Ginsenoside Rg1 protects against Sca-1$^+$ HSC/HPC cell aging by regulating the SIRT1-FOXO3 and SIRT3-SOD2 signaling pathways in a γ-ray irradiation-induced aging mice model

YAN-LONG TANG[1], YUE ZHOU[1], YA-PING WANG[2], YING-HONG HE[1], JI-CHAO DING[1], YUAN LI[1] and CUI-LI WANG[1]

[1]Department of Histology and Embryology, Key Laboratory of Cell Biology, Dali University, Dali, Yunnan 671000; [2]Laboratory of Stem Cell and Tissue Engineering, Department of Histology and Embryology, Chongqing Medical University, Chongqing 400016, P.R. China

Received November 4, 2017; Accepted September 10, 2019

DOI: 10.3892/etm.2020.8810

Abstract. Aging is characterized by a progressive deterioration in metabolic functions. The present study aimed to investigate the antagonistic effects of ginsenoside Rg1 (Rg1) on the γ-ray irradiation-induced aging of mixed hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). C57BL/6 mice were divided into a control group, a γ-ray irradiation group that served as an aging mouse model, and an Rg1 group. The Rg1 group was treated with Rg1 at dosage of 20 mg/kg/day for 7 days prior to γ-ray irradiation. The aging mouse model was established by exposing the mice to 6.5-Gy γ-ray total-body irradiation. Stem cell antigen 1 positive (Sca-1$^+$) HSC/HPCs isolated from the mice were examined using a senescence-associated β-galactosidase (SA-β-Gal) staining assay. The cell cycle of the HSC/HPCs was examined using flow cytometry. A mixed hematopoietic progenitor cell colony-forming unit (CFU-mix) assay was also conducted. The mRNA and protein expression levels of sirtuin 1 (SIRT1), SIRT3, forkhead box O3 (FOXO3) and superoxide dismutase (SOD2) were evaluated using western blot and reverse transcription-quantitative PCR assays. The results indicated that Rg1 treatment significantly increased white blood cell, red blood cell and platelet counts in peripheral blood compared with those in the γ-ray irradiation group ($P<0.05$). However, Rg1 significantly attenuated the senescence of Sca-1$^+$ HSC/HPCs in the γ-ray irradiation aging mice model. The proportion of SA-β-Gal stained HSC/HPCs was significantly decreased and CFU-Mix counts were significantly increased in the Rg1 group compared with the γ-ray irradiation group ($P<0.05$). Rg1 significantly increased the mRNA and protein levels of SIRT1, SIRT3, FOXO3 and SOD2 in the Sca-1$^+$ HSC/HPCs compared with those in the γ-ray irradiation group ($P<0.05$). The percentage of Sca-1$^+$ HSC/HPCs arrested at the G1 phase in the Rg1 group was significantly decreased compared with that in the γ-ray irradiation group ($P<0.05$). In conclusion, the present study indicates that Rg1 exerts anti-aging effects via the regulation of SIRT1-FOXO3 and SIRT3-SOD2 signaling pathways, and triggering the progression of Sca-1$^+$ HSC/HPCs from the G1 phase to the S phase in γ-ray irradiation-induced aging mice.

Correspondence to: Dr Yue Zhou, Department of Histology and Embryology, Key Laboratory of Cell Biology, Dali University, 2 Hongsheng Road, Dali, Yunnan 671000, P.R. China
E-mail: zhouxille@yeah.net

Key words: aging, senescence, hematopoietic stem cells, hematopoietic progenitor cells, ginsenoside Rg1, SIRT1, SIRT3, γ-ray irradiation

Introduction

Aging is mainly characterized by a progressive dysfunction of metabolism and various physiological roles, such as maintaining metabolic rate and delaying cell aging (1,2). Clinically, abnormalities of the metabolism and physiological functions can affect the morbidity and mortality of patients. A previous study reported that injury- or damage-associated risk factors are able to cause obvious aging of stem cells (3). Reactive oxygen species (ROS) have been shown to cause injury or damage to cells (4,5).

Recently, radiotherapy has been extensively applied for the treatment of cancers. Although radiotherapy inhibits the proliferation of tumor cells, it also suppresses the growth of hematopoietic stem cells (6). Therefore, the discovery of drugs that could effectively prevent the radiation-induced damage of cells in the hematopoietic system undergoing radiotherapy would be important. Previous studies have investigated the anti-oxidant capacity of drugs by exploring the progression of aging and associated oxidative injuries (7,8); however, studies on the effects of antioxidants on radiation-induced hematopoietic hypofunction are lacking.

Ginsenoside Rg1 (Rg1) is the most active ingredient of *Panax ginseng*, and is characterized by radiation resistance, anti-aging and anti-oxidation effects (9). A previous study conducted by the present research team showed that Rg1 could attenuate the aging processes of hematopoietic stem

cells (HSCs) and hematopoietic progenitor cells (HPCs) (10). Chen *et al* (11) also reported that Rg1 provided resistance against the radiation-induced aging of mouse HSCs/HPCs. However, the anti-aging mechanism ofRg1 and the associated regulatory molecules have not yet been discovered.

Sirtuins (SIRTs) area highly conserved family of proteins, consisting of seven NAD^+-dependent deacetylases, namely SIRT1-7 (12,13). Among these deacetylases, SIRT1 and SIRT3, as the primary deacetylases, participate in cell proliferation, apoptosis, aging and energy metabolism (14,15). A previous study (16) reported that SIRT3 directly regulates ROS production in mitochondria, and further affects cell aging processes. Libert and Guarente (17) reported that SIRT1 serves a critical function in several molecular processes, including inflammation, senescence/aging and intracellular transcription. SIRT1 has also been shown to protect against cellular senescence by deacetylating forkhead box O3 (FOXO3) transcription factors (18). Another previous study demonstrated that SIRT3 could regulate ROS levels by changing the expression of superoxide dismutase 2 (SOD2) (19). A deficiency of SOD2 has also been shown to be associated with aging (20).

