· Basic Research ·

SUMOylation and deacetylation affect NF-κB p65 activity induced by high glucose in human lens epithelial cells

Xiao Han[1], Xiao-Xuan Dong[2], Ming-Yu Shi[1], Li Feng[1], Xin-Ling Wang[1], Jin-Song Zhang[1], Qi-Chang Yan[1]

[1]Department of Ophthalmology, the Fourth Affiliated Hospital of China Medical University; Key Laboratory of Lens Research of Liaoning Province; Eye Hospital of China Medical University, Shenyang 110005, Liaoning Province, China
[2]Department of Ophthalmology, the Fourth People's Hospital of Shenyang, Shenyang 110031, Liaoning Province, China
Correspondence to: Qi-Chang Yan. Department of Ophthalmology, the Fourth Affiliated Hospital of China Medical University; Key Laboratory of Lens Research of Liaoning Province; Eye Hospital of China Medical University, 11Xinhua Road, Heping District, Shenyang 110005, China. cmu4h_yqc@163.com
Received: 2018-12-25 Accepted: 2019-05-07

Abstract

● AIM: To explore the effects of IκBα SUMOylation and NF-κB p65 deacetylation on NF-κB p65 activity induced by high glucose in cultured human lens epithelial cells (HLECs).

● METHODS: HLECs (SRA01/04) were cultured with 5.5, 25, and 50 mmol/L glucose media for 24h, and with 50 mmol/L glucose media for 0, 12, and 24h respectively. SUMO1 and SIRT1 expressions were detected by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot (WB). IκBα and NF-κB p65 expressions were detected by WB. With NAC, DTT, MG132 or Resveratrol (RSV) treatment, SUMO1 and SIRT1 expressions were detected by WB. Protein expression localizations were examined by immunofluorescence and co-immunofluorescence. The effects of SUMO1 or SIRT1 overexpression, as well as MG132 and RSV, on the nuclear expression and activity of IκBα and NF-κB p65 were analyzed by immunoblot and dual luciferase reporter gene assay.

● RESULTS: SUMO1 and SIRT1 expressions were influenced by high glucose in mRNA and protein levels, which could be blocked by NAC or DTT. SUMO1 was down-regulated by using MG132, and SIRT1 was up-regulated under RSV treatment. IκBα nuclear expression was attenuated and NF-κB p65 was opposite under high glucose, while IκBα and NF-κB p65 location was transferred to the nucleus. SUMO1 or SIRT1 overexpression and MG132 or RSV treatment affected the nuclear expression and activity of IκBα and NF-κB p65 under high glucose condition.

● CONCLUSION: IκBα SUMOylation and NF-κB p65 deacetylation affect NF-κB p65 activity in cultured HLECs under high glucose, and presumably play a significant role in controlling diabetic cataract.

● **KEYWORDS:** SUMOylation; deacetylation; NF-κB p65; IκBα; diabetic cataract; high glucose; lens epithelial cells

DOI:10.18240/ijo.2019.09.01

Citation: Han X, Dong XX, Shi MY, Feng L, Wang XL, Zhang JS, Yan QC. SUMOylation and deacetylation affect NF-κB p65 activity induced by high glucose in human lens epithelial cells. *Int J Ophthalmol* 2019;12(9):1371-1379

INTRODUCTION

Diabetic cataract (DC) is a common complication of type 1 diabetes mellitus[1]. High blood glucose can obviously accelerate cataract progression in age-related cataract patients[2] and promote cataract formation in type 2 diabetes mellitus patients[3]. High glucose treatment can cause oxidative stress to damage the human lens epithelial cells (HLECs) and form cataractogenesis[4]. Meanwhile, it is widely acknowledged that oxidative stress is one of major mechanisms of DC[5-6]. In previous studies, it is clarified that oxidative stress can motivate various signal pathways, as well as posttranslational modification (PTM), such as SUMOylation[7-8] and deacetylation[9-10]. Nuclear factor κB (NF-κB) as a sensitive transcriptional factor plays an important role in regulating oxidative stress[11] which contributes to DC[12-13]. However, whether SUMOylation and deacetylation has relationship with NF-κB signal pathway involving in DC in HLECs is still unknown.

SUMOylation is one of PTM, which has been validated to protect target proteins from degradation by ubiquitination[14-15]. There are four various isoforms of SUMO grouped into three classes, SUMO1, SUMO2/3 and SUMO4 mediating SUMOylation in mammals[16]. SUMO1, the most well-known SUMO family member, is involved in kinds of cellular processes and resulted in different diseases. It establishes an essential role of regulating transcriptional factors[17], protecting cell[18], inhibiting oxidative stress reaction[19], repairing DNA damage[20], preventing apoptosis[21] and so on. Nevertheless, there is few study of cataract to date have focused on SUMO1 and SUMOylation.

SIRT1, the most crucial sirtuin family member, modifies deacetylation (another significant PTM) and brings on various physiological and pathological processes. SIRT1, as a longevity gene, is participated in reducing oxidative stress[22], preventing apoptosis[23], resisting aging[24] and all that. It has demonstrated the relationship between SIRT1 and age-related cataract in others studies[25-26]. Increasing evidence suggests that Resveratrol (RSV), a famous antioxidant and anti-aging agent, can accelerate the expression and activity of SIRT1[27]. But the influence of SIRT1 and RSV in HLECs under high glucose remains poorly understood.

NF-κB is a significant stress responsive transcriptional factor located in the cytoplasm in nonactivated state and its activity can be affected by various external stimuli. NF-κB p65, as a prominent member of NF-κB family, is concerned with various stimuli stress, especially oxidative stress[28]. When stimulated, NF-κB is activated and translocated into the nucleus. Its transcriptional activity is controlled by inhibitor IκB proteins[29] and IκBα is a main member of IκBs. NF-κB activity is determined by degradation of IκB which is mediated by ubiquitin-proteasome pathway (UPP). Therefore, in another study, it has verified the proteasome inhibitor MG132 reversed IκBα degradation and decreased NF-κB expression and activity which was induced by high glucose in rat mesangial cells[30]. MG132 treatment could also accumulate the conjugations of SUMO and target proteins[31]. Liu *et al*[32] found that ubiquitin and SUMO competed for the same target lysine on $K^{21/22}$ of IκBα in previous study. It was cleared that acetylation sites of NF-κB p65 were found on lysines K^{221}, K^{310}, and $K^{122/123}$, although there were different effects on different lysines[33].

