Targeting the Cardiac Myofibroblast Secretome to Treat Myocardial Fibrosis in Heart Failure

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Raising the Issue and Proposing a Potential Solution

Therapeutic strategies for heart failure (HF) should increasingly consider measures directly targeting the myocardium itself rather than strictly focusing on agents that unload the heart or target systemic neurohormones. In this regard, a new view is emerging that proposes the direct intervention on the pathological structural remodeling of the myocardium as part of HF therapy. One hallmark myocardial lesion in HF is the diffuse accumulation of collagen fibers (namely, collagen type I) within the interstitium and around the microvasculature of the myocardium (ie, myocardial fibrosis [MF]). MF is the result of the predominance of collagen synthesis over its degradation as a result of the actions of a population of persistent metabolically active cells: the myofibroblasts. MF impairs cardiac function and contributes to the development and worsening of HF, in addition to facilitating arrhythmias and ischemia, and thus adversely influences the evolution and outcome of nearly all cardiac diseases.[1] Therefore, MF regression is an unmet medical need, and the search for new drugs aiming its reversal is active. However, the efficacy and safety of novel antifibrotic strategies currently under investigation seem questionable.[2] We thus propose a therapeutic approach based on targeting the cardiac myofibroblast secretome with already available drugs, which have proven to be both efficient in reducing MF and safe in protecting cardiac function. In addition, we will discuss how to identify those HF patients in whom these therapies can be more beneficial and thus should be personalized.

Reviewing Some Fibrogenic and Fibrolytic Axes of the Cardiac Myofibroblast Secretome

Cardiac myofibroblasts are cells expressing α-smooth muscle actin microfilaments that originate from the differentiation of several cell types (including resident cardiac fibroblasts) under the influence of many local and systemic factors (Figure 1). Myofibroblasts exhibit a synthetic phenotype that includes a secretome consisting of molecules requisite to modify the quantity and quality of the myocardial collagen network and facilitate MF (Figure 1).[3] Although few large-scale proteomics studies have focused on the whole myofibroblast secretome, Abonnenc et al[4] reported that mouse cardiac myofibroblasts grown in vitro can secrete ≤245 proteins. Whereas some of these proteins regulate the activity of the myofibroblast in an autocrine–paracrine manner, others are involved directly in the extracellular processing of collagen (Figure 1). Following are examples of these 2 types of proteins.

Among the best-known proteins secreted by the myofibroblast are angiotensin peptides. In particular, a fibrogenic axis consists of angiotensin converting enzyme (ACE)/angiotensin II/angiotensin type 1 receptor, with angiotensin II/angiotensin type 1 receptor representing the most upstream signal stimulating procollagen types I and III synthesis and secretion by the myofibroblast via a downstream transforming growth factor-β_1/Smad pathway.[5] In conditions of MF, this axis predominates over a counter-regulatory, fibrolytic ACE2/Angiotensin-(1–7)/Mas receptor axis, where ACE2-based hydrolysis of angiotensin II leads to Ang-(1–7) formation. Ang-(1–7)/Mas receptor signaling induces myofibroblast apoptosis through inactivation of antiapoptotic proteins.[3,5] Pharmacological interruption of the fibrogenic axis by the ACE inhibitor lisinopril[6] or the AT_1R antagonist losartan[7] has been used to reduce the established MF found in patients with hypertensive heart disease together with improvement in myocardial stiffness and left ventricular function. Additionally, these agents each upregulate the fibrolytic ACE2/Ang-(1–7)/Mas receptor axis,[8] whose contribution to the regression of MF must also be considered. Direct targeting of cardiac myofibroblasts broadens the traditional view of ACE inhibitors and AT_1R antagonists beyond their simply opposing systemic neurohormonal activation and supports that their marked beneficial effects in HF patients may be, at least in part, because of regression of MF.

