Effects of Endothelial Progenitor Cells Used for Autograft Transplantation in Acute Myocardial Infarction Pig Model

Didi Wen, Jian Xu, Yi Huan, Mengqi Wei, Minwen Zheng

Department of Radiology, Xijing Hospital, Xi'an, China

Objective: Use magnetic resonance imaging (MRI) to explore the effects of superparamagnetic iron oxide (SPIO) nanoparticle-labeled endothelial progenitor cells (EPCs) in an acute myocardial infarction (AMI) pig model.

Materials and Methods: After the AMI pig models were established, SPIO-labeled EPCs were transplanted. Cardiac functions were evaluated by serial echocardiography immediately after transplantation and at 1-, 2-, 3-, and 4-weeks post-transplantation. 3T whole-body MRI examination (Siemens Magnetom TrioTim) was also performed using different sequences to assess the most ideal procedure for high-quality imaging. The fate of the EPCs after transplantation was investigated by histological analysis.

Results: Fifteen out of 27 pigs with AMI survived. After transplantation, SPIO-labeled EPCs were visualized on MRI scans as persistent, hypo-intense regions. At 4 weeks post-transplantation, the myocardial infarct size in Groups B1 and B2 was significantly decreased, and the ventricular posterior wall thickness at diastole and the ejection fraction were both improved compared to the control group. Furthermore, left ventricular fractional shortening had improved significantly ($p < 0.050$). Also, histology of SPIO-labeled EPC grafts showed that the myocardial infarct size decreased with time.

Conclusion: EPC therapy not only improved cardiac functions but also resulted in significantly decreased myocardial infarct size in an AMI pig model.

Key words Endothelial progenitor cells · Transplantation · Myocardial infarction · Superparamagnetic iron oxide · Magnetic resonance imaging.

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Corresponding author

Minwen Zheng, MD

Department of Radiology, Xijing Hospital,

127# West Changle Road, Xi'an, China

Tel: 86-29-84771938

Fax: 86-29-84773732

E-mail: zhengmw2007@163.com

Mengqi Wei, MD

Department of Radiology, Xijing Hospital,

127# West Changle Road, Xi'an, China

Tel: 86-29-84771938

Fax: 86-29-84773732

E-mail: weimengqi2008@163.com

INTRODUCTION

Acute myocardial infarction (AMI), an endpoint of complicated atherosclerotic plaques or thrombotic events, is a major cause of mortality worldwide [1]. The loss of viable myocardium during AMI serves as a main predictor for contractile ventricular dysfunction, the occurrence of acute complications, and the subsequent development of chronic heart failure. Therefore, a timely and accurate treatment of coronary occlusion and its sequelae is of particular importance for improving both survival and quality of life after AMI. AMI is commonly treated with medications or surgery, but the emergence of stem

cell-based therapeutic strategies may represent a promising outlook for patients with cardiovascular disease, especially in the setting of AMI [2-6]. In the traditional view, revascularization of ischemic tissue was thought to occur through the migration and proliferation of mature endothelial cells in nearby tissues, a process called "angiogenesis." However, mature endothelial cells are terminally differentiated and have low proliferative potential so their capacity to replace damaged endothelial cells and create new vessels is limited [7]. Thus, effective endothelial repair and angiogenesis appear to require the involvement of other angiogenic processes. There has been an accumulation of evidence in recent years indicating that the peripheral blood of adults contains bone marrow (BM)-derived cells with properties similar to those of embryonic angioblasts. These precursor cells have the potential to differentiate into mature endothelial cells and are

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collectively referred to as endothelial progenitor cells (EPCs).

