Impaired cardioprotective function of transplantation of mesenchymal stem cells from patients with diabetes mellitus to rats with experimentally induced myocardial infarction

Yu Liu1,2, Zhi Li1, Tao Liu2, Xiaodong Xue2, Hui Jiang2, Jianhua Huang3 and Huishan Wang2*

Abstract

Background: Diabetes mellitus (DM) exacerbates coronary artery disease (CAD) morbidity and mortality. Mesenchymal stem cells (MSCs) play an important therapeutic role in myocardial ischemic injury. However, little is known about changes in the cardioprotective characteristics of MSCs from patients with DM.

Methods: Sternal bone marrow aspirates were taken at the time of coronary artery bypass graft surgery. The morphology and growth characteristics of hMSCs were observed in passage 3. Differences in gene expression profiling were measured by Affymetrix GeneChipHuman Genome U133 Plus 2.0 Arrays. Forty two adult male rats with experimentally CAD were randomized into three groups. MSCs from patients with CAD+DM or CAD were injected into the infarcted myocardium. Control animals received culture medium. Echocardiography, TUNEL, immunohistochemistry and Western-blot analysis were performed 4 weeks after transplantation.

Results: Growth curves showed that proliferation of hMSCs in the CAD+DM group was significantly lower than in the CAD group. Nine transcripts of genes related to apoptosis containing Bcl-2 were found to differentiate the two groups. Transplantation of hMSCs in the infarcted border zone improved cardiac function, but DM partly impaired this effect. Similar results were observed from TUNEL, immunohistochemistry and Western-blot analysis.

Conclusions: hMSCs from patients with CAD+DM and CAD alone both have proliferative properties. Transplantation of hMSCs ameliorate heart function, but proliferative ability and myocardial protection decrease significantly in MSCs obtained from patients with CAD+DM compared with cultures from patients with CAD alone, possibly as a result of differences in Bcl-2 protein expression and reduced anti-apoptosis.

Keywords: Mesenchymal stem cells, Coronary artery disease, Diabetes mellitus, Myocardial infarction, Bcl-2

Background

Myocardial infarction (MI) and diabetes mellitus (DM) are serious diseases with high morbidity and mortality. It was estimated in 2010 that there were almost 1,000,000 patients with MI in the USA, with a 3-year mortality of nearly 25% [1]. Diabetes mellitus has been widely recognized as a major risk factor for cardiovascular disease, and carries the same risk of mortality as MI itself [2]. Previous studies have demonstrated that the prognosis of the coronary artery disease (CAD) in patients with DM is significantly worse than in patients with CAD alone [3]. However, the mechanisms that underlie this observation are not fully understood.

Mesenchymal stem cells (MSCs) transplantation has been widely used as an effective method of protecting the damaged myocardium and improving cardiac function [4]. MSCs can be readily isolated from bone marrow, adipose tissue, and umbilical cord blood. Ex vivo experiments indicate that MSCs express CD105, CD73, CD90, CD29 and CD166, but lack expression of CD45, CD34, CD14, CD11b, CD79a, CD19 and HLA-DR [5]. It has also been shown that MSCs transplantation...
improves heart function through various mechanisms, including angiogenesis, myogenesis, and inhibition of left ventricular (LV) remodeling [6]. The paracrine effects of MSCs have also been highlighted as an important mechanism responsible for improving cardiac function [7].

MSCs have been shown to secrete various cytokines, including vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), etc [8]. These cytokines play vital roles in paracrine function and contribute to cardiac repair through mechanisms involving cytoprotection, neovascularization and inhibition of apoptosis, all of which minimize ischemic reperfusion injury [9,10]. The beneficial effects of MSCs transplantation are well documented in both animal and clinical studies [11-13]. However, it is possible that age and other risk factors, such as the presence of DM might reduce the potential for MSCs differentiation and proliferation [14,15]. Age-related changes in MSCs function have previously been reported [16-18], but to date little is known about the effects of DM on MSCs proliferation.

Previous studies have described the biological characteristics of MSCs in a rat model [19] and in patients with CAD [13]. The present study was undertaken to investigate the differences in the gene expression profile of MSCs obtained from human subjects with CAD and DM, and with CAD alone, and to determine if this had the potential to alter the cardioprotective effect of hMSCs transplantation against MI in patients with CAD + DM.

