Journal of Molecular and Cellular Cardiology 92 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Increased fibroblast chymase production mediates procollagen autophagic digestion in volume overload

Lianwu Fu [a,c], Chih-Chang Wei [a,b], Pamela C. Powell [a], Wayne E. Bradley [b], Sarfaraz Ahmad [d], Carlos M. Ferrario [d], James F. Collawn [c], Louis J. Dell'Italia [a,b,c,*]

[a] Birmingham Veteran Affairs Medical Center, University of Alabama at Birmingham, Birmingham, AL, United States
[b] Division of Cardiovascular Disease, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, United States
[c] Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, United States
[d] Division of Surgical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, United States

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 20 January 2016
Accepted 21 January 2016
Available online 22 January 2016

Keywords:
Volume overload
Cardiac fibroblast
Chymase
Autophagy
Intracellular procollagen

ABSTRACT

Background: Previous work has identified mast cells as the major source of chymase largely associated with a profibrotic phenotype. We recently reported increased fibroblast autophagic procollagen degradation in a rat model of pure volume overload (VO). Here we demonstrate a connection between increased fibroblast chymase production and autophagic digestion of procollagen in the pure VO of aortocaval fistula (ACF) in the rat.
Methods and results: Isolated LV fibroblasts taken from 4 and 12 week ACF Sprague–Dawley rats have significant increases in chymase mRNA and chymase activity. Increased intracellular chymase protein is documented by immunocytochemistry in the ACF fibroblasts compared to cells obtained from age-matched sham rats. To implicate VO as a stimulus for chymase production, we show that isolated adult rat LV fibroblasts subjected to 24 h of 20% cyclical stretch induces chymase mRNA and protein production. Exogenous chymase treatment of control isolated adult cardiac fibroblasts demonstrates that chymase is internalized through a dynamin-dependent mechanism. Chymase treatment leads to an increased formation of autophagic vacuoles, LC3-II production, autophagic flux, resulting in increased procollagen degradation. Chymase inhibitor treatment reduces cyclical stretch-induced autophagy in isolated cardiac fibroblasts, demonstrating chymase's role in autophagy induction.
Conclusion: In a pure VO model, chymase produced in adult cardiac fibroblasts leads to autophagic degradation of newly synthesized intracellular procollagen I, suggesting a new role of chymase in extracellular matrix degradation.

Published by Elsevier Ltd.

1. Introduction

Previous work has identified mast cells as the major source of chymase that has been largely associated with a profibrotic phenotype. In a pure volume overload (VO) induced by aortocaval fistula (ACF) or primary mitral regurgitation (MR), we identified increased chymase and mast cell degranulation at early and late stages in the progression to left ventricular (LV) dilatation, LV wall thinning, cardiomyocyte elongation and thinning, and heart failure [1–4]. We sought to understand the reasons for the net extracellular matrix (ECM) breakdown and lack of replacement of ECM in the pure VO, despite of the upregulation of chymase and other renin-angiotensin system components. In addition to being the major Ang II-forming mechanism in the heart [5–7], chymase activates MMPs [8–11], degrades fibronectin [12] and causes apoptosis of vascular smooth muscle cells and cardiomyocytes through disruption of the focal adhesion complex [13,14]. Until recently, these chymase actions have been assigned to the interstitial compartment within the extracellular matrix. However, we recently demonstrated intracellular chymase in cardiomyocytes during ischemia reperfusion injury in the dog and that the chymase uptake in cardiomyocytes is dynamin-mediated [15].

More recently, we reported increased fibroblast autophagic procollagen degradation in a pure VO rat model [16]. Such a role of autophagy in intracellular procollagen degradation has been demonstrated after TGF-β treatment in primary mesangial cells from mouse kidney [17]. Other studies have demonstrated that chymase is produced in neonatal cardiac fibroblasts under glucose stimulation and is an important Ang II-forming mechanism within the fibroblast [18–20]. In addition, Husain and coworkers [21] showed evidence of chymase production in human cardiac interstitial cells and emphasized the importance of chymase production by endothelial cells and fibroblasts rather than just mast cells alone. Verification of this finding would then elevate the presence and location of chymase from the relatively sparse sites of mast cells in the heart to a more prominent role in juxtaposition to cardiomyocytes throughout the cardiac interstitium.