EPCs were originally described in the late 1990s as BM-derived circulating immature cells involved in the process of vasculogenesis in adults [5]. The first pioneering papers inspired a large body of literature over the subsequent 15 years, providing a broad understanding of the identity, function, and regulation of EPCs. EPCs, as well as mature endothelial cells, are detectable in the peripheral circulation [8]. EPCs may appear in the circulation by detaching from activated or damaged vessels. An increase in circulating EPCs has been described in several pathologic conditions that involve vascular injury or instability, such as myocardial infarction and cancer [9,10]. Emerging evidence suggests that circulating EPCs may provide an endogenous repair mechanism to counteract ongoing risk factor-induced endothelial injury and therefore protect against the development of radiation pneumonitis [11]. Furthermore, many reports have demonstrated that cell therapy performed with culture-expanded EPCs can successfully promote neovascularization in ischemic tissue without co-administration of angiogenic growth factors [5]. Thus, due to their general involvement in the vascular health maintenance, EPCs have begun to be considered an integral component of the cardiovascular system [12].

Largely because of its high contrast-noise ratio (CNR), reduced operator dependence, and lack of ionizing radiation, magnetic resonance imaging (MRI) is effective in examining and tracking the distribution of transplanted cells *in vivo*. Tracking is enabled by labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles, which has a low risk of toxicity [13].

However, it is not clear whether the SPIO-labeled EPCs could be an important tool for the accurate diagnosis of microvascular obstruction and for following its evolution in clinical practice. The objective of the present study is to investigate whether and to what extent the labeled EPCs affect AMI.

MATERIALS AND METHODS

Ethics statement

All protocols using animals in this study were approved by the Institutional Animal Care and Use Committee of our institution and conformed to Institutional Guidelines for the Care and Use of Laboratory Animals (KY20182082-1).

Establishment of acute myocardial infarction pig models

All pigs were purchased and maintained under standard conditions according to institutional guidelines. Twenty-seven mature Chinese pigs, weighing between 30–35 kg were used for this study. Pigs were housed one animal per cage under pathogen-free conditions with 12 h dark-light cycles, temperature held between 20–24°C, and free access to sterilized pig chow.

All pig models were fasted for 4 hours before the interventional operation to create an acute myocardial ischemia/reperfusion injury. The pigs were pre-sedated with an intramuscular injection of stressnil (1 mL/kg) and midazolam (1 mL/kg). After induction of anesthesia with intravenous propofol (5 mg/kg) and endotracheal intubation, anesthesia was maintained with isoflurane (2.5%) in oxygen and a continuous rate of fentanyl infusion (3 mg/kg/hr). The pigs were mechanically ventilated with a tidal volume of 450 mL (respiratory rate 12/min).

In each pig, the right common femoral artery was exposed by a surgical cut down, and a 6 F introducer sheath was inserted into the artery followed by a bolus injection of heparin (2000 IU/kg) through the sheath. Coronary occlusion was induced by placing a 2.5×20 mm angioplasty balloon in the left anterior descending artery (LAD) distal to the second diagonal branch artery and then inflating the balloon to 4 atm. Sacculus was used to block the LAD for 90 min. A standard 12-lead electrocardiogram (ECG) was used to monitor ECG changes during the experiment. After 90 min, the balloon was deflated and removed. Angiograms were performed after balloon inflation and deflation to confirm coronary occlusion and coronary reperfusion, respectively. To prevent ventricular fibrillation, 2 mL of 2% lidocaine was administered through the sheath prior to the induction of malignant tachyarrhythmia throughout the experiment. If ventricular fibrillation occurred, non-synchronized direct current defibrillation was performed. In addition, ECG was used to examine left ventricular (LV) volume and wall movement during interventional operation, 2 weeks and 4 weeks after transplantation.

Cell culture and transplantation

EPCs labeled with SPIO were maintained at 37°C in a 5% CO₂ environment. After 24 hours of culture, the cells were collected by removing the free SPIO and washing three times with phosphate buffer solution (PBS). The samples were digested by trypsin, transferred into a tube, and then centrifugated at 1500 rpm for 15 min. Finally, the cell pellet was resuspended in saline at a concentration of 1×10^5 cells/mL. A 10 mL cell suspension was used for each transplantation animal.

