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Ablation of biglycan attenuates cardiac hypertrophy and fibrosis after left ventricular pressure overload☆

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ABSTRACT

Aims: Biglycan, a small leucine-rich proteoglycan, has been shown to play an important role in stabilizing fibrotic scars after experimental myocardial infarction. However, the role of biglycan in the development and regression of cardiomyocyte hypertrophy and fibrosis during cardiac pressure overload and unloading remains elusive. Thus, the aim of the present study was to assess the effect of biglycan on cardiac remodeling in a mouse model of left ventricular pressure overload and unloading.

Methods and results: Left ventricular pressure overload induced by transverse aortic constriction (TAC) in mice resulted in left ventricular dysfunction, fibrosis and increased biglycan expression. Fluorescence- and magnetic-assisted sorting of cardiac cell types revealed upregulation of biglycan in the fibroblast population, but not in cardiomyocytes, endothelial cells or leukocytes after TAC. Removal of the aortic constriction (rTAC) after short-term pressure overload (3 weeks) improved cardiac contractility and reversed ventricular hypertrophy but not fibrosis in wild-type (WT) mice. Biglycan ablation (KO) enhanced functional recovery but did not resolve cardiac fibrosis. After long-term TAC for 9 weeks, ablation of biglycan attenuated the development of cardiac hypertrophy and fibrosis. *In vitro*, biglycan induced hypertrophy of neonatal rat cardiomyocytes and led to activation of a hypertrophic gene program. Putative downstream mediators of biglycan signaling include *Rcan1*, *Abra* and *Tnfrsf12a*. These genes were concordantly induced by TAC in WT but not in biglycan KO mice.

Conclusions: Left ventricular pressure overload induces biglycan expression in cardiac fibroblasts. Ablation of biglycan improves cardiac function and attenuates left ventricular hypertrophy and fibrosis after long-term pressure overload. *In vitro* biglycan induces hypertrophy of cardiomyocytes, suggesting that biglycan may act as a signaling molecule between cell types to modulate cardiac remodeling.

1. Introduction

Cardiovascular disease remains the leading cause of mortality globally. A hallmark of cardiovascular disease ultimately leading to heart failure is myocardial adverse remodeling, which includes cardiomyocyte death, changes of cardiac morphology, myocardial fibrosis, and cardiac dysfunction [1,2]. Well-established pharmacological therapy, including beta-blockers [3], angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists and mineralocorticoid receptor antagonists [4,5] as well as device therapy such as cardiac resynchronization [6], aim at improving cardiac function, influencing neurohumoral

Abbreviations: LVAD, left ventricular assist device; NRCM, neonatal rat cardiomyocytes; rTAC, reversible transverse aortic constriction; TAC, transverse aortic constriction.

☆ Beetz: biglycan and cardiac remodeling.

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maladaptive activation, and halting or reversing remodeling. Still, numerous patients in end-stage heart failure will ultimately require heart transplantation [7,8]. Due to the limited number of donor organs, left ventricular assist devices (LVAD) are frequently used as a bridge to transplantation. Interestingly, in a number of patients, this approach led to myocardial recovery, by a process called reverse remodeling, to an extent eventually allowing LVAD explantation [9].

However, despite this improvement in cardiac function after LVAD implantation in individual patients, the degree of myocardial remodeling might be restricted by the amount of extracellular collagen deposition and scar formation [10]. Thus, strategies aiming at reducing the amount or modulating the stability of myocardial fibrosis during the development or management of heart failure are promising approaches to enhance cardiac reverse remodeling on top of standard therapy.

Biglycan, together with decorin, lumican and others, belongs to the group of small leucine-rich proteoglycans, which are known to play major roles during development and disease [11–13]. Biglycan has been shown to interact with collagen to promote collagen stability [14]. Mice lacking biglycan were more prone to ventricular ruptures due to inappropriate scar formation after myocardial infarction [15].

In this study, we tested if a targeted deletion of biglycan in mice influences the development and regression of pressure overload-induced cardiac remodeling. Our results show that biglycan-deficiency attenuates the development of fibrosis and cardiac hypertrophy and preserves cardiac function during long-term cardiac pressure overload. However, genetic loss of biglycan *in vivo* did not facilitate the regression of fibrosis after cardiac unloading in mice. Soluble biglycan was able to induce cardiomyocyte hypertrophy *in vitro* and activated pro-hypertrophic pathways. We conclude that, besides its role as a structural extracellular matrix protein, biglycan may serve as a signaling molecule during cardiac remodeling.

2. Materials and methods

2.1. Animal procedures

All animal procedures were approved by the responsible Committees on the Ethics of Animal Experiments at the University of Freiburg (Regierungspräsidium, Freiburg, Germany, permit number: G12/30) and Düsseldorf, respectively and they conformed to the Guide for the Care and Use of Laboratory Animals (8th edition, 2011). The generation of biglycan-deficient mice was described previously [16]. Animals were maintained at a 12 h dark/light cycle and permitted access to food and water *ad libitum*. Experiments were performed using wild-type and male and female knockout littermates ($Bgn^{-/0}$ and $Bgn^{+/0}$, $Bgn^{-/-}$ and $Bgn^{+/+}$) at 2–3 months of age.

