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## Stem cell factor/c-kit signaling mediated cardiac stem cell migration via activation of p38 MAPK

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■ **Abstract** *Objective* It was reported that there are cardiac stem cells (CSCs) in the rat heart, and they could reconstitute well-differentiated myocardium that are formed by blood-carrying new vessels and myocytes. However, how do the CSCs migrate into the peri-infarcted areas after myocardial infarction (MI)? It remains entirely unknown about the signal transduction involved in the migration of CSCs. *Methods and results* Rat heart MI was induced by left coronary artery ligation. Both immunohistochemical staining and Western blotting analysis was performed to detect the expression of SCF protein, and RT-PCR was conducted for the expression of SCF mRNA. Cardiac stem cells were isolated from rat hearts, and a cardiac stem cell migration assay was performed using a 48-well chemotaxis chamber system. On day 5 after MI in rats, the expression of stem cell factor (SCF) mRNA and protein was significantly increased in the peri-infarcted area, which was matched with more accumulation of CSCs in the region and improvement of cardiac function, which was blocked by p38 MAPK selective inhibitor SB203580. In *in vitro* experiments, SCF induced CSC migration in a concentration-dependent manner, and the antibody against SCF receptor (c-kit) blocked the SCF-induced CSC migration. Western blot analysis showed that the phosphorylated p38 MAPK (Phospho-p38 MAPK) was highly increased in the SCF-treated CSCs, and the inhibition of p38 MAPK activity significantly attenuated SCF-induced the migration of CSCs. *Conclusion* It demonstrated that SCF/c-kit signaling may mediate the migration of CSCs via activation of p38 MAPK.

■ **Key words** cardiac stem cell – stem cell factor – migration – myocardial infarction

### Introduction

Myocardial infarction (MI) is a leading cause of heart failure. Because of the ingrained concept of the myocardium as a terminally differentiated organ, it has been assumed that any attempt to replace the lost myocytes using cellular therapy would require the

introduction of exogenous cells into the myocardium. However, it has recently been reported that the adult heart harbors primitive stem/progenitor cells [4, 7, 8, 18, 24]. Beltrami et al. [4] demonstrated that cardiac stem cells (CSCs) are a pure population of cloned c-kit-positive cells that can be differentiated into cardiomyocytes, smooth muscle cells and vascular endothelium. Previous studies have documented the

ability of CSCs to regenerate infarcted myocardium when CSCs were injected intramyocardially in the peri-infarct region after a permanent coronary occlusion. Dawn et al. [8] also reported that after intracoronary administration, CSCs could traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. Despite these encouraging observations, the mechanism of CSCs to repair the heart remains unclear. Especially, how do the CSCs migrate into the peri-infarcted areas when myocardial infarction occurs? Little has been reported on the migration of CSCs, and it remains entirely unknown about the signal transduction involved in the migration of CSCs.

Stem cell factor (SCF, also called c-kit ligand) is a critical factor for the development, survival, proliferation and migration of mast cells [12, 32]. By binding to c-kit, a tyrosine kinase-containing receptor, SCF can induce cell migration, adhesion to extracellular matrix components and secretion of mediators [17, 39]. Our previous study has shown that SCF induced the bone marrow stem cell migration and improved the cardiac function [40]. However, whether SCF is involved in the cardiac stem cell migration remains unknown.

It was well known that the family of MAPKs plays a key role in cellular response to cytokine stimulation. The main extracellular signal-regulated kinase of MAPKs consists of ERK1/2, p38 MAPK and JNK. However, the JNK and p38 MAPK pathways have been reported to be responsible for the cytokine-induced signaling of cell migration [21].

In the present study, we reported the identification of SCF as a cytokine that is highly expressed in peri-infarcted areas after MI, and mediated the migration of CSCs via activation of p38 MAPK.

## Materials and methods

### ■ Rat myocardial infarction model

A rat myocardial infarction model leading to left ventricle dysfunction was used in present study. Male Wistar rats (250–300 g) were randomized to coronary artery ligation or sham-operated groups. At day 5 after ligation, heart tissues were prepared for the detection of SCF mRNA and protein. All procedures were performed in accordance with the Guidelines of the Hubei Council of Animal Care and approved by the Animal Use Subcommittee at the Huazhong University of Science and Technology, China.

### ■ Hemodynamic measurement

Three weeks after coronary artery ligation or sham operation, rats were re-anesthetized with sodium

pentobarbital. The right carotid artery was cannulated with a Millar microtip pressure transducer catheter. After arterial blood pressure and heart rate measurements were obtained, the catheter was advanced into the left ventricle (LV) for the measurement of LV systolic and end-diastolic pressures as well as the maximal rate of pressure development ( $+dP/dt_{\max}$ ) and rate of relaxation ( $-dP/dt_{\min}$ ) of LV.

### ■ Immunohistochemical staining

To detect the expression of SCF in the heart tissues, the rabbit antibody (1:100, Pepro Tech EC Ltd) against murine SCF was used. Endogenous peroxidase was blocked by 0.3%  $H_2O_2$  for 20 min. The secondary antibody for immunostaining was biotin-conjugated anti-rabbit immunoglobulin (1:200, Dako).