Methods
Isolation and culture of human MSCs
Bone marrow collection for research purpose was approved by the Ethics Committee of the Shenyang Northern Hospital, Shenyang City, China. All patients provided informed consent and the study was conducted according to the ethical guidelines of the Declaration of Helsinki (1975).

MSCs were isolated and cultured as previously reported [13]. Briefly, 3 to 5 mL of bone marrow was aspirated from the sternum of patients with CAD who were undergoing coronary artery bypass graft surgery (CABG). Patients were divided into CAD+DM and CAD groups (n = 10 per group). The study inclusion and exclusion criteria are shown in Table 1, and patient demographic and clinical data are summarized in Table 2. The diagnosis of DM was based on The American Diabetes Association (ADA) diagnostic criteria [20].

Bone marrow aspirates were placed in a 5 mL tube containing phosphate-buffered saline (PBS) and 1250U of heparin. The marrow samples were washed twice with PBS twice after centrifugation at 900 × g for 10 min to discard the fat layer. The residual cells were added into the equal volume of 1.073 g/mL Percoll solution in a 50 mL conical tube and centrifuged at 1100 × g/min for 30 min. The mononuclear cells were collected from the upper layer and interface, diluted with two volumes of PBS, and collected by centrifugation at 1100 × g/min. The nucleated cells were plated into 100 mm plastic culture dishes (Beckton Dickinson, San Jose, CA, USA) and incubated at 37°C in 5% CO₂ and 95% humidity. The culture medium was replaced by new medium every 3 days. On each occasion, floating cells or non-adherent hematopoietic cells were removed. After 12 to 15 days of primary culture, the adherent cells were nearly 80% confluent. The cells were dissociated using 0.25% trypsin, replated at a ratio of 1:4 in 100 mm plastic culture dishes and grown to near confluence to expand the cells through successive passages.

Growth curves of MSCs from patients were depicted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazlium bromide (MTT) assay. Briefly, cells from Passage 3 were cultured in 96-well plastic culture dishes at a density of 2.5 × 10³ 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. These were dissolved in dimethyl sulfoxide (DMSO) (Gibco/Invitrogen, NY, USA). Optical density was measured at a wave length of 490 nm using a Bio-Rad 680 microplatereader (Bio-Rad, Califronia, USA).

Phenotype analysis of hMSCs
The hMSCs from Passage 3 were trypsinized, incubated and stained with mouse anti-human antibody for 30 min

| Table 1 Inclusion and exclusion criteria |
|----------------------------------------|
| **Inclusion criterion** | **Male** |
| Age between 50 and 60 years old | Previous myocardial infarction with multiple vessels involved |
| Previous myocardial infarction with multiple vessels involved | Type II diabetes mellitus for over 10 years |
| Type II diabetes mellitus for over 10 years | Infectious, systemic immunologic diseases, malignancy, hepatic and nephritic dysfunction |

Liu et al. Cardiovascular Diabetology 2013, 12:40
http://www.cardiab.com/content/12/1/40
at room temperature. The cells were then rinsed twice with PBS and resuspended in 500 μL PBS after centrifugation at 900 × g. The cells were analyzed using a flow cytometer (Beckton Dickinson, San Jose, CA, USA). The antibodies used in this experiment were: CD34-PE, CD44-PE, CD29-PE and CD45-PE (Beckton Dickinson, San Jose, CA, USA). The experiments of gene expression profiling were authorized to be carried out by CapitalBio Corporation (Beijing, China).

Gene expression profiling and protein validation
The hMSCs from Passage 3 were used for transplantation. The hMSCs from different areas. Intramuscular penicillin G benzathine (100,000 U/kg) was used to prevent infection. The hMSCs from different patients were injected respectively. Immunosuppression was provided by daily intramuscular administration of cyclosporine (10 mg/kg/day; Novartis, Switzerland) for 4 weeks post transplantation.

Echocardiography
A blinded investigator performed transthoracic echocardiographic studies on the anesthetized rats. Left ventricle dimension and function were assessed immediately prior to myocardial infarction, and at 1 and 4 weeks after hMSCs transplantation. Images were recorded using a 12-MHz high frequency linear phased-array transducer (Philips SONOS 5500, Bothell, WA, USA). Left ventricular end diastolic and systolic dimensions were derived from two-dimensional echocardiographic images. The left ventricular internal dimension in diastole and systole were measured from parasternal long axis views. A sample volume was placed at the mid-cavity level in the left ventricular outflow tract. The reported data were derived from the averaged measurements from five cardiac cycles.

