Journal of Molecular and Cellular Cardiology 100 (2016) 54–63

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

S100A4 protects the myocardium against ischemic stress☆

Shirin Doroudgar, PhD [a,b,c], Pearl Quijada, PhD [a], Mathias Konstandin, MD [a,b,c], Kelli Ilves, BS [a], Kathleen Broughton, PhD [a], Farid G. Khalafalla, PhD [a], Alexandria Casillas [a], Kristine Nguyen [a], Natalie Gude, PhD [a], Haruhiro Toko, MD [a], Luis Ornelas, BS [a], Donna J. Thuerauf, MS [a], Christopher C. Glembotski, PhD [a], Mark A. Sussman, PhD [a], Mirko Völkers, MD [a,b,c,*]

[a] The San Diego State Heart Institute and Department of Biology, San Diego State University, San Diego, CA 92182, USA
[b] University Hospital Heidelberg, Internal Medicine III, Heidelberg, Germany
[c] DZHK (German Centre for Cardiovascular Research), partner site Heidelberg/Mannheim, Germany

ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 13 September 2016
Accepted 4 October 2016
Available online 6 October 2016

Keywords:
S100A4
Myocardial infarction
Remodeling

ABSTRACT

Background: Myocardial infarction is followed by cardiac dysfunction, cellular death, and ventricular remodeling, including tissue fibrosis. S100A4 protein plays multiple roles in cellular survival, and tissue fibrosis, but the relative role of the S100A4 in the myocardium after myocardial infarction is unknown. This study aims to investigate the role of S100A4 in myocardial remodeling and cardiac function following infarct damage.
Methods and results: S100A4 expression is low in the adult myocardium, but significantly increased following myocardial infarction. Deletion of S100A4 increased cardiac damage after myocardial infarction, whereas cardiac myocyte-specific overexpression of S100A4 protected the infarcted myocardium. Decreased cardiac function in S100A4 Knockout mice was accompanied with increased cardiac remodeling, fibrosis, and diminished capillary density in the remote myocardium. Loss of S100A4 caused increased apoptotic cell death both in vitro and in vivo in part mediated by decreased VEGF expression. Conversely, S100A4 overexpression protected cells against apoptosis in vitro and in vivo. Increased pro-survival AKT-signaling explained reduced apoptosis in S100A4 overexpressing cells.
Conclusion: S100A4 expression protects cardiac myocytes against myocardial ischemia and is required for stabilization of cardiac function after MI.

1. Introduction

The progressive nature of ischemic cardiomyopathy appears to be dictated partly by the continuous loss of cardiac myocytes and the inability of the myocardium to regenerate [1]. New treatments that target the disease mechanism at the cellular and whole-organ level are needed to halt and reverse the devastating consequences of this disease. S100A4 is a calcium-binding cardiac protein shown to protect cardiac myocytes from cell death [1,2]. S100 proteins constitute of EF-hand calcium-binding proteins that regulates biological processes, such as cardiac contractility, proliferation, migration, and cell differentiation [3]. S100A4 was first described as a factor promoting metastasis and angiogenesis [4,5]. The mechanisms of these effects are not fully elucidated, but it is well known that the protein has both intracellular and extracellular functions, and that the molecular pathways and mechanism are mediated, at least partly, by activation of the pro-survival kinase AKT [6,7]. Importantly, in the last years S100A4 was related to multiple functions including cardiomyogenesis, growth and also survival promoting activity on cardiac myocytes [1,2,8]. Moreover, S100A4-Cre-recombinase reporter mice have been used for fibroblast lineage tracing in the myocardium, although specificity of S100A4 for cardiac fibroblasts has been recently questioned [2,9,10]. More recently it has been shown that S100A4 knockout mice showed reduced interstitial fibrosis, decreased myofibroblasts, suppressed expressions of collagens and pro-fibrotic cytokines in the left ventricle after pressure overload [3,11]. However, no studies have examined the effects of increased S100A4 protein levels in the ischemic myocardium. Therefore, acute myocardial infarction (MI) was used as the setting to assess the impact of S100A4 on pathologic injury using knockout and overexpression models, with implications for S100A4 as a novel therapeutic target for cardiac ischemia.

2. Methods

2.1. Animal procedures, echocardiography and invasive hemodynamics assessment

Generation of S100A4 null mice was previously reported [4,5,12]. Eight-ten week-old male S100A4 KO and wild-type (WT) mice of an A/Sn genetic background were used for the present study. The mice

☆ Subject codes: Basic Science Research (130), Other Myocardial Biology (108).

* Corresponding author at: University Hospital Heidelberg, Department of Internal Medicine III, Im Neuenheimer Feld 669, 69120 Heidelberg, Germany.
E-mail address: Mirko.voelkers@med.uni-heidelberg.de (M. Völkers).

http://dx.doi.org/10.1016/j.yjmcc.2016.10.001
0022-2828/

were housed 8–10 animals/cage with a 12-hour light and dark cycle and were fed standard laboratory chow and water ad libitum. Myocardial infarction was produced by ligating the left anterior descending (LAD) branch of the coronary artery using a 8-0 suture (Ethicon). Control mice underwent a sham operation. Echocardiography was performed under mild isoflurane sedation (0.5%–1.5%) using a Vevo 770 high-resolution system. Cardiac function was analyzed in the parasternal long axis view by tracking the endocardium with the supplied analysis software to obtain end systolic volume, end diastolic volume, ejection fraction, and heart rate. Consecutive noninvasive assessment of cardiac function in the parasternal long axis view has been performed 1, 2, 4, and 6 weeks after surgery. Closed chest hemodynamic assessment was performed on mice anesthetized with 3% chloral hydrate (10 μL per 1 mg body weight) before insertion of microtip pressure transducer (FT111B, Scisense) into the right carotid artery and advancement into left ventricle. The catheter was connected to an A/D converter (FV892A, Scisense) for data collection. After hemodynamic measurements, hearts were arrested in diastole using high potassium solution