The pigs with acute myocardial ischemia were divided into three groups by randomized block design. To determine the therapeutic efficacy of labeled EPCs in acute myocardial ischemia, the labeled EPCs (1×10^6 cells per pig) were suspended in 10 mL of saline and injected into the infarction zone through the coronary artery. In the first group (Group A), which served as the control group, 6 pigs were injected 1 hour after myocardial injury with the same amount of saline into the same region of the ischemic myocardium. In the second group (Group B1), 6 pigs were transplanted with the labeled EPCs 1 hour after myocardial injury. In the third group (Group B2), 6 pigs were trans-

planted with the labeled EPCs 1 week after acute myocardial ischemia. Cells were infused via an over-the-wire balloon catheter advanced into the stent, which was previously implanted during the acute myocardial ischemia procedure. The catheter was also inflated with low pressure to completely block blood flow for 3 minutes in order to allow adhesion and potential transmigration of the infused cells through the endothelium. This maneuver was repeated 3 times to accommodate infusion of the 10 mL progenitor cell suspension and interrupted by 3 minutes of reflow by deflating the balloon to minimize extensive ischemia. Needle dead space was cleared with saline before each needle retraction. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency and unimpeded flow of contrast material.

Echocardiography and cardiac performance

To assess the effects of EPC transplantation, the cardiac function of control and transplanted animals was evaluated by a series of echocardiography: immediately after transplantation (day 0) and at 1, 2, 3, and 4 weeks after transplantation. We used a VV7 color Doppler ultrasound developing-out unit equipped with a cardiac probe 7V3 and a high-frequency 6 MHz linear array transducer to evaluate cardiac performance. The end-diastolic and end-systolic dimensions of the left ventricle cavity, end-diastolic volume, and end-systolic volume of the left ventricle were recorded. The LV fractional shortening (LVFS, %), LV wall thickness, and the ejection fraction (EF, %) were calculated.

Magnetic resonance imaging of animal models undergoing autograft transplantation with SPIO-labeled EPCs *in vivo*

On day 0, 1, 7, 14, 21, and 28 after EPC therapy, MRI was performed using a clinical 3T whole-body MRI system (Siemens Magnetom Triotim, Erlangen, Germany) with a twelve-element phased array cardiac surface coil. All pigs were scanned in the left supine position. Peripheral or ECG gating was obtained using an *in vivo* Magnitude TM physiological monitor through a plethysmography trace placed on the pig's tail or through electrodes placed on the anterior chest wall of the animal. For this study, the MRI protocol included the routine and specific magnetic resonance (MR) sequences, T2-weighted True FISP Imaging of Myocardium and CV-3DRAD-400C, respectively (Siemens Magnetom Triotim). The routine MR heart sequence imaging parameters were: repetition time (TR)/echo time (TE)=649/52 ms; flip angle=160°; field of view (FOV)=380 mm; slice thickness=6 mm; matrix size=256×256; acquisition window=673 ms; trigger pulse=2; and trigger delay=24 ms. The T2-weighted True FISP Imaging of Myocardium imaging parameters were: TR/TE=281.95/1.09 ms; flip angle=90°; FOV=360 mm;

slice thickness=6 mm; matrix size=256×256; acquisition window=715 ms; trigger pulse=2; and trigger delay=433 ms. The CV-3DRAD-400C sequence parameters were: TR/TE=599/73 ms; flip angle=60°; FOV=256 mm; slice thickness=6 mm; matrix size=256×256; acquisition window=750 ms; trigger pulse=2; and trigger delay=300 ms. Images from this specific sequence were of stem cells containing Fe ions. Animals in the control group were scanned by the same 3T MR machine on the same days as the transplanted groups.

