Pre-Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(2) 893–904

DOI: 10.1177/0300060518805302
journals.sagepub.com/home/imr
SAGE

Ku86 alleviates human umbilical vein endothelial cellular apoptosis and senescence induced by a low dose of ionizing radiation

Kai Wu[1,2,3], Zejin Chen[1], Qing Peng[2], Guojian Chen[1], Weihong Yan[1] and Xiaoping Chen[2]

Abstract

Objective: The aim of this study was to observe the effect of Ku86 on cellular senescence and apoptosis induced by various doses of ionizing radiation in human umbilical vein endothelial cells (HUVECs).

Methods: Senescence-associated β-galactosidase activity was detected to evaluate cell senescence. Apoptosis was determined by flow cytometry and a caspase enzyme determination kit. $p16^{Ink4a}$, Sirt1, superoxide dismutase 2 (SOD2), xanthine oxidase (XOD), and Bcl-2 protein expression levels were measured by western blotting.

Results: Low doses of ionizing radiation induced cellular senescence and apoptosis in a dose-dependent manner. The Ku86 protein was negatively correlated with ionization intensity. After transfection of Ku86 with a vector (pcDNA 3.1), or interference with siRNA (si-Ku86), apoptosis/senescence and related protein expression were observed. Western blot results revealed that this induction of senescence was associated with activated Sirt1 and SOD2, and downregulation of $p16^{Ink4a}$ and XOD in 0.2 Gy ionizing radiation. The expression levels of apoptosis-associated proteins, such as Bcl-2, cleaved caspase-3, caspase-8, and caspase-9, were significantly altered in both the presence and absence of Ku86 with ionizing radiation (0.2 Gy).

[1]Department of Cardiovascular Medicine, 903 Hospital, and Center for Medical Radiation Biology, Institute of Materials, China Academy of Engineering Physics, Mianyang, China
[2]Department of Cardiovascular Medicine, West China Hospital, Mianyang, China
[3]Department of Cardiovascular Medicine, Guangyuan Central Hospital, Guangyuan, China

Corresponding author:
Xiaoping Chen, Department of Cardiovascular Medicine, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu 610041, Sichuan, China.
Email: flyxxiang@yeah.net

Conclusions: Our study revealed that Ku86 overexpression inhibits HUVEC apoptosis and senescence induced by low doses of ionizing radiation.

Keywords
Senescence, apoptosis, Ku86, ionizing radiation, HUVEC, DNA damage, DNA repair, reactive oxygen species

Date received: 15 March 2018; accepted: 14 September 2018

Introduction

Ionizing radiation is defined as radiation with sufficient energy to displace electrons from molecules. Substantial evidence shows that exposure to high doses of ionizing radiation gives rise to deterministic effects that can ultimately lead to illness or fatality. Other health effects of ionizing radiation include cardiovascular disease, teratogenesis, cognitive decline, heart disease, and stroke.[1] Long-term studies on atomic bomb survivors in Japan revealed that the risk of cardiovascular disease-related death in these survivors was increased by 17% per Gy after 0–4 Gy total-body irradiation.[2]

Senescence and apoptosis are two important physiological processes that have been studied extensively in cell biology. Cells undergo senescence and apoptosis after exposure to various stimuli, such as oxidative stress, ionizing radiation, serum deprivation, heat shock, and anticancer chemotherapy.[3] Among these, ionizing radiation is a key potent inducer of apoptosis and senescence in a variety of cells. Previous reports revealed that radiation can increase the production of reactive oxygen species[4] while antioxidant administration has the opposite effect.[5] The exposure of chronic low-dose γ radiation was found to strongly induce cellular senescence in human umbilical vein endothelial cells (HUVECs).[6] Senescence is considered to be an irreversible state of growth cessation after several continuous passages, and the senescent cells are generally characterized by permanent cell cycle arrest, telomere shortening, morphological changes,[7] tumor suppressor network activation, and senescence-associated β-galactosidase (SA-β-gal) activity enhancement, but the cells remain metabolically active.[8]

Definitive evidence has revealed that the main reason for the loss of function that is associated with senescence is the accumulation of DNA damage that can dysregulate gene expression and cell function, impair transcription, cause cell cycle arrest, and trigger programmed cell apoptosis.[9] The DNA-dependent protein kinase (DNA-PK) plays a critical role in repairing DNA damage induced by ionizing radiation, which is composed of a serine/ammonia subunit (DNA-PKcs) and proteins regulating a heterodimeric DNA end-binding complex that consist of 70- and 86-kDa subunits (Ku70 and Ku86). Ku86 is an important member of the Ku family that was originally identified as an autoantigen and was detected in the serum of patients with autoimmune disorders.[10] Ku86 is reportedly involved in the repair of DNA double-strand breaks (DSB) that can arise through exposure to chemotherapeutic agents or ionizing radiation. The Ku86/70 proteins recruit and activate the catalytic subunit of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and then identify and combine with the free end of broken DNA. Repair of the damaged

DNA is facilitated by regulation of a series of phosphorylation reactions.[11] However, the potential role of Ku86 in ionizing radiation related-senescence and apoptosis remains unclear. The purpose of this study is to explore the functional mechanism of Ku86 in the process of cell senescence and apoptosis induced by radiation.

