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Myofibroblast secretome and its auto-/paracrine signaling

Ritin Bomb, MD[1], **Mark R. Heckle, MD**[2], **Yao Sun, MD, PhD**[1], **Salvatore Mancarella, PhD**[3], **Ramareddy V. Guntaka, PhD**[4], **Ivan C. Gerling, PhD**[5], and **Karl T. Weber, MD**[1]

[1]Division of Cardiovascular Diseases, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A

[2]Department of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A

[3]Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A

[4]Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A

[5]Division of Endocrinology, University of Tennessee Health Science Center, Memphis, Tennessee, U.S.A

Summary

Myofibroblasts (myoFb) are phenotypically transformed, contractile fibroblast-like cells expressing α-smooth muscle actin microfilaments. They are integral to collagen fibrillogenesis with scar tissue formation at sites of repair irrespective of the etiologic origins of injury or tissue involved. MyoFb can persist long after healing is complete, where their ongoing turnover of collagen accounts for a progressive structural remodeling of an organ (a.k.a. fibrosis, sclerosis or cirrhosis). Such persistent metabolic activity is derived from a secretome consisting of requisite components in the *de novo* generation of angiotensin (Ang) II. Autocrine and paracrine signaling induced by tissue AngII is expressed via AT_1 receptor ligand binding to respectively promote: *i*) regulation of myoFb collagen synthesis via the fibrogenic cytokine TGF-β_1-Smad pathway; and *ii*) dedifferentiation and protein degradation of atrophic myocytes immobilized and ensnared by fibrillar collagen at sites of scarring.

Several cardioprotective strategies in the prevention of fibrosis and involving myofibroblasts are considered. They include: inducing myoFb apoptosis through inactivation of antiapoptotic proteins; AT_1 receptor antagonist to interfere with auto-/paracrine myoFb signaling or to induce counterregulatory expression of ACE2; and attacking the AngII-AT_1R-TGF-β_1-Smad pathway by antibody or the use of triplex-forming oligonucleotides.

Correspondence: Karl T. Weber, M.D., Division of Cardiovascular Diseases, University of Tennessee Health Science Center, 956 Court Ave., Suite A312, Memphis, TN 38163, Tel: (901) 448-5750, Fax: (901) 448-8084, KTWeber@uthsc.edu.

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Keywords

cardiac fibrosis; myofibroblast secretome; auto-/paracrine signaling; angiotensin II; cardioprotection

Introduction

Fibrosis is a fundamental component of the pathological remodeling found in the failing heart irrespective of its etiologic origins. In hypertensive heart disease, for example, a diffuse interstitial fibrosis and microscopic scarring are present in association with cardiomyocyte hypertrophy and atrophy which is found at sites of fibrosis [1, 2]. In ischemic cardiomyopathy, an important morphologic finding remote to an infarct scar is the widely scattered foci of microscopic scars, indicative of ongoing bouts of necrosis with reparative fibrosis [1, 3], and with an attendant atrophy of cardiomyocytes ensnared by fibrillar collagen. Scarring is also a feature of hypertrophic cardiomyopathy involving both the hypertrophied left and nonhypertrophied right ventricles and where heterogeneity in myocyte size is again seen [4, 5].