Table 2 Patient demographic data

| Target vessel, n | CAD+DM (n = 10) | DM (n = 10) | P-value |
|-----------------|----------------|-------------|---------|
| LM              | 3              | 2           | 0.158   |
| LAD             | 10             | 10          | 1.000   |
| LCX             | 5              | 6           | 0.247   |
| RCA             | 5              | 6           | 0.247   |
| OM              | 5              | 4           | 0.247   |
| Diagonal        | 4              | 3           | 0.572   |

Values are presented as n, or mean ± SD.
BMI body mass index; LVEF left ventricular ejection fraction; LM left main coronary artery; LAD left anterior descending coronary artery; RCA right coronary artery; OM obtuse marginal.

12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis 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 (1:2000 dilution; Abcam, Cambridge, MA, USA). Housekeeping protein β-actin was employed as loading control. Antibody binding was detected using horseradish peroxidase conjugated secondary antibody, and visualized by an ECL kit (Amersham Biosciences, Piscataway, NJ, USA).

Myocardial infarction formation and hMSCs transplantation
Male Sprague-Dawley (SD) rats weighing 280 to 300 g were divided into three groups (CAD+DM group; CAD group; control group, n = 14 per group). All animals received humane care, and all animal protocols complied with the institution’s guidelines.

Rats were anesthetized by intraperitoneal injection with pentobarital (50 mg/kg), intubated via an endotracheal cannula and mechanically ventilated. A left lateral thoracotomy was performed. The proximal portion of the left anterior descending artery was ligated with a 6-0 Prolene (Ethicon, Somerville, NJ, USA) suture. MSCs from Passage 3 were used for transplantation. The MSCs were dissociated from the culture dishes with 0.25% trypsin, neutralized with culture medium, washed with PBS and collected by centrifugation at 900 × g for 5 min at room temperature. The cells were then suspended in culture medium at a concentration of 2 × 10⁶ cells in 50 μL and were kept on ice until transplantation. Animals in the CAD+DM (n = 11) and CAD (n = 9) groups received a sub-epicardial injection of hMSCs obtained from patients with CAD+DM and CAD respectively. The 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 (100,000U/kg) was used to prevent infection. The hMSCs from different patients were injected respectively. Immunosuppression was provided by daily intramuscular administration of cyclosporine (10 mg/kg/day; Novartis, Switzerland) for 4 weeks post transplantation.
from two-dimensionally targeted M-mode tracings obtained along the para-sternal short-axis view of the left ventricle at the papillary muscle level. Ejection fraction (EF) and fractional shortening (FS) were calculated. All measurements were performed and averaged over three consecutive cardiac cycles.

**TUNEL (Terminal Deoxynucleotidyl Transferase-mediated dUTP-x Nick End Labeling) staining**
The histochemical detection of apoptotic cells was performed as previously reported [22]. 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 Corporation, Madison, WI, USA). 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 and Western-blot analysis**
Myocardial tissue was embedded in paraffin and cut into 5 μm sections. Detection of Bcl-2 expression was performed as described previously [23]. Tissue sections were exposed overnight to rabbit Bcl-2 protein polyclonal antibody (1:2000 dilution; Abcam, Cambridge, MA, USA) 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 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 (++++). Myocardial tissue samples were homogenized in RIPA buffer and the protein expression of Bcl-2 and VEGF were identified by Western blotting as described above.

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

**Results**

**Growth characteristics of hMSCs**
The hMSCs were tightly attached to the culture dishes after 24 h. They appeared as spindle shaped cells after 3 to 4 days’ culture of the primary passage, after which they proliferated rapidly. After 12 to 15 days of primary culture, the hMSCs reached nearly 80% confluences. The hMSCs in passage 3 from the CAD+DM group had a more flattened appearance were larger in size than those from the CAD group (Figure 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 3 to 7 day) during which cells divided at exponential rates. This was followed by a plateau phase after Day 8. The proliferative potential of hMSCs obtained from patients with CAD+DM was significantly impaired relative to that seen in cells from patients with CAD (P <0.05). These differences were apparent at each time point after Day 2 (Figure 1B).
Phenotype of human MSCs
Human MSCs contained a unique phenotypic population which was identified by flow cytometric analysis of expressed surface antigens. All hMSCs were uniformly positive for CD29, CD44 and negative for CD34 and CD45 (Figure 2).

