BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 24(4), 371-379 (2016)

Inhibition of Store-Operated Calcium Entry Protects Endothelial Progenitor Cells from H_2O_2-Induced Apoptosis

Yan-Wei Wang, Ji-Hang Zhang, Yang Yu, Jie Yu and Lan Huang*

Institute of Cardiovascular Diseases of PLA, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, People's Republic of China

Abstract

Store-operated calcium entry (SOCE), a major mode of extracellular calcium entry, plays roles in a variety of cell activities. Accumulating evidence indicates that the intracellular calcium ion concentration and calcium signaling are critical for the responses induced by oxidative stress. The present study was designed to investigate the potential effect of SOCE inhibition on H_2O_2-induced apoptosis in endothelial progenitor cells (EPCs), which are the predominant cells involved in endothelial repair. The results showed that H_2O_2-induced EPC apoptosis was reversed by SOCE inhibition induced either using the SOCE antagonist ML-9 or via silencing of stromal interaction molecule 1 (STIM1), a component of SOCE. Furthermore, SOCE inhibition repressed the increases in intracellular reactive oxygen species (ROS) levels and endoplasmic reticulum (ER) stress and ameliorated the mitochondrial dysfunction caused by H_2O_2. Our findings provide evidence that SOCE inhibition exerts a protective effect on EPCs in response to oxidative stress induced by H_2O_2 and may serve as a potential therapeutic strategy against vascular endothelial injury.

Key Words: Endothelial progenitor cells, Oxidative stress, SOCE, STIM 1, ML-9

INTRODUCTION

Endothelial dysfunction plays a central role in the development and progression of vascular diseases. Endothelial progenitor cells (EPCs), which are mobilized from bone marrow to the vascular endothelium, are vital for endothelial repair, functioning directly through differentiation and indirectly through paracrine effects. Oxidative stress caused by free radicals, oxygen anions and peroxides is closely associated with the initiation, development and progression of vascular diseases (Heitzer *et al*., 2001; Yoshii *et al*., 2006; Schleicher and Friess, 2007; Cachofeiro *et al*., 2008; Battelli *et al*., 2014) and has direct cytotoxic effects on the endothelium (Griendling and FitzGerald, 2003; Harrison *et al*., 2003) and EPCs (Hung *et al*., 2003; Urbich *et al*., 2005; Thum *et al*., 2006; Thum *et al*., 2007; Watson *et al*., 2008). However, the specific mechanism of these effects is not clear, and the roles of calcium and calcium signaling in this mechanism are undefined.

Calcium is an important ion that regulates a variety of activities, including muscle contraction, skeleton formation, and cell proliferation, migration and apoptosis. Store-operated calcium channels (SOCs; also known as SOCCs) mediate store-operated calcium entry (SOCE) and are expressed ubiquitously in non-excitable cells, such as EPCs (Lewis, 2007). Previous studies have demonstrated that SOCE in embryonic fibroblasts is critical for the oxidative stress response (Henke *et al*., 2012). Numerous studies have suggested that oxidative stress enhances the inward flow of calcium, leading to intracellular calcium overload (Caron *et al*., 2007) and ultimately, cell apoptosis (Henke *et al*., 2013). Therefore, we hypothesized that SOCE may play a significant role in oxidative stress-induced apoptosis in EPCs. Inhibition of SOCE may protect EPCs from oxidative stress-induced apoptosis, thus making SOCE a potential target for the treatment of various vascular diseases.

Stromal interaction molecule (STIM) proteins are localized to the endoplasmic reticulum (ER) and act as sensors of ER calcium. These proteins play a direct role in the ER-plasma membrane (PM) junction (Rebecchi and Pentyala, 2000; Liou *et al*., 2005). They regulate extracellular calcium via SOCE or capacitative calcium entry (Roos *et al*., 2005). Studies by our group have indicated that both shRNA (shSTIM1) and ML-9, an SOCE antagonist, protect EPCs from injuries induced by oxidative stress. Further work has suggested that this protection may involve interference with multiple factors associated with intracellular reactive oxygen species (ROS), ER stress

Open Access **http://dx.doi.org/10.4062/biomolther.2015.130**

Received Aug 17, 2015 **Revised** Nov 9, 2015 **Accepted** Nov 17, 2015
Published Online Jul 1, 2016

