JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2015 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.
VOL. 66, NO. 12, 2015
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2015.07.035

A Novel Collagen Matricryptin Reduces Left Ventricular Dilation Post-Myocardial Infarction by Promoting Scar Formation and Angiogenesis

Merry L. Lindsey, PhD,*†‡ Rugmani Padmanabhan Iyer, PhD,*† Rogelio Zamilpa, PhD,† Andriy Yabluchanskiy, MD, PhD,*† Kristine Y. DeLeon-Pennell, PhD,*† Michael E. Hall, MD,§ Abdullah Kaplan, MD,*† Fouad A. Zouein, PhD,*† Dustin Bratton, BSc,*† Elizabeth R. Flynn, BSc,*† Presley L. Cannon, BSc,*† Yuan Tian, PhD,*† Yu-Fang Jin, PhD,†‖ Richard A. Lange, MD,†¶ Dorota Tokmina-Roszyk, MSc,†# Gregg B. Fields, PhD,†# Lisandra E. de Castro Brás, PhD*†**

ABSTRACT

BACKGROUND Proteolytically released extracellular matrix (ECM) fragments, matricryptins, are biologically active and play important roles in wound healing. Following myocardial infarction (MI), collagen I, a major component of cardiac ECM, is cleaved by matrix metalloproteinases (MMPs).

OBJECTIVES This study identified novel collagen-derived matricryptins generated post-MI that mediate remodeling of the left ventricle (LV).

METHODS Recombinant collagen Ia1 was used in MMPs cleavage assays, the products were analyzed by mass spectrometry for identification of cleavage sites. C57BL6/J mice were given MI and animals were treated either with vehicle control or p1158/59 matricryptin. Seven days post-MI, LV function and parameters of LV remodeling were measured. Levels of p1158/59 were also measured in plasma of MI patients and healthy controls.

RESULTS In situ, MMP-2 and -9 generate a collagen Iα1 C-1158/59 fragment, and MMP-9 can further degrade it. The C-1158/59 fragment was identified post-MI, both in human plasma and mouse LV, at levels that inversely correlated to MMP-9 levels. We synthesized a peptide beginning at the cleavage site (p1158/59, amino acids 1159 to 1173) to investigate its biological functions. In vitro, p1158/59 stimulated fibroblast wound healing and robustly promoted angiogenesis. In vivo, early post-MI treatment with p1158/59 reduced LV dilation at day 7 post-MI by preserving LV structure ($p < 0.05$ vs. control). The p1158/59 stimulated both in vitro and in vivo wound healing by enhancing basement membrane proteins, granulation tissue components, and angiogenic factors.

CONCLUSIONS Collagen Iα1 matricryptin p1158/59 facilitates LV remodeling post-MI by regulating scar formation through targeted ECM generation and stimulation of angiogenesis. (J Am Coll Cardiol 2015;66:1364–74) © 2015 by the American College of Cardiology Foundation.

From the *Mississippi Center for Heart Research, Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi; †San Antonio Cardiovascular Proteomics Center, University of Texas Health Science Center, San Antonio, Texas; ‡Research Service, G.V. (Sonny) Montgomery Veterans Affairs Medical Center, Jackson, Mississippi; §Division of Cardiology and Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi; ‖Department of Electrical and Computer Engineering, University of Texas at San Antonio, San Antonio, Texas; ¶Paul L. Foster School of Medicine, Texas Tech University Health Science Center, El Paso, Texas; #Florida Atlantic University, Department of Chemistry and Biochemistry, Jupiter, Florida; and the **Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, North Carolina. This work was supported by the National Institutes of Health, Heart, Lung, and Blood Institute, Bethesda, Maryland: HHSN268201000036C (N01-HV-00244), HL075360, HL051971, GM104357; Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development: 5I01BX000505 (to Dr. Lindsey); American Heart Association, Dallas, Texas: 13POST14350034 (to Dr. DeLeon-Pennell), 14POST18770012 (to Dr. Iyer), 14SDG18860050 (to Dr. de Castro Brás); and Bernard and Audre Rapoport Foundation, Waco, Texas (to Dr. Lange). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.
Listen to this manuscript's audio summary by *JACC* Editor-in-Chief Dr. Valentin Fuster.

Manuscript received February 2, 2015; revised manuscript received June 24, 2015, accepted July 14, 2015.

Despite current treatment strategies, progression to heart failure can occur in up to one-third of myocardial infarction (MI) patients as a result of adverse remodeling of the collagenous scar that replaces the necrotic myocardium (1). Although excessive collagen deposition may result in a stiff and noncompliant left ventricle (LV), insufficient collagen deposition may produce LV thinning and dilation. Therefore, collagen levels are a critical determinant of LV remodeling.

Cardiac extracellular matrix (ECM) dynamically interacts with cells to regulate cell-cell and cell-ECM interactions (2). ECM proteins modulate cell proliferation, migration, adhesion, differentiation, and survival (3). Although ECM protein synthesis is controlled by growth factors, degradation is regulated by proteases, particularly matrix metalloproteinases (MMPs) (2). Collagen Iα1 is a major cardiac ECM component of the post-MI scar and a substrate for several MMPs, including MMP-1, -2, -8, and -9 (4).

SEE PAGE 1375

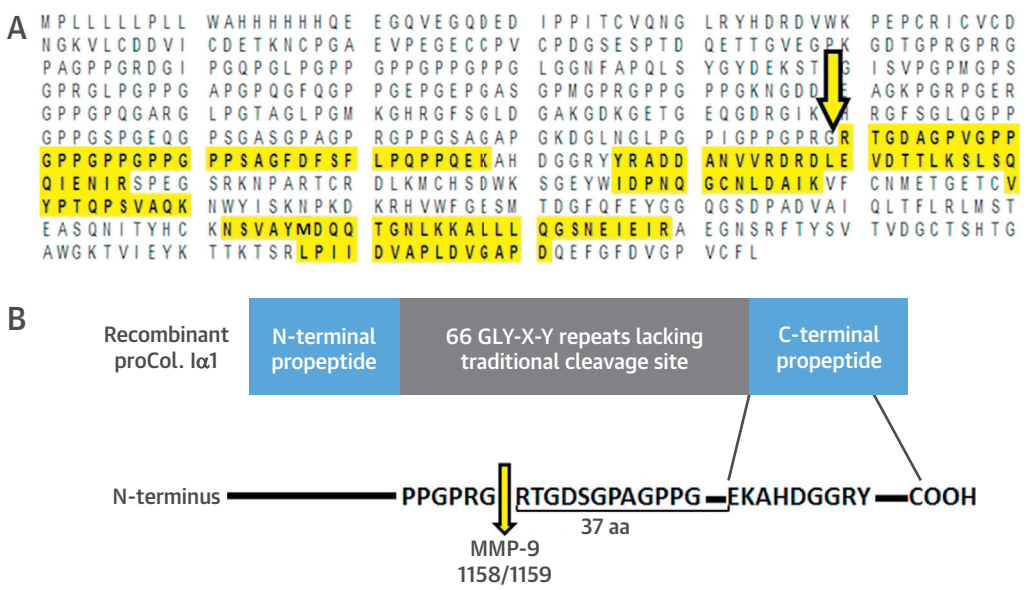
Davis et al. (5) introduced the term *matricryptin* to describe proteolytically released biologically active ECM fragments. Elastin-derived matricryptins induce leukocyte activation, smooth muscle cell proliferation, and MMP expression (6–8). Hyaluronan-derived matricryptins interact with Toll-like receptor-2 and -4 to modulate the inflammatory and fibrotic responses (9). The collagen Iα1 matricryptin DGGRYY, amino acids 1200 to 1205, is a potent neutrophil activator, and the tripeptide GHK (derived from both collagen Iα2 and secreted protein acidic and rich in cysteine [SPARC]) is chemotactic for monocytes, macrophages, and mast cells and has proangiogenic properties (10–12). Endostatin, an MMP-9-released C-terminal fragment from collagen XVIII, has antiangiogenic properties, whereas matricryptins from collagen IV are both pro- and antiangiogenic (13,14). MMP-9 cleaves collagen IVα3 to produce tumstatin, which inhibits angiogenesis (15). These reports suggest that ECM-generated matricryptins may have important biological activity during the healing cascade that follows MI.

