Int J Clin Exp Pathol 2017;10(9):10047-10055
www.ijcep.com /ISSN:1936-2625/IJCEP0050851

Original Article

Effects of mitochondrial uncoupling protein 2 inhibition by Genipin on rat bone marrow mesenchymal stem cells under hypoxia and serum deprivation (H/SD) conditions

Qi Zhang[1,2], Shi-Peng Wang[1], Li-Li Li[1], Yao Zhang[1]

[1]*Department of Cardiology, The Second Affiliated Hospital of Harbin Medical University, Heilongjiang Province, China;* [2]*First Department of Cardiology, Central Hospital of Jiamusi City, Heilongjiang Province, China*

Received February 14, 2017; Accepted March 20, 2017; Epub September 1, 2017; Published September 15, 2017

Abstract: Bone marrow-derived mesenchymal stem cells (BMSCs) have shown great promise for ischemic tissue repair. However, poor viability of transplanted BMSCs within ischemic tissues has limited their therapeutic potential. Numerous evidences suggested that reactive oxygen species (ROS) generated and apoptosis play an important role in regulation BMSCs loss at the ischemic site. Uncoupling protein 2 (UCP2), a member of the anion carrier superfamily of mitochondrial inner membrane and high expression in stem cells, has been reported to influence mitochondrial ROS production and regulate the energy metabolism. However the exact roles of UCP2 in regulation the BMSCs apoptosis are still not clear. In our study, we determined the functions of UCP2 in BMSCs from SD rats. Genipin, a special UCP2 inhibitor, was added into the cultural medium to reduce the UCP2 expression in BMSCs. Apoptosis was induced by the specific apoptotic insult hypoxia and serum deprivation (SD). There was no significant differences in ATP level in BMSCs from Genipin treatment group as compared with other treatment groups. But, the levels of Reactive oxygen species (ROS) and malondialdehyde (MDA) content in BMSCs treated with Genipin were significant higher than other groups ($P<0.05$). Furthermore, the level of BMSCs apoptosis was much higher in H/SD and 50 μM Genipin treatment group (31.93% ± 0.16) than H/SD treatment (17.59 ± 0.69) or control group (5.79 ± 0.04) ($P<0.05$). In addition, Bax and caspase3 activation were elevated after treatment with Genipin ($P<0.05$). However, the level of anti-apoptotic protein Bcl2 was significantly declined after treatment with Genipin ($P<0.05$). Taken together, our findings indicate that inhibitionof UCP2 by Genipin enhanced the BMSCs apoptosis under H/SD conditions.

Keywords: BMSCs, UCP2, serum deprivation, hypoxia, apoptosis

Introduction

Bone marrow-derived mesenchymal stem cells (BMSCs) are self-renewing progenitor cells that can differentiate into many types of cells such as osteoblasts, chondrocytes astrocytes, neurons, and skeletal muscle cells [1, 2]. In addition to their differentiating potentials, autologous BMSCs can be acquired from bone marrow and expanded, which make BMSCs as a conceivable source of stem cells for repairing damaged tissues. However, other studies have exhibited that transplanted BMSCs do not survive well within diseased tissues [3]. Numerous reports indicated that reactive oxygen species (ROS) generated plays a key role in regulating BMSCs loss at the ischemic site [3, 4]. Furthermore, Previous reports have shown that apoptosis is the main reason for the transplant cells death [3, 4]. Thus, the methods of enhancing tolerance to apoptotic damages may significantly affect the efficiency of BMSCs transplantation therapy.

The uncoupling protein 2 (UCP2), which is located in mitochondrial innermembrane, involves in oxidative phosphorylation, cellular autophagy, maintaining energy balance and regulating ATP concentration [5]. Recently, some studies have also showed that UCP2 taken part in regulating ROS elimination and apoptosis [6-7]. UCP2 has been detected in various tissues including liver, heart, brain, pancreas, and adipose tissue [8-10]. The wide tissue distribution of UCP2

means that UCP2 play an important role in regulating several physiologic or pathologic events based on the specific tissue or organ implicated. In addition, some studies utilized knockdown or mutagenesis methods showed UCP2 might have many deleterious effects and play an important role in pathogenesis of some diseases (e.g.cardiovascular diseases, type 2 diabetes mellitus and various cancers) [11-13].

Ge *et al* found that the levels of ROS and Mn-SOD were significantly elevated in human cumulus cells after UCP2 inhibited, but, the ratio of reduced GSH to GSSG significantly declined [14]. Furthermore, overexpression of UCP2 attenuates ROS generation and prevents mitochondrial Ca^{2+} overload, revealing a novel mechanism of cardioprotection [15]. At present, the functions of UCP2 in BMSCs are still not clearly understood. In our study, to analyze the roles of UCP2 played in BMSCs apoptosis and ROS elimination, we utilized Genipin, a special UCP2 inhibitor to inhibit UCP2 functions. Previous studies have shown that Genipin can decrease the protein level of UCP2, inhibit mitochondrial proton leak and increase ATP synthesis [16]. Meanwhile, apoptosis was induced by the specific apoptotic insult hypoxia and serum deprivation (H/SD). In addition, effects of UCP2 inhibition by Genipin on BMSCs cellular viability and the contents of ATP, MDA was explored. Furthermore, apoptosis related protein, caspase-3, Bax and Bcl2 are quantified in cultured BMSCs *in vitro* treated with Genipin and H/SD. Taken together, our findings will add new knowledge about effects of UCP2 inhibition by Genipin on BMSCs apoptosis and ROS levels.