***Corresponding Author**
E-mail: huanglan260@126.com
Tel: +86-02368755601, Fax: +86-02368755601

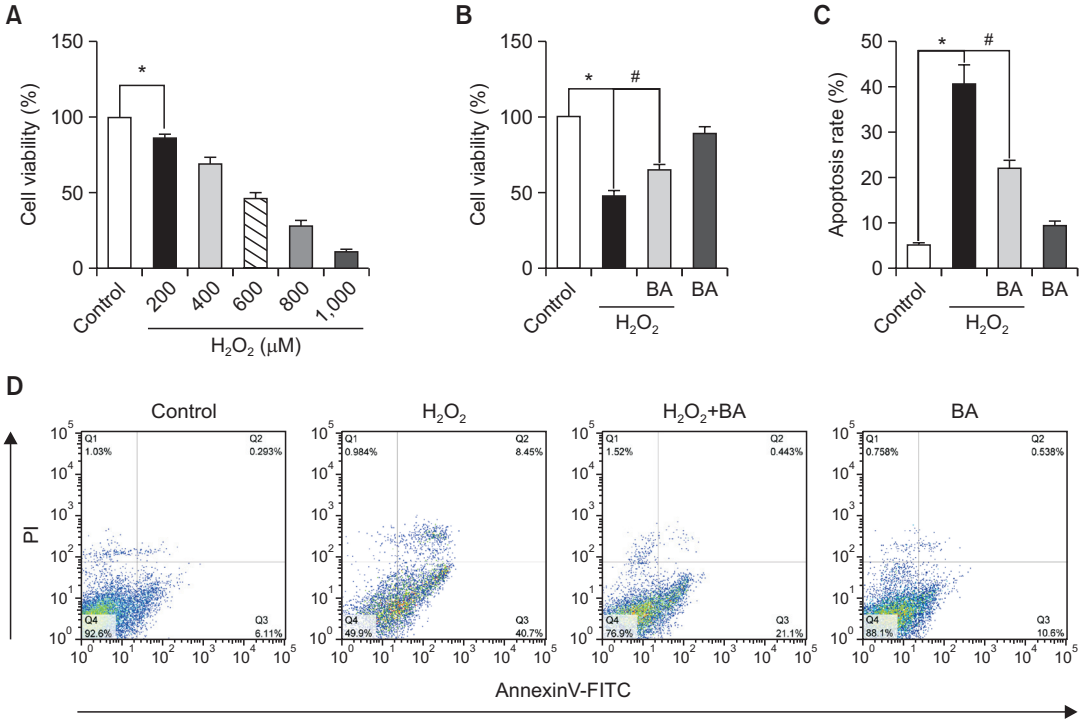

Fig. 1. H_2O_2-induced cytotoxicity and apoptotic cell death involve disruption of intracellular calcium homeostasis. After treatment with different concentrations of H_2O_2 for 6 h, cell viability was measured using a CCK-8 kit (A). After pretreatment with BAPTA-AM (BA), cells were treated with H_2O_2 for 6 h, and cell viability was measured (B). EPCs were pretreated with BAPTA-AM (BA) before exposure to H_2O_2 for 6 h. The cells were then stained with Annexin V-FITC and propidium iodide (PI) and analyzed via flow cytometry (D). Quantitative data for Annexin V-FITC/PI staining is presented (C). *p<0.05; $^{\#}p$<0.05.

and mitochondrial dysfunction.

MATERIALS AND METHODS

Cell culture and characterization of EPCs

Male Sprague-Dawley rats were sacrificed by injection of an overdose of sodium pentobarbital (100 mg/kg) into the peritoneal cavity (Zhao *et al*., 2008), according to the standards of the American Veterinary Medical Association Panel on Euthanasia. Bone marrow-derived EPCs (BM-EPCs) were isolated from the femurs and tibias of rats through density-gradient centrifugation (Sigma-aldrich, St. Louis, USA) according to established methods. Isolated EPCs were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, USA) at 37°C under 5% CO_2 and 100% humidity. EPCs were cultured for 7 days before being utilized in experiments. To confirm the identities of EPCs, cells were incubated with acLDL-DiI (10 mg/ml) for 6 h and then fixed with 4% paraformaldehyde for 15 min, incubated with fluorescein isothiocyanate-labeled lectin (UEA-1; 10 mg/ml) for 1 h and examined under a laser confocal scanning microscope (Leica, Wetzlar, Germany) (Kuang *et al*., 2012). Cells that were double positive for acLDL-DiI and UEA-1 were identified as EPCs. Approximately 85% of cells were positive for both markers.

ShRNA transfection

STIM1 expression was silenced using a lentiviral vector carrying stromal interaction molecule 1 (STIM1) shRNA constructed by GenePharma (Shanghai, China). EPCs were cultured in 6-well plates 2 days before transfection. Cells were infected with the lentiviral vector at a multiplicity of infection of 100 for 48 h. The STIM1 shRNA sequence was GCGACTTCTGAAGAGTCTACC. The negative control shRNA sequence was TTCTCCGAACGTGTCACGT. The efficiency of shRNA transfection was confirmed by Western blot analysis.

Cell viability assay

A cell viability assay was performed using a Cell Counting Kit-8 (CCK-8) (Beyotime, Jiangsu, China) in accordance with the manufacturer's protocol. After 7 days of culturing, EPCs were trypsinized (HyClone, Utah, USA) and seeded into 96-well plates (approximately 5,000 cells/well). Cell viability was quantified by measuring absorbance at 450 nm with a microplate reader (Molecular Devices, Sunnyvale, USA) after cells were treated with processing factors and incubated with CCK-8. The results were expressed as a percentage of the control group (Hou *et al*., 2007; Zhang and Sun, 2010).

Apoptosis assay

EPC apoptosis was evaluated with a FITC-Annexin V Apoptosis Detection Kit (Neo Bioscience, Beijing, China). After 7 days of culturing, EPCs were treated with processing factors and then trypsinized and treated with Annexin V-FITC and propidium iodide (PI). The treated cells were finally counted via flow cytometry (Beckman coulter, Indianapolis, USA) following the manufacturer's protocol. The results were expressed as the percentages of cells in the second quadrant