Differences of genes expression profile of hMSCs between CAD+DM and CAD groups
Three transcripts were dramatically up-regulated (TNFRSF10B, TNFRSF21, NGF) and six transcripts that were down-regulated (EPR1, BIRC5, HELLS, BCL2, TNFRSF1B, CASP1) in the CAD+DM group relative to the CAD group (Table 3). In addition, expression of Bcl-2 mRNA was significantly lower in the CAD+DM group than in the CAD group (Figure 3A).

To confirm the gene expression profile results, Bcl-2 protein was evaluated in vitro and Western bolt analysis was performed on cell samples at the same time points. These results also showed that the expression of Bcl-2 protein was significantly lower in the CAD+DM group than in the CAD group (Figure 3B).

Evaluation of myocardial function
Transplantation of hMSCs into the infarcted border of zone of rats with experimentally induced CAD significantly improved left ventricular function. There were no remarkable differences in EF and FS between the three groups preinfarction or at 1 week postinfarction.

In the CAD+DM and CAD groups, both EF and FS increased significantly, relative to the control group, after MSC transplantation. However, EF and FS were significantly lower in the CAD+DM group than in the CAD group.

Contractile function was impaired in all three groups after infarction, but significantly improved after 4 weeks in animals transplanted with hMSCs (CAD+DM and CAD groups) relative to values in the control group. Improvements in contractile function were significantly less marked in the CAD+DM group than in the CAD group (Figure 4).

Myocardial apoptosis after hMSCs transplantation
The degree of apoptosis 4 week after hMSCs transplantation was significantly lower in the CAD+DM and CAD groups than in the control group. However, myocardial apoptosis was significantly higher in the CAD+DM group than in the CAD group (Figure 5).

Protein expression in infarcted myocardium
Protein expression of Bcl-2 following hMSC transplantation in the CAD+DM and CAD groups were both significantly higher than in the control group, as evidenced by immunohistochemical staining and Western-blot analysis. However, Bcl-2 levels in the CAD+DM were significantly lower than in the CAD group. Meanwhile, VEGF levels in the CAD+DM were also significantly lower than in the CAD group (Figure 6).

Discussion
The present study demonstrated that hMSCs, isolated from the sternum had a unique appearance and

Figure 2 The phenotypic nature of human MSCs. The phenotype of human MSCs from CAD+DM and CAD groups were positive for CD29, CD44 and negative for CD34, CD45.
phenotype similar to the MSCs isolated from iliac bone marrow. A previous study demonstrated that hMSCs from Passage 1 to 5 had significantly greater proliferative potential than those from later passages [13]. We therefore chose hMSCs from Passage 3 for all our in vitro and in vivo experiments.

We demonstrated there was a significant difference in proliferation and gene expression profiling of hMSCs derived from patients with CAD+DM relative to those derived from patients with CAD only. These findings

| ProbeSet ID | Gene title                                                                 | Gene symbol | CAD+DM/CAD   |
|-------------|-----------------------------------------------------------------------------|-------------|--------------|
| 206814_at   | Nerve growth factor(beta polypeptide)                                       | NGF         | 4.1658       |
| 209295_at   | Tumor necrosis factor receptor superfamily, member 10b                      | TNFRSF10B   | 2.3979       |
| 218856_at   | Tumor necrosis factor receptor superfamily, member 21                       | TNFRSF21    | 2.2182       |
| 155526_at   | Effector cell peptidase receptor 1 (non-protein coding)                     | EPR1        | 0.2846       |
| 202094_at   | Baculoviral IAP repeat-containing 5                                         | BIRC5       | 0.1978       |
| 203508_at   | Tumor necrosis factor receptor superfamily, member 1B                       | TNFRSF1B    | 0.3262       |
| 203685_at   | B-cell CLL/lymphoma 2                                                       | BCL2        | 0.4131       |
| 209970_x_at | Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase) | CASP1 | 0.3542 |
| 220085_at   | Helicase, lymphoid-specific                                                 | HELLS       | 0.4298       |

Table 3 Differential apoptosis-related genes from hMSCs obtained from CAD+DM and CAD

---

**Figure 3** Bcl-2 expression in human MSCs. (A) Levels of Bcl-2 expression estimated by Affymetrix microarray analysis show a nearly 2.5-fold down-regulation in CAD+DM-MSCs compared with CAD-MSCs. (B) Western blot validation of protein levels demonstrated a great than 2-fold down-regulation in CAD+DM-MSCs compared with CAD-MSCs. (*P < 0.05 vs. CAD group).

