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Prolyl Hydroxylase Inhibitor Dimethyloxalylglycine Enhances Mesenchymal Stem Cell Survival

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ABSTRACT

Mesenchymal stem cell (MSC) transplantation is a promising approach in the therapy of ischemic heart or CNS diseases; however, the poor viability of MSCs after transplantation critically limits the efficacy of this new strategy. Prolyl hydroxylase inhibition followed by HIF-1α up-regulation participates in the regulation of apoptosis and cell survival, which have been shown in cancer cells and neurons. The role of prolyl hydroxylase inhibition by dimethyloxalylglycine (DMOG) in regulation of cell survival has not been investigated in MSCs. In the present investigation with MSCs, apoptosis and cell death induced by serum deprivation were assessed by caspase-3 activation and trypan blue staining, respectively. The mitochondrial apoptotic pathway and PI3K/Akt cell survival pathway were evaluated. DMOG significantly attenuated apoptosis and cell death of MSCs, stabilized HIF-1α and induced downstream glucose transport 1 (Glut-1) synthesis. DMOG treatment reduced mitochondrial cytochrome *c* release, nuclear translocation of apoptosis inducing factor (AIF), and promoted Akt phosphorylation. A specific PI3K inhibitor, wortmannin, blocked Akt phosphorylation and abrogated the beneficial effect of DMOG. These data suggest that the DMOG protection of MSCs may provide a novel approach to promote cell survival during cell stress. J. Cell. Biochem. 106: 903–911, 2009.

KEY WORDS: DIMETHYLOXALYLGLYCINE; MESENCHYMAL STEM CELLS; APOPTOSIS; PI3K/Akt PATHWAY; HIF-1α

Despite great strides in medical strategies aimed at post-infarct remodeling, the development of aggressive reperfusion strategies such as interventional diagnosis and therapy, as well as common drug therapy, heart failure persists as an emerging public health concern in the developing world and remains the leading cause of death and hospitalization in industrialized countries [Schuleri et al., 2007]. In recent years, cell-based therapy has emerged as a promising therapeutic approach for restoration of heart function after myocardial infarction [Sharma and Raghubir, 2007]. Circumventing ethical and immune rejection issues, transplantation of autologous mesenchymal stem cells (MSCs) is an ideal option for clinical cell therapy. It has been demonstrated that MSCs are capable of ameliorating the cardiac function after myocardial infarction [Wang et al., 2005; Miyahara et al., 2006; Hu et al., 2007]. However, the majority of transplanted cells usually die a few days after transplantation into the harsh host environment. It was reported that over 90% of transplanted MSCs died in the first 24 h [Tang et al., 2005] and only 0.44% survived 4 days after transplantation to the ischemic heart [Toma et al., 2002]. Poor cell viability after transplantation into infarcted myocardium obviously restricts the efficacy of this attractive therapy. Therefore, strategies to enhance MSCs survival are important and urgently needed.

The hypoxia inducible factor-1 (HIF-1) is a heterodimer composed of HIF-1α and HIF-1β, both belonging to the basic-helix-loop-helix PER-ARNT-SIM family of transcriptional factors. HIF-1β is constitutively expressed in the nucleus and HIF-1α is an oxygen sensitive subunit [Wang et al., 1995]. In the presence of oxygen, modification of HIF-1α by the oxygen dependent prolyl hydroxylases allows an interaction between HIF-1α and the von Hippel Lindau (VHL) complex, resulting in rapid degradation through the ubiquitin-proteasome pathway [Salceda and Caro, 1997]. In addition, asparagine hydroxylation by Factor Inhibiting HIF-1 (FIH-1) blocks the interaction of HIF-1α with the transcriptional co-activator p300/CBP leading to inhibition of HIF-1-mediated gene transcription in the presence of oxygen [Lando et al., 2002]. Under hypoxic conditions, prolyl hydroxylase activity decreases because it requires oxygen as a co-factor and the HIF-1α protein is stabilized. HIF-1α stabilization is followed by activation

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of the transcription of numerous target genes such as vascular endothelial growth factor (VEGF), erythropoietin (EPO), and glucose transporter 1 (Glut-1) that are involved in angiogenesis, erythropoiesis, energy metabolism and cell survival [Iyer et al., 1998; Kerendi et al., 2006].

Prolyl hydroxylases play an important role in heart diseases. Inhibition of prolyl hydroxylase activity or expression can be experimentally effected using competitive inhibitors such as dimethyloxalylglycine (DMOG) or siRNA, leading to the stabilization of HIF and other beneficial factors. In ischemia-reperfusion (I/R) injury and acute myocardial infarction animal models, inhibition of prolyl hydroxylases prevents deleterious remodeling and attenuates the acute inflammatory response [Ockaili et al., 2005; Natarajan et al., 2006, 2007; Philipp et al., 2006]. Additionally, prolyl hydroxylase inhibition followed by HIF-1α up-regulation participates in the protection of cell survival and regulation of apoptosis which have been shown in cancer cells and neurons under hypoxic conditions, serum deprivation or trophic factor deprivation [Alvarez-Tejado et al., 2001; Piret et al., 2004; Sasabe et al., 2005; Lomb et al., 2007]. The precise role of HIF-1α in the regulation of cell death and apoptosis remains controversial because of the concern that HIF-1α may promote cell death [Carmeliet et al., 1998; Sowter et al., 2001]. Specifically, the role of prolyl hydroxylase inhibition by DMOG in regulation of cell survival has not been investigated in MSCs. The purpose of this study is to evaluate the effect of DMOG on apoptosis and cell death induced by serum deprivation in MSCs and to delineate the underlying signaling mechanism.

MATERIALS AND METHODS

MATERIALS

Dimethyloxalylglycine was purchased from Frontier Scientific (Logan, UT). Antibodies against p-Akt, Akt, AIF and wortmannin were obtained from Cell Signaling Technology (Boston, MA), Caspase-3 and Cytochrome-c from Millipore (Billerica, MA), HIF-1α, Tubulin and Actin from Santa Cruz Biotechnology (Santa Cruz, CA), and V-DAC from Abcam (Cambridge, MA). Trypan blue and Hoechst 33342 were from Sigma (St. Louis, MO) and Molecular Probes (Carlsbad, CA), respectively.

