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Stem cells with FGF4-bFGF fused gene enhances the expression of bFGF and improves myocardial repair in rats

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ABSTRACT

The aim of this study was to investigate whether the modification of bone marrow-derived mesenchymal stem cells (BMSCs) with the fused FGF4 (fibroblast growth factor 4)-bFGF (basic fibroblast growth factor) gene could improve the expression and secretion of BFGF, and increase the efficacies in repairing infarcted myocardium. We used In-Fusion technique to construct recombinant lentiviral vectors containing the individual gene of bFGF, enhanced green fluorescent protein (EGFP), or genes of FGF4-bFGF and EGFP, and then transfected these lentiviruses into rat BMSCs. We conducted an *in vitro* experiment to compare the secretion of bFGF in BMSCs infected by these lentiviruses and also examined their therapeutic effects in the treatment of myocardial infraction in a rodent study. Sixty rats were tested in the following five conditions: Group-SHAM received only sham operation as controls; Group-AMI received only injection of placebo PBS buffer; Group-BMSC, Group-bFGF and Group-FGF4-bFGF received implantation of BMSCs with empty lentivirus, bFGF lentivirus, and FGF4-bFGF lentivirus, respectively. Our results found out that the transplanted FGF4-bFGF BMSCs had the highest survival rate, and also the highest myocardial expression of bFGF and microvascular density as evidenced by Western blotting and immunohistochemistry, respectively. As compared to other groups, the Group-FGF4-BFGF rats had the lowest myocardial fibrotic fraction, and the highest left ventricular ejection fraction. These results suggest that the modification of BMSCs with the FGF4-bFGF fused gene can not only increase the expression of bFGF but also improve its secretion. The FGF4-bFGF BMSCs thus can enhance the survival of the transplanted cells, diminish myocardial fibrosis, promote myocardial angiogenesis, and improve cardiac functions.

1. Introduction

Bone marrow-derived mesenchymal stem cells (BMSCs) have been shown to have great potential for the treatment of ischemic heart disease because they have the ability to differentiate in to cardiomyocyte or cardiomyocyte-like cells and repair myocardial infarction [1]. However, previous studies have found that the effectiveness of BMSCs transplantation is often limited because the transplanted cells have low chance to survive and differentiate in the infracted myocardium due to adverse cardiac micro-environment, for example, the hypoxia and ischemia, acute and chronic inflammatory activation, and impaired myocardial structures associated with or following myocardial infarction [2,3]. Accordingly, considerable efforts have been focusing on improving the cardiac micro-environment (soil), the stem cell itself (seed), or both [4,5]. For example, pretreatments of rats with acute myocardial infarction by using statins (exogenous intervention) or ischemic postconditioning (an endogenous cardio-protective mechanism) have been shown to improve the local cardiac micro-environment, and thus enhance the survival of transplanted cells [6,7]. Still, their clinical utilities remain unclear. Another approach is to modifying donor cells with hypoxic stimulus, growth factors, or anti-apoptotic and pro-survival gene transfection. However, the effectiveness of this means has not been determined, and concerns remain about the potential tumorigenic risk generated. Therefore, it is desirable to explore a new approach that can improve not only the donor cells but the host micro-environment as well.

It has been suggested that genetic modification of BMSC with basic fibroblast growth factor (bFGF) can enhance the stem cell therapies [8] which While it has been shown that bFGF promotes cell division and angiogenesis [9], the effectiveness of bFGF gene therapy is greatly limited by the fact that bFGF is a non-secreted protein and bFGF exists only in the cytoplasm of live cells. Therefore, it cannot be secreted into the extracellular space to promote

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cell growth [10]. Therefore, the effectiveness of bFGF gene therapy is often unsatisfied due to low efficiencies of gene transfection and expression. One approach to improve the transfection of bFGF is to use the lentiviral vector and gene targeting method to combine the bFGF gene with a secretion-promoting signal peptide, fibroblast growth factor 4 (FGF4), to promote protein secretion [11,12]. Therefore, in this study, we fused the bFGF gene to that of FGF4, transfected the FGF4-bFGF gene to BMSCs, and then tested if such modification helped to improve the treatment effectiveness of myocardial infarction.

We hypothesized that the combination of BMSCs with the fusion gene of FGF4-BFGF may further promote the therapeutic effects of the transplanted cells in a rat model with myocardial infarction.

2. Materials and methods

2.1. Animals

Sixty male Sprague–Dawley (SD) rats (Shanghai Slac Laboratory Animal Co. Ltd., China) were used in this study. The animal protocol was in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Experimental Animal Care Committee of Fujian Medical University Union Hospital.

