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

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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.

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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).

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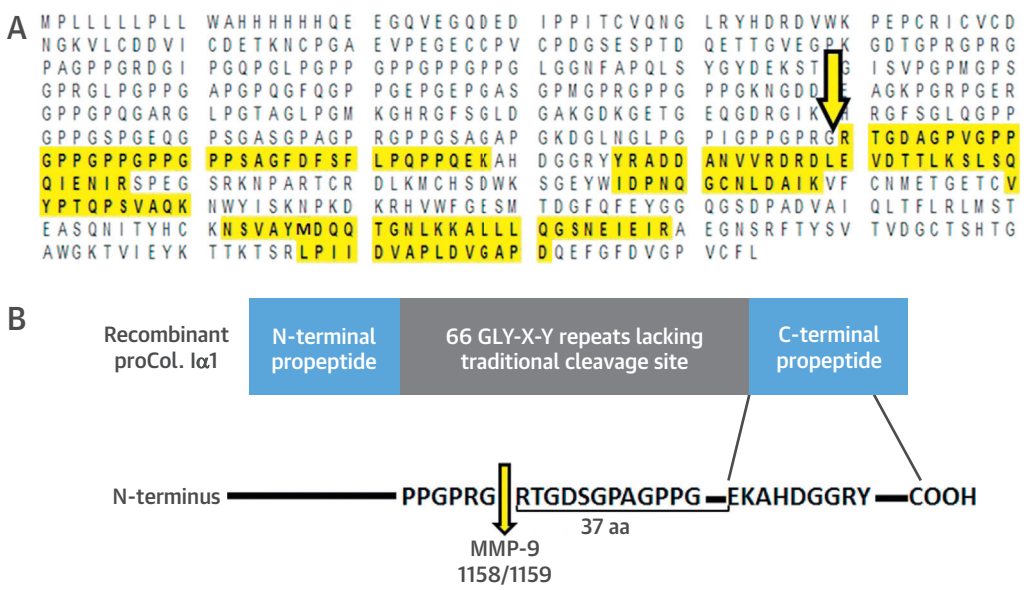
Davis et al. (5) introduced the term *matricryptin* to describe proteolytically released biologically active ECM fragments. Elastin-derived matricryptins induce leukocyte activation, smooth muscle cell proliferation, and MMP expression (6–8). Hyaluronan-derived matricryptins interact with Toll-like receptor-2 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.