In the present study, Rg1 was used to treat a γ-irradiation-induced aging mouse model. In addition, the antagonistic effects of Rg1 on the radiation-induced aging of stem cell antigen 1 positive ($Sca\text{-}1^+$) HSC/HPCs were also explored. Furthermore, the regulative role of SIRT1/SIRT3 signaling pathways in the anti-aging effects of Rg1 on $Sca\text{-}1^+$ HSCs/HPCs derived from the γ-ray irradiation aging mouse model was also investigated.

Materials and methods

Mice. A total of 90 clean C57BL/6 mice (weighing 20-25 g, 6-8 weeks old, random selection of 43 males and 47 females) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). The mice were housed in an environment with a 12-hlight/dark cycle, 40% humidity at room temperature with free access to the food and water. All experiments were approved by the Ethics Committee of the Key Laboratory of Cell Biology (Kunming, China).

Animal grouping. The mice were divided into three groups, namely the control group, γ-ray irradiation group and Rg1 group. The mice in the control group (n=30) were intraperitoneally injected with normal saline and not exposed to γ-ray irradiation. The mice in the γ-ray irradiation group (n=30) were intraperitoneally injected with normal saline for 7 days, followed by exposure to 6.5-Gy γ-ray total-body irradiation. The γ-rays were delivered by a linear accelerator at a dose rate of 57.28 Gy/min (11). The mice in the Rg1 group (n=30) were subjected to the same processes as the γ-ray irradiation group, but with the replacement of normal saline by the same volume of Rg1 at dose of 20 mg/kg/day. The time interval between the final injection and irradiation was 24 h. The specific processes for the treatment of each group were conducted according to a previously published study (11). The Rg1 (cat. no. 060427; purity >95%) was purchased from Jilin Hongjiu Biological Technology Co., Ltd.

Sample preparation. Prior to irradiation treatment, the mice were anesthetized by the intraperitoneal injection of 55 mg/kg pentobarbital. Following the 7 day treatment protocol, mice were sacrificed via the intraperitoneal injection of 150 mg/kg pentobarbital (Altana AG) on day 8. The $Sca\text{-}1^+$ HSC/HPCs were harvested using an immunomagnetic separation method as described in a previous study (21). The harvested $Sca\text{-}1^+$ HSC/HPCs were used in several experiments, including senescence-associated β-galactosidase (SA-β-Gal) cytochemical staining, flow cytometry (FCM), mixed hematopoietic progenitor cell colony-forming unit (CFU-Mix), reverse transcription-quantitative PCR (RT-qPCR) and western blot assays.

Blood routine analysis. Mice were sacrificed as described above, and blood was collected from the eyeball. Blood routine tests, including white blood cell (WBC), red blood cell (RBC) and blood platelet (PLT) counts were conducted using an XE-2100 hematology analyzer (Sysmex Corporation).

SA-β-Gal staining. $Sca\text{-}1^+$ HSC/HPCs were collected on day 7 from every group. The HSC/HPCs were stained using an Senescence β-Galactosidase Staining kit (cat. no. 9860; Cell Signaling Technology, Inc.) according to the manufacturer's instructions. In brief, the purified cells ($1x10^5$) were washed three times using PBS for 5 min each time, and fixed using 4% paraformaldehyde (Beyotime Institute of Biotechnology) for 10 min at room temperature. Then, the $Sca\text{-}1^+$ HSC/HPCs were stained using staining solution at 37˚C for 12 h. The cells were centrifuged at 1,000 x g at room temperature for 10 min and cytospin slides were prepared. Subsequently, the slides were sheet-sealed using 70% glycerol (Sigma-Aldrich; Merck KGaA). In order to observe and calculate the percentage of blue-stained positive cells under bright field illumination, ~$1x10^4$ cells were separated on a slide, and 400 cells on each slide were selected randomly and counted.

CFU-mix culture. The CFU-mix assay was performed according to a previously published study (22) with a few modifications. Briefly, the cells ($1x10^4$ cells/group) were incubated with DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with horse serum (Gibco; Thermo Fisher Scientific, Inc.), 2-mercaptoethanol ($1x10^{-4}$ mol/l; Sigma-Aldrich; Merck KGaA), 3% L-glutamine (Sigma-Aldrich; Merck KGaA), recombinant human erythropoietin (Kyowa Hakko Kirin China Pharmaceutical Co., Ltd.), recombinant human granulocyte macrophage colony stimulating factor (Kyowa Hakko Kirin China Pharmaceutical Co., Ltd.), interleukin-3 (IL-3; Sigma-Aldrich; Merck KGaA) and 2.7% methylcellulose. The components were mixed intensively, seeded into 96-well plates (0.2 ml/well) and then cultured at 37˚C with 5% CO_2 for 7 days. The CFU-Mix and multiple-differentiation capacities were evaluated according to the CFU-Mix numbers and percentage of $Sca\text{-}1^+$ HSC/HPCs.

FCM assay of the cell cycle. $Sca\text{-}1^+$ HSC/HPCs were collected from all three groups and washed using PBS. $Sca\text{-}1^+$ HSC/HPCs were incubated with 70% iced ethanol overnight, and washed with PBS three times (5 min/time). The $Sca\text{-}1^+$ HSC/HPCs were then incubated with bovine pancreatic ribonuclease (cat. no. R2638; Sigma-Aldrich; Merck KGaA;

Table I. Primers used for quantitative PCR.

Primers	Forward (5'-3')	Reverse (5'-3')	Gene length (bp)	Tm (˚C)
SIRT1	AAAGTGATGACGATGACAGAACG	GCCAATCATGAGATGTTGCTG	104	64
SIRT3	GCTTCTGCGGCTCTATACACAG	CACCCTGTCCGCCATCAC	169	66
FOXO3	CAGTCACCCATGCAGACTATCC	GTCGCTGTGGCTGAGTGAGTC	117	68
SOD2	GAGGCTATCAAGCGTGACTTTG	GCAATGGGTCCTGATTAGAGC	157	67
β-actin	GAGACCTTCAACACCCCAGC	ATGTCACGCACGATTTCCC	263	61

SIRT, sirtuin; FOXO3, forkhead box O3; SOD2, superoxide dismutase 2; Tm, melting temperature.

