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Transplantation with survivin-engineered mesenchymal stem cells results in better prognosis in a rat model of myocardial infarction

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Aims To investigate the effect of survivin (SVV)-engineered mesenchymal stem cells (MSCs) on post-infarction cardiac performance and remodelling in rats.

Methods and results Mesenchymal stem cells from male Sprague–Dawley rat bone marrow were infected with the self-inactive lentiviral vector GFP-wre-CMV/LTR and Flap-Ubiqutin promoter (GCFU) carrying green fluorescent protein (GFP) gene and SVV recombinant vector (GCFU-SVV). *In vitro*, modification with SVV increased the secretion of vascular endothelial growth factor (VEGF) by 1.28-fold under hypoxic conditions. *In vivo*, after permanent left anterior descending artery occlusion, rats were randomized ($n = 18$ per group) to receive intra-myocardial injections of 100 μL of phosphate-buffered saline without cells (group vehicle) or containing 2 million MSC_{GFP} (group MSC_{GFP}) or MSC_{SVV} (group MSC_{SVV}) cells. Cellular survival assessed by reverse transcriptase–polymerase chain reaction for GFP in the MSC_{SVV} group was 2.5-fold higher at 7 days and 4.3-fold higher at 28 days after transplantation than in the MSC_{GFP} group. When compared with transplantation with MSC_{GFP}, transplantation with MSC_{SVV} further upregulated VEGF expression at 7 and 28 days after myocardial infarction (MI), increased capillary density by 38%, reduced the infarct size by 12.7%, significantly inhibited collagen deposition, and further improved cardiac function at 28 days after MI.

Conclusion Transplantation with SVV-engineered MSCs by lentiviral vector leads to better prognosis for MI by enhancing cellular survival.

Keywords Myocardial infarction • Mesenchymal stem cells • Gene therapy • Survivin • Lentiviral vector

Introduction

Transplantation with mesenchymal stem cells (MSCs) has been extensively investigated as a potential therapeutic strategy for myocardial infarction (MI). However, only marginal recovery of cardiac function has been achieved because of considerable cell death following transplantation into the infarcted myocardium.[1,2] In recent years, some groups have demonstrated that gene modification can enhance the survival of transplanted MSCs, which in turn results in more successful functional recovery in the setting of animal experiments.[3,4] However, a non-viral vector was used for the modification of MSCs in these studies. This may be insufficient to maximize the benefits of stem cell-based therapy because of the poor transduction efficiency of non-viral vectors, which has been identified in a comparative study of viral and non-viral vectors used for gene transfer into rat MSCs.[5] Recently, Mangi *et al.*[6] have reported that a direct intra-myocardial injection of 5×10^6 Akt-engineered MSCs by a retroviral vector almost normalized post-infarction cardiac function. The lentiviral vector, as a class of retroviral vector, possesses some unique advantages, in particular, an ability to transduce both dividing and non-dividing cells. Furthermore, lentiviral vector has lower risk of

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replication-competent recombinant propagation than conventional retroviral vector.[7] Thus, the lentiviral vector may be a better alternative and has been successfully used for transgenic delivery into stem cells.[8,9] In addition, the 16.5 kDa survivin (SVV) protein, an X-linked inhibitor of apoptosis (XIAP) family member, possesses broadly cytoprotective actions.[10–12] The SVV gene has been found to be upregulated in infarcted and failing hearts and to prevent programmed cell death of ventricular myocytes in previous studies.[13,14]

Thus, we generated SVV-engineered MSCs with a lentiviral vector and evaluated cell survival, angiogenesis, collagen deposition, and functional improvement in a rat model of MI.

Methods

Generation of GCFU-survivin plasmid and lentivirus production

Detailed methodology is shown in the Supplementary material online.

Animals

Male Sprague–Dawley rats (80 and 200 g) were used with the approval of the Animal Care and Use Committee, Fujian Medical University. In this study, the 80 g rats were used for cell isolation, characterization, and transplantation,[15] and the 200 g animals were used for the *in vivo* study. The investigation conformed to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication, No. 86-23, revised 1985). The investigators responsible for molecular, histological, and functional studies were blinded to the treatment groups.

Cell isolation, culture, and identification

Mesenchymal stem cells were prepared from the bone marrow of rats as described by Friedenstein *et al.*[16] All MSCs were cultured in low-glucose Dulbecco's modified Eagle's medium containing 10% foetal bovine serum (Gibco). The non-adherent cells were removed by medium changing at 24 h and every 72 h thereafter. The passage 3 MSCs were analysed by fluorescence-activated cell sorter (Beckman Coulter) for their surface marker expression. Briefly, after blocking for non-specific binding with buffer containing 1% bovine serum albumin, the cells were incubated for 20 min at 4°C with the following antibodies: anti-CD44.FITC, anti-CD45.FITC, anti-C90.FITC, anti-CD14.FITC (AbD Serotec), anti-CD34.PE, and anti-CD29.PE (Biolegend). The matched isotype controls were purchased from AbD Serotec or Biolegend. At least 1×10^4 cells per sample were acquired and analysed.