This study is the first to demonstrate whether high glucose could induce SUMO1 and SIRT1 expression owing to oxidative stress in cultured HLECs, and whether IκBα SUMOylation and NF-κB p65 deacetylation could affect NF-κB p65 activity *in vitro*. The results showed SUMO1 or SIRT1 overexpression could influence the nuclear expression of IκBα and NF-κB p65, as well as the activity of NF-κB p65 in cultured HLECs. Meanwhile, it was the first time to investigate the effects of MG132 and RSV on protecting lens transparency from oxidative damage induced by high glucose through regulation of NF-κB p65 activity in HLECs.

MATERIALS AND METHODS

Cell Culture and Treatments Human lens epithelial cells (SRA01/04) were gifts from Key Lens Laboratory of Lens Research of Liaoning Province. The cells were cultured in Dulbecco's modified Eagle's media (DMEM; Hyclone) with 5.5 mmol/L glucose, 10% fetal bovine serum (FBS; Invitrogen), 100 mg/mL streptomycin (Hyclone) and 100 IU/mL penicillin (Hyclone) in a 5% CO_2 humidified atmosphere at 37°C. The SRA01/04 cells were grown to 75%-80% confluence and divided randomly into several groups: normal control glucose group (NC; media with 5.5 mmol/L glucose), high glucose 1 group (HG1; media with 25 mmol/L glucose), high glucose 2 group (HG2; media with 50 mmol/L glucose), and osmotic pressure control group (OP; media with 50 mmol/L mannitol). N-acetyl cysteine (NAC; Sigma) 5 mmol/L for 4h or the thiol-reducing agent dithiothreitol (DTT; Sigma) 10 mmol/L for 1h was as anti-oxidant addressed in high glucose media. MG132 (Sigma) 10 μmol/L as the proteasome inhibitor added in media for 4h. RSV (Sigma) 10 μmol/L as SIRT1 activator was participated in media for 4h.

Reverse Transcription-Polymerase Chain Reaction Total RNA from SRA01/04 cells was extracted using TRIzol (TaKaRa) and reverse transcribed using M-MLV First Kit (Invitrogen) to get cDNA, which was amplified using Taq DNA polymerase Recombinant Kit (Invitrogen). The results were determined using chemiluminescent gel imaging system and normalized to β-actin gene expression. The primer sequences were as followed: SUMO1 (forward: 5'-tgg aca gga tag cag tga ga-3'; reverse: 5'-tct tcc tcc att ccc agt tct-3'; product size: 174 bp), SIRT1 (forward: 5'-cca gcc atc tct ctg tca ca-3'; reverse: 5'-tcc tcg tac agc ttc aca gt-3'; product size: 193 bp), β-actin (forward: 5'-cat ccg taa aga cct cta tgc caa c-3'; reverse: 5'-atg gag cca ccg atc cac a-3'; product size: 171 bp).

Western Blot Total proteins from SRA01/04 cells were extracted using RIPA lysis buffer with PMSF and protease inhibitor cocktail set (Calbiolchem, Germany). Nuclear proteins from SRA01/04 cells were extracted with nuclear protein extraction kit (Beyotime, China). The lysates were separated by NuPAGE 10% Bis-Tris Gel (Invitrogen), and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, USA). Primary antibodies against SUMO1 (Abcam), SIRT1 (Abcam), NF-κB p65 (Bioss, China), IκBα (Bioss, China), and β-actin (Proteintech, USA) were used, as well as peroxidase-conjugated affinipure goat anti-rabbit IgG and peroxidase-conjugated affinipure goat anti-mouse IgG secondary antibodies (Jackson immunoresearch, USA). The proteins were detected by enhanced hemagglutinin (Thermo), quantified by chemiluminescent gel imaging system, and normalized to β-actin protein expression.

Immunofluorescence The SRA01/04 cells were cultured on cover lips in 24-well plates and were treated with 5.5 mmol/L (NC) and 50 mmol/L (HG2) glucose in media for 24h. The cells were fixed with 4% paraformaldehyde (PFA, Invitrogen) solubilized in 0.1% Triton×100-PBS for 20min, and were blocked with 5% BSA-PBS (Sigma) for 1h. The cells were incubated with anti-IκBα and anti-NF-κB p65 antibodies in 2% BSA-PBS overnight at 4°C. Alex Fluor 596 goat anti-rabbit IgG (H+L) (Invitrogen) in 2% BSA-PBS was as secondary antibody incubated for 1h in the dark room. DAPI (Beyotime, China) used to stain the nucleus for 1min. Images were taken with fluorescence microscope.

SUMO1 or SIRT1 Overexpression and Immunoblot Analysis GFP-SUMO1 (gift from Prof. Chen[34]), GFP-SIRT1 (gift from Doctor Jiang) and empty GFP-vector (Invitrogen) were transfected with lipofectamin 2000 (Invitrogen) into cells for 6h. After cultured 40h, the cells were incubated with 5.5 mmol/L (NC) and 50 mmol/L (HG2) glucose media respectively for 24h. The nuclear protein of transfected SUMO1 or SIRT1 cells was extracted and detected the nuclear protein expressions of IκBα and NF-κB p65 by immunoblot. The cells transfected with empty GFP-vector were as a blank group.

MG132 or RSV Treatment and Immunoblot Analysis SRA01/04 cells were cultured with normal (NC) or high glucose (HG2) media for 24h. Then, cells were treated with 10 μmol/L MG132 or 10 μmol/L RSV for 4h respectively. The nuclear proteins of treated cells were extracted and the nuclear expression of IκBα and NF-κB p65 was detected by immunoblot.

Dual Luciferase Reporter Gene Assay SRA01/04 cells were cultured in 6 well plates and transiently transfected with pNF-κB-TA-luc, the control pGL6-TA (Beyotime, China) reporters, GFP-SUMO1, GFP-SIRT1, GFP-vector, and together with Renilla luciferase control plasmid (pRL-TK) as internal control plasmid. After 24h co-transfection, cells were treated with or without high glucose, and MG132 or RSV 10 μmol/L treatment for 4h. Absolute luminescence was measured according to the Dual-Luciferase Reporter Assay protocol (Beyotime, China). The relative NF-κB dual luciferase activities were measured and firefly values were normalized by Renilla values.

Statistical Analysis All data were presented as the mean±SD for at least 3 independent experiments and statistical analysis was evaluated using one-way ANOVA of SPSS program version 19.0. $P<0.05$ was considered statistically significant.