1 mg/ml; 100 μl) at 37˚C for 30 min. Then, the cells were stained using propidium iodide (PI; 50 μg/ml) for 30 min at room temperature in the dark. Finally, the stained cells were analyzed using FCM with a FACSVantage SE instrument (BD Biosciences). The proportion of cells in each cell cycle phase was analyzed using Cell Quest software version 3.3 (BD Biosciences).

RT-qPCR assay. Total cellular RNAs of Sca-1$^+$ HSC/HPCs were extracted using TRIzol reagent (cat. no. 15596018; Thermo Fisher Scientific, Inc.). ABeyoRTIII First Strand cDND Synthesis kit (cat. no. D7178S; Beyotime Institute of Biotechnology) was used to synthesize cDNAs from the RNA. All processes of the reverse transcription were conducted according to the manufacturer's protocol. The synthesized cDNAs were amplified using SYBR-Green I as the fluorescent dye. RT-qPCR was performed using a Fast real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following conditions were used for amplification: 94˚C for 4 min, 94˚C for 20 sec, 60˚C for 30 sec and 72˚C for 30 sec, for 35 cycles. The primers for SIRT1, SIRT3, FOXO3, SOD2 and β-actin are listed in Table I. The relative mRNA expression of the target gene was normalized to that of β-actin using the comparative threshold cycle ($2^{-\Delta\Delta Cq}$) method (23).

Western blot assay. Proteins were isolated from Sca-1$^+$ HSC/HPCs by lysing cells with lysis buffer (Beyotime Institute of Biotechnology). The concentration of isolated proteins was calculated with a BCA protein quantification kit (Beyotime Institute of Biotechnology). Equal amounts of protein (0.2 μg) were separated using 15% SDS-PAGE (Sigma-Aldrich; Thermo Fisher Scientific, Inc.) and electrotransferred onto PVDF membranes (Pierce; Thermo Fisher Scientific, Inc.). The PVDF membranes were blocked using 5% defatted milk at 4˚C overnight. Then, the PVDF membranes were incubated with rabbit anti-mouse SIRT1 polyclonal antibody (cat. no. sc-15404, 1:3,000; Santa Cruz Biotechnology, Inc.), rabbit anti-mouse SIRT3 polyclonal antibody (cat. no. sc-99143, 1:3,000; Santa Cruz Biotechnology, Inc.), rabbit anti-mouse FOXO3 polyclonal antibody (cat. no. ab47285, 1:3,000; Abcam), rabbit anti-mouse SOD2 polyclonal antibody (cat. no. sc-30080; 1:2,000; Santa Cruz Biotechnology, Inc.) and rabbit anti-mouse GAPDH polyclonal antibody (cat. no. sc-25778; 1:2,000; Santa Cruz Biotechnology, Inc.) for 2 h at room temperature. The PVDF membranes were then washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. sc-2030; 1:1,000; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h. Finally, the western blot bands were visualized using an enhanced chemiluminescence kit (Pierce; Thermo Fisher Scientific, Inc.). The western blotting density was qualified using the NIH ImageJ software version 1.46 (National Institutes of Health).

Table II. Effects of Rg1 treatment on peripheral blood (mean ± SD; n=6).

Groups	WBC (x10^9/l)	RBC (x10^{12}/l)	PLT (x10^{12}/l)
Control	8.67±0.45	11.34±1.02	17.08±0.73
γ-ray irradiation	1.06±0.54[a]	3.87±0.92[a]	5.25±0.67[a]
Rg1	3.24±1.26[a,b]	5.09±0.7[4a,b]	6.12±1.02[a,b]

[a]P<0.01 vs. the control group; [b]P<0.05 vs. the γ-ray irradiation group. Rg1, ginsenoside Rg1; WBC, white blood cell; RBC, red blood cell; PLT, platelet.

Statistical analysis. Data are presented as the mean ± standard deviation. Data were analyzed using SPSS software 11.0 (SPSS, Inc.). All data were obtained from at least three independent tests or experiments. Tukey's post hoc test was used following ANOVA to compare continuous data among multiple groups. P<0.05 was considered to indicate statistical significance.

Results

Rg1 improves peripheral blood levels in γ-ray irradiated aging mice. In order to investigate Sca-1$^+$ HSC/HPC-induced hematopoietic functions, the WBC, RBC and PLT levels in the peripheral blood of the mice were examined. The results indicated that the WBC, RBC and PLT counts in the γ-ray irradiation group were significantly decreased compared with those in the control group (P<0.01; Table II). Notably, Rg1 treatment significantly increased the WBC, RBC and PLT counts compared with those in the γ-ray irradiation group (P<0.05; Table II). However, the WBC, RBC and PLT counts in the Rg1 group were significantly lower compared with those in the control group (P<0.01; Table II).

Rg1 affects the senescence of Sca-1$^+$ HSC/HPCs in γ-ray irradiated aging mice. SA-β-Gal is an extensively applied