* Corresponding author at: Birmingham VA Medical Center, 700 South 19th Street, Birmingham, AL 35233, United States.
E-mail address: louis.dellitalia@va.gov (L.J. Dell'Italia).

http://dx.doi.org/10.1016/j.yjmcc.2016.01.019
0022-2828/Published by Elsevier Ltd.

Here, we demonstrate that chymase is produced within adult cardiac fibroblasts themselves in response to the chronic stress of VO of ACF and in isolated adult cardiac fibroblasts after cyclical stretch. *In vitro* studies in fibroblasts show that chymase addition alone is sufficient to induce autophagy in cardiac fibroblasts and promote intracellular degradation of procollagen without affecting collagen mRNA transcript levels. These studies report a heretofore unrecognized mechanism for chymase production and uptake by fibroblasts and subsequent autophagic degradation of intracellular procollagen. This process exacerbates the ECM homeostatic imbalance by decreasing procollagen production in the face of interstitial collagen degradation and results in net ECM loss and adverse LV remodeling in a pure VO.

2. Experimental methods

2.1. Animal studies

Adult male Sprague–Dawley rats (200–250 g) at 10 weeks old of age were subjected to either sham or ACF surgery as described previously in our laboratory [1–3,22]. Briefly, for induction of ACF, rats were anesthetized with isoflurane (2% O_2 at 2 L/min) 10 min prior to the surgery. A ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava 1.5 cm below the renal arteries. The overlying adventitia was removed by blunt dissection to expose the two vessels, taking care not to disrupt the tissue connecting the vessels. Both vessels were then occluded proximal and distal to the intended puncture site, and an 18-guage needle was inserted into the exposed abdominal aorta and advanced through the medial wall into the vena cava to create the fistula. The needle was withdrawn and the ventral aortic puncture was sealed with cyanoacrylate. Creation of the ACF was visualized by the pulsatile flow of oxygenated blood into the vena cava. The abdominal musculature and skin incisions were closed by standard techniques with absorbable suture and auto clips. The control animals, sham, underwent general anesthesia and an abdominal incision without ACF. Rats were medicated with buprenorphine (0.05 mg/kg; IP) preoperatively and at the end of the day of surgery. Every effort was made to minimize any discomfort to the animals used in these studies. The animals were euthanized following anesthesia with isoflurane and exsanguination by rapid removal of the heart. This method is consistent with the recommendations of the Panel of Euthanasia of the American Veterinary Medical Association. All procedures were approved and performed according to the guidelines of the Institutional Animal Care and Use Committees of the University of Alabama at Birmingham (Animal Resource Program, Protocol 140909,251) and followed the National Institute of Health's "Guide for the Care and Use of Laboratory Animals".

2.2. Cardiac fibroblast isolation

4 or 12 weeks after sham or ACF surgery, adult rat LV fibroblasts were isolated by recirculating perfusion buffer supplemented with 1 mg/ml collagenase type II (Invitrogen, CA) as previously described [1]. Briefly, the heart was perfused with a buffer (120 mM NaCl, 15 mM KCl, 0.5 mM KH_2PO_4, 5 mM $NaHCO_3$, 10 mM HEPES, and 5 mM glucose, at pH 7.0) for 5 min and digested with perfusion buffer containing 1 mg/ml collagenase II for 30 min at 37 °C. The right ventricle and atria were removed before the perfused-heart was minced. The cell suspension was then mixed with stop buffer (perfusion buffer containing 10 mg/ml bovine serum albumin) to prevent further digestion. The cell suspension was added to a mesh cell collector and the flow-through was centrifuged at 80 *g* for 3 min to remove most of the cardiomyocytes. The supernatant containing mainly cardiac fibroblasts was centrifuged at 400 *g* for 8 min then resuspended in DMEM supplemented with antibiotics (penicillin/streptomycin, 1%), L-glutamine ascorbate and 10% FBS. Cells were subjected to differential plating on uncoated cell culture dishes (10 cm diameter) for 90 min. Non-adherent cells (mostly cardiomyocytes, endothelial and smooth muscle cells) were removed. We have demonstrated >95% purity of the prep with very little myofibroblast differentiation [1]. Cultured cells by this protocol routinely showed positive staining for anti-vimentin (Millipore #AB5733; 1:500) and no immunostaining with antibodies to anti-smooth muscle alpha-actin or fibronectin (1:100 dilutions, Sigma-Aldrich, MO). Adult rat cardiac fibroblasts were used at passages 1 and 2 in the current study.