RESULTS

SUMO1 and SIRT1 Expression Influenced by High Glucose-Induced Oxidative Stress The relative SUMO1 expression of HG1 and HG2 groups were higher than that of NC group in both mRNA and protein levels. In contrast, the expression of SIRT1 was decreased in HG1 and HG2 groups, which compared with NC group in both mRNA and protein levels (Figure 1A, 1C). Compared with 0h group, SUMO1 expression in mRNA and protein levels were enhanced in 12h and 24h group. There were also had different results for SIRT1 (Figure 1B, 1D). Importantly, it was confirmed that SUMO1 and SIRT1 expression changed by high glucose owing to oxidative stress rather than osmotic pressure stress (compared with NC, $P>0.05$, Figure 1A, 1C). The increase in SUMO1

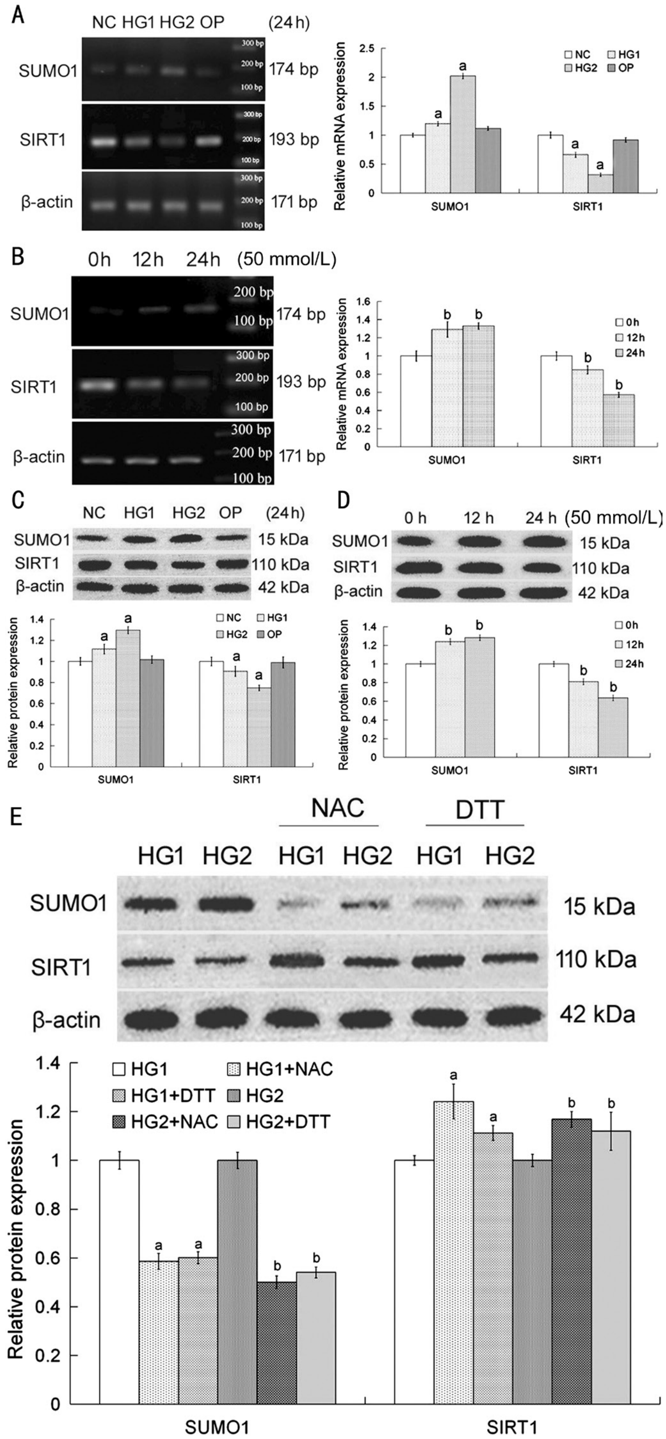

Figure 1 High glucose induced oxidative stress and influenced SUMO1 and SIRT1 expression in HLECs The expressions of SUMO1 and SIRT1 in the mRNA (A, B) and protein (C, D) levels in SRA01/04 cells with different concentrations of glucose media (A, C) and treated various times (B, D). There was no obvious change in OP group (A, C). Compared with NC or 0h group, ${}^{a}P<0.05$ or ${}^{b}P<0.05$. SRA01/04 cells were cultured with high glucose media for 24h, as well as 5 mmol/L NAC for 4h or 10 mmol/L DTT for 1h respectively (E). Compared with HG1 or HG2 group, ${}^{a}P<0.05$ or ${}^{b}P<0.05$. The data were normalized to β-actin and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 3 times with the same results. NC: Media with 5.5 mmol/L glucose; HG1: Media with 25 mmol/L glucose; HG2: Media with 50 mmol/L glucose; OP: Media with 50 mmol/L mannitol; NAC: Media with 5 mmol/L NAC; DTT: Media with 10 mmol/L DTT.

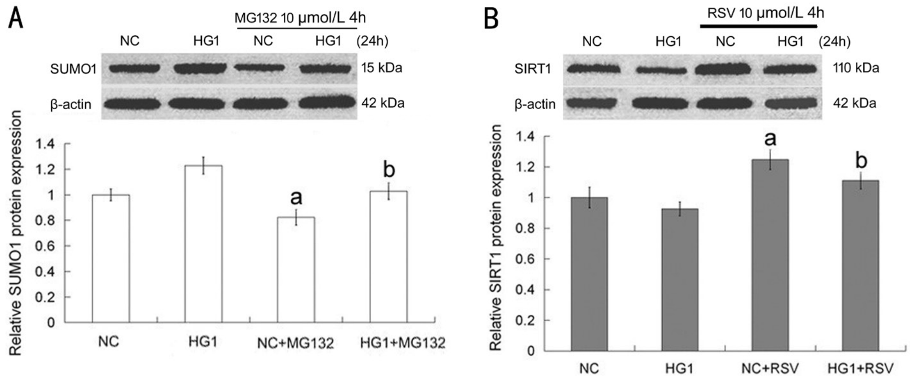

Figure 2 MG132 and RSV could influence SUMO1 and SIRT1 expression in HLECs A: With or without high glucose added to the media for 24h, cells were treated with 10 μmol/L MG132 for 4h; B: With or without high glucose added to the media for 24h, cells were treated with 10 μmol/L RSV for 4h. Compared with NC or HG1 group, ${}^{a}P<0.05$ or ${}^{b}P<0.05$. The data were normalized to β-actin and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 3 times with the same results. NC: Media with 5.5 mmol/L glucose; HG1: Media with 25 mmol/L glucose; MG132: Media with 10 μmol/L MG132; RSV: Media with 10 μmol/L RSV. ·

