Editorial

“Fibroblast” pharmacotherapy – Advancing the next generation of therapeutics for clinical cardiology

The extracellular matrix (ECM) is the dynamic non-cellular compartment of the heart that provides biochemical and biophysical support. The importance of the ECM is well recognized in the pathogenesis of heart failure, as both a cause and consequence of this disease. The ECM interacts with diverse cells of the heart to regulate differentiation, contractile function, cell migration and transmit force. The matrix has a latent reserve of substrates and growth factors; its physicochemical structure and diversity are regulated by matrix metalloproteinases [1]. This matrix is maintained by “fibroblasts” that synthesize, cross-link and align secreted protein-polymers to create the model architecture of the heart. The ECM is so refined that when stripped of all cells, the residual matrix guides de novo cardiomyocyte regeneration while remnant blood vessels remain patent [2,3]. Yet, despite the importance of ECM in cardiac structure and function, we know very little about this proteome in healthy hearts as most research is focused on end-stage disease remodeling.

The term “fibroblast” has become an umbrella term to describe a heterogeneous population of resident mesenchymal cells, medullary fibrocytes, fibroblasts, myofibroblasts, chordal fibroblasts and valvular interstitial cells. Surprisingly, while the cardiomyocyte occupies the majority of the heart by volume, it is the fibroblasts that are the most abundant [4], foreshadowing an equal importance to the regulation and maintenance of cardiac function. Further, the population of fibroblasts grows substantially in response to all cardiovascular diseases aetiology. This canonical expansion occurs by unrestricted proliferation of the fibroblast population or non-canonically by (trans-)differentiation of medullary or vascular/epithelial populations [5]. The incipient events in ECM dysregulation are associated with impregnation of the cardiac interstitium by circulating matrix (e.g. ED-A fibronectin) in response to excess neurohormonal activation, inflammation, metabolic or hemodynamic stress [6–10]. With injury or chronic stress, fibroblasts can be driven to a myofibrotic phenotype (i.e., myofibroblasts) as they adapt, or are cued by, inflammatory or mechanical loads to alter both ECM and their own intracellular contractile proteins and signalling networks. Loss of essential ECM or excess matrix-proteases can result in cardiac aneurysm or wall rupture (i.e., MI). In this way, remodeling has a role in adaptive repair, but is more often viewed as both a cause and consequence of the underlying pathology—contributing to systolic and/or diastolic dysfunction. Loss of tensile strength and elasticity in the ECM by proteolysis or misaligning of matrix synthesis results in myocyte slippage and dilatation of the left ventricle, a distinct feature of systolic dysfunction. Excess ECM synthesis (reactive fibrosis) is often associated directly with decreases in cardiac compliance and diastolic dysfunction, but there are exceptions [11,12]. Since fibrotic contributions to systolic dysfunction differ from that causing diastolic dysfunction, ECM remodeling isn’t necessarily the same in genetic, aging, metabolic, pressure or volume overload heart failure. Importantly, therapeutic interventions affecting the ECM remodeling can be curative, regressive or intractable in relation to the timing of intervention for optimal outcomes. As well, the proportions of ECM constituents (e.g. collagen, fibronectin, hyaluronan, tenascin, periostin) altered by the remodeling process, with due spatial and temporal consideration, are as variable as the disease aetiologies themselves. It is imperative to recognize ECM remodeling as both predictive and determinant to patient outcome. Where there is reactive fibrotic remodeling in one organ—such as the heart—others, such as the kidney are often concomitant (i.e. cardiorenal syndrome). Finally, fibroblasts of many organs are increasingly being recognized for their complex contributions to homeostasis beyond the ECM, such as their endocrine and metabolic function.

Intervening in ECM remodeling remains focused on pharmacological targeting of the renin–angiotensin–aldosterone–system (RAS) [13]. Two drug classes are standard in clinical practice, the angiotensin converting enzyme (ACE) inhibitor (e.g. enalapril) and the angiotensin receptor-1 (AT1R) antagonists (ARB, e.g. valsartan). These drugs arrest or regress cardiac fibrosis in many, but not all, disease states. The timing of intervention and the drug class (or combinations thereof) determine the degree of benefit [14–16]. Systemic features of RAS targeting include lowering sympathetic drive, blood pressure and volume, which is attributed to reduced circulating angiotensin-II [1–8] (AngII) or activation of AT1R. However, this can also be attributed to changes in other downstream effectors, such as bradykinin or endothelin [17,18]. Yet, it has been recognized for some time that tissue levels (i.e., ~10–750 nM) of AngII are significantly greater than circulating levels (i.e., ~17–27 pM) in health and following sodium depletion [19]. Since that time, much has been gained about angiotensinogen (AGT) enzymatic processing (angiotensin escape). This is the processing of at least 10 distinct angiotensin fragments (Fig. 1) by other enzymes than the classical ACE—notably for clinical pharmacologists and cardiologists, nephrilysin (NEP) [20] and chymase [21,51]. With a greater understanding of the complex RAS network, there are now eight distinct RAS drug-classes that pharmacologically could be used to target fibrosis (Fig. 1). To date, only ACE and/or ARB are used broadly, and it is still unlikely that we know the best-in-class selection or combination for
optimal outcomes [22]. Indeed, the recent SPRINT-trial provided clues to poly-pharmacological targeting of RAS [23]. Though guided by a blood pressure target (i.e., 120 mm Hg), the beneficial clinical outcomes were astonishing. This begs the questions: were additional drugs or higher doses lowering tissue RAS, and could this system be more important than the reduction in blood pressure per se? In mice over-expressing AT1R, premature heart failure occurred with high interstitial collagen and hypertrophy without any changes in blood pressure or heart rate [24], which can occur even in the absence of AngII [25,26] since the receptors are mechanosensitive [25,27]. The effective treatment of experimental hypertension by ACE therapy is dependent on time of day and not relevant to the actual lowering of blood pressure [28]. With this knowledge, we begin to appreciate an eclipsing importance of tissue RAS over blood pressure. This is particularly relevant to exploring how tissue RAS relates to aging, metabolic disease and sex differences in cardiovascular diseases of various aetiologies. There is an urgent need to deliver further evidence and mechanisms as to the role of tissue RAS in pathological ECM remodeling in all disease states.

