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Morphological and functional changes in bone marrow mesenchymal stem cells in rats with heart failure

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Abstract. The changes in bone marrow-derived mesenchymal stem cells (BMSCs), in terms of cell morphology and protein expression in rats with heart failure, were studied. Pressure overload chronic heart failure rat model was induced with partial constriction of the abdominal aorta. BMSCs from the model and the sham operation groups were isolated and cultured (cell density, 10^8 cells/l), and supernatant was collected after 72 h. Enzyme-linked immunosorbent assay was used to measure HGF, IGF-1, PDGF, SCF, FGF and VEGF levels in the supernatant. Results showed that in the model group, the minimum cell diameter, the average cell area and the protein expression in single BMSCs were significantly less than those in the sham operation group. In the model group, SCF and PDGF levels were significantly lower than those in the sham operation group. VEGF concentration in the model group was significantly higher than that in the sham operation group. Compared with normal rats, the morphology of BMSCs in rats with heart failure changed considerably, the protein expression of a single cell and the ability to secrete cytokines decreased in a meaningful way.

Introduction

Heart failure is the final stage in different types of cardiovascular diseases (1), and chronic pressure overload is known to be an important cause of heart failure (2). Cellular cardiomyoplasty is a new way to improve the impaired cardiac function. It employs stem cell therapy to regenerate myocardium. Characterized by their potential for proliferation, differentiation and capacity for self-renewal, stem cells are ideally suited for use in regenerative medicine (3,4). With the development of stem cell technology, it is now possible to increase the number of myocardial cells with intact contractile function by cell transplantation.

The use of stem cells for regeneration purposes has been broadly studied, and bone marrow-derived mesenchymal stem cells (BMSCs) remain the most extensively investigated cells. Several advantages have been reported, however, the most dominant advantages associated with the use of BMSCs are the following: i) They have high differentiation potential; ii) they are easy to collect; iii) there is no risk of rejection after autologous transplantation; and iv) there is no ethical controversy. BMSCs are now considered as one of the hot spots in the field of transplantation research (3-13), however, there are still some questions about BMSCs that need to be answered. We do not yet know whether BMSCs in patients with heart failure have the same form and function as those in the healthy people, or whether BMSCs can play a constructive role in healthy individuals.

In the present study, the pressure overload chronic heart failure rat model was induced with partial constriction of the abdominal aorta (3). BMSCs from normal rats and heart failure rats were isolated, and all changes in cell morphology, protein expression and the ability to secrete cytokines were studied. Through this study, we provided a theoretical basis for clinical application of BMSCs.

Materials and methods

Materials

Experimental animals and cell lines. Sprague-Dawley male rats (body weight range, 200-220 g) were obtained from the Experimental Animal Center of the Chinese Academy of Sciences in Shanghai, China.

Main reagents. Fetal bovine serum (FCS) was purchased from HyClone (Logan, UT, USA), L-glutamine from Sigma-Aldrich (St. Louis, MO, USA), DMEM/F12 culture media from Gibco (Grand Island, NY, USA). Mouse monoclonal CD29 antibody (dilution, 1:200; cat. no. 368511) and mouse monoclonal CD45 antibody (dilution, 1:200; cat. no. 102216) were from BioLegend, Inc. (San Diego, CA, USA). Mouse monoclonal CD44 antibody (dilution, 1:200; cat. no. MCA643FA) was from Serotec (Oxford, UK), enzyme-linked immunosorbent assay (ELISA) kit and AEC color reagent kit from Wuhan Boster Biological Technology,

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Table I. Comparison of the hemodynamics indexes between the sham group and the model group (mean ± SD).

Group	n	LVSP (mmHg)	LVEDP (mmHg)	+dp/dt (mmHg/sec)	-dp/dt (mmHg/sec)
Sham	20	121.22±11.78	1.47±1.04	4016.29±513.72	3828.81±642.37
Model	30	108.94±10.36	13.30±6.53[a]	2541.10±378.36[b]	2475.80±327.21[b]

[a]P<0.05, [b]P<0.01 vs. Sham group.

