Life Sciences 191 (2017) 59–67

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

PP2A regulates SCF-induced cardiac stem cell migration through interaction with p38 MAPK

Ying Wang[a,b], Yanli Xia[a,b], Dong Kuang[a], Yaqi Duan[a,b,*], Guoping Wang[a,b,*]

[a] Institute of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China
[b] Department of Pathology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China

ARTICLE INFO

Keywords:
Protein phosphatase 2A
Stem cell factor
Cardiac stem cells
Migration
p38 MAPK
Cofilin

ABSTRACT

Aims: Previous studies have shown that stem cell factor (SCF) induces the migration of cardiac stem cells (CSCs) and helps to repair myocardial infarctions. Earlier studies on the migration mechanism only focused on the activation of kinases; here, we aimed to explore the functional role of protein phosphatase 2A (PP2A) in SCF-induced CSC migration.

Main methods: CSCs were treated with SCF, PP2A enzymatic activity was measured, the phosphorylation levels of PP2A, p38 MAPK and cofilin were evaluated using western blot. Transwell assay was used to determine the migratory ability of CSCs.

Key findings: In vitro, SCF induced the phosphorylation of p38 MAPK and cofilin, leading to the migration of CSCs. Cofilin acted as a downstream signal of p38 MAPK. PP2A was involved in this process. Further studies revealed that PP2A was inactivated via phosphorylation at Tyr307 by SCF and the inactivation/phosphorylation was mediated by activated p38 MAPK, as p38 MAPK inhibitor SB203580 or siRNA prevented SCF-induced inactivation and phosphorylation of PP2A. When CSCs were pretreated with PP2A inhibitor (okadaic acid, OA), SCF-induced CSC migration and the downstream signals were enhanced, and the enhancement was reversed when p38 MAPK was blocked. Additionally, co-immunoprecipitation showed a direct interaction of PP2A with p38 MAPK.

Significance: Our results indicated that PP2A regulated the SCF-induced activation of p38 MAPK/cofilin signaling pathway and subsequent migration of CSCs by interaction with p38 MAPK.

1. Introduction

After myocardial infarction (MI), the heart is repaired with fibrous scar tissues to compensate for the loss of functional cardiomyocytes, resulting in a lifelong effect on cardiac function. c-Kit$^+$ cardiac stem cells (CSCs) are capable of differentiating into myocytes, smooth muscle and endothelial cells [1], and makes it a promising candidate for stem cell therapy. Despite the current controversy over stem cell therapy, clinical trials still proceed [2,3]. In stem cell therapy, intramyocardial injection of exogenous CSCs [1,4–6], intracoronary infusion [7,8] and local activation of endogenous CSCs [9,10] are effective treatments. In all of these therapeutic methods, CSCs need to mobilize to the peri-infarction area; thus, it is important to explore the underlying mechanism of CSC migration.

c-Kit is usually regarded as a stem cell marker. As the ligand for c-kit, stem cell factor (SCF) leads to receptor dimerization and initiation of signaling cascades involved in mediating cell survival, migration and proliferation by binding to c-kit [11]. So far the well-established signal transductions downstream of c-kit are PI3-kinase [12], Src family kinases [13] and MAP kinases [14,15]. In contrast to the intensive investigation into kinases in SCF/c-kit signaling, the role and regulation of phosphatases in this process have remained underexplored.

Phosphatases are divided into two broad categories: protein serine/threonine phosphatases and protein tyrosine phosphatases [16]. Protein phosphatase 2A (PP2A) is the main soluble Ser/Thr phosphatase and most abundant in the heart [17]. Recently some studies suggested that PP2A may have a negative effect on heart function after MI, but the mechanisms are still unknown [18–21].

The PP2A holoenzyme contains a scaffolding subunit, a catalytic subunit and a variable regulatory subunit. The enzymatic activity of PP2A is modified post-translationally in two ways: phosphorylation and methylation. Phosphorylation of the catalytic subunit at Tyr307 leads to the loss of > 90% of the activity of PP2A; PP2A phosphorylation is usually a result of activation of Src family kinases, EGFR or insulin receptor [22,23].