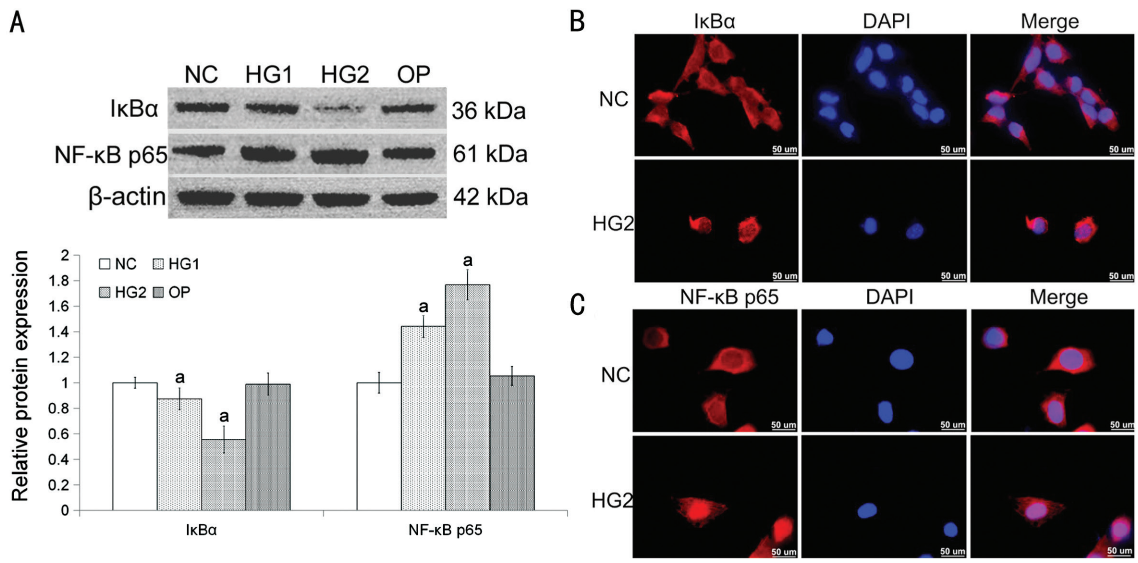

Figure 3 High glucose affected the nuclear expression of IκBα and NF-κB p65 in HLECs A: The nuclear expression of IκBα and NF-κB p65 in HLECs with different concentrations of glucose media for 24h. Compared with NC group, ${}^{a}P<0.05$. Osmotic pressure had little effect on the expressions of IκBα and NF-κB p65. B, C: Immunofluorescence staining for IκBα (red) and NF-κB p65 (red) in NC and HG2 group, nuclei with DAPI (blue), Bar=50 μm. The data were normalized to β-actin and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 3 times with the same results. NC: Media with 5.5 mmol/L glucose; HG1: Media with 25 mmol/L glucose; HG2: Media with 50 mmol/L glucose; OP: Media with 50 mmol/L mannitol.

protein could be blocked by NAC or DTT (antioxidant) treatment under high glucose condition. In the same way, the decrease in SIRT1 protein could be reversed by NAC or DTT addition in high glucose media (Figure 1E).

MG132 and RSV Influenced SUMO1 and SIRT1 Expression The SUMO1 expression was decreased in whether normal (NC) or high glucose (HG1) condition when it was treated with MG132 (Figure 2A). As shown in Figure 2B, SIRT1 had an opposite situation. After RSV was participated in normal or high glucose media, the expression of SIRT1 was enhanced obviously.

High Glucose Affected the Nuclear Expression of IκBα and NF-κB p65 High glucose could attenuate IκBα nuclear expression, while increase the nuclear expression of NF-κB p65 (Figure 3A). However, osmotic pressure had little effect on the nuclear expressions of IκBα and NF-κB p65 (compared with NC, $P>0.05$; Figure 3A). Immunofluorescence (Figure 3B, 3C) showed the locations of IκBα and NF-κB p65 were transferred to nucleus from cytoplasm induced by high glucose.

SUMO1 or SIRT1 Overexpression Influenced IκBα Nuclear Expression and NF-κB p65 Activity SRA01/04 cells were highly efficient transfected with GFP-SUMO1 or SIRT1 respectively and cultured with high glucose media for 24h. Compared with transfected empty GFP-vector cells, IκBα nuclear expression was increased and NF-κB p65 nuclear expression was decreased in transfected SUMO1 or SIRT1 cells under high glucose condition (Figure 4A). SRA01/04 cells were highly efficient transfected with GFP-SUMO1, GFP-SIRT1, GFP-vector, pNF-κB-TA-luc, pGL6-TA, and pRL-TK respectively and cultured with high glucose media for 24h. Compared with transfected empty GFP-vector cells, NF-κB p65 activity was decreased in transfected SUMO1 or SIRT1 cells under high glucose condition (Figure 4B). There was no obvious change in pGL6-TA control groups (P>0.05; Figure 4B).

MG132 and RSV Influenced IκBα Nuclear Expression and NF-κB p65 Activity After cultured with or without high glucose for 24h, the nuclear expression of IκBα and NF-κB p65 was changed by MG132 and RSV treatment. Both MG132 and RSV could enhance IκBα nuclear expression; in contrast, reduce the nuclear expression of NF-κB p65. The effects of MG132 and RSV were just like SUMO1 and SIRT1 overexpression on the nuclear expression of IκBα and NF-κB p65 in HLECs (Figure 5A). SRA01/04 cells were highly efficient transfected with GpNF-κB-TA-luc, pGL6-TA and pRL-TK respectively. After cultured with or without high glucose for 24h, the activity of NF-κB p65 was changed by MG132 and RSV treatment. Compared with HG2 group, both MG132 and RSV could reduce the activity of NF-κB p65 under high glucose condition. The effects of MG132 and RSV were just like SUMO1 and SIRT1 overexpression on the activity of NF-κB p65 in HLECs (Figure 5B). There was no obvious change in pGL6-TA control groups (P>0.05; Figure 5B).

DISCUSSION

To date, there was no previous experimental evidence for the function of SUMOylation and deacetylation in HLECs or pathology of diabetic cataract. In the previous work, we have discussed the expression and function of SUMO1-4 and SUMO E3 (Cbx4 and PIASy) under high glucose environment in HLECs[35]. The increasing studies have reported the regulation of SUMOylation and deacetylation by cellular stress, suggesting a key role of SUMOylation and deacetylation in the cellular response. Therefore, it is significant to explore study the relative proteins of SUMOylation and deacetylation under stress in HLECs.