**Figure 4** Evaluation of myocardial function. Ejection fraction EF (A) and fractional shortening FS (B) were not significantly changed in any of the three groups at 1 week post infarction. However, EF and FS increased significantly in CAD+DM and CAD groups after MSC transplantation. EF and FS were significantly lower in the CAD+DM group than in the CAD group after 4 weeks. (*P < 0.05 CAD+DM and CAD groups compared with control group, #P < 0.05 CAD+DM group compared with CAD group).
Myocardial apoptosis 4 weeks after cells transplantation. Myocardial apoptosis was detected by TUNEL staining. TUNEL-positive cells (TP indicated) decreased significantly in CAD+DM and CAD groups compared with control group. However, the number of TUNEL-positive cells was significantly higher in the CAD+DM group than in the CAD group. (*p < 0.05 vs. control group, #p < 0.05 vs. CAD group).

Protein expression in infarcted myocardium. (A) Immunohistochemical detection of Bcl-2 expression in infarcted myocardium from control (+), CAD+DM (++) and CAD (+++) groups. (B) Western-blot analysis shows that hMSCs transplantation from patients with CAD+DM and CAD significantly increased Bcl-2 expression in the myocardium compared with the control group. However, Bcl-2 protein level was significantly lower in the CAD+DM group than in the CAD group. (C) Western-blot analysis shows that VEGF expression after hMSCs transplantation in CAD+DM group was significantly lower than in the CAD group. (*p < 0.05 vs. control group, #p < 0.05 vs. CAD group).
provided initial evidence that DM reduces the proliferation of hMSCs in vitro. The current results were consistent with previous reports in which they found that endothelial progenitor cells were depleted even in DM patients without clinical evidence of macrovascular disease [24]. We also showed that Bcl-2 as well as other differential genes may play a crucial role in hMSC proliferation. The other important finding was that transplantation of hMSCs from CAD patients into rats with experimentally induced myocardial infarction improved cardiac contractility and attenuated apoptosis of cardiomyocytes. These effects were also weakened in MSCs derived from patients with CAD+DM, possibly due in part to reduced expression of Bcl-2 in these cells. These results were consistent with those from a previous study using a rat model [19].

In our study, we identified several differentially expressed genes related to apoptosis. From differential genes, TNFRSF10B, TNFRSF21 and TNFRSF1B are a member of the TNF-receptor superfamily, which deliver signals for cell death, survival, proliferation and differentiation. However, their effects on apoptosis are diverse. TNFRSF10B, TNFRSF21 can be activated by tumor necrosis factor-related apoptosis inducing ligand and transducer apoptosis signals. In contrast, TNFRSF1B plays a vital role in preventing apoptosis [25]. Moreover, BIRC5, which positively correlated with the expression of Bcl-2, is a member of the inhibitor of apoptosis gene family and take part in the prevention of apoptotic cell death [26]. From all the differential genes related to apoptosis, Bcl-2 was selected for further study since our previous studies demonstrated that Bcl-xl gene transfer has a cardioprotective function against ischemia/reperfusion injury [27,28]. Both Bcl-2 and Bcl-xl belong to the Bcl-2 family, and are overexpressed in B-cell lymphoma [29]. Bcl-2 family of proteins acts as critical regulators of pathways involved in anti-apoptosis and inhibition of cell death [30]. It has also been shown that Bcl-2 contributes to cardiac protection during ischemic conditions, where it acts as one of the regulators of the metabolic functions of mitochondria [31]. In the present study, mRNA and protein expression of Bcl-2 were significantly lower in the CAD+DM group than in the CAD group, suggesting that Bcl-2 expression in patients with CAD might be impaired by DM.