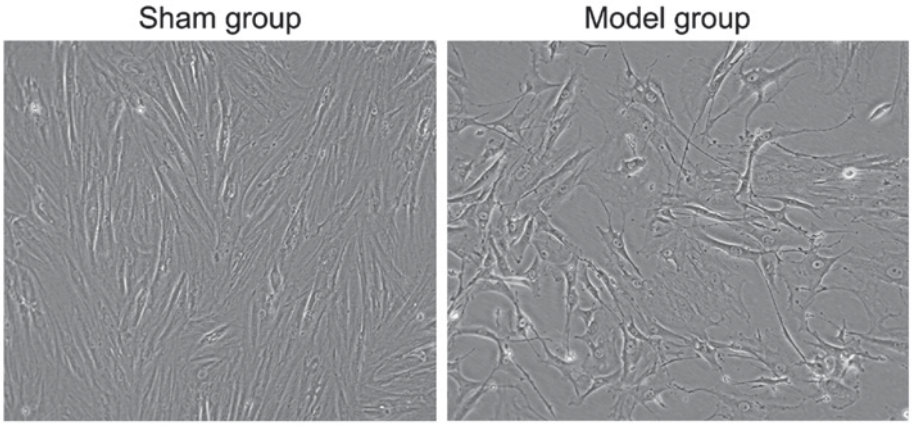

Figure 1. Sixth generation of BMSC cell culture: BMSCs in the Sham operation group rats became adherent in spindle shaped with fibroblastic morphology; BMSC morphology in the model group became irregular. BMSCs, bone marrow-derived mesenchymal stem cells.

Ltd. (Wuhan, China). ELISA kits for HGF, IGF-1, PDGF, SCF, FGF and VEGF were purchased from the US UCL Company.

Instruments. We used CO_2 cell incubator (Thermo Fisher Scientific, Waltham, MA, USA), Micro-camera system (version 2.0; Olympus, Tokyo, Japan), and enzyme standard instrument (Tecan Group Ltd., Männedorf, Switzerland).

Methods

Preparation of the animal model. Rat model of pressure overload chronic heart failure was induced with partial constriction of abdominal aorta, using the protocol reported by Wang *et al* (13). Rats were routinely fed after operation, and hemodynamic monitoring was conducted 12 weeks after the operation.

Hemodynamic monitoring. We used the protocol reported by Wang *et al* for hemodynamic monitoring (13). After the operation, rats were routinely fed and hemodynamic monitoring was performed 12 weeks later. After hemodynamic monitoring, BMSCs were rapidly isolated.

Isolation and culture of BMSCs. Isolated BMSCs in the model group and sham operation group were cultured following protocols provided by Gong *et al* and Wang *et al* (3,13).

BMSC surface markers detected by immunocytochemistry. The expression of BMSC surface markers, CD29 and CD44, and the hematopoietic stem cell surface marker CD45 were evaluated using immunocytochemistry. PBS was used in the control group. For immunocytochemistry we used the protocol reported by Gong *et al* and Wang *et al* (3,13,14).

Collection of BMSC culture supernatant. The sixth generation of BMSCs (cell density, 10^8 cells/l) was transferred to 60 mm cell culture flasks. Supernatant was collected after 72 h and stored at -80˚C.

Table II. Comparison of BMSC forms in heart failure rats and normal rats.

Groups	n	Maximum diameter of cells, l/μm	Minimum diameter of cells, l/μm	Average area of cells, r/μm^2
Sham	20	109.98±26.07	55.85±19.33	4847.70±611.46
Model	30	117.74±59.79	26.21±12.93[a]	2803.35±395.48[a]

[a]P<0.01 vs. Sham group. BMSCs, bone marrow-derived mesenchymal stem cells.

HGF, IGF-1, PDGF, SCF, FGF, and VEGF levels. ELISA was used to measure various cytokines using the instructions provided by the kit manufacturer.

Statistical analysis. Image Pro-Plus (IPP) 6.0 image analysis system was used to measure the maximum and minimum cell diameters, the average single cell area and the average gray value of a single cell. SPSS 12.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The data are presented by mean ± standard deviation. Comparison between groups was conducted using t-test. One-way ANOVA was used in for comparison among groups. P<0.05 was set as the statistically significant difference.

Results

Hemodynamic changes. LVDEP in the model group was significantly higher than the sham operation group. Compared with the control group the $\pm dp/dt_{max}$ decreased significantly in