Materials and methods

Rat BMSCs culture and Genipin treatment

BMSCs were isolated from SD rats as previously described [17]. In brief, BMSCs were obtained from the femoral and tibial bones of rats. Cells were flushed from the femurs and tibias of rats using a 25-guage needle. Mononuclear cells were suspended in culture medium (DMEM/F12 Hyclone, USA) containing 10% of fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin then cultured into 50 cm^2 cell culture flask. Cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide. After 24 h, non-adherent cells were discarded, and adherent cells were washed two times with phosphate-buffered saline solution (PBS). Fresh complete medium was added and replaced every 3 days. Each primary culture was sub-cultured 1:2 when BMSCs grew to 80% confluency, and study them between passages 3 and 5. The Genipin powder (Purifa Bio, China) was dissolved in DMSO. BMSCs were randomly divided into control group and treatment group. The control group was treated with equivalent amounts of complete medium/DMSO while the treatment group was treated with Genipin.

Cell viability measurement

Cellular viability was measured using a CCK-8 kit (CCK-8, Dojingo, Japan) according to manufacturer's instruction. The cultured BMSCs were seeded in 96-well plates (10^5 cells/well), at the end of the treatments, were rinsed twice with PBS, and then F12 with 10% CCK-8 was added to these samples in a separate volume of 0.11 mL. After incubation for one hour, the absorbance was measured at 450 nm under a microplate reader (Bio-TEK, USA). Five parallel replicates of each sample at each time point were prepared during this cell viability assay. Three independent experiments were performed for each assay condition.

BMSCs hypoxia/serum deprivation (H/SD) injury

BMSCs were randomly divided into four groups as followed: BMSCs group (BMSC), BMSCs+H/SD (BMSC+H/SD), BMSCs+Genipin+H/SD (BMSC+Genipin+H/SD), BMSCs+DMSO+H/SD (DMSO). Briefly, BMSCs treated with fresh complete medium maintained in normoxia conditions (95% air-5% CO_2) were considered as control group. BMSCs treated with fresh complete medium, Genipin (50 μM), or DMSO for 24 h,then replaced with F12 without FBS and exposed to hypoxia (approximately 95% N_2 and 5% CO_2) in an anaerobic system (Thermo Forma) at 37°C for 24 h, were considered as H/SD injury groups.

Measurement of ATP, ROS and MDA

The amount of ATP was measured by the ATP detection kit (Beyotime, China). BMSCs were lysed on ice with 100 μL lysis buffer from ATP detection kit. After being centrifuged at 12,000 g for 5 min at 4°C, the supernatant was trans-

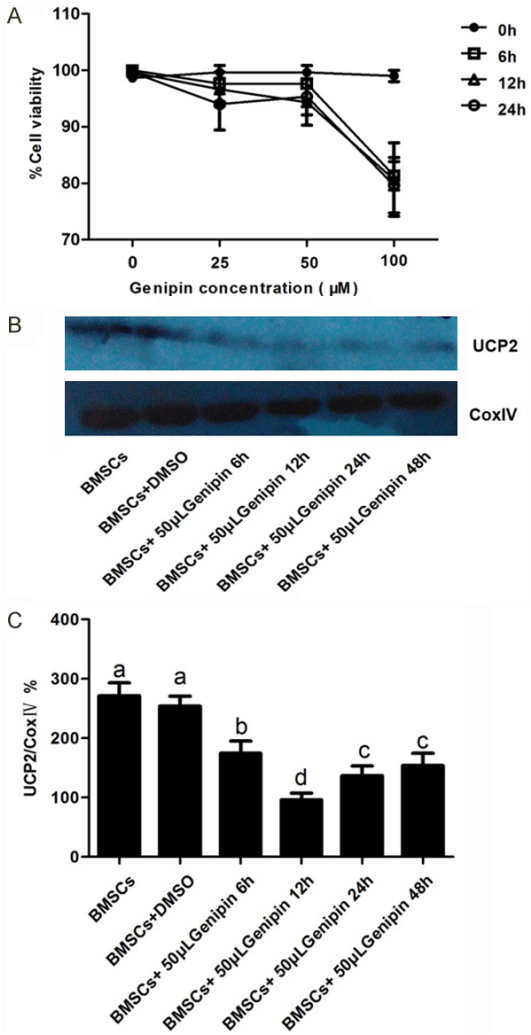

Figure 1. The effects of UCP2 inhibition by Genipin on cell viability (A), UCP2 protein level changes (B). The protein level of UCP2 was normalized to cytochrome c oxidase subunit IV (C). BMSCs were cultivated in Genipin concentrations 0, 25, 50, and 100 uM and treated with 6, 12, 24 and 48 h respectively. Data are expressed as the mean ± SE of three independent experiments. Different letters indicated significantly different at $P<0.05$.

ferred to a new 1.5 mL tube for ATP test. Protein concentrations were determined by using a BCA Protein Assay Reagent Kit (Beyotime, China). The luminescence from a 100 μL sample was assayed in a luminometer together with 100 μL of ATP detection buffer from the ATP detection kit. The standard curve of ATP concentration was prepared from a known amount (1 nM-10 μM).