Plasma MMP-9 levels directly correlate with LV dysfunction post-MI, in both human and animal models. In mouse models, MMP-9 deletion improves LV remodeling and cardiac function post-MI (16). Analysis of the wild-type LV infarct proteome showed increased levels of a low-molecular-weight collagen Iα1 fragment, compared with baseline day 0 levels

ABBREVIATIONS AND ACRONYMS

ECM = extracellular matrix
LV = left ventricle
LVI = infarcted left ventricle
MI = myocardial infarction
MMP = matrix metalloproteinase
TIMP = tissue inhibitor of metalloproteinase

FIGURE 1 MMP-9 Cleaved Collagen Iα1



(A) Mass spectrometry (MS) identified a naturally occurring collagen fragment in the infarcted left ventricle (LV), which was generated by cleavage of the C-terminus region of collagen Iα1 **(arrow)**. The sequence **highlighted in yellow** represents the sequence covered by MS and the unique peptides identified. **(B)** Matrix metalloproteinase (MMP)-9 cleaved collagen Iα1 at amino acids 1158/59 **(arrow)**, which are upstream of the helical C-terminal propeptide region.

(17). Collagen and collagen fragments are known MMP-9 substrates, and MMP-9-null mice have better LV function post-MI (16). However, whether this is a result of accumulation of collagen matricryptins has not been investigated. In this report, we evaluated the mechanisms of generating a novel collagen fragment and its roles in post-MI remodeling.

METHODS

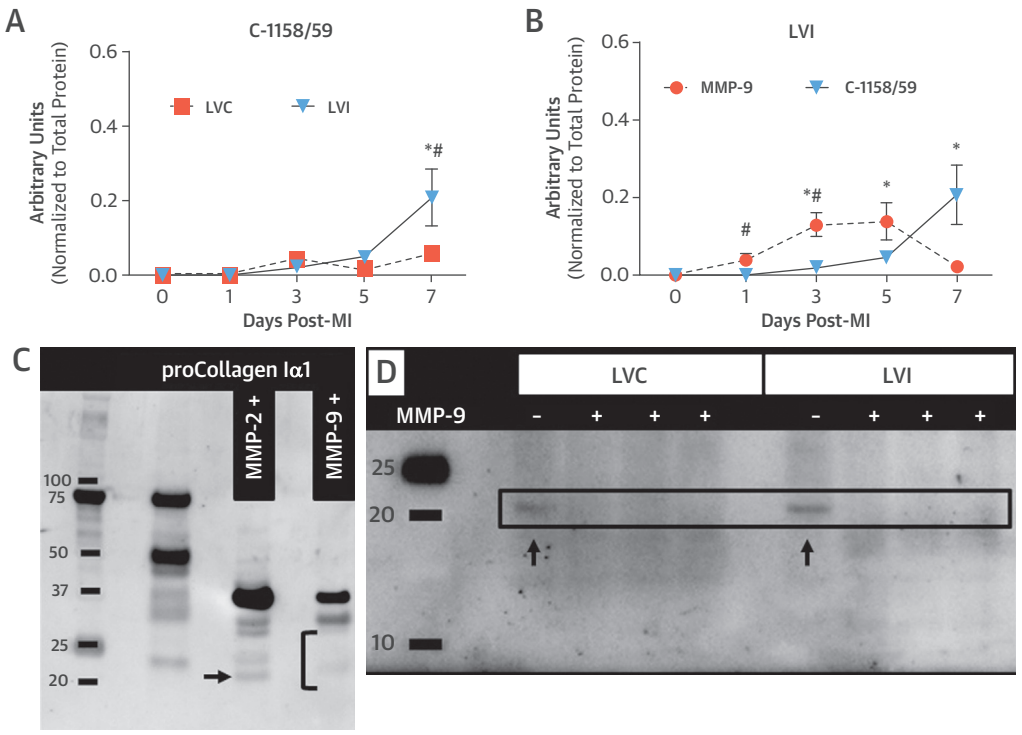
Animal studies were performed according to protocols approved by the institutional animal care and use committee at the University of Mississippi Medical Center. Written informed consent was collected from all patients under protocols approved by the University of Mississippi Medical Center institutional review board committee. Experimental details are provided in the Online Appendix.

RESULTS AND DISCUSSION

Previously, we identified an ~30-kDa C-terminal collagen Iα1 fragment in the infarcted LV (LVI) at 7 days post-MI (**Figure 1A**) (17). Because MMP-9 is prominent post-infarction, we performed cleavage assays using a recombinant collagen Iα1 that contains the N- and C-terminal regions and 66 Gly-X-Y triplet repeats (residues Gln23-Lys277 + Gly1094-Leu1464). Mass spectrometry analysis of the cleavage products revealed a new MMP-9 cleavage site between amino acids 1158/59 in the C-terminal region, 37 amino acids upstream of the collagen C-telopeptide (**Figure 1B**). The generated C-collagen Iα1 fragment (C-1158/59) is downstream of the canonical collagenase cleavage site at Gly775-Ile776 (18).

Using an antibody specific for the 1158/59 cleavage site, we evaluated the temporal generation of

FIGURE 2 C-1158/59 Collagen Fragment Generated Post-MI



The C-1158/59 fragment levels were higher at day 7 post-myocardial infarction (MI), as detected by immunoblotting. **(A)** C-1158/59 time course in the infarcted (LVI) and remote (LVC) regions. n = 4/sex/genotype/time point; *p < 0.05 versus other days; #p < 0.05 versus same day LVC. **(B)** The C-1158/59 LVI time course with matrix metalloproteinase (MMP)-9. The C-1158/59 fragment accumulated in the LVI at day 7, when MMP-9 was at its lowest post-MI level. Values are mean ± SEM; n = 8/group; *p < 0.05 versus days 0 and 1; #p < 0.05 versus same day C-1158/59. **(C)** By cleavage assays, both MMP-2 and MMP-9 generated the C-1158/59 collagen fragment; increased doses and prolonged exposure to enzyme resulted in fragment degradation only by MMP-9. The **arrow** indicates the C-1158/59 fragment after cleavage by MMP-2; **bracket** indicates fragment degradation by MMP-9. **(D)** LVI protein extracts from MMP-9-null mice at day 7 post-MI showed presence of C-1158/59 collagen fragment **(arrows)**. The **plus sign** indicates protein extracts (n = 3 biological replicates) incubated with MMP-9; the **minus sign** indicates protein extracts without enzyme (n = 3 biological replicates).