PP2A is involved in many essential cellular functions – including

* Corresponding authors at: Institute of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China.
E-mail addresses: yqduan@hust.edu.cn (Y. Duan), wanggp@hust.edu.cn (G. Wang).

http://dx.doi.org/10.1016/j.lfs.2017.10.006
Received 12 July 2017; Received in revised form 22 September 2017; Accepted 2 October 2017
Available online 03 October 2017

development, cell mobility, cell cycle and cell growth [24–26]–by reversing the effects of protein kinases. For migration, PP2A likely functions at many different levels: receiving external stimuli by enhancing CXCR2 recycling [27]; signal transduction through dephosphorylation of ERK, p38 MAPK and AKT [28,29], and reorganization of the cytoskeleton by associating with paxillin and cofilin [30–32]. Although the previous studies showed a negative effect of PP2A on p38 MAPK, the role of PP2A and this relationship with p38 MAPK in CSC migration are still unclear. In general, cell migration requires significant change in cell shape which is modulated by the cytoskeleton; cofilin is an essential regulator of the cytoskeleton as cofilin severs and reorganizes filamentous-actin [33] and ultimately regulates cell migration. Subcellular localization, pH and phosphorylation all regulate the activity of cofilin [34–36]. The phosphorylation of cofilin is modified by different signals in different cells, and these signals include LIMKs, TESKs, PIP2, PP2A, SSHs, and CAP1 [37].

In this study, we aimed to explore the role of PP2A in SCF-induced CSC migration and found that PP2A regulated CSC migration through interaction with p38 MAPK.

2. Materials and methods

2.1. Reagents

Antibodies against β-actin (4967), phospho-p38 MAPK (Thr180/Thr182) (4511), phospho-cofilin (Ser3) (3313), p38 MAPK (9212), and cofilin (5175) were obtained from Cell Signaling Technology. The PP2A antibody, C subunit (05-421) was purchased from Millipore. The phospho-PP2A (Y307) (ab32104) antibody was from Abcam. The PE-CD117 (553355) and PE-IgG2b, κ isotype control (553989) antibodies were purchased from BD Pharmingen. The p38 MAPK inhibitor SB203580 (S8307) was purchased from Sigma-Aldrich. The PP2A inhibitor okadaic acid potassium salt (OA) (S1786) was obtained from Beyotime Biotechnology. The PP2A activator FTY720 (sc-202161) was obtained from Santa Cruz Biotechnology. Recombinant murine SCF (250-03-10) was purchased from Peprotech.

2.2. CSC isolation and culture

The protocol was approved by the Tongji Hospital, Huazhong University of Science and Technology Institutional Review Board of Experimental Animals. The hearts of 8-week-old C57BL/6 mice were used in CSC isolation as described previously [5,38] with minor modifications. In summary, mice were sacrificed and hearts were isolated. The hearts were minced into very small pieces and washed twice with cold PBS. The tissue fragments were digested with 5 ml of Liberase TH (Roche, 05401135001) for 10 min, and then were planted and cultured in Ham's F12 medium containing 10% FBS, 0.1 mM 2-ME, 2 mM L-Glu and 100 U/ml antibiotics. After two weeks, cells migrated from the explants were collected and selected by magnetic bead selection (Stem Cell, 18556) according to the manufacturer's manual. These obtained cells were cultured in DMEM/F-12 medium supplemented with 10% FBS, 10 ng/ml LIF, 20 ng/ml bFGF, 10 ng/ml EGF, insulin-transferrin-selenite and 100 U/ml antibiotics, and were used for subsequent experiments.

2.3. Flow cytometry

A PE-c-kit monoclonal antibody was added to the single cell suspension, the PE-IgG2b, κ isotype was used as negative control. After incubation for 30 min on ice in the dark, cells were then washed and resuspended in 200 μl cold PBS. The stained cell samples were analyzed by flow cytometry (BD Biosciences, FACSCalibur).

