Advanced Age Impairs Cardioprotective Function of Mesenchymal Stem Cell Transplantation from Patients to Myocardially Infarcted Rats

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Key Words

Mesenchymal stem cells · Coronary artery disease · Advanced age · Myocardial infarction

Abstract

Objectives: Mesenchymal stem cells (MSCs) have limited clinical therapeutic effects in older myocardial infarction (MI) patients. Thus, whether younger MSCs might confer greater protection is worth investigating. **Methods:** Human MSCs (hMSCs) were isolated before coronary artery bypass graft surgery and growth characteristics of hMSCs at passage 3 were observed. Vascular endothelial growth factor (VEGF) and Bcl-2 mRNA and protein expression from hMSCs were measured. In vivo, 45 adult male rats with MI were randomized to receive one of three treatments: old hMSCs, young hMSCs or culture medium (control) transplanted into infarcted myocardium. Echocardiography, TUNEL, immunohistochemistry and Western blot were used to assess results. **Results:** hMSC proliferation in the old group was significantly lower than the young group. VEGF decreased 35% and Bcl-2 decreased more than 60% at the mRNA level; VEGF and Bcl-2 protein were decreased in the old versus the young group. hMSC transplantation may improve cardiac function, but MSC source may affect therapeutic efficacy. Similar data were obtained from TUNEL, immunohistochemistry and

Western blot. **Conclusion:** Transplantation of hMSCs improves heart function, but proliferative ability and myocardial protection decrease with older MSCs, likely due to differences between VEGF and Bcl-2 expression and reduced anti-apoptosis.

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Instruction

Coronary heart disease (CHD) is a leading cause of cardiovascular mortality worldwide, with more than 4.5 million deaths in the developing world [1]. For instance, approximately 13–22% of cardiovascular patients die annually because of CHD in China, making this the second leading cause of cardiovascular death in this country [2]. In the developed world, the mortality of CHD is still unacceptably high. In the USA, nearly 1 million Americans suffer from CHD, almost 25% of whom will die from this condition [3]. Many therapeutic methods have been used to treat CHD, including drug therapy, interventional therapy and coronary artery bypass grafting. Also, mesenchymal stem cell (MSCs) transplantation is reported to be an effective

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method for protecting the damaged myocardium and improving cardiac function [4].

MSCs expressing CD105, CD90 and CD29 (and rarely CD45, CD34 and HLA-DR) can be isolated from bone marrow, adipose tissue and umbilical cord blood [5]. Because MSCs are multipotent, they may differentiate into various cell lineages, such as osteoblasts, adipocytes, chondrocytes, myoblasts and connective tissue, among other tissues [6]. Heart function is improved when MSC transplantation is used to promote angiogenesis, myogenesis, inhibition of left ventricular (LV) remodeling, and certain paracrine effects [7, 8]. Of these mechanisms for improving cardiac function, paracrine effects hold great significance [8]. Paracrine-mediated secretion of various cytokines such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor [9] from MSCs may contribute to cardiac repair by minimizing ischemic reperfusion injury via cytoprotection, neovascularization and inhibition of apoptosis [10, 11]. Although both animal and clinical studies have confirmed that MSC transplantation may benefit CHD patients, the efficacy of this therapeutic intervention is uncertain. Most CHD patients from whom MSCs can be obtained are older patients who have fewer MSCs and less MSC differentiation/regeneration and mobilization capacities [12, 13]. For example, the biological characteristics of MSCs were previously described in a rat model and in patients with CHD. In this report, MSCs had impaired therapeutic transplant efficacy for myocardial infarction (MI) when obtained from old mice, and this was related to pigment epithelium-derived factor (PEDF). Previous studies have focused on the biological characteristics of MSCs in a rat model [14] and in patients with CHD [15]. Thus, aging may influence the efficacy of MSC transplantation [6, 16], but at present the importance of MSC donor age on therapeutic efficacy is uncertain [17]. Therefore, in this work, we focused on the differences in biological characteristics of MSCs obtained from young and old CHD patients to determine whether older MSCs can be optimized sufficiently to provide post-MI therapy in older patients.