After intravenous application of contrast medium (0.1 mmol/kg body/wt), "late enhancement" imaging was performed with a delay time of 7–10 minutes. Two dimensional True-FISP sequences using an individually optimized inversion time of 170 ms to 280 ms were acquired. The parameters were: TR/TE=2.3/1.3 ms; flip angle 18°; spatial resolution 2.8×3.0×10 mm; FOV range 360 mm; and 3 slices were acquired in the LV short-axis using a 10 mm interslice gap.

Pathology

Histological analysis was performed on ischemic heart samples from each group at 4 weeks post-cell transplantation to investigate the effects of the labeled EPC transplantation. After completion of MR imaging, pigs were kept under anesthesia and transported directly to the operating room. The animals were then euthanized, and the heart was excised immediately at baseline, 3 weeks, and 4 weeks after the magnetic-labeled EPC autograft transplantation. The heart was fixed in 10% paraformaldehyde in PBS, dehydrated, and embedded in paraffin. The embedded specimens were cut into 5 µm serial sections and stained with prussian blue. The sections were detected to identify LV volumes by fluorescence microscope.

Statistical analysis

Data are presented as the mean±standard deviation for continuous variables. Comparisons of continuous variables were performed using an independent sample t-test or one-way analysis of variance with Dunnett's test, as appropriate. In all cases, statistical significance was defined as $p<0.050$ (two-tailed). All analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA).

RESULTS

Twenty-seven pigs were subjected to the experimental protocol; 9 of the pigs died of ventricular fibrillation during the intervention operation (26% mortality rate). Thus, 18 pigs were available for both MR examinations and pathological procedures. All MR examinations and pathological procedures were successfully performed.

Labeled EPCs improved cardiac function

Cardiac function measurements are summarized in Table 1. When the labeled EPCs were transplanted immediately after myocardial injury, ventricular function of the transplanted hearts was similar to that of the saline injected hearts in the control group. After 4 weeks, there was a significant decrease in myocardial infarct size in groups B1 and B2, and the hearts had better ventricular function than those in the control group (Fig. 1). Compared with the control group, LVFS had improved significantly ($p<0.050$) after EPC therapy in both experimental groups. Furthermore, the ventricular posterior wall thickness at

diastole and the EF also improved, but there were no significant differences between groups B1 and B2.

In accordance with myocardial infarct size reduction, intramyocardial injection of labeled EPCs significantly improved cardiac function, and this improvement was comparable to that in the control group.

In vivo MRI

The CV-3DRAD-400C sequence was sensitive to Fe ions and also retained the background signal. In this study, the signal from the cardiac muscle and chambers was weaker while the

Table 1. Measurement of two dimensional echocardiographies in pigs at 4 weeks

Parameters	Group A	Group B1	Group B2
Myocardial thickness of infraction zone (mm)	3.1 ± 0.2	5.5 ± 0.6	6.0 ± 0.4
LV posterior wall thickness at diastole (mm)	0.6 ± 0.2	2.8 ± 0.6	2.5 ± 0.3
End-diastolic volume of the left ventricle (mm^3)	3.4 ± 0.3	6.2 ± 0.4	5.5 ± 0.2
End-systolic volume of the left ventricle (mm^3)	2.0 ± 0.1	4.9 ± 0.3	4.4 ± 0.5
LV fractional shortening (%)	10.6 ± 1.1	21.0 ± 1.6	19.7 ± 1.2
LV ejection fraction (%)	48.5 ± 12.7	53.1 ± 0.2	51.4 ± 1.1

LV: left ventricular

Fig. 1. Evaluation of cardiac function by echocardiography between the three groups. Four weeks after transplantation, endocardial echocardiography in Groups B1 (arrows) and B2 was significant lower than that of Group A (arrow). Cardiac function was better than Group A.

autograft EPC signal was stronger. Combined with the delayed perfusion scan, the autograft EPCs were oriented in the area of the myocardial infarction. The imaging of labeled EPCs retained good contrast after one month, which suggested that SPIO was an ideal tool for labeling EPCs for MRI. LV volumes were assessed by MRI before EPC transplantation and 4 weeks post-transplantation. LV volumes did not differ significantly between the control and EPC groups prior to transplantation (Fig. 2). Four weeks after transplantation, the myocardial infarct size significantly differed between the control and transplantation groups, although the differences were not statistically significant between control and transplantation groups.