Gene modification of mesenchymal stem cells

The passage 1 MSCs were infected with lentivirus at multiplicity of infection of 8 (as detailed in the Supplementary material online). The MSCs infected with SVV recombinant lentivirus were named as MSC_{SVV}, and the MSCs infected with mock lentivirus were named as MSC_{GFP} (GFP—green fluorescent protein). To achieve optimal gene transfer, polybrene (a final concentration of 8 μg/mL) was used. All MSCs were expanded to passage 3 and then used for *in vitro* and *in vivo* studies. The efficiency of gene transduction was assessed with FASC.

Paracrine effects of mesenchymal stem cells under hypoxic conditions

Cells were planted in 6 cm plates at a primary density of 1×10^6 per dish (triplicates per dish). After complete adherence, cells were incubated for 24 h at 37°C in a humidified modular hypoxia chamber (Billups Rothenberg) containing 95% nitrogen and 5% carbon dioxide. Subsequently, the supernatants were collected for the assay of vascular endothelial growth factor (VEGF) with an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the instructions of the supplier. Such experiments were repeated three times, and all samples and standards measured in duplicate.

Surgical procedures

Myocardial infarction was generated as described previously.[17] Briefly, rats were anaesthetized using 10% chloral hydrate (4 mL/kg) by intraperitoneal injection, and a left thoracotomy was performed under backup ventilation. The heart was exposed through the fifth intercostal space, the pericardial sac was cut, and the left anterior descending coronary artery (LAD) was permanently occluded. Immediately afterwards, the animals ($n = 18$ per group) were randomized to receive intra-myocardial injections of 100 μL of phosphate-buffered saline (PBS) without cells (group vehicle) or containing 2×10^6 MSC_{SVV} (group MSC_{SVV}) or MSC_{GFP} (group MSC_{GFP}). The intra-myocardial injections were performed at five sites in the ischaemic region of the left ventricle 60 min after ligation. Six rats per group were sacrificed under anaesthesia 7 and 28 days after cell transplantation. The heart tissue below the occlusion site was collected and transversely cut into three parts. The middle-level tissue samples (2 mm thickness) were embedded in OCT medium for the preparation of frozen sections. Six thin (8 μm) and then one thick (100 μm) heart sections were cut consecutively for histological study. After removing the right ventricular myocardium, the remaining heart tissue was used for the preparation of homogenate and further used for molecular study. All tissue samples were prepared identically. Another six rats in each group were used for the assessment of cardiac function.

Reverse transcriptase–polymerase chain reaction for green fluorescent protein and human survivin

Total RNA was extracted from the cardiac tissue of rats 7 and 28 days after cell transplantation, with TRIZOL reagent (Invitrogen). First-strand cDNA synthesis was carried out using primer oligo(dT) and reverse transcription with Superscript II (Invitrogen) in accordance with the manufacturer's recommended conditions.

Human SVV (255 bp)	forward 5′-CTGAGAACGAGCCAGACTTG-3′, reverse 5′-GGCGCACTTTCTCCGCAGTTT-3′
GFP (397 bp)	forward 5′-CCCACCCTCGTGACCACCCT-3′, reverse 5′-CGCCGATGGGGGTGTTCTGC-3′
Rat GAPDH (738 bp)	forward 5′-TTCTTGTGCAGTGCCAGCCTCGTC-3′, reverse 5′-TAGGAACACGGAAGGCCATGCCAG-3′

Polymerase chain reaction (PCR) conditions were: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, 30 cycles for human SVV; denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 1 min, 32 cycles for GFP; denaturation at 94°C for 30 s, annealing at 58°C for 50 s, and extension at 72°C for 1 min, 22 cycles for GAPDH. Polymerase chain reaction products were subjected to electrophoresis on 1.5%

agarose gels. All bands from reverse transcriptase (RT)–PCR were analysed using ImageJ analysis software (version 1.6; NIH) to verify the relative level of GFP or SVV expression defined as the optical density ratio of GFP or SVV over GAPDH.

Immunofluorescence

To ensure that the transplanted MSCs had enough time to differentiate into cardiomyocyte-like cells, the thin sections from hearts of 28-day rats were used for assessing cellular differentiation. Briefly, frozen tissue sections were co-incubated with a goat anti-GFP antibody conjugated with FITC (AbCam) and a rabbit anti-cTnI primary antibody (Santa Cruz) overnight at 4°C. On the following day, the sections were washed with PBS and then incubated with a mouse anti-rabbit secondary antibody conjugated with TEXAS-red (Santa Cruz) at 37°C for 30 min. Finally, nuclei were labelled with DAPI (Sigma). These sections were scanned using a laser confocal scanning microscope (Zeiss Co., LSM510) to identify differentiation of MSCs into cardiomyocyte-like cells or fusion with the host cardiomyocytes.

Myocardial vascular endothelial growth factor level after cell transplantation

Proteins were extracted from tissue samples of 7- and 28-day rats, and their concentrations were determined by bicinchoninic acid protein assay (Beyotime Biotechnology, P.R. China). Subsequently, protein (100 μg) was loaded on a 12% of SDS–polyacrylamide gel for electrophoresis and then transferred onto polyvinylidene fluoride membrane (Invitrogen). Western blot was performed with a 1:1000 dilution of rabbit anti-VEGF antibody (Santa Cruz) and a 1:2000 dilution of horseradish peroxidase-conjugated anti-rabbit secondary antibody. β-Tubulin was used as an internal control. Finally, the results of western blot were analysed using the same method as that for RT–PCR.