MSCs exhibit the property of immune-tolerance whereby they express low levels of major histocompatibility complex (MHC) and co-stimulant molecules [32]. This means that MSCs are generally safe and effective when used for allo-transplantation [11]. However, post-transplant rejection has previously been reported in a xenogenic model [33], and for this reason we used, cyclosporine to suppress the immune response in our study.

We also showed that hMSCs transplantation improved myocardial but that the improvement was significantly more marked with cells derived from patients with CAD than with those derived from patients with CAD+DM. The findings that MSCs transplantation improves heart function after myocardial infarction and that DM may weaken myocardial protective function of hMSCs transplantation significantly are in accordance with previous studies [13,34]. In our study, myocardial tissue from the infarcted zone and border was determined by TUNEL, immunohistochemistry and Western-blot analysis. We found that apoptosis of myocardial cells increased dramatically in CAD+DM group compared with CAD group, in accordance with a previous study in a rat model [19]. We also showed that, expression of Bcl-2 decreased markedly in the CAD+DM group compared with CAD group. Previous studies revealed a reduced expression of VEGF in the myocardium in diabetes [35]. However, there is no difference between the two groups at mRNA level in Gene Chip results of cultured hMSCs. On the contrary, protein expression of VEGF after hMSCs transplantation decreased significantly in the CAD+DM group compared with CAD group. This might be due to the elevated VEGF secretion induced by high-level expression of Bcl-2 in response to hypoxic condition [36]. Thus, different levels between protein and mRNA expression existed in cell culture under normoxic condition and infarcted myocardium under hypoxic condition. Thus, inhibition of Bcl-2 expression may result in increased apoptosis which in turn decreases myocardial protection; decreased secretion of VEGF may one of the possible reasons. It has been shown that the function of MSCs can be modulated by introduction of specific genes. [36]. It is therefore possible that hMSCs from CAD+DM patients modified with Bcl-2 gene may have improved expression levels of Bcl-2 protein and enhanced ability to protect the ischemic myocardium. At the same time, the method of human stem cell based three dimensional microtissues may also represent a translational therapy strategy which may enhance cellular retention, survival and integration [37]. Both of above can be studied in deep-going way in the future.

Limitations
Previous studies have demonstrated that aging impairs the quantity, quality and mobilization capacity of MSCs [14,38]. Thus, hMSCs were obtained from patients 50 to 60 years of age, which limited the number of relative younger patients with CAD+DM. This may have compromised the capacity of MSCs to offer myocardial protection. The relatively small sample size in our study may also have impacted our results to some extent. Our findings therefore need to be substantiated in larger populations of patients of different age groups.
Conclusions
The present study indicates that hMSCs from patients with CAD+DM and CAD have proliferative properties, and that transplantation of hMSCs from all patients improved heart function in rats with experimentally-induced myocardial infarction. The ability to proliferate and preserve myocardial function decreased significantly in MSCs obtained from patients with CAD+DM compared with those obtained from patients with CAD. The differential effects of transplantation of different human MSCs might result from differences of Bcl-2 protein expression, which determine the extent of anti-apoptosis.

Abbreviations
MI: Myocardial infarction; DM: Diabetes mellitus; CAD: Coronary artery disease; MSCs: Mesenchymal stem cells; L.V.: Left ventricular; VEGF: Vascular endothelial growth factor; HGFF: Hepatocyte growth factor; hMSCs: Human MSCs; CABG: Coronary artery bypass graft surgery; ADA: American diabetes association; PBS: Phosphate buffered saline; DMEM-LG: Low-glucose Dulbecco’s modified Eagle Medium; MITT: 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; DMSO: Dimethyl sulfoxide; EF: Ejection fraction; FS: Fractional shortening; TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-X Nick End Labeling.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HW and JH made substantial contributions to the conception and design of this study. XX participated in designing the study, performing the experiments. HJ participated in acquisition of hMSCs. YL carried out the cells culture studies, acquisition of data, performed the statistical analysis and drafted the manuscript. TL carried out animal experiments and performed Immunohistochemistry analysis. ZL carried out TUNEL staining and Western blot analysis. All authors read and approved the final manuscript.

Acknowledgments
This study was funded in part by the National Science Foundation of China (81071267).

Author details
1. Department of Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, Xian, PR China. 2. Department of Cardiovascular Surgery, Shenyang Northern Hospital, 83 Wenhu Rd, Shenyang, Shenyang, Liaoning 110016, China. 3. Department of Cardiothoracic Surgery, Ningxia Shenyang Northern Hospital, 83 Wenhua Rd, Shenhe District, Shenyang, Liaoning, 110016, China.