Methods

Isolation and Culture of Human MSCs

Bone marrow collection for research purposes was approved by the Ethics Committee of the Shenyang Northern Hospital, Shenyang City, China. MSCs were isolated and cultured as previously reported [18]. Briefly, at the time of coronary artery bypass

Table 1. Inclusion and exclusion criteria

Inclusion criterion	Male Age >60 years or <45 years. Acute MI. Multiple vessels involved.
Exclusion criterion	Infectious, systemic immunologic diseases, malignancy, hepatic and nephritic dysfunction, diabetes mellitus.

Table 2. Patient demographics

Categories	Old (n = 10)	Young (n = 10)	p value
Age, years	69.90 (5.72)	42.12 (1.81)	0.000
BMI, kg/m ²	25.32 (2.76)	27.44 (1.92)	0.084
LVEF, %	43.93 (5.57)	43.88 (4.16)	0.982
Smoker	6	4	0.656
Hypertension	5	3	0.650
Hyperlipidemia	2	3	1.000
Target vessel			
LM	4	3	1.000
LAD	10	10	1.000
LCX	5	3	0.650
RCA	4	7	0.370
OM	3	2	1.000
Diagonal	3	1	0.582

Values are presented as n, or mean with SD in parentheses. LVEF = Left ventricular ejection fraction; LM = left main coronary artery; LAD = left anterior descending coronary artery; RCA = right coronary artery; OM = obtuse marginal.

graft surgery (CABG), bone marrow was aspirated from the sternum and then mixed immediately with heparin. Patients were divided into young (<45 years old) and old (>60 years old) groups (n = 10). Table 1 depicts the inclusion and exclusion criteria. Patient demographic and clinical data are summarized in table 2. All patients provided informed consent and the study was conducted according to the ethical guidelines of the Declaration of Helsinki (1975).

The marrow samples were washed twice with PBS after centrifugation at 900 g for 10 min to discard the fat layer. The residual cells were added into an equal volume of 1.073 g/ml Percoll solution in a 50-ml conical tube and centrifuged at 1,100 g/min for 30 min. Mononuclear cells were collected from the upper layer and interface, diluted with two volumes of PBS, and collected by centrifugation at 1,100 g/min. The cells were seeded in low-glucose Dulbecco's Modified Eagle Medium (Gibco, Grand Island, N.Y., USA) supplemented with 10% fetal bovine serum (Gibco, Mulgrave, Victoria, Australia), 100 U/ml penicillin and 100 mg/ml streptomycin. The nucleated cells were plated into 100-mm plastic culture dishes (Beckton Dickinson, San Jose, Calif., USA) and in-

cubated at 37°C in 5% CO₂ and 95% humidity. The culture medium was replaced by new medium every 2–3 days. Adherent cells were cultured until they reached 80% confluence. The cells were harvested by 0.25% trypsin and passaged. Passage 3 cells were used for future experiments.

Growth curves of human MSCs (hMSCs) from patients were depicted using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, hMSCs were isolated from both patient groups and cultured in 96-well plastic culture dishes at a density of 2.5×10^3 cells/well. After 1, 2, 3, 4, 5, 6, 7 and 8 days of culture, MTT (Sigma, St. Louis, Mo., USA) dissolved in PBS was added to each well at a final concentration of 5 mg/ml, and the samples were incubated at 37°C for 4 h. Water-insoluble dark blue formazan crystals formed during MTT cleavage in actively metabolizing cells and these crystals were dissolved in dimethyl sulfoxide (DMSO; Gibco/Invitrogen, Grand Island, N.Y., USA). Optical density was measured ($\lambda = 490$ nm) using a Bio-Rad 680 microplate reader (Bio-Rad, Hercules, Calif., USA).