Evaluation of infarct size, immunohistochemical studies, and collagen deposition

At least six thin tissue sections from each heart of 28-day rats were stained by Masson's trichrome for visualizing the thickness of the left ventricle wall and further analysing the infarct size with ImageJ analysis software (version 1.6; NIH) as described previously.[18] Thin tissue sections from the hearts of rats 28 days after cell transplantation were also immunostained using a specific antibody for von Willebrand factor (vWF) (rabbit anti-vWF, Santa Cruz) and detected with a goat anti-rabbit secondary antibody (Santa Cruz) and visualized with AEC kit. The number of vWF-positive blood vessels was counted in the infarct zone. Collagen deposition of 28-day hearts was detected by a laser confocal scanning microscope on the basis of the theory of the second harmonic generation (SHG) image.[19,20] During this procedure, a sapphire femtosecond laser (Zeiss Co.) was used for the detection of SHG signals from collagen in the infarcted myocardium. The following parameters were selected: an incident power of 3 mW to avoid the tissue specimen being photobleached, an excitation wavelength of 850 nm, and an emission wavelength of 420–430 nm.

Assessment of cardiac function by echocardiography

Transthoracic echocardiographic studies were performed by an experienced cardiologist at 28 days after cell transplantation on a GE Vivid Seven ultrasound machine (GE Medical System) with a 10.0 MHz transducer. The echo transducer was placed on the left hemithorax, and short-axis views were recorded. Two-dimensional images were obtained at the midpapillary level.[21,22] The M-mode tracing of left ventricular (LV) contraction was also obtained at the same level as the short-axis view. Left ventricular end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) were measured with the M-mode tracing. Left ventricular end-diastolic volume (LVEDV) was calculated as $7.0 \times \mathrm{LVEDD}^3/(2.4 + \mathrm{LVEDD})$, LV end-systolic volume (LVESV) as $7.0 \times \mathrm{LVESD}^3/(2.4 + \mathrm{LVESD})$, and LV ejection fraction (EF) as $\mathrm{EF}(\%) = (\mathrm{LVEDV} - \mathrm{LVESV})/\mathrm{LVEDV} \times 100$ (%). Two images were obtained in each view and each parameter was measured from 3 consecutive beats in each image.

Statistical analysis

For tissue specimens, data were averaged on at least six microscopic fields from each section and five sections from each heart. Capillary density was defined as the number of capillaries, and collagen deposition was defined as the peak value of SHG signal intensity (derived from an emission wavelength of 425 nm), per high-power magnification field (HPF) (×400), respectively. All data were expressed as mean ± SD. Differences among groups were compared using one-way ANOVA with Bonferroni Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Mesenchymal stem cells purification and efficiency of gene transduction

The isolated cells were 99.9% pure for CD29, 99.8% for CD44, and 100% for CD90. The contaminated population of haematopoietic stem cells positive to CD14, CD34, and CD45 was 0.7, 0.6, and 2.3%, respectively (see Supplementary material online, *Figure S3*). After infection with SVV recombinant lentivirus, >90% of MSCs overexpressed GFP, and the efficiency of gene transduction was similar to that of mock lentivirus (95.2 vs. 95.3%) (see Supplementary material online, *Figure S4*).

Cellular survival, differentiation, and survivin expression *in vivo*

The survival of the transplanted MSCs was quantified by assaying the relative level of GFP expression. A specific amplified band corresponding to an expected 397 bp PCR product of GFP was observed in group $\mathrm{MSC}_{\mathrm{SVV}}$ and group $\mathrm{MSC}_{\mathrm{GFP}}$ but not in group vehicle (*Figure 1A*). Semi-quantitative analysis showed that the relative level of GFP expression in group $\mathrm{MSC}_{\mathrm{SVV}}$ was ~2.5-fold higher (the ratio of optical density of GFP over GAPDH: 0.63 ± 0.08 vs. 0.25 ± 0.04, $P < 0.001$) at 7 days after MSCs transplantation and ~4.3-fold higher (0.53 ± 0.08 vs. 0.12 ± 0.02, $P < 0.001$) at 28 days after MSCs transplantation than that of group $\mathrm{MSC}_{\mathrm{GFP}}$. Although GFP expression at 28 days after MSCs transplantation was lower than that at 7 days in group $\mathrm{MSC}_{\mathrm{SVV}}$, the difference was not significant ($P = 0.056$) (*Figure 1C*). Long-term expression of human SVV *in vivo* was also assessed by RT–PCR with human SVV-specific primer. Thus, a specific amplified band corresponding to an expected 255 bp PCR product of human SVV was observed in group $\mathrm{MSC}_{\mathrm{SVV}}$ but not in group $\mathrm{MSC}_{\mathrm{GFP}}$ or group vehicle (*Figure 1B*). The change in the relative level of SVV expression (the ratio of optical density of GFP over GAPDH: 0.46 ± 0.07 at