2.2. Preparation of BMSCs

BMSCs were prepared from rats weighing 60–80 g. Flow cytometry was used to detect the markers of BMSCs (P3) that included the cells positive for CD29, CD44, and CD90 and negative for CD34 and CD45. Adipogenic induction medium and osteogenic induction medium were then added to induce differentiation into adipocytes or osteoblasts, respectively.

2.3. Construction of recombinant lentiviral vector and cell transfection

The In-Fusion technique and the pGC-FU lentiviral vector system that carries EGFP were used to construct a recombinant lentiviral vector containing bFGF fused to the FGF4 signal peptide. The optimal multiplicity of infection (MOI) and bFGF protein secretion were detected by ELISA.

2.4. Experimental protocol and cell transplantation

Sixty male SD rats, weighting 250–300 g, were used in the experiment. A rat model of myocardial infarction was established by the ligation of the left anterior descending coronary artery (LAD) for 45 min to induce sustained ischemia [13]. Rats ($n = 60$) were randomly divided into five experimental groups ($n = 12$ per group): Group-SHAM, received sham operation only by an open chest surgery without LAD ligation. Group-AMI, received a placebo injection of 100 μl 1× PBS buffer in the rat MI model; Group-BMSC, received an injection of 100 μl 1× PBS buffer containing 2× 106BMSCs transfected with empty carrying only EGFP in the rat MI model; Group-BFGF, received an injection of 100 μl 1× PBS buffer containing 2× 106 BMSCs transfected with lentivirus carrying BFGF and EGFP; and Group-FGF4-BFGF, received an injection of 100 μl 1× PBS buffer containing 2× 106 BMSCs transfected with lentivirus carrying FGF4-BFGF fusion gene and EGFP. All the injections were completed within 30–60 min after the LAD ligation.

2.5. Echocardiographic study

Echocardiography was performed to evaluate the cardiac function at the baseline and at the end of the experimental protocol (28 days after the cell transplantation) by using a digital imaging system equipped with a 10 MHz electronic-phased-array transducer. The left ventricular end-systolic diameter (LVESD, mm) and end-diastolic diameter (LVEDD, mm) were measured. The left ventricular fractional shortening (FS, %) and the left ventricular ejection fraction (EF, %) were calculated by cubic method. All echocardiographic measurements were analyzed for at least 3 consecutive cardiac cycles by two experienced echocardiographer blind to each other and to the treatment of the animals, and were then averaged.

2.6. Preparation of tissue specimen

All rats were sacrificed after completing the experimental protocol (28 days after the cell transplantation) with intravenous injection of 10% KCl solution, and the hearts arrested at diastole were harvested.

2.7. Histological studies

The general pathology was observed in sections using H&E and Masson's trichrome staining. The mean wall thickness (mm) was assessed and the myocardial fibrotic fraction (%) was quantified as the percentage of the myocardial fibrotic area in the section area of the LV myocardial ring. Microvascular regeneration in myocardium was detected using immunohistochemical staining. The microvascular density for the microvascular regeneration was quantified as the number of anti-factor VIII-positive vessels (diameter $\leqslant$ 10 μm) per high power field (400×).

The survival (differentiation or fusion) of the transplanted green fluorescent protein (GFP) positive cells were detected with immunofluorescence staining. The number of the surviving transplanted cells was quantified as the number of GFP-positive cells per high power field (400×).

2.8. Western-blotting assays

The myocardial expression level of bFGF was also assessed by western blotting. The myocardial expression level was determined by the target signals, which were normalized relative to the GAPDH expression and assessed by ImageJ (Version 1.36, NIH).

2.9. Statistical analysis

All results were presented as mean ± SD. Statistical analysis were performed with SPSS (version 13.0) to compare among groups by using one-way ANOVA and Student–Newman–Keuls test. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Identification of BMSCs

The cultured BMSCs were positive for CD29 (95.4%), CD44 (97.6%), and CD90 (96.2%); and negative for CD34 (96.4%) and CD45 (97.5%). After 2 weeks of adipogenic induction, lipid droplets appeared in P3 BMSCs. Oil red O staining showed a large amount of lipid deposition (Fig. 1A). Three weeks after osteogenic induction, formation of calcium nodule was obvious in P3 BMSCs, and were presented as red nodules after alizarin red staining (Fig. 1B).