For the first time, this finding was demonstrated that SUMO1 and SIRT1 expression was influenced by high glucose in mRNA and protein levels in HLECs. They were also changed time-dependently in mRNA and protein levels. Huang *et al*'s[30] study clarified that SUMO1-3 expression was also enhanced by high glucose in rat mesangial cells. High glucose-induced oxidative stress represses SIRT expression and increases histone acetylation leading to neural tube defects[36]. We tried to explore the reason for the changes of SUMO1 and SIRT1 under high glucose microenvironments, because osmotic pressure stress had little effect on regulating SUMO1 and

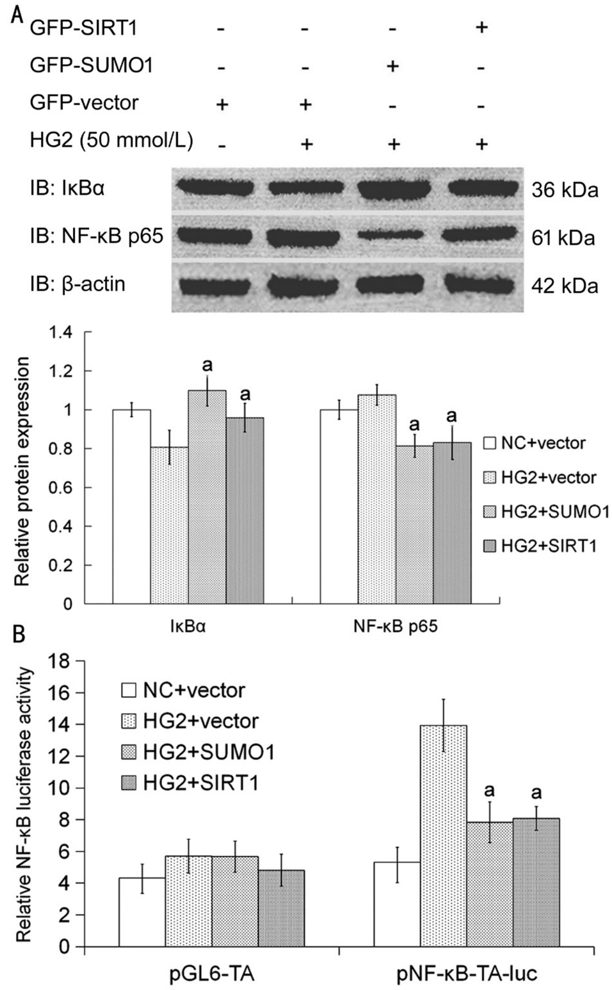

Figure 4 SUMO1 or SIRT1 overexpression influenced the nuclear expression of IκBα and affected the expression and activity of NF-κB p65 in HLECs A: The cells transfected with SUMO1 or SIRT1 were cultured with high glucose media to detect the nuclear expression of IκBα and NF-κB p65. Compared with HG2+vector group, ^aP<0.05. The data were normalized to β-actin and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 3 times with the same results. B: The cells transfected with GFP-SUMO1, GFP-SIRT1, GFP-vector, pNF-κB-TA-luc, pGL6-TA and pRL-TK respectively were cultured with high glucose media to detect the relative NF-κB luciferase activity. Compared with transfected HG2+GFP-vector group, ^aP<0.05. There was no obvious change in pGL6-TA control groups. The value of fluorescence was normalized by Renilla values and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 9 times with the same results. NC: Media with 5.5 mmol/L glucose; HG2: Media with 50 mmol/L glucose.

SIRT1 expression in HLECs. We found the change of SUMO1 and SIRT1 expression under high glucose could be blocked and reversed by anti-oxidants, NAC or DTT. It was guessed that high glucose could regulate SUMO1 and SIRT1 protein expression owing to oxidative stress reaction, which allowed for a new SUMOylation/deSUMOylation and acetylation/deacetylation balance in response to oxidative stress. We

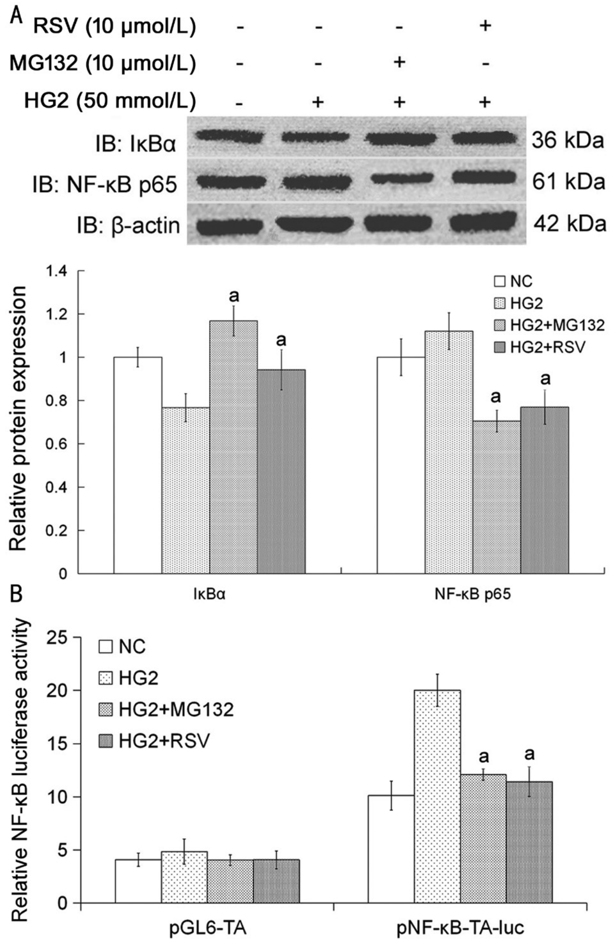

Figure 5 MG132 and RSV influenced the nuclear expression of IκBα and affected the expression and activity of NF-κB p65 in HLECs A: The cells were cultured with or without high glucose media for 24h, and were treated with MG132 and RSV 10 μmol/L for 4h respectively. Compared with HG2 group, [a]$P<0.05$. The data were normalized to β-actin and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 3 times with the same results. B: The cells transfected with pNF-κB-TA-luc, pGL6-TA and pRL-TK were cultured with or without high glucose media for 24h, and were treated with MG132 and RSV 10 μmol/L for 4h respectively. The relative NF-κB luciferase activity compared with HG2 group, [a]$P<0.05$. There was no obvious change in pGL6-TA control groups. The value of fluorescence was normalized by Renilla values and expressed as mean±SD of triplicates in an independent experiment, which was repeated at least 9 times with the same results. NC: Media with 5.5 mmol/L glucose; HG2: Media with 50 mmol/L glucose; MG132: Media with 10 μmol/L MG132; RSV: Media with 10 μmol/L RSV.

hypothesized that high glucose-mediated oxidative stress might decline the conjugation of SUMO1 and its target proteins, leading to endogenous free SUMO1 proteins increase. In our results, MG132 (as antioxidant) might improve SUMO1 conjugating with target proteins, which could lead to decrease of endogenous free SUMO1 under MG132 treatment in HLECs. In another study, MG132 could induce accumulation of SUMO2/3 conjugates, while reduce the expression of free endogenous SUMO2/3 in HEK 293T cells[31]. According to the change of SIRT1 under high glucose, we hypothesized that oxidative stress might reduce SIRT1 expression and activity mediated deacetylation under high glucose. It has been recognized that H_2O_2-mediated oxidative stress reduces the expression and activity of SIRT1 in human lung epithelial cells[37]. In our study, it was striking that RSV was still SIRT1 activator in HLECs.