Phenotype Analysis of hMSCs

hMSCs were trypsinized, incubated and stained with mouse anti-human antibody for 30 min at room temperature. The cells were then rinsed twice with PBS and resuspended in 500 μ l of PBS after centrifugation at 900 g. The cells were analyzed using a flow cytometer (Beckton Dickinson). The antibodies used in this experiment were: CD34-PE, CD45-PE, CD29-PE and CD44-PE (Beckton Dickinson). Approximately 5×10^5 cells per 100 μ l were labeled with primary mouse antibodies against human CD29, CD34, CD44 and CD45. Cells were incubated at 4°C for 30 min and washed. Mouse IgG1-PE (Beckton Dickinson) was used as an isotype control [19].

Expression of mRNA and Protein in vitro

RT-PCR analysis was performed as previously described. Briefly, hMSCs were incubated for 48 h prior to experimentation. Total RNA isolated from hMSCs was used for cDNA synthesis with SuperScript III RNase H-reverse transcriptase (Invitrogen). The cDNA template was amplified with Taq DNA polymerase (Invitrogen). The primers used are shown in table 3.

VEGF concentrations released by hMSCs were measured by ELISA. Briefly, 5×10^4 hMSCs were plated in 24-well plates in 1 ml of medium. The supernatant was collected after incubation for 48 h. Production of VEGF in supernatant was determined with ELISA using a human VEGF ELISA kit (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's instructions.

Bcl-2 protein expression was measured by Western blot. Briefly, hMSCs were treated with cell lysis buffer (Promega, Madison, Wisc., USA). Protein concentration was measured using a BCA protein assay (Pierce, Rockford, Ill., USA). Total proteins (20 μ g) were separated using 12.5% sodium SDS-PAGE and transferred to a 0.2-mm nitrocellulose membrane. The membrane was blocked in PBS buffer containing 0.2% Tween 20 and 5% non-fat milk for 1 h. The membrane was then incubated overnight at 4°C with rabbit Bcl-2 protein polyclonal antibody (1:2,000 dilution; Abcam, Cambridge, Mass., USA). Housekeeping protein β -actin was used as a loading control. Antibody binding was detected using horseradish peroxidase conjugated secondary antibody and visualized by an ECL kit (Amersham Biosciences, Piscataway, N.J., USA).

Table 3. RT-PCR primers used

Gene	Primer
VEGF(F)	5'-CTA CCT CCA CCA TGC CAA GT-3'
VEGF(R)	5'-GCA GTA GCT GCG CTG ATA GA-3'
Bcl-2(F)	5'-ATC GCC CTG TGG ATG ACT GAG-3'
Bcl-2(R)	5'-CAG GCC CCA GGA GAA ATC AAA CAG AGG-3'
β -Actin(F)	5'-TCT GGC ACC ACA CCT TCT ACA ATG-3'
β -Actin(R)	5'-AGC ACA GCC TGG ATA GCA ACG-3'

MI Formation and hMSC Transplantation

To prevent immunorejection, all animals received cyclosporine (10 mg/kg/day; Novartis, Basel, Switzerland) for 4 weeks post-transplantation. Male Sprague-Dawley rats (280–300 g) were divided into three groups (old, young and control groups, $n = 15$ per group). All animals received humane care and all animal protocols complied with the institution's guidelines.

Rats were anesthetized using pentobarbital (50 mg/kg, i.p.), and respiration was assisted with 100% O₂ via a ventilator. Through a left lateral thoracotomy, the proximal portion of the left anterior descending artery was ligated with a 6-0 Prolene suture (Ethicon, Somerville, N.J., USA). MSCs were dissociated and suspended in culture medium at a concentration of 2×10^6 cells in 50 μ l and were kept on ice until transplantation. Animals in the old ($n = 12$) and young ($n = 9$) groups received a subepicardial injection of hMSCs obtained from old and young patients, respectively. Cells were injected into the infarcted scar and adjacent myocardium. The control group ($n = 11$) received injections of culture medium into the same area. Intramuscular penicillin G benzathine (100,000 U/kg) was used to prevent infection.