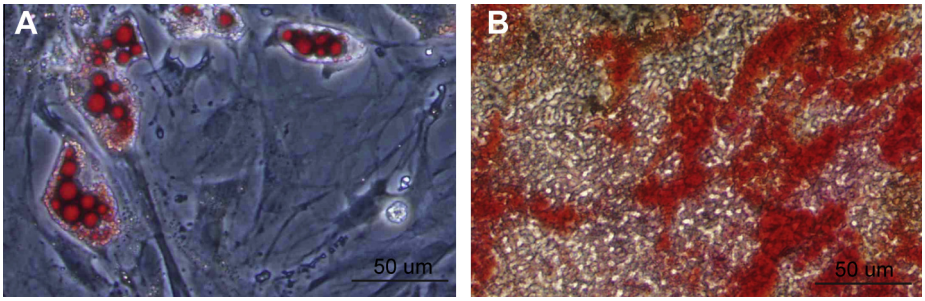

Fig. 1. Identification of the differentiated BMSCs. (A) Oil red staining of the lipid deposition in isolated BMSCs cultured in adipogenic medium. (B) Alizarin red staining of the calcium nodules (red) in isolated BMSCs cultured in osteogenic medium. Magnification 200×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Improved cell survival of the transplanted BMSCs modified with FGF4-bFGF fusion gene

PCR and fluorescence expression sequence analysis showed that we had successfully constructed lentiviral constructs that carried bFGF and FGF4-bFGF genes, and determined by the expression of EGFP of at least 1×10^8 TU/ml.

Fluorescence microscopy showed that the fluorescence intensity of BMSCs transfected with FGF4-bFGF and EGFP recombinant lentivirus became similar to that of BMSCs infected with empty lentivirus at MOI of 10, indicating that almost all the cells has been transfected. Beyond that, no specific increase in the fluorescence intensity or cell infection rate was noticed (Fig. 2A). ELISA analysis indicated that bFGF had been secreted. *In vitro* bFGF