Methods

Cell culture and transfection

The HUVECs were obtained from Shanghai Cellular Research Institute (Shanghai, China) and cultured in Endothelial Cell Medium (ScienCell, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) in a humidified incubator maintained at 37°C with 5% CO. Ku86 siRNA (si-Ku86) and Ku86 pcDNA 3.1 (Ku86-pcDNA3.1) were designed and synthesized by GenePharma (Shanghai, China). The HUVECs were transfected with a pcDNA3.1 vector using the previously reported packaging method.[12] The Ku86 receptor was silenced by siRNA using the Lipofectamine 2000 transfection reagent kit (Invitrogen, NY, USA). The Institute Research Ethics Committee of West China Hospital, Sichuan University approved this study. All experiments were rigorously conducted according to the approved guidelines.

Irradiation treatment

Caesium-137 gamma source (HWM-D 2000, Waelischmüller, Germany) was adopted to irradiate cells. After 48 h of transfection, all cells were subjected to ionizing irradiation for continuous 7 days with cumulative doses of 0, 0.1, 0.2, 0.3, and 0.5 Gy.

Galactosidase (β-gal) staining

HUVECs were seeded at $0.8–1.0 \times 10^4$ cells per 35-mm dish containing coverslips (about 50%–70% confluence) and incubated overnight, before irradiation or sham irradiation. At the indicated time points after irradiation, cells were washed twice with ice-cold phosphate-buffered saline (PBS), and fixed in 2% formaldehyde and 0.2% glutaraldehyde in PBS for 5 minutes at room temperature. In accordance with the manufacturer's protocol, cells were then stained using a galactosidase (β-gal) staining kit (Beyotime, Shanghai, China). The entire cell population in three random microscopic fields (with at least 100 cells) were analyzed for perinuclear blue staining, which indicates senescence-associated β-galactosidase (SA-β-gal) activity.

Western blotting

Transfected cells were incubated in fresh medium for 24 hours before being exposed to 0.2 Gy of irradiation. The cellular protein samples were subjected to SDS-PAGE, and the gels were transferred onto a polyvinylidene difluoride membrane (Millipore) and initially probed with specific primary antibodies. Secondary horseradish peroxidase-conjugated antibodies were used as follows: Anti-Ku86 (Gibco, NY, USA); Bcl-2 antibody (BD Transduction Laboratories, San Jose, CA, USA); anti-caspases (Upstate, NY, USA); anti-$p16^{Ink4a}$ and anti-SirT1 (Abcam Inc., Cambridge, MA, USA); anti-superoxide dismutase (SOD)2 and anti-xanthine oxidase (XOD; Santa Cruz Biotechnology, Santa Cruz, CA, USA); and anti-acetylglyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Sigma, St. Louis, MO, USA). Proteins were visualized using an enhanced chemiluminescence solution.

Annexin V/7-AAD assay

Apoptosis was measured by flow cytometry using an annexin V/7-AAD apoptosis detection kit (Sigma-Aldrich, Shanghai, China). Transfected cells were treated with

0.2 Gy of ionizing radiation and harvested at 48 hours after irradiation. Subsequently, cells were treated in accordance with the manufacturer's instructions that were supplied with the annexin V/7-AAD apoptosis detection kit. The apoptosis rate was calculated as follows: number of early apoptotic cells (annexin V^+/7-AAD^-) + number of late apoptotic cells (annexin V^+/7-AAD^+).

Statistical analysis

Statistical analysis was performed using PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Software (GraphPad Software, Inc., San Diego, CA, USA). All comparisons were performed using a one-way analysis of variance (ANOVA). $P < 0.05$ was considered to indicate a significant difference. Each experiment was performed at least three times and the results were expressed as the mean ± standard deviation (SD).

Results

Low doses of ionizing radiation induced senescence and apoptosis in HUVECs

The senescence level of HUVECs was first investigated via staining with SA-β-gal.[13] HUVECs treated with low doses of ionizing radiation showed a significant increase in SA-β-gal^+ cells in a dose-dependent manner ($p < 0.05$), suggesting that the low doses of ionizing radiation could cause cell senescence (Figure 1a). Additionally, flow cytometry analysis revealed that the percentage of apoptotic cells gradually increased as the intensity of the ionizing radiation increased, and the Ku86 level was negatively correlated with low doses of ionization intensity ($p < 0.05$, Figure 1b). We also found that the increased ionization caused a significant reduction in Ku86 expression and a significant increase in $p16^{Ink4a}$ expression in a dose-dependent manner ($p < 0.05$, $p < 0.01$, Figure 1c). The above results suggested that low doses of ionizing radiation can induce senescence and apoptosis in HUVECs. A dose of 0.2 Gy of ionizing radiation significantly increased cellular apoptosis and senescence compared with the other radiation doses. Therefore, 0.2 Gy of ionizing radiation was selected for the subsequent experiments.