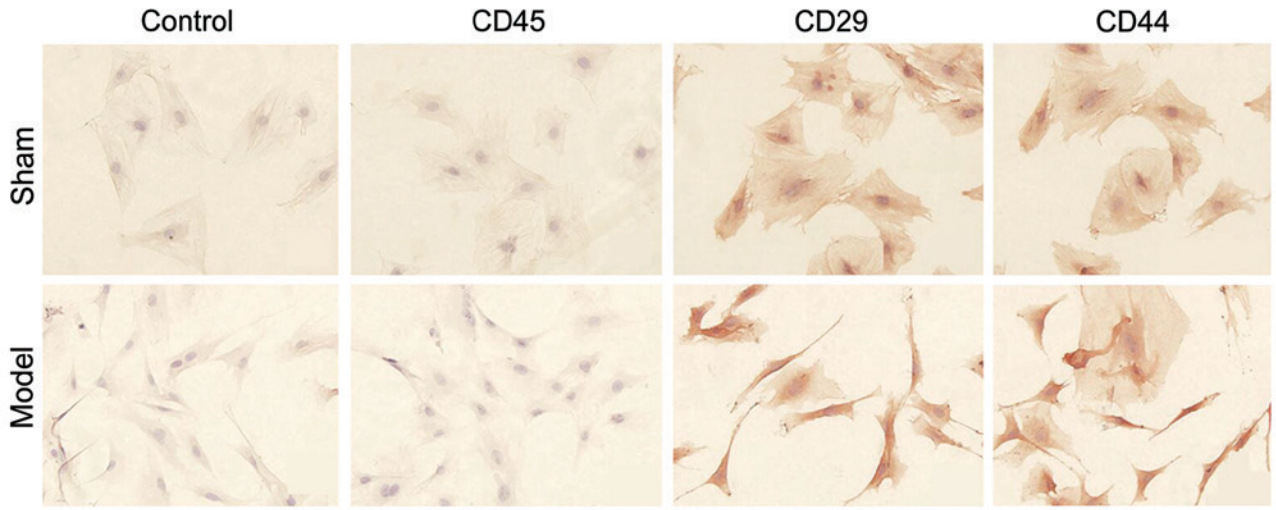

Figure 2. The identification of BMSCs by immunocytochemical staining of CD45, CD29 and CD44 in two groups (magnificaton, x400). BMSCs, bone marrow-derived mesenchymal stem cells.

Table III. Comparison of BMSCs protein expression in heart failure rats and normal rats.

Group	n	Grey value	Average area of cells, r/μm^2	Grey value/area
Sham	20			
Control		216.26±1.10		0.0446±0.0018
CD29		203.13±7.08[a]	4847.70±611.46	0.0419±0.0116
CD44		196.76±5.06[a]		0.0406±0.0083
Model	30			
Control		215.21±3.74		0.0768±0.0095
CD29		185.85±10.90[b]	2803.35±395.48	0.0663±0.0276[b]
CD44		170.24±6.51[b]		0.0607±0.0165[b]

[a]P<0.01 vs. control group, [b]P<0.01 vs. Sham group. BMSCs, bone marrow-derived mesenchymal stem cells.

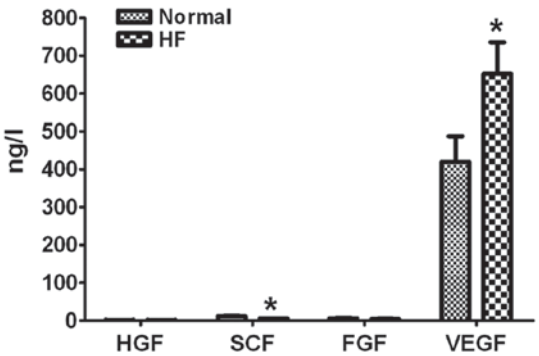

Figure 3. Comparison of VEGF, FGF, HGF and SCF secretion in BMSCs between the heart failure and normal rats. BMSCs, bone marrow-derived mesenchymal stem cells. *Compared with normal group, P<0.05.

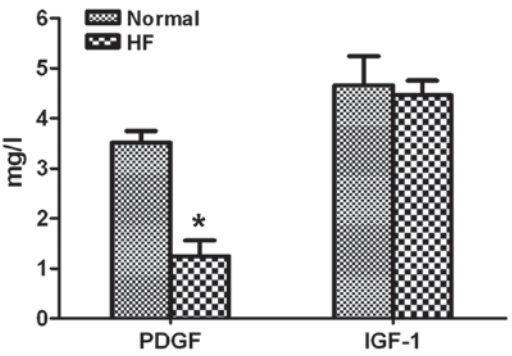

Figure 4. Comparison of PDGF and IGF-1 secretion in BMSCs between heart failure and normal rats. BMSCs, bone marrow-derived mesenchymal stem cells. *Compared with normal group, P<0.05.

the model group. All differences were statistically significant (P<0.05) (Table I).

Separation and culture of BMSCs. Culture media were changed after seven days, and at this time BMSCs in the primary culture of sham operation group were in clonal distribution with various morphologies. One week later, cells reached fusion stage and when they reached P6, cell confluence was in swirling or parallel growth. Cells in the model group needed 2 weeks to fuse and reach P6, these cells were irregular in shape (large, flattened, spindle form, and cells with poles extending process-like substance). When compared with the sham operation group, there was no significant changes in the BMSC maximum cell diameter in the model group, but the minimal cell diameter and the average area of single cell were reduced. Difference was statistically significant (P<0.01) (Fig. 1 and Table II).

BMSC identification. Cell surface antigens were detected in both groups using immunocytochemistry staining. Positive expression for CD29 and CD44 and negative for CD45 were detected. Compared with the sham operation group, the expression of CD29 and CD44 in the model group was significantly higher, and the differences were statistically significant (P<0.01). Nevertheless, due to the significant reduction in cell size, the amount of protein expressed by an individual cell per unit area significantly reduced (Fig. 2 and Table III).