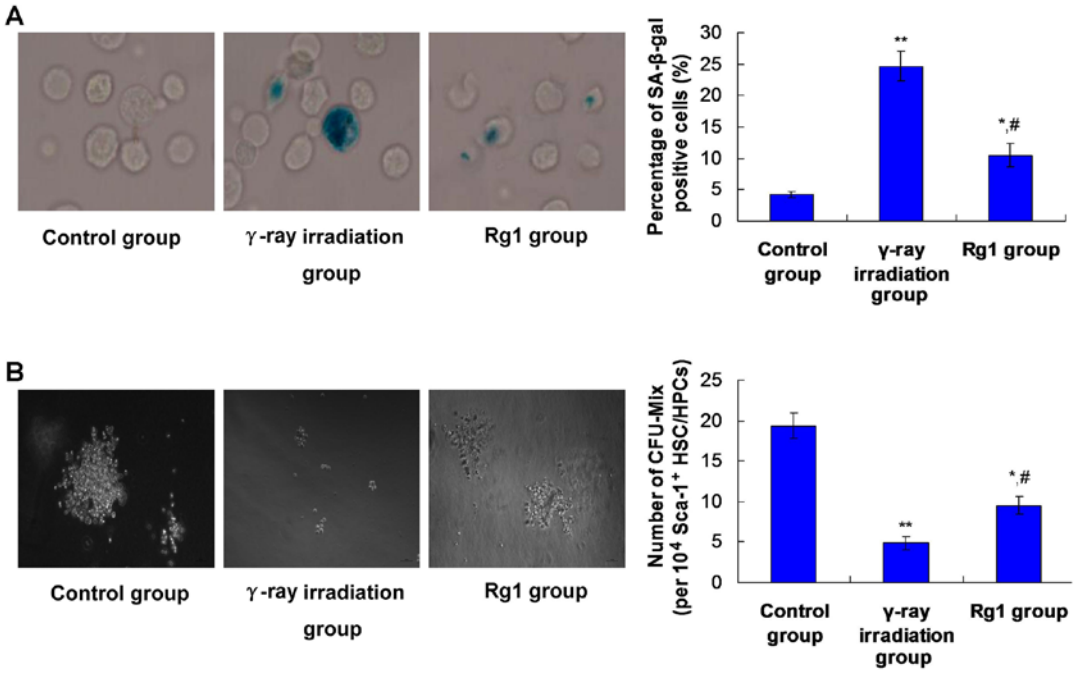

Figure 1. Effects of Rg1 treatment on (A) SA-β-gal staining and (B) CFU-mix counts of Sca-1$^+$ HSC/HPCs in a γ-ray irradiation aging mice model. Results are presented as mean ± SD (n=6). For SA-β-gal staining, magnification x400. For CFU-mix counts, magnification, x100. *P<0.05 and **P<0.01 vs. the control group; $^\#$P<0.05 vs. the γ-ray irradiation group. Rg1, ginsenoside Rg1; SA-β-gal, senescence-associated β-galactosidase; CFU-Mix, mixed hematopoietic progenitor cell colony-forming unit; Sca-1$^+$, stem cell antigen 1 positive; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell.

biomarker for aging cells (24). A decreased CFU-Mix formation capacity is also an evaluative marker for the senescence of HSCs (25). Therefore, an SA-β-Gal staining assay and CFU-Mix formation assay were performed to evaluate the effects of Rg1 on the senescence of Sca-1$^+$ HSC/HPCs.

The results showed that γ-ray irradiation induced a significant increase in the percentage of SA-β-Gal stained Sca-1$^+$ HSC/HPCs (P<0.01; Fig. 1A) and a significant reduction in the CFU-Mix counts of Sca-1$^+$ HSC/HPCs, compared with the control group (P<0.01; Fig. 1B). In the Rg1 group, the proportion of SA-β-Gal stained HSC/HPCs was significantly decreased (Fig. 1A; P<0.05) and the CFU-Mix count was significantly increased (Fig. 1B; P<0.05), compared with the respective values in the γ-ray irradiation group. However, the percentage of SA-β-Gal stained HSC/HPCs and CFU-Mix numbers in the γ-ray irradiation group were also significantly higher and significantly lower, respectively, compared with those in the control group (Fig. 1; P<0.05).

Rg1 increased the mRNA levels of SIRT1/SIRT3 signaling molecules in Sca-1$^+$ HSC/HPCs. Cell proliferation-associated SIRT1 and SIRT3 molecules were examined using RT-qPCR. Compared with the control group, SIRT1 (Fig. 2A) and SIRT3 (Fig. 2B) mRNA levels in the γ-ray irradiation group were significantly decreased (both P<0.01). However, Rg1 treatment (Rg1 group) significantly upregulated SIRT1 (Fig. 2A) and SIRT3 (Fig. 2B) mRNA levels compared with those in the γ-ray irradiation group (P<0.05).

The downstream molecule of SIRT1 (FOXO3) and the downstream molecule of SIRT3 (SOD2) were also examined. The results indicated that FOXO3 (Fig. 2C) and SOD2 (Fig. 2D) mRNA levels were significantly decreased in the γ-ray irradiation group compared with the control group (both P<0.01). However, Rg1 treatment significantly increased the mRNA levels of FOXO3 (Fig. 2C) and SOD2 (Fig. 2D) compared with those in the γ-ray irradiation group (both P<0.05).

Rg1 increased the expression of SIRT1/SIRT3 signaling molecules in Sca-1$^+$ HSC/HPCs. The SIRT1/SIRT3 signaling pathway-associated molecules, namely SIRT1, SIRT3, FOXO3 and SOD2, were also examined using western blotting (Fig. 3A). The results showed that SIRT1, SIRT3, FOXO3 and SOD2 expression levels in the γ-ray irradiation group were significantly decreased compared with their respective levels in the control group (Fig. 3B; all P<0.01). However, when compared with the γ-ray irradiation group, the SIRT1, SIRT3, FOXO3 and SOD2 expression levels in the Rg1 group were significantly increased (Fig. 3B; all P<0.05).

Rg1 impacted the cell cycle distribution of Sca-1$^+$ HSC/HPCs in the γ-ray irradiation aging mouse model. Previous studies (26,27) reported that SIRT1 and SIRT3 participate in cell proliferation and cell cycle regulation. Furthermore, HSCs undergoing aging are arrested at the G1 stage (28). Therefore, the cell cycle distribution of Sca-1$^+$ HSC/HPCs was analyzed using FCM. The significant G1 phase arrest of Sca-1$^+$ HSC/HPCs was observed, and the percentages of cells in the S and M phases were significantly decreased in the γ-ray irradiation and Rg1 groups, compared with those in the control group (Fig. 4; all P<0.05). However, the percentage of Sca-1$^+$ HSC/HPCs arrested at the G1 phase in the Rg1 group was significantly decreased compared with that in the γ-ray irradiation group (Fig. 4; P<0.05).