Ku86 inhibits cellular senescence induced by a low dose of ionizing radiation in HUVECs

We then assessed whether Ku86 was involved in the progression of cellular senescence and apoptosis. As shown in Figure 2a, the number of SA-β-gal^+ (positive) cells was significantly reduced, indicating that pcDNA3.1-Ku86 significantly inhibited the increase in the number of SA-β-gal^+ cells when co-incubated with HUVECs ($p < 0.01$). To further evaluate the impact of Ku86 on cellular senescence, $p16^{Ink4a}$, Sirt1, XOD, and SOD2 expression was detected using western blotting. Sirt1 ($p < 0.01$) and SOD2 ($p < 0.05$) levels were significantly higher in Ku86-overexpressing cells, and $p16^{Ink4a}$ and XOD levels were reduced in overexpressing cells, compared with control cells ($p < 0.01$, Figure 2b). To confirm whether reduced Ku86 activity was directly associated with a low dose of ionizing radiation-induced cellular senescence, we inhibited Ku86 activity using a specific siRNA. The SA-β-gal^+ cell population was significantly increased by Ku86 knockdown ($p < 0.05$, Figure 3a). Additionally, the expression of senescence-related proteins in HUVECs lacking Ku86 showed the opposite trend, compared with that observed in Ku86-overexpressing cells ($p < 0.01$, Figure 3b).

Ku86 inhibits cellular apoptosis induced by a low dose of ionizing radiation in HUVECs

Ku86 overexpression inhibited low-dose ionizing radiation-induced cellular senescence in

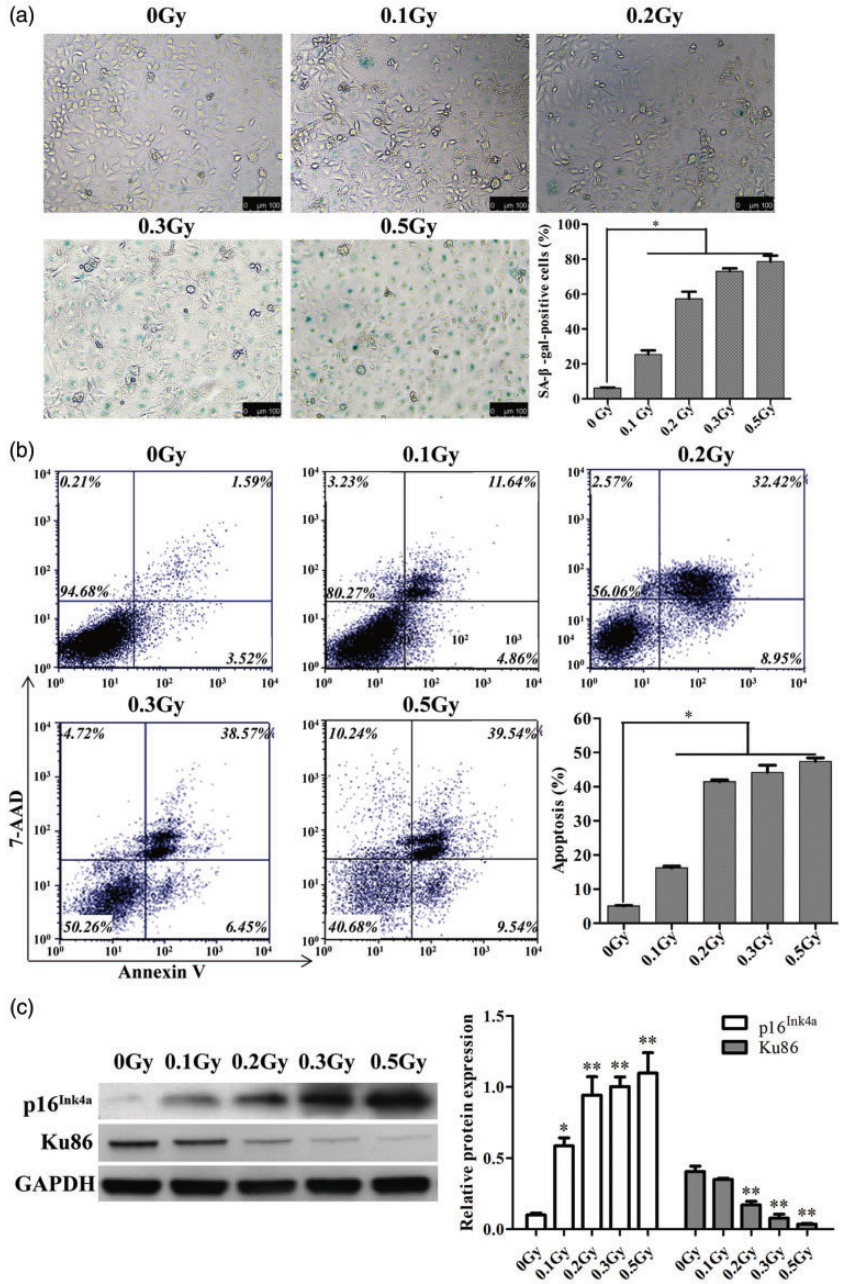

Figure 1. Ku86 expression was downregulated in HUVECs following exposure to various low doses of ionizing radiation for 7 consecutive days. The cells were harvested at 48 hours after irradiation. (a) Cellular senescence was quantified by calculating the percentage of SA-β-gal^{+} cells. (b) Cell apoptosis was determined by flow cytometry in HUVECs subjected to various low doses of ionizing radiation. (c) p16^{Ink4a} and Ku86 protein expression under various low doses of ionizing radiation. *$p < 0.05$, **$p < 0.01$.