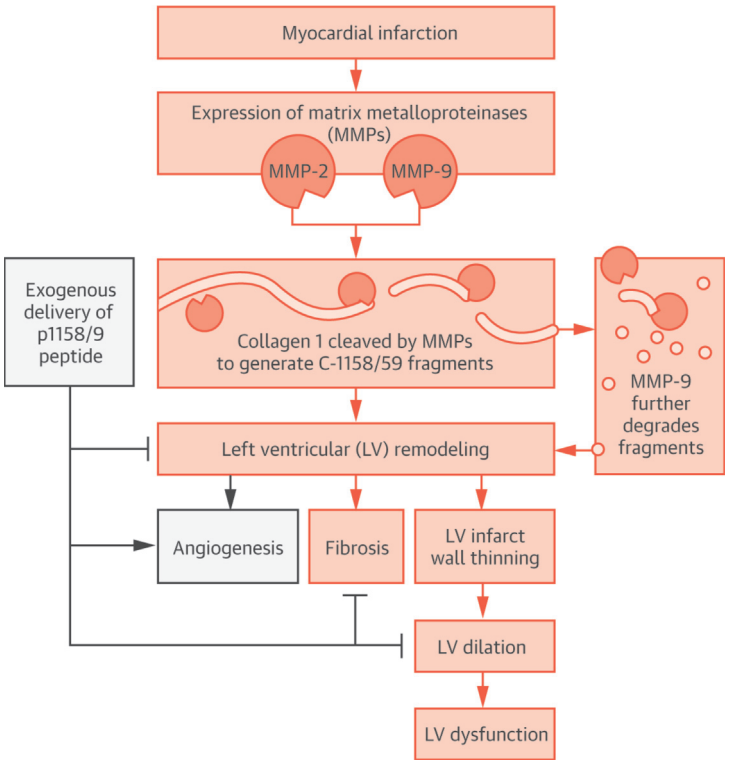
C-1158/59 fragment in the LVI and noninfarcted remote control regions in a mouse MI model. C-1158/59 LVI levels robustly increased at day 7 post-MI; it was >4-fold higher versus all earlier time-points (all $p < 0.05$) and 3.5-fold higher than the day 7 LV remote noninfarcted control ($p < 0.05$) (**Figure 2A**, Online Figure S1A). Post-MI, loss of myocytes and contractility in the infarct region increases wall stress in the remote LV and induces compensatory hypertrophy. Our data suggest a small degree of collagen turnover in the remote noninfarcted region in response to LV remodeling post-MI. In the LVI, MMP-9 protein concentrations increased significantly in the first week post-MI, returning to baseline (day 0) levels by day 7 (**Figure 2B**). This increase was consistent with our past reports and with the timing of the generation of the C-1158/59 fragment (19).

Because C-1158/59 fragment levels were higher at day 7 post-MI, when MMP-9 levels were returning to baseline, and were generated in the remote region where MMP-9 expression is low, we expanded our search to other MMPs that could cleave collagen at this site. We tested the gelatinase MMP-2 that shares highest homology and several substrates with MMP-9 and the collagenase MMP-8. MMP-11 was used as a negative control, because this stromelysin does not cleave collagen. Analysis of cleavage assays for MMP-2, -8, -9, and -11 showed that both MMP-2 and -9, but not MMP-8 or -11, can generate the C-1158/59 fragment (Online Figures S1B and S1C). Our results show, for the first time, that MMP-2 and -9 cleaved collagen Iα1 in the C-terminus at the amino acids 1158/1159.

Of note, increasing incubation times or MMP-9 doses (but not MMP-2) resulted in degradation of the C-1158/59 fragment (**Figure 2C**). To confirm this in vivo, we quantified C-1158/59 levels from LVI protein extracts of MMP-9-null mice at day 7 post-MI, incubated in the presence or absence of MMP-9. We established that C-1158/59 is generated in the MMP-9-null mice and incubation with MMP-9 degraded the fragment (**Figure 2D**). This confirmed that C-1158/59 can be generated by other MMPs and suggested that decreased C-1158/59 levels at days 3 and 5 post-MI in the wild-type mice might be caused by MMP-9 degradation.

IN VITRO TREATMENT WITH THE P1158/59 PEPTIDE. To study the biological activity of the C-1158/59 fragment (**Central Illustration**), we synthesized a short peptide containing the 15 amino acids downstream of the cleavage site, beginning at amino acid 1159 (p1158/59; molecular weight 1,360.4 g/mol). The use of small synthetic peptides to mimic the biological activities of naturally occurring molecules has great potential in overcoming difficulties associated with synthesis of larger peptides. A short peptide is easier to synthesize, purify, and concentrate. Although technological advances have improved peptide synthesis strategies, the purity and yield of synthesized peptides can be limited by the sequence length. Additionally, a small size peptide limits response variability, which can occur with a longer peptide that may possess multiple activities; by using a small peptide, we ensured that cellular effects occurred as a direct response to the cleavage site sequence and not peptide structural properties. Cardiac fibroblasts were stimulated with increasing doses of p1158/59 or the spanning peptide negative control. The peptides did not induce cell apoptosis or affect cell proliferation (n = 6 to 10/dose/assay) (Online Figures S2A and S2B).