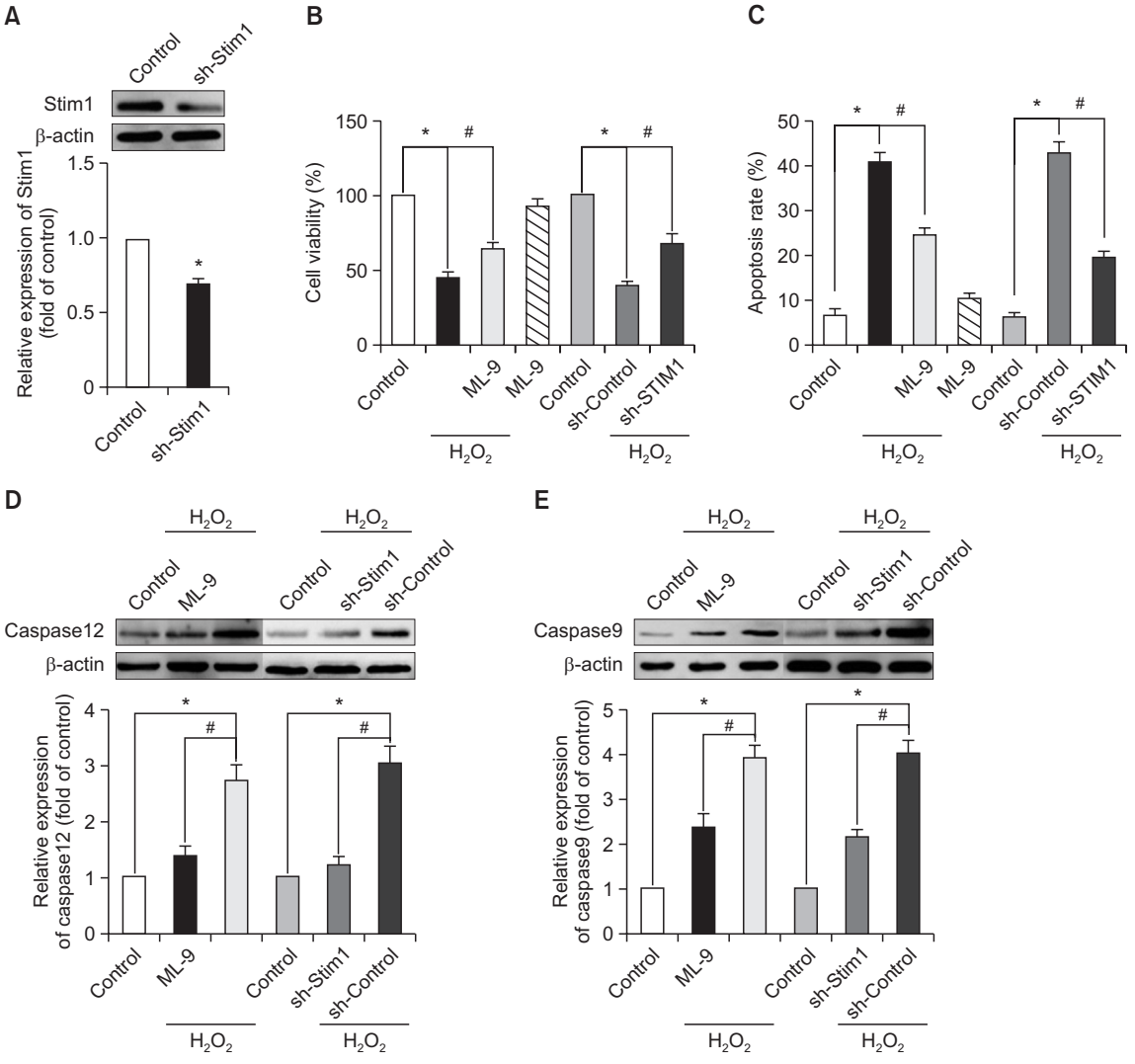

Fig. 2. Inhibition of SOCE attenuates the detrimental effects of H_2O_2 on EPCs. Representative immunoblots (top) and quantification (bottom) of STIM1 expression 48 h after transfection (A). EPCs that had been cultured for 7 days were pretreated with ML-9 for 20 min or with STIM1 shRNA before the addition of H_2O_2 for 6 h. Cell viability (B) and apoptosis (C) were then measured. The relative expression levels of caspase 12 (D) and caspase 9 (E) were measured by Western blot analysis. *p<0.05; $^{\#}p$<0.05.

(early apoptosis) and third quadrant (late apoptosis).

Calcium imaging

Intracellular calcium concentration was measured with the Fluo-3AM calcium indicator. EPCs were pretreated with 50 μM ML-9 or shSTIM1, incubated with Fluo-3AM for 30 min in the dark, washed 3 times with phosphate-buffered saline (PBS), incubated in medium for 30 min, washed with PBS three times and placed in a calcium-containing or calcium-free solution. The cells were then observed under a laser scanning confocal microscope at an excitation wavelength of 488 nm and emission wavelengths alternating between 525 nm and 530 nm. The results were analyzed with specific software (Li *et al*., 2009).

Detection of ROS in EPCs

To detect intracellular ROS in EPCs, we used a Reactive Oxygen Species Assay Kit (Beyotime), which detects changes in intracellular ROS using the DCFH-DA fluorescent probe. EPCs were treated with processing factors and then incubated with DCFH-DA and observed under a laser scanning confocal microscope at a 488 nm excitation wavelength and a 525 nm emission wavelength. The results were evaluated based on the intensity of the intracellular fluorescence (Jia *et al*., 2006).

Measurement of the mitochondrial membrane potential (MMP)

The MMP was measured using the fluorescent dye rhodamine123 (Rh123) (Beyotime). Rh123 rapidly passes through the PM and is sequestered by active mitochondria within a few minutes. In our experiments, EPCs were cultured with 2 μM Rh123 for 30 min at 37°C in the dark and then washed with PBS. The results were estimated by determining the gray values of the cells.

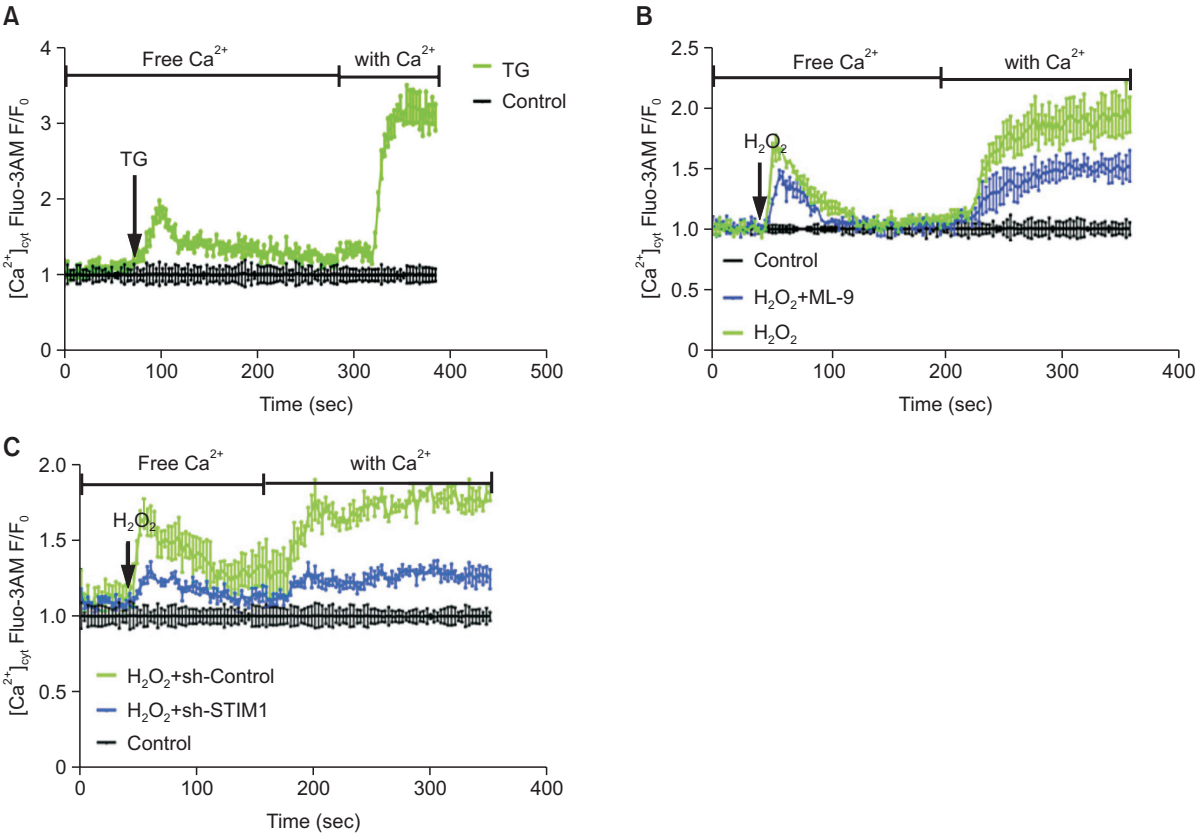

Fig. 3. Inhibition of SOCE restores intracellular calcium homeostasis. EPCs that had been cultured for 7 days were treated with thapsigargin (TG) (A) or pretreated with ML-9 for 20 min (B) or with STIM1 shRNA (C) before exposure to H_2O_2 in a calcium-free solution, followed by treatment with 2 mM $CaCl_2$. The intracellular calcium concentration was then measured using Fluo-3AM, a calcium indicator.