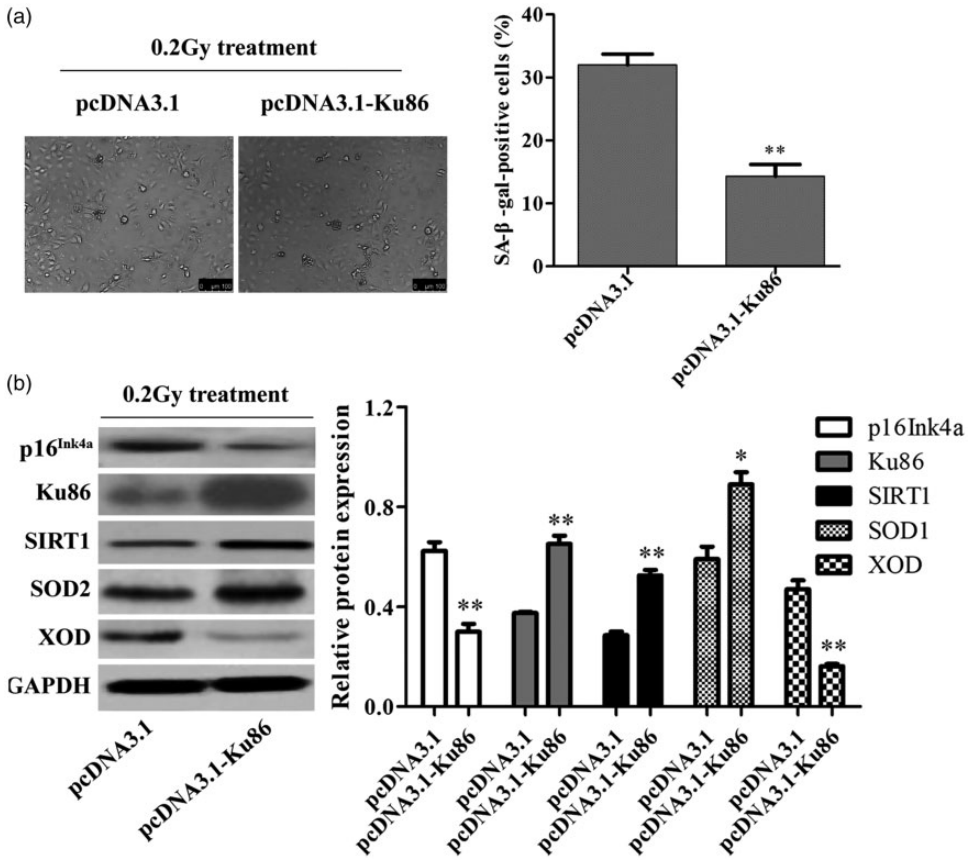

Figure 2. Overexpression of Ku86 reduced ionizing radiation-induced cellular senescence in culture. The cells were harvested at 48 hours after irradiation. (a) Ku86 overexpression reduced the population of SA-β-gal$^+$ cells. (b) Proteins associated with senescence in the Ku86 overexpression group were detected by western blotting. *$p < 0.05$, **$p < 0.01$.

HUVECs, but its effect on cellular apoptosis was unknown. We tested whether overexpression or loss of Ku86 activity affects cellular apoptosis in the presence of 0.2-Gy ionizing radiation. The extent of apoptosis in transfected cells was analyzed using flow cytometry. The data showed that Ku86 overexpression markedly reduced the percentage of annexin V^+ apoptotic cells ($p < 0.001$, Figure 4a). To determine the relationship between the apoptotic proteins and Ku86, we transfected cells with pcDNA3.1 (overexpressing Ku86 protein) and then detected the level of cleaved caspase-3, 8, and 9, and Bcl-2 in HUVECs. As expected, the Ku86 protein level was increased in the cells transfected with pcDNA3.1-Ku86 compared with those transfected with the empty vector ($p < 0.01$, Figure 4b). Moreover, Ku86 overexpression (pcDNA3.1-Ku86) significantly downregulated caspase 3, 8, and 9 ($p < 0.01$) expression and upregulated Bcl-2 expression ($p < 0.01$, Figure 4b). However, the population of annexin V^+ apoptotic cells was significantly increased in the Ku86 interference group ($p < 0.001$, Figure 5a). The cleavage of cleaved caspase-3, 8, and 9 and Bcl-2 in