MSC CULTURE

Bone marrow mesenchymal stem cells were isolated and harvested as previously described [Campagnoli et al., 2001]. In brief, bone marrow samples were collected by flushing the cavities of femurs and tibias from green fluorescent protein (GFP) transgenic mice (Charles River laboratories, Wilmington, MA) with basal Dulbecco's Modified Eagle's medium (DMEM). Whole marrow cells were seeded at $1 \times 10^6/cm^2$ into flasks with DMEM supplemented with 10% fetal bovine serum (FBS). The cultures were maintained at 37°C in a 5% CO_2 and humidified incubator. After 24 h, non-adherent cells were removed and small number of spindle-shaped cells were found to adhere the bottom of flasks. Cells were subcultured 1:2 or 1:3 when they reached approximately 80% confluence. To avoid hematopoietic cell contamination in earlier passages and the presence of differentiated or senescent MSCs in later passages, we used MSCs of four to seven passages.

Using CD90, CD34 and CD45 cell surface markers, cells were identified by fluorescence-activated cell sorting analysis before the experiments and identified as CD90+/CD34−/CD45− cells [Hu et al., 2008].

SERUM DEPRIVATION

Cells were plated in 60 mm dishes and allowed to attach overnight. Cells were washed three times with phosphate buffered saline, and then administered serum-free DMEM for serum deprivation (SD) or normal culture media as control. DMOG or vehicle were co-applied at the time of serum withdrawal for cell death/apoptosis assays, and western blotting of active caspase 3, HIF-1α, GLUT-1, cytochrome *c*, and AIF. For p-AKT and p-ERK detection, cells were treated with 21 h of SD to induce a decrease in phosphorylated AKT or ERK and then DMOG was added for an additional 3 h of SD.

APOPTOSIS ASSESSMENT

Cell morphology was examined by a phase contrast microscope and Hoechst33342 at the concentration of 5 μg/ml was used for chromosomal condensation and nuclear fragmentation analysis under a fluorescence microscope. Apoptotic cells were characterized by caspase-3 activation and morphological changes, such as cellular shrinkage, nuclear condensation and fragmentation.

Cleaved caspase-3 staining was applied to detect and quantify MSCs apoptosis induced by serum deprivation. MSCs were fixed with 10% formalin for 5 min, permeabilized with 0.2% Trition X-100 for 5 min, blocked with 1% Fish gelatin (Sigma) for 1 h at room temperature, and then incubated with primary antibody overnight. After incubation with Cy3-conjugated second antibody (Jackson ImmunoResearch, West Grove, CA) for 1 h at room temperature, Hoechst 33342 was used to stain the nucleus. Dishes were mounted and analyzed under a fluorescent microscope (BX51, Olympus, Japan).

CELL DEATH EVALUATION

Trypan blue staining was performed to assess serum deprivation induced cell death. Trypan blue at a final concentration of 0.05% was added to the medium at the end of treatment. Subsequently, pictures in five different randomly chosen fields per well were taken under a phase contrast microscope after 10 min incubation. Cell death was calculated by the ratio of trypan blue positive cells to total number of cells.

WESTERN BLOT ANALYSIS

According to the time points in experimental design, cells were harvested into 1.5 ml Eppendorf tubes and then lysed with modified RIPA buffer (50 mM HEPES, PH 7.3, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 1 mM Na3VO4, 1 mM NaF, and protease inhibitor cocktail (Roche, Nutley, NJ)). Cells were vortexed until lysed completely, followed by centrifugation at 12,000 rpm for 20 min. Protein concentrations of the supernatant were determined by the Bicinchoninic Acid Assay (Sigma). All procedures were performed at 4°C.

Proteins (30–50 μg) were then electrophoresed on a 6–18% gradient gel by SDS–PAGE in a Hoefer Mini-Gel system (Amersham Biosciences, Piscataway, NJ) and transferred in a Hoefer Transfer

Tank (Amersham Biosciences) to PVDF membrane (BioRad, Hercules, CA). After blocking the membranes with 5% milk in Tris-buffered saline with 0.1% Tween (TBS-T) at room temperature for 2 h, membranes were incubated with specific primary antibodies by gentle agitation overnight at 4°C. Conjugation of primary antibodies with AP-marked or HRP-marked secondary antibodies were incubated at room temperature for 2 h. Finally, protein expression signals were detected by using BCIP/NBT solution (Sigma) or film and analyzed by Image-Pro Plus analysis software.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (SEM) and were analyzed with SPSS13.0. Student's two-tailed *t*-test was used for comparison of two independent groups and one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Statistical significance was defined as $P < 0.05$.

RESULTS

DMOG STABILIZED HIF-1α EXPRESSION AND UP-REGULATED DOWNSTREAM Glut-1 SYNTHESIS WITH NO OBVIOUS TOXICITY

Compared with control group, DMOG showed no toxicity to MSCs at the concentration of 1 mM (3.6 ± 0.2% and 3.8 ± 0.2% trypan blue positive cells, respectively, n = 5 independent assays). Increasing DMOG concentration to 5 mM resulted in a trend of cytotoxicity (4.3 ± 0.3% trypan blue positive cells, n = 5). We therefore tested DMOG at 1 mM (1,000 μM) or lower concentrations in following experiments.

MSCs were exposed to the apoptotic insult serum deprivation (SD) along with increasing doses of DMOG. DMOG markedly increased HIF-1α protein expression in a dose-dependent manner. HIF-1α protein expression was significantly up-regulated at 500 and 1,000 μM DMOG treatments. A trend of increased expression of HIF-1α was observed with 100 μM DMOG (Fig. 1A). Glucose transporter 1 (Glut-1) is one of the downstream genes that depends on HIF-1 activation [Iyer et al., 1998]. Glut-1 is normally highly expressed in endothelial cells [Gerhart et al., 1989]. In our study, increased Glut-1 expression verifies the increase in HIF-1 transcriptional activity, and represents an increase in a vascular protein. An increase in vascular proteins may indicate an increased angiogenic potential of these MSCs, which could be beneficial for transplantation studies. As expected, there was a significant increase of Glut-1 expression observed in DMOG-treated cells compared to SD-only group (Fig. 1B). Thus, the HIF-1α protein is not only upregulated, but also transcriptionally active.