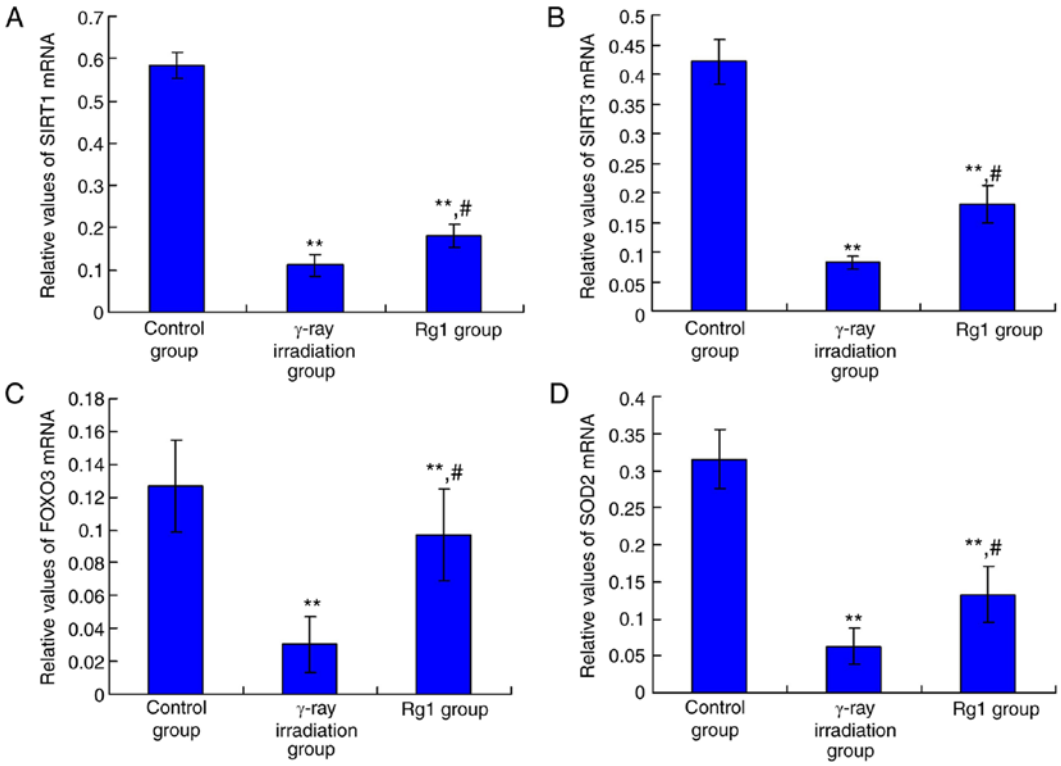

Figure 2. Effects of Rg1 treatment on SIRT1, SIRT3, FOXO3 and SOD2 mRNA expression in Sca-1$^+$ HSC/HPCs. Relative mRNA expression of (A) SIRT1, (B) SIRT3, (C) FOXO3 and (D) SOD2. Results are presented as mean ± SD (n=6). **P<0.01 vs. the control group; $^{\#}$P<0.05 vs. the γ-ray irradiation group. Rg1, ginsenoside Rg1; SIRT, sirtuin; FOXO3, forkhead box O3; SOD, superoxide dismutase 2; Sca-1$^+$, stem cell antigen 1 positive; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell.

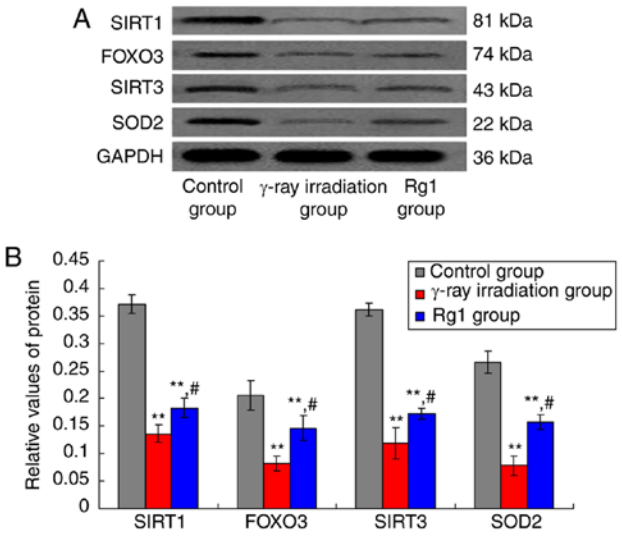

Figure 3. Effects of Rg1 treatment on SIRT1, SIRT3, FOXO3 and SOD2 protein expression in Sca-1$^+$ HSC/HPCs. (A) Western blot bands for SIRT1, SIRT3, FOXO3 and SOD2. (B) Quantification of relative SIRT1, SIRT3, FOXO3 and SOD2 expression. Results are presented as mean ± SD (n=6). **P<0.01 vs. the control group; $^{\#}$P<0.05 vs. the γ-ray irradiation group. Rg1, ginsenoside Rg1; SIRT, sirtuin; FOXO3, forkhead box O3; SOD, superoxide dismutase 2; Sca-1$^+$, stem cell antigen 1 positive; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell.

The proliferative index (PI; S + G2/M) in the γ-ray irradiation group was significantly decreased compared with that in the control group (Fig. 4; P<0.01). However, the PI of Sca-1$^+$ HSC/HPCs in the Rg1 group was significantly increased compared with that in the γ-ray irradiation group (Fig. 4; P<0.05).

Discussion

Scientific studies have demonstrated that the Chinese herbal drug ginseng is characterized by anti-injury, anti-aging and anti-oxidant functions, and the ability to enhance immunity (29,30). Zhu *et al* (31) found that Rg1 could prolong the life of mice and delay the aging processes of human lung fibroblasts. In the aging processes of cells, HSC/HPCs in the hematopoietic system exhibit progressive and morphological changes (32). Zhou *et al* (33) reported that Rg1 delays the tert-butyl hydroperoxide-induced aging of HSCs.

A previous study (34) reported that radiation induces damage or injury in HSC/HPCs, causes cells to undergo apoptosis and senescence, and reduces the hematological reconstitution function of HSC/HPCs. Chen *et al* (11) found that Rg1 promotes the proliferation of hematopoietic cells; however, further evaluation of the mechanism is required. The present study showed that γ-ray irradiation significantly decreased the WBC, RBC and PLT counts of mice compared with those in un-irradiated controls; however, Rg1 was able to attenuate these reductions in blood cell levels. Therefore, the present findings confirm that Rg1 prevents irradiation-induced hematopoietic dysfunction.