The intracellular ROS was measured by flow cytometry using a cell-based ROS assay kit (Beyotime, China) following the manufacturer's instruction. At the end of H/SD treatment, the BMSCs were trypsinized, washed with PBS, and then incubated with DCFH-DA at a final concentration of 10 mM DCHF-DA in F12 medium for 30 min at 37°C to allow cellular incorporation and then washed and resuspended in PBS. The ROS level was detected by and the DCF florescence was measured by a flow cytometer with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. (Becton Dickinson FACScan, USA). Data were analyzed using Cellquest software (Becton Dickinson). For each analysis, 10,000 events were recorded. All data are presented as the mean of three independent experiments.

The MDA content was detected by MDA Assay Kit (Beyotime, China) as manufacture's instruction. BMSCs were lysed and centrifuged at 15,000 g at 4°C for 15 min and the supernatants were retained. A 100 μL of thiobarbituric acid (TBA) and 50 μL of the suspension were added into 96-well plates. The reaction mixture was treated at 100°C for 15 min, and then the absorbance was measured at 530 nm by a microplate reader (Bio-TEK, USA).

Measurement of BMSCs apoptosis

Apoptosis of BMSCs induced by hypoxia and serum deprivation (H/SD) was assessed using hoechst 33258 staining following the manufacturer's instruction (Beyotime, China) and flow cytometry with Annexin-V-FITC/PI dual staining (Invitrogen USA). The emitted green fluorescence of annexin-V (FL1) and red fluorescence of PI (FL2) were detected by a flow cytometer (FACS Calibur, BECTON-DICKINSON, USA) with an excitation wavelength of 488 nm and an emission wavelength of 630 nm, respectively. For each sample, 10,000 events were recorded. The amount of early apoptosis, late apoptosis, and necrosis were determined as the percentage of annexin-V+/PI-; annexin-V+/PI+; and annexin-V-/PI+ cells, respectively.

Measurement of apoptosis related protein

Proteins from BMSCs were isolated with RIPA lysis buffer (Beyotime, China). The mixture was centrifuged at 12000 g for 20 min at 4°C and the supernatant transferred into 1.5 mL Eppendorf tube. Protein concentrations were determined using a BCA Protein Assay Reagent Kit (Beyotime, China). Bax, Bcl2, Caspase-3