DMOG DECREASED MSC APOPTOSIS

MSCs were treated with SD in the absence or presence of different concentrations of DMOG. After 24 h SD, MSCs underwent obvious cell volume decreases, nuclear condensation and fragmentation. This apoptotic morphology was prevented by co-applied DMOG in a dose-dependent manner (Fig. 2). In a fluorescent staining assay for caspase-3 cleavage, DMOG treatment again showed a dose-dependent reduction of the caspase-3 activation (Fig. 3). The anti-apoptotic property of DMOG was confirmed by Western blot

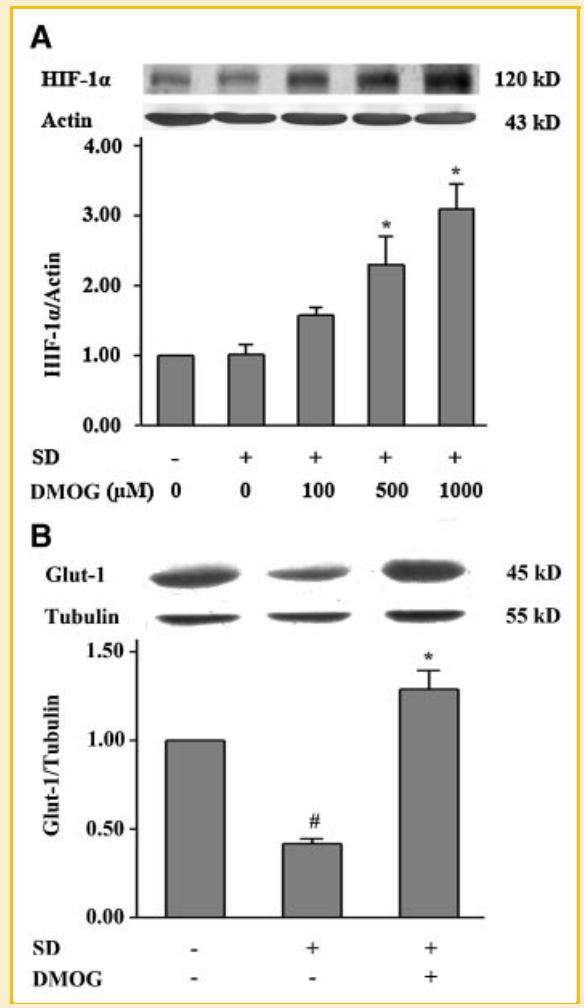

Fig. 1. DMOG induced HIF-1α stabilization and downstream Glut-1 expression. A: MSCs were cultured in the presence or absence of DMOG for 24 h, and dose dependent effect on HIF-1α was determined (n = 4). B: Glut-1 expression was evaluated at the concentration of 1,000 μM (n = 4). $^{\#}P < 0.001$ for SD versus control group; $^{*}P < 0.01$ for SD + DMOG versus SD group.

analysis (Fig. 4). In apoptotic cells, caspase-3 activation was detected at 6, 12, and 24 h after the onset of SD, reaching the highest level at 24-h time point (Fig. 4). DMOG (1,000 μM) significantly prevented the SD-induced caspase-3 activation (Fig. 4).

To assess the influence of DMOG on cell death, trypan blue staining was applied. Similar to caspase-3 activation, DMOG diminished the SD-induced cell death of MSCs in a dose-dependent manner. At 1,000 μM, DMOG reduced the ratio of trypan blue positive cells from 29.4 ± 1.0% to 12.7 ± 0.5% ($P < 0.05$; Fig. 5). Cell death was detectable at 12 h after SD and gradually increased in later time points up to 72 h in SD. DMOG (1,000 μM) attenuated cell death at all time points (Fig. 5).

DMOG REDUCED CYTOCHROME *c* RELEASE AND AIF NUCLEAR TRANSLOCATION

Cytochrome *c* release from mitochondria is a key step in the caspase-dependent apoptotic cascade [Green and Reed, 1998]. DMOG