Other less-known proteins present in the myofibroblast secretome are enzymes that directly intervene in the extracellular synthesis, deposition, and degradation of collagen type I and III fibers (Figure 2).[9] For instance, the enzymes procollagen type I amino-terminal proteinase (PNP) and procollagen type I carboxy-terminal proteinase (PCP, also termed bone morphogenetic protein-1) cleave the terminal propetides converting the procollagen precursor secreted by the myofibroblast

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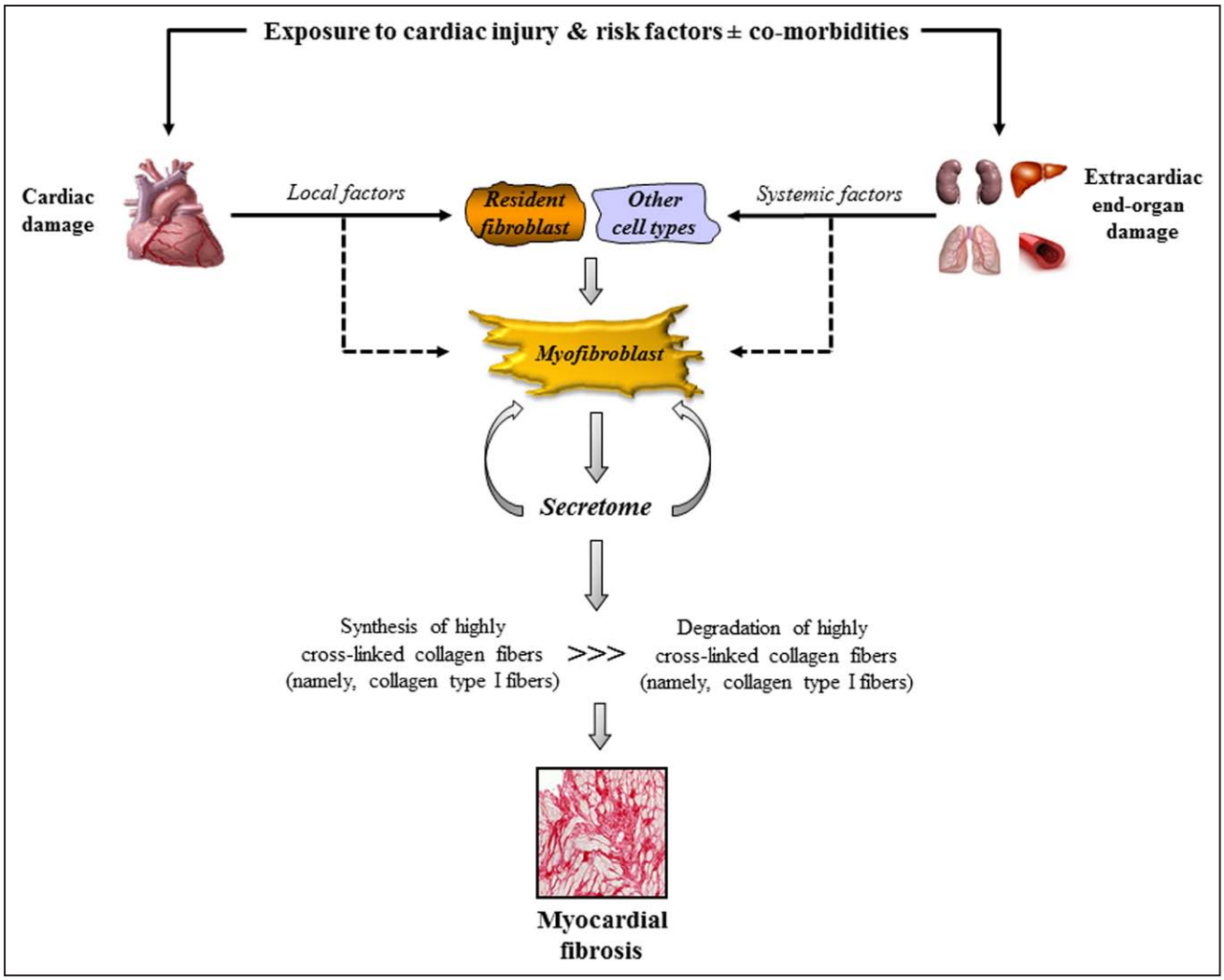

Figure 1. General view of the cause of diffuse myocardial fibrosis highlighting the major role played by the myofibroblast secretome.

in mature fibril-formig collagen molecule. Subsequently, the copper-dependent enzyme lysyl oxidase (LOX) that catalyzes the formation of covalent bonds between polypeptide chains of adjacent fibrils (ie, cross-links) forming the final collagen type I fiber which is deposited in the interstitium and around the microvessels (Figure 2). The enzyme matrix metalloproteinase-1 (MMP-1) initiates the degradation of collagen type I molecules within the fiber that results in 2 peptides: 1 small carboxy-terminal telopeptide (CITP), which is released into the blood stream, and 1 large telopeptide, which is further degraded by matrix metalloproteinase-2 and -9 to final fragmented peptides termed matrikines (Figure 2). In conditions of fibrosis, the fibrogenic PCP-PNP/LOX axis predominates over the fibrolytic MMP-mediated axis thus offering an opportunity for therapies against MF.