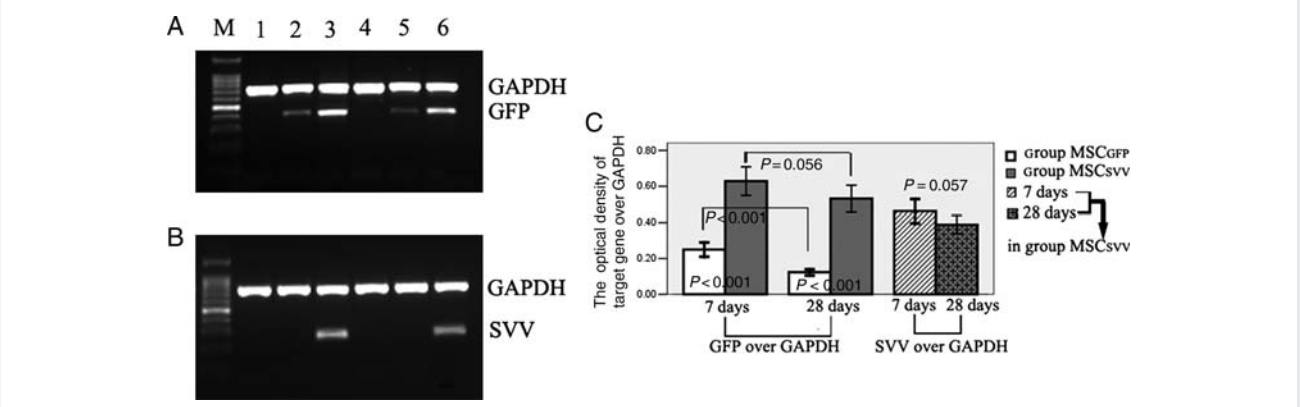

Figure 1 Green fluorescent protein (GFP) or survivin (SVV) expression after mesenchymal stem cell (MSC) transplantation. (*A* and *B*) Respective electrophoresis graph of PCR product of green fluorescent protein or survivin; M: 1000 bp DNA ladder; lane 1: 7 days in group vehicle; lane 2: 7 days in group MSC_{GFP}; lane 3: 7 days in group MSC_{SVV}; lane 4: 28 days in group vehicle; lane 5: 28 days in group MSC_{GFP}; lane 6: 28 days in group MSC_{SVV}. (*C*) Semi-quantitative analysis of green fluorescent protein or survivin expression *in vivo*.

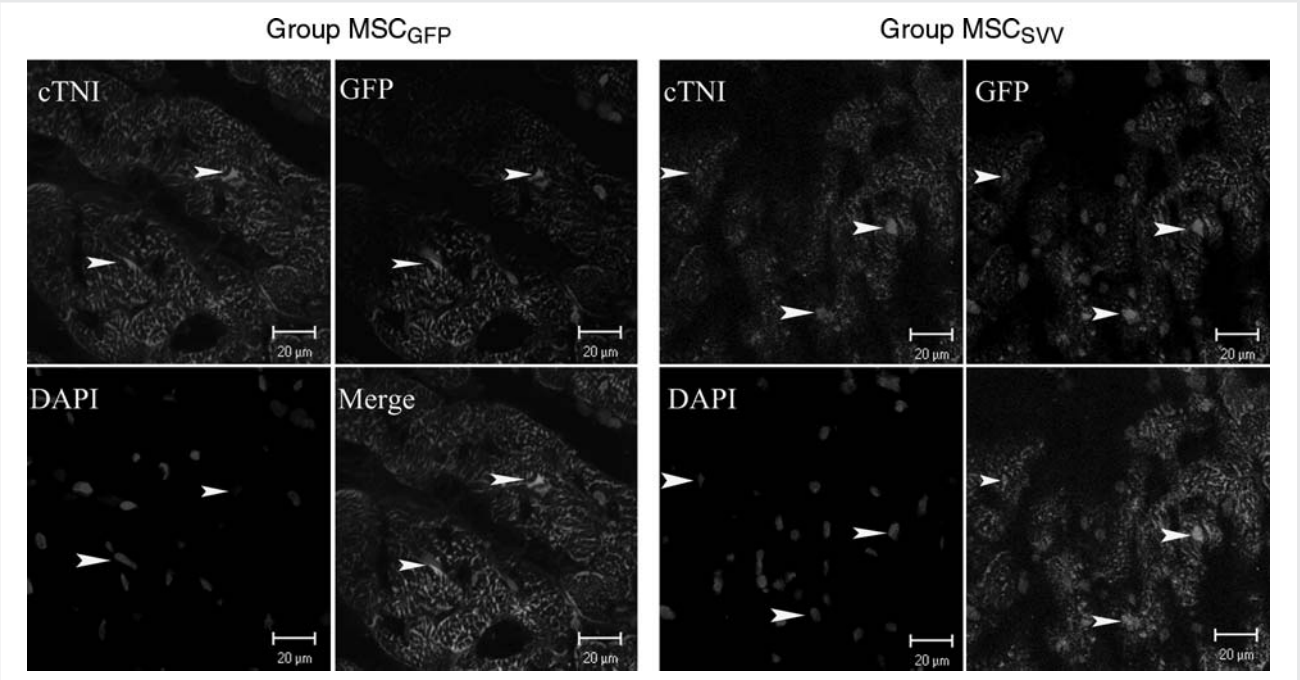

Figure 2 Confocal images of myocardial tissue sections from rats 28 days after mesenchymal stem cell (MSC) transplantation. There was no differentiation into cardiomyocyte-like cells but some mesenchymal stem cell fusion (white arrows) with host cardiomyocytes was observed in the two groups. Scale bar: 20 μm.

