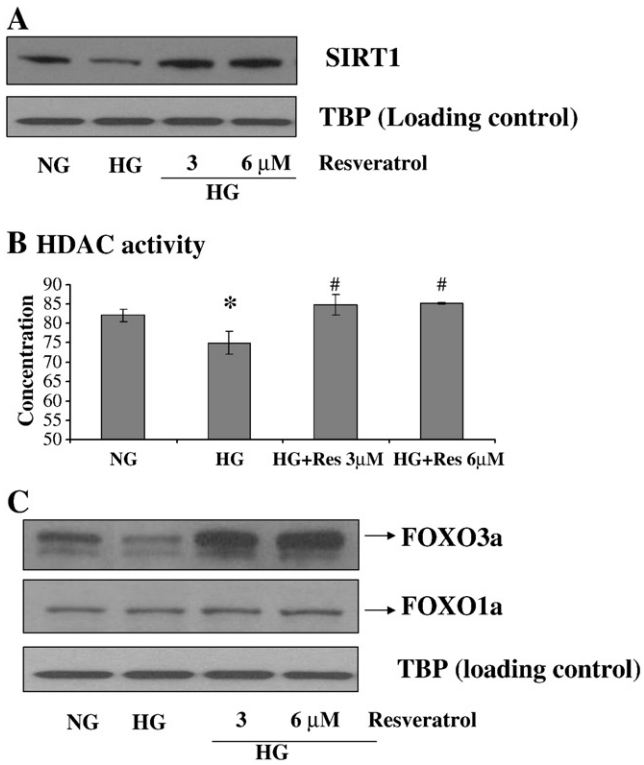

Fig. 2. (A) Resveratrol treatment significantly induced *SIRT1* gene expression and HDAC activity in HG-induced THP-1 cells *in vitro*. (B) Induced HDACs activity by resveratrol treatment in HG-induced THP-1 cells. (C) Resveratrol treatment induces *FOXO3a* gene expression in HG-induced THP-1 cells. (A, C) For the Western blot analysis, protein was subjected to SDS-PAGE and used SIRT1, FOXO3a and FOXO1a antibodies (1:1000 dilutions) as detailed in Materials and Methods. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for TATA binding protein (TBP). The immunoblot shown here are representative of three independent experiments with similar results. (B) Following treatment of cells with 3 and 6 μmol/l of reveratrol for 48 h as described in Materials and Methods, cells were harvested, nuclear lysates were prepared and 10 μg of nuclear lysate protein from each group were taken for determination of HDAC activity. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 405 and 440 nm using an ELISA reader. Results were shown as mean±S.D. of five different experiments. *P<.05 compared with NG; **P<.01 compared with HG.