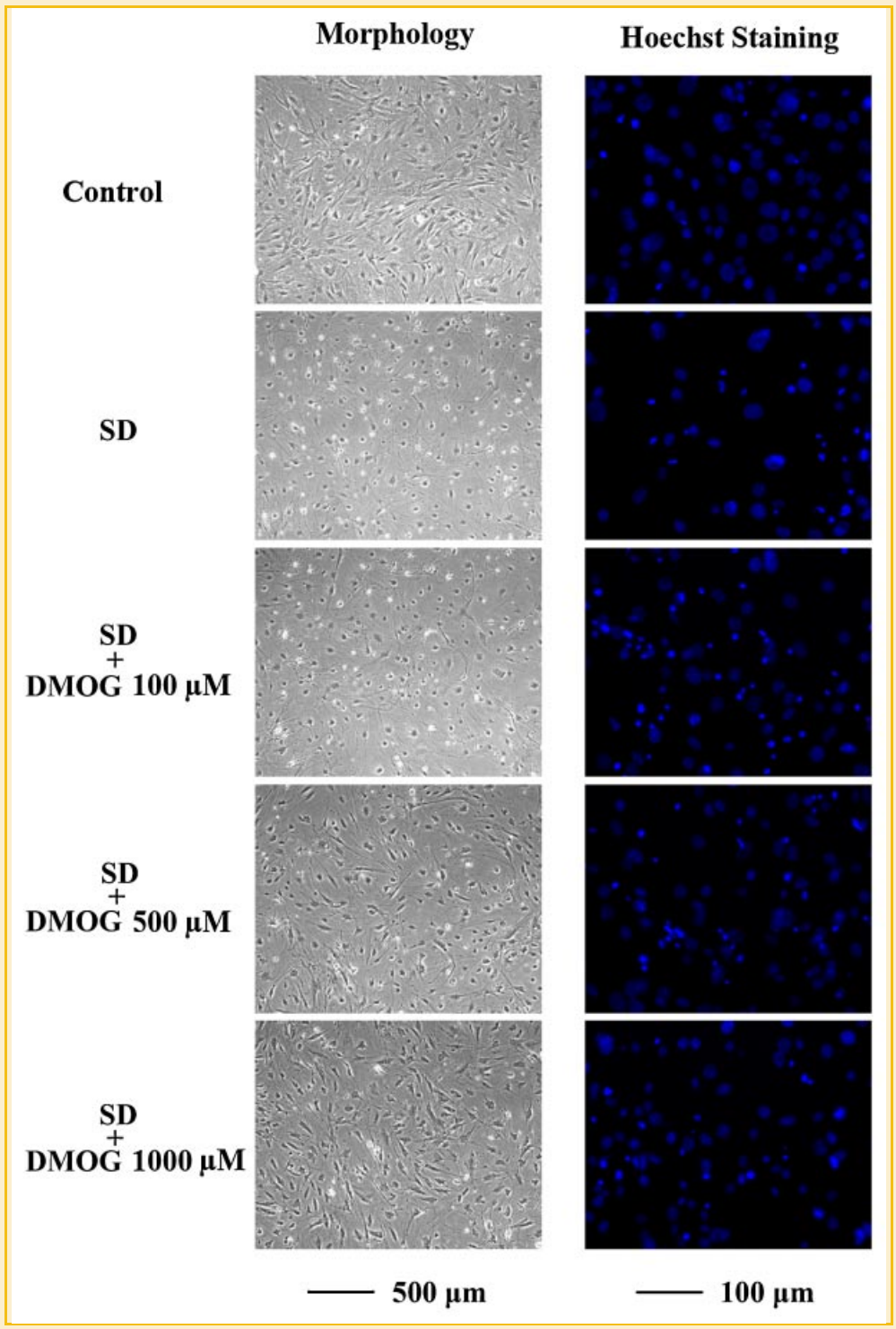

Fig. 2. Effect of DMOG on the morphological changes of MSCs undergoing apoptosis. DMOG at concentrations of 100, 500, and 1,000 μM was co-applied with serum deprivation. Images were taken 24 h after the treatments. DMOG showed dose-dependent effect against cell volume decrease. Fluorescent immunostaining of Hoechst 33342 revealed nuclear changes in volume, condensation and integrality of MSCs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

treatment significantly decreased cytochrome *c* release compared to cells in SD-only group (Fig. 6A). Apoptosis inducible factor (AIF) is also released from mitochondria, its nuclear translocation mediates a caspase-independent DNA fragmentation and apoptotic pathway [Broker et al., 2005]. In the SD-induced apoptotic MSCs death, we identified noticeable AIF translocation into the nucleus of cells undergoing apoptosis, while DMOG significantly diminished this caspase-independent event (Fig. 6B).