Echocardiography

Cardiac function was evaluated by echocardiography prior to and at 1 and 4 weeks after MI, MSC or medium injection. Images were recorded using a 12-MHz high-frequency liner phased-array transducer (Philips SONOS 5500, Bothell, Wash., USA). LV end diastolic and systolic dimensions were derived from two-dimensionally targeted M-mode tracings obtained along the parasternal short-axis view of the left ventricle at the papillary muscle level. The ejection fraction (EF) and fractional shortening (FS) were calculated. EF was calculated on the basis of the Teichholz formula, while FS was calculated as follows: $(LVD_d - LVD_s)/LVD_d \times 100$, where LVD_d is LV diastolic dimension and LVD_s is LV systolic dimension. All measurements were performed and averaged over three consecutive cardiac cycles.

TUNEL Staining

The histochemical detection of apoptotic cells was performed as previously reported [20]. The tissue blocks were fixed in 4% paraformaldehyde and incubated with proteinase K. Fragments of DNA in the tissue sections were analyzed using a TUNEL detection kit (Promega). For each slide, color images of 10 separate fields were captured randomly and digitized. Cells with clear nuclear labeling were defined as TUNEL-positive cells. The apoptotic index was calculated as the number of TUNEL-positive cells/total number of myocytes.

Immunohistochemistry

Myocardial tissue was embedded in paraffin and cut into 5- μ m sections. Detection of Bcl-2 and Bax expression was performed as described previously [21]. Tissue sections were exposed overnight to rabbit Bcl-2 protein polyclonal antibody (1:2,000 dilution; Abcam) and rabbit Bax protein polyclonal antibody (1:2,000 dilution; Abcam) at 4°C, washed in PBS and incubated with biotinylated goat anti-rabbit IgG for 60 min at 37°C. After two washing steps, sections were exposed to a streptavidin-horseradish-peroxidase complex for 30 min at 37°C and visualized with 3,3'-diaminobenzidine, embedded in glycerol gelatin. Images were captured digitally and analyzed using IPP version 6.0. Cytoplasmic staining was considered positive, and scored as: absent (-); weakly positive (+), moderately positive (++) or strongly positive (+++).

Measurement of Capillary Density

The number of capillary vessels was counted as described previously [22] using paraffin-embedded sections stained with anti-factor VIII antibody (U0034; Dako A/S, Glostrup, Denmark). The number of capillaries in five randomly selected high-power fields in the LV-free wall was averaged and expressed as the number of capillary vessels per high-power field (0.2 mm²).

Western Blot Analysis

Myocardial tissue samples were homogenized in RIPA buffer, and then total protein was collected. Bcl-2 and VEGF protein expression were measured with Western blot as described above.

Statistical Analysis

Data were analyzed using SPSS version 12.0 for Windows (SPSS, Chicago, Ill., USA). All variables were presented as means and standard deviations. The t test was used to compare treatments in the in vitro experiments. The results of hMSC transplantation into rat models were tested using one-way analysis of variance. Tukey's method was used for post hoc analysis. $p < 0.05$ was considered statistically significant.

Results

Growth Characteristics of hMSCs

hMSCs were tightly attached to the culture dishes after 24 h. They appeared as spindle-shaped cells after 3–4 days of culture of the primary passage, after which they proliferated rapidly. After 11–13 days of primary culture the hMSCs reached nearly 80% confluences. The hMSCs in passage 3 from the old group had a more flattened appearance and were larger in size than those from the young group (fig. 1a).

Proliferative Abilities of MSCs from Each Group

Growth curves were characterized by an initial lag phase (during the first 2 days) followed by a log phase (from day 3 to 7) during which cells divided at exponential rates. This was followed by a plateau phase after day 8.