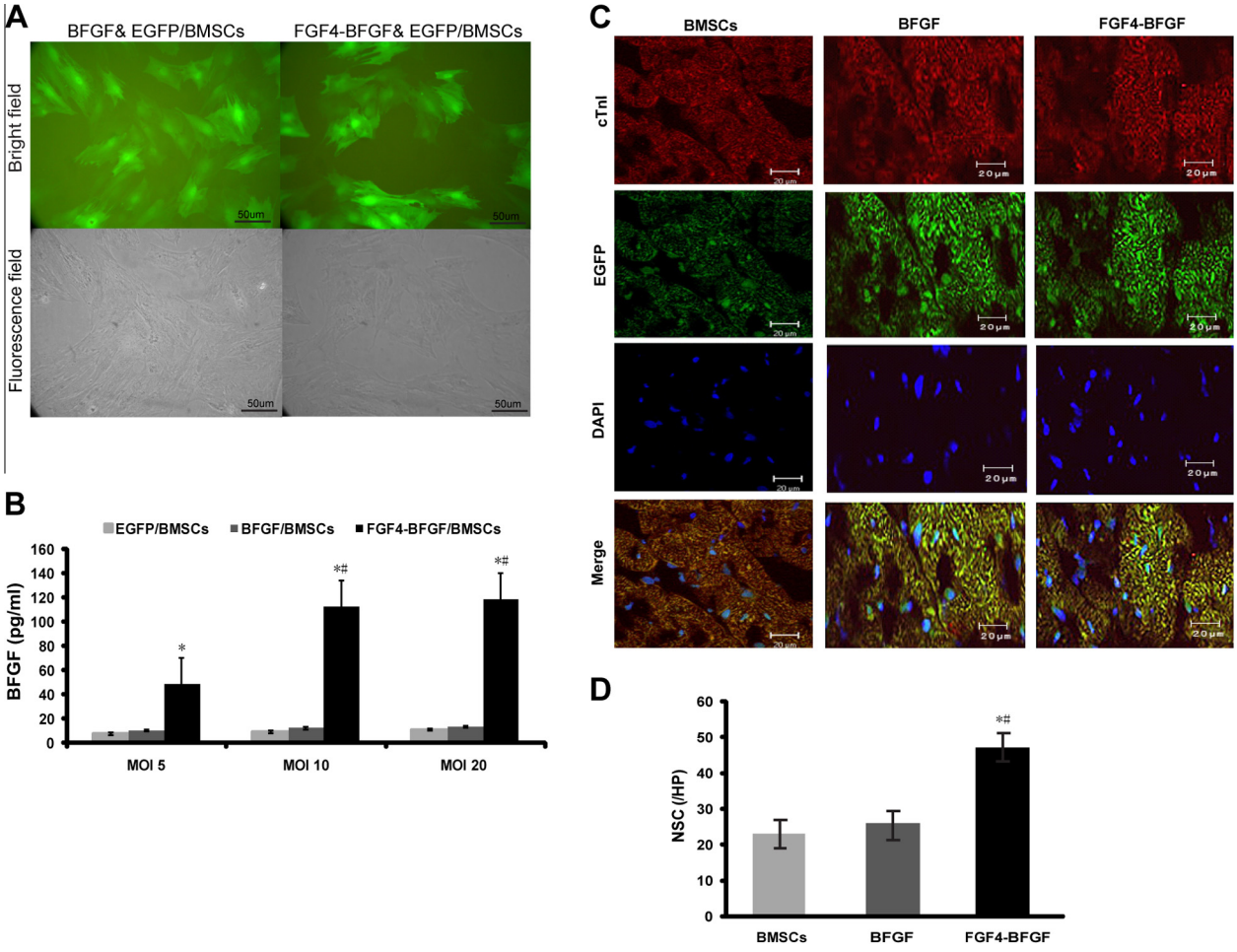

Fig. 2. Improved cell survival of the transplanted BMSCs modified with FGF4-bFGF fusion gene. (A) Fluorescence microscopy showed that the fluorescence intensity of BMSCs transfected with FGF4-bFGF and EGFP recombinant lentivirus became similar to that of BMSCs infected with empty lentivirus at MOI of 10, indicating that almost all the cells were transfected. After that, no specific increase in the fluorescence intensity or cell infection rate was noticed. (B) At all MOI values, the level of bFGF protein secreted from BMSCs in FGF4-bFGF and EGFP group were significantly higher than that in the other groups. At MOI = 10, $^{*}p < 0.05$ compared to EGFP/BMSC and bFGF/BMSC, $^{\#}p < 0.05$ compared to FGF4-bFGF/BMSC. (C) Qualitative immunohistochemical analysis under laser scanning confocal microscopy showed that the lentiviral infected BMSCs were present in the myocardium, equipped with blue argon (for DAPI), green argon (for GFP) and red krypton (for cTnI) lasers. (D) The number of survived transplanted cells (NSC, n/HP) stained with immunohistochemistry was detected by laser scanning confocal microscopy. $^{*}p < 0.05$ as compared to Group-BMSC; $^{\#}p < 0.05$ as compared to Group-BFGF.

secretion in those BMSCs infected by FGF4-bFGF & EGFP recombinant lentivirus was significantly higher than that in BMSCs infected only by bFGF & EGFP recombinant lentivirus or in BMSCs infected by empty lentivirus for all MOI values (when MOI = 5: 48.2 ± 1.3 pg/ml vs. 10.2 ± 0.7 pg/ml, 7.3 ± 1.1 pg/ml, $p < 0.05$; when MOI = 10: 112.0 ± 3.4 pg/ml vs. 12.4 ± 1.4 pg/ml, 8.9 ± 1.1 pg/ml, $p < 0.05$; and when MOI = 20: 118.1 ± 3.3 pg/ml vs. 13.3 ± 1.7 pg/ml, 11.0 ± 1.2 pg/ml, $p < 0.05$), with no further increase of the secretion when MOI increased from 10 to 20 ($p > 0.05$, for each modified BMSCs) (Fig. 2B).