7 days after MSCs transplantation vs. 0.39 ± 0.05 at 28 days after MSCs transplantation, $P = 0.057$) was similar to that of GFP expression (*Figure 1C*). Furthermore, although at least 200 tissue sections from 1228-day hearts were examined thoroughly, very few transplanted MSCs (only ~1–2%) were found with co-expression of cTnI and GFP, with cells inlaid into the host cardiomyocytes. Conversely, most transplanted MSCs were located around the injured cardiomyocytes and some were attached to the host cardiomyocytes, with irregular spherical phase, which resembled that before transplantation (*Figure 2*).

Survivin-engineered mesenchymal stem cells enhanced secretion of mesenchymal stem cells for vascular endothelial growth factor *in vitro* and expression of vascular endothelial growth factor *in vivo*

In vitro, there was no difference in VEGF concentration between group MSC_{GFP} and group uninfected MSCs (710.3 ± 80.8 vs. 739.9 ± 86.0 pg/mL, $P > 0.05$). But overexpression of SVV increased the secretion of VEGF by over one-fold (1620.3 ± 127.4 pg/mL, $P < 0.001$) when compared with group MSC_{GFP} and group uninfected MSCs. *In vivo*, when compared with transplantation with MSC_{GFP}, SVV-engineered MSCs upregulated expression of VEGF in the ischaemic myocardium at all time points, especially at day 7 after transplantation ($P < 0.001$). Although transplantation with MSC_{GFP} also upregulated VEGF at 7 days after the induction of MI ($P < 0.05$), there was no difference in the level of VEGF at 28 days after surgery compared with group vehicle (*Figure 3*).

Survivin-engineered mesenchymal stem cells reduced infarct size and enhanced angiogenesis

In contrast to significant thinning of the LV free wall in group vehicle, the thickness of the wall was maintained better in group MSC_{GFP} and especially in group MSC_{SVV} (*Figure 4A*). The infarct size of group MSC_{SVV} ($22.5 \pm 2.7\%$) was significantly less than that of group MSC_{GFP} ($35.2 \pm 2.6\%$, $P < 0.01$) and group vehicle ($41.8 \pm 4.6\%$, $P < 0.001$) despite the fact that infarct size was reduced in group MSC_{GFP} when compared with group vehicle ($P < 0.05$) (*Figure 4C*). The angiogenic effect of SVV-engineered MSCs was determined by immunohistochemical staining for vWF. Capillary density observed in the infarct zone in group MSC_{SVV} (35.0 ± 4.5 per HPF, $P < 0.01$) and group MSC_{GFP} (25.3 ± 2.6 per HPF, $P < 0.05$) was significantly higher than that of group vehicle (20.3 ± 2.3 per HPF). When compared with group MSC_{GFP}, MSC_{SVV} transplantation increased capillary density by ~38% ($P < 0.05$) (*Figure 4B* and *D*).

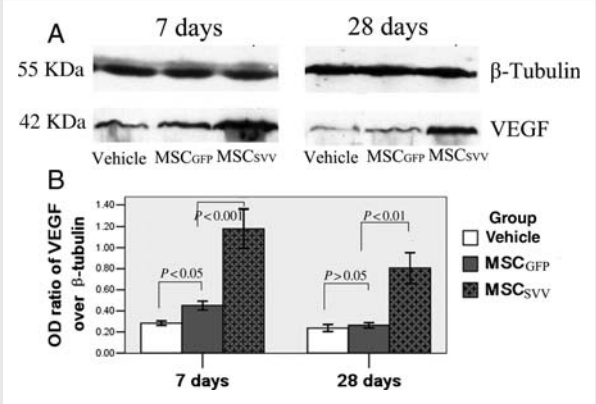

Figure 3 Transplantation with survivin (SVV)-engineered mesenchymal stem cells (MSCs) further upregulated protein expression of vascular endothelial growth factor (VEGF) in ischaemic myocardium. (*A*) Representative electrophoresis graph of vascular endothelial growth factor assayed by western blot. (*B*) Quantitative analysis for vascular endothelial growth factor.