In addition to regulating SUMO1 and SIRT1, oxidative stress is also regulated by SUMO1 and SIRT1. Researchers have revealed that SUMO1 conjugated to proteins involving in the regulation of diverse cellular events, including transcriptional regulation, stress resistance, cellular senescence, apoptosis, responses to extracellular stimuli, and especially oxidative stress. SUMOylated lysines cannot be ubiquitinated, which contribute to the stabilization of target proteins[38]. Deacetylation of target proteins mediated by SRIT1 involved in activity/inactivity of substance[33], which also contributes to regulating aging, inflammation, metabolic processes, oxygen sensing, redox-dependent cellular processes, among others[39]. SUMO1 mediated SUMOylation to prevent degradation of IκBα from ubiquitin-proteasome pathway, and to stabilize IκBα of IκB by SUMO, which results in the inhibition of NF-κB transcriptional activity[40]. Moreover, the activation of NF-κB p65 was reduced through deacetylation mediated by SIRT1[41]. SIRT1 interacts with NF-κB p65 leading to its deacetylation and resulting in decreased NF-κB-dependent transcription[42]. As a transcriptional factor, NF-κB has function for many target genes that control various cellular responses such as apoptosis, stress and inflammation. More importantly, clinical and laboratory studies have demonstrated NF-κB pathway participated in diverse human diseases, such as myocardial disease[43], diabetic nephropathy[44], cataract[45] and cancer[46]. A crucial observation made in this study was NF-κB p65 nuclear expression induced by high glucose in HLECs, while IκBα nuclear expression was opposite. It was confirmed that the changes of NF-κB p65 and IκBα was due to oxidative stress rather than osmotic pressure stress. The finding suggested NF-κB p65 and IκBα were mainly located in the cytoplasm normally, but were transferred into nucleus by high glucose mediated oxidative stress. It also evidenced that high glucose activated and translocated NF-κB p65 and IκBα protein. Redox derived by high glucose led to covalent post-translational modifications, SUMOylation and deacetylation.

SUMOylated IκBα as inhibitor of NF-κB has been demonstrated in attenuating NF-κB activation. It was verified SUMO1 overexpression promoted IκBα SUMOylation and attenuated the activity of NF-κB p65 in HLECs. We also recognized that SIRT1 overexpression induced NF-κB p65

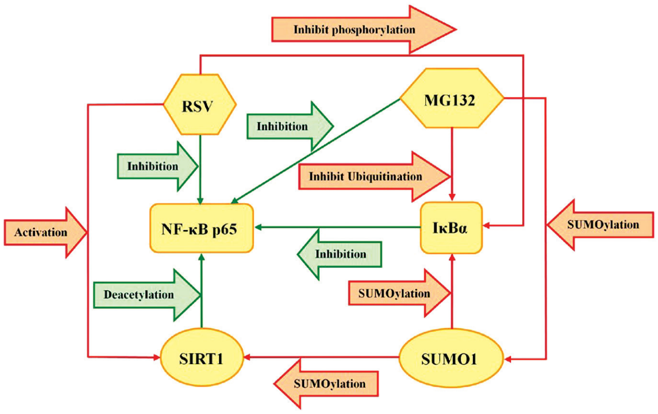

Figure 6 The relationship of SUMO1, SIRT1, NF-κB p65 and IκBα in HLECs.

deacetylation, which could also reduce the activity of NF-κB p65. On the other side, a study reported that SUMO1 mediated SUMOylation of SIRT1[47-48], and stabilized the deacetylase activity of SIRT1[49]. Therefore, SUMO1 overexpression might activate SIRT1 deacetylase at the same time, which also promoted SIRT1 which mediated deacetylation of NF-κB p65 in HLECs. There was complicated correlate among SUMO1, SIRT1, NF-κB p65 and IκBα shown in Figure 6. All the results above-mentioned suggest that SUMO1 and SIRT1 might be new potential therapeutic targets for the treatment of DC. Furthermore, we also found MG132 and RSV could also influence the expression of SUMO1 and SIRT1 respectively in some degree, which meant MG132 and RSV could regulate SUMOylation and deacetylation. Therefore, we chose MG132 and RSV as antioxidant to treat HLECs under normal or high glucose condition. The fact proved that MG132 and RSV, both could influence the activity of NF-κB p65 and IκBα. In addition, MG132 is one kind of proteasome inhibitor which inhibits modification of ubiquitination and accumulates conjugations of SUMO and its target proteins. It is universal that IκBα degradation is attenuated through UPP. Meanwhile, MG132 can improve the conjugation of SUMO1 and IκBα. Therefore, stabilization of IκBα can promote its conjugating with NF-κB p65, and inhibit the activation of NF-κB p65. We also found RSV had effect on reducing the activation of NF-κB p65. As an antioxidant, RSV might influence cellular stress response through kinds of pathway. In our study, as SIRT1 activator, RSV might regulate SIRT1 mediated deacetylation to affect NF-κB p65 activity in HLECs. RSV could also inhibit the degradation of IκBα in our study. The mechanism was discovered that RSV inhibited IκBα phosphorylation to maintain the expression level of IκBα[50]. The relationship of MG132, RSV, IκBα and NF-κB p65 was described as shown in Figure 6. In previous studies, there have been reports to identified that MG132 or RSV was involved in controlling high glucose in diabetes respectively[51-52]. It was full proof that MG132 and RSV as antioxidant might play a significant role in protecting lens from DC.

In conclusion, the study was first to found that SUMO1 and SIRT1 expression was influenced by high glucose due to oxidative stress in cultured HLECs. SUMO1 or SIRT1 overexpression could enhance the modifications of IκBα SUMOylation and NF-κB p65 deacetylation, and then influence the activation of NF-κB p65. In the same time, MG132 and RSV as antioxidant also regulated NF-κB p65 activity through influencing SUMOylation and deacetylation respectively. They had the potential to protect HLECs from oxidative damage and maintain lens transparency. This study supported that IκBα SUMOylation and NF-κB p65 deacetylation may be involved in the pathogenesis of DC through affecting NF-κB p65 activity under high glucose conditions. SUMO and SIRT signaling molecules may be potential therapeutic targets for the treatment of DC.

ACKNOWLEDGEMENTS

We are grateful to Prof. Chen of Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education (Shanghai Jiao-Tong University School of Medicine) and Dr. Zhong-Xiu Jiang of Department of Oncology (Shengjing Affiliated Hospital of China Medical University) supporting plasmids for the gifts.

Foundations: Supported by the National Natural Science Foundation of China (No.81170836; No.81570838).