Qualitative immunohistochemical analysis under laser scanning confocal microscope showed that the lentiviral infected BMSCs were present in the myocardium in Group-FGF4-BFGF, causing substantially increased expression of FGF4-bFGF and EGFP. DAPI nuclear staining revealed that the transplanted BMSCs were scattered throughout the myocardium and became oriented in a similar direction as the host myocardial cells. In contrast, the expression of EGFP in the myocardium in Group-BMSC and Group-BFGF were weak or absent, with sparse bFGF expression from these transplanted cells and mainly located in the space between myocardial cells. No differentiated cells with positive cTnI staining were found to have EGFP expression. Few fusions between BMSCs and myocardial cells were observed (Fig. 2C).

Quantitative immunohistochemical analysis under laser scanning confocal microscope showed that the number of surviving transplanted cells in Group-FGF4-BFGF (47.50 ± 3.83/HP) was highest and significantly higher than that in Group-BFGF (vs. 25.67 ± 3.56/HP, $p < 0.01$) or Group-BMSC (vs. 23.83 ± 3.19/HP, $p < 0.01$), and with no significant difference of the number of surviving transplanted cells between the latter two groups (25.67 ± 3.56/HP vs. 23.83 ± 3.19/HP, $p > 0.05$) (Fig. 2D).

3.3. While the FGF4-bFGF fusion gene modified the BMSCs, the modification caused the bFGF expression, myocardial fibrosis and angiogenesis changes

Western blotting demonstrated that the myocardial expression level of bFGF in Group-FGF4-BFGF (0.66 ± 0.04) was significantly higher than that in Group-SHAM (0.13 ± 0.01, $p < 0.01$), Group-AMI (0.31 ± 0.02, $p < 0.01$), Group-BMSC (0.42 ± 0.05, $p < 0.05$) or Group-BFGF (0.45 ± 0.05, $p < 0.05$). The myocardial expression level in Group-BFGF was significantly higher than that in Group-SHAM or Group-AMI, but similar to Group-BMSC (0.42 ± 0.05 vs. 0.45 ± 0.05, $p > 0.05$) (Fig. 3A and B).