2.1.1. Transverse aortic constriction

C57BL6/J mice were obtained from Charles River (Sulzfeld, Germany). Transverse aortic constriction was applied at the age of 8–12 weeks. Mice were anesthetized with isoflurane (2 vol% in O_2, 1 l/min). A sternotomy was performed and the aortic arch was visualized after displacing the thymus lobes. A 7–0 polypropylene suture was looped around the transversal part of the aortic arch and a stenosis was placed using a slip-knot technique and a 27G needle as space holder. Sternum and skin were sutured using a 5–0 polypropylene suture and mice recovered on a heating plate. After three weeks of pressure overload (TAC group) mice underwent a second surgery for removal of stenosis. A sternotomy was performed and the knot was removed by pulling on the movable free end of the thread. Mice were followed-up for 9 weeks. The same protocol (3 weeks pressure overload/9 weeks follow-up after relief of stenosis) was applied to biglycan-deficient animals and littermate controls. For inducing pressure overload for 10 weeks, biglycan-deficient animals and littermate controls were used and aortic banding was performed using 6–0 silk suture. Mice were sacrificed at the indicated time points by cervical dislocation, hearts were excised and used for histology and biochemistry.

2.1.2. Left ventricular catheterization

Invasive hemodynamic measurements were performed at 5–6 months of age after 10 weeks of transverse aortic constriction as described [17]. Mice were anesthetized with isoflurane (2 vol% in O_2, 1 l/min). A 1.4 F Millar microtip catheter was inserted into the right carotid artery and pushed forward to the aortic arch and into the left ventricular cavity to allow measurement of systemic blood pressure and heart rate as well as cardiac contractility. Data were acquired and analyzed using LabChart v7.1.2 (ADInstruments). The isovolumetric time constant tau (τ) was calculated from LV pressure curves as described previously [18].

2.1.3. Echocardiography

Transthoracic echocardiography was performed in anesthetized mice (2 vol% isoflurane in O_2, 1 l/min) at various time points using a GE Healthcare Vivid 7 ultrasound machine and a 14 MHz transducer placed in a parasternal short-axis view position. Images acquired in two-dimensionally-guided M-mode were used to evaluate left ventricular anterior to posterior wall motion and thickness as well as cardiac cavity dimensions. The Teichholtz formula was used to calculate fractional shortening and ejection fraction. Pulsed wave Doppler was applied to visualize aortic constriction. After TAC, images of the aortic arch were carefully checked for signs of band internalization [19].

2.1.4. Isolation of adult cardiac cells

To obtain cardiomyocyte, endothelial cell, leukocyte, and fibroblast populations from adult mouse hearts, mice were sacrificed by cervical dislocation. Heparin (500 IE, Liquemin® Roche) was injected intraperitoneally 15 min before isolation of the cardiopulmonary tract. Hearts were mounted in a Langendorff apparatus by connecting the ascending aorta to the perfusion cannula. Hearts were retrogradely perfused with an enzyme solution modified from the Alliance for Cell Signaling protocol (Protocol ID PP00000125, http://www.signaling-gateway.org) containing 0.8 mg/ml collagenase B, 0.4 mg/ml hyaluronidase and 3 μg/ml trypsin. The resulting single cell suspension was centrifuged at 20 × *g* for 10 s. The pellet was re-suspended in phosphate-buffered saline and stained with Draq5. Rod-shaped cardiac myocytes ($Draq5^{pos}$, FSC^{high}) were purified using a BioRad S3 FACS sorter. The supernatant containing the non-myocyte fraction was incubated for 30 min with anti-CD45 magnetic beads (1:10, Miltenyi) and FITC-labeled griffonia simplicifolia lectin I (1:200, Sigma). $CD45^{pos}$ leukocytes were depleted from the cell suspension by magnetic assisted cell sorting according to the manufacturer's protocol. Endothelial cells ($CD45^{neg}$, $Draq5^{pos}$, $FITC^{pos}$) and the non-cardiomyocyte, non-leukocyte, non-endothelial cell fraction ($CD45^{neg}$, $Draq5^{pos}$, $FITC^{neg}$) containing cardiac fibroblasts were isolated by FACS.

2.2. Proteomic analysis

Proteomic analysis was performed from whole heart tissue lysed with 0.5% Igepal CA-630 and mixed 1:1 with cell lysates from three SILAC-labeled (Lys8/Arg10) mouse cell lines (C2C12, B16F10 and 3T3, ATCC) as internal standard. Sample preparation, LC-MS/MS experiments, and data analysis were performed as described [20]. In brief, samples were reduced with β-mercaptoethanol, alkylated with iodoacetamide, and subjected to SDS-PAGE. Gel lanes were excised and proteins were digested with trypsin. Peptides were extracted and desalted manually with reversed phase C-18 STAGE tips. Peptides were entered into an LC-MS/MS experiment using an Agilent 1200 nanoflow HPLC (Agilent) coupled on-line to a LTQ OrbiTrap XL (Thermo Scientific). Spectra were analyzed using MaxQuant by comparison with UniProt sequence database (release 2016_08, September 2016). The protein list was filtered for contaminants and reverse database hits. Only proteins with ≥1 unique peptides were considered. Biglycan was