It is speculated that the improvement of hematopoietic function observed in the mice might be triggered by increased levels of Sca-1$^+$ HSC/HPCs. SA-β-Gal accumulates in aging cells and reflects the dysfunction of cells (24). Furthermore,

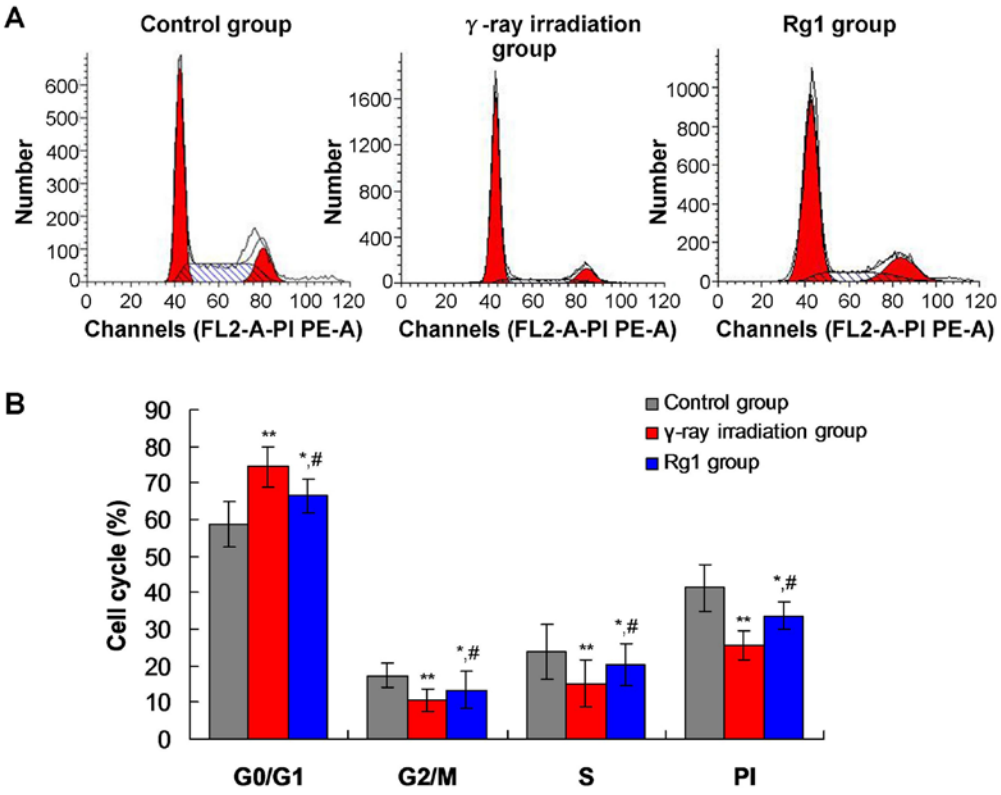

Figure 4. Effects of Rg1 treatment on the cell cycle distribution of Sca-1$^+$ HSC/HPCs. (A) Flow cytometry histograms for the cell cycle. (B) Quantification of the cell cycle data. Results are presented as mean ± SD (n=6). *P<0.05 and **P<0.01 vs. the control group; $^\#$P<0.05 vs. the γ-ray irradiation group. Rg1, ginsenoside Rg1; Sca-1$^+$, stem cell antigen 1 positive; HSC, hematopoietic stem cell; HPC, hematopoietic progenitor cell.

the ability of mixed hematopoietic progenitor cells to form colonies decreases following the regression of self-renewal potential in aging Sca-1$^+$ HSC/HPCs (35). Therefore, the aging or senescence of Sca-1$^+$ HSC/HPCs was evaluated using the SA-β-Gal method and CFU-Mix assay in the present study. The results demonstrated that the percentage of SA-β-Gal stained HSC/HPCs was significantly increased and CFU-Mix counts were significantly decreased following γ-ray irradiation compared with those in the control group. Notably, Rg1 treatment significantly attenuated the γ-ray irradiation-induced and aging/senescence-associated changes. These findings are consistent with a previous study (11), which reported that Rg1 delays the irradiation-induced senescence of Sca-1$^+$ HSC/HPCs. Therefore, the present data suggest that Rg1 effectively counteracts the γ-ray irradiation-induced persistent senescence/aging of HSC/HPCs.

At present, several signaling pathways have been demonstrated to participate in cell senescence or aging; these include SIRT1 and SIRT3 pathways, which may inhibit cellular senescence by regulating the cell cycle (26,27,36). SIRT3 upregulates the self-renewal and multi-differentiation capacity of HSCs, and further delays their aging processes (37). SIRT3 also enhances the activity of SOD2 by triggering deacetylation, which plays an important role in the negative regulation of organism aging (38). Therefore, SIRT3 and SOD2 expression in Sca-1$^+$ HSC/HPCs were examined in the present study. The results indicated that irradiation significantly induced the downregulation of SIRT3 and SOD2 in Sca-1$^+$ HSC/HPCs. This suggests that irradiation might initiate the progression of aging and oxidative stress. SOD2 is also considered to be an important anti-oxidant defense enzyme; therefore, it is speculated that γ-ray irradiation might result in the oxidative damage of Sca-1$^+$ HSC/HPCs via the reduction of SOD2 activity. However, the results of the present study revealed that Rg1 significantly inhibited the irradiation-induced downregulation of SIRT3 and SOD2 expression. This suggests that Rg1 is able to inhibit the aging of Sca-1$^+$ HSC/HPCs and increaseSOD2 activity, which is critical to prevent Sca-1$^+$ HSC/HPCs from undergoing irradiation-induced aging or oxidative stress.

It has previously been reported that SIRT1 can delay cardiovascular and neuron aging (39). Morris *et al* (40) reported that FOXO3 is an important gene for human longevity. It is well known that the SIRT1 gene can interact with FOXO3 and regulate FOXO3 expression in the mitochondria of cells (41). Therefore, it is hypothesized that the effects of SIRT1 on aging might be triggered by FOXO3 expression. Therefore, SIRT1 and FOXO3 expression were examined in the present study, and it was found that irradiation treatment significantly downregulated SIRT1 and FOXO3 levels in Sca-1$^+$ HSC/HPCs. However, Rg1 treatment significantly attenuated the irradiation-induced SIRT1 and FOXO3 downregulation. These results suggest that Rg1 may also suppress the aging of Sca-1$^+$ HSC/HPCs via the activation of SIRT3 and FOXO3 expression.