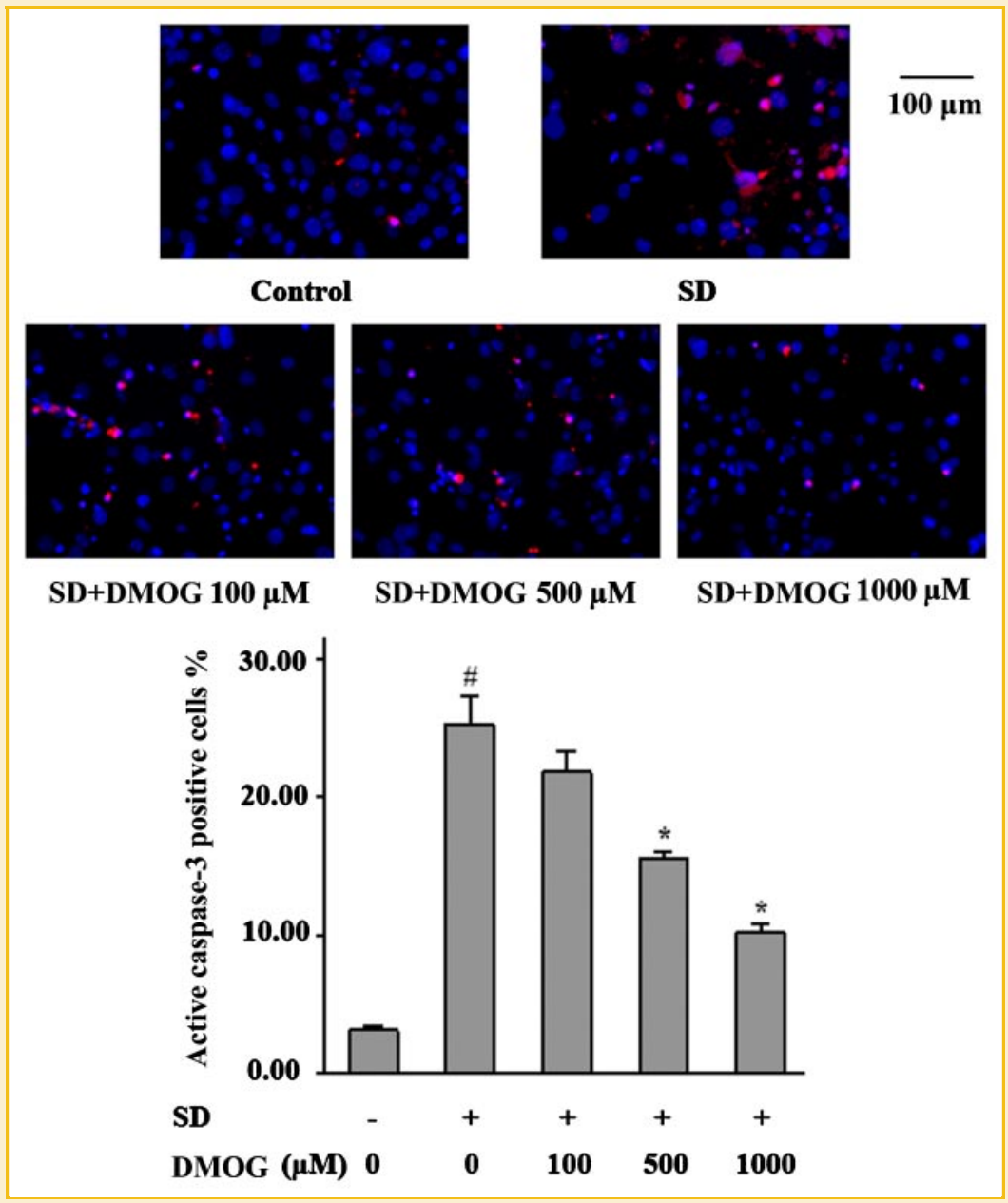

Fig. 3. Effect of DMOG on caspase-3 activation. Images illustrate immunostaining of cleaved caspase-3 (red) in MSCs under control, 24-h SD and SD plus DMOG. Hoechst 33342 staining (blue) shows nuclei of total cells. The percentage of apoptotic cells that were positive to caspase-3 staining was calculated and summarized in the bar graph. $^{\#}P < 0.001$ for SD versus control group; $^{*}P < 0.01$ for SD + DMOG versus SD group. N = 3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PI3K/Akt BUT NOT ERK PATHWAY INVOLVED IN THE DMOG PROTECTIVE EFFECT

To test the roles of cell survival pathways in DMOG protective effect, PI3K/Akt pathway and MEK/ERK1/2 pathway were evaluated. Compared with control groups, SD decreased the activation of both Akt and ERK. Three hours DMOG treatment at the end of the 24 h SD, reversed the reduction of phosphorylated Akt, however, DMOG showed no effect on ERK activation (Fig. 7). Administration of a typical PI3K inhibitor, wortmannin (100 nM), blocked Akt phosphorylation activated by DMOG. Consistently, wortmannin applied throughout the 24 h SD treatment abrogated the DMOG protective effect on MSCs (Fig. 8).

DISCUSSION

This investigation provides the first evidence that the prolyl hydroxylase inhibitor DMOG has an anti-apoptotic activity in MSCs. At the tested concentrations up to 1 mM, DMOG showed no toxic effect on MSCs. DMOG induced stabilization of HIF-1α protein and

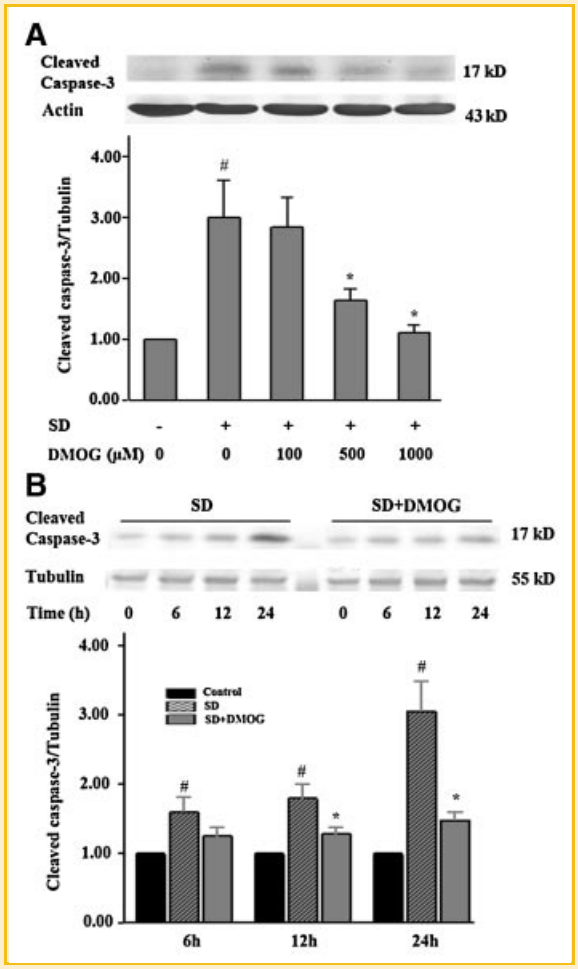

Fig. 4. DMOG protected MSCs against SD-induced apoptosis. A: Western blot analysis confirmed a dose-dependent effect of DMOG on caspase-3 activation in MSCs subjected to SD. DMOG was co-applied with SD for 24 h. B: The time course of SD-induced caspase-3 activation in MSCs and the inhibitory effects by 1,000 μM DMOG. $^{\#}P < 0.01$ for SD versus control group; $^{*}P < 0.05$ SD + DMOG versus SD group. N = 3.