Pathology

From MR imaging, the results showed that the myocardial infarct size was smaller than that of the control group. In Fig. 3, prussian blue staining shows numerous blue-stained iron particles in the blood vessel lumen of the anterior LV wall and interventricular septum right after transplantation and 24 hours post-transplantation. At 2 weeks post-transplantation, the transplanted EPCs had enhanced neovascularization of the ischemic myocardium, resulting in blue-stained iron particles in the vascular endothelial cells. Microvessel networks were detected in the infarction zone 3 weeks after transplantation. There were also a few

blue-stained iron particles in the myocardial cells 4 weeks after transplantation. These observations suggest that the EPC transplantation had an important role in AMI therapy.

DISCUSSION

Due to its capacity to regenerate tissues and organs, stem cell transplantation has become a potential strategy for the treatment of many human diseases. Compared to organ transplantation, cell transplantation has advantages of lower cost and risk [14,15]. Furthermore, autologous cell transplantation has no risk of immunological rejection. Among the different cell types, EPCs have a capacity for multi-directional differentiation and have been previously demonstrated to possess better characteristics for transplantation. While the efficacy and methods of transplantation are very important, it is equally significant to evaluate cell delivery, which has been intensively investigated in several animal models [16,17]. The three routes of stem cell delivery that have been used in clinical trials are intracoronary or intramyocardial injection, or peripherally through systemic circulation. Intramyocardial injection, which has high-operability and less injury, has proven to be the most effective stem cell delivery method regardless of the model and was chosen for further research in this study [18]. In our study, we were able to use this

Fig. 2. Evaluation of myocardial infarct size by magnetic resonance imaging immediately after transplantation and four weeks post-transplantation. In the control group, the myocardial infarct size (arrows) was not obviously different. In the transplantation group, the myocardial infarct size (arrows) decreased significantly after therapy.

Fig. 3. Analysis of labeled EPC differentiation after transplantation into the acute myocardial infarction pigs using prussian blue staining. (A) and (B) showed most blue particles were distributed in the vessel periphery and vessel intracavity. (C) shows that the labeled EPCs promoted neoangiogenesis in the infarcted zone 2 weeks after transplantation (red arrows). (D) shows that a few blue particles were observed in the intramyocardial cells 4 weeks after transplantation (red arrow). The magnification was $\times 200$ and the staining method was prussian blue. EPC: endothelial progenitor cell.

method to inject EPCs into the infarct zone and administer treatment to the target areas. However, there were difficulties in the lesion orientation.

A previous study had reported that the number of circulating EPCs began to increase and reached peak levels at 6 h and then decreased to normal levels after 24 h [19]. Mechanistically, when the body's stress response causes injury to tissue, emergency EPCs are released into the peripheral blood and home to the injured area. After 24 hours, slower homing EPCs are released in the peripheral blood. Thus, it is reasonable to assume that direct delivery of EPCs into the injured area will accelerate wound healing. That is to say, the effect of EPCs engraftment in the AMI was positive with the timing of cell administration post-AMI. In this study, the infarct areas significantly decreased in the two transplanted groups as compared to the control group. The cardiac functions in the AMI pigs were not significantly different from 1 hour to 1 week after labeled-EPC transplantation. This result was not in line with previous reports [20,21]. There could be a few possible explanations for this discrepancy. First, the method used for the establishment of AMI animal models was different from other reports. Second, the EPC transplantation was conducted with an intramyocardial injection method, which can directly deliver cells into the infarcted myocardium

and negate the need for EPC uptake from the circulation. The common intracoronary delivery method is to directly inject cells into an area of good blood supply rich in nutrients and oxygen, which is essential for cell survival. Even after infarction, however, the absolute number of EPCs detected in the heart is very low, but intracoronary infusion of EPCs may enhance local accumulation and homing compared to intravenous injection [22]. Also, the sample size in this experiment was relatively small. Further experiments are needed to confirm the results in this study and before clinical application.