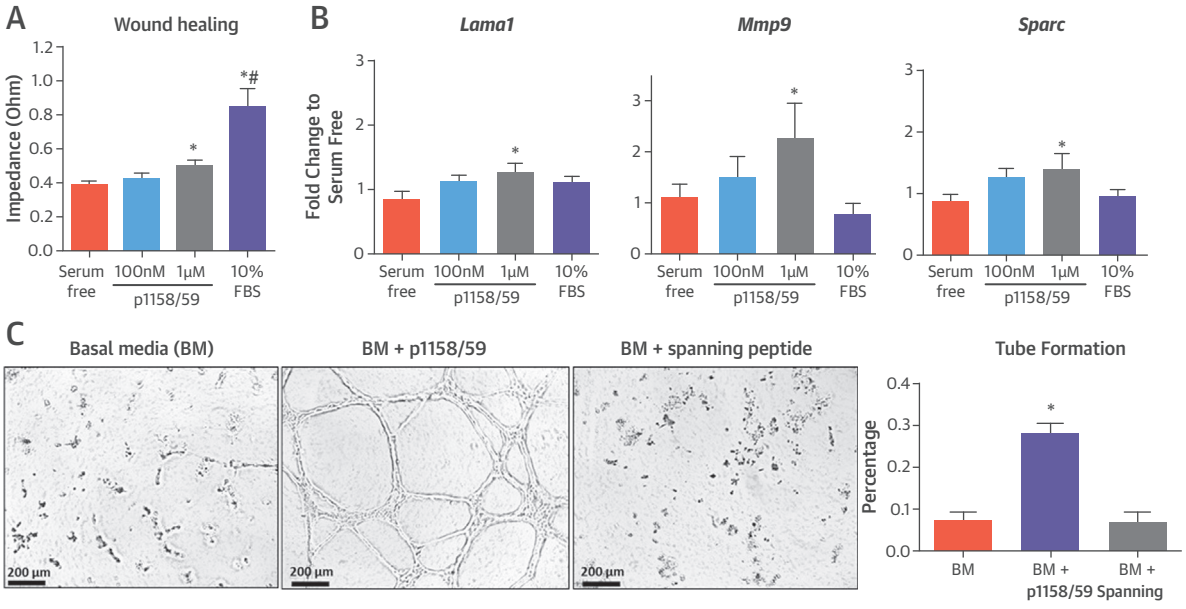
CENTRAL ILLUSTRATION Matricryptin Reduces LV Dilation Post-MI: Diagram Depicting the Effects of Exogenous Delivery of p1158/59 Peptide to Mice Post-MI



Lindsey, M.L. et al. J Am Coll Cardiol. 2015; 66(12):1364–74.

Following myocardial infarction (MI), the collagen fragment C-1158/59 is generated by both matrix metalloproteinase (MMP)-2 and -9; however, it is degraded by MMP-9 when MMP-9 levels peak. Endogenous delivery of the p1158/59 peptide, beginning at 3 h post-MI and continuing for 7 days, improved the quality of extracellular matrix scar formed, reduced fibrosis, and promoted angiogenesis. This resulted in preserved left ventricular (LV) geometry and reduced LV dilation post-MI.

FIGURE 3 Fibroblast Migration and Angiogenesis In Vitro



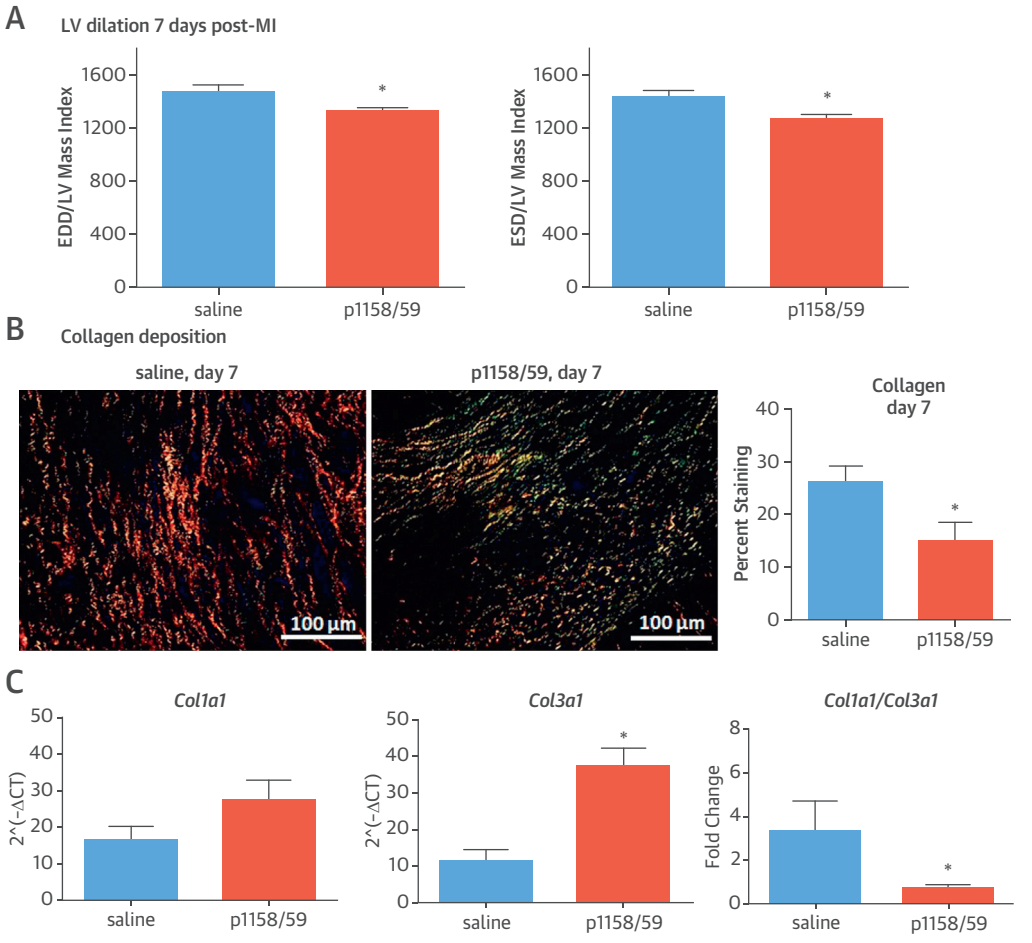
(A) An automated wound healing assay showed increased migration of cardiac fibroblasts after incubation with p1158/59. n = 6/group; *p < 0.05 versus serum free; #p < 0.05 versus all other groups. **(B)** p1158/59 stimulated overexpression of the genes *Lama1*, *Mmp-9*, and *Sparc*. n = 6/group; *p < 0.05 versus serum free. **(C)** Stimulation with the p1158/59 peptide displayed strong proangiogenic properties by enhancing human umbilical vein endothelial cell alignment and formation of a 3-dimensional tube network. n = 3/condition, with each experiment run in duplicate, figures show representative images. *p < 0.05 versus all other groups. FBS = fetal bovine serum; MMP = matrix metalloproteinase.

We performed automated wound healing assays and observed that wound closure rates were enhanced with 1 μmol/l p1158/59 treatment and peaked at 22 h compared with untreated controls (**Figure 3A**). Because proliferation rates were not different, the increase in wound healing rates was due to increased cell migration. Gene array analysis on the treated cells revealed that *Lama1* (laminin α1), *Mmp-9*, and *Sparc* expression were all increased in cells treated with p1158/59 (**Figure 3B**) (all $p < 0.05$). Laminin α1 is a glycoprotein involved in basement membrane assembly and architecture, and plays an important role in cell migration through α3β1 and α6β1 integrins (20). MMP-9 is also known to facilitate migration by cleaving adhesion molecules, and SPARC has been suggested to have antiadhesive properties, therefore promoting cell migration by reducing cell-substrate adhesion (21,22). Cell migration can be enhanced by a directional cue, such as a chemoattractant, disruption of cell-cell contact (mechanical cue), internal signaling cascades, and extracellular cues such as ECM proteins, growth factors, and ligands (23). Our data suggest that the p1158/59 matricryptin acts as an extracellular cue and stimulates cell migration by inducing overexpression of migration-mediator factors. Similarly, others have reported collagen, fibronectin, and elastin-derived matricryptins that stimulate directed cell migration of leukocytes and fibroblasts (24,25). Because p1158/59 induced fibroblast MMP-9 expression, we looked at collagen levels in the conditioned media to evaluate if MMP-9 increased collagen degradation. The p1158/59 peptide did not affect levels of collagen secreted in vitro (Online Figure S2C).