Conflicts of Interest: Han X, None; **Dong XX,** None; **Shi MY,** None; **Feng L,** None; **Wang XL,** None; **Zhang JS,** None; **Yan QC,** None.

REFERENCES

1 Geloneck MM, Forbes BJ, Shaffer J, Ying GS, Binenbaum G. Ocular complications in children with diabetes mellitus. *Ophthalmology* 2015;122(12):2457-2464.

2 Ghaem Maralani H, Tai BC, Wong TY, Tai ES, Li J, Wang JJ, Mitchell P. Metabolic syndrome and risk of age-related cataract over time: an analysis of interval-censored data using a random-effects model. *Invest Ophthalmol Vis Sci* 2013;54(1):641-646.

3 Tan NC, Barbier S, Lim WY, Chia KS. 5-Year longitudinal study of determinants of glycemic control for multi-ethnic Asian patients with type 2 diabetes mellitus managed in primary care. *Diabetes Res Clin Pract* 2015;110(2):218-223.

4 Mirsky N, Cohen R, Eliaz A, Dovrat A. Featured Article: Inhibition of diabetic cataract by glucose tolerance factor extracted from yeast. *Exp Biol Med (Maywood)* 2016;241(8):817-829.

5 Kruk J, Kubasik-Kladna K, Aboul-Enein HY. The role oxidative stress in the pathogenesis of eye diseases: current status and a dual role of physical activity. *Mini Rev Med Chem* 2015;16(3):241-257.

6 Kandarakis SA, Piperi C, Topouzis F, Papavassiliou AG. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog Retin Eye Res* 2014;42:85-102.

7 Lee YJ, Bernstock JD, Nagaraja N, Ko B, Hallenbeck JM. Global SUMOylation facilitates the multimodal neuroprotection afforded by quercetin against the deleterious effects of oxygen/glucose deprivation and the restoration of oxygen/glucose. *J Neurochem* 2016;138(1):101-116.

8 Wang T, Xu W, Qin M, Yang Y, Bao P, Shen F, Zhang Z, Xu J. Pathogenic mutations in the valosin-containing protein/p97(VCP) N-domain inhibit the SUMOylation of VCP and lead to impaired stress response. *J Biol Chem* 2016;291(27):14373-14384.

9 Ding YW, Zhao GJ, Li XL, Hong GL, Li MF, Qiu QM, Wu B, Lu ZQ. SIRT1 exerts protective effects against paraquat-induced injury in mouse type II alveolar epithelial cells by deacetylating NRF2 *in vitro*. *Int J Mol Med* 2016;37(4):1049-1058.

10 Li S, Zhao G, Chen L, Ding Y, Lian J, Hong G, Lu Z. Resveratrol protects mice from paraquat-induced lung injury: The important role of SIRT1 and NRF2 antioxidant pathways. *Mol Med Rep* 2016;13(2):1833-1838.

11 Akyol S, Ugurcu V, Balci M, Gurel A, Erden G, Cakmak O, Akyol O. Caffeic acid phenethyl ester: its protective role against certain major eye diseases. *J Ocul Pharmacol Ther* 2014;30(9):700-708.

12 Nambu H, Kubo E, Takamura Y, Tsuzuki S, Tamura M, Akagi Y. Attenuation of aldose reductase gene suppresses high-glucose-induced apoptosis and oxidative stress in rat lens epithelial cells. *Diabetes Res Clin Pract* 2008;82(1):18-24.

13 Kim J, Kim CS, Sohn E, Kim H, Jeong IH, Kim JS. Lens epithelial cell apoptosis initiates diabetic cataractogenesis in the Zucker diabetic fatty rat. *Graefes Arch Clin Exp Ophthalmol* 2010;248(6):811-818.

14 Ahner A, Gong X, Frizzell RA. Divergent signaling via SUMO modification: potential for CFTR modulation. *Am J Physiol Cell Physiol* 2016;310(3):C175-C180.

15 Vishwamitra D, Curry CV, Shi P, Alkan S, Amin HM. SUMOylation confers posttranslational stability on NPM-ALK oncogenic protein. *Neoplasia* 2015;17(9):742-754.

16 Enserink JM. Sumo and the cellular stress response. *Cell Div* 2015;10:4.

17 Nishida T, Yamada Y. SUMOylation of the KRAB zinc-finger transcription factor PARIS/ZNF746 regulates its transcriptional activity. *Biochem Biophys Res Commun* 2016;473(4):1261-1267.

18 Hudson JJ, Chiang SC, Wells OS, Rookyard C, El-Khamisy SF. SUMO modification of the neuroprotective protein TDP1 facilitates chromosomal single-strand break repair. *Nat Commun* 2012;3:733.

19 Lee A, Jeong D, Mitsuyama S, Oh JG, Liang L, Ikeda Y, Sadoshima J, Hajjar RJ, Kho C. The role of SUMO-1 in cardiac oxidative stress and hypertrophy. *Antioxid Redox Signal* 2014;21(14):1986-2001.

20 Marchiani S, Tamburrino L, Ricci B, Nosi D, Cambi M, Piomboni P, Belmonte G, Forti G, Muratori M, Baldi E. SUMO1 in human sperm: new targets, role in motility and morphology and relationship with DNA damage. *Reproduction* 2014;148(5):453-467.

21 Meinecke I, Pap G, Mendoza H, Drange S, Ender S, Strietholt S, Gay RE, Seyfert C, Ink B, Gay S, Pap T, Peters MA. Small ubiquitin-like modifier 1 [corrected] mediates the resistance of prosthesis-loosening fibroblast-like synoviocytes against Fas-induced apoptosis. *Arthritis Rheum* 2009;60(7):2065-2070.

22 Emamgholipour S, Hossein-Nezhad A, Sahraian MA, Askarisadr F, Ansari M. Evidence for possible role of melatonin in reducing oxidative stress in multiple sclerosis through its effect on SIRT1 and antioxidant enzymes. *Life Sci* 2016;145:34-41.

23 Zhang C, Qu S, Wei X, Feng Y, Zhu H, Deng J, Wang K, Liu K, Liu M, Zhang H, Xiao X. HSP25 down-regulation enhanced p53 acetylation by dissociation of SIRT1 from p53 in doxorubicin-induced H9c2 cell apoptosis. *Cell Stress Chaperones* 2016;21(2):251-260.

24 Wang RH, Zhao T, Cui K, Hu G, Chen Q, Chen W, Wang XW, Soto-Gutierrez A, Zhao K, Deng CX. Negative reciprocal regulation between Sirt1 and Per2 modulates the circadian clock and aging. *Sci Rep* 2016;6:28633.