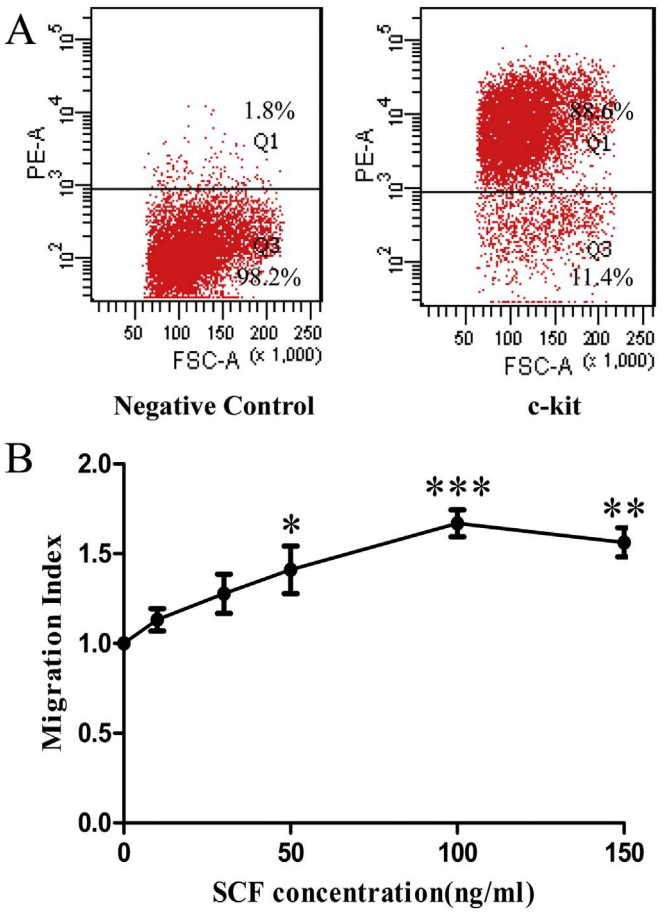

Fig. 1. SCF induces CSC migration. (A) Identification of c-kit$^+$ CSCs with a purity of 88.6% by flow cytometry. (B) The chemotaxis of SCF on CSCs was measured with transwell migration assay. The results are presented as the mean ± SEM. n = 3. *P < 0.05, **P < 0.01 and ***P < 0.001 vs. control.

2.4. Cell transfection

The cofilin small interfering RNA (siRNA) (4390771) and Silencer Select Negative Control siRNA (4390843) were from Ambion. The p38 MAPK siRNA (siG0957171300) was obtained from Ribobio. CSCs were transfected with siRNA at 50–70% confluence using RNAiMAX (Invitrogen, 13778-150) and were harvested 48 h after transfection.

2.5. Western blot

CSCs were lysed with RIPA buffer (Sigma, R0278) containing Inhibitor Cocktails. Lysates were collected and centrifuged for 15 min at 4 °C to obtain the supernatant. The protein concentrations were then determined with BCA Protein Assay Kit (Pierce, 23227). Samples were separated by 12% SDS–PAGE gel electrophoresis, transferred onto NC Membranes (Millipore, HATF000010), and incubated with the following antibodies: antibodies against phospho-p38 MAPK (1:1000), phospho-PP2A (1:5000), phospho-cofilin (1:1000), p38 MAPK (1:1000), PP2A (1:3000), cofilin (1:1000), β-actin (1:1000) overnight at 4 °C. After incubating with corresponding HRP-labeled secondary antibodies, protein signals were visualized by a chemiluminescence imaging system (Syngene, GeneGnomeXRQ).

2.6. Transwell migration assay

To determine the migratory ability of CSCs, 24-well transwell plates (Corning, 3422) were used; 600 μl of medium alone or with 10, 30, 50, 100, and 150 ng/ml recombinant murine SCF was placed in the 24-well