Human umbilical vein endothelial cells were used to assess p1158/59 angiogenic properties. After 6 h, cells cultured in basal media and with the negative control spanning peptide showed initial stages of alignment and elongation, whereas cells treated with p1158/59 showed robust induction of a network of 3-dimensional capillary-like tubular structures ($p < 0.05$) (**Figure 3C**). Our data indicated that p1158/59 had strong proangiogenic in vitro properties.

SYSTEMIC TREATMENT WITH THE P1158/59 PEPTIDE. The in vitro data suggested that increased levels of p1158/59 post-MI could lead to accelerated fibroblast migration into the infarcted area, which in turn would

FIGURE 4 Post-MI LV Dilation and Geometry



Compared with the saline group, animals treated with p1158/59 post-MI showed reduced LV dilation, as evidenced by reduced end-diastolic (EDD) and end-systolic (ESD) dimensions **(A)**, and collagen deposition at day 7 post-MI **(B)**. Using picrosirius red staining under polarized light, saline produced thick yellow/red fibers, and p1158/59 mostly thin green collagen fibers. p1158/59 treatment did not affect collagen 1α1 expression **(C)**, but stimulated collagen 3α1 overexpression, which reduced the col 1/col 3 ratio. n = 12/group; *p < 0.05 versus saline. Abbreviations as in Figures 1 and 2.

result in earlier deposition of granulation tissue that could provide a sounder support for scar formation and maturation. Thus, we treated mice with p1158/59 or saline vehicle control 3 h post-MI for a total of 7 days. Our aim was to determine whether an early intervention strategy would stimulate scar formation and angiogenesis to attenuate LV remodeling. There was no difference in infarct size between groups ($p = 0.15$), indicating equal initial injury. This was expected, because treatment was started 3 h post-MI, after myocardial necrosis was fulminant.

In vivo, p1158/59 treatment resulted in attenuated increased LV end-diastolic diameter and end-systolic diameter post-MI compared with the saline group ($p < 0.05$) (Figure 4A), indicating that p1158/59 treatment preserved LV structural integrity and reduced LV dilation in the infarct region. Lower end-diastolic dimensions indicate decreased stretching of the LV muscle fibers during preload, which in turn would result in preserved LV structure. A study evaluating chronic effects of the peptide would be beneficial to fully understand p1158/59 effects on cardiac function post-MI. After MI, a person immediately encounters decreased cardiac output and increased strain on the heart. Prolonged inflammation and/or delayed scar formation result in progressive LV wall thinning and LV dilation, both associated with increased incidence of heart failure and patient mortality.

TABLE 1 ECM and Adhesion Molecule Genes Overexpressed Following p1158/59 Peptide Treatment*

Gene Name	Protein Encoded	Fold Change	p Value
Adamts2	A disintegrin-like and metallopeptidase with thrombospondin type-1 motif, 2	1.9	0.021
Cd44	CD44 antigen	2.4	0.003
Col3a1	Collagen type III, alpha-1	3.3	0.002
Col4a1	Collagen type IV, alpha-1	2.2	0.005
Col4a2	Collagen type IV, alpha-2	2.3	0.025
Col5a1	Collagen type V, alpha-1	4.5	<0.001
Ctgf	Connective tissue growth factor	1.8	0.035
Ctnnb1	Catenin (cadherin associated protein), beta-1	1.4	0.037
Ecm1	Extracellular matrix protein-1	3.0	0.015
Emilin1	Elastin microfibril interface-1	2.8	0.011
Entpd1	Ectonucleoside triphosphate diphosphohydrolase-1	2.0	0.043
Fn1	Fibronectin-1	3.1	0.014
Icam1	Intercellular adhesion molecule-1	1.9	0.013
Itga3	Integrin, alpha-3	2.9	0.028
Itga4	Integrin, alpha-4	3.0	0.005
Itga5	Integrin, alpha-5	3.0	0.010
Itgam	Integrin, alpha-m	2.1	0.008
Itgb1	Integrin, beta-1	1.4	0.018
Itgb2	Integrin, beta-2	4.1	0.005
Itgb3	Integrin, beta-3	2.8	0.005
Lama1	Laminin, alpha-1	2.1	0.048
Lamc1	Laminin, gamma-1	3.0	0.005
Mmp8	Matrix metallopeptidase-8	10.1	0.004
Mmp11	Matrix metallopeptidase-11	2.6	0.001
Mmp14	Matrix metallopeptidase-14	2.2	0.004
Sele	Selectin, endothelial cell	2.6	0.015
Selp	Selectin, platelet	3.0	0.021
Sgce	Sarcoglycan, epsilon	2.8	<0.001
Sparc	Secreted acidic cysteine rich glycoprotein	2.4	0.002
Spp1	Secreted phosphoprotein-1	6.5	0.014
Tgfbi	Transforming growth factor, beta-induced	4.8	0.003
Thbs3	Thrombospondin-3	2.4	0.003
Tnc	Tenascin C	3.7	0.013
Vcam1	Vascular cell adhesion molecule-1	2.2	0.009
Vcan	Versican	1.4	0.029

*Treatment ran 7 days post-myocardial infarction; values compared with saline vehicle control (n = 6/group).
ECM = extracellular matrix.

Preserved chamber geometry and reduced dilation, compared with the saline group, indicated better LV contractility. Overall, our data demonstrated that treatment with p1158/59 reduced LV dilation 7 days post-MI, which opens therapeutic avenues for this and other matricryptins. The literature describes several studies in which matricryptins or other peptides are used in MI models with different rates of success. In a rat model, treatment with B-type natriuretic peptide for 7 days improved LV function at 8 weeks post-MI (26). Ladage et al. (27) delivered recombinant periostin peptide into the pericardial space post-MI in a pig model and observed a decrease in infarct size and improved LV function compared with controls.