### ■ Isolation and culture of CSCs from the adult rat heart

CSCs were isolated from the hearts of male Wistar rats (250–300 g) by a method described previously with a minor modification [4]. Briefly, the rat was injected with heparin (5,000 IU/kg, i.p.) 20 min prior to the experimental protocol, and then was killed by cervical dislocation. The heart was excised and the aorta was cannulated rapidly. The cannulated heart was mounted on a Langendorff perfusion apparatus with constant flow and perfusion pressure was monitored. The heart was firstly perfused with  $Ca^{2+}$ -free Tyrode solution for 10 min to remove the blood and then digested by 0.5 mg/ml collagenase (Sigma) and 0.05 mg/ml trypsin (Difoo) at 37°C for 30 min. After the enzymatic digestion was terminated, the heart tissue was chopped and cell suspension collected was filtered with a strainer (Becton Dickson). Afterward, cells were incubated with a rabbit anti-c-kit antibody (Santa Cruz) and separated by using sheep anti-rabbit immunomagnetic microbeads (Dynal Biotech). Small round cells, containing most of the c-kit<sup>pos</sup> population, were separated. These c-kit<sup>pos</sup> cells were cultured for 3–5 days with Dulbecco's MEM (Invitrogen) containing fetal calf serum, bFGF, and LIF at 37°C. After recovery, they were used for subsequent experiments.

### ■ Western immunoblotting analysis

The protein level of SCF in heart tissues was determined by Western immunoblotting analysis. Briefly, a total of 50  $\mu$ g of protein in each sample were separated by SDS polyacrylamide gel (12.5%) electrophoresis followed by electrophoretic transfer of proteins from the gel to a nitrocellulose membrane. The membrane

was probed with rabbit anti-SCF antibody (Santa Cruz Biotechnology). Bands corresponding to SCF protein were visualized using enhanced chemiluminescence reagents and analyzed with a gel documentation system. For the detection of phosphorylation of p38 MAPK, the anti-phosphotyrosin antibody (Cell Signaling Technology, Inc., USA) against P<sup>38</sup> was used. To ensure similar amounts of proteins in each sample, the same membrane was stripped off, and reprobed with the antibodies against p38 MAPK.

### ■ Reverse transcription (RT)-PCR analysis

Total RNA was extracted from the left ventricle with Trizol reagent. RT-PCR was performed to analyze mRNA expression of SCF or c-kit with the primers and conditions as described previously [14, 41]. PCR products were separated on 1.5% of agarose gel.

### ■ Chemotaxis assay

Chemotaxis experiments were performed using a 48-well chemotaxis chamber technique (Neuro Probe) as previously described with a minor modification [29]. Briefly, 25  $\mu$ l of medium (RPMI 1640, Gibco) alone or medium containing 5, 10, 30, 50, 100 ng/ml SCF (Santa Cruz) was placed in the lower chamber. A polycarbonate membrane with a 5- $\mu$ m pore size separated the upper and lower chamber. CSCs resuspended in RPMI 1640 (50  $\mu$ l) were placed in each well of the upper chamber. For inhibition experiment, CSCs were preincubated with these inhibitors for 20 min, and then added into the upper chamber. The chamber was then incubated for 3 h at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and then disassembled. The membrane was removed and scraped to remove non-migrating CSCs from the upper surface. Then the membrane was fixed and stained. The numbers of CSCs that had migrated to the lower surface of the membrane were counted in ten random high-power fields (HPFs) by light microscopy, and a chemotactic index (CI) was calculated to express stimulated migration. Each assay was performed in triplicate wells.

$$CI = \frac{\text{Stimulated migration (CSCs number per HPF)}}{\text{Random migration (CSCs number per HPF)}}$$

### ■ Cell labeling and assessment of cell migration in vivo

To detect the response of CSCs to SCF in vivo, the cultured CSCs were labeled with the thymidine ana-

logue 5-bromo-2'-deoxyuridine (BrdU; Zymed) as described previously [3]. The labeled cells ( $1 \times 10^5$ ) were injected into AV-groove followed by a coronary ligation. For the inhibition experiment, the labeled cells were suspended in the medium containing 10  $\mu$ M SB203580, then were injected into AV-groove. At day 5 after ligation, histological examination was performed in peri-infarcted regions to localize the BrdU-labeled cells which migrated from AV-groove after myocardial infarction by using mouse anti-BrdU (Zymed) antibody and fluorescent antibody (secondary antibody) in the heart. For the cardiac function, at three weeks, the hemodynamic parameters were measured.

### ■ Statistics analysis

All data are expressed as mean  $\pm$  SEM. For analysis of differences between two groups, Student's *t* test was performed. For multiple groups, ANOVA was carried out followed by Student–Newman–Keuls test. The level of statistical significance was set at  $P < 0.05$ .