HGF, IGF-1, PDGF, SCF, FGF, and VEGF levels. ELISA was used to measure the level of cytokines in BMSC culture supernatant in both groups. In the model group, the average concentrations of SCF and PDGF were 6.06±1.18 ng/l and 1.25±0.32 mg/l, respectively. These levels were significantly lower than levels detected in the sham operation group which were 12.56±1.15 ng/l and 3.52±0.23 mg/l, respectively. Average VEGF concentration in the model group was 652.44±83.19 ng/l, which was significantly higher than 419.97±6.785 ng/l measured in the sham operation group, and the difference had statistical significance ($P<0.01$) (Figs. 3 and 4).

Discussion

Relative or absolute reduction in functional integrity of myocardial cells is the common characteristics in coronary heart disease, rheumatic heart disease and idiopathic cardiomyopathy. This condition may eventually lead to ventricular remodeling or deformation and heart failure (13-15).

Cellular cardiomyoplasty is a new method for treating patients with heart failure. Research has shown that cellular cardiomyoplasty can improve heart contractility by repairing damaged areas with new cells, and improving cardiac function (3,16). Results obtained from several experimental studies and clinical trials have shown that BMSC transplantation can restore cardiac function within the myocardial damaged area (3,8,17). The peak period for BMSCs in human body is during the neonatal period, and decreases with age (8). This may explain why the vast majority of patients with congestive heart failure are the elderly. In these cases, it is interesting to know whether the disease can affect the number of BMSCs or if the cells morphology and function are influenced by it.

We, in this study, have successfully prepared animal models of heart failure and discovered that significant morphological changes occurred in BMSCs in rats with chronic heart failure. The cells appeared in irregular shapes and the average area of single cell was reduced significantly. Average gray value of single BMSC in rats with heart failure was reduced significantly (smaller grey value represented stronger positive immunoreaction). Protein expression in the heart failure group increased significantly, and due to significant reduction in cell area during the heart failure, protein expression carried by unit area decreased significantly.

It has been shown that several factors such as FGF, VEGF, IGF-1, PDGF, TGF-β, Ang, EGF, G-CSF, PGF and HGF can promote cardiovascular regeneration (18). Results obtained from a prior study proved that VEGF, FGF-b, PDGF-AB/PDGFRa signaling pathway was essential for the stem cell to differentiate into cardiomyocyte-like cells (17). IGF-1 is a cytokine that promotes cardiac myocyte growth and survival, IGF-1 also promotes blood vessel regeneration in infarcted area, reduces the degree of myocardial necrosis, maintains the myocardial structure, stimulates cardiac fibroblasts proliferation, and inhibits the degradation of matrix proteins. It can effectively prevent myocardial ischemia reperfusion injury (13,18-20). BMSCs have the capacity to produce and secrete several paracrine factors, which can affect BMSC migration, promote blood vessel generation and reduce the rate of cell apoptosis (21). The BMSC capacity to repair myocardial tissue depends on the secretion of nutrient factors and not on their differentiation potential (22-24). BMSCs secrete nutrient factors to improve cardiac function through a variety of complex mechanisms, such as reducing tissue damage, inhibiting fibrosis formation, promoting angiogenesis, mobilization of host tissue stem cells and reducing inflammation. It has been shown that in the course of treatment with BMSCs, VEGF is the major secreted nutritional factor (25). VEGF promotes stem cell differentiation into myocardial cells and endothelial cells (26,27), and can mobilize bone marrow progenitor cells to participate in muscle formation and angiogenesis (28).

We discovered that PDGF and SCF levels decreased significantly in the heart failure group, while VEGF level surged significantly in this group. Usually, VEGF expression happens under both physiological and pathological conditions. In physiological conditions, many normal tissues express low levels of VEGF, however, VEGF expression is generally more elevated in myocardium, prostate and adrenal cortex. In pathological conditions, VEGF transcription and translation in tumor cells are highly upregulated. Elevated expression of VEGF has been also reported in non-neoplastic lesions, such as wound healing, ischemic myocardial cells, psoriasis, chlamydia, diabetic retinopathy and rheumatoid arthritis (29). In most cases of heart failure, patients suffer from myocardial remodeling and myocardial fibrosis, myocardial remodeling reduces the number of capillaries in the unit weight of myocardium and increases oxygen diffusion, so the myocardium is relatively hypoxia. Thus, during heart failure, due to myocardial hypoxic, VEGF is over-expressed, which corresponds well with our results showing that BMSCs intensify the VEGF secretion during heart failure.

In conclusion, we showed that heart failure had a profound impact on: i) BMSC morphology; ii) protein expression; and iii) the ability to secrete cytokines. Although BMSC transplantation has broad application prospects for the treatment of heart failure, it is yet unknown whether the BMSCs can help those patients in poor condition.

Acknowledgements

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