The new manuscript, “Increased fibroblast chymase production mediates procollagen autophagic digestion in volume overload [29],” we gain further insight into tissue RAS regulation with a heightened awareness of fibroblast autoregulation of ECM remodeling. Here, Fu et al. prove that adult cardiac fibroblasts are a novel site of chymase synthesis in pure volume-overload heart failure, and regulate ECM degradation by autophagy through a heretofore unknown intracellular mechanism. These data provide a watershed moment, highlighting the importance of the fibroblast to over-all cardiac function in relation to RAS.

Independence between systemic and tissue RAS has previously been established [17,30] with chymase featuring prominently therein [31, 32]. The importance of ECM dysfunction in ventricle chamber enlargement following MI has been recognized for some time [33], though a means for pharmacological intervention was far less so. In addition to ECM degradation by MMPs, there is concurrent degradation by cardiac fibroblasts through autophagy [34]. Previous reports demonstrated an important role for autophagy in human cardiac fibroblasts [35]. The problem eliminated by the current study, is a clear link to clinically translatable drug targeting of tissue RAS to influence pathological fibrosis involving autophagy.

What has been accepted to date is that mast cells are the principle culprit in angiotensin escape by tissue chymase [17,30]. This was of limited interest to cardiologists who had few options to diagnose cardiac mast cell accumulation or otherwise prevent their degranulation. Further, it is difficult to reconcile that a few scarce mast cells are solely capable of driving wide-spread pathological remodeling of the ECM, notwithstanding that circulating AngII is often the lone scapegoat for fibrotic remodeling. What is now readily apparent and supported by the findings of Fu et al., is that the major cell of the heart—the fibroblast—is capable of intra- and inter-cellular RAS signalling via chymase. Further, in volume-overload, which is burdened by weakening ECM, fibroblasts are degrading procollagen by autophagy and denying the heart of this reinforcing matrix. As well, the authors describe that chymase is produced in response to mechanotransduction of fibroblasts, which alters our view of the mast-cell dominant contribution of chymase henceforth. Whether fibroblasts alter circulating AngII levels now remains to be

**Fig. 1.** Fibroblasts are essential modulators of RAS and ECM remodeling. Fibroblasts are a contributing source of cardiac chymase, converting AngI into AngII both intra- and extracellularly. (MMP: matrix metalloproteinase; AGT: angiotensinogen; Ang: angiotensin; ACE: angiotensin converting enzyme; NEP: nephrilysin; FB: fibroblast; ECM: extracellular matrix; ROS: reactive oxygen species; CM: cardiomyocyte; RAS: renin–angiotensin system; AT1R: angiotensin receptor-1; AT2R: angiotensin receptor-2; REN: renin; CMA1: chymase).
determined. Also, whether a parallel exists in fibroblasts of other organs (e.g., kidney, limb muscles, vasculature) is of interest. Mast cells release chymase in response to an acute inflammatory-mediated degranulation. In contrast, fibroblast release of chymase would be wide-spread throughout the heart and more persistent with chronic disease and progressive mechanical load. Direct comparative studies of chymase in mast cells and fibroblasts may offer further novel insight to the quantitative contributions in acute and chronic disease states.

While not discounting the contribution of acute chymase release from mast cells, which is well established in heart failure, they are a small minority sub-population of cells. Distinguishing mast cell contributions from the cardiovascular progenitor and cardiomyocyte in the heart has been a challenge in recent years because they both express CD117 (cKit) and both reside in the perivascular and interstitial spaces of the heart. It remains to be determined whether mast cell-to-fibroblast cross-talk is occurring in this model and their relative spatial-temporal contributions to in vivo ECM degradation. For example, are mast cells more important towards initiating chymase signalling with perpetuation by fibroblasts (in other words, mast cells start the fire while fibroblasts stoke the fire)? The use of mast cell-specific animal models will provide further insight to this as well [36].