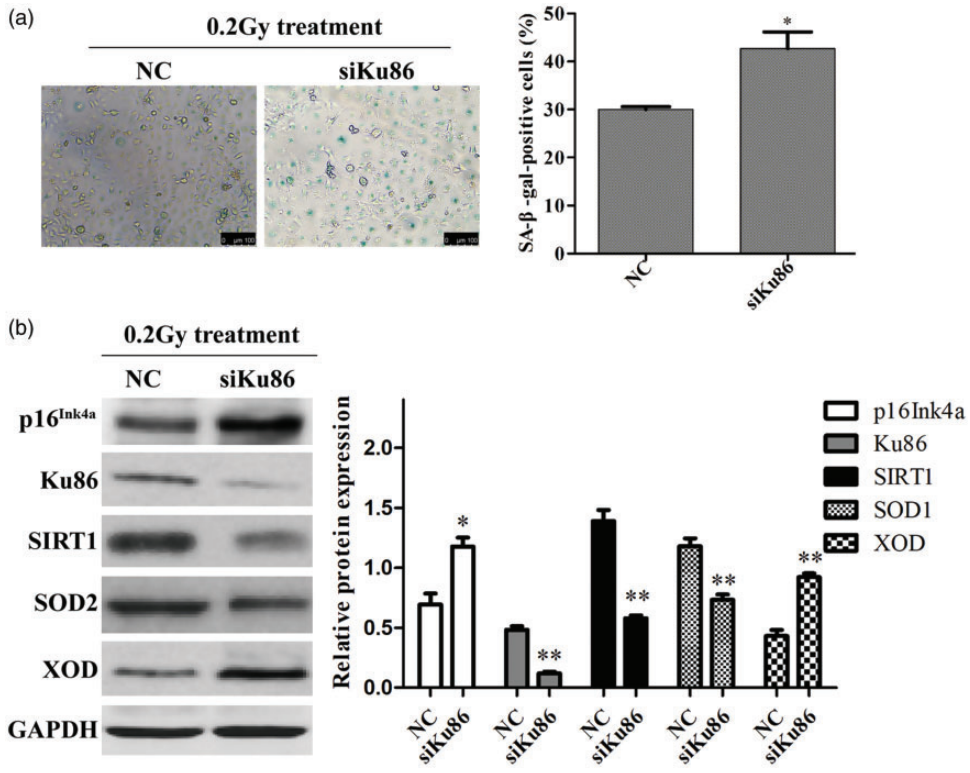

Figure 3. Ku86 deficiency accelerates ionizing radiation-induced cellular senescence in culture. The cells were harvested at 48 hours after irradiation. (a) Ku86 deficiency increased the population of SA-β-gal^{+} cells. (b) Proteins associated with senescence in the Ku86 deficiency group were detected by western blotting. $^{*}p < 0.05$, $^{**}p < 0.01$.

Ku86-defective HUVECs showed the opposite trend to that observed in Ku86-overexpressing cells ($p < 0.01$, Figure 5b).

Discussion

Ionizing radiation has negative effects on health. Ischemic heart disease and cardiovascular disease are the most common conditions caused by ionizing irradiation.[14] An increasing amount of evidence indicates that ionizing radiation is involved in cellular senescence and apoptosis.[15] Accumulation of DNA damage is identified as the main reason for loss of function that is associated with senescence.[9] Thus, most premature aging syndromes or progeroid syndromes result from defects in DNA repair genes,[16] which indicates that the balance between DNA damage and repair determines the rate of aging. The *Ku86* gene is associated with the repair of damaged DNA, and an increase in its expression in multiple myeloma cells could accelerate the process of DNA repair.[17] We reasonably assumed that the process of DNA repair has a close relationship with Ku86's anti-apoptotic/senescence effects.

In this study, we revealed that a low dose of ionizing radiation triggers senescence and apoptosis in HUVEC cells. There was a direct correlation between apoptosis/senescence and various low doses of ionizing radiation, as exhibited by the increase of

