Heart, Lung and Circulation (2014) **23,** 75–81
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2013.05.652

ORIGINAL ARTICLE

Regulation of c-kit+ Progenitor Cells by Stromal Cell Derived Factor-1α in Adult Murine Heart

Zhongpu Chen, MD, PhD, Xiaodong Pan, MD, PhD, Yuyu Yao, PhD, Fengdi Yan, MD, PhD, Long Chen, MD, PhD, Rong Huang, MM, Genshan Ma, MD, PhD*

Department of Cardiology, Zhongda Hospital, Medical School of Southeast University, Nanjing, Jiangsu 210009, China

Received 8 March 2013; received in revised form 29 May 2013; accepted 30 May 2013; online published-ahead-of-print 23 July 2013

Background c-kit-positive cardiac progenitor cells (CPCs) have been proven suitable for stem cell therapy. CPCs marker c-kit and its ligand, the stem cell factor (SCF), are associated with the functions of proliferation and differentiation. In our previous study, we found that stromal cell-derived factor-1α (SDF-1α) could enhance the expression of c-kit. However, the mechanism is unknown.

Methods and results CPCs were isolated from adult mouse hearts, and c-kit-positive CPCs were purified by magnetic-activated c-kit cell sorting magnetic beads. The cells were cultured with SDF-1α, c-kit expression was measured by western blotting and qPCR, the proliferation and migration of cells were measured by CCK-8 and transwell assay, DNA methyltransferase (DNMT) mRNA were measured by qPCR, global DNMT activity was measured by DNMT activity assay kit, and DNA methylation was analysed using Sequenom's MassARRAY platform. Results showed that SDF-1α could enhance the expression of c-kit, which results in the promoting of c-kit-positive CPCs proliferation and migration. SDF-1α stimulation inhibited the expression of DNMT1, DNMT3β, and global DNMT activity, which led to significant demethylation in c-kit-positive CPCs.

Conclusions SDF-1α signalling, via CXCR4 activation, up-regulated c-kit expression by inhibiting DNMT1 and DNMT3β expression and global DNMT activity, and by subsequent demethylation of the c-kit gene.

Keywords Cardiac progenitor cells • c-kit • Stromal cell derived factor-1α • CXCR4 • DNA methylation • DNA methyltransferase

Introduction

Recent studies have shown significant evidence that not only the adult heart [1], but also the aged [2] and diseased heart [3] have regeneration potential. In 2003, cardiac progenitor cells (CPCs) were first reported to reside in the adult heart [4–6]. The heart has several populations of CPCs, which are self-renewing, clonogenic and multi-potent [5,7]. CPCs have more advantages than other stem cells [8,9], especially c-kit-positive CPCs, which are present in large numbers, are proliferative and capable of migration [10].

c-kit is a proto-oncogene, expressed on several types of cells, including CPCs [11,12], with the stem cell factor (SCF) as its ligand. c-kit expression is related to the regulation of cell proliferation, and migration [13]. A member of the CXC chemokine family, stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4, are expressed in a variety of cell types, including CPCs [14,15]. SDF-1α has been reported to be increased after acute myocardial infarction [16]. SDF-1 activation of CXCR4 has been demonstrated to prompt stem cell homing to injured cardiac tissue [17]. AMD3100 is a specific antagonist to SDF-1α, which binds to CXCR4

*Corresponding author at: Department of Cardiology, Zhongda Hospital, Medical School of Southeast University, 87 Dingjiaqiao, Nanjing, Jiangsu 210009, China. Tel.: +86 25 83272595; fax: +86 25 83272038., Email: magenshan@hotmail.com

competitively for preventing the combination of SDF-1α and CXCR4. Recent studies have indicated that SDF-1α/CXCR4 and c-kit/SCF axes are closely linked [18], and our study found that SDF-1α could enhance c-kit expression. However, limited information is known on the regulation of SDF-1α on c-kit.