A previous study (26) reported that SIRT3 overexpression disrupts mitochondrial proteostasis, induces cell cycle arrest and inhibits the proliferation of cells. In another study, SIRT1 was reported to contribute to delay of the epithelial cell cycle in diabetic corneas (42). Therefore, the cell cycle status of Sca-1$^+$ HSC/HPCs was investigated in the present study to determine

the proportion of cells in the G1, S, G2 and M phases. The results indicated that irradiation triggered cell cycle arrest at the G1 phase and that cell senescence was induced. However, as discussed above, Rg1 treatment significantly increased SIRT1 and SIRT3 expression in irradiated Sca-1$^+$ HSC/HPCs. The increased SIRT1 and SIRT3 expression may have inhibited cells from arresting in the G1 phase and suppressed cell senescence. Irradiation-induced arrest of the cell cycle at the G1 phase, without entry to the S phase, is mainly induced by the gradual loss of mitosis reactivity and DNA synthesis activity in aging cells (28). The cell cycle results in the present study suggest that Rg1 treatment significantly attenuated the irradiation-induced G1 arrest by regulating the expression of SIRT1 and SIRT3. This may have triggered the progression of cells from the G1 phase to the S phase, and thereby delayed the senescence of Sca-1$^+$ HSC/HPCs.

In conclusion, Rg1 decreased the percentage of SA-β-Gal stained Sca-1$^+$ HSC/HPCs, and increased the ability of HSC/HPCs to form colonies. These results suggest that Rg1 treatment attenuates γ-ray irradiation-induced aging in a mouse model. Rg1 may exert anti-aging effects via the enhancement of SOD2 activity and reduction of SIRT3, SIRT1 and FOXO3 expression. This study provides a promising hypothesis for the mechanism by which Rg1 application delays the aging of Sca-1$^+$ HSC/HPCs.

Acknowledgements

Not applicable.

Funding

This study was supported by grants from the National Natural Science Foundation of China (grant nos. 81660731 and 81673748).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YLT and YZ designed this study and wrote the manuscript. YLT, YZ, YPW, YHH and JCD performed the experiments or tests. YL and CLW analyzed the data. CLW reviewed the literature. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Ethics Committee of the Key Laboratory of Cell Biology (Kunming, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Xu YF, Liang ZJ, Kuang ZJ, Chen JJ, Wu J, Lu XE, Jiang WW, Fan PL, Tang LY, Li YT, *et al*: Effect of Suo Quan Wan on the bladder function of aging rats based on the β-adrenoceptor. Exp Ther Med 13: 3424-3432, 2017.
2. Li YN, Guo Y, Xi MM, Yang P, Zhou XY, Yin S, Hai CX, Li JG and Qin XJ: Saponins from Aralia taibaiensis attenuate D-galactose-induced aging in rats by activating FOXO3a and Nrf2 pathways. Oxid Med Cell Longev 2014: 320513, 2014.
3. Bustos ML, Huleihel L, Kapetanaki MG, Lino-Cardenas CL, Mroz L, Ellis BM, McVerry BJ, Richards TJ, Kaminski N, Cerdenes N, *et al*: Aging mesenchymal stem cells fail to protect because of impaired migration and antiinflammatory response. Am J Respir Crit Care Med 189: 787-798, 2014.
4. Montezano AC and Touyz RM: Reactive oxygen species, vascular Noxs, and hypertension: Focus on translational and clinical research. Antioxid Redox Signal 20: 164-182, 2014.
5. Liochev SI: Reactive oxygen species and the free radical theory of aging. Free Radic Biol Med 60: 1-4, 2013.
6. Milano F, Merriam F, Nicoud I, Li J, Gooley TA, Heimfeld S, Imren S and Delaney C: Notch-expanded murine hematopoietic stem and progenitor cells mitigate death from lethal radiation and convey immune tolerance in mismatched recipients. Stem Cells Transl Med 6: 566-575, 2017.
7. Sadowska-Bartosz I and Bartosz G: Effect of antioxidants supplementation on aging and longevity. Biomed Res Int 2014: 404680, 2014.
8. Guerra-Araiza C, Alvarez-Mejia AL, Sanchez-Torres S, Farfan-Garcia E, Mondragon-Lozano R, Pinto-Almazan R and Salgado-Ceballos H: Effect of natural exogenous antioxidants on aging and on neurodegenerative diseases. Free Radic Res 47: 451-462, 2013.
9. Chu SF and Zhang JT: New achievements in ginseng research and its future prospects. Chin J Integr Med 15: 403-408, 2009.
10. Xu FT, Li HM, Yin QS, Cui SE, Liu DL, Nan H, Han ZA and Xu KM: Effect of ginsenoside Rg1 on proliferation and neural phenotype differentiation of human adipose-derived stem cells in vitro. Can J Physiol Pharmacol 92: 467-475, 2014.
11. Chen C, Mu XY, Zhou Y, Shun K, Geng S, Liu J, Wang JW, Chen J, Li TY and Wang YP: Ginsenoside Rg1 enhances the resistance of hematopoietic stem/progenitor cells to radiation-induced aging in mice. Acta Pharmacol Sin 35: 143-150, 2014.
12. Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K and Auwerx J: Sirtuins: The 'magnificent seven', function, metabolism and longevity. Ann Med 39: 335-345, 2007.
13. Imai S, Johnson FB, Marciniak RA, McVey M, Park PU and Guarente L: Sir2: An NAD-dependent histone deacetylase that connects chromatin silencing, metabolism, and aging. Cold Spring Harb Symp Quant Biol 65: 297-302, 2000.
14. Buler M, Aatsinki SM, Izzi V and Hakkola J: Metformin reduces hepatic expression of SIRT3, the mitochondrial deacetylase controlling energy metabolism. PLoS One 7: e49863, 2012.
15. D'Aquila P, Rose G, Panno ML, Passarino G and Bellizzi D: SIRT3 gene expression: A link between inherited mitochondrial DNA variants and oxidative stress. Gene 497: 323-329, 2012.
16. Finley LW, Haas W, Desquiret-Dumas V, Wallace DC, Procaccio V, Gygi SP and Haigis MC: Succinate dehydrogenase is a direct target of sirtuin 3 deacetylase activity. PLoS One 6: e23295, 2011.
17. Libert S and Guarente L: Metabolic and neuropsychiatric effects of calorie restriction and sirtuins. Annu Rev Physiol 75: 669-684, 2013.
18. Yao H, Chung S, Hwang JW, Rajendrasozhan S, Sundar IK, Dean DA, McBurney MW, Guarente L, Gu W, Ronty M, *et al*: SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. J Clin Invest 122: 2032-2045, 2012.
19. Chen Y, Zhang J, Lin Y, Lei Q, Guan KL, Zhao S and Xiong Y: Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Rep 12: 534-541, 2011.
20. Miao L and St Clair DK: Regulation of superoxide dismutase genes: Implications in disease. Free Radic Biol Med 47: 344-356, 2009.
21. Liwski CJ, Padley DJ, Gustafson MP, Winters JL, Gastineau DA and Jacob EK: Discordant CD34$^+$ cell results in peripheral blood and hematopoietic progenitor cell-apheresis product: Implications for clinical decisions and impact on patient treatment. Transfusion 54: 541-544, 2014.