We further investigated which changes were occurring at the molecular level during scar formation that could explain preserved LV structure after peptide delivery. We determined that p1158/59-treated mice showed less fibrosis in the LVI, evidenced by lower levels of collagen deposition in the infarcted area at day 7 post-MI ($p < 0.05$ vs. saline) (**Figure 4B**). We used picrosirius red staining for histological visualization of collagen I and III fibers (27). Sirius red dye molecules align parallel to the long axis of collagen molecules to enhance their natural birefringence (28). Under polarized light, interstitial collagens show different colors and intensities of birefringence. Collagen I displays yellow or red thick, strongly birefringent fibers that are highly cross-linked, whereas collagen III appears as thin, more compliant green fibers with lower birefringence (28). Of note, although the saline group showed thick, strongly birefringent fibers consistent with collagen I deposition, p1158/59 treatment resulted in thin green fibers with lower birefringence, suggestive of increased collagen III deposition. By gene array, we confirmed that p1158/59 stimulated *Col3a1* expression by 3.3-fold (**Table 1**). Collagen III is the first fibrillar collagen produced by fibroblasts during the proliferative phase after injury, later being replaced by collagen I, which is stiffer and less compliant than collagen III (29). These data suggest that p1158/59 stimulated collagen III deposition, resulting in a decreased ratio of collagen I/collagen III, which would generate a less stiff scar and may explain the better LV geometry (**Figure 4C**). Fetal tissues show increased expression of collagen III compared with adult tissue, which has led some to hypothesize that collagen III is a main contributor to the scarless phenotype observed in the early stages of gestation (30). We further looked at the expression of MMPs and tissue inhibitors of metalloproteinases (TIMP), because MMP activity and MMP/TIMP ratios can determine ECM turnover and remodeling (31). At 7 days post-MI, only MMP-8, -11, and -14 expression were increased in the peptide-treated group compared with saline (Online Tables S1 and S2). Our data demonstrated that p1158/59 treatment improved scar formation through reduced stiffness and fibrosis, and preserved LV structure post-MI.

Treatment with p1158/59 induced 35 ECM- and adhesion molecule-associated genes in the infarct region compared with saline-treated MI controls, out of 85 genes examined (**Table 1**) (Online Tables S1 and S2 shows the 50 unaltered genes). Expression of *Adamts2* was increased 2-fold in the treated group

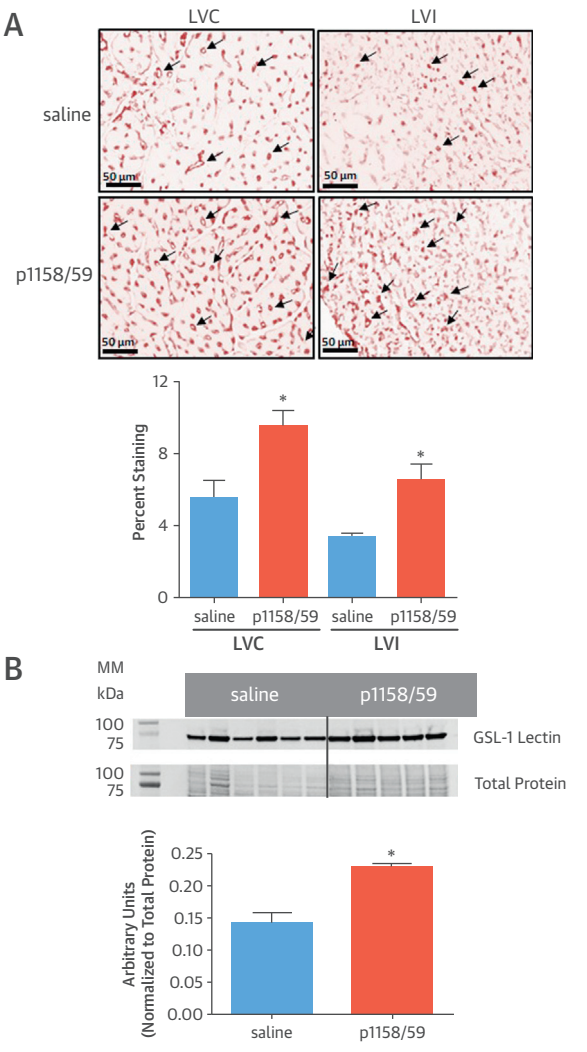
compared with the saline control ($p < 0.05$). ADAMTS2 cleaves the N-terminal propeptides of collagen types I and III during collagen maturation, therefore playing a key role in collagen turnover. Connective tissue growth factor (CTGF) expression was elevated in the treated group, CTGF promotes fibroblast proliferation, migration, adhesion, and ECM and vessel formation (32). CTGF directly binds to integrin receptors and proteoglycans, and thus participates in signal transduction events. Most biological activities of matricryptins are mediated by integrins, proteoglycans, and growth factor receptors (14). Endostatin (MMP-2 generated) and tumstatin (MMP-9 generated) exert their angiogenic effects by interacting through αvβ3 integrins, whereas angiogenic laminin peptides bind to αvβ3 and α5β1 integrins (33,34). **Table 1** shows overexpression of *Itga5* (integrin alpha-5), *Itgb1* (integrin beta-1), and *Itgb3* by p1158/59 treatment, suggesting that this matricryptin may be mediated by these integrins.

Our data suggest p1158/59 reduced the stiffness of the provisional matrix post-MI by enhancing expression of collagen III and CTGF, which would reduce fibrosis during early wound healing. The accelerated formation of a stable matrix may further support cell migration, adhesion, and vessel formation, which would contribute to preserved LV structure and reduced LV dilation.

Animals that received p1158/59 for 7 days post-MI showed enhanced expression of *Col4a1*, *Col4a2*, and *Lama1* compared with the saline MI group (**Table 1**) ($p < 0.05$ for all). These genes are components of the basement membrane and stimulate angiogenesis. Collagen IV provides a stable substrate for vessel formation and promotes and regulates the formation, extension, and stabilization of microvessels during angiogenesis. Similarly, Lama1 stimulates angiogenesis and is critical for vessel integrity. Therefore, increased collagen IV and Lama1 expression by p1158/59 may promote angiogenesis and quicker formation of adaptive capillary growth during LV remodeling. Extracellular matrix protein-1 (*Ecm1*), a secretory glycoprotein that promotes angiogenesis by inducing endothelial cell proliferation and neovessel formation, was overexpressed in the p1158/59 group (**Table 1**). Interestingly, this protein has been shown through high-affinity protein-protein interactions to inhibit MMP-9 activity (35). *Mmp-11*, also significantly elevated in the p1158/59-treated animals (**Table 1**), is highly associated with cell migration and invasion (36).

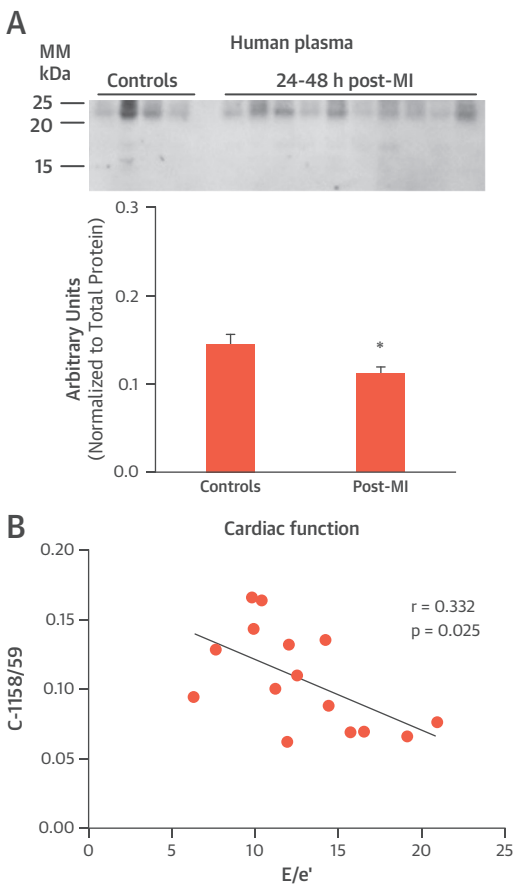
To validate what we observed at the gene level, we quantified by immunoblotting and immunohistochemistry *Griffonia* (*Bandeiraea*) *simplicifolia* lectin I (GSL-1), which specifically binds to endothelial cells (37). Mice treated with p1158/59 displayed higher binding of GSL-1 and increased vessel numbers in both the remote and infarct regions compared with saline (**Figures 5A and 5B**) ($p < 0.05$), indicative of increased vascularization. The remote region maintains perfusion after MI; however, remodeling occurs due to changes in wall strain patterns that lead to rapid regression of cardiac mass through cardiomyocyte