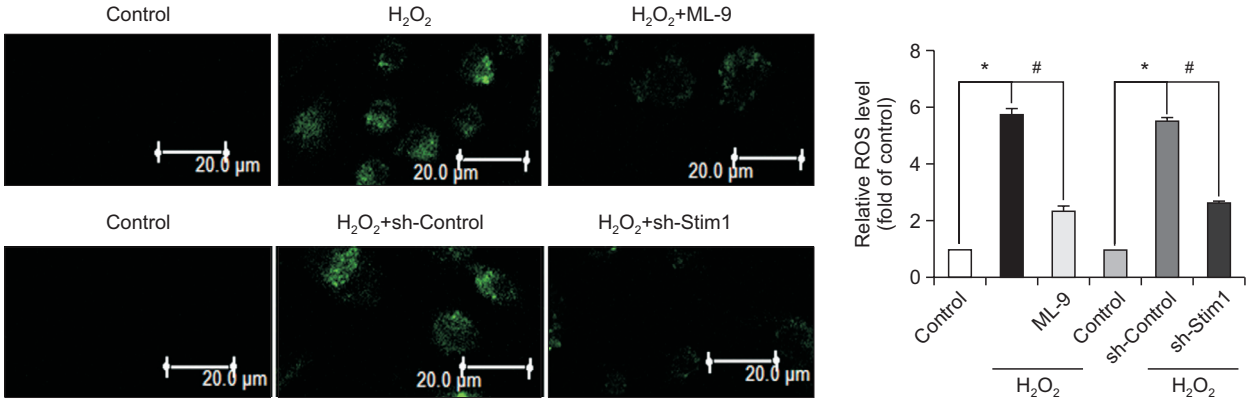

Fig. 4. Inhibition of SOCE prevents intracellular ROS accumulation. EPCs that had been cultured for 7 days were pretreated with ML-9 for 20 min or with STIM1 shRNA before exposure to H_2O_2 for 6 h, and intracellular ROS levels were evaluated using a fluorescence probe (DCFHDA). *p<0.05; #p<0.05.

Western blot analysis

After the various treatments were performed, proteins were extracted from EPCs using a lysis buffer (RIPA) containing phenylmethanesulfonyl fluoride (PMSF). Protein concentrations were determined with a BCA protein assay kit. Equal amounts of protein (30 μg per lane) were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline containing 0.5% Tween 20 and then incubated with specific primary antibodies. The membranes were subsequently incubated with appropriate secondary antibodies and then visualized with a luminescent image analyzer (GE, Connecticut, USA).

Statistical analysis

Statistical analyses were performed, and the data were expressed as the mean ± standard deviation (SD) of five experiments. SPSS 16.0 was utilized for data analysis. The t-test was employed to compare two groups, and one-way analysis of variance (ANOVA) was used to compare multiple groups. A *p*-value<0.05 was regarded as statistically significant.

RESULTS

H_2O_2-induced cytotoxicity and apoptotic cell death involve disruption of intracellular calcium homeostasis

EPCs (cultured for 7 days) were exposed to various concentrations (200, 400, 600, 800 and 1000 μM) of H_2O_2 for 6 h, and cytotoxicity was determined with a CCK-8 kit. As shown in Fig. 1A, H_2O_2 induced cytotoxicity in a dose-dependent manner. When the H_2O_2 concentration was increased to 600 μM, cell viability was approximately 50%. This concentration was used in the following experiments. H_2O_2-induced apoptosis was detected via flow cytometry. To assess whether intracellular calcium was involved in the H_2O_2-induced cytotoxicity and apoptosis of EPCs, we pretreated EPCs with BAPTA-AM (BA), an intracellular free calcium chelator (Fig. 1B-1D). The addition of BA (10 μM) attenuated the H_2O_2-induced injury of EPCs, suggesting that H_2O_2-induced injury may be related to disruption of intracellular calcium homeostasis.

Inhibition of SOCE attenuates the detrimental effects of H_2O_2 on EPCs

To investigate the effects of SOCE inhibition, we utilized ML-9 (50 μM), an antagonist of SOCE, and silenced the expression of STIM1 by shRNA (Fig. 2A). This method was used for SOCE in the following experiments. As hypothesized, the H_2O_2-induced decrease in EPC viability and increase in EPC apoptosis were reversed by the inhibition of SOCE (Fig. 2B, 2C). In addition, the H_2O_2-induced up-regulation of caspase 12 , which is responsible for ER stress-induced apoptosis (Rasheva and Domingos, 2009), and caspase 9, which is closely related to the mitochondrial apoptotic pathway, was significantly attenuated by SOCE inhibition (Fig. 2D, 2E).

Inhibition of SOCE restores intracellular calcium homeostasis

Numerous data have demonstrated that calcium entry through SOCE channels is a common and ubiquitous mechanism of regulating calcium entry and that SOCE is the main mode of calcium entry into EPCs. Thus, we performed calcium imaging to determine whether H_2O_2 affects calcium signaling in EPCs through SOCE. H_2O_2 stimulation of EPCs in a calcium-free buffer led to an increase in cytosolic calcium due to calcium release from the ER (left peaks in Fig. 3B, 3C). The addition of calcium after cytosolic calcium level slowed down resulted in calcium influx through the PM (right peaks in Fig. 3B, 3C) due to the depletion of ER stores. These results were considered typical calcium imaging results for SOCE and were similar to thapsigargin (TG; 2 μM)-induced calcium fluxes (Fig. 3A). Therefore, H_2O_2-induced calcium changes potentially occur mainly through SOCE. Furthermore, the inhibition of SOCE resulted in a significant decrease in cytosolic calcium in response to H_2O_2 insult (Fig. 3B, 3C).