DNA methylation is a covalent modification, which cytosine is methylated in a reaction catalysed by DNA methyltransferases (DNMT), with S-adenosyl methionine (SAM) as a methyl donor [19]. DNA methylation occurs in a CpG dinucleotide in adult somatic tissues, whereas non-CpG methylation occurs in embryonic stem cells [20]. In mammalian cells, three DNMTs, namely, DNMT1, DNMT3α, and DNMT3β, are responsible for DNA methylation [19–21]. DNA methylation is an important method in the regulatory mechanisms of gene expression [19–21]. A recent study has found that TGFβ1 could regulate CD133 expression through the inhibition of DNMT1 and DNMT3β expressions, and subsequently, the demethylation of promoter-1 [22]. However, as the influence of SDF-1α on the expression of c-kit by DNA methylation is unknown, the aim of the present study was to examine whether SDF-1α alters DNMT1 and DNMT3β expression and induces c-kit expression through demethylation of its promoter, in c-kit positive cardiomyocytes isolated from C57BL/6 adult mouse hearts.

Materials and Methods

Isolation and Culture of CPCs

Following the standard method described previously [23], the obtained cells were purified by magnetic-activated c-kit cell sorting magnetic beads (Miltenyi Biotec Inc., GER) following the instructions of the manufacturers.

Characterisation of CPCs

c-kit-positive CPCs were characterised by phase-contrast microscopy that evaluates morphology and flow cytometric analysis to examine the expression of stem cell surface markers. Flow cytometric analysis was performed as previously described [13].

Quantitative Real-time PCR

c-kit-positive CPCs were stimulated with 100 ng/ml SDF-1α and 5 μg/ml AMD3100 for 48 h, and qPCR was performed as previously described [13]. Sequences of each primer were designed as follows: GAPDH forward primer: CAAGGTCATCCATGACAACTTTG and reverse primer: GTCCACCACCCTGTTGCTGTAG, c-kit forward primer: ACATCGCCAGAGCCAACG and reverse primer: ATCCACTTTAATTTCGGGTCAA, DNMT1 forward primer: GAGCCCAGCAAAGAGTAT and reverse primer: ATGGTAGAAGGAGGAACAG, DNMT3α forward primer: CTGTCCCATCCAGGCAGTAT and reverse primer; CTTAGCGGTGTCTTGGAAGC, DNMT3β forward primer: AGATGATGGGAATGGCTCTG and reverse primer: TGCTGAAGATGATGCTCGAC.

Western Blotting

c-kit-positive CPCs were stimulated with 100 ng/ml SDF-1α and 5 μg/ml AMD3100 for 48 h, and western blotting was performed as previously described [13].

CPCs Proliferation and Migration

Cell proliferation assay was performed using CCK-8 kit (cell counting kit-8) (Dojido, Japan), according to the manufacturer's instruction.

A cell migration assay was performed in 24-well Transwell plates (Millipore, Billerica), according to the manufacturer's instruction.

Nuclear DNMT Activity Assay

c-kit-positive CPCs were stimulated with 100 ng/ml SDF-1α for 48 h. Nuclear protein was extracted using a nuclear extraction kit (Epigentek, Brooklyn, NY). Approximately 5 μg of nuclear protein was applied for the DNMT activity assay, which was performed using an EpiQuik DNMT activity assay kit (Epigentek), according to the instructions of the manufacturer.

Bisulfite Sequencing Analysis

Bisulfite treatment: Genomic DNA sodium bisulfate conversion was performed using the EZ-96 DNA methylation kit (Zymo Research). The instruction of the manufacturer was followed, using 1 μg of genomic DNA and the alternative conversion method.

Methylation analysis: Quantitative methylation analysis was carried out with Sequenom's MassARRAY platform, using MALDI-TOF mass spectrometry combined with RNA base-specific cleavage (MassCLEAVE), performed as described previously [24]. Mass spectra was acquired by a MassARRAY Compact MALDI-TOF (Sequenom) and their methylation ratios were generated using the Epityper software v1.0 (Sequenom).

Statistical Analysis

Statistical analysis was performed using SPSS (v 11.5, SPSS Inc.). All values were presented as mean ± SD. The differences between the two groups were analysed using the student's *t*-test. All tests were two-tailed and statistical significance was accepted if $P < 0.05$.

Results

CPCs Generation and Phenotypic Characterisation

CPCs were obtained with mild enzymatic digestion from adult C57BL/6 mouse hearts, and enriched for c-kit-positive cells by using magnetic-activated cell sorting. After 10 days in culture, a layer of fibroblast-like cells emerged from adherent plated adult mouse heart tissues (named cardiac explants) and small, round and phase-bright cells migrated (Fig. 1A and B). Inverted phase contrast microscope