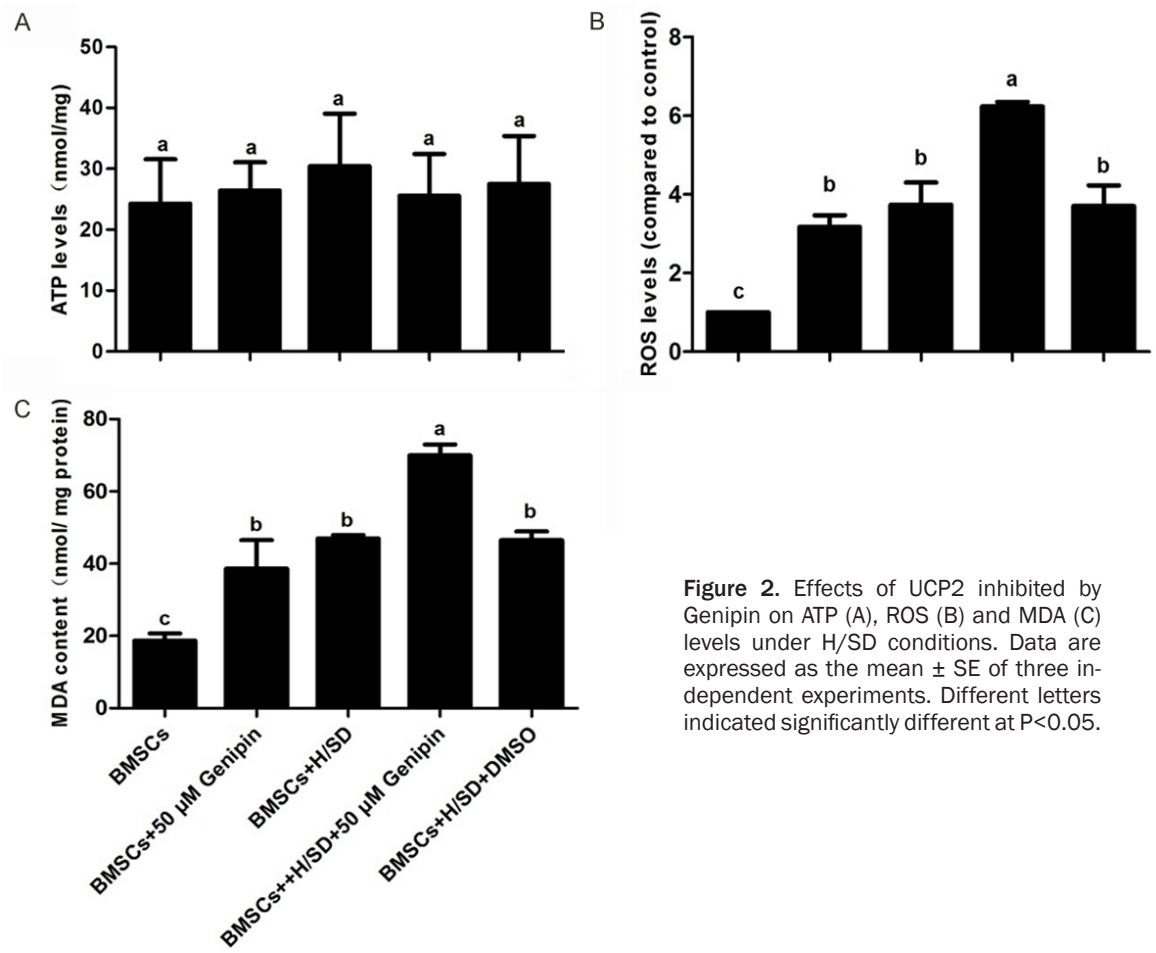

Figure 2. Effects of UCP2 inhibited by Genipin on ATP (A), ROS (B) and MDA (C) levels under H/SD conditions. Data are expressed as the mean ± SE of three independent experiments. Different letters indicated significantly different at P<0.05.

and cleaved Caspase-3 protein were quantified by Western blotting. The primary rabbit polyclonal antibody to Bax and Bcl2 were used at a concentration of 1:1000 (Cell Signaling Technology, USA). The primary antibody to caspase-3 was used at a concentration of 1:1000 (Cell Signaling Technology, USA). The primary antibody to cleaved Caspase-3 was used at a concentration of 1:500 (Wanleibio, China). The primary antibody to β-actin was used at a concentration of 1:1000 (Bioss, China). Secondary antibodies were conjugated with horseradish peroxidase were purchased from Zhongshanbio of Beijing

Statistical analysis

All experiments were performed in triplicate. Data are presented as the mean ± SE. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA). A level of P<0.05 was considered statistically significant.

Results

Effects of UCP2 inhibition by Genipin on BMSCs cell viability

A CCK8 assay was used to determine the appropriate concentration and time of Genipin choice in the study, which would not result in significant cytotoxicity on BMSCs. In addition, Immunoblot analysis was used to assess the inhibition effect of Genipin on UCP2. As shown in **Figure 1A**, BMSCs were cultivated in Genipin concentrations 0, 25, 50, and 100 μM and treated with 6, 12, 24 and 48 h respectively. There were not significantly impacts on cellular survival at 50 μM. But the cell viability was changed significantly when the Genipin concentration reached 100 μM (**Figure 1A**). So, the 50 μM Genipin was choice in this study. Furthermore, the level of UCP2 protein was the most decreased at 12 h treatment (**Figure 1B**, **1C**).