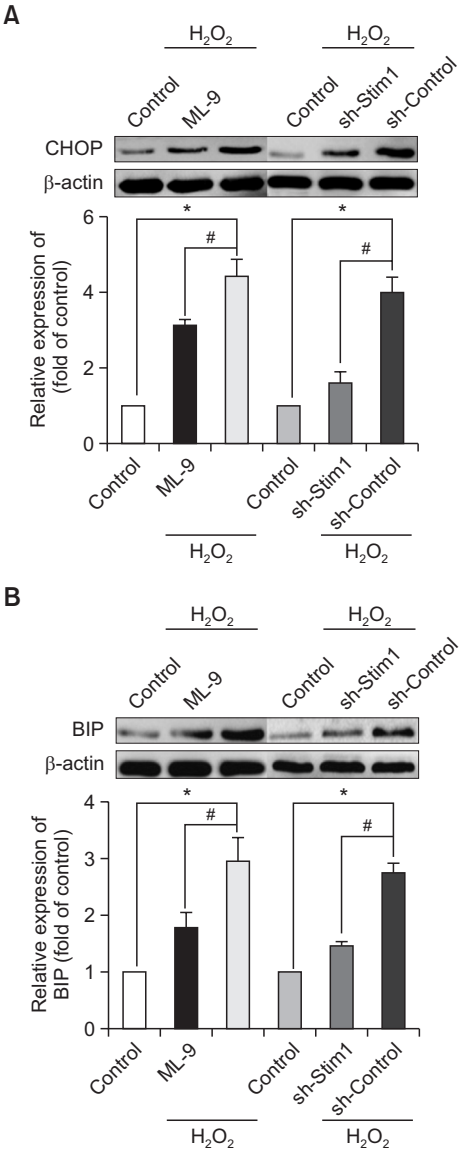

Fig. 5. Inhibition of SOCE protects EPCs from ER stress. EPCs that had been cultured for 7 days were pretreated with ML-9 for 20 min or with STIM1 shRNA before treatment with H_2O_2 for 6 h. The expression of the ER stress-related proteins CHOP (A) and BIP (B) was evaluated by Western blot analysis. **p*<0.05; #*p*<0.05.

Inhibition of SOCE prevents intracellular ROS accumulation

To investigate the role of ROS in the protective effects of SOCE inhibition, we used the fluorescent probe DCFH-DA to detect intracellular ROS production. Compared with the control group, the H_2O_2 treatment group exhibited significantly increased ROS accumulation, and the inhibition of SOCE markedly blunted this effect (Fig. 4).

Inhibition of SOCE protects EPCs from ER stress

Many studies have demonstrated that ER stress is often accompanied by increased ROS production. To assess the protective effect of SOCE inhibition, we measured the expression of ER stress-related proteins. As shown in Fig. 5A, 5B, the expression levels of C/EBP homologous protein (CHOP) and

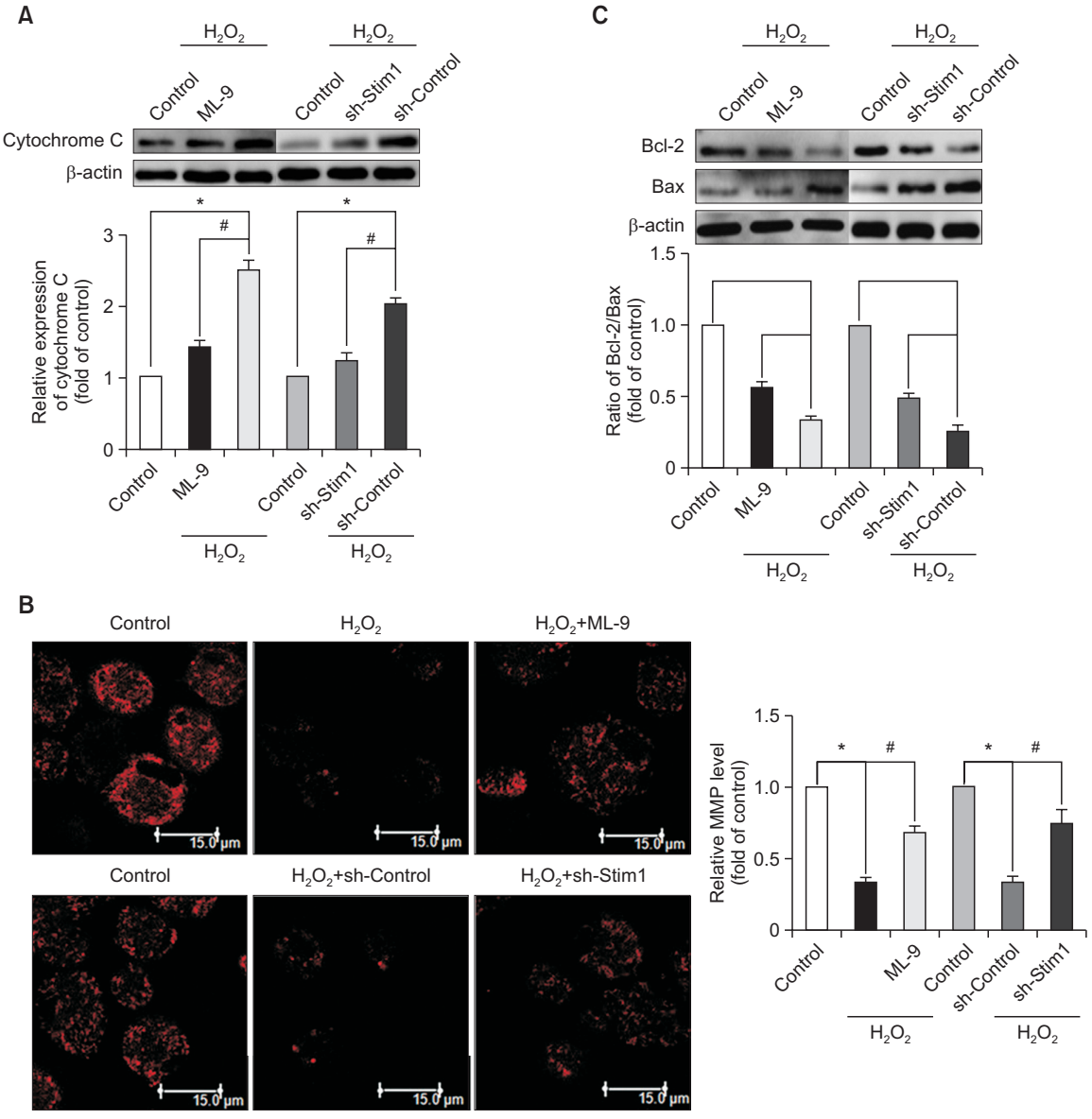

Fig. 6. Inhibition of SOCE ameliorates H_2O_2-induced mitochondrial dysfunction. EPCs that had been cultured for 7 days were pretreated with ML-9 for 20 min or with STIM1 shRNA before treatment with H_2O_2 for 6 h. The release of cytochrome C into the cytoplasm (A) and the Bcl-2/Bax ratio were measured by Western blot analysis (C). The MMP was measured via rhodamine 123 staining (B). $^{*}p<0.05$; $^{\#}p<0.05$.