Received: 6 January 2013 Accepted: 27 February 2013

Published: 3 March 2013

References
1. Bolson BA, Gulati R: Stem cell therapy for the heart: a perspective. Transf Res 2010, 155(1):3–5.
2. Kanaya AM, Grady D, Barrett-Connor E: Explaining the sex difference in coronary heart disease mortality among patients with type 2 diabetes mellitus: a meta-analysis. Arch Intern Med 2002, 162(15):1737–1745.
3. Mazzone T, Chait A, Plutzky J: Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. Lancet 2008, 371(9626):1800–1809.
4. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, Pattany PM, Zambrowo JP, Hu Q, McNiece I, et al: Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. Proc Natl Acad Sci U S A 2009, 106(33):14022–14027.
5. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prokop D, Horvitz E: Minimal criteria for defining multipotential mesenchymal stromal cells, The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8(4):315–317.

6. Krause U, Harter C, Seckinger A, Wolf D, Reinhard A, Bea F, Dengler T, Hardt S, Ho A, Katus HA, et al: Intravenous delivery of autologous mesenchymal stem cells limits infarct size and improves left ventricular function in the infarcted porcine heart. Stem Cells Dev 2007, 16(1):31–37.
7. Caplan AI, Dennis JE: Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006, 98(5):1076–1084.
8. Wang M, Criostomo PR, Herring C, Meldrum KK, Meldrum DR: Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. Am J Physiol Regul Integr Comp Physiol 2006, 291(4):R880–R884.
9. Rupatt C, Horstkotte J, Vlastos GA, Plosser A, Lebherz C, Semisch M, Thalgott M, Buttners K, Browarczuk C, Mages J, et al: Embryonic endothelial progenitor cells expressing a broad range of proangiogenic and remodeling factors enhance vascularization and tissue recovery in acute and chronic ischemia. FASEB J 2005, 19(11):1576–1578.
10. Gnecci M, Zhang Z, Ni A, Dzau VJ: Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008, 103(1):1204–1219.
11. Hare JM, Traverse JH, Henry TD, Dib N, Stumpf RK, Schulman SP, Gentenbith G, DeMara AN, Dentakes A, Gammon RS, et al: A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009, 54(24):2277–2286.
12. Cui X, Wang H, Guo H, Wang C, Ao H, Liu X, Tan YZ: Transplantation of mesenchymal stem cells preconditioned with diazoxide, a mitochondrial ATP-sensitive potassium channel opener, promotes repair of myocardial infarction in rats. Tokerubu J Exp Med 2010, 220(2):139–147.
13. Hou M, Yang KM, Zhang H, Zhu WQ, Duan FJ, Wang H, Song YH, Wei YJ, Hu SS: Transplantation of mesenchymal stem cells from human bone marrow improves damaged heart function in rats. Int J Cardiol 2007, 115(2):220–228.
14. Seth S, Scutt A, Stolzing A: Aging of mesenchymal stem cells. Ageing Res Rev 2006, 5(1):91–116.
15. Tepper OM, Galiano RD, Capla JM, Kalta C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002, 106(22):2781–2786.
16. Takova SV, Gruener S, Schindler S, Wolf G, Baumann M, Abolmaali N: Age-related changes in the frequency of mesenchymal stem cells in the bone marrow of rats. Stem Cells Dev 2007, 16(3):439–446.
17. Scheubel RI, Zorn H, Silber RE, Kuss O, Morawietz H, Holtz J, Simm A: Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting. J Am Coll Cardiol 2003, 42(12):2073–2085.
18. Xin Y, Wang YM, Zhang H, Li J, Wang W, Wei YJ, Hu SS: Aging Adversely Impacts Biological Properties of Human Bone Marrow-derived Mesenchymal Stem Cells: Implications for Tissue Engineering Heart Valve Construction. Arq Orgens 2009.
19. Jin P, Zhang X, Wu Y, Li L, Yin Q, Zheng L, Zhang H, Sun C: Strepptozotocin-induced diabetic rat-derived bone marrow mesenchymal stem cells have impaired abilities in proliferation, paracrine, antiapoptosis, and myogenic differentiation. Transplant Proc 2010, 42(7):2745–2752.
20. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care 2010, 33(Suppl 1):S52–S59.
21. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM: Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001, 98(8):2396–2402.
22. Gao F, Gong B, Christopher TA, Lopez BL, Karawara A, Ma XL: Anti-apoptotic effect of bendipine, a long-lasting vasodilating calcium antagonist, in ischemic/reperfused myocardial cells. Br J Pharmacol 2001, 132(4):869–878.
23. Tian Y, Zhang W, Xia D, Moddi P, Liang D, Wei M: Postconditioning inhibits myocardial apoptosis during prolonged reperfusion via a JM2K-STAT3-Bad2-pathway. J Biomed Sci 2011, 18(5).
24. Zhao CT, Wang M, Siu CW, Hou YL, Wang T, Tse HF, Yiu KH: Myocardial dysfunction in patients with type 2 diabetes mellitus: role of endothelial progenitor cells and oxidative stress. Cardiovase Diabetol 2012, 11:147.
25. Lockley RM, Killeen N, Lenardo MJ: The TNF and TNF receptor superfamily: integrating mammalian biology. Cell 2001, 104:487–501.
26. Gao Q, Yang S, Kang MQ: Influence of survivin and Bcl-2 expression on the biological behavior of non-small cell lung cancer. Mol Med Report 2012, 5:1409–1414.