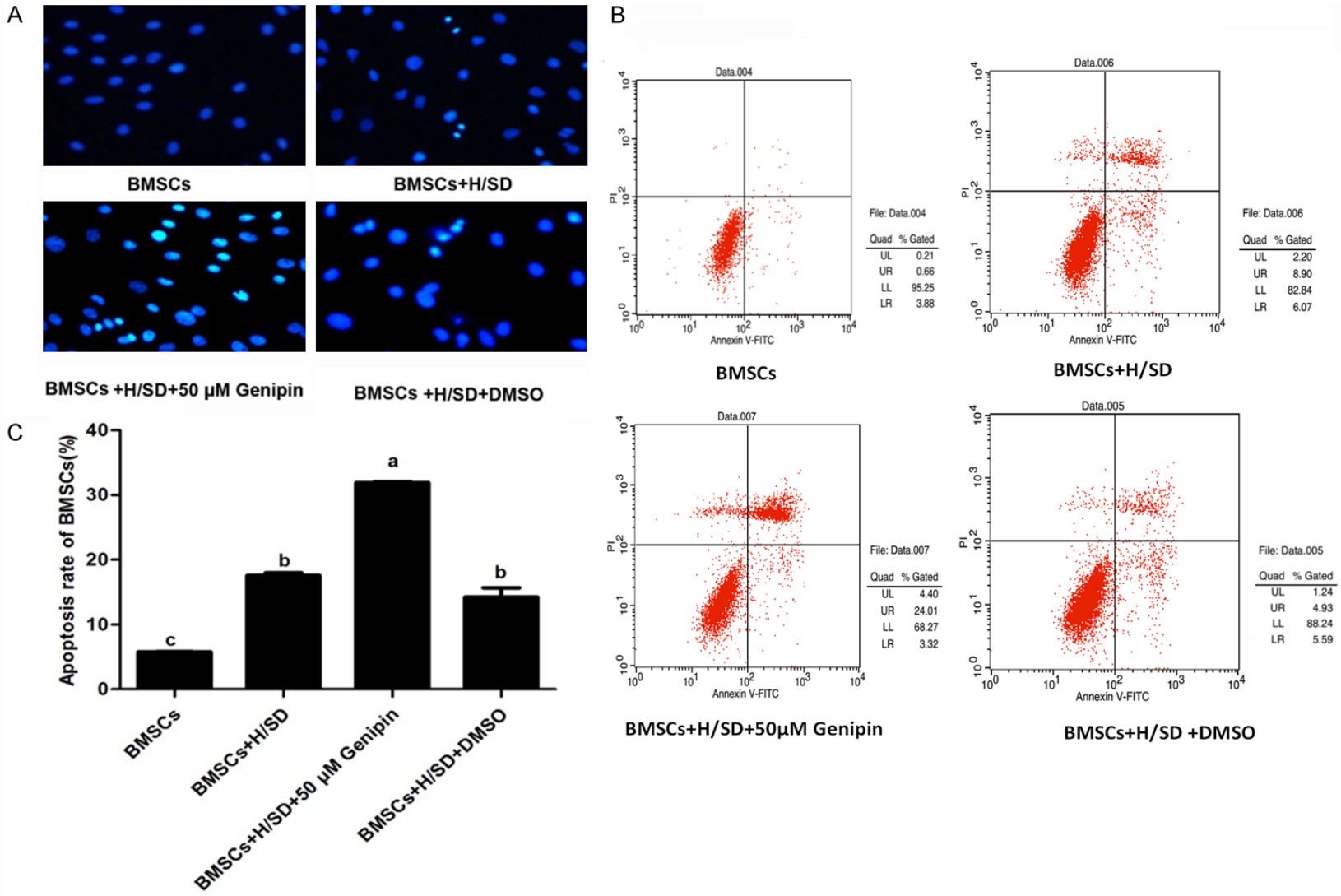

Figure 3. Effects of UCP2 inhibition by Genipin on BMSCs apoptosis. A. Representative images of hoechst 33258 staining in BMSC treated with H/SD, H/SD+50 μM Genipin or H/SD+DMSO. B and C. Quantitative results of flow cytometry with Annexin-V-FITC/PI dual staining assays. Similar results were obtained from 3 independent experiments. The bar graph inserted shows the percentage of apoptosis and necrosis cells at the end of scanning. Data are expressed as the mean ± SE of three independent experiments. Different letters indicated significantly different at $P<0.05$.