## Results

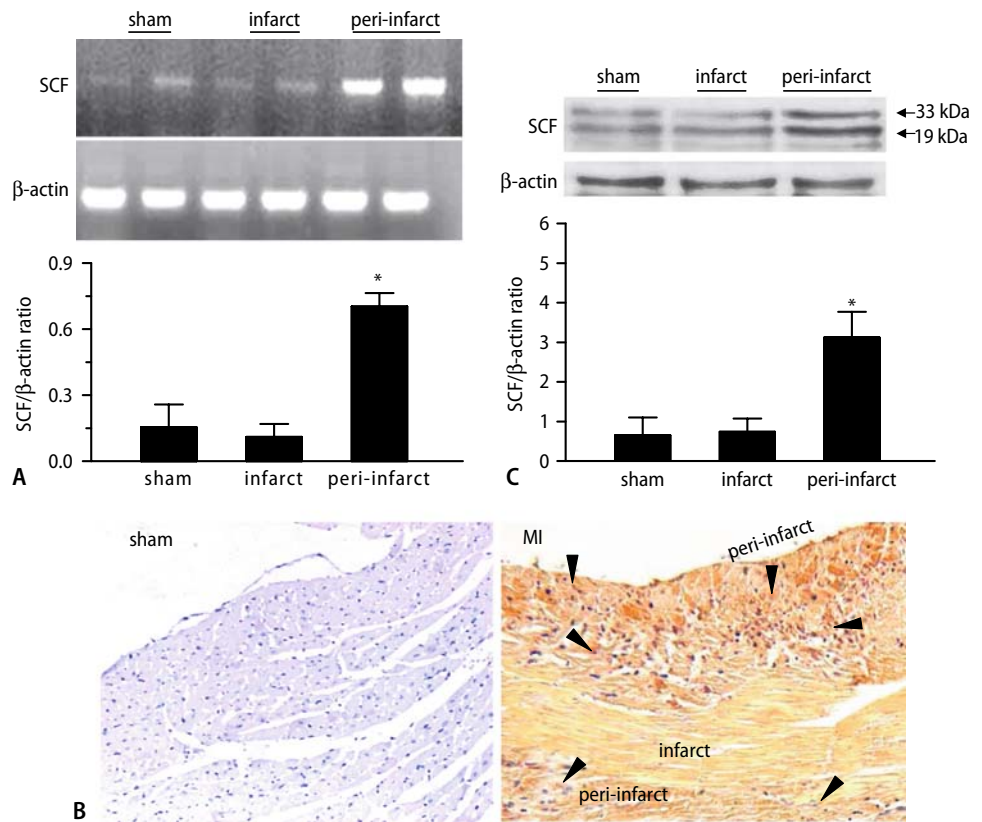
### ■ SCF expression in rat left ventricle myocardium after MI

The expression of SCF mRNA in LV myocardium was studied at day 5 after MI in rats. As shown in Fig. 1A, quantitative analysis of SCF mRNA expression to  $\beta$ -actin was illustrated. The result showed that the sham-operated heart tissue exhibited weak expression of SCF mRNA, however, after MI, SCF mRNA expression was significantly increased in the peri-infarcted area. To further confirm this result, we detected SCF expression at protein level by immunohistochemical staining and Western blotting analysis. The results showed that SCF protein expression was markedly increased in peri-infarcted area compared to the sham (Fig. 1B, C).

### ■ CSC migration to the peri-infarcted regions after MI

In order to understand whether the increased SCF expression in the peri-infarcted area led to more accumulation of CSCs after MI, the assessment of CSC migration in vivo was performed by using BrdU-labeled CSCs, which were injected into AV-groove. As shown as in Fig. 2A, the increased SCF expression was matched with more accumulation of CSCs in the peri-infarcted regions at 5 days after MI. The administra-

**Fig. 1** SCF expression in left ventricle tissues at day 5 following myocardial infarction in rats. **A** SCF mRNA expression by RT-PCR. **B** SCF protein expression by immunohistochemical staining (▼ indicates expression of SCF in peri-infarcted areas). **C** SCF protein levels by Western blot analysis in left ventricle tissues following myocardial infarction or sham operation.  $N = 5-7$  per group. Results are mean  $\pm$  SEM from four independent experiments. \* Means  $P < 0.05$  versus corresponding sham



tion of SB203580 resulted in a significant reduction of the accumulation of CSCs in the peri-infarcted regions (Fig. 2B) at day 5 after MI, which demonstrated that p38 MAPK may play important roles in SCF-induced CSC migration.

### ■ The effect of SB203580 on cardiac function

In order to understand the functional relevance of the reduction of CSC migration into the peri-infarcted regions via the inhibition of p38 MAPK, the cardiac function was measured at 3 weeks after MI. Treatment with SB203580 significantly decreased LV  $+dP/dt$  and  $-dP/dt$  to a greater extent in the rats compared to no treatment after MI (Fig. 2C, D), however, there were no significant differences in heart rate, mean arterial pressure, or LV systolic pressure and LV end-diastolic pressure between SB203580-treated and non-treatment.