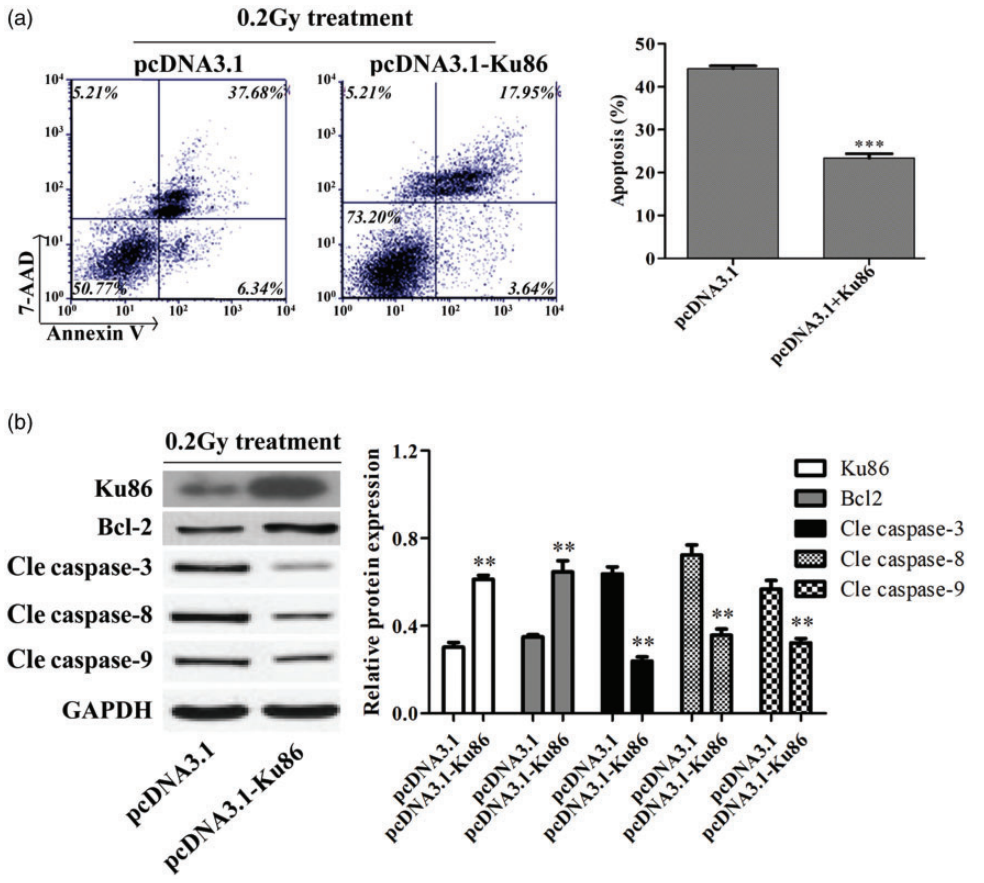

Figure 4. Ku86 overexpression reduced ionizing radiation-induced cellular apoptosis in culture. The cells were harvested at 48 hours after irradiation. (a) Ku86 overexpression reduced the population of apoptotic cells. (b) Proteins associated with apoptosis in the Ku86 overexpression group were detected by western blotting. $^{**}p < 0.01$, $^{***}p < 0.001$.

β-galactosidase^{+} and apoptotic cells following administration of a low dose of ionizing radiation, in a dose-dependent manner. This is in partial agreement with a previous study showing that ionizing radiation induces apoptosis by increasing the expression of apoptosis-related proteins, such as caspases.[18] However, ionization significantly reduced Ku86 expression, which is consistent with a previous report that confirmed a close association between Ku86 and radiation sensitivity.[19] The above findings indirectly suggest that Ku86 may be associated with the induction of apoptosis in HUVECs following ionizing radiation. To verify this, we used pcDNA 3.1 or siRNA to overexpress or silence Ku86, respectively, and observed the effects on HUVECs. Ku86 overexpression inhibited the increase in the number of SA-β-gal^{+} cells when co-incubated with HUVECs; Sirt1 and SOD2 were both activated, and this caused a downregulation of $p16^{Ink4a}$ and XOD. Functioning as a nicotinamide adenine dinucleotide $(NAD)^{+}$-dependent deacetylase, Sirt1 might prevent stimuli-induced HUVEC senescence and dysfunction, and it is involved in the regulation of some

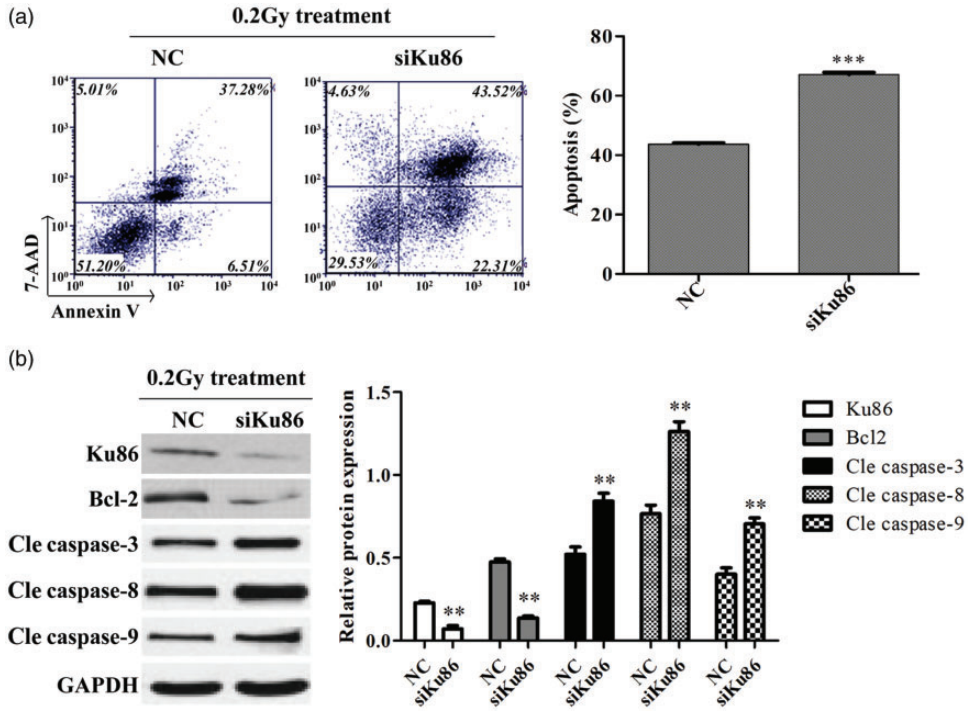

Figure 5. Ku86 deficiency accelerated ionizing radiation-induced cellular apoptosis in culture. The cells were harvested at 48 hours after irradiation. (a) Ku86 deficiency increased the population of apoptotic cells. (b) Proteins associated with apoptosis in the Ku86 deficiency group were detected by western blotting. $^{**}p < 0.01$, $^{***}p < 0.001$.