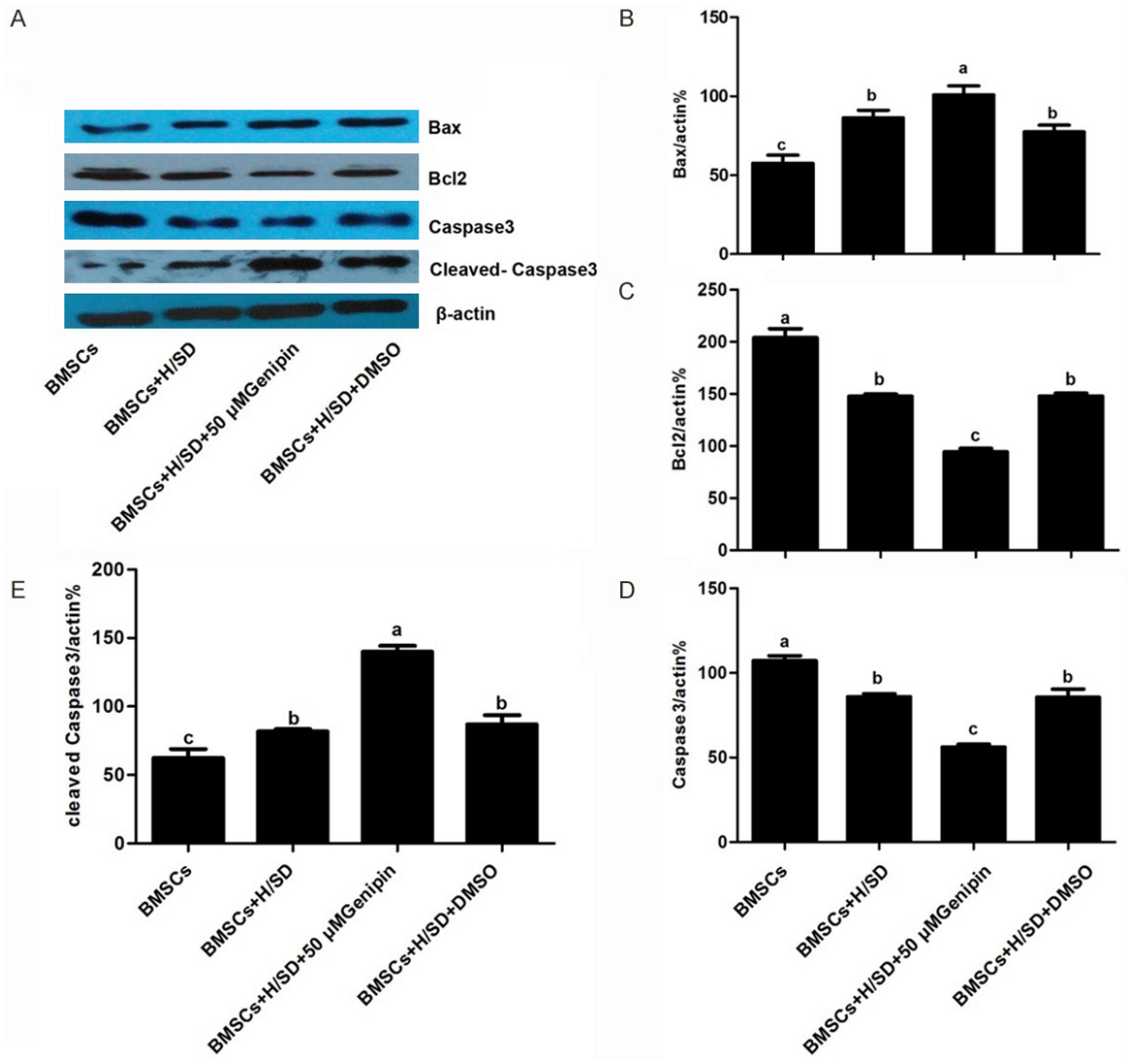

Figure 4. Effects of UCP2 inhibited by Genipin on apoptosis related protein. (A) The Bax, Bcl2, caspase-3 and cleaved caspase-3 protein were quantified by Western blotting. The protein levels of Bax (B), Bcl2 (C), caspase-3 (D) and cleaved caspase-3 (E) were normalized to β-actin. Data are expressed as the mean ± SE of three independent experiments. Different letters indicated significantly different at $P<0.05$.

Effects of UCP2 inhibition by Genipin on ATP, ROS and MAD in BMSCs under H/SD conditions

In our study,we explored effects of inhibition UCP2 on ATP contents in BMSCs. As shown in **Figure 2A**, there were no significant differences in ATP contents were found in the different treatment groups (**Figure 2A**). In addition, the intracellular ROS generation in BMSCs was markedly elevated after BMSCs were treated with H/SD or 50 μM Genipin (**Figure 2B**). Furthermore, ROS levels also showed an increasing trend in BMSCs treated with H/SD and 50 μM Genipin at the same time compared with BMSCs only treated with H/SD or 50 μM Genipin (**Figure 2B**). Meanwhile, the MDA concentration increased significantly after being treated with H/SD and 50 μM Genipin as compared with other groups (**Figure 2C**). This change trend was similar to the change of ROS between different groups.

Effects of Genipin on BMSCs apoptosis

In order to determine the effects of UCP2 inhibition on BMSCs apoptosis, Hoechst 33258 nuclear staining examined morphological changes of BMSC nuclei. In our study we found H/SD treatment caused nuclear condensation

(**Figure 3A**). When adding 50 μM Genipin into the H/SD treatment, the degree of nuclear condensation showed increasing compared with BMSCs only treated with H/SD group (**Figure 3A**). Furthermore, flow cytometry with Annexin-V-FITC/PI dual staining assays were also used to evaluate the effects of Genipin on BMSCs apoptosis. The amount of early apoptosis, late apoptosis, and necrosis were determined as the percentage of annexin-V+/PI-; annexin-V+/PI+; and annexin-V-/PI+ cells, respectively. As shown in **Figure 3B**, **3C**, the rate of BMSCs apoptosis was much higher in H/SD and 50 μM Genipin treatment group than H/SD treatment or control group.

Effect s of Genipin on apoptosis related protein

To confirm the effects of UCP2 inhibition on BMSCs apoptosis related protein, the protein of Bax, Bcl2, caspase-3 and Cleaved-Caspase3, were, respectively, assessed by western blot. The levels of Bax and Bcl2 were significantly increased under the H/SD condition. The content of these proteins reached the highest in H/SD and 50 μM Genipin treatment group (**Figure4A-C**). At the same time, the expression of Cleaved-Caspase3 (17 kD) was obviously increased, which was associated with the decreasing of Caspase-3 (35 kD) in H/SD and Genipin treated group as compared with other groups (**Figure 4A**, **4D**, **4E**).

Discussion

Many of studies showed that UCP2 plays an important role in regulating ATP generation and keeping cellular energy balance. Previous reports exhibited that down regulation of UCP2 expression can change ATP levels in several cell types [12, 18]. But, in our study, we found that inhibition of UCP2 expression by Genipin in BMSCs could not alter ATP contents. This result is in accordance with other reports, which showed that overexpression of UCP2 did not affect the ATP levels in brain cell [19]. Maybe the major functions of UCP2 in BMSCs were not involved in ATP production, and UCP2 may play roles priority in reducing ROS generation and regulating apoptosis.