### ■ Features and identification of c-kit<sup>pos</sup> cells from the adult rat heart

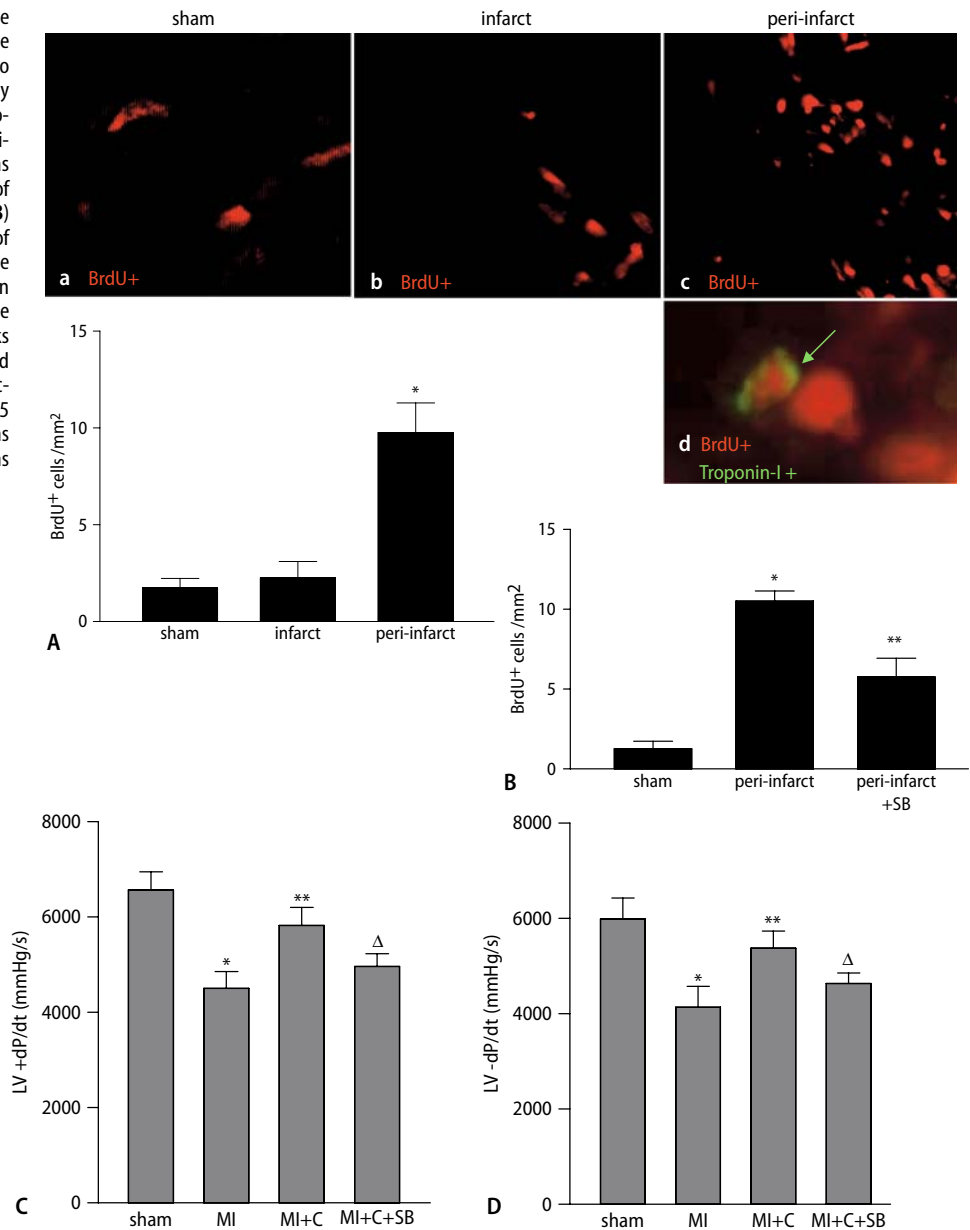
By using immunomagnetic microbeads, the c-kit<sup>pos</sup> cells were isolated and collected from adult rat hearts [4]. This can finally result in the c-kit<sup>pos</sup> cells with a

purity of 92%. Under light microscopy, they were small, round, phase-bright, and suspended in the medium. The expression of c-kit was detected by RT-PCR. As shown in Fig. 3A, c-kit mRNA was detectable in isolated CSCs, however, as a negative control, the NIH 3T3 cells cannot express c-kit mRNA. These results confirmed that these isolated cell were really c-kit<sup>pos</sup> CSCs.

### ■ Chemoattractant effects of SCF on CSC migration

During myocardial infarction, SCF was rapidly induced by the peri-infarcted area, and the increased SCF was matched with more accumulation of CSCs. We next asked if the SCF upregulation in the injured myocardium was functionally relevant for CSC migration. A Boyden chamber-based migration assay was established to quantitatively evaluate CSC migration in vitro. The lower chamber was placed with medium (RPMI 1640) alone or medium containing 5, 10, 30, 50, 100 ng/ml SCF, while the upper chamber was placed with CSCs. As shown in Fig. 3B, compared with the control group, the averaged numbers of migrated CSCs increased significantly as the concentration of SCF increased, which reach a peak at 30 ng/ml ( $n = 30$ ,  $P < 0.05$  compared with medium alone). As c-kit mRNA was not detectable in the NIH 3T3 cells [30], therefore we included NIH 3T3

**Fig. 2** The effect of SB203580 on the migration of BrdU labeled CSCs into the peri-infarcted regions. The migrated CSCs to the peri-infarcted areas was detected by using mouse anti-BrdU antibody and fluorescent-labeled antibody (secondary antibody) **A**, the BrdU-labeled cell was troponin-I positive (**A d**). The effect of SB203580 on the migration was assessed (**B**) at day 5 after MI, the administration of SB203580 was conducted to detect the blockage of SB203580 on the accumulation of CSCs in the peri-infarcted regions. The cardiac function was measured at 3 weeks after MI, the changes of LV  $+dP/dt_{max}$  and  $-dP/dt_{min}$  were shown in **C** and **D**, respectively.  $N = 7-9$  per group \* Means  $P < 0.05$  versus corresponding sham; \*\* means  $P < 0.05$  versus peri-infarct or MI;  $\Delta$  means  $P < 0.05$  versus group MI + C



cells in the Boyden chamber assay as a negative control, which did not induce migration of CSCs regardless of the concentration of SCF used. However, SCF resulted in a significant chemoattractant effect on CSCs in a dose dependent manner in vitro. This result documented that SCF could serve as a chemoattractant of CSCs in acute MI.