Immunohistochemical study showed that the microvascular density in Group-FGF4-BFGF (29.5 ± 0.73/HP) was significantly higher than that in Group-SHAM (10.4 ± 2.49/HP, $p < 0.05$), Group-AMI (15.4 ± 1.02/HP, $p < 0.05$), Group-BMSC (20.7 ± 1.55/HP, $p < 0.05$), Group-BFGF(21.7 ± 1.83/HP, $p < 0.05$). The microvascular density in Group-BFGF was also significantly higher than that in Group-SHAM or Group-AMI, but similar to Group-BMSC (21.7 ± 1.83/HP vs. 20.7 ± 1.55/HP, $p > 0.05$) (Fig. 3C and D).

The FGF4-BFGF BMSCs were found to be able to considerably alleviate the pathological consequence of myocardial infarction. As compared to Group-AMI, the Group-FGF4-BFGF showed smaller infracted area with less (usually patchy) myocardial fibrosis, less regional wall thinning, and less global LV dilatation. Whereas, Group-BMSC and Group-BFGF had medium changes between those of Group-FGF4-BFGF and Group-AMI (Fig. 3A). Quantitative analysis further showed that the mean wall thickness in Group-FGF4-BFGF (1.28 ± 0.14 mm), though thinner than that in Group-SHAM (1.50 ± 0.17 mm, $p < 0.05$), was significantly thicker than that in Group-AMI (0.65 ± 0.12 mm, $p < 0.01$), and Group-BMSC (0.85 ± 0.15 mm, $p < 0.05$). No significant difference was found between Group-BFGF (0.92 ± 0.12 mm) and Group-AMI or Group-BMSC ($p > 0.05$). Quantitative analysis showed that the myocardial fibrotic fraction in Group-FGF4-BFGF (27.16 ± 3.40%), though higher than that in Group-SHAM (25.62 ± 2.25%, $p < 0.05$), was much lower than that in Group-AMI (38.78 ± 3.75%, $p < 0.05$), Group-BMSC 31.40 ± 3.56%, $p < 0.05$), and Group-BFGF (33.04 ± 4.29%, $p < 0.05$). The myocardial fibrotic fraction in Group-BGFG was not significantly different from that in Group-AMI and Group-BMSC ($p > 0.05$) (Fig. 3E and F).

3.4. Improved LV function by the BMSCs modified with FGF4-bFGF fusion gene

Qualitative echocardiographic study showed that Group-FGF4-BFGF had less LV structural and functional changes, typically manifested by less regional wall thinning, segmental wall motion abnormality and global LV dilatation as compared to Group-AMI. These structural and functional parameters in Group-BMSC and Group-BFGF were located between the ranges of Group-FGF4-BFGF and Group-AMI (Fig. 4A). Further quantitative analysis showed that in comparison with Group-AMI, Group-BMSC, Group-BFGF and Group-FGF4-BFGF all had smaller LVESD (6.43 ± 0.19 mm vs. 5.18 ± 0.17 mm, 5.07 ± 0.12 mm and 4.03 ± 0.19 mm, $p < 0.05$ among groups) and LVEDD (7.40 ± 0.13 mm vs. 6.63 ± 0.12 mm, 6.57 ± 0.11 mm and 5.85 ± 0.10 mm, $p < 0.05$ among groups), higher LVFS (13.02 ± 2.80% vs. 21.95 ± 1.96%, 22.84 ± 1.81%, and 30.99 ± 3.24%, $p < 0.05$ for all comparison), and higher LVEF (26.93 ± 5.33% vs. 43.27 ± 3.30%, 44.79 ± 2.88% and 57.75 ± 4.63%, $p < 0.05$ among groups). Moreover, the improvement of these cardiac indices in Group-FGF4-BFGF was more prominent than that in Group-BMSC or Group-BFGF ($p < 0.05$ for these comparisons) (Fig. 4B).

4. Discussion

In this study, we first recombined a lentiviral vector with the fused genes of FGF4-bFGF and successfully modified BMSCs with the vector. We confirmed that the bFGF secretion in BMSCs was significantly enhanced by the gene modification with FGF4-bFGF but not bFGF alone. Our study also demonstrated that implantation of FGF4-bFGF modified BMSCs could further improve the regional and global LV function via reducing myocardial fibrosis and enhancing angiogenesis in the infarcted zone in a rat model, as compared to transplantation of unmodified BMSCs, or BMSCs modified solely by bFGF gene. Such enhanced therapeutic benefits might be attributed mainly to the fused gene of FGF4-bFGF through its functions of not only co-expression but also secretion of bFGF. To the best of our knowledge, this is the first study to confirm the promoted bFGF expression and secretion by modifying BMSCs with the fused gene of FGF4-bFGF.