Cell-based therapy is an exciting, and emerging treatment for ischemic diseases. Stem cell-based therapies administered following the ischemic heart diseases are intended to improve the long-term outcomes and represent the current focus of multiple clinical trials. Although Bone mesenchymal stem cells are being proposed as a promising cell population for cell therapy and repairing ischemia-damaged tissues in the heart and brain, its therapeutic exploitation has been limited bymost of BMSCs death in ischemic tissue [20]. Apoptosis induced by ischemic insults is a main reason for the death of transplanted BMSCs [20]. Thus, regulating BMSCs apoptosis may a supplemental strategy to improve the survival of BMSCs in transplantation therapy. Mild mitochondrial uncoupling has been proposed as a mechanism to decrease ROS generation, in which, (UCP2), a member of the anion carrier superfamily of mitochondrial inner membrane and high expression in stem cells, has been shown to diminish mitochondrial reactive oxygen species (ROS) formation and regulate the energy metabolism [21-23]. However, little is known regarding the precise role of UCP2 in regulating BMSCs ROS levels or apoptosis. In order to confirm the function of UCP2 in BMSCs apoptosis, its expression was inhibited in BMSCs by Genipin under H/SD treatment. We found that UCP2 inhibited by 50 μM Genipin markedly increase mitochondrial ROS production and the MDA concentration. These results indicated that UCP2 inhibition can aggravate the oxidative damage in BMSCs. Our result is in accordance with a previous report [24], which showed a UCP2-ROS mutual-regulation system existedin mural granulosa cells. In addition, it is reported that over-expression of UCP2 has decreased ROS levels [25-27]. Furthermore, level of apoptosis followed increasing trend under 50 μM Genipin treatment compared with that of the other group cells. In addition, the significant increasing of active form of caspase-3 and Bax and the decline of Bcl2 also indicated that UCP2 inhibition by Genipin promotes apoptosis in BMSCs connection of UCP2 with apoptosis has also been reported in several type cells, where UCP2 showed anti-apoptotic properties [28, 29]. Deng et al found that over-expression of UCP2 had alleviated apoptosis by inhibiting ROS-mediated apoptosis in A549 cells [30]. Other research [31] proved that UCP2 expression in human colon cancer cells decreased apoptosis induced by mechanisms involving regulation of p53 pathway, but whether inhibition of UCP2 on inhibition apop-

tosis of by modulation of p53 pathway in BMSCs is also need to prove.

In conclusion, the findings of this study have shown that the content of ATP in BMSCs from Genipin treatment group is no change compared with control. However, the levels of ROS and MDA content in BMSCs treated with Genipin were significant higher than other groups. In addition, the degree of BMSCs apoptosis was much higher in H/SD and 50 μM Genipin treatment group than H/SD treatment. Finally, the protein levels of Bax and caspase3 activation were increasing under Genipin treatment. In contrast, the level of anti-apoptotic protein Bcl2 was significantly declined after treatment with Genipin. Taken together, our findings indicate that inhibition UCP2 by Genipin enhanced the BMSCs apoptosis under H/SD conditions.

Acknowledgements

This work was supported by Natural Science Foundation of Heilongjiang Province (H2013-104).

Disclosure of conflict of interest

None.

Address correspondence to: Yao Zhang, Department of Cardiology, The Second Affiliated Hospital of Harbin Medical University, 148 Baojian Road, Harbin 150081, Heilongjiang Province, China. Tel: 86-451-86296230; Fax: 86-451-86605180; E-mail: zhangyao09372@sina.com

References

[1] Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, Bagasra O, Prockop DJ. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci U S A 1995; 92: 4857-4861.

[2] Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats--similarities to astrocyte grafts. Proc Natl Acad Sci U S A 1998; 95: 3908-3913.

[3] Geng YJ. Molecular mechanisms for cardiovascular stem cell apoptosis and growth in the hearts with atherosclerotic coronary disease and ischemic heart failure. Ann N Y Acad Sci 2003; 1010: 687-697.

[4] Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. J Mol Cell Cardiol 2001; 33: 907-921.

[5] Sluse FE. Uncoupling proteins: molecular, functional, regulatory, physiological and pathological aspects. Adv Exp Med Biol 2012; 942: 137-156.

[6] Ježek P, Olejár T, Smolková K, Ježek J, Dlasková A, Plecitá-Hlavatá L, Zelenka J, Špaček T, Engstová H, PajueloReguera D, Jabůrek M. Antioxidant and regulatory role ofmitochondrial uncoupling protein UCP2 in pancreatic beta-cells. Physiol Res 2014; 63 Suppl 1: S73-91.

[7] Merhi Z, Irani M, Doswell AD, Ambroggio J. Follicular fluid soluble receptor for advanced glycation end-products (sRAGE): a potential indicator of ovarian reserve. J Clin Endocrinol Metab 2014; 99: E226-233.

[8] Ricquier D, Bouillaud F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J 2000; 345 Pt 2: 161-179.

[9] Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D, Warden CH. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 1997; 15: 269-272.

[10] Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, Woolf EA, Tartaglia LA. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. Diabetes 1997; 46: 900-906.

[11] Gioli-Pereira L, Santos PC, Sugaya LS, Ferreira NE, Krieger JE, Pereira AC, Hueb WA. Association between UCP2 A55V polymorphism and risk of cardiovascular events in patients with multi-vessel coronary arterial disease. BMC Med Genet 2013; 14: 40.