22. Zhou Y, Yang B, Yao X and Wang Y: Establishment of an aging model of Sca-1^{+} hematopoietic stem cell and studies on its relative biological mechanisms. In Vitro Cell Dev Biol Anim 47: 149-156, 2011.
23. Schmittgen TD, Zakrajsek BA, Mills AG, Gorn V, Singer MJ and Reed MW: Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: Comparison of endpoint and real-time methods. Anal Biochem 285: 194-204, 2000.
24. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I and Pereira-Smith O: A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 92: 9363-9367, 1995.
25. Tang YL, Zhou Y, Wang YP, Wang JW and Ding JC: SIRT6/NF-κB signaling axis in ginsenoside Rg1-delayed hematopoietic stem/progenitor cell senescence. Int J Clin Exp Pathol 8: 5591-5596, 2015.
26. Giralt A and Villarroya F: SIRT3, a pivotal actor in mitochondrial functions: Metabolism, cell death and aging. Biochem J 444: 1-10, 2012.
27. Atkins KM, Thomas LL, Barroso-Gonzalez J, Thomas L, Auclair S, Yin J, Kang H, Chung JH, Dikeakos JD and Thomas G: The multifunctional sorting protein PACS-2 regulates SIRT1-mediated deacetylation of p53 to modulate p21-dependent cell-cycle arrest. Cell Rep 8: 1545-1557, 2014.
28. Stein GH, Beeson M and Gordon L: Failure to phosphorylate the retinoblastoma gene product in senescent human fibroblasts. Science 249: 666-669, 1990.
29. Chen X, Zhang J, Fang Y, Zhao C and Zhu Y: Ginsenoside Rg1 delays tert-butyl hydroperoxide-induced premature senescence in human WI-38 diploid fibroblast cells. J Gerontol A Biol Sci Med Sci 63: 253-264, 2008.
30. Asadullina NR, Usacheva AM and Gudkov SV: Protection of mice against X-ray injuries by the post-irradiation administration of inosine-5'-monophosphate. J Radiat Res 53: 211-216, 2012.
31. Zhu J, Mu X, Zeng J, Xu C, Liu J, Zhang M, Li C, Chen J, Li T and Wang Y: Ginsenoside Rg1 prevents cognitive impairment and hippocampus senescence in a rat model of D-galactose-induced aging. PLoS One 9: e101291, 2014.
32. Hu W, Jing P, Wang L, Zhang Y, Yong J and Wang Y: The positive effects of Ginsenoside Rg1 upon the hematopoietic microenvironment in a D-Galactose-induced aged rat model. BMC Complement Altern Med 15: 119, 2015.
33. Zhou Y, Yang B, Jiang R, Yao X and Wang YP: Mechanism of ginsenoside Rg1 in the delayed senescence of hematopoietic stem cell. Zhonghua Yi Xue Za Zhi 90: 3421-3425, 2010 (In Chinese).
34. Wang Y, Liu L, Pazhanisamy SK, Li H, Meng A and Zhou D: Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. Free Radic Biol Med 48: 348-356, 2010.
35. Kamminga LM, van Os R, Ausema A, Noach EJ, Weersing E, Dontje B, Vellenga E and de Haan G: Impaired hematopoietic stem cell functioning after serial transplantation and during normal aging. Stem Cells 23: 82-92, 2005.
36. Kwon Y, Kim J, Lee CY and Kim H: Expression of SIRT1 and SIRT3 varies according to age in mice. Anat Cell Biol 48: 54-61, 2015.
37. Brown K, Xie S, Qiu X, Mohrin M, Shin J, Liu Y, Zhang D, Scadden DT and Chen D: SIRT3 reverses aging-associated degeneration. Cell Rep 3: 319-327, 2013.
38. Lu J, Cheng K, Zhang B, Xu H, Cao Y, Guo F, Feng X and Xia Q: Novel mechanisms for superoxide-scavenging activity of human manganese superoxide dismutase determined by the K68 key acetylation site. Free Radic Biol Med 85: 114-126, 2015.
39. Chang HC and Guarente L: SIRT1 and other sirtuins in metabolism. Trends Endocrinol Metab 25: 138-145, 2014.
40. Morris BJ, Willcox DC, Donlon TA and Willcox BJ: FOXO3: A major gene for human longevity-a mini-review. Gerontology 61: 515-525, 2015.
41. Das S, Mitrovsky G, Vasanthi HR and Das DK: Antiaging properties of a grape-derived antioxidant are regulated by mitochondrial balance of fusion and fission leading to mitophagy triggered by a signaling network of Sirt1-Sirt3-Foxo3-PINK1-PARKIN. Oxid Med Cell Longev 2014: 345105, 2014.
42. Gao J, Wang Y, Zhao X, Chen P and Xie L: MicroRNA-204-5p-mediated regulation of SIRT1 contributes to the delay of epithelial cell cycle traversal in diabetic corneas. Invest Ophthalmol Vis Sci 56: 1493-1504, 2015.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.