25 Mimura T, Kaji Y, Noma H, Funatsu H, Okamoto S. The role of SIRT1 in ocular aging. *Exp Eye Res* 2013;116:17-26.

26 Kang L, Zhao W, Zhang G, Wu J, Guan H. Acetylated 8-oxoguanine DNA glycosylase 1 and its relationship with p300 and SIRT1 in lens epithelium cells from age-related cataract. *Exp Eye Res* 2015;135:102-108.

27 Zhang E, Guo Q, Gao H, Xu R, Teng S, Wu Y. Metformin and resveratrol inhibited high glucose-induced metabolic memory of endothelial senescence through SIRT1/p300/p53/p21 pathway. *PLoS One* 2015;10(12):e0143814.

28 Pei H, Yang Y, Cui L, Yang J, Li X, Yang Y, Duan H. Bisdemethoxycurcumin inhibits ovarian cancer via reducing oxidative stress mediated MMPs expressions. *Sci Rep* 2016;6:28773.

29 Hochrainer K, Pejanovic N, Olaseun VA, Zhang S, Iadecola C, Anrather J. The ubiquitin ligase HERC3 attenuates NF-κB-dependent transcription independently of its enzymatic activity by delivering the RelA subunit for degradation. *Nucleic Acids Res* 2015;43(20):9889-9904.

30 Huang W, Xu L, Zhou X, Gao C, Yang M, Chen G, Zhu J, Jiang L, Gan H, Gou F, Feng H, Peng J, Xu Y. High glucose induces activation of NF-κB inflammatory signaling through IκBα sumoylation in rat mesangial cells. *Biochem Biophys Res Commun* 2013;438(3):568-574.

31 Castorálová M, Březinová D, Svéda M, Lipov J, Ruml T, Knejzlík Z. SUMO-2/3 conjugates accumulating under heat shock or MG132 treatment result largely from new protein synthesis. *Biochim Biophys Acta* 2012;1823(4):911-919.

32 Liu Q, Li J, Khoury J, Colgan SP, Ibla JC. Adenosine signaling mediates SUMO-1 modification of IkappaBalpha during hypoxia and reoxygenation. *J Biol Chem* 2009;284(20):13686-13695.

33 Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic Biol Med* 2013;61:95-110.

34 Li J, Xu Y, Long XD, Wang W, Jiao HK, Mei Z, Yin QQ, Ma LN, Zhou AW, Wang LS, Yao M, Xia Q, Chen GQ. Cbx4 governs HIF-1α to potentiate angiogenesis of hepatocellular carcinoma by its SUMO E3 ligase activity. *Cancer Cell* 2014;25(1):118-131.

35 Han X, Wang XL, Li Q, Dong XX, Zhang JS, Yan QC. HIF-1α SUMOylation affects the stability and transcriptional activity of HIF-1α in human lens epithelial cells. *Graefes Arch Clin Exp Ophthalmol* 2015;253(8):1279-1290.

36 Yu J, Wu Y, Yang P. High glucose-induced oxidative stress represses sirtuin deacetylase expression and increases histone acetylation leading to neural tube defects. *J Neurochem* 2016;137(3):371-383.
37 Caito S, Rajendrasozhan S, Cook S, Chung S, Yao H, Friedman AE, Brookes PS, Rahman I. SIRT1 is a redox-sensitive deacetylase that is post-translationally modified by oxidants and carbonyl stress. *FASEB J* 2010;24(9):3145-3159.
38 Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, Holsboer F, Arzt E. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* 2007;131(2):309-323.
39 Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. *Antioxid Redox Signal* 2013;18(10):1208-1246.
40 Kracklauer MP, Schmidt C. At the crossroads of SUMO and NF-kappaB. *Mol Cancer* 2003;2:39.
41 Shinozaki S, Chang K, Sakai M, Shimizu N, Yamada M, Tanaka T, Nakazawa H, Ichinose F, Yamada Y, Ishigami A, Ito H, Ouchi Y, Starr ME, Saito H, Shimokado K, Stamler JS, Kaneki M. Inflammatory stimuli induce inhibitory S-nitrosylation of the deacetylase SIRT1 to increase acetylation and activation of p53 and p65. *Sci Signal* 2014; 7(351):ra106.
42 Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 2004;23(12):2369-2380.
43 Li X, Luo R, Chen R, Song L, Zhang S, Hua W, Chen H. Cleavage of IκBα by calpain induces myocardial NF-κB activation, TNF-α expression, and cardiac dysfunction in septic mice. *Am J Physiol Heart Circ Physiol* 2014;306(6):H833-H843.
44 Sun Y, Peng R, Peng H, Liu H, Wen L, Wu T, Yi H, Li A, Zhang Z. miR-451 suppresses the NF-kappaB-mediated proinflammatory molecules expression through inhibiting LMP7 in diabetic nephropathy. *Mol Cell Endocrinol* 2016;433:75-86.
45 Tang X, Yao K, Zhang L, Yang Y, Yao H. Honokiol inhibits H(2)O(2)-induced apoptosis in human lens epithelial cells via inhibition of the mitogen-activated protein kinase and Akt pathways. *Eur J Pharmacol* 2011;650(1):72-78.
46 Keshk WA, Zineldeen DH, Wasfy RE, El-Khadrawy OH. Fatty acid synthase/oxidized low-density lipoprotein as metabolic oncogenes linking obesity to colon cancer via NF-kappa B in Egyptians. *Med Oncol* 2014;31(10):192.
47 Bossis G, Melchior F. SUMO: regulating the regulator. *Cell Div* 2006;1:13.
48 Tong C, Morrison A, Mattison S, Qian S, Bryniarski M, Rankin B, Wang J, Thomas DP, Li J. Impaired SIRT1 nucleocytoplasmic shuttling in the senescent heart during ischemic stress. *FASEB J* 2013;27(11): 4332-4342.
49 Yang Y, Fu W, Chen J, Olashaw N, Zhang X, Nicosia SV, Bhalla K, Bai W. SIRT1 sumoylation regulates its deacetylase activity and cellular response to genotoxic stress. *Nat Cell Biol* 2007;9(11):1253-1262.
50 Zhang X, Jiang A, Qi B, Ma Z, Xiong Y, Dou J, Wang J. Resveratrol protects against Helicobacter pylori- associated gastritis by combating oxidative stress. *Int J Mol Sci* 2015;16(11):27757-27769.
51 Liu H, Yu S, Xu W, Xu J. Enhancement of 26S proteasome functionality connects oxidative stress and vascular endothelial inflammatory response in diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2012;32(9):2131-2140.
52 Pereira TM, Pimenta FS, Porto ML, Baldo MP, Campagnaro BP, Gava AL, Meyrelles SS, Vasquez EC. Coadjuvants in the diabetic complications: nutraceuticals and drugs with pleiotropic effects. *Int J Mol Sci* 2016;17(8):pii:E1273.