The proliferative potential of hMSCs obtained from old patients was significantly impaired relative to that observed in cells from young patients ($p < 0.05$). These differences were apparent at each time point after day 3 (fig. 1b).

Phenotype of hMSCs

hMSCs from both the old and young groups contained a unique phenotypic population which was identified by flow cytometric analysis of expressed surface antigens. All hMSCs were uniformly positive (fig. 1c, d).

Expression of VEGF and Bcl-2 at mRNA and Protein in vitro

After hMSCs were cultured, VEGF and Bcl-2 gene expression in old and young hMSCs were measured. Compared with young hMSCs, VEGF mRNA expression in old hMSCs decreased, on average, nearly 35% and Bcl-2 mRNA expression was reduced by more than 60% (fig. 2a).

To confirm the gene expression results, VEGF and Bcl-2 protein was evaluated in vitro, and ELISA and Western blot was performed on the supernatant and cell samples at the same time points as mentioned above. Data revealed that VEGF protein expression was significantly lower in the old cells compared to the young group, and expression of Bcl-2 protein was similarly reduced in older cells (fig. 2b, c).

Evaluation of Myocardial Function

Transplantation of hMSCs into the infarcted border zone of rats with experimentally induced MI improved EF significantly, but FS improved insignificantly. There were no remarkable differences in EF and FS among the three groups pre-infarction or at 1 week post-infarction.

In the old and young groups, EF increased significantly relative to the control group after MSC transplantation. However, EF was significantly lower in the old group compared to the young group. However, FS increased significantly in the young group compared with the control and old groups, and there were no significant differences in FS between the old and control groups (fig. 2d, e).

Myocardial Apoptosis after hMSC Transplantation

The degree of apoptosis 4 weeks after hMSC transplantation was significantly lower in the old and young groups compared to the control group. However, myocardial apoptosis was significantly higher in the old cell group compared to the young group (fig. 3a).

Fig. 1. Characteristics of cultured hMSCs. **a** In passage 3, the hMSCs from the old group were flatter in appearance and larger than those in the young group. **b** An MTT assay indicated that the proliferative potential of hMSCs from the old group decreased significantly compared with the young group at each time point at

both the log and plateau phase of cell growth after the first 3 days. **c** Quantification revealed no differences in the percentages of old and young hMSCs. **d** The phenotype of hMSCs from the old and young groups was negative for CD34 and CD45, and positive for CD29 and CD44. * $p < 0.05$ vs. young group.

Immunohistochemistry in Infarcted Myocardium

Bcl-2 was upregulated and Bax was downregulated by immunohistochemistry in both the old and young groups compared to the control group. Furthermore, compared with the young group, Bcl-2 (fig. 3b) was downregulated and Bax (fig. 3c) was upregulated in the old group.

Capillary Densities

Blood vessel density was detected with factor VIII (fig. 4a–d). The capillary density stained with a brown color in the LV free wall was significantly higher in the

old and young groups compared to the control group. Furthermore, young cell groups had significantly greater capillary density than the old group.

Protein Expression in Infarcted Myocardium

Protein expressions of VEGF and Bcl-2 after hMSC transplantation in the old and young groups were both significantly higher than in the control group, as evidenced by Western blot. However, VEGF and Bcl-2 expression in the old group was significantly lower than in the young group (fig. 4e, f).

Fig. 2. Cytokine expression in hMSCs and evaluation of myocardial function after hMSC transplantation. **a** VEGF and Bcl-2 expression estimated by RT-PCR indicates downregulation in the old group compared with the young group. **b** Protein expression of VEGF validated by ELISA indicated downregulation in the old group compared with the young group. **c** Western blot validation of Bcl-2 protein revealed downregulation in the old group compared with the young group. EF (**d**) and FS (**e**) in the three groups

were not significantly different before and 1 week after operation. EF in the old and young groups was increased significantly after MSC transplantation, while FS increased only in the young group. Furthermore, EF and FS decreased significantly in the old group compared with the young group 4 weeks post-operation. ⁺ $p < 0.05$ compared with the young group; * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with the old group.