FIGURE 5 p1158/59-Stimulated Vessel Formation In Vivo



p1158/59 peptide treatment increased vessel number in the LV 7 days post-MI, as indicated by increased binding of lectin 1 (GSL-1). **(A)** Endothelial cell numbers were quantified by immunohistochemistry in 5 random fields per section. n = 5 to 6/group; *$p < 0.05$ versus saline. **Arrows** indicate positive cells. **(B)** Protein levels were quantified by densitometry (**top** immunoblot) and normalized to the total protein (**bottom** immunoblot) in each respective lane. n = 5 to 6/group; *$p < 0.05$ versus saline. Other abbreviations as in **Figures 1 and 2**.

FIGURE 6 C-1158/59 Collagen Fragment Levels



(A) Human plasma displayed lower levels of C-1158/59 in myocardial infarction (MI) patients 24 to 48 h post-infarction compared with healthy controls (*$p < 0.05$; $n = 8$ controls, $n = 20$ patients). **(B)** C-1158/59 fragment levels negatively correlated with early diastolic transmitral flow velocity (E) and mitral annular velocities (e′) ratios in MI patients.

atrophy. The cardiomyocytes respond to the increased load by increasing protein synthesis, and the resulting hypertrophic response may lead to relative insufficient vascular growth. To assess whether the increased vessel numbers observed with p1158/59 treatment were due to myocyte atrophy, we quantified the expression of the Forkhead Box O3 (*Foxo3*), a transcription factor that, when activated, promotes myocyte atrophy and autophagy (38,39). No difference was observed in *Foxo3* activation between groups (Online Table S2), giving evidence that p1158/59 treatment stimulated angiogenesis did not occur through *Foxo3* signaling. Increased angiogenesis in both remote and infarct regions may offer the necessary cardioprotection to mitigate LV dysfunction post-MI. Taken together, our results established that p1158/59 matricryptin had robust proangiogenic properties in vivo. Endorepellin, a perlecan-derived matricryptin, displayed proangiogenic and neuroprotective properties in a rodent model of ischemic stroke and has shown promising results for the treatment of stroke (40). It would be interesting to determine whether the use of proangiogenic matricryptins such as p1158/59 in the settings of ischemia and compromised healing, such as diabetic wounds, could delay or even reverse the tissue necrosis caused by poor vascularization and chronic inflammation. Further studies are necessary for a deeper understanding of the modes of action. For example, doses to be administered in vivo require careful optimization because many angiogenic factors display biphasic response curves. Another critical issue is the dual activities of some matricryptins, which depend on environment, concentration, and/or cell type. Moreover, time and duration of intervention is also important. In our study, we delivered the matricryptin for 7 days, beginning very early post-MI, but long-term effects will need to be studied.

Finally, we quantified the C-1158/59 fragment in the plasma of healthy subjects and patients at 24 to 48 h post-MI. Patient characteristics are in Online Table S3. Of interest, the post-MI patients showed reduced levels of C-1158/59 (**Figure 6A**) ($p < 0.05$ vs. healthy controls), consistent with high MMP-9 levels at this time. Plasma MMP-9 levels peak at day 1 in post-MI patients, which may increase collagen fragment degradation (41). This decrease may also reflect a difference between sampling from the tissue and plasma. In post-MI patients, the plasma levels of the C-1158/59 fragment negatively correlated with E/e′ ratios ($r = 0.332$; $p = 0.025$) (**Figure 6B**). An E/e′ ratio <8 is indicative of normal filling pressures, whereas an E/e′ >15 is indicative of markedly elevated filling pressures (42). Thus, our data suggest that lower levels of C-1158/59 collagen fragment correlated with reduced cardiac function.

STUDY LIMITATIONS. One limitation was the time difference in sampling between the mouse and human MI studies. Another was that we collected peripheral venous plasma samples, but did not collect coronary sinus levels, which would have allowed us to evaluate the myocardium as a direct source of the peptide. Further studies will be necessary to investigate the natural occurrence of this collagen fragment at later time points and its effects on long-term cardiac remodeling.

CONCLUSIONS

We have identified a novel collagen Iα1 cleavage site and the generation of a matricryptin downstream of

this site, p1158/59, stimulated in vitro and in vivo wound healing. Our data show that p1158/59 is a biologically active molecule with potent proangiogenic properties and has direct effects on the quality of scar tissue post-MI by promoting early ECM deposition. Together, these effects preserved LV structure and geometry, resulting in reduced LV dilation post-MI. These data strongly promote this matricryptin as a molecule of interest to treat adverse LV remodeling.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Lisandra E. de Castro Brás, Department of Physiology, The Brody School of Medicine, East Carolina University, 600 Moye Boulevard, Greenville, North Carolina 27834. E-mail: decastrobrasl14@ecu.edu.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Following MI, collagen I, a major component of the cardiac extracellular matrix, is proteolytically cleaved by matrix metalloproteinases into fragments known as matricryptins that mediate left ventricular remodeling in a mouse model by enhancing fibroblast activity, basement membrane and granulation tissue integrity and angiogenesis.

TRANSLATIONAL OUTLOOK: Clinical studies are needed to assess the safety and efficacy of matricryptins and other fragments that modulate cardiac remodeling at the molecular level in patients recovering from MI.

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. Circulation 2014;129:e28–292.

2. Jarvelainen H, Sainio A, Koulu M, Wight TN, Penttinen R. Extracellular matrix molecules: potential targets in pharmacotherapy. Pharmacol Rev 2009;61:198–223.

3. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008;121:255–64.

4. Mann DL, Spinale FG. Activation of matrix metalloproteinases in the failing human heart: breaking the tie that binds. Circulation 1998;98:1699–702.

5. Davis GE, Bayless KJ, Davis MJ, Meininger GA. Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules. Am J Pathol 2000;156:1489–98.

6. Fulop T Jr., Jacob MP, Varga Z, Foris G, Leovey A, Robert L. Effect of elastin peptides on human monocytes: Ca2+ mobilization, stimulation of respiratory burst and enzyme secretion. Biochem Biophys Res Commun 1986;141:92–8.