Animal and human studies have clearly shown that stem cell engraftment into the myocardium is associated with improved cardiac function; however, determining the optimal population of cells remains a challenge [23,24]. A previous study had shown that the number of cells administered to the animals (BM) ranged from 1×10^4 to 2×10^5 per animal. There was no association between the number of cells administered and vessel density or neuroblast extension. Therefore, increasing the number of cells should not increase the regenerative potential, at least for the tested range of cells [25]. From previously tested ranges of cells, increasing the number of cells did not increase the regenerative potential. Therefore, 1×10^6 cells were administered into the AIM pigs, and the results showed that cardiac functions were signifi-

cantly improved after EPC therapy. However, there was no association between the number of cells administered and the timing of cell administration post-AMI.

Preliminary clinical trials have shown that stem cell transplantation could help with the recovery of cardiac functions after myocardial infarction. However, during AMI, whether EPCs could survive in infarct areas, or differentiate into neovessels, cardiomyocytes, or scar tissue in their respective micro-environments is still an open question. In this study, we showed that transplanted EPCs differentiated into cardiomyocytes by prussian blue staining. These cells not only improved angiogenesis and myocardium with microvessel networks but also replaced the necrotic myocardium and helped with recovery of normal myocardium structures. All of these effects led to a reduction in the areas of the infarct regions and an improvement in cardiac functions and prognosis.

Primarily due to the high CNR, high spatial resolution, no isotopic labeling, and a lack of ionizing radiation, MRI is an effective technique in molecular imaging. Because MRI cannot differentiate transplantation cells from the target tissues, some reports have shown that MRI can track the distribution of transplanted cells *in vivo* via SPIO labeling. Due to its paramagnetism, our results have demonstrated the MRI can be used to assess infarct zone, revealing cells as hypo-intense signals using the T2 shortening effect caused by the elevated myocardial densities of the labeled EPCs. This hypo-intense signal represents the distribution and accumulation of transplantation cells *in vitro*. Routine MRI sequences have limitations in producing images with good CNR. Here, after the CV-3DRAD-400C sequence application, which was sensitive to Fe ion imaging, background signal was well maintained. Furthermore, when combined with a delayed perfusion scan, SPIO labeled EPCs were observed as stippled hypo-intense signals clearly located in the infarct regions.