Discussion

Many factors may impair the effect of MSC therapy, such as diabetes [23], myocarditis [20] and antineoplastic drug treatment [21]. Thus, advanced age is only one risk factor [24]. Therefore, we used rigid inclusion and exclusion criteria to rule out the influence of other factors except advanced age.

In the present study, we used hMSCs from the third passage of culture for in vitro and in vivo experiments. hMSCs from passages 1 to 5 had significantly greater proliferative potential than those from later passages [15]. Initially, we discovered that proliferation of hMSCs derived from old patients were significantly different than those derived from young patients, offering prelim-

inary evidence that senescence reduces the proliferation of hMSCs in vitro. Our data were consistent with previous reports that an important degenerative factor in senescence is the accumulation of cell damage [25]. However, the differentiation potential of MSCs did not change with age [26]. Experiments in vitro also indicated that VEGF and Bcl-2 mRNA level and protein expression decreased in the old group compared with the young group. Both of these cytokines may play an important role in hMSC proliferation [27, 28]. Also, transplantation of hMSCs from old patients and young patients into a rat MI model improved cardiac contractility and attenuated apoptosis of cardiomyocytes. However, these effects diminished in hMSCs derived from old patients. Furthermore, the capillary density in the LV free wall was mea-

Fig. 3. Myocardial apoptosis at 4 weeks after hMSC transplantation. **a** Myocardial apoptosis was detected by TUNEL staining. TUNEL-positive cells (arrow) decreased significantly in the old and young groups compared with the control group. Furthermore, TUNEL-positive cells increased significantly in the old group in contrast to the young group. Immunohistochemical detection of

apoptosis-related protein including Bcl-2 (**b**) and Bax (**c**) expression in infarcted myocardium from the control (+), old (++) and young (+++) groups, and control (+++), old (++) and young (+) groups, respectively. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. CAD group.

sured and the young group had the greatest capillary density, and the capillary density in the old group was also higher than in the control group. These findings may be explained by decreased expression of VEGF and Bcl-2, which may weaken the effect of angiogenesis and anti-apoptosis.

In our study, we measured VEGF and Bcl-2 expression in hMSCs. VEGF is a cell-signaling protein produced that stimulates vasculogenesis and angiogenesis [29], and this is mediated partially through the anti-apoptotic effects of the PI3K/AKT-dependent pathway [30]. Bcl-2 (of the Bcl-2 family) and VEGF are overex-

pressed in B cell lymphoma [31]. Bcl-2 family proteins are critical regulators of pathways involved in anti-apoptosis and cell death inhibition [32]. In the present study, mRNA and protein expression of VEGF and Bcl-2 were significantly lower in the old group compared to the young group, suggesting that VEGF and Bcl-2 expression in patients with CAD might be impaired by senescence, which may decrease the anti-apoptotic function of hMSCs.

Previous reports have emerged that MSCs are immune-tolerant and express low levels of major histocompatibility complex and co-stimulant molecules [33]. This

Fig. 4. Angiogenesis and protein expression in infarcted myocardium. **a** Representative sections from old (**a**), young (**b**) and control (**c**) hearts are stained with anti-factor VIII antibody (original magnification $\times 200$) and capillary densities are shown. Capillary density in the old group decreased compared with the young group, but increased compared with the control group (**d**). West-

ern blot revealed that hMSC transplantation from the old and young significantly elevated VEGF (**e**) and Bcl-2 (**f**) expression in the myocardium compared with the control group. Moreover, both VEGF and Bcl-2 protein decreased significantly in the old group compared with the young group. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. young group.

means that MSCs are generally safe and effective when used for allotransplantation [12]. However, we used heterotransplantation which made post-transplant rejection a concern [34]. Therefore, cyclosporine was used to suppress the immune response in our study. However, the cardioprotective function of reduced infarct size from cyclosporine is controversial, especially in swine [35, 36]. Still, all treated animals were administered cyclosporine at the same time and dose in order to prevent any potential influence from it.