Survivin-engineered mesenchymal stem cells attenuated collagen deposition

Severe collagen deposition was found in group vehicle. In contrast to group MSC_{GFP}, transplantation with MSC_{SVV} further inhibited myocardial fibrosis (*Figure 5A–C*). Collagen deposition was further quantified by the signal intensity of SHG. Thus, the signal intensity of SHG in group vehicle was 961.2 ± 122.3 a.u. per HPF, and higher than that of group MSC_{GFP} (709.3 ± 62.2 a.u. per HPF, $P < 0.05$) and group MSC_{SVV} (119.3 ± 10.0 a.u. per HPF, $P < 0.001$). The signal intensity of SHG in group MSC_{SVV} was significantly lower than in group MSC_{GFP} ($P < 0.01$) (*Figure 5D*).

Survivin-engineered mesenchymal stem cell transplantation further improved cardiac function

Twenty-eight days after cell transplantation, LVESV and LVEF showed significant improvements in group MSC_{SVV} (0.15 ± 0.04 mL, $P < 0.01$; $69.1 \pm 3.3\%$, $P < 0.01$) and in group MSC_{GFP} (0.40 ± 0.05 mL, $P < 0.01$; $44.1 \pm 4.3\%$, $P < 0.01$) when compared with group vehicle (0.58 ± 0.03 mL, $33.6 \pm 3.5\%$). There were also significant differences in LVESV and LVEF between group MSC_{SVV} and group MSC_{GFP} ($P < 0.01$). Left ventricular end-diastolic volume was significantly reduced in group MSC_{SVV} (0.49 ± 0.09 mL, $P < 0.01$) and group MSC_{GFP} (0.72 ± 0.09 mL, $P < 0.05$) compared with group vehicle (0.87 ± 0.04 mL), with a lower trend in group MSC_{SVV} compared with group MSC_{GFP} ($P < 0.01$). These data clearly demonstrate that SVV-engineered MSCs transplantation can improve cardiac performance.

Discussion

In this study, we showed that overexpression of SVV promoted MSCs survival in the infarcted myocardium and also enhanced the secretion effect of MSCs for VEGF *in vitro* and *in vivo*. This led to angiogenesis in the infarcted myocardium and ultimately reduced the infarct size, inhibited myocardial remodelling, and resulted in substantial recovery of cardiac function after MI.

Previous studies have demonstrated that donor cell death after transplantation is a major negative factor impacting on the outcome of cell therapy for MI.[1,2] Several major factors may contribute to implanted cell death, including loss of survival signals from cell–cell contact, bouts of intensive inflammatory response, various proapoptotic or cytotoxic factors derived from ischaemia or ischaemia/reperfusion, and high sensitivity of the implanted cells to the hypoxic and inflammatory environment in the infarcted myocardium.[2,4,23,24] Hence, modification of stem cells aimed at circumventing these various negative factors is important for enhancing the efficacy of cell therapy for MI. Although the modification of MSCs with some genes (such as Bcl-2,[3] HO-1,[4] and AKt[6]) can enhance the survival of the implanted MSCs in the infarcted