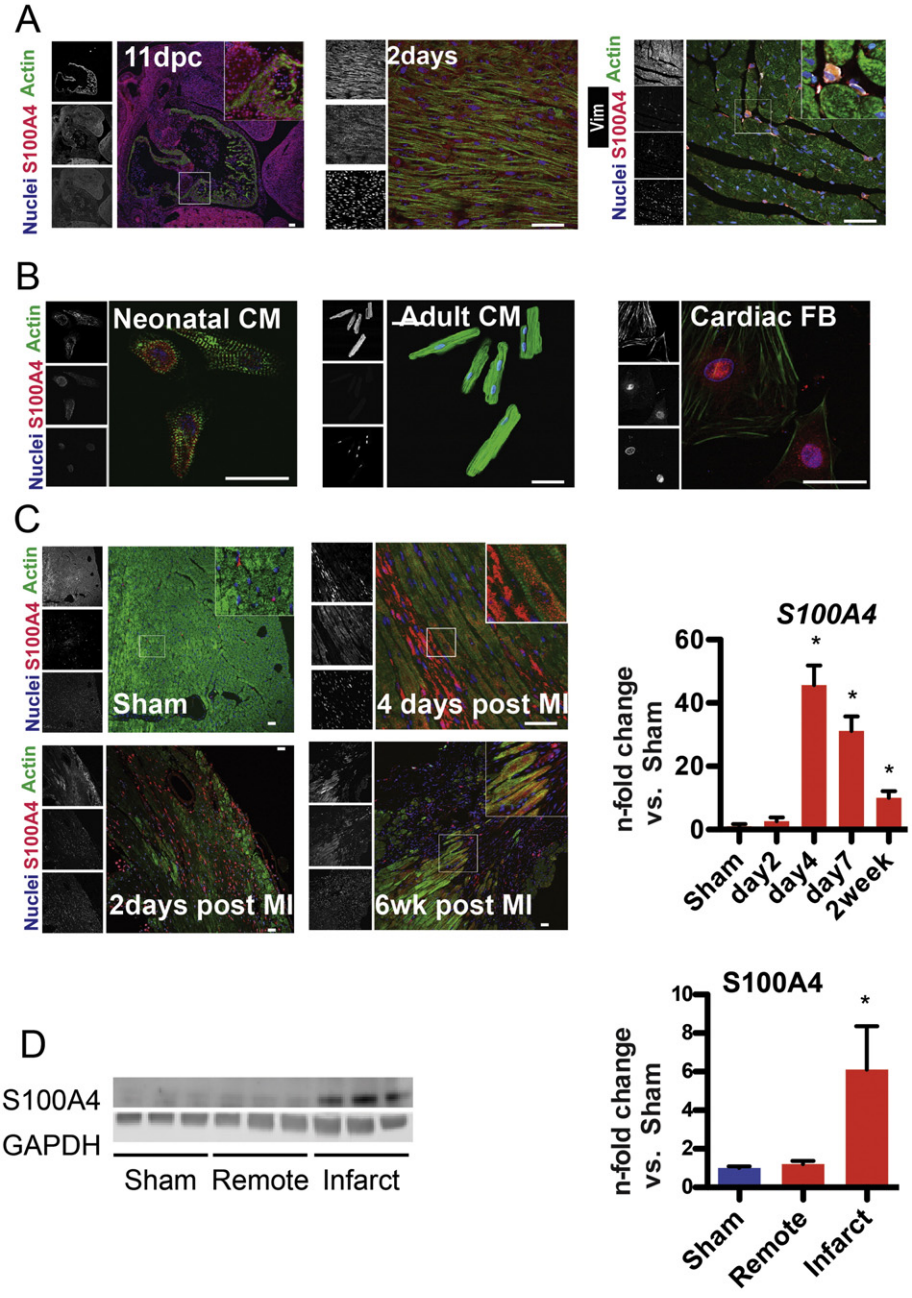

Fig. 1. S100A4 in development and after myocardial infarction. (A) Confocal microscopy of paraffin-embedded sections from wild-type mouse hearts at 11 dpc, 2 days and 3 months stained for S100A4 (red), Actin (green), Vimentin (white) and nuclei (blue) at different developmental stage. S100A4 is highly expressed in embryonic hearts, but only expressed in interstitial cells in adult myocardium. Scale bar 25 μM. (B) Immunofluorescence in neonatal cardiomyocytes (CM) (left panel) show robust expression of S100A4 whereas adult myocytes (middle panel) are negative for S100A4. Strong S100A4 expression is observed in cardiac Fibroblast (FB - right panel). Scale bar 25 μM. (C) Confocal microscopy of paraffin-embedded sections from wild-type mouse hearts at sham operated, 2 days, 4 days and 6 weeks post MI stained for S100A4 (red), Actin (green), Scale bar 25 μM. Right: S100A4 transcription in hearts of mice of the indicated group after surgery. *$p < 0.01$ versus Sham. $n = 4$ per group. Scale bar 25 μM. (D) Immunoblot and quantification for S100A4 in infarcted myocardium (6 weeks after Infarction). *$p < 0.01$ versus Sham. $n = 3$ per group.