### ■ Role of c-kit antibody in SCF-induced CSC migration

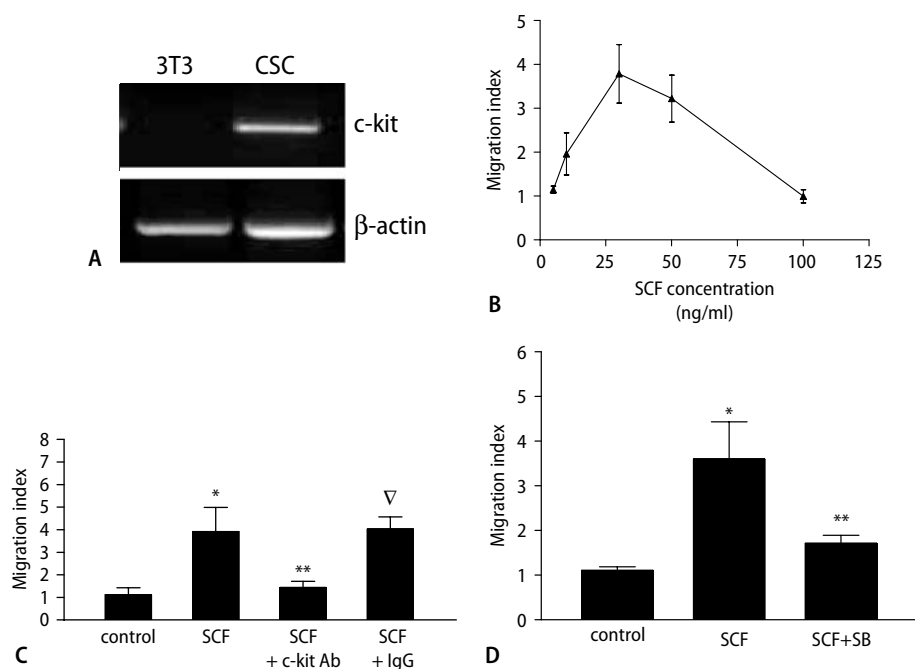
SCF-induced CSC migration could be abolished by the pretreatment of the CSCs with c-kit blocking anti-

body, as shown in Fig. 3C. This migration inhibition did not occur after pretreatment with a control IgG

### ■ P<sup>38</sup> MAPK was involved in the SCF-induced CSC migration

To explore whether SCF induced migration through the P<sup>38</sup>-dependent pathways in CSCs, the specific inhibitor SB203580, an agent that selectively inhibits p38 MAPK, was tested for the effect on SCF-induced CSC migration. CSCs were preincubated with SB203580 (10  $\mu$ M) for 30 min, the cells were then

**Fig. 3** CSC migration induced by SCF. CSCs were isolated from adult rat heart and RT-PCR analysis of *c-kit* mRNA was performed in the isolated cells **A**, NIH 3T3 cells were used as a negative control, and  $\beta$ -actin as an internal control for RT-PCR. In vitro CSC chemotaxis assays were performed on a 48-well Boyden chamber system. The lower chamber was placed with medium (RPMI 1640) alone or medium containing 5, 10, 30, 50, 100 ng/ml SCF, while CSCs were added in the upper chamber. The medium alone was used in negative control experiments. Data are mean  $\pm$  SEM, from four independent experiments. **B** Showed SCF-induced CSC migration with a dose dependent manner. **C** CSCs were pre-incubated with *c-kit* antibody (or IgG non-specific antibody) for 20 min, then were added into upper chamber for the migration assay (30 ng/ml of SCF). **D** The cells were pre-incubated with the  $P^{38}$  MAPK selective inhibitor SB203580 for 20 min, then added into upper chamber for the migration assay. \* Means  $P < 0.05$  versus corresponding control, \*\* means  $P < 0.05$  versus corresponding SCF,  $\nabla$  means  $P > 0.05$  versus corresponding SCF