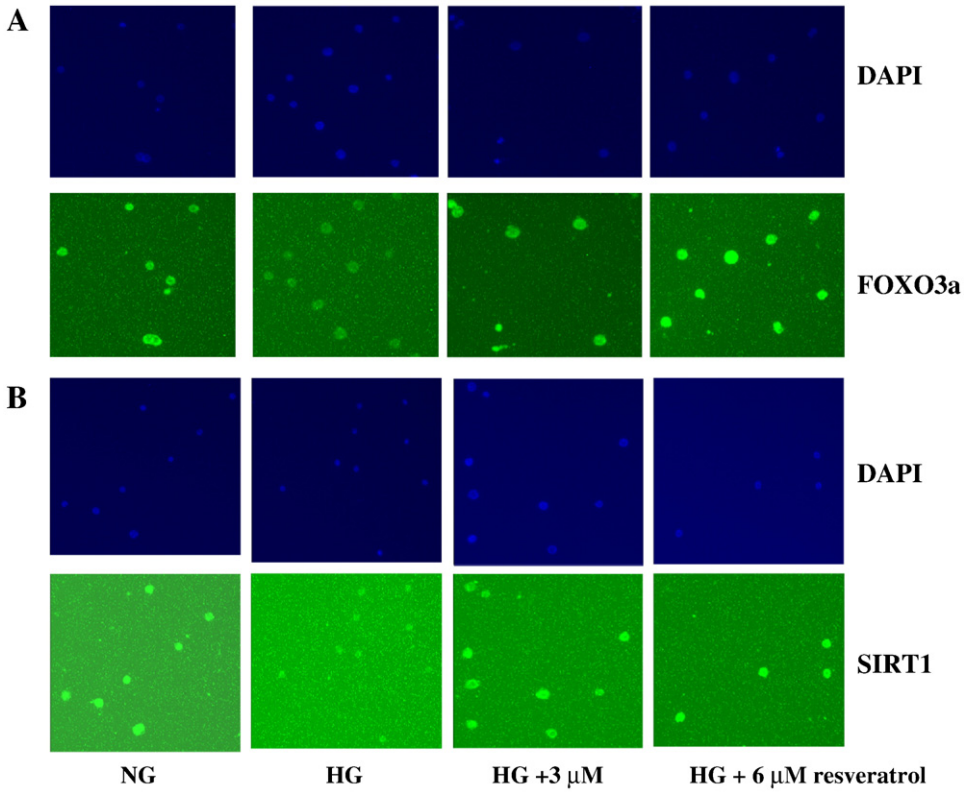

Fig. 3. Resveratrol treatment induced FOXO3a in HG-induced monocytes by immunofluorescence. The cells (50%–60% confluent) were treated with 3–6 μmol/l resveratrol for 48 h. Cells were fixed with 4% formaldehyde for 30 min at 4°C and stained overnight at 4°C with SIRT1 and FOXO3a as described in Materials and Methods. Data from five typical experiments are shown (original magnification ×400).

employed an additional approach of RNA interference-mediated knockdown of the *SIRT1* gene. As shown in Fig. 5A and B, SIRT1 siRNA inhibited FOXO3a but not p47phox expression in HG-induced monocytic cells. Furthermore, in the presence of resveratrol, there was no effect in SIRT1 siRNA-treated cells, showing that resveratrol primarily acts via SIRT1. In these cells, under HG conditions, resveratrol did not affect FOXO1A or p53 expression (data not shown).

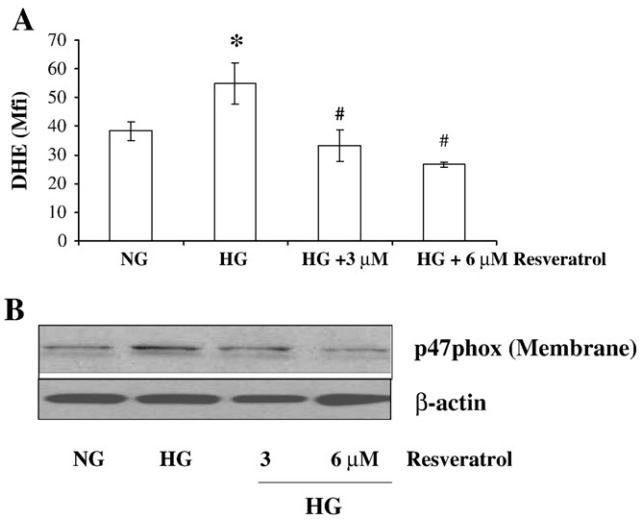

Fig. 4. Effect of resveratrol on superoxide production and *p47phox* gene expression level in HG-induced THP-1 cells. (A) NG, HG and HG–resveratrol-treated cells were incubated with 5 μmol/l DHE. Cells (1×10^5) were stained with 5 μmol/l DHE for 30 min at 37°C and were washed and resuspended in PBS. The oxidative conversion of DHE to ethidium was measured by flow cytometry. (B) For the Western blot analysis, protein was subjected to SDS-PAGE and used p47phox antibody (1:1000 dilution) as detailed in Materials and Methods. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for β-actin. The immunoblot shown here is representative of three independent experiments with similar results.

4. Discussion

Diabetic patients have increased O_2^- production in monocytes and neutrophils [2,7,9]. Hyperglycemia-induced production of ROS is a key event in the development of diabetic complications [2,7,8,10]. Dandona *et al.* [55] elucidated the role of ROS in diabetes by showing increased oxidative DNA damage in diabetics compared with controls. Previously, we have shown that p47phox is an essential component of monocyte NADPH oxidase production and required for ROS generation under high-glucose conditions *in vitro* [11] and Mohanty *et al.* [56] have observed increased p47phox in humans *in vivo*. So far, the exact mechanism in diabetes is not known at present. Recently, sirtuins have been implicated in metabolic processes and stress resistance. Recent studies have been suggested a key role for the mammalian SIRT1 in the adequate cellular response to metabolic stress events such as nutrient overload or nutrient deprivation [57]. In fact, the effects of SIRTs appear to be beneficial. SIRT1 knockout and overexpression mouse models have demonstrated metabolic benefits of Sirt1 activation. Recently, it has been proposed that SIRT1 is an important regulator of many factors influencing obesity and T2DM [58] and also a possible target for the treatment of metabolic syndrome [59,60].