Targeting the Fibrogenic PCP-PNP/LOX Axis to Treat MF in the HF Patient

As the degree of cross-linking determines both the stiffness and the resistance to degradation of collagen type I fibers, the enzyme LOX may play an important role in MF. Some clinical evidence supports this possibility. It has been shown that LOX mRNA and active protein expression were increased in the fibrotic myocardium of patients with dilated cardiomyopathy and end-stage HF compared with normal hearts.[10] Of interest, the excess of LOX was associated with both increased transforming growth factor-β_1 expression and collagen content in dilated cardiomyopathy hearts.[10] We have reported that the active form of LOX was highly expressed in myofibroblasts and areas of interstitial and perivascular fibrosis in the myocardium of patients with HF of hypertensive cause compared with the myocardium of healthy control subjects.[11,12] In addition, LOX was independently associated with the degree of collagen cross-linking[11] and with the deposition of collagen type I fibers[12] in HF patients. In another study, both the expression of active LOX and the degree of collagen cross-linking were enhanced in the myocardium of patients with HF of hypertensive cause with elevated pulmonary capillary wedge pressure compared with patients with normal pulmonary capillary wedge pressure.[13] In addition, LOX was independently associated with pulmonary capillary wedge pressure in HF patients, as was the degree of collagen cross-linking. Furthermore, myocardial active LOX was associated with left ventricular stiffness in patients with HF of hypertensive cause.[12]

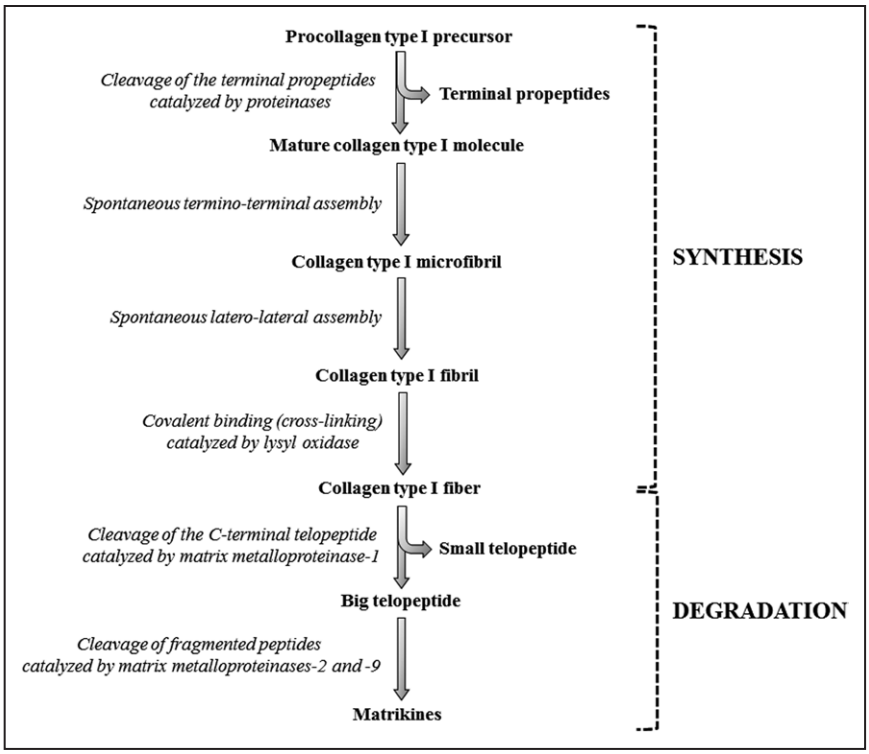

Figure 2. Schematic representation of the different steps involved in the extracellular synthesis and degradation of collagen type I in the myocardium.