critical metabolic processes, including stress responses, aging, and apoptosis. Upregulation of Sirt1 was shown to significantly extend the mean and maximum lifespan in mice.[20] Sirt1 was reported to potentially prevent stress-induced endothelial cell senescence and dysfunction by deacetylating p53.[21] Thus, Sirt1 can inhibit senescence/apoptosis and promote survival in various cell types. SODs are a group of metal-containing enzymes that play a pivotal role in oxidative stress resistance by binding to a superoxide anion, such as a metallic zipper. Decreased SOD1 levels have been found in senescent endothelial cells isolated from irradiated mouse hearts[22] and decreased SOD2 levels were found in the hearts of plutonium workers.[23] Both Sirt1 and SOD can interact with each other to amplify the anti-senescence effect.[24] Additionally, Sirt1 has been shown to increase manganese superoxide dismutase (MnSOD) expression and increase oxidative stress resistance in human retinal microvascular endothelial cells.[24] XOD has been shown to be associated with cell differentiation and proliferation in various life forms including bacteria, mammals, and birds,[25] suggesting that it is involved in senescence-related responses, such as the production of free radicals and oxidative stress mediators, and that it plays an important role in the process of senescence by regulating free radicals.[26] Therefore, Sirt1 activation and subsequent SOD upregulation in this study may ameliorate endothelial anti-apoptosis. However, the SA-β-gal^{+} cell population was significantly increased

following Ku86-silent expression. Thus, si-Ku86 in HUVECs leads to an upregulation of $p16^{Ink4a}$ and XOD and a reduction in Sirt1 and SOD2 levels. These findings indicate that Ku86 can inhibit cellular senescence induced by a low dose of ionizing radiation in HUVECs.

Caspase 9 activation leads to cleavage of caspase 3, and this was mediated by the release of cytochrome c from the mitochondria. Separate signaling pathways are initially activated by various pro-apoptotic signals that eventually converge into a unique set of cysteine proteases, which are now called caspases. The Bcl2 protein blocks a specific step that leads to the activation of caspases that in turn prevents cell death.[27] In mitochondria, the Bcl family plays an important role in regulating apoptosis. Depleting Bcl-2 could enhance the DNA-binding activity of the Ku86/DNA-PKcs complex to accelerate DNA repair. Additionally, Bcl-2 protein expression can suppress apoptosis, while caspase 8 participates in both the initiation and progression of apoptosis. Activated caspase 8 cleaves poly (ADP-ribose) polymerase DNA repair enzyme, and it is associated with the interleukin-1 converting enzyme (ICE) family of proteases, such as in the activation of caspases 3 and 7.[28] Therefore, our observations revealed that Ku86 inhibition increased XOD and caspase-3, 8, and 9 levels, and reduced Sirt1, SOD2, and Bcl-2 levels. However, overexpressed Ku86 showed the opposite effects, suggesting that Ku86 can indirectly modulate these proteins. These results revealed that Ku86 plays an anti-apoptotic role in ionizing radiation-treated HUVECs by regulating the expression level of apoptosis-related genes. Thus, Ku86 had an anti-senescence and anti-apoptosis effect that was induced by a low dose of ionizing radiation in HUVECs.

The explanation of why Ku86 simultaneously exerts anti-senescence and anti-apoptosis functions remains unknown, and no conclusion can be reached because there is no definitive agreement on the relationship between cell aging and apoptosis, based on the existing research results. An increasing number of studies have reported the relationship between Ku86 and cellular senescence. For example, mice with homozygous defects in Ku86 were found to experience an early onset of senescence.[29,30] Ku86 levels are also correlated with species longevity because homozygous mutant mice have a severely reduced life span and heterozygous mutant mice do not live as long as their wild type counterparts.[31] Therefore, our present research provides valuable supplemental information in the investigation of Ku86's role in the process of senescence and anti-apoptosis.

In summary, our findings demonstrate that Ku86 can inhibit low-dose ionizing radiation-induced apoptosis and senescence in HUVECs, acting as a pivotal determinant of cell fate in low-dose ionizing radiation-exposed HUVEC cells.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This study was supported by Youth Innovation and Medical Research of Sichuan Province (Grant No.: Q15069) and a Scientific Research Project of Sichuan Provincial Health Department (Grant No.: 120394).