Myofibroblasts are responsible for scar tissue formation at every site of cardiomyocyte necrosis. Myocyte apoptosis elicits neither a wound-healing response nor scarring and therefore leaves no morphologic footprint [6]. Metabolic signaling from the myofibroblast secretome regulates ongoing fibrillar collagen production integral to the scar tissue formation and its subsequent turnover. During the early stages of wound repair, inflammatory cell-derived TGF-β_1 induces the *de novo* expression of angiotensinogen, renin and angiotensin-converting enzyme (ACE) and the secretory phenotype of myofibroblasts and which, in turn, serves to generate angiotensin II at the site of repair (see Figure 1) [7-9]. The *autocrine* signaling invoked by this tissue peptide is mediated via AT_1 receptor binding with the resultant expression of the fibrogenic cytokine transforming growth factor (TGF)-β_1 [10]. Together with activation of downstream connective tissue growth factor and Smad-signaling pathway, the deposition of fibrillar collagen types I and III follows with scar tissue formation. An active interplay also exists between myofibroblasts and the extracellular structural protein matrix, including incorporation of latent TGF-β_1 with its binding protein and its release and activation by proteases under the influence of reactive oxygen species [11]. The heterocellular *paracrine* signaling between myofibroblast-derived AngII and neighboring cardiomyocytes (see Figure 2) raises myocyte cytosolic $[Ca^{2+}]_i$ to induce oxidative stress and activate redox-sensitive proteolytic ligases of the ubiquitin proteasome system (UPS) with resultant protein degradation leading to cell atrophy. Myofibroblasts also promote the dedifferentiation of these atrophic myocytes with re-expression of fetal genes, including β-myosin heavy chain and natriuretic peptides [12-20]. The re-expression of these fetal genes in atrophic myocytes, as well as in hypertrophied myocytes, is mediated by reduced intracellular thyroid hormone signaling. This localized hypothyroid state arises from the increased degradative activity of deiodinase-3 and its metabolism of T_3 and T_4 into inactive compounds [21-23].

Given their diverse roles in cardiac remodeling, myofibroblasts and their secretome are targeted in the prevention of fibrosis. The purpose of this mini-review is to provide a perspective that addresses the role of myofibroblasts in cardiac repair, their secretome and its auto- and paracrine signaling by angiotensin II in leading to adverse myocardial remodeling, and finally several myofibroblast-directed cardioprotective strategies. A full discourse on the many aspects of myofibroblast biology and antifibrotic strategies that could be utilized in cardioprotection is beyond the scope of this report. The interested reader is referred to reviews found elsewhere [24-27].

Myofibroblasts and Tissue Repair

Myofibroblast-mediated scar tissue formation appears following cardiomyocyte necrosis, irrespective of whether such cell loss involves a segment of myocardium with a macroscopic infarct scar or as a microscopic scar with the loss of individual myocytes. The origins of these fibroblast-like cells remain uncertain. Pericytes of the microvasculature, circulating fibrocytes derived from bone marrow stem cells, and usual interstitial fibroblasts have each been implicated [24, 28]. Valvular interstitial cells, normal residents of heart valve leaflets and having a myofibroblast phenotype, are another possible source [29]. An endothelial-mesenchymal cell transition has also been suggested [30].

In response to inflammatory cell-derived TGF-β_1 activation, myofibroblasts produce angiotensin II and fibronectin [31], creating a provisional scaffolding for the subsequent angiotensin II-AT_1R-TGF-β_1-Smad-mitogen-activated-protein-kinase-mediated deposition of fibrillar collagen types I and III [11, 32], the major structural proteins forming scar tissue [33, 34]. Stiff, cross-linked type I fibrillar collagen confers strength to resist scar deformation and, in turn, to resist myocardial thinning and ventricular chamber dilatation. And while the structural integrity of myocardium is preserved, its architecture, mechanical and electrical characteristics have been disrupted.

Myofibroblasts dominate the regulation of collagen turnover in the injured heart [35, 36]. Fibrillogenesis is self-regulated through myofibroblast expression of molecules requisite to the formation of angiotensin II and its subsequent autocrine signaling via AT_1 receptor binding [10, 37-40]. At each site of scarring with their resident myofibroblasts, is the high-density expression of ACE and AT_1 receptor binding involving these cells irrespective of the injured tissue or the cause of injury [37-41]. The *de novo* generation of angiotensin peptides has been demonstrated in α-SMA-positive valvular interstitial cells and myofibroblasts harvested from a 4-wk-old infarct scar [29, 42]. Their ACE activity and responsiveness to ACE inhibition remains intact at each site, including that of the fibrosed visceral pericardium as detected by monitoring angiotensin II in the superfusate of the isolated perfused heart [43].