Collectively, these findings are consistent with the notion that in the failing human heart, the quality of collagen (specifically the degree of cross-linking) plays a key role in translating collagen quantity into mechanical stiffness and functional impairment of the left ventricle, thus highlighting the potential of targeting LOX for treatment of MF. This possibility has been preliminarily confirmed by studies showing that in patients with HF of different causes and with either reduced or preserved ejection fraction, administration of torasemide in addition to standard HF therapy (including either an ACE inhibitor or an AT_1R antagonist) was associated with reductions in myocardial expression of active LOX, the degree of collagen cross-linking, and collagen type I deposition.[11,14] Additionally, treatment with torasemide was accompanied by normalization of left ventricular stiffness and improvement of function in 80% of the patients, without left ventricular enlargement.[11] Of note, none of these effects were observed in furosemide-treated HF patients.[11,14]

LOX can be regulated at several levels including the extracellular conversion of the protein precursor into the active enzyme because of the action of PCP.[15] We reported that torasemide, but not furosemide, decreased myocardial PCP activation in HF patients.[16] It thus is tempting to speculate that the reduction in myocardial LOX observed in torasemide-treated HF patients can be related to the ability of this compound to deactivate PCP. Thus, beyond its capacity to promote diuresis by inhibiting the renal Na^+, K^+, Cl^- cotransport protein, torasemide emerges as an antifibrotic agent by interfering with the cardiac PCP-(PNP)/LOX axis.

Identifying HF Patients to Be Treated With Torasemide to Reduce MF

A major unmet need in HF therapy is the ability to identify homogeneous subsets of patients whose underlying disease is driven by a specific mechanism that can be solely targeted using personalized treatment. In this regard, the question is how to identify those HF patients with increased myocardial LOX-mediated collagen cross-linking and thus who would be susceptible to torasemide treatment (associated with either an ACE inhibitor or an AT_1R antagonist)? Recently, we have demonstrated that a low serum CITP:MMP-1 ratio identifies with good sensitivity and specificity HF patients with histologically proven excessive myocardial collagen type I cross-linking, which account for approximately half of all HF patients.[17] Furthermore, the serum CITP:MMP-1 ratio is associated with the risk of presenting with hospitalization for HF.[17] The pathophysiological basis of this biomarker relies on the consideration that a diminished circulating level of CITP (corrected by circulating MMP-1) reflects reduced collagen type I fiber degradation by MMP-1 because a high LOX-mediated cross-linking augments the resistance of the fiber to proteolysis by MMP-1.

Whether HF patients with a low serum CITP:MMP-1 ratio would benefit more from the antifibrotic properties of torasemide than the remaining HF patients is a hypothesis that remains to be tested in an adequately designed trial. Interestingly, in 2 open-label, randomized trials performed in patients with chronic HF under standard therapy, the addition of torasemide was associated with a lesser rate of hospitalization for HF than the addition of furosemide in one[18] and with a similar rate in another.[19] Whether this discrepancy can be related to differences in the frequencies of collagen cross-linking phenotypes between the 2 studies is unknown. Anyway, this example highlights that identification of well-phenotyped patients (using chemical and imaging biomarkers) for highly focused clinical trials investigating mechanistically oriented agents is critical to advance the

field of MF therapy. Furthermore, we suggest that phenotyping and treatment strategies based on the myofibroblast secretome may offer success in the regression of MF. Thus, research of the human cardiac myofibroblast secretome emerges as one area of interest to develop antifibrotic therapies for HF, namely, for HF with preserved ejection fraction and for worsening HF.

Disclosures

Javier Díez has consulted for Novartis, Bayer, Merck, Sharp and Dohme, and Abbvie.