CRP78/BIP were up-regulated by H_2O_2 stimulation compared with that of the control group. Moreover, the inhibition of SOCE suppressed the expression of ER stress-related proteins.

Inhibition of SOCE ameliorates H_2O_2-induced mitochondrial dysfunction

To demonstrate the effects of SOCE inhibition on the repair of mitochondrial dysfunction, we measured the MMP by Rh123 staining. After incubation with H_2O_2, the MMP was reduced compared with that of the control group, and this effect was ameliorated by SOCE inhibition (Fig. 6B). Moreover, the results of Western blot analysis suggested that H_2O_2 treatment up-regulated the cytosolic cytochrome C level compared with that of the control group (Fig. 6A). As predicted, SOCE inhibition significantly reduced the detrimental effects induced by H_2O_2. Furthermore, we observed the effects of SOCE inhibition on Bcl-2 family members, which play a vital role in the mitochondrial pro-apoptotic signaling pathway. Exposure to H_2O_2 led to a decrease in the Bcl-2/Bax ratio compared with that of the control group (Fig. 6C), and this decrease was ameliorated by SOCE inhibition.

DISCUSSION

This study has shown for the first time that SOCE plays key roles in the H_2O_2-induced decrease in EPC cell viability and increase in EPC apoptosis. We have also demonstrated that SOCE inhibition attenuates H_2O_2-induced EPC cytotoxicity and apoptosis. First, H_2O_2 insult activated the process known

as calcium release-activated calcium influx. Second, SOCE inhibition suppressed H_2O_2-induced calcium influx. Third, SOCE inhibition attenuated H_2O_2-induced EPC cytotoxicity and apoptosis. Finally, SOCE inhibition significantly reversed the H_2O_2-mediated induction of several key pro-apoptotic signaling pathways mediated by ER stress and mitochondrial dysfunction. Taken together, our results demonstrate that SOCE inhibition protects EPCs from H_2O_2-induced apoptosis.

It is generally recognized that H_2O_2 insult leads to calcium influx and disruption of intracellular calcium homeostasis, followed by an increase in ROS production (Ohyama *et al*., 2012). These changes further activate downstream apoptotic signaling pathways and ultimately result in cell death. Accumulating evidence suggests that oxidative stress-induced injuries might be attenuated by stabilizing intracellular calcium homeostasis (Godfraind, 2005; Ohyama *et al*., 2012). Moreover, some studies have indicated that antioxidants diminish increases in intracellular calcium levels (Pieper and Dondlinger, 1998; Su *et al*., 1999). Although the specific mechanism of the reduction of intracellular calcium following antioxidant application is still not clear, we hypothesized that the decrease in intracellular ROS production in response to antioxidant insult might lead to a change in the intracellular calcium level. In the present study, we demonstrated that H_2O_2 insult resulted in calcium influx and increase in intracellular ROS levels. In addition, we found that SOCE inhibition prevented intracellular ROS accumulation. To date, there have been no reports of the effect of antioxidants on calcium regulation in EPCs. Thus we will pay more attention to this aspect in our future study.

SOCE, which occurs ubiquitously in non-excitable cells, plays an essential role in the regulation of intracellular calcium homeostasis. Accumulating evidence has demonstrated that SOCE is involved in oxidative stress (Törnquist *et al*., 2000; Martín-Romero *et al*., 2008; Henke *et al*., 2013; Plomaritas *et al*., 2014). However, the effect of SOCE on oxidative stress remains to be elucidated. Several studies have demonstrated that H_2O_2 insult weakens SOCE (Törnquist *et al*., 2000; Plomaritas *et al*., 2014). Other studies have shown that H_2O_2 insult augments SOCE and the influx of calcium ions (Rao *et al*., 2013). These contradictory results may be explained by the activation of different signaling pathways in response to oxidative stress in different cell types. For example, the oxidative stress-induced changes in calcium influx and SOCE are mediated by the activation of protein kinase C (PKC) in thyroid FRTL-5 cells (Törnquist *et al*., 2000) and by regulation of STIM1 expression in the HT22 cells (Rao *et al*., 2013). In the present study, the H_2O_2 insult-induced disruption of intracellular calcium homeostasis was attenuated by inhibiting SOCE using ML-9 or via STIM1 silencing. These findings suggest that SOCE plays a critical role in oxidative stress.

Accumulating evidence supports the notion that calcium and calcium signaling are involved in ER and mitochondrial activities (Arnaudeau *et al*., 2001; Pizzo and Pozzan, 2007). The ER, a site of major calcium stores, plays a critical role in a variety of cellular activities, such as protein synthesis, post-translational modifications, protein folding and apoptosis. A prolonged decrease in the ER calcium level (Hooper *et al*., 2013) or accumulation of unfolded or misfolded proteins within the ER (Hammadi *et al*., 2013) leads to ER stress. Prolonged ER stress further promotes the expression of CHOP, a CEBP family transcription factor, and caspase 12, thereby activating downstream apoptotic pathways. Moreover, ER stress can result in sustained mitochondrial calcium accumulation (Deniaud *et al*., 2008). The mitochondria, which are also important calcium-buffering organelles, are physically and functionally interconnected with the ER. Mitochondria are often located near calcium release sites in the ER or at calcium influx channels at the PM, where they buffer intracellular calcium changes by modulating the rate of capacitative calcium entry through calcium release-activated channels (CRACs) or the rate of calcium release by IP3 receptors (Arnaudeau *et al*., 2001). Disruption of mitochondrial calcium homeostasis may result in dysfunction of the mitochondria, resulting in up-regulation of caspase 9 expression and enhanced release of cytochrome c from the mitochondria into the cytoplasm. Cytochrome c and caspase 9 form apoptotic bodies through the multiple polymerization of apoptotic protease activating factor 1 (Apaf-1), thereby activating downstream apoptotic pathways (Hikita *et al*., 2015). Several published reports have suggested that various apoptotic pathways are involved in the cross-talk between the ER and mitochondria (Wang and El-Deiry, 2004; Pizzo and Pozzan, 2007). Cytochrome c, which plays a vital role in this cross-talk, translocates to the ER to bind inositol 1,4,5-triphosphate receptors, leading to sustained calcium release. This calcium release in turn triggers the release of cytochrome c. Importantly, cytochrome c is involved in these apoptotic pathways. The present study demonstrated that SOCE inhibition restored intracellular calcium homeostasis and suppressed the pro-apoptotic effect by reducing ER stress and preserving mitochondrial function. Taken together, these data show that communication between the ER and mitochondria is involved in the protective effect of SOCE inhibition against H_2O_2 insult.