In this study, after AMI, precisely targeted catheter-based implantation of EPCs into the infarct myocardium is feasible, accurate, and can be reliably and noninvasively identified *in vivo* by cardiac MRI. A special sequence showed the infarct myocardium and could track transplantation EPCs *in vitro*. Furthermore, intramyocardial injection of labeled-EPCs into the infarct myocardium could significantly improve ventricular function. However, there are no data on the potential toxicity of the direct injections of iron-loaded cells into the human myocardium. Therefore, there is a need to perform a large-scale clinical trial to establish whether EPC therapy can indeed translate into meaningful clinical benefits.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Park JH, Yoon JY, Ko SM, Jin SA, Kim JH, Cho CH, et al. Endothelial progenitor cell transplantation decreases lymphangiogenesis and adverse myocardial remodeling in a mouse model of acute myocardial infarction. *Exp Mol Med* 2011;43:479-485.
2. Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, et al. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* 2012;489:322-325.
3. Hong KS, Byun KH, Seo J, Lee HJ, Choi JJ, Kim KS, et al. Modification to the injection needle to a screw needle improves effective cell delivery in acute myocardial infarction. *Biotechnol Lett* 2014;36:859-868.
4. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 2003;9:1195-1201.
5. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-967.
6. Comas GM, Esrig BC, Oz MC. Surgery for myocardial salvage in acute myocardial infarction and acute coronary syndromes. *Heart Fail Clin* 2007;3:181-210.
7. Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. *J Mol Med (Berl)* 2014;92:387-397.
8. Jujo K, Li M, Losordo DW. Endothelial progenitor cells in neovascularization of infarcted myocardium. *J Mol Cell Cardiol* 2008;45:530-544.
9. Ribatti D. The involvement of endothelial progenitor cells in tumor angiogenesis. *J Cell Mol Med* 2004;8:294-300.
10. Thal MA, Krishnamurthy P, Mackie AR, Hoxha E, Lambers E, Verma S, et al. Enhanced angiogenic and cardiomyocyte differentiation capacity of epigenetically reprogrammed mouse and human endothelial progenitor cells augments their efficacy for ischemic myocardial repair. *Circ Res* 2012;111:180-190.
11. Giannoni E, Taddei ML, Parri M, Bianchini F, Santosuosso M, Grifantini R, et al. EphA2-mediated mesenchymal-amoeoid transition induced by endothelial progenitor cells enhances metastatic spread due to cancer-associated fibroblasts. *J Mol Med (Berl)* 2013;91:103-115.
12. Yin M, Liao Z, Yuan X, Guan X, O'Reilly MS, Welsh J, et al. Polymorphisms of the vascular endothelial growth factor gene and severe radiation pneumonitis in non-small cell lung cancer patients treated with definitive radiotherapy. *Cancer Sci* 2012;103:945-950.
13. Gazeau F, Wilhelm C. Magnetic labeling, imaging and manipulation of endothelial progenitor cells using iron oxide nanoparticles. *Future Med Chem* 2010;2:397-408.
14. Stutchfield BM, Forbes SJ, Wigmore SJ. Prospects for stem cell transplantation in the treatment of hepatic disease. *Liver Transpl* 2010;16:827-836.
15. Shabbir A, Zisa D, Suzuki G, Lee T. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. *Am J Physiol Heart Circ Physiol* 2009;296:H1888-H1897.
16. Sun JH, Zhang YL, Nie CH, Qian SP, Yu XB, Xie HY, et al. In vitro labeling of endothelial progenitor cells isolated from peripheral blood with superparamagnetic iron oxide nanoparticles. *Mol Med Rep* 2012;6:282-286.
17. Stamm C, Kleine HD, Choi YH, Dunkelmann S, Lauffs JA, Lorenzen B, et al. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg* 2007;133:717-725.
18. Patel AN, Geffner L, Vina RF, Saslavsky J, Urschel HC Jr, Kormos R, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J Thorac Cardiovasc Surg* 2005;130:1631-1638.
19. Jiao LF, Zhang JN. Marking of rat EPCs and changes in circulating rat

- EPCs after TBI. Tianjin Medical University master's graduation thesis-abstract III 2008.
20. Terrovitis J, Lautamäki R, Bonios M, Fox J, Engles JM, Yu J, et al. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. *J Am Coll Cardiol* 2009;54:1619-1626.
 21. Li RK, Mickle DA, Weisel RD, Rao V, Jia ZQ. Optimal time for cardiomyocyte transplantation to maximize myocardial function after left ventricular injury. *Ann Thorac Surg* 2001;72:1957-1963.
 22. Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, et al. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 2002;105:732-738.
 23. Smits AM, van Vliet P, Hassink RJ, Goumans MJ, Doevendans PA. The role of stem cells in cardiac regeneration. *J Cell Mol Med* 2005;9:25-36.
 24. Davani S, Deschaseaux F, Chalmers D, Tiberghien P, Kantelip JP. Can stem cells mend a broken heart? *Cardiovasc Res* 2005;65:305-316.
 25. Rosell A, Morancho A, Navarro-Sobrino M, Martínez-Saez E, Hernández-Guillamon M, Lope-Piedrafita S, et al. Factors secreted by endothelial progenitor cells enhance neurorepair responses after cerebral ischemia in mice. *PLoS One* 2013;8:e73244.