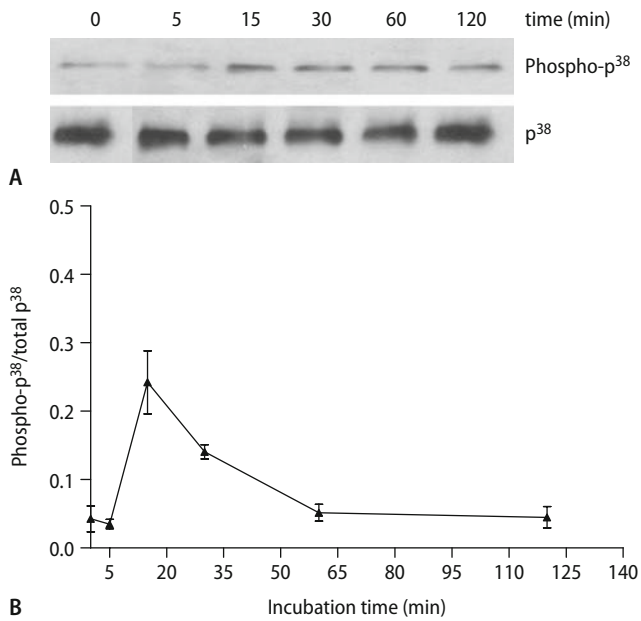
added to the upper chambers for the CSC migration assay. As shown in Fig. 3D, application of the inhibitor significantly attenuated the effect of SCF on CSC migration. However, the number of migrated cells in the presence of inhibitor remains significantly greater than the control group which suggests that other signaling pathways may be involved in SCF-induced CSC migration. Western blot analysis showed that the levels of phospho-p38 MAPK were significantly increased in SCF-treated CSCs, however the total protein levels of p38 MAPK were not markedly altered in SCF-treated cells. The phospho-p38 MAPK reached a peak after a 15 min-incubation with SCF, and returned to basal level 60 min after application of SCF (Fig. 4).

## Discussion

One of the contributing factors in the progression of heart failure is the loss of cardiomyocytes after myocardial infarction, combined with the absence of an adequate endogenous repair mechanism. A fibrous scar finally replaces the injured myocardium. The significance of cardiac *c-kit* positive cells has recently demonstrated that the result from Dr. Li's lab showed that cardioprotective *c-kit*<sup>+</sup> cells were from the bone marrow and regulated the myocardial balance of angiogenic cytokines in infarcted myocardium, thereby driving efficient cardiac repair [11]. Stem cell factor receptor induced progenitor and natural killer cell-mediated cardiac survival and repair after MI, which contributed to improved remodeling and car-

diac function after MI [2]. Recently, stem cell based therapy has become a realistic option to replace damaged cardiomyocytes [13, 34]. Several cardiac stem/progenitor cells have been identified, which expressed the stem cell-related antigens *c-kit*, MDR1, Sca-1 or islet-1 [1, 4-7, 22]. In vitro data suggest that the growth potential of *c-kit*-positive CSCs is greater than that of others, although these cell categories give rise to all cardiac cell lineages [19]. The extraordinary clinical potential of myocardial regeneration makes the dissection of the biology of CSCs a challenging and exciting endeavor.

Since in fetal life, *c-kit*-positive cells colonize the yolk sack, liver, and probably other organs. The colonized organs express SCF, the ligand of the *c-kit* receptor [16, 33], so it was reasonable to assume that stem like cells are present in the heart from fetal life. Recently, CSC clusters have been found in the adult heart [5, 23, 37]. Although CSC clusters are scattered throughout the myocardium, they accumulate in the atria and apex, and are less numerous at the base and mid-portion of the left ventricle [1]. The cardiac niches are expected to control the physiological turnover of myocardial cells and the growth, migration, and commitment of primitive cells leaving the niches to replace old dying cells in the myocardium. However, it remains unknown how these CSCs migrate into the injured myocardium after MI. Anversa's group has reported that HGF plays a critical role in migration and proliferation of *c-kit* positive CSC [36]. Our results showed an increased expression of SCF in the peri-infarcted area at the levels of mRNA and



**Fig. 4** Western blot analysis of p<sup>38</sup> MAPK after SCF stimulation for 0–120 min in CSCs. **A** showed the original gels (P<sup>38</sup> and phosphor-P<sup>38</sup>). **B** Showed the quantified data from three independent experiments. The results are mean ± SEM

protein, which was matched with more accumulation of CSCs in the region. It drew the hypothesis that SCF may be involved in the activation of resident primitive *c-kit*-positive cells which migrate into the peri-infarcted area, thereby, in the increased formation of myocytes in the acutely infarcted heart, but it remains undetermined whether SCF could play a crucial role in this process.

For the purpose in the present study, the CSCs were isolated from heart tissues, and the migration assay was carried out by using 48-well chemotaxis chamber. Our data documented that SCF could attract migration of CSCs in a dose-dependent manner in vitro, which suggests that SCF may attract CSCs to injured myocardium and participate in the repair of heart in acute MI.

C-kit is a receptor tyrosine kinase (RTK), which constitutes a type III RTK subfamily with the receptors for platelet-derived growth factor (PDGF), colony-stimulating factor 1 (CSF-1), and flt-3 ligand [26, 43]. C-kit and its ligand stem cell factor (SCF) play an important role in hematopoiesis, melanogenesis, and gametogenesis [42]. Upon ligand stimulation, c-kit receptors dimerize, activate its intrinsic tyrosine kinase, and autophosphorylate. The phosphorylated c-kit receptor generates binding sites for SH2 domain-containing proteins, which include proteins of the p21 Ras-mitogen-activated protein kinase (MAPK) pathway [9], the p85 subunit of