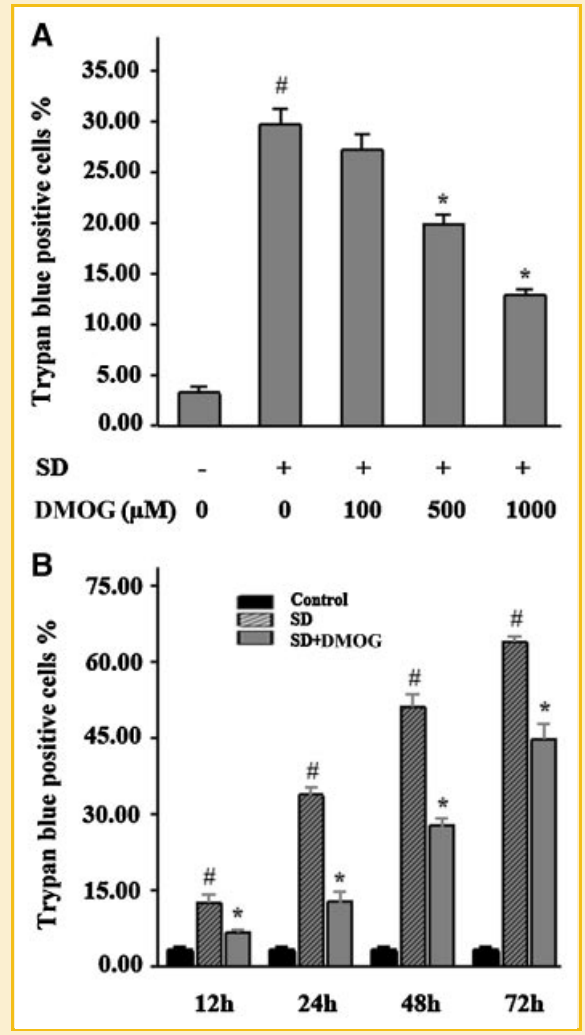

Fig. 5. Effect of DMOG on SD-induced cell death. A: MSCs death was detected by using Trypan blue staining 24 h after SD exposure. DMOG co-applied with SD showed dose-dependent effects of reducing Trypan blue-positive cells. B: Time course of SD-induced cell death and the protective effect of DMOG (1,000 μM). $^{\#}P < 0.001$ for SD versus control group; $^{*}P < 0.01$ for SD + DMOG versus SD group. N = 3.

augmented downstream Glut-1 synthesis. The mechanisms of the protective effect of DMOG were mediated through mitochondrial pathways involving reduced cytochrome *c* release and caspase-3 activation. Interestingly, we also identified a protective role for DMOG against caspase-independent apoptotic pathway, in blocking the key step of AIF translocation into nucleus. We further demonstrated that the PI3K/Akt signaling pathway but not ERK activation mediates the DMOG-associated cell survival.

HIF-1α stabilization induced by prolyl hydroxylase inhibition participates in the protection against apoptosis in different cell types under different insults [Alvarez-Tejado et al., 2001; Piret et al., 2004; Sasabe et al., 2005]. However, until now, the role of hydroxylase inhibition in MSCs was unclear. The present investigation supports the notion that prolyl hydroxylase inhibition, which stabilizes HIF-1α, is highly protective against apoptosis and cell death. As shown in Figure 5, DMOG exerted its protective effect in a dose dependent manner and delayed cell death at least 72 h after serum deprivation. Glut-1, one of the HIF-1 target genes, may have anti-apoptotic effects [Lin et al., 2000] and Glut-1 antibodies induced apoptosis in human cancer cell lines [Rastogi et al., 2007]. Consistently, we observed an increase in Glut-1 expression in cells treated with DMOG.

A recent study demonstrated that hypoxia and serum deprivation induced apoptosis of MSCs via mitochondrial pathway but not the extrinsic death receptor pathway [Zhu et al., 2006]. Based on this understanding, we examined the mitochondria-associated cytochrome *c* release, caspase 3 activation, and AIF translocation to the nucleus. DMOG effectively inhibited cytochrome *c* release into the cytosol. This result is consistent with other studies showing the effect of DMOG on neuronal cell death induced by trophic factor deprivation [Lomb et al., 2007]. There is evidence in a neuronal cell line that HIF-1α stabilization by removal of the prolyl hydroxylase

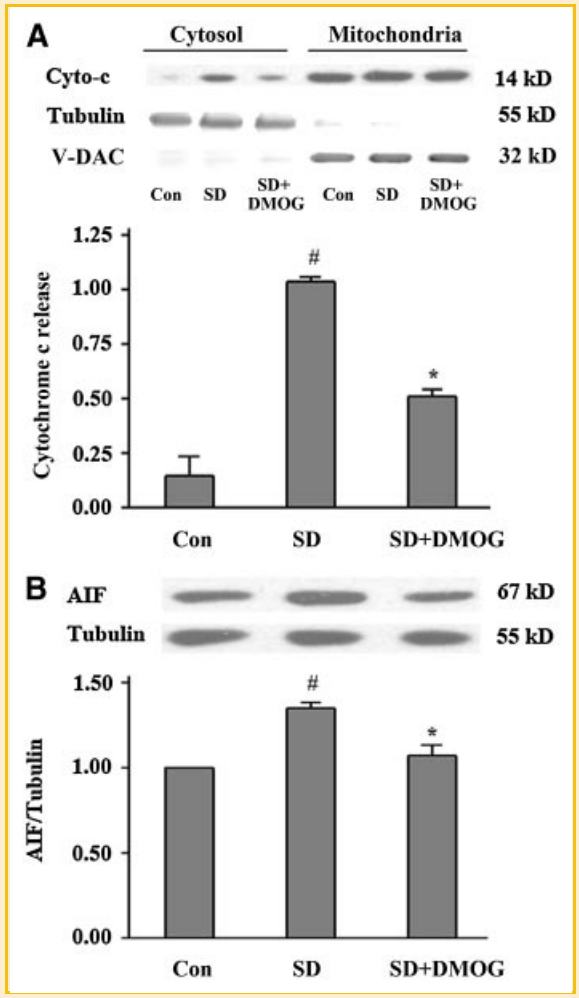

Fig. 6. Effect of DMOG on mitochondrial apoptotic pathway. Cells were cultured in the absence or presence of 1,000 μM DMOG for 12 h, and then cytosol protein, mitochondrial protein or nuclear protein was isolated and analyzed by using Western blot, respectively. A: Decrease of cytochrome c release into cytosol by DMOG treatment. Tubulin and voltage-dependent anion channel (V-DAC) were used as cytosol marker and mitochondrial marker, respectively. B: Reduction of AIF translocation into nucleus by DMOG treatment. $^{\#}P < 0.01$ for SD versus control group; $^{*}P < 0.01$ for SD + DMOG versus SD group. N = 3.