BMSC transplantation is generally recognized to be the most promising stem cell therapy in treatment of ischemic heart disease due to its advantages of self-replicating ability, multi-directional differentiation potential, easily obtaining, no immune rejection, and no ethical conflicts [14,15]. However, simple BMSC transplantation has limitations because of limited survival and poor differentiation or maturation of the transplanted cells [4,16]. More importantly, the adverse host micro-environment in infarction foci such as hypoxia and ischemia, ischemia–reperfusion injury with subsequent excessive inflammation in acute or subacute phase, and local structural alterations (e.g. myocardial fibrosis and micro-vascular damage) in chronic phase is not favorable to transplanted cell surviving [14,15,17]. Accordingly, it is desirable to explore a newly approach that modify not only the donor cells but the host micro-environment as well.

As a potent growth factor, bFGF has pleiotropic biological effects, such as promotion of cell division and differentiation,

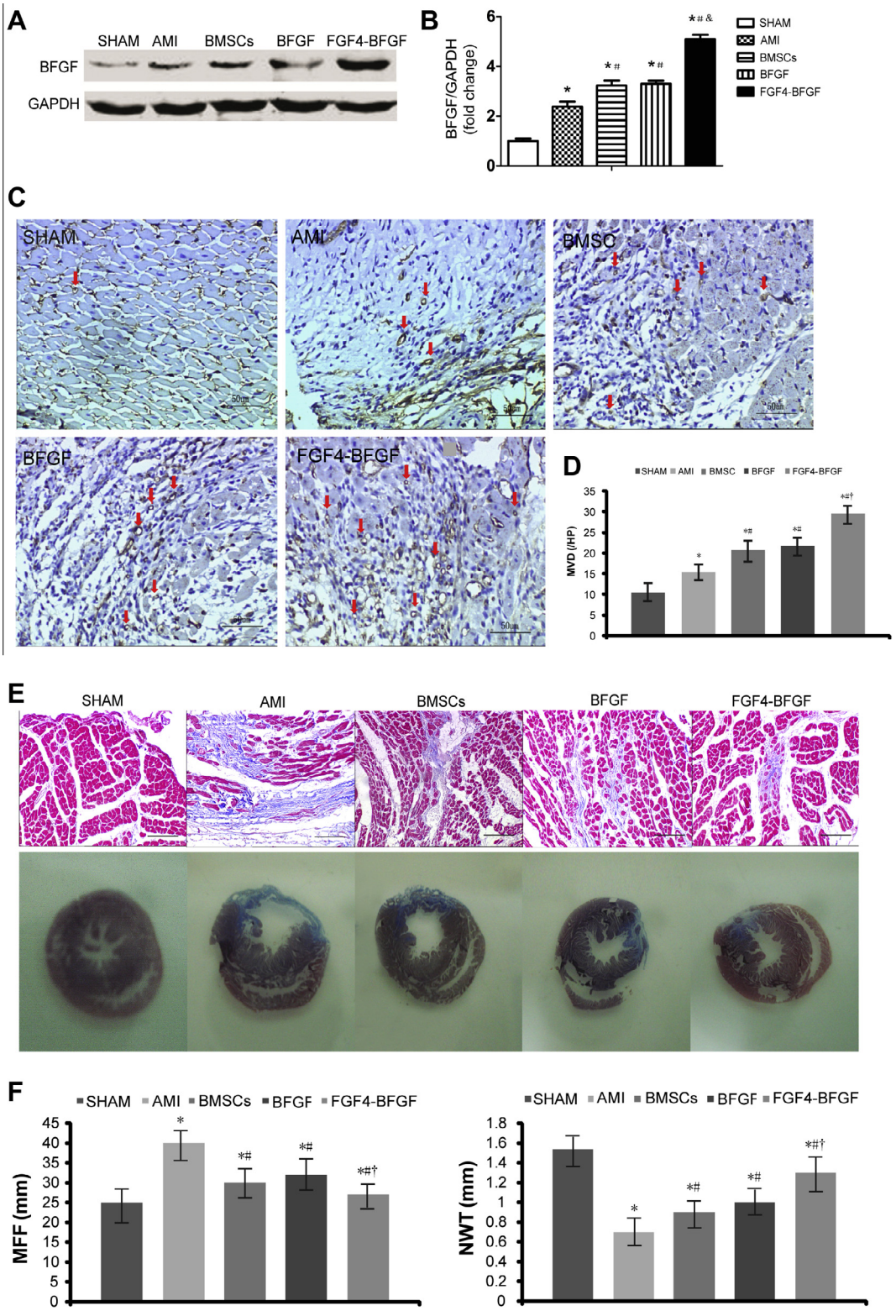

Fig. 3. While the FGF4-bFGF fusion gene modified the BMSCs, the modification caused the bFGF expression, myocardial fibrosis and angiogenesis changes. (A) The myocardial expression of bFGF was detected using Western blotting. (B) The myocardial expression level (MEL) of bFGF was quantitatively analyzed by densitometry (lower lanes). $^{*}p < 0.05$ as compared to Group-AMI; $^{\#}p < 0.05$ as compared to Group-BMSC; $^{\dagger}p < 0.05$ as compared to Group-BFGF. (C) The myocardial microvascular density (MVD, n/HP) was evaluated with immunohistochemistry. (D) Data were presented as the mean ± SD. $^{*}p < 0.05$ as compared to Group-AMI; $^{\#}p < 0.05$ as compared to Group-BMSC; $^{\dagger}p < 0.05$ as compared to Group-BFGF. (E) The myocardial fibrotic fraction (MFF, %) of the whole left ventricular sectional ring was evaluated with Masson's staining. (F) Data were presented as the mean ± SD. $^{*}p < 0.05$as compared to Group-AMI; $^{\#}p < 0.05$ as compared to Group-BMSC; $^{\dagger}p < 0.05$ as compared to Group-BFGF.

maintenance of cell survival, stimulation of angiogenesis and collateral vessel formation with increasing myocardial perfusion, anti-apoptosis of stem cells, and enhancement of chemotaxis of BMSCs [18–22]. Therefore, modification with bFGF gene may promote the survival of donor cells and improve the host micro-environment as well. Unfortunately, as a non-secreted protein,