References

1. Wang Y, Boerma M and Zhou D. Ionizing radiation-induced endothelial cell senescence and cardiovascular diseases. *Radiat Res* 2016; 186: 153–161.
2. Preston DL, Shimizu Y, Pierce DA, et al. Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer

disease mortality: 1950-1997. *Radiat Res* 2003; 160: 381–407.
3. Kuilman T, Michaloglou C, Mooi WJ, et al. The essence of senescence. *Genes Dev* 2010; 24: 2463–2479.
4. Yamamori T, Yasui H and Yamazumi M. Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. *Free Radic Bio Med* 2012; 53: 260–270.
5. Kitagawa Y, Suzuki K, Yoneda A, et al. Effects of oxygen concentration and antioxidants on the in vitro developmental ability, production of reactive oxygen species (ROS), and DNA fragmentation in porcine embryos. *Theriogenology* 2004; 62: 1186–1197.
6. Yentrapalli R, Azimzadeh O, Barjaktarovic Z, et al. Quantitative proteomic analysis reveals induction of premature senescence in human umbilical vein endothelial cells exposed to chronic low-dose rate gamma radiation. *Proteomics* 2013; 13: 1096–1107.
7. Parrinello S, Samper E, Krtolica A, et al. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nat Cell Biol* 2003; 5: 741–747.
8. Dimri GP, Lee X, Basile G, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 1995; 92: 9363–9367.
9. Hayflick L. Biology of aging: observations and principles by Robert Arking. Sinauer Associates, 2006.
10. Mimori T, Akizuki M, Yamagata H, et al. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. *J Clin Invest* 1981; 68: 611–620.
11. Kubota Y, Takanami T, Higashitani A, et al. Localization of X-ray cross complementing gene 1 protein in the nuclear matrix is controlled by casein kinase II-dependent phosphorylation in response to oxidative damage. *DNA Repair* 2009; 8: 953–960.
12. Li YC, Kong J, Wei M, et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002; 110: 229–238.
13. Parzonko A and Naruszewicz M. Silymarin inhibits endothelial progenitor cells' senescence and protects against the antiproliferative activity of rapamycin: preliminary study. *J Cardiovasc Pharmacol* 2010; 56: 610–618.
14. Adams MJ, Lipsitz SR, Colan SD, et al. Cardiovascular status in long-term survivors of Hodgkin's disease treated with chest radiotherapy. *J Clin Oncol* 2004; 22: 3139–3148.
15. Goud SN. Effects of sublethal radiation on bone marrow cells: induction of apoptosis and inhibition of antibody formation. *Toxicology* 1999; 135: 69–76.
16. de Magalhães JP and Faragher RG. Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity. *Bioessays* 2008; 30: 567–578.
17. Tai YT, Teoh G, Lin B, et al. Ku86 variant expression and function in multiple myeloma cells is associated with increased sensitivity to DNA damage. *J Immunol* 2000; 165: 6347–6355.
18. Zhang ZY, Fu SL, Xu SQ, et al. By downregulating Ku80, hsa-miR-526b suppresses non-small cell lung cancer. *Oncotarget* 2015; 6: 1462–1477.
19. Sriharshan A, Boldt K, Sarioglu H, et al. Proteomic analysis by SILAC and 2D-DIGE reveals radiation-induced endothelial response: four key pathways. *J Proteomics* 2012; 75: 2319–2330.
20. Godoy JA, Zolezzi JM, Braidy N, et al. Role of Sirt1 during the ageing process: relevance to protection of synapses in the brain. *Mol Neurobiol* 2014; 50: 744–756.
21. Ota H, Akishita M, Eto M, et al. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. *J Mol Cell Cardiol* 2007; 43: 571–579.
22. Azimzadeh O, Sievert W, Sarioglu H, et al. Integrative proteomics and targeted transcriptomics analyses in cardiac endothelial cells unravel mechanisms of long-term radiation-induced vascular dysfunction. *J Proteome Res* 2015; 14: 1203–1219.
23. Azimzadeh O, Azizova T, Merl-Pham J, et al. A dose-dependent perturbation in cardiac energy metabolism is linked to

radiation-induced ischemic heart disease in Mayak nuclear workers. *Oncotarget* 2017; 8: 9067–9078.
24. Zhang W, Huang Q, Zeng Z, et al. Sirt1 inhibits oxidative stress in vascular endothelial cells. *Oxid Med Cell Longev* 2017; 2017: 7543973.
25. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? *Free Radic Biol Med* 2002; 33: 774–797.
26. Chung HY, Baek BS, Song SH, et al. Xanthine dehydrogenase/xanthine oxidase and oxidative stress. *Age (Omaha)* 1997; 20: 127–140.
27. Tsujimoto Y. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? *Genes Cells* 1998; 3: 697–707.
28. Kerr JF, Winterford CM and Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. *Cancer* 1994; 73: 2013–2026.
29. Vogel H, Lim DS, Karsenty G, et al. Deletion of Ku86 causes early onset of senescence in mice. *Proc Natl Acad Sci U S A* 1999; 96: 10770–10775.
30. Reiling E, Dollé MET, Youssef SA, et al. The Progeroid Phenotype of Ku80 Deficiency Is Dominant over DNA-PKCS Deficiency. *Plos One* 2014; 9: e93568.
31. Lorenzini A, Johnson FB, Oliver A, et al. Significant correlation of species longevity with DNA double strand break recognition but not with telomere length. *Mech Ageing Dev* 2009; 130: 784–792.