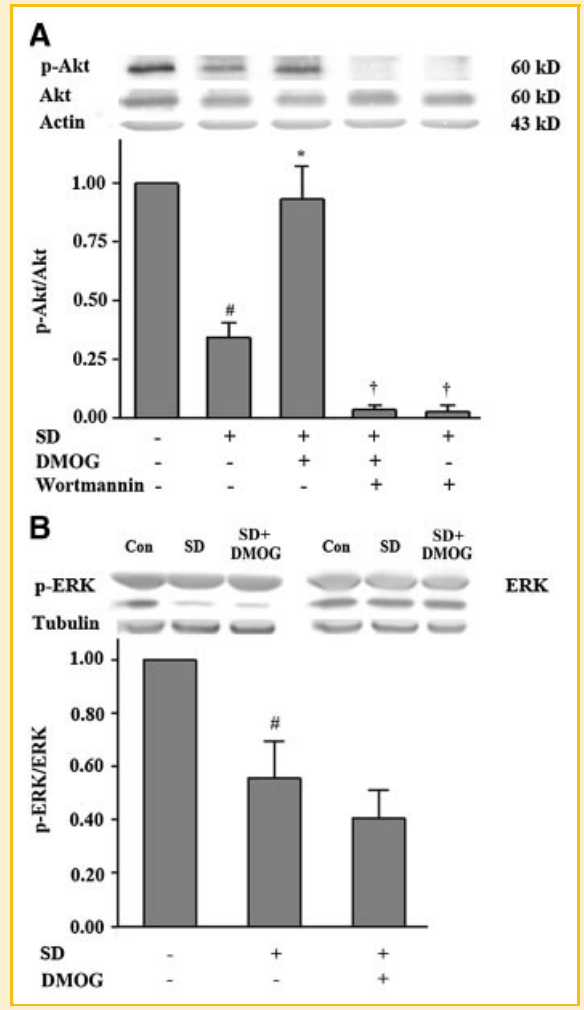

Fig. 7. Effect of DMOG on Akt and ERK activation in MSCs. MSCs were exposed to SD for 21 h before exposed to DMOG (1,000 μM) for 3 h. Wortmannin (100 nM) was added to MSCs 1 h before DMOG application. A: Ratio of phosphorylated Akt to total Akt affected by different treatments. B: Ratio of phosphorylated ERK to total ERK affected by SD and SD plus DMOG. $^{\#}P < 0.001$ for SD versus control group; $^{*}P < 0.01$ for SD + DMOG versus SD group; $^{\dagger}P < 0.001$ for SD + DMOG + wortmannin or SD + wortmannin versus SD + DMOG group. N = 3.

target sites reduces apoptosis without prevention of cytochrome *c* released from mitochondria [Xie et al., 2005]. This suggests that there may be multiple mechanisms of protection by DMOG including prolyl hydroxylase-dependent up-regulation HIF-1α.

It is well known that the PI3K/Akt pathway is very important in regulation of cell survival. Increased Akt activity in MSCs protects against SD- and hypoxia-induced apoptosis [Mangi et al., 2003]. Hypoxia was reported to activate Akt phosphorylation and show a protective effect on PC12 cells [Alvarez-Tejado et al., 2001; Beitner-Johnson et al., 2001]. Prolyl hydroxylase inhibitors or hypoxia mimicking agents were reported to decrease the apoptosis of PC12 cells in vitro [Alvarez-Tejado et al., 2001] and myocardial apoptosis in vivo [Kerendi et al., 2006] by activation of the PI3K/Akt pathway. Consistent with these studies, we found that DMOG significantly increased Akt phosphorylation. Wortmannin, a specific inhibitor of PI3K, inhibited Akt activation and abrogated the protective effect of DMOG, indicating that the PI3K/Akt pathway played an important role in the protective effect of DMOG. However, the exact mechanism by which DMOG induces PI3K/Akt signaling is unknown and further study is needed.

In conclusion, we demonstrated that the prolyl hydroxylase inhibitor DMOG inhibited apoptosis induced by serum deprivation in MSCs concurrent with HIF-1α stabilization, mitochondrial protection, and activation of the PI3K/Akt pathway. This study provides a new approach for enhancing MSCs viability in vitro and further investigations will evaluate whether prolyl hydroxylase

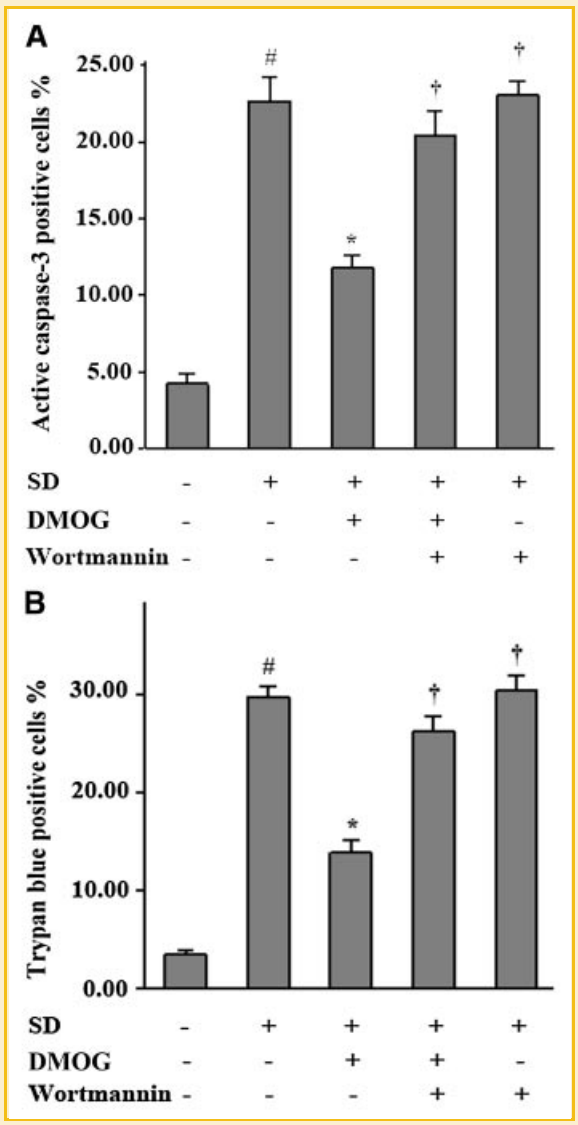

Fig. 8. Role of PI3K/Akt pathway in the protective effect of DMOG. SD-induced caspase-3 activation (A) and MSCs death (B) were decreased by DMOG (1,000 μM); the DMOG protective effect was antagonized by wortmannin (100 nM) applied 1 h before and during SD. $^{\#}P < 0.001$ for SD versus control group; $^{*}P < 0.001$ for SD + DMOG versus SD group; $^{\dagger}P < 0.001$ for SD + DMOG + wortmannin or SD + wortmannin versus SD + DMOG group. N = 3.

inhibitors may be applied to protect MSCs after transplantation into the ischemic heart.