SIRT1, belongs to class III HDACs, the most extensively studied of the sirtuins. SIRT1, through its deacetylase activity, is regarded as a key regulator of cell defense and survival under various stress conditions by deacetylating the p53 [18–21] and forkhead transcription

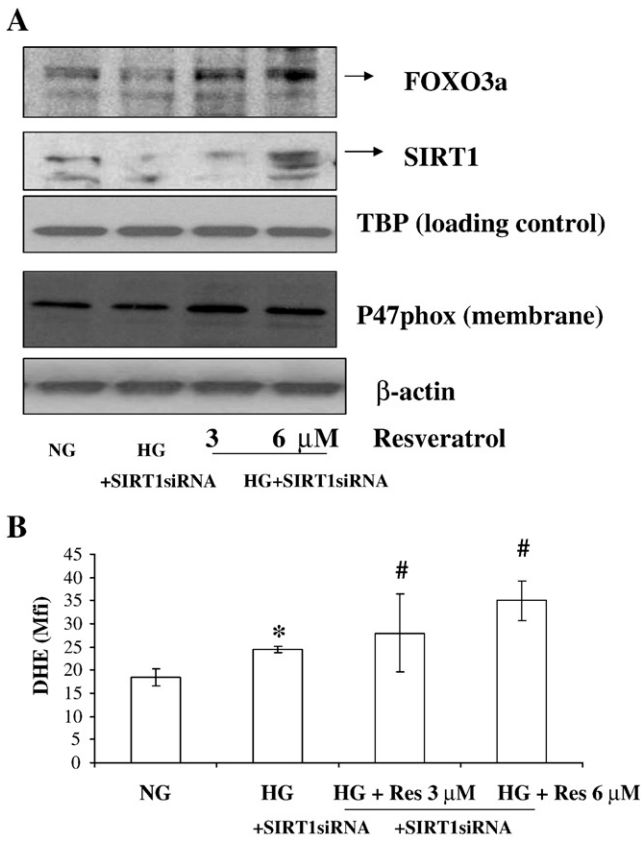

Fig. 5. Modulation of Foxo3a and p47phox using siRNA-SIRT1 transfection under high-glucose conditions. Cell were transfected using lipofectamine with prevalidated siRNA-SIRT1. Subsequently, human monocytic cells (1×10^5 cells/ml) were cultured in the presence of NG and HG conditions with or without resverarol (3–12 μmol/l) for 48 h. Then, cells were washed with PBS and then harvested. (A) NG, HG and HG–resveratrol-treated cells (transfected with siRNA to SIRT1) were incubated with 5 μmol/l DHE. Cells (1×10^5) were stained with 5 μmol/l DHE for 30 min at 37°C and were washed and resuspended in PBS. The oxidative conversion of DHE to ethidium was measured by flow cytometry. (B) For the Western blot analysis, protein was subjected to SDS-PAGE and used p47phox antibody (1:1000 dilution) as detailed in Materials and Methods. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for β-actin. The immunoblot shown here are representative of three independent experiments with similar results.

factor [14,22,37]. The effect of SIRT1 might be linked on FOXO function. Several studies have shown that SIRT1 can regulate mammalian FOXO transcription factors through direct binding and/or deacetylation. However, the mechanism of the interactions between SIRT1 and FOXO under diabetic conditions is not well understood. As one possible mechanism, SIRT1 enhances the activity of the FOXO by their nuclear translocation [22] and probably regulates the gene-specific transcription [24,35,42,44]. The translocation of FOXO3a from the cytoplasm to the nucleus is induced by deacetylation by SIRT1 in response to oxidative stress [22,61]. Among FOXO family, FOXO3a (also known as FKHR-L1) have been known to protect quiescent cells from oxidative stress by directly increasing their quantities of MnSOD messenger RNA and protein [44]. Recently, van der Horst *et al.* [62] and Barthel [62] have suggested that the FOXO3a genotype was significantly associated with plasma insulin levels as well as coronary heart disease, cancer and T2DM prevalence. The results of recent reports reveal that FOXO3a is an important regulator influencing obesity and diabetes. As an important downstream mechanism, FOXO3a might, at least in part, influence human metabolic diseases through modification of oxidative stress [62,63].

Resveratrol is a polyphenol existing in grapes, berries and peanuts. It has been reported to activate SIRT1 and extends life span in multiple model organisms [60,64]. In addition, It has been suggested that resveratrol is a promising new therapeutic approach for preventing cardiovascular diseases in T2DM and aging [47,63]. In addition, resveratrol has been reported to confer vasoprotection, improving endothelial function and preventing complications of diabetes in animal models [46,48–51,65–67].

Diabetes and aging are characterized by increased cellular ROS production, yet the effects of resveratrol on oxidative stress in human monocytes remain incompletely understood. Recently, there is evidence to suggest that resveratrol suppresses ROS generation by inhibiting NADPH oxidase activity [68]. Resveratrol suppresses ROS generation via FOXO3a [69] and up-regulates MnSOD expression via SIRT1 activation [70]. Recently, Orimo *et al.* [71] have suggested that hyperglycemia decreases SIRT1 expression and activation of SIRT1 prevents the hyperglycemia-induced endothelial senescence and thereby protects against vascular dysfunction in mice with diabetes [71]. As a mechanism, they have been suggested that the Akt/FOXO pathway plays a crucial role in the down-regulation of SIRT1 expression by high-glucose conditions [71]. Previous studies have demonstrated that high glucose reduced SIRT1 expression, leading to increased p53 and FOXO3a acetylation [57]. Also, very recently, de Kreutzenberg *et al.* [33] have shown that insulin resistance and metabolic syndrome affects *SIRT1* gene. Despite these previous reports, correlation with FOXO3a, SIRT1 and monocyte p47phox on ROS generation under HG conditions has not been not studied and its mechanism is still unclear.