Persistent Myofibroblasts and their Activity

Myofibroblasts persist for months [32] and even years [44] within an infarct scar. Their anticipated disappearance through programmed cell death when tissue repair is complete fails to occur [44]. Beyond their persistence is a secretory phenotype with ongoing scar

tissue collagen turnover to confer stability and strength to the infarct scar of this muscular pump. However, the mobility of α-smooth muscle actin-containing myofibroblasts in migrating to distant sites and the soluble signals they generate at these sites invokes a progressive fibrosis of myocardium. The pathophysiological mechanisms accounting for this *progressive fibrosis*, however, remain unclear. Several possibilities, each driven by reactive oxygen species derived from mitochondria and/or NADPH oxidase of these cells [45-47], could be theorized. A persistent highly synthetic myofibroblast phenotype with an excessive generation of type I collagen driven by angiotensin II-induced intracellular Ca^{2+} overloading with oxidative stress [48]. Another is repeated episodes of nonischemic cardiomyocyte necrosis, with each bout eliciting a wound-healing response. Such a repetition of the wound-healing response would suggest myofibroblasts are never quiescent, instead remaining in an unrelenting profibrogenic phenotype.

Myofibroblast Secretome: Autocrine Signaling

The *de novo* generation of the angiotensin II by myofibroblasts is a self-regulating autocrine signal that can lead to ongoing fibrosis via protracted AT_1 receptor binding. Also involved in this self-sustained myofibroblast collagen synthesis is TGF-β_1 [49] and Smad-dependent and Smad-independent proteins with connective tissue growth factor (see Figure 1) [50]. Downstream to AT_1 receptor binding signal transduction pathways involve TGF-β_1, Smad proteins, and connective tissue growth factor. Interleukins 1, 6, and 13, and noncoding RNA molecules are involved in regulating myofibroblast collagen synthesis [51]. Intracellular signaling pathways regulating the myofibroblast secretome and collagen turnover are under investigation [52]. TGF-β_1, for example, regulates myofibroblast expression of scleraxis, a profibrotic transcription factor which stimulates collagen synthesis via a Smad-independent pathway [53]. TGF-β_1 suppresses myofibroblast gene expression of matrix metalloproteinases involved in collagen degradation [54]. Because myofibroblasts are persistent and have ongoing auto-/paracrine activity, scar tissue is metabolically active [35].

Myofibroblast Secretome: Paracrine Signaling

Fibrosis is a “crucial determinant” of the tissue heterogeneity found within the diseased myocardium of the failing heart [55]. Myocyte size is normally variable [56]; this variability is accentuated in the failing heart because tendrils of fibrillar collagen, emanating from scar tissue to secure it within this contractile organ, ensnare and immobilize neighboring cardiomyocytes (see Figure 2). In so doing, the work of these myocytes is reduced and disuse atrophy ensues [57].

Myofibroblast angiotensin II signaling has paracrine properties on neighboring myocytes again mediated by AT_1 receptor binding with increments in cytosolic $[Ca^{2+}]_i$ taking place via store-operated Ca^{2+} channels that induce oxidative stress and myocyte dedifferentiation with re-expression of β-MHC and ANP, a fetal gene program also found in hypertrophied myocytes. The important role of oxidative stress in promoting protein degradation with atrophy is further suggested by the response to antioxidants in its prevention [12-17, 19, 20, 58-60]. Angiotensin II and oxidative stress are similarly involved in skeletal muscle atrophy [61]. Activation of redox-sensitive, proteolytic ligases (MuRF1 and atrogin-1) of the

ubiquitin-proteasome system with attendant protein degradation is essential to atrophy of immobilized myocytes ensnared by fibrillar collagen (see Figure 3) [20].

The loss of cardiomyocytes through necrotic and apoptotic forms of cell death, together with atrophic cardiomyocytes contribute to the progressive nature of heart failure. Collectively, the structural remodeling of myocardium by progressive accumulation of an excessive fibrillar collagen matrix has led to the concept of interstitial heart disease [62, 63]. Molecular signaling emanating from myofibroblasts are therefore logical targets for developing cardioprotective strategies to prevent fibrosis.