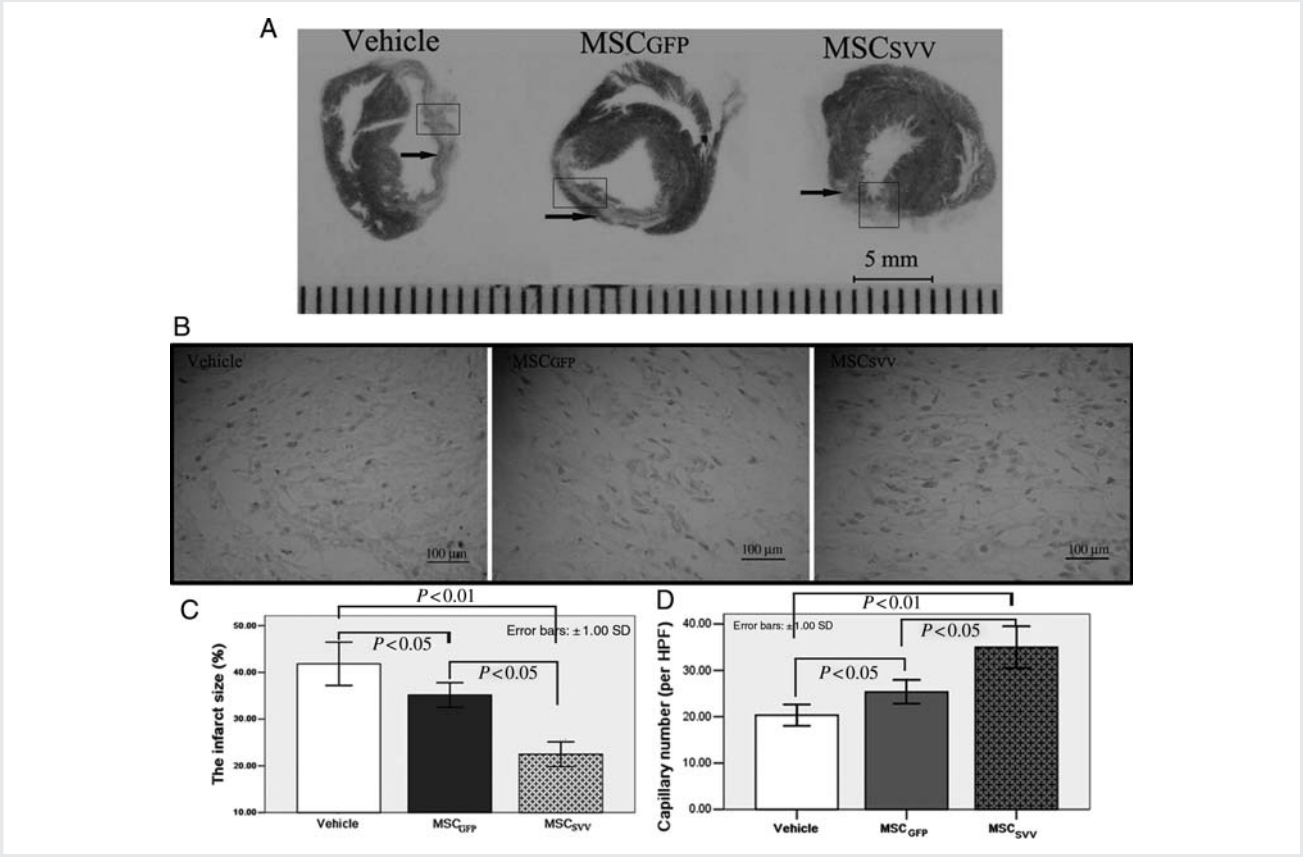

Figure 4 Transplantation with survivin (SVV)-engineered mesenchymal stem cells (MSCs) reduced the infarct size and collagen deposition. (*A*) Thinning of the left ventricular wall (indicated by the black arrow) and enlarging of the left ventricular chamber were noted in group vehicle and group MSC_{GFP} but not in group MSC_{SVV} at day 28 after left anterior descending coronary artery ligation. Rectangular marker in (A) indicated as a region that was chosen for vessel quantification. (*B*) Representative photomicrographs of the infarct zone obtained after immunostaining for vWF antibody. (*C*) Quantitative analysis for the infarct size on the basis of Masson staining. (*D*) Quantitative analysis for capillary density on the basis of vWF immunostaining. Scale bar: 100 μm.

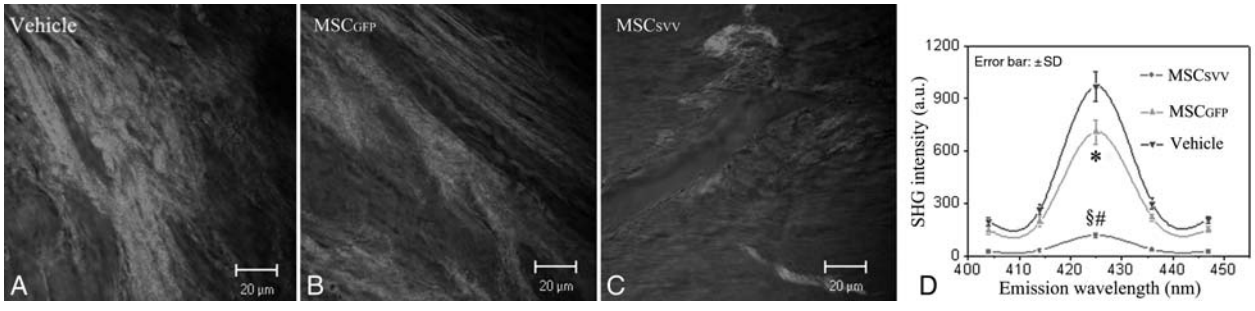

Figure 5 Transplantation with survivin (SVV)-engineered mesenchymal stem cells (MSCs) further inhibited collagen deposition. (*A*–*C*) The second harmonic generation (SHG) imaging for collagen (green) in the infarct zone at 28 days following left anterior descending coronary artery dilation. (*D*) Quantitative analysis for the peak value of second harmonic generation signal intensity (derived from an emission wavelength of 425 nm). $^{*}P < 0.05$ vs. group vehicle, $^{\S}P < 0.001$ vs. group vehicle, $^{\#}P < 0.01$ vs. group MSC_{GFP}.

myocardium, their actions against donor cell death appear to be powerful only at the early stage after transplantation. For instance, manipulation of MSCs with Bcl-2 only resulted in a 1.2-fold increase at week 6 after transplantation,[3] and even, modification with AKt did not lead to much more of an increase in MSCs survival at day 28 after transplantation.[6] These strategies for short-term survival may be insufficient to protect MSCs against loss of nutrition resulting from myocardial scarring at the late stage of

MI and to obtain maximal functional recovery. The results from semi-quantitative analysis with RT–PCR for GFP in our study showed that modification with SVV by a lentiviral vector increased MSCs survival at the early stage and, importantly, at the later stage after transplantation. The biological characteristics of the lentiviral gene delivery system and the SVV gene may contribute to the long-term survival of MSC_{SVV} in the infarct environment. First, the results from detection of SVV mRNA with RT–PCR confirmed that the lentiviral gene delivery system possesses a capacity to integrate the exogenous gene into the host genome and ensures stable and long-term expression of the SVV gene. Second, SVV possesses powerful direct anti-apoptotic action by binding to caspase-3 or casepase-7[12] and upregulates expression of heat shock protein 70 and inhibitors of multiple apoptotic pathways (e.g. BTG2, TIA1, DUSP1) and in turn indirectly protects target cells from apoptosis or death.[10] The present data also confirm that transplantation with SVV-engineered MSCs could further limit infarct size, attenuate collagen deposition, and improve cardiac performance. The better prognosis could be related to the survival of more MSCs resulting from modification with SVV.