phosphatidylinositol 3' kinase (PI3K) [28], phospholipase C- $\gamma_1$ , the Grb2 adaptor protein, the Src family kinases [20], Cbl, CRKL [27], p62Dok-1 [38], SHP1, and SHP2 [15]. Those proteins are subsequently activated or phosphorylated and further transduce signaling cascades that lead to various cellular responses. Migration is one of the unique and important cell functions undertaken by SCF/c-kit system. Much effort has been done to clarify signal transduction leading to SCF/c-kit-mediated proliferation and survival, but the signaling mechanism in SCF-mediated cell migration has not been clarified yet. Recently, a few reports indicated that JNK or p38 MAPK plays an important role in cell migration [21, 25, 31]. Shuji Ueda et al. [35] has provided evidence that SFK and PI<sub>3</sub>K cooperatively contribute to SCF-mediated cell migration through Ca<sup>2+</sup> mobilization, and the signaling of SFK is transduced sequentially from p38 MAPK, Ca<sup>2+</sup> influx, to Erk1/2. The JNK and p38 MAPK pathways have been reported to be responsible for the cytokine-induced signaling of cell migration [21]. We hypothesized that these signaling molecules may also play roles in SCF-mediated CSCs migration. To test the hypothesis, c-kit antibody and the specific inhibitors of JNK and p38 MAPK were used to block the SCF-induced migration of CSCs. Our present results showed that SCF-induced CSC migration was blocked by the antibody against c-kit, and significantly attenuated by a specific inhibitor of p38 MAPK in vivo and in vitro, and the functional relevance of the reduction of CSC migration into the peri-infarcted regions via the inhibition of p38 MAPK was assessed. The improvement of cardiac function after MI was significantly blocked by administration of p38 MAPK inhibitor. However, Engel et al. [10] reported that FGF1/p38 MAPK inhibitor therapy improves recovery after myocardial infarction, a long term observation on systemic administration of p38 MAPK inhibitor should be further carried out. Additionally, in the present study, the SCF-induced migration of CSCs cannot be influenced by specific inhibitor of JNK, although a higher concentration of JNK inhibitor was used (data not shown). However, the number of migrated cells in the presence of inhibitor remains significantly greater than the control group even if a higher concentration of SB203580 was used, which suggests that other signaling pathways may be involved in SCF-induced CSC migration. Further stimulation of CSCs with SCF markedly increased the phosphorylation of proteins of p38 MAPK, however the total protein levels of p38 MAPK did not change. It indicated that phosphorylation of proteins of p38 MAPK may be involved in SCF-induced migration of CSCs.

In summary, our study showed that myocardial infarction of rat heart led to an increased expression of SCF, which mediated migration of CSCs via stimulation of c-kit and activation of p38 MAPK.

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## References

- Anversa P, Kajstura J, Nadal-Ginard B, Leri A (2003) Primitive cells and tissue regeneration. *Circ Res* 92:579–582
- Ayach BB, Yoshimitsu M, Dawood F, Sun M, Arab S, Chen M, Higuchi K, Siatskas C, Lee P, Lim H, Zhang J, Cukerman E, Stanford W, Medin JA, Liu PP (2006) Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction. *Proc Natl Acad Sci USA* 103:2304–2309
- Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration and body distribution. *Circulation* 108:863–868
- Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P (2001) Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 344:1750–1757
- Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Nadal-Ginard B, Anversa P (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114:763–776
- Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S (2003) Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 5:877–889
- Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, Di Meglio F, Nadal-Ginard B, Frustaci A, Leri A, Maseri A, Anversa P (2003) Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res* 93:604–613
- Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R, Torella D, Tang XL, Rezazadeh A, Kajstura J, Leri A, Hunt G, Varma J, Prabhu SD, Anversa P, Bolli R (2005) Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA* 102:3766–3771
- Duronio V, Welham MJ, Abraham S, Dryden P, Schrader JW (1992) p21ras activation via hemopoietin receptors and c-kit requires tyrosine kinase activity but not tyrosine phosphorylation of p21ras GTPase-activating protein. *Proc Natl Acad Sci USA* 89:1587–1591
- Engel FB, Hsieh PC, Lee RT, Keating MT (2006) FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction. *Proc Natl Acad Sci USA* 103:15546–15551
- Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, Verma S, Weisel RD, Keating A, Li RK (2006) Cardio-protective c-kit<sup>+</sup> cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J Clin Invest* 116:1865–1877
- Galli SJ, Zsebo KM, Geissler EN (1994) The kit ligand, stem cell factor. *Adv Immunol* 55:1–96
- Histov M, Weber C (2006) The therapeutic potential of progenitor cells in ischemic heart diseases—past, present and future. *Basic Res Cardiol* 101:1–7
- Konard L, Munir Keilani M, Cordes A, Voulck-Badouin E, Laible L, Albrecht M, Renneberg N, Aumoullier G (2005) Rat sertoli cells express epithelial but also mesenchymal genes after immortalization with SV40. *Biochim Biophys Acta* 1722:6–14
- Kunisada T, Yoshida H, Yamakazi H, Miyamoto A, Hemmi H, Nishimura E, Shultz LD, Nishikawa S, Hayashi S (1998) Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. *Development* 125:2915–2923
- Laflamme MA, Myerson D, Saffitz JE, Murry CE (2002) Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. *Circ Res* 90:634–640
- Lam V, Kalesnikoff J, Lee CW, Hernandez-Hansen V, Wilson BS, Oliver JM, Krystal G (2003) IgE alone stimulates mast cell adhesion to fibronectin via pathways similar to those used by IgE + antigen but distinct from those used by Steel factor. *Blood* 102:1405–1413
- Leri A, Kajstura J, Anversa P (2005) Cardiac stem cells and mechanisms of myocardial regeneration. *Physiol Rev* 5:1373–416
- Linke A, Mueller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Bohm M, Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, Anversa P (2005) Cardiac stem cells in the dog heart regenerate infarcted myocardium improving cardiac performance. *Proc Natl Acad Sci USA* 102:8966–8971
- Linnekin D, DeBerry CS, Mou S (1997) Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells. *J Biol Chem* 272:27450–27455
- Lewis TS, Shapiro PS, Ahn NG (1998) Signal transduction through MAP kinase cascades. *Adv Cancer Res* 74:49–139
- Lyngbaek S, Schneider M, Hansen JL, Sheikh SP (2007) Cardiac regeneration by resident stem and progenitor cells in the adult heart. *Basic Res Cardiol* 102:101–114
- Nadal-Ginard B, Kajstura J, Anversa P, Leri A (2003) A matter of life and death: cardiac myocyte apoptosis and regeneration. *J Clin Invest* 111:1457–1459
- Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Poicus J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA* 100:12313–12318



