Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Transplantation of CREG modified embryonic stem cells improves cardiac function after myocardial infarction in mice

Jian Zhang [a, 1], Xiaoxiang Tian [a, 1], Chengfei Peng [a, 1], Chenghui Yan [a], Yang Li [a], Mingyu Sun [a], Jian Kang [a], Erhe Gao [b], Yaling Han [a, *]

[a] Department of Cardiology, Institute of Cardiovascular Research, General Hospital of Shenyang Military Region, Shenyang 110016, China
[b] Center for Translational Medicine, Temple University School of Medicine, Philadelphia, PA 19140, USA

ARTICLE INFO

Article history:
Received 16 April 2018
Accepted 19 April 2018
Available online xxx

Keywords:
CREG
Embryonic stem cell
Myocardial infarction
Apoptosis

ABSTRACT

Engraftment of embryonic stem cells (ESC) has been proposed as a potential therapeutic approach for post-infarction cardiac dysfunction. However, only mild function improvement has been achieved due to low survival rate and paracrine dysfunction of transplanted stem cells. Cellular repressor of E1A stimulated genes (CREG) has been reported to be a secreted glycoprotein implicated in promoting survival and differentiation of many cell types. Therefore we hypothesized that transplantation of genetically modified ESC with CREG (CREG-ESC) can improve cardiac function after myocardial infarction in mice. A total of 2×10^5 CREG-ESC or EGFP-ESC were engrafted into the border zone in a myocardial infarction model in mice. Cardiac function, infarct size and fibrosis at 4 weeks, survival of transplanted ESC, apoptosis and cytokine level of heart tissue, and teratoma formation were assessed in vivo. Apoptosis of ESC under inflammatory stimuli and cardiac differentiation of ESC were investigated in vitro. After 4 weeks, we found transplantation of CREG-ESC could significantly improve cardiac function, ameliorate cardiac remodeling, and reduce infarct size and fibrosis area. Transplantation of CREG-ESC remarkably increased ESC survival in the border zone and inhibited apoptosis of cardiomyocytes. Furthermore, the decrease of inflammatory factors (IL-1β, IL-6 and TNF-α) and increase of anti-inflammatory factors (TGF-β, bFGF and VEGF165) in the border zone were higher in CREG-ESC transplanted hearts. Safety evaluation showed that all transplantation at 2×10^5 per heart dose produced no teratoma. Surprisingly, the mice with 3.0×10^6 CREG-ESC transplantation was demonstrated teratoma free without cardiac rhythm disturbances in contrast to 100% teratoma formation and rhythm abnormality for the same dose of EGFP-ESC transplantation. In addition, overexpression of CREG inhibits ESC apoptosis and enhanced their differentiation into cardiomyocytes in vitro. Transplantation of CREG-modified ESC exhibits a favorable survival pattern in infarcted hearts, which translates into a substantial preservation of cardiac function after acute myocardial infarction.

1. Introduction

Myocardial infarction (MI) remains a major cause of morbidity and mortality and is responsible for about one third of heart failure worldwide [1]. Loss of cardiomyocytes and vasculature leads to progressive fibrosis replacement of myocardium, hypertrophic growth of the spared myocardium, and left ventricular (LV) dilatation. Although coronary intervention, pharmacological thrombolytic therapy, and coronary bypass surgery have led to a salient decrease in AMI mortality, none of these treatments have been shown to regenerate the dead myocardium. A major reason for the high mortality is the limited intrinsic regeneration capacity of the adult heart after myocardial infarction [2].

Cellular repressor of E1A-stimulated genes (CREG) is a recently identified secreted glycoprotein that antagonizes transcription activation and cellular transformation induced by the adenovirus E1A oncoprotein. CREG has been used in the studies due to its cytoprotective and antiapoptotic properties on smooth muscle cells, endothelial cells, cardiomyocytes and bone marrow-derived mesenchymal stem cell in ischemic and inflammatory environments, as well as its ability to induce neovascularization processes

* Corresponding author.
E-mail address: hanyaling2014@126.com (Y. Han).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.04.160
0006-291X/

(under review) and to reduce fibrosis and inflammatory responses, we try to explored the potential of CREG-modified ESC (CREG-ESC) to preserve cardiac function following MI.

In this study, we report that implantation of mouse ESC with CREG overexpression improves cardiac function by maintaining survival and paracrine function of engrafted ESC. Our data suggest that CREG modified ESC represent a novel therapeutic choice for ischemic heart disease with advantages of bypassing limitations of routing stem cell transplantation.

2. Methods

All of the experimental procedures were approved by Northern Hospital's Animal Care and Use Committee.

2.1. Myocardial infarction and cell transplantation

C57BL/6 mice were maintained under pathogen-free conditions in the animal facility of our laboratory. Seven-to nine-week-old male C57BL/6 mice were anesthetized with isoflurane (3%), the pericardium opened, and a 6.0 silk suture placed at the distal 1/3 of the left anterior descending coronary artery (LAD). Cells were then injected into the peri-ischemic area. Cells (2×10^5 CREG-ESCs or EGFP-ESCs) were injected in 20 μl PBS without Ca^{++} or Mg^{++}. After the injection, the heart was immediately placed back into the intra-thoracic space, the thorax was closed, and manual evacuation of pneumothoraxes was performed. In the sham group, the LAD was left unligated as described.