27. Huang J, Ito Y, Morikawa M, Uchida H, KOshino M, Sasaki K, Abe T, Hamada H: Bcl-xL gene transfer protects the heart against ischemia/reperfusion injury. Biochem Biophys Res Commun 2003, 311(1):64–70.

28. Huang J, Nakamura K, Ito Y, Uzuka T, Morikawa M, Hira S, Tomihara K, Tanaka T, Masuta Y, Ishi K, et al: Bcl-xL gene transfer inhibits Bax translocation and prolongs cardiac cold preservation time in rats. Circulation 2005, 112(1):76–83.

29. Gross A, McDonnell JM, Korsmeyer SJ: BCL-2 family members and the mitochondria in apoptosis. Genes Dev 1999, 13(15):1899–1911.

30. Willis S, Day CL, Hinds MG, Huang DC: The Bcl-2-regulated apoptotic pathway. J Cell Sci 2003, 116(Pr 20):4053–4056.

31. Imahashi K, Schneider MD, Steenbergen C, Murphy E: Transgenic expression of Bcl-2 modulates energy metabolism, prevents cytosolic acidification during ischemia, and reduces ischemia/reperfusion injury. Circ Res 2004, 95(7):734–741.

32. Atoui R, Shum-Tim D, Chiu RC: Myocardial regenerative therapy: immunologic basis for the potential "universal donor cells". Ann Thorac Surg 2008, 86(1):327–334.

33. Nauta AJ, Fibbe WE: Immunomodulatory properties of mesenchymal stromal cells. Blood 2007, 110(10):3499–3506.

34. Yi W, Sun Y, Gao E, Wei X, Lau WB, Zheng Q, Wang Y, Yuan Y, Wang X, Tao L, et al: Reduced cardioprotective action of adiponectin in high-fat diet-induced type II diabetic mice and its underlying mechanisms. Antioxid Redox Signal 2011, 15(7):1779–1788.

35. Keats EC, Khan ZA: Vascular stem cells in diabetic complications: evidence for a role in the pathogenesis and the therapeutic promise. Cardiovasc Diabetol 2012, 11:37.

36. Li W, Na N, Ong LL, Nesselmann C, Kloppisch C, Lailov Y, Furlani D, Piechaczek C, Moebius JM, Lutzow K, et al: Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells 2007, 25(8):2118–2127.

37. Emmert MY, Wolint P, Winklhofer S, Stolzmann P, Cesarovic N, Fleischmann T, Nguyen TD, Frauenfelder T, Boni R, Scherman J, et al: Transcatheter based electromechanical mapping guided intramyocardial transplantation and in vivo tracking of human stem cell based three dimensional microtissues in the porcine heart. Biomaterials 2013, 34(10):2428–2441.

38. Assmus B, Fischer-Rasokat U, Horold J, Seeger FH, Fichtscheer S, Tonn T, Seifried E, Schachinger V, Dommelh S, Zethner AE: Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. Circ Res 2007, 100(8):1234–1241.