In conclusion, these findings have confirmed the role of SOCE in oxidative stress. H_2O_2-induced EPC apoptosis was shown to be alleviated by SOCE inhibition. The probable underlying mechanisms may be related to intracellular ROS levels, ER stress and mitochondrial dysfunction. Therefore, SOCE inhibition may serve as a potential therapeutic strategy for EPC injury in oxidative stress.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant 81370211). We would like to thank Prof. J.H.Z. and Prof. Y.Y. for the critical comments on this manuscript.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

REFERENCES

Arnaudeau, S., Kelley, W. L., Walsh, J. V., Jr. and Demaurex, N. (2001) Mitochondria recycle Ca^{2+} to the endoplasmic reticulum and prevent the depletion of neighboring endoplasmic reticulum regions. *J. Biol. Chem.* **276**, 29430-29439.

Battelli, M. G., Polito, L. and Bolognesi, A. (2014) Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. *Atherosclerosis* **237**, 562-567.

Cachofeiro, V., Goicochea, M., de Vinuesa, S. G., Oubiña, P., Lahera, V. and Luño, J. (2008) Oxidative stress and inflammation, a link be-

tween chronic kidney disease and cardiovascular disease. *Kidney Int. Suppl.* S4-S9.
Caron, A. Z., Chaloux, B., Arguin, G. and Guillemette, G. (2007) Protein kinase C decreases the apparent affinity of the inositol 1,4,5-trisphosphate receptor type 3 in RINm5F cells. *Cell calcium* **42**, 323-331.
Deniaud, A., Sharaf el dein, O., Maillier, E., Poncet, D., Kroemer, G., Lemaire, C. and Brenner, C. (2008) Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene* **27**, 285-299.
Godfraind, T. (2005) Antioxidant effects and the therapeutic mode of action of calcium channel blockers in hypertension and atherosclerosis. *Philos. Trans. R. Soc. Lond B, Biol. Sci.* **360**, 2259-2272.
Griendling, K. K. and FitzGerald, G. A. (2003) Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation* **108**, 2034-2040.
Hammadi, M., Oulidi, A., Gackière, F., Katsogiannou, M., Slomianny, C., Roudbaraki, M., Dewailly, E., Delcourt, P., Lepage, G., Lotteau, S., Ducreux, S., Prevarskaya, N. and Van Coppenolle, F. (2013) Modulation of ER stress and apoptosis by endoplasmic reticulum calcium leak via translocon during unfolded protein response: involvement of GRP78. *FASEB J.* **27**, 1600-1609.
Harrison, D., Griendling, K. K., Landmesser, U., Hornig, B. and Drexler, H. (2003) Role of oxidative stress in atherosclerosis. *Am. J. Cardiol.* **91**, 7A-11A.
Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T. and Münzel, T. (2001) Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* **104**, 2673-2678.
Henke, N., Albrecht, P., Bouchachia, I., Ryazantseva, M., Knoll, K., Lewerenz, J., Kaznacheyeva, E., Maher, P. and Methner, A. (2013) The plasma membrane channel ORAI1 mediates detrimental calcium influx caused by endogenous oxidative stress. *Cell Death Dis.* **4**, e470.
Henke, N., Albrecht, P., Pfeiffer, A., Toutzaris, D., Zanger, K. and Methner, A. (2012) Stromal interaction molecule 1 (STIM1) is involved in the regulation of mitochondrial shape and bioenergetics and plays a role in oxidative stress. *J. Biol. Chem.* **287**, 42042-42052.
Hikita, H., Kodama, T., Tanaka, S., Saito, Y., Nozaki, Y., Nakabori, T., Shimizu, S., Hayashi, Y., Li, W., Shigekawa, M., Sakamori, R., Miyagi, T., Hiramatsu, N., Tatsumi, T. and Takehara, T. (2015) Activation of the mitochondrial apoptotic pathway produces reactive oxygen species and oxidative damage in hepatocytes that contribute to liver tumorigenesis. *Cancer Prev. Res. (Phila.)* **8**, 693-701.
Hooper, R., Samakai, E., Kedra, J. and Soboloff, J. (2013) Multifaceted roles of STIM proteins. *Pflugers Arch.* **465**, 1383-1396.
Hou, G., Xue, L., Lu, Z., Fan, T., Tian, F. and Xue, Y. (2007) An activated mTOR/p70S6K signaling pathway in esophageal squamous cell carcinoma cell lines and inhibition of the pathway by rapamycin and siRNA against mTOR. *Cancer Lett.* **253**, 236-248.
Hung, Y. C., Sava, V. M., Blagodarsky, V. A., Hong, M. Y. and Huang, G. S. (2003) Protection of tea melanin on hydrazine-induced liver injury. *Life Sci.* **72**, 1061-1071.
Jia, S. J., Jiang, D. J., Hu, C. P., Zhang, X. H., Deng, H. W. and Li, Y. J. (2006) Lysophosphatidylcholine-induced elevation of asymmetric dimethylarginine level by the NADPH oxidase pathway in endothelial cells. *Vascul. Pharmacol.* **44**, 143-148.
Kuang, C. Y., Yu, Y., Wang, K., Qian, D. H., Den, M. Y. and Huang, L. (2012) Knockdown of transient receptor potential canonical-1 reduces the proliferation and migration of endothelial progenitor cells. *Stem Cells Dev.* **21**, 487-496.
Lewis, R. S. (2007) The molecular choreography of a store-operated calcium channel. *Nature* **446**, 284-287.
Li, B., Xiao, L., Wang, Z. Y. and Zheng, P. S. (2014) Knockdown of STIM1 inhibits 6-hydroxydopamine-induced oxidative stress through attenuating calcium-dependent ER stress and mitochondrial dysfunction in undifferentiated PC12 cells. *Free Radic. Res.* **48**, 758-768.