In vivo experiments, we found that hMSC transplantation improved myocardial function but that the improvement was significantly more marked with cells derived from young patients than with those derived from old patients. The myocardial protective function of hMSC transplantation may be diminished by aging and this is a finding that is in accordance with previous studies [37]. In our study, myocardial tissue from the infarcted zone and border was studied using TUNEL, immunohistochemistry and Western blot. We found that apoptosis of myocardial cells increased dramatically, while the capillary density decreased significantly in the old group compared with the young group. We also showed that expression of VEGF and Bcl-2 decreased markedly in the old group compared with the young group. VEGF expression in vitro at the mRNA and protein level was reduced in the old group compared with the young group. However, in vivo, protein expression of VEGF after hMSC transplantation decreased dramatically in the old group compared with the young group. This might be due to elevated VEGF secretion in response to hypoxia [38]. Thus, dramatically different levels of VEGF were observed in cells cultured under normoxic conditions versus infarcted myocardium under hypoxic conditions. In contrast, Bcl-2 expression was similar under both normoxic and hypoxic conditions. Thus, inhibition of Bcl-2 expression may result in increased apoptosis of donor cells, whereas decreased secretion of VEGF may weaken the angiogenesis function of cell transplantation and induce apoptosis of myocardial cells through the PI3K/Akt-dependent pathway. A combination these events may impair the cardioprotective function of hMSCs used for transplantation. In addition, previous work suggests that genetic modification with the anti-apoptotic *Bcl-2* gene increased VEGF expression in response to hypoxia [39], which means that high Bcl-2 expression partially contributes to increased VEGF secretion in hypoxia. Our data agree with this study.

To enhance the cardioprotective effect of old MSC transplantation, some techniques may be useful. For ex-

ample, modification of hMSCs from old patients using VEGF and/or Bcl-2 gene expression may improve VEGF and Bcl-2 protein expression and enhance the ability of hMSCs to protect the ischemic myocardium. Previously, we confirmed that PEDF can impair the therapeutic efficacy of MSCs in the attenuation of MI injury [37], and this impairment can be suppressed by VEGF [40]. Thus, optimization of VEGF and PEDF concentrations may be a genetically modifiable target for increasing the therapeutic function of MSCs from old patients. Also, because of poor stem cell transplant viability due to tissue ischemia and local inflammation, modulation of the myocardial microenvironment may offer an alternative method. A recent study suggests that rosuvastatin enhanced survival and viability of MSCs after transplantation, protecting against ischemia-reperfusion cardiac injury, suppressing inflammation and reducing apoptosis of cardiomyocytes [41]. Thus, combined drug therapy with statins may be beneficial for MSC transplantation.

Limitations

Previous studies indicate that aging impairs the quantity, quality and mobilization capacity of MSCs [6]. Thus, hMSCs were obtained from patients older than 60 years of age (the old group) and younger than 40 years of age (the young group) according to young and old WHO criteria. However, retrospective studies indicated that only 1 or 2 patients younger than 40 years of age received CABG in our heart center (of 500–600 total CABG cases per year). Future studies will require selection of even more patients younger than 45 years of age to capture more ‘young’ patients. Thus, the relatively small sample size used here may influence the data. Our findings must to be substantiated in larger populations of patients.

Conclusions

The present study indicates that hMSCs from young and old patients have proliferative properties, and that transplantation of hMSCs from all patients improved heart function in rats with MI. The ability to proliferate and ameliorate myocardial function decreased significantly in MSCs obtained from old patients compared with those obtained from young patients. These differences may be attributed to differences in VEGF and Bcl-2 protein expression, which determine the extent of anti-apoptosis.

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Conflict of Interest

The authors declare that they have no competing interests.

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