In the manuscript by Fu et al., they used post-hoc dissociated fibroblasts from pure volume-overload. While this approach was necessary to definitively establish the mechanistic role of the fibroblast in chymase production, this is biased towards those cells most able to be enzymatically freed from the fibrotic heart. Further work is necessary to investigate the extent of fibroblast-mediated chymase production. For example, are all fibroblasts equally producing chymase or are these cells confined to specific regions (e.g., atria vs ventricle, left vs right ventricle, epicardium vs endocardium). Whether similar mechanisms are retained in human cells and whether these mechanisms are retained in vivo are not yet completely clear. MI is another clinically relevant volume-overload model that is beset with reparative, adaptive and pathological ECM remodeling. Investigations in other models will ultimately shed more light on the general use of chymase treatment in other relevant cardiovascular conditions that are absolutely necessary to guide successful clinical trials.

A critical barrier to understanding and distinguishing the dependency and mechanisms of fibroblasts from other cardiovascular cell types is the frustrating fact that there are no reliable transgenic tools to do so. No gene to date has offered fibroblast specificity (e.g. Postn, Fsp1, Ddr1, Cdh11, Fap) that alone cardiac fibroblast specificity with inducible regulation. In contrast, many cardiomyocyte specific genes offer natal, post-natal and inducible cre-regulated expression (e.g., Minc, oMH, Nks2.5, Gata4) with some genes offering chamber specificity (i.e., Hand1). In an era of rising fibroblast importance to the regulation of cardiovascular function, this is extremely limiting. Moreover, we are in a position to initiate several translational and clinical studies based on chymase, but are doing so in a basic science deficit, which substantially increases the risk of clinical failure and/or harm.

In failing hearts, we do appreciate that as much as 75% of tissue AngII is derived from chymase [37]. Animal studies show that chymase inhibition reduces MMP activity, diastolic dysfunction and late stage fibrotic remodeling [38–40]. Chymase inhibition decreases plasma and tissue AngII levels and pathological remodeling without affecting blood pressure [41,42]). Chymase inhibition also prevents vascular remodeling and improves survival in the stroke-prone spontaneously hypertensive rat [43] suggesting chymase activity is not restricted to the heart but whether this is related to mast cells and/or fibroblasts remains to be determined. Taken together, the clinical end-point of RAS therapy should not necessarily be dependent on a target blood pressure but also on tissue and plasma AngII levels.

There are distinct differences between the animal and human chymase. Rodent chymase has a higher affinity for big-endothelin-1 [44] that could influence clinical expectations. Further, patients requiring therapy are older and of both sexes which is not typically accounted for in preclinical studies. Fu et al., show some age-dependency in their study with increasing chymase activity over time in healthy male rodents. The ECM, c-kit positive cell content/function and chymase levels change with sex-associated aging and chymase also activates pro-collagenases, MMPs and catalyses thrombin and plasmin [45–49]. Expecting human parallels to animal studies should be approached with caution and requires further translational studies in age and sex appropriate models.

The traditional endocrine function of RAS is well characterized, but with increasing intricacy for unique paracrine and intracrine effects as well [50]. The heart is composed of many different cell types: cardiomyocytes, fibroblast, vascular, epithelial, medullary and the purported cardiovascular progenitor/stem cell populations. They are highly integrated and interdependent with a growing number of studies similar to Fu et al, showing a new age for comprehending the enigmatic fibroblast's roles in affecting cardiac function. The fibroblast orchestrates much of the ECM, but it is also an equal contributor to chemical, mechanical, electrical, metabolic and endocrine function of the cardiomyocyte. It is an important regulator of cardiomyocyte proliferation and neovascularisation in neonatal and adult life. As the traditional black sheep of the cardiovascular system to scientists, we must commit to further investigation to understand its active regulation and contribution to disease aetiologies. With an emerging intracrine RAS system being characterized, first for AngII and now for chymase, there are finer basic science points that must emerge to inform cardiologists and clinical pharmacologists as we further RAS pharmacology. These are exciting times for the fibroblast.

**Abbreviations**

ACE angiotensin converting enzyme
AGT angiotensigen
Ang angiotensin
ARB angiotensin receptor blocker
AT,R angiotensin receptor-1
AT,R angiotensin receptor-2
ECM extracellular matrix
MI myocardial infarction
MMP matrix metalloproteinase
NEP nephrisin
RAS renin-angiotensin-system
SPRINT systolic blood pressure intervention

**Disclosures**

Dr. Keith R. Brunt holds equity in NB-BioMatrix Inc.

**Acknowledgments**

The authors acknowledge the financial support of the Saint John Regional Hospital Foundation (#4101-4173214) and National Science and Engineering Research Council (#05520). Dr. Simpson is an early career investigator of the Heart & Stroke Foundation of Canada.

**References**


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