Cardioprotective Strategies

Given the adverse impact of fibrosis on tissue stiffness, arrhythmogenesis, and the conversion of adaptive to pathologic hypertrophy, antifibrotic strategies are of marked interest. Several are briefly discussed.

Subsarcolemmal Mitochondria

One strategy proposed to attenuate the appearance of cardiac fibrosis is mitochondria-targeted interventions aimed at preventing cardiomyocyte necrosis, a requisite that initiates tissue repair with scarring. This mitochondria-targeted cardioprotective strategy has focused on interrupting the mitochondriocentric signal-transducer-effector pathway to necrosis by preventing subsarcolemmal mitochondrial Ca^{2+} overload. This includes the use of targeted antioxidants or inhibition of the opening of the mitochondrial inner membrane permeability transition pore which is less resistant in these organelles as contrasted to interfibrillar mitochondria [64-66]. Such strategies have included nutriceuticals (flavonoids) [67], pharmaceuticals (cyclosporine A or third-generation β-adrenergic-receptor antagonists) [68], inhaled hydrogen gas [69], or microRNAs [70-72].

Myofibroblast Survival

Therapies that disrupt myofibroblast survival are another potential direction in cardioprotection. Apoptotic clearance of myofibroblasts would serve to remove the ongoing cellular supply of collagen. Nuclear factor kappa B (NF-κB), is integral to fibrogenesis and myofibroblast survival. Transcriptional inhibition of NF-κB, a redox-sensitive transcription factor, could inactivate antiapoptotic proteins [73] as may epigenetic modifications of myofibroblast gene expression and survival [74]. A DNA methylation inhibitor has shown promise in the treatment of hypertension-induced hypertrophy with fibrosis [75].

Myofibroblast-Derived Angiotensin II

Angiotensin II and its AT_1 receptor binding play a pivotal role in the autocrine regulation of collagen turnover and paracrine myocyte signaling. Losartan and valsartan, AT_1 receptor blockers, have each proven cardioprotective [10, 40]. Other AT_1 receptor antagonists likewise prevent reactive fibrosis and attenuate reparative fibrosis in the infarcted heart, in hypertensive heart disease, and in cardiomyopathy induced by rapid atrial pacing [37, 40, 76-79]. Efficacy of AT_1 receptor blockade in suppressing the myofibroblast-based ACE-

angiotensin II-TGF-β_1 signaling axis to fibrosis also has been demonstrated in other diseased cardiovascular tissues, including systemic arterioles and aortic aneurysm [80-82].

ACE2 Expression

ACE2, a homologue of ACE, offers a counter-regulatory approach to the control of tissue angiotensin II. ACE2 hydrolyzes the octapeptide angiotensin II into angiotensin (1–7) which exerts its actions via binding to a Mas receptor. The ACE2-angiotensin (1–7)-Mas signaling axis is in equilibrium with the ACE-angiotensin II-AT_1 receptor axis and angiotensin (1–7) is counter-regulatory to angiotensin II. Angiotensin (1–7) formation is dependent on angiotensin II as its substrate [83]. Increased levels of cardiac ACE2 and angiotensin (1–7)-forming activity are found in the failing heart and its cardiomyocytes [84, 85] and ACE2 activity is insensitive to ACE inhibitors [83]. AT_1-receptor antagonists, on the other hand, increase cardiac expression of ACE2 [86, 87]. Loss of ACE2 augments maladaptive angiotensin II-based remodeling, including upregulation and activation of tissue matrix metalloproteinase [88]. These effects can be blocked by an AT_1-receptor antagonist [88]. By contrast, overexpression of ACE2 and upregulated angiotensin (1–7) attenuate pathological remodeling [89, 90]. Chronic inhibition of ACE2 results in increased fibrosis [91].

TGF-β_1 Pathway

The TGF-β_1 signaling pathway to collagen synthesis can be blocked using a TGF-β_1 antibody, antisense oligonucleotide, or its soluble truncated receptor [73]. The Smad 2/3 pathway