2.2. Echocardiography

Transthoracic echocardiography was performed 4w after coronary ligation and cell grafting, using the Vevo 2100 Imaging system (MS-400 transducer, VisualSonic Inc.). M-mode interrogation was performed in the parasternal short-axis view at the level of the greatest LV end-diastolic dimension. LV end-diastolic dimension, LV end-systolic dimension, and septal and LV posterior wall thicknesses were determined and used to calculate the percentage of fractional shortening and ejection fraction. All measurements were averaged for 3 cardiac cycles and performed by an experienced operator blinded to the treatment group.

2.3. Hemodynamic parameters

After 28 days, mice were studied for hemodynamic parameters. Briefly, after full anesthesia with intra-peritoneal injections of 2.5% avertin, the right carotid artery was exposed. A PE-10 catheter connected to a pressure transducer was inserted retrograde from the carotid artery to the left ventricular cavity. The mean arterial blood pressure (MABP), left ventricular pressure (LVP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), first derivative of the LVP (+dP/dtmax and −dP/dtmax), and heart rate (HR) were measured using computer algorithms and an interactive video graphics program. Four groups of mice were analyzed for hemodynamic parameters 4w after MI and injection.

2.4. Quantification of infarct size

Four weeks after LAD ligation and injection, hearts were quickly excised, atria and right ventricular free wall were removed, and the left ventricle (LV) was sliced into 1-mm-thick sections perpendicular to the long axis of the heart; TTC (1% 2,3,5-triphenyltetrazolium chloride; Sigma-Aldrich) staining was performed as described. Normal myocardium was identified by brick-red coloration and infarcted areas by lack of dehydrogenase activity (white coloration). The circumference of necrotic and viable tissue was measured using SigmaScan Pro 5: percentage of myocardial infarction was calculated as the total length of circumference of infarction area divided by total length of circumference of LV (circumference of infarct + alive tissue). Slices were photographed with a digital camera.

2.5. Determination of myocardial apoptosis

Terminal deoxynucleotidyl-transferase-mediated dUTP nick-end labeling (TUNEL) (Roche No. 12 156 792 910) was used to detect the apoptosis in situ. The LV sections were treated according to the manufacturer's instructions, and slides were covered with a glass cover slide applied with mounting media containing 4,6-diamidino-2-phenylindole. An Olympus confocal microscope was used to visualize the cells, and NIS Elements software was used to record images. The percentage of TUNEL-positive nuclei (of 3000 total nuclei per heart) was determined in the myocardium remote from the infarct zone of each animal.

2.6. Immunostaining procedures

The hearts were harvested at 24 h to 4 weeks after grafting, frozen in liquid nitrogen, and cryo-sectioned (4-μm sections). Immunostaining was performed using monoclonal antibodies to α-actinin (Sigma-Aldrich) antigen. Preparations were incubated with secondary antibodies at 1:100 dilutions and analyzed by confocal microscopy.

2.7. Western blotting

Western blot was performed as described before. Briefly, frozen heart tissue was pulverized in a liquid-nitrogen-cooled mortar, and protein was extracted from powdered tissue using an ice-cold 0.15 mmol/L NaCl/0.05 mmol/L Tris-HCl, pH 7.2/1% Triton X-100/1% sodium deoxycholate/0.1% SDS (RIPA buffer) and quantified using the bicinchoninic acid assay method. For Western blot analysis, 30 μg of total protein was electrophoresed on Bis-Tris 5–12% gradient gels (Bio-Rad), and wet transferred onto polyvinylidene fluoride membrane (PVDF) membranes. Bands were visualized by chemiluminescence (Super West Dura kit; Pierce Biotechnologies) with a 16-bit camera imaging system (Fuji LAS-3000; Fuji film) until a saturated pixel was observed and densitometry performed using Image Pro plus software. Antibodies to Flag, CREG, EGFP, caspase-3, cleaved-caspase-3 and β-actin were all obtained from Cell Signaling.

2.8. ELISA analysis for cytokines

The levels of IL-1β, IL-6, TNF-α, VEGF, bFGF, TGF-β in the myocardium of infarcted border zone were determined by ELISA using commercially available Quantikine kits (R&D) according to the manufacturer's instruction. All samples and standards were measured in duplicates.

2.9. RT-PCR analysis

GATA-4: forward 5′-GGTTCCCAGGCCTCTTGCAATGCGG-3′ and reverse 5′-AGT GGCATTGCTGGAGTTACCGCTG-3’; Mef2C: forward 5′-AGATACCCACAACACACCACGC GCC and reverse 5′-ATCCTTCA-GAGAGTCGCATGCGCTT-3’; Tbx3: forward 5′- ATTTCA-CAATTCTCGGTGGA-3′ and reverse 5′-TATAATTCCCCTGCCACGTA-3′ and GAPDH forward 5′-GAAGGTGAAGGTCGGAGTC-3′ and reverse 5′-GAAGATGGTGATGGGATTTC-3’. All reactions were performed in

Please cite this article in press as: J. Zhang, et al., Transplantation of CREG modified embryonic stem cells improves cardiac function after myocardial infarction in mice, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.160