Li, H., Wang, L., Ye, L., Mao, Y., Xie, X., Xia, C., Chen, J., Lu, Z. and Song, J. (2009) Influence of Pseudomonas aeruginosa quorum sensing signal molecule N-(3-oxododecanoyl) homoserine lactone on mast cells. *Med. Microbiol. Immunol.* **198**, 113-121.
Li, X., Chen, W., Zhang, L., Liu, W. B. and Fei, Z. (2013) Inhibition of store-operated calcium entry attenuates MPP(+)-induced oxidative stress via preservation of mitochondrial function in PC12 cells: involvement of Homer1a. *PLoS ONE* **8**, e83638.
Liou, J., Kim, M. L., Heo, W. D., Jones, J. T., Myers, J. W., Ferrell, J. E., Jr. and Meyer, T. (2005) STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. *Curr. Biol.* **15**, 1235-1241.
Martín-Romero, F. J., Ortíz-de-Galisteo, J. R., Lara-Laranjeira, J., Domínguez-Arroyo, J. A., González-Carrera, E. and Alvarez, I. S. (2008) Store-operated calcium entry in human oocytes and sensitivity to oxidative stress. *Biol. Reprod.* **78**, 307-315.
Ohyama, T., Sato, K., Kishimoto, K., Yamazaki, Y., Horiguchi, N., Ichikawa, T., Kakizaki, S., Takagi, H., Izumi, T. and Mori, M. (2012) Azelnidipine is a calcium blocker that attenuates liver fibrosis and may increase antioxidant defence. *Br. J. Pharmacol.* **165**, 1173-1187.
Pieper, G. M. and Dondlinger, L. A. (1998) Antioxidant pyrrolidine dithiocarbamate prevents defective bradykinin-stimulated calcium accumulation and nitric oxide activity following exposure of endothelial cells to elevated glucose concentration. *Diabetologia* **41**, 806-812.
Pizzo, P. and Pozzan, T. (2007) Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics. *Trends Cell Biol.* **17**, 511-517.
Plomaritas, D. R., Herbert, L. M., Yellowhair, T. R., Resta, T. C., Gonzalez Bosc, L. V., Walker, B. R. and Jernigan, N. L. (2014) Chronic hypoxia limits H_2O_2-induced inhibition of ASIC1-dependent store-operated calcium entry in pulmonary arterial smooth muscle. *Am. J. Physiol. Lung Cell Mol. Physiol.* **307**, L419-L430.
Rao, W., Zhang, L., Su, N., Wang, K., Hui, H., Wang, L., Chen, T., Luo, P., Yang, Y. F., Liu, Z. B. and Fei, Z. (2013) Blockade of SOCE protects HT22 cells from hydrogen peroxide-induced apoptosis. *Biochem. Biophys. Res. Commun.* **441**, 351-356.
Rasheva, V. I. and Domingos, P. M. (2009) Cellular responses to endoplasmic reticulum stress and apoptosis. *Apoptosis* **14**, 996-1007.
Rebecchi, M. J. and Pentyala, S. N. (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol. Rev.* **80**, 1291-1335.
Roos, J., DiGregorio, P. J., Yeromin, A. V., Ohlsen, K., Lioudyno, M., Zhang, S., Safrina, O., Kozak, J. A., Wagner, S. L., Cahalan, M. D., Veliçelebi, G. and Stauderman, K. A. (2005) STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J. Cell Biol.* **169**, 435-445.
Schleicher, E. and Friess, U. (2007) Oxidative stress, AGE, and atherosclerosis. *Kidney Int. Suppl.* **72**, S17-S26.
Su, J. Y., Duffy, S. and Murphy, T. H. (1999) Reduction of H_2O_2-evoked, intracellular calcium increases in the rat N18-RE-105 neuronal cell line by pretreatment with an electrophilic antioxidant inducer. *Neurosci. Lett.* **273**, 109-112.
Thum, T., Fraccarollo, D., Galuppo, P., Tsikas, D., Frantz, S., Ertl, G. and Bauersachs, J. (2006) Bone marrow molecular alterations after myocardial infarction: Impact on endothelial progenitor cells. *Cardiovasc. Res.* **70**, 50-60.
Thum, T., Fraccarollo, D., Schultheiss, M., Froese, S., Galuppo, P., Widder, J. D., Tsikas, D., Ertl, G. and Bauersachs, J. (2007) Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes* **56**, 666-674.
Törnquist, K., Vainio, P. J., Björklund, S., Titievsky, A., Dugué, B. and Tuominen, R. K. (2000) Hydrogen peroxide attenuates store-operated calcium entry and enhances calcium extrusion in thyroid FRTL-5 cells. *Biochem. J.* **351**, 47-56.
Urbich, C., Knau, A., Fichtlscherer, S., Walter, D. H., Brühl, T., Potente, M., Hofmann, W. K., de Vos, S., Zeiher, A. M. and Dimmeler, S. (2005) FOXO-dependent expression of the proapoptotic protein Bim: pivotal role for apoptosis signaling in endothelial progenitor cells. *FASEB J.* **19**, 974-976.
Wang, S. and El-Deiry, W. S. (2004) Cytochrome c: a crosslink between the mitochondria and the endoplasmic reticulum in calcium-dependent apoptosis. *Cancer Biol. Ther.* **3**, 44-46.
Watson, T., Goon, P. K. and Lip, G. Y. (2008) Endothelial progenitor cells, endothelial dysfunction, inflammation, and oxidative stress in

hypertension. *Antioxid. Redox Signal.* **10**, 1079-1088.
Yoshii, T., Iwai, M., Li, Z., Chen, R., Ide, A., Fukunaga, S., Oshita, A., Mogi, M., Higaki, J. and Horiuchi, M. (2006) Regression of atherosclerosis by amlodipine via anti-inflammatory and anti-oxidative stress actions. *Hypertens. Res.* **29**, 457-466.
Zhang, H. Y. and Sun, H. (2010) Up-regulation of Foxp3 inhibits cell proliferation, migration and invasion in epithelial ovarian cancer. *Cancer Lett.* **287**, 91-97.
Zhao, X., Huang, L., Yin, Y., Fang, Y., Zhao, J. and Chen, J. (2008) Estrogen induces endothelial progenitor cells proliferation and migration by estrogen receptors and PI3K-dependent pathways. *Microvasc. Res.* **75**, 45-52.