Theoretically, the survival of more MSCs would result in more myocardial regeneration, which has been demonstrated in previous studies,[1,3,4,25] to replace the infarcted tissue. However, no evidence of cellular differentiation was found in our observations. Inversely, the confocal images showed that very few transplanted MSCs co-expressed GFP and cTnI, and their morphous was not the same as the new cardiomyocyte-like cells' depicted in previous studies[3,4] but like that of MSCs before transplantation. Thus, it is likely that this phenomenon resulted from cellular fusion as described previously.[26–28] On the one hand, it may seem unfair that the absence of cellular differentiation is ascribed to a shortage of time, because all of the heart sections observed were from rats 28 days after MSC transplantation. On the other hand, although the role of cellular fusion in cardiac repair was not investigated further in this study, it is unreasonable that the recovery of cardiac function is ascribed to cellular fusion so readily. Thus, the paracrine effect of MSCs may contribute to cardiac repair, primarily. Here, we focused only on VEGF considering direct secretion of VEGF by MSCs and that SVV may indirectly increase expression of VEGF by upregulating the promoter of VEGF[10]; however, other cytokines are also associated with the paracrine effect of MSCs, such as basic fibroblast growth factor, hepatocyte growth factor, and angiopoietin-2.[29,30] To verify whether SVV-engineered MSCs could enhance secretion of VEGF, we conducted a preliminary *in vitro* experiment in which MSCs were cultured under hypoxic conditions. The results showed that the concentration of VEGF in conditioned medium from MSC_{SVV} was over one-fold higher than that from MSC_{GFP}. Furthermore, our results also demonstrated that expression of VEGF was significantly increased in the infarct regions treated with MSC_{SVV} from 7 to 28 days after cell transplantation. Thus, the enhancement of secretion of VEGF may be a major reason for the reduction in infarct size. First, the angiogenic effects of VEGF may contribute to the salvaging of ischaemic myocardium and the reduction of the infarct size by improving the blood supply. Second, VEGF could protect cardiomyocytes against ischaemia[31] to further prevent loss of cardiomyocytes in the infarct region. Third, VEGF may also induce smooth muscle cell differentiation and proliferation[32] to partially replace the infarct tissue. Finally, it cannot be excluded that the small-scale replacement of the infarct tissue with new cardiomyocytes derived from the differentiation of resident stem cells owing to improvements in the infarct micro-environment induced by high expression of VEGF may be involved in the reduction in infarct size. Ultimately, smaller infarct size resulting from enhanced secretion of VEGF derived from improved survival of transplanted MSCs owing to SVV modification could be translated into histo-structural and functional benefits, including reduced collagen disposition or cardiac remodelling, and better cardiac performance, in turn.

In terms of cell-based gene therapy, there does not seem to be much difference between the present strategy and previous studies.[3,4,6,17,22,23] However, SVV-engineered MSCs transplantation still has some advantages. First, modification with SVV successfully enhanced the angiogenic effects of MSCs with a capillary density of 35.0 ± 4.5 per 400-power magnification field in the infarct zone, which was not significantly lower than that resulting from modification with Akt and Ang-1 (on average 76 per 200-power magnification field in the infarct zone).[22] This suggests that single-gene modification may achieve a similar therapeutic effect as that of multi-gene modification if a suitable gene and a high-efficiency gene delivery system were selected. Importantly, in contrast to multi-gene modification, single-gene modification is technically simpler and may minimize the potential risk. Second, as a major index of cardiac remodelling, myocardial fibrosis is a key cause of post-infarction heart failure. Conventionally, Masson's trichrome staining or Sirius Red staining was used for the examination of myocardial fibrosis, and then collagen deposition was quantified using image analysis software. However, accurate quantification may be limited for poor specificity for collagen or the variability in the staining protocols.[20] In this study, collagen deposition was quantified by a laser confocal scanning microscope on the basis of SHG theory. During detection, it is only necessary to select suitable parameters including excitation and emission wavelength and laser power and then collect and analyse the SHG signal intensity of auto-fluorescence from the heart tissue specimen. Thus, accurate quantification for collagen deposition could be achieved without the pitfalls of conventional pathological techniques. Of course, we also recognize that there are some limitations in this study: (i) the effects of SVV on differentiation characteristics of MSCs and how SVV increases VEGF expression were not investigated; (ii) the observational time was not long enough to enable an evaluation of the safety of SVV-engineered MSCs transplantation, although no tumorigenesis due to modification with SVV was observed over almost 1 month.

In summary, engineering MSCs genetically by SVV using a lentiviral vector is an effective strategy for improving prognosis of MI in rats. This novel strategy of cell transplantation may be of significance for the treatment of similar diseases in humans in the future.

Supplementary Material

Supplementary material is available at *European Journal of Heart Failure* online.

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