Based on a previous study, we suggest that SIRT1 could serve as a target toward developing novel strategies for diabetes. We focused on its regulatory mechanism involving FOXO3a, SIRT1 and p47phox under HG-mediated oxidative stress of human monocytes. We investigated the effect of resveratrol on ROS production via SIRT1–FOXO3a signaling in HG-induced monocytes. Thus, we determined whether resveratrol can be used as a therapeutic agent for diabetic complications.

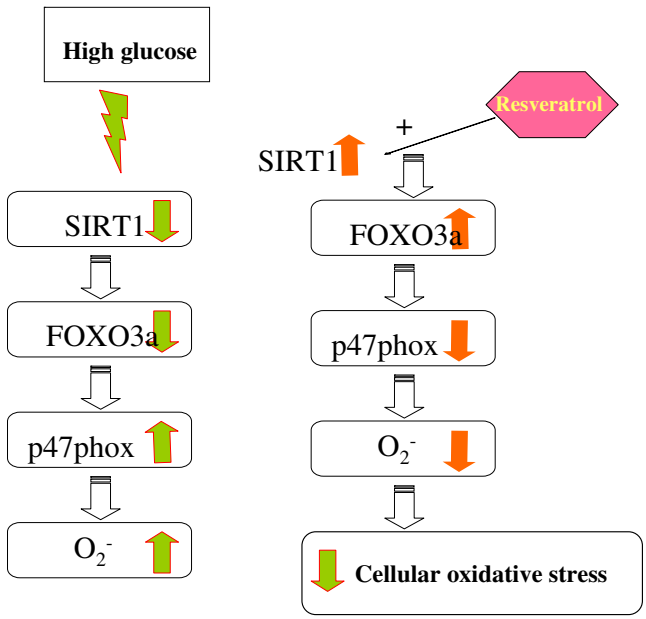

Fig. 6. Proposed mechanisms by which resveratrol regulates SIRT1–FOXO3a leading to decreased p47phox expression and ROS production. High glucose suppresses SIRT1–FOXO3a pathway, leading to increased *p47phox* gene expression and ROS production. Resveratrol treatment of HG-induced cells activates the HDACs activity and induces SIRT1 and FOXO3a expression, subsequently suppressing production of ROS and p47phox activation.

In the present study, first of all, we observed decreased SIRT1 and increased p47phox expression in monocytes of T1DM patients compared with normal healthy controls. The results of the present study, exposure of human monocytes to a high concentration of glucose, led to a decrease in nuclear SIRT1 and FOXO3a and a parallel increase in membrane p47phox expression and superoxide production. Interestingly, in this study, there was no change in FOXO1a expression or p53 by HG-induced oxidative, and moreover, there was no change by resveratrol.

When we employed an additional approach of RNA interference-mediated knockdown of the *SIRT1* gene, we observed that SIRT1 siRNA inhibited FOXO3a in HG-induced monocytes. Resveratrol failed to inhibit intracellular superoxide when SIRT 1 is knocked down, these results indicate that resveratrol primarily acts by inducing SIRT1, inducing FOXO3a and decreasing p47phox, resulting in decreased superoxide production under HG conditions in monocytes.

Thus, in our study, we show that HG suppresses SIRT1–FOXO3a pathway, leading to increased p47phox expression and monocytic ROS production. Resveratrol treatment of HG-induced cells activates HDACs and induces SIRT1 and FOXO3a expression, subsequently suppressing production of ROS and p47phox activation in monocytes (Fig. 6). Ghanim *et al.* [72] previously showed that a polygonum extract containing resveratrol significantly reduced ROS generation, the expression of p47phox and intranuclear NF-B binding in mononuclear cells of healthy subjects when compared with the baseline and the placebo but had no effect on SIRT1. Furthermore, resveratrol resulted in decreased PTP-1B, SOCS-3, JNK-1 and IKK-β, and it has been shown that these proteins interfere with insulin signal transduction. However, this study was not under hyperglycemia, and the extract contained only 20% resveratrol; thus. other products may have also contributed to these findings. Our study indicates that activation of FOXO3a pathway is a key protective pathway against oxidative stress in high-glucose conditions. However, resveratrol will need extensive evaluation in clinical trials before it can be recommended for retarding oxidative stress and diabetic complications.

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