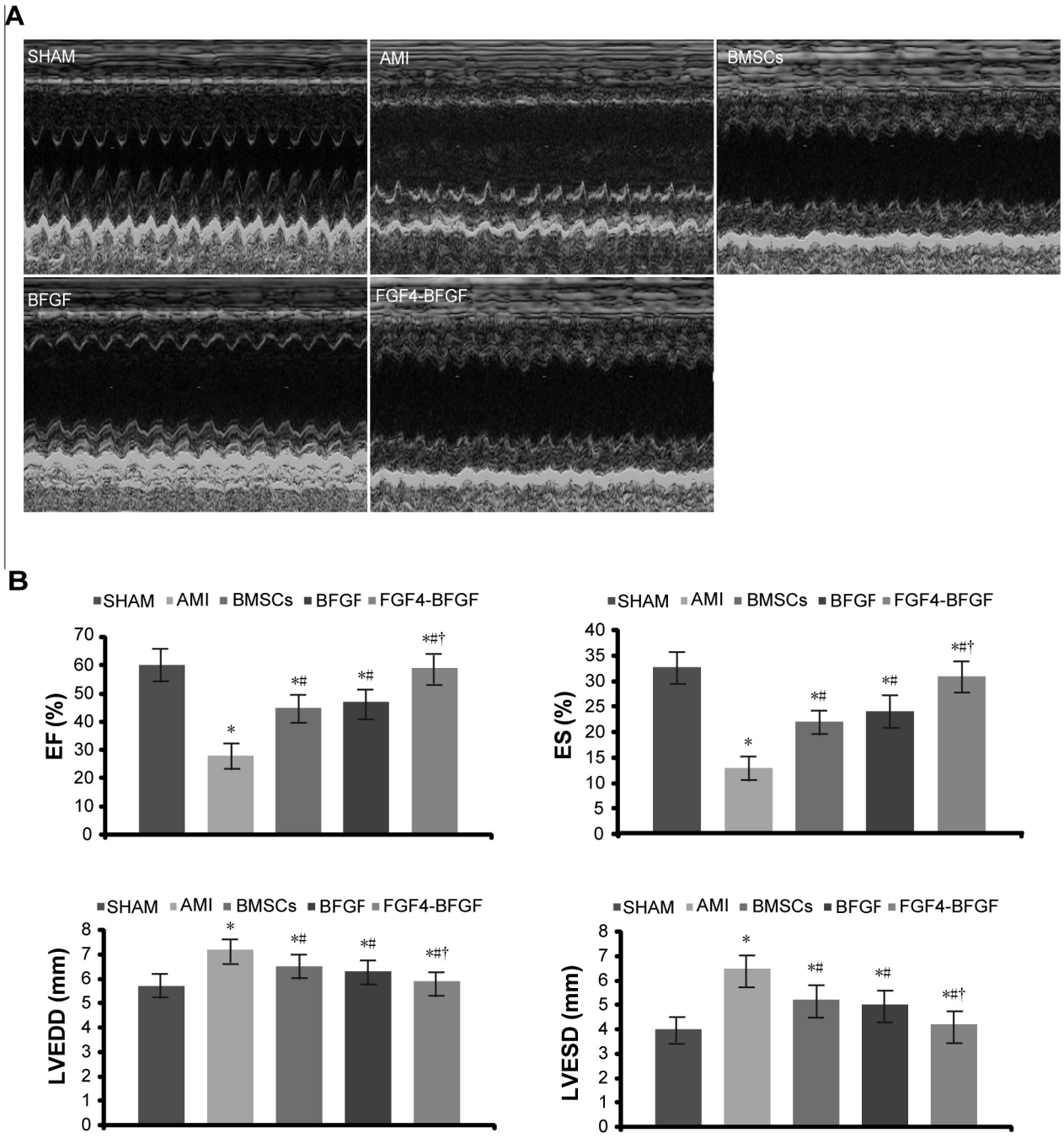

Fig. 4. Improved cardiac performance after transplanting the BMSCs modified with FGF4-bFGF fusion gene. (A) Typical echocardiograms and cardiac performance from various treatments. (B) The left ventricular function parameters (LVFS, %; LVEF, %; LVEDD, %; LVEDS, %) were measured echocardiographically 28 days after the cell transplantation. $^{*}p < 0.05$ as compared to Group-AMI; $^{\#}p < 0.05$ as compared to Group-BMSC; $^{\dagger}p < 0.05$ as compared to Group-BFGF.

bFGF exists only in the cytoplasm of live cells and cannot be secreted into the extracellular space to promote cell growth [8]. This is a key barrier to be overcome. In present study, we first recombined bFGF gene with FGF4 gene, a pro-secretion petite gene of bFGF, to produce a fused gene of FGF4-bFGF, and then transferred it into lentivirus in order to obtain a stable and high efficient gene transfection and expression. Our *in vitro* data show that we had successfully recombined a lentiviral vector with the fused gene and effectively modified BMSCs, as evidenced by that the fluorescence intensity of BMSCs modified with the fused gene became similar to that of BMSCs infected with empty lentivirus, and also by that the bFGF expression and secretion in the BMSCs modified with the fused gene was much higher than that in pure BMSCs or BMSCs modified with bFGF gene only.

To further explore the roles and mechanisms of the BMSCs modified with FGF4-bFGF fused gene after transplanting them into myocardial infarction zone, the survival of transplanted cells, fibrosis and angiogenesis and bFGF expression in the myocardium were all investigated *in vivo*. As compared with Group-AMI, Group-BMSC or Group-BFGF, the major findings were: (1) Group-FGF4-BFGF had a higher survival rate of the transplanted cells as shown in immunohistochemistry and confocal laser scanning fluorescence microscopy, evidenced by the large number of transplanted cells migrating and gathering in the myocardial infarction zone and

the surrounding area. The survival rate of the transplanted cells was similar in Group-BMSC and Group-BFGF. (2) Group-FGF4-BFGF had a smaller infarcted area with less (usually patchy) myocardial fibrosis, regional wall thinning and global LV dilatation, whereas the pathological change was indifferent between Group-BMSC and Group-BFGF. (3) The microvascular density in Group-FGF4-BFGF was the highest among all groups. The microvascular density in Group-BFGF, though higher than that in Group-AMI, was similar to Group-BMSC. (4) The myocardial expression level by Western blotting in Group-FGF4-BFGF was the highest; while the myocardial expression level in Group-BFGF, though higher than that in Group-AMI, was similar to Group-BMSC. Accordingly, it is reasonable to postulate that genetically modified BMSCs with FGF4-bFGF was able to express bFGF with high efficiency and secret it freely into the extracellular space to promote cell division and growth as well, a crucial role that can be transferred not only to promote the survival of the transplanted cells (seed modification), but also to diminish myocardial fibrosis and enhance myocardial angiogenesis in the infracted zone (soil modification).

Although both *in vitro* and *in vivo* data have demonstrated BMSCs modified with the fused gene of FGF4-bFGF had above-mentioned capabilities, it remained unclear whether such abilities could be translated into the therapeutic benefits. Our echocardiographic data showed that Group-FGF4-BFGF had less LV structural and functional impairment, as characterized by less regional wall thinning, less segmental wall motion abnormality and less global LV dilatation as compared to Group-AMI. We also found that the LV structural and functional impairment in Group-BFGF, although less than that in Group-AMI, was similar to Group-BMSC. Therefore, transplantation of BMSCs could diminish myocardial fibrosis and preserve the LV function, and such therapeutic benefits could be further enhanced by genetically modified BMSCs with FGF4-bFGF instead of bFGF only.

In conclusions, in comparison with transplantation of BMSCs only or BMSCs modified with bFGF gene only, the BMSCs modified with the FGF4-bFGF fused gene showed extra abilities to express and secret bFGF more efficiently. This modification could further enhance the survival of the cells after being transplanted into infarcted myocardium, diminish myocardial fibrosis, promote myocardial angiogenesis, and thus improve cardiac function.

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