25. O'Laughlin-Bunner B, Radosevic N, Taylor ML, Shivakrupa, DeBerry C, Metcalfe DD, Zhou M, Lowell C, Linn-ekin D (2001) Lyn is required for normal stem cell factor-induced proliferation and chemotaxis of primary hematopoietic cells. *Blood* 98:343–350
26. Qiu FH, Ray P, Brown K, Barker PE, Jhanwar S, Ruddle FH, Besmer P (1988) Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family—oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. *EMBO J* 7:1003–1011
27. Sattler M, Salgia R, Shrikhande G, Verma S, Pisick E, Prasad KV Griffin JD (1997) Steel factor induces tyrosine phosphorylation of CRKL and binding of CRKL to a complex containing ckit, phosphatidylinositol 3-kinase, and p120(CBL). *J Biol Chem* 272:10248–10253
28. Serve H, Hsu YC, Besmer P (1994) Tyrosine residue 719 of the c-kit receptor is essential for binding of the P85 subunit of phosphatidylinositol (PI) 3-kinase and for c-kit-associated PI 3-kinase activity in COS-1 cells. *J Biol Chem* 269:6026–6030
29. Song ZH, Zhong M (2000) CB1 cannabinoid receptor-mediated cell migration. *J Pharmacol Exp Ther* 294:204–209
30. Sun L, Lee J, Fine HA (2004) Neuronally expressed stem cell factor induces neural stem cell migration to areas of brain injury. *J Clin Invest* 113:1364–1374
31. Sundstrom M, Alfredsson J, Olsson N, Nilsson G (2001) Stem cell factor-induced migration of mast cells requires p38 mitogen-activated protein kinase activity. *Exp Cell Res* 267:144–151
32. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ (2006) Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* 176:4102–4112
33. Teyssier-Le Discorde M, Prost S, Nandrot E, Kirzenbaum M (1999) Spatial and temporal mapping of c-kit and its ligand, stem cell factor expression during human embryonic haemopoiesis. *Br J Haematol* 107:247–253
34. Templin C, Kotlarz D, Marquart F, Faulhaber J, Brendecke V, Schaefer A, Tsikas D, Bonda T, Hilfiker-Kleiner D, Ohl L, Naim HY, Foerster R, Drexler H, Limbourg FP (2006) Transcoronary delivery of bone marrow cells to the infarcted murine myocardium: feasibility, cellular kinetics, and improvement in cardiac function. *Basic Res Cardiol* 101:301–310
35. Ueda S, Mizuki M, Ikeda H, Tsujimura T, Matsumura I, Nakano K, Daino H, Honda Zi Z, Sonoyama J, Shibayama H, Sugahara H, Machii T, Kanakura Y (2002) Critical roles of c-Kit tyrosine residues 567 and 719 in stem cell factor-induced chemotaxis: contribution of src family kinase and PI3-kinase on calcium mobilization and cell migration. *Blood* 99:3342–3349
36. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosota T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, Anversa P (2005) Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res* 97:663–673
37. Urbanek K, Torella D, Sheikh F, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P (2005) Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc Natl Acad Sci USA* 102:8692–8697
38. van Dijk TB, van Den Akker E, Amelsvoort MP, Mano H, Lowenberg B, von Lindern M (2000) Stem cell factor induces phosphatidylinositol 3-kinase-dependent Lyn/Tec/Dok-1 complex formation in hematopoietic cells. *Blood* 96:3406–3413
39. Vosseller K, Stella G, Yee NS, Besmer P (1997) c-kit receptor signaling through its phosphatidylinositide-3'-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion, and membrane ruffling. *Mol Biol Cell* 8:909–922
40. Wang G, Lei M, Lu X, Feng Q (2004) Bone marrow stem cell migration to the infarcted myocardium: role of TNF. *Faseb J* 18:A1286
41. Wang R, Li J, Yashpal N (2004) Phenotypic analysis of c-kit expression in epithelial monolayers derived from postnatal rat pancreatic islets. *J Endocrinol* 182:113–122
42. Witte ON (1990) Steel locus defines new multipotent growth factor. *Cell* 63:5–6
43. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J* 6:3341–3351