REFERENCES

Alvarez-Tejado M, Naranjo-Suarez S, Jimenez C, Carrera AC, Landazuri MO, del Peso L. 2001. Hypoxia induces the activation of the phosphatidylinositol 3-kinase/Akt cell survival pathway in PC12 cells: Protective role in apoptosis. J Biol Chem 276:22368–22374.

Beitner-Johnson D, Rust RT, Hsieh TC, Millhorn DE. 2001. Hypoxia activates Akt and induces phosphorylation of GSK-3 in PC12 cells. Cell Signal 13:23–27.

Broker LE, Kruyt FA, Giaccone G. 2005. Cell death independent of caspases: A review. Clin Cancer Res 11:3155–3162.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. 2001. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98:2396–2402.

Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E. 1998. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 394:485–490.

Gerhart DZ, LeVasseur RJ, Broderius MA, Drewes LR. 1989. Glucose transporter localization in brain using light and electron immunocytochemistry. J Neurosci Res 22:464–472.

Green DR, Reed JC. 1998. Mitochondria and apoptosis. Science 281:1309–1312.

Hu X, Wang J, Chen J, Luo R, He A, Xie X, Li J. 2007. Optimal temporal delivery of bone marrow mesenchymal stem cells in rats with myocardial infarction. Eur J Cardiothorac Surg 31:438–443.

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

Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. 1998. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 12:149–162.

Kerendi F, Kirshbom PM, Halkos ME, Wang NP, Kin H, Jiang R, Zhao ZQ, Kanter KR, Guyton RA, Vinten-Johansen J. 2006. Thoracic Surgery Directors Association Award. Cobalt chloride pretreatment attenuates myocardial apoptosis after hypothermic circulatory arrest. Ann Thorac Surg 81:2055–2062; discussion 2062.

Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. 2002. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 16:1466–1471.

Lin Z, Weinberg JM, Malhotra R, Merritt SE, Holzman LB, Brosius FC III. 2000. GLUT-1 reduces hypoxia-induced apoptosis and JNK pathway activation. Am J Physiol Endocrinol Metab 278:E958–E966.

Lomb DJ, Straub JA, Freeman RS. 2007. Prolyl hydroxylase inhibitors delay neuronal cell death caused by trophic factor deprivation. J Neurochem 103:1897–1906.

Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. 2003. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nat Med 9:1195–1201.

Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. 2006. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 12:459–465.

Natarajan R, Salloum FN, Fisher BJ, Kukreja RC, Fowler AA III. 2006. Hypoxia inducible factor-1 activation by prolyl 4-hydroxylase-2 gene silencing attenuates myocardial ischemia reperfusion injury. Circ Res 98: 133–140.

Natarajan R, Salloum FN, Fisher BJ, Ownby ED, Kukreja RC, Fowler AA III. 2007. Activation of hypoxia-inducible factor-1 via prolyl-4 hydoxylase-2 gene silencing attenuates acute inflammatory responses in postischemic myocardium. Am J Physiol Heart Circ Physiol 293:H1571–H1580.

Ockaili R, Natarajan R, Salloum F, Fisher BJ, Jones D, Fowler AA III, Kukreja RC. 2005. HIF-1 activation attenuates postischemic myocardial injury: Role for heme oxygenase-1 in modulating microvascular chemokine generation. Am J Physiol Heart Circ Physiol 289:H542–H548.

Philipp S, Jurgensen JS, Fielitz J, Bernhardt WM, Weidemann A, Schiche A, Pilz B, Dietz R, Regitz-Zagrosek V, Eckardt KU, Willenbrock R. 2006.

Stabilization of hypoxia inducible factor rather than modulation of collagen metabolism improves cardiac function after acute myocardial infarction in rats. Eur J Heart Fail 8:347–354.

Piret JP, Lecocq C, Toffoli S, Ninane N, Raes M, Michiels C. 2004. Hypoxia and CoCl2 protect HepG2 cells against serum deprivation- and t-BHP-induced apoptosis: A possible anti-apoptotic role for HIF-1. Exp Cell Res 295:340–349.

Rastogi S, Banerjee S, Chellappan S, Simon GR. 2007. Glut-1 antibodies induce growth arrest and apoptosis in human cancer cell lines. Cancer Lett 257:244–251.

Salceda S, Caro J. 1997. Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 272:22642–22647.

Sasabe E, Tatemoto Y, Li D, Yamamoto T, Osaki T. 2005. Mechanism of HIF-1alpha-dependent suppression of hypoxia-induced apoptosis in squamous cell carcinoma cells. Cancer Sci 96:394–402.

Schuleri KH, Boyle AJ, Hare JM. 2007. Mesenchymal stem cells for cardiac regenerative therapy. Handb Exp Pharmacol 180:195–218.

Sharma R, Raghubir R. 2007. Stem cell therapy: A hope for dying hearts. Stem Cells Dev 16:517–536.

Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL. 2001. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res 61:6669–6673.

Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. 2005. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. J Am Coll Cardiol 46:1339–1350.

Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. 2002. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105:93–98.

Wang GL, Jiang BH, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92:5510–5514.

Wang JA, Fan YQ, Li CL, He H, Sun Y, Lv BJ. 2005. Human bone marrow-derived mesenchymal stem cells transplanted into damaged rabbit heart to improve heart function. J Zhejiang Univ Sci B 6:242–248.

Xie L, Johnson RS, Freeman RS. 2005. Inhibition of NGF deprivation-induced death by low oxygen involves suppression of BIMEL and activation of HIF-1. J Cell Biol 168:911–920.

Zhu W, Chen J, Cong X, Hu S, Chen X. 2006. Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Stem Cells 24: 416–425.