2.3. Immunocytochemistry (ICC) of cardiac fibroblasts

Immunocytochemistry was performed on adult cardiac fibroblasts isolated from sham or ACF rats, or normal fibroblasts subjected to mechanical stretch (24 h, 20% stretch, 1 Hz) or exposed to recombinant human chymase (2.5 μg/ml for 2 h at 37 °C, Sigma-Aldrich #C8118). The cells were fixed in 4% formaldehyde (Tousimis, Rockville, MD) for 20 min at room temperature (RT) and washed 3 times in PBS, and permeabilized with 0.1% Triton-X-100 (Fisher #BP-151) for 15 min at RT. 10% normal serum (in 1% bovine serum/PBS) for 1 h at RT was used for blocking, followed by overnight incubation at 4 °C with a chymase monoclonal (Abcam #ab2377; 1:50) and a vimentin polyclonal antibody (Millipore #AB5733; 1:500). Alexa Fluor 488- and 594-conjugated secondary antibodies (1:700, Life Technologies/Invitrogen, OR) with the appropriate host combinations were incubated for 1 h to stain. Nuclei were stained with DAPI (1.5 μg/ml; Vector Laboratories, CA). Image acquisition and analyzing were performed using a Leica DM6000 epifluorescence microscope and SimplePCI software as described previously [16].

2.4. Cardiac fibroblast chymase activity measurements

Cardiac chymase activity in LV fibroblasts was calculated based on adding 1 nmol/L of highly purified ^{125}I-Ang-(1–12) substrate to the fibroblast plasma membranes and determining the amount of ^{125}I-Ang II product formation as previously described [29]. Briefly, isolated cardiac fibroblasts were homogenized in assay buffer (containing 25 mM HEPES, 125 mM $NaCl_2$ and 10 μM $ZnCl_2$, pH 7.4) and centrifuged at 44,000 *g* for 60 min to collect the native plasma membranes. The plasma membrane pallet was resuspended in assay buffer. Protein content was measured using the BCA kit. For chymase activity and inhibition studies, the plasma membranes (50–100 μg) were preincubated for 10 min with or without the chymase inhibitor-chymostatin (50 μM) in assay buffer. Besides chymostatin, other inhibitors (Lisinopril for ACE, SCH39373 for neprilysin, MLN-4760 for ACE2, amastatin for aminopeptidases, bestatin for aminopeptidases, benzyl succinate for carboxypeptidases and PCMB for cysteine proteases, each 50 μM) were also added to inhibit the renin-angiotensin enzymes (ACE/ACE2/neprilysin), aminopeptidases and cysteine proteases. After preincubation of plasma membrane with the inhibitor cocktail, radiolabeled ^{125}I-Ang-(1–12) substrate was added into the reaction medium and incubated for an additional 60 min at 37 °C. At the end of the incubation time, the reaction was stopped by adding equal volume of 1% phosphoric acid, mixed well and centrifuged at 28,000 *g* for 20 min to remove the plasma membranes. The clear supernatants were filtered through 0.2 μm PVDF membrane and injected on HPLC C-18 column and ^{125}I-Ang II product generation from ^{125}I-Ang-(1–12) by chymase was measured using an in-line flow-through gamma detector (BioScan Inc., Washington, DC). The enzyme activity was defined as fmoles of Ang II product formed from ^{125}I-Ang-(1–12) substrate per min per mg of protein (fmol Ang II formation/min/mg protein).

2.5. Application of extrinsic mechanical load

Cardiac fibroblasts (50,000 cells/cm^2) were cultured on non-coated Flexcell plates (Flexcell International Corp., Hillsborough, NC, USA) in DMEM medium containing 10% FBS, 2 mM glutamine, 10 U/mL penicillin, and 100 μg/ml streptomycin. The media was changed 24 h before initiation of the experiment and cells were subjected to cyclic strain (1 Hz) on the Flexcell Strain apparatus (model FX-5000; Flexcell International, Hillsborough, NC, USA) at a level of distension sufficient to

Download English Version:

https://daneshyari.com/en/article/8473907

Download Persian Version:

https://daneshyari.com/article/8473907

Daneshyari.com