References

1. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis: functional significance and regulatory factors. *Cardiovasc Res*. 1993;27:341–348.
2. Roubille F, Busseuil D, Merlet N, Kritikou EA, Rhéaume E, Tardif JC. Investigational drugs targeting cardiac fibrosis. *Expert Rev Cardiovasc Ther*. 2014;12:111–125. doi: 10.1586/14779072.2013.839942.
3. Bomb R, Heckle MR, Sun Y, Mancarella S, Guntaka RV, Gerling IC, Weber KT. Myofibroblast secretome and its auto-/paracrine signaling. *Expert Rev Cardiovasc Ther*. 2016;14:591–598. doi: 10.1586/14779072.2016.1147348.
4. Abonnenc M, Nabeebaccus AA, Mayr U, Barallobre-Barreiro J, Dong X, Cuello F, Sur S, Drozdov I, Langley SR, Lu R, Stathopoulou K, Didangelos A, Yin X, Zimmermann WH, Shah AM, Zampetaki A, Mayr M. Extracellular matrix secretion by cardiac fibroblasts: role of microRNA-29b and microRNA-30c. *Circ Res*. 2013;113:1138–1147. doi: 10.1161/CIRCRESAHA.113.302400.
5. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. *Nat Rev Cardiol*. 2013;10:15–26. doi: 10.1038/nrcardio.2012.158.
6. Brilla CG, Funck RC, Rupp H. Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. *Circulation*. 2000;102:1388–1393.
7. Díez J, Querejeta R, López B, González A, Larman M, Martínez Ubago JL. Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chamber stiffness in hypertensive patients. *Circulation*. 2002;105:2512–2517.
8. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111:2605–2610. doi: 10.1161/CIRCULATIONAHA.104.510461.
9. Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol*. 2011;3:a004978. doi: 10.1101/cshperspect.a004978.
10. Sivakumar P, Gupta S, Sarkar S, Sen S. Upregulation of lysyl oxidase and MMPs during cardiac remodeling in human dilated cardiomyopathy. *Mol Cell Biochem*. 2008;307:159–167. doi: 10.1007/s11010-007-9595-2.
11. López B, Querejeta R, González A, Beaumont J, Larman M, Díez J. Impact of treatment on myocardial lysyl oxidase expression and collagen cross-linking in patients with heart failure. *Hypertension*. 2009;53:236–242. doi: 10.1161/HYPERTENSIONAHA.108.125278.
12. López B, González A, Lindner D, Westermann D, Ravassa S, Beaumont J, Gallego I, Zudaire A, Brugnolaro C, Querejeta R, Larman M, Tschöpe C, Díez J. Osteopontin-mediated myocardial fibrosis in heart failure: a role for lysyl oxidase? *Cardiovasc Res*. 2013;99:111–120. doi: 10.1093/cvr/cvt100.
13. López B, Querejeta R, González A, Larman M, Díez J. Collagen cross-linking but not collagen amount associates with elevated filling pressures in hypertensive patients with stage C heart failure: potential role of lysyl oxidase. *Hypertension*. 2012;60:677–683. doi: 10.1161/HYPERTENSIONAHA.112.196113.
14. López B, Querejeta R, González A, Sánchez E, Larman M, Díez J. Effects of loop diuretics on myocardial fibrosis and collagen type I turnover in chronic heart failure. *J Am Coll Cardiol*. 2004;43:2028–2035. doi: 10.1016/j.jacc.2003.12.052.
15. Pischon N, Babakhanlou-Chase H, Darbois L, Ho WB, Brenner MC, Kessler E, Palamakumbura AH, Trackman PC. A procollagen C-proteinase inhibitor diminishes collagen and lysyl oxidase processing but not collagen cross-linking in osteoblastic cultures. *J Cell Physiol*. 2005;203:111–117. doi: 10.1002/jcp.20206.
16. López B, González A, Beaumont J, Querejeta R, Larman M, Díez J. Identification of a potential cardiac antifibrotic mechanism of torasemide in patients with chronic heart failure. *J Am Coll Cardiol*. 2007;50:859–867. doi: 10.1016/j.jacc.2007.04.080.
17. López B, Ravassa S, González A, Zubillaga E, Bonavila C, Bergés M, Echegaray K, Beaumont J, Moreno MU, San José G, Larman M, Querejeta R, Díez J. Myocardial collagen cross-linking is associated with heart failure hospitalization in patients with hypertensive heart failure. *J Am Coll Cardiol*. 2016;67:251–260. doi: 10.1016/j.jacc.2015.10.063.
18. Murray MD, Deer MM, Ferguson JA, Dexter PR, Bennett SJ, Perkins SM, Smith FE, Lane KA, Adams LD, Tierney WM, Brater DC. Open-label randomized trial of torsemide compared with furosemide therapy for patients with heart failure. *Am J Med*. 2001;111:513–520.
19. Müller K, Gamba G, Jaquet F, Hess B. Torasemide vs. furosemide in primary care patients with chronic heart failure NYHA II to IV–efficacy and quality of life. *Eur J Heart Fail*. 2003;5:793–801.

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