7. Wachi H, Seyama Y, Yamashita S, et al. Stimulation of cell proliferation and autoregulation of elastin expression by elastin peptide VPGVG in cultured chick vascular smooth muscle cells. FEBS Lett 1995;368:215–9.

8. Brassart B, Fuchs P, Huet E, et al. Conformational dependence of collagenase (matrix metalloproteinase-1) up-regulation by elastin peptides in cultured fibroblasts. J Biol Chem 2001;276:5222–7.

9. Jiang D, Liang J, Fan J, et al. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med 2005;11:1173–9.

10. Garnotel R, Monboisse JC, Randoux A, Haye B, Borel JP. The binding of type I collagen to lymphocyte function-associated antigen (LFA) 1 integrin triggers the respiratory burst of human polymorphonuclear neutrophils. Role of calcium signaling and tyrosine phosphorylation of LFA 1. J Biol Chem 1995;270:27495–503.

11. Sage EH, Reed M, Funk SE, et al. Cleavage of the matricellular protein SPARC by matrix metalloproteinase 3 produces polypeptides that influence angiogenesis. J Biol Chem 2003;278:37849–57.

12. Zetter BR, Rasmussen N, Brown L. An in vivo assay for chemoattractant activity. Lab Invest 1985;53:362–8.

13. Zhang H, Wang Z, Peng Q, et al. Tumor refractoriness to endostatin anti-angiogenesis is associated with the recruitment of CD11b+Gr1+ myeloid cells and inflammatory cytokines. Tumori 2013;99:723–33.

14. Ricard-Blum S, Salza R. Matricryptins and matrikines: biologically active fragments of the extracellular matrix. Exp Dermatol 2014;23:457–63.

15. Hamano Y, Zeisberg M, Sugimoto H, et al. Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin. Cancer Cell 2003;3:589–601.

16. Ducharme A, Frantz S, Aikawa M, et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest 2000;106:55–62.

17. Zamilpa R, Lopez EF, Chiao YA, et al. Proteomic analysis identifies in vivo candidate matrix metalloproteinase-9 substrates in the left ventricle post-myocardial infarction. Proteomics 2010;10:2214–23.

18. Lauer-Fields JL, Juska D, Fields GB. Matrix metalloproteinases and collagen catabolism. Biopolymers 2002;66:19–32.

19. DeLeon-Pennell KY, de Castro Bras LE, Iyer RP, et al. P. gingivalis lipopolysaccharide intensifies inflammation post-myocardial infarction through matrix metalloproteinase-9. J Mol Cell Cardiol 2014;76:218–26.

20. Tzu J, Marinkovich MP. Bridging structure with function: structural, regulatory, and developmental role of laminins. Int J Biochem Cell Biol 2008;40:199–214.

21. Fiore E, Fusco C, Romero P, Stamenkovic I. Matrix metalloproteinase 9 (MMP-9/gelatinase B) proteolytically cleaves ICAM-1 and participates in tumor cell resistance to natural killer cell-mediated cytotoxicity. Oncogene 2002;21:5213–23.

22. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest 2001;107:1049–54.

23. Woodham EF, Machesky LM. Polarised cell migration: intrinsic and extrinsic drivers. Curr Opin Cell Biol 2014;30C:25–32.

24. Clark RA, Wikner NE, Doherty DE, Norris DA. Cryptic chemotactic activity of fibronectin for human monocytes resides in the 120-kDa fibroblastic cell-binding fragment. J Biol Chem 1988;263:12115–23.

25. Albini A, Adelmann-Grill BC. Collagenolytic cleavage products of collagen type I as chemoattractants for human dermal fibroblasts. Eur J Cell Biol 1985;36:104–7.

26. George I, Xydas S, Klotz S, et al. Long-term effects of B-type natriuretic peptide infusion after acute myocardial infarction in a rat model. J Cardiovasc Pharmacol 2010;55:14–20.

27. Ladage D, Yaniz-Galende E, Rapti K, et al. Stimulating myocardial regeneration with periostin peptide in large mammals improves function post-myocardial infarction but increases myocardial fibrosis. PLoS One 2013;8:e59656.

28. Montes GS, Junqueira LC. The use of the Picrosirius-polarization method for the study of the biopathology of collagen. Mem Inst Oswaldo Cruz 1991;86 Suppl 3:1–11.

29. Wan C, Hao Z, Wen S, Leng H. A quantitative study of the relationship between the distribution of different types of collagen and the mechanical

behavior of rabbit medial collateral ligaments. PLoS One 2014;9:e103363.

30. Goldberg SR, Quirk GL, Sykes VW, Kordula T, Lanning DA. Altered procollagen gene expression in mid-gestational mouse excisional wounds. J Surg Res 2007;143:27–34.

31. Webb CS, Bonnema DD, Ahmed SH, et al. Specific temporal profile of matrix metalloproteinase release occurs in patients after myocardial infarction: relation to left ventricular remodeling. Circulation 2006;114:1020–7.

32. Moussad EE, Brigstock DR. Connective tissue growth factor: what's in a name? Mol Genet Metab 2000;71:276–92.

33. Faye C, Moreau C, Chautard E, et al. Molecular interplay between endostatin, integrins, and heparan sulfate. J Biol Chem 2009;284:22029–40.

34. Malinda KM, Wysocki AB, Koblinski JE, et al. Angiogenic laminin-derived peptides stimulate wound healing. Int J Biochem Cell Biol 2008;40:2771–80.

35. Fujimoto N, Terlizzi J, Aho S, et al. Extracellular matrix protein 1 inhibits the activity of matrix metalloproteinase 9 through high-affinity protein/protein interactions. Exp Dermatol 2006;15:300–7.

36. Wu D, Li M, Wang L, et al. microRNA-145 inhibits cell proliferation, migration and invasion by targeting matrix metallopeptidase-11 in renal cell carcinoma. Mol Med Rep 2014;10:393–8.

37. Alroy J, Goyal V, Skutelsky E. Lectin histochemistry of mammalian endothelium. Histochemistry 1987;86:603–7.

38. Cao DJ, Jiang N, Blagg A, et al. Mechanical unloading activates FoxO3 to trigger Bnip3-dependent cardiomyocyte atrophy. J Am Heart Assoc 2013;2:e000016.

39. Schips TG, Wietelmann A, Hohn K, et al. FoxO3 induces reversible cardiac atrophy and autophagy in a transgenic mouse model. Cardiovasc Res 2011;91:587–97.

40. Bix GJ. Perlecan domain V therapy for stroke: a beacon of hope? ACS Chem Neurosci 2013;4:370–4.

41. Squire IB, Evans J, Ng LL, Loftus IM, Thompson MM. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. J Card Fail 2004;10:328–33.

42. Geske JB, Sorajja P, Nishimura RA, Ommen SR. Evaluation of left ventricular filling pressures by Doppler echocardiography in patients with hypertrophic cardiomyopathy: correlation with direct left atrial pressure measurement at cardiac catheterization. Circulation 2007;116:2702–8.

KEY WORDS collagen, extracellular matrix, matricyrptin, MMP, proteomics, remodeling

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this article.