[12] Mailloux RJ, Adjeitey CN, Harper ME. Genipin-induced inhibition of uncoupling protein-2 sensitizes drug-resistant cancer cells to cytotoxic agents. PLoS One 2010; 5: e13289.

[13] Liu Y, Jiang H, Xing FQ, Huang WJ, Mao LH, He LY. Uncoupling protein 2 expression affects androgen synthesis in polycystic ovary syndrome. Endocrine 2013; 43: 714-723.

[14] Ge H, Zhang F, Shan D, Chen H, Wang X, Ling C, Xi H, Huang J, Zhu C, Lv J. Effects of Mitochondrial Uncoupling Protein 2 Inhibition by Genipin in Human Cumulus Cells. Biomed Res Int 2015; 2015: 323246.

[15] Teshima Y, Akao M, Jones SP, Marbán E. Uncoupling protein-2 overexpression inhibits mitochondrial death pathway in cardiomyocytes. Circ Res 2003; 93: 192-200.

[16] Zhang CY, Parton LE, Ye CP, Krauss S, Shen R, Lin CT, Porco JA Jr, Lowell BB. Genipin inhibits

UCP2-mediated proton leak and acutely reverses obesity- and high glucose-induced beta cell dysfunction in isolated pancreatic islets. Cell Metab 2006; 3: 417-427.

[17] Hu X, Yu SP, Fraser JL, Lu Z, Ogle ME, Wang JA, Wei L. Transplantation of hypoxiapreconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg 2008; 135: 799-808.

[18] Cheng G, Polito CC, Haines JK, Shafizadeh SF, Fiorini RN, Zhou X, Schmidt MG, Chavin KD. Decrease of intracellular ATP content downregulated UCP2 expression in mouse hepatocytes. Biochem Biophys Res Commun 2003; 308: 573-580.

[19] Peixoto PM, Kim HJ, Sider B, Starkov A, Horvath TL, Manfredi G. UCP2 overexpression worsens mitochondrial dysfunction and accelerates disease progression in a mousemodel of amyotrophic lateral sclerosis. Mol Cell Neurosci 2013; 57: 104-110.

[20] Zeng X, Yu SP, Taylor T, Ogle M, Wei L. Protective effect of apelin on cultured rat bonemarrow mesenchymal stem cells against apoptosis. Stem Cell Res 2012; 8: 357-367.

[21] Wang P, Li B, Cai G, Huang M, Jiang L, Pu J, Li L, Wu Q, Zuo L, Wang Q, Zhou P. Activation of PPAR-γby pioglitazone attenuates oxidative stress in aging rat cerebral arteries throughupregulating UCP2. J Cardiovasc Pharmacol 2014; 64: 497-506.

[22] Lou J, Wang Y, Wang X, Jiang Y. Uncoupling protein2 regulates palmitic acid-induced hepatoma cell autophagy. Biomed Res Int 2014; 2014: 810401.

[23] Andrews ZB, Liu ZW, Walllingford N, Erion DM, Borok E, Friedman JM, Tschöp MH, Shanabrough M, Cline G, Shulman GI, Coppola A, Gao XB, Horvath TL, Diano S. UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. Nature 2008; 454: 846-851.

[24] Fu ZH, Zhu WJ, Li XM, Chen XM, Xing FQ. Uncoupling protein-2 expression in granular cells and embryo development in invitro fertilization-embryo transfer cycle. Nan Fang Yi Ke Da Xue Xue Bao 2007; 27: 1077-1079.

[25] Chen XL, Tang WX, Tang XH, Qin W, Gong M. Downregulation of uncoupling protein-2 by genipin exacerbates diabetes-induced kidney proximal tubular cells apoptosis. Ren Fail 2014; 36: 1298-1303.

[26] Park D, Han CZ, Elliott MR, Kinchen JM, Trampont PC, Das S, Collins S, Lysiak JJ, Hoehn KL, Ravichandran KS. Continued clearance of apoptotic cells critically depends on the phagocyte Ucp2 protein Nature 2011; 477: 220-224.

[27] Collins P, Jones C, Choudhury S, Damelin L, Hodgson H. Increased expression of uncoupling protein 2 in HepG2 cells attenuates oxidative damage and apoptosis. Liver Int 2005; 25: 880-887.

[28] Bodyak N, Rigor DL, Chen YS, Han Y, Bisping E, Pu WT, Kang PM. Uncoupling protein2 modulates cell viability in adult rat cardiomyocytes. Am J Physiol Heart Circ Physiol 2007; 293: H829-835.

[29] Namgaladze D, Preiss S, Dröse S, Brandt U, Brüne B. Phospholipase A2-modified low density lipoprotein induces mitochondrial uncoupling and lowers reactive oxygen species in phagocytes. Atherosclerosis 2010; 208: 142-147.

[30] Deng S, Yang Y, Han Y, Li X, Wang X, Li X, Zhang Z, Wang Y. UCP2 inhibits ROS-mediated apoptosis in A549 under hypoxic conditions. PLoS One 2012; 7: e30714.

[31] Derdak Z, Mark NM, Beldi G, Robson SC, Wands JR, Baffy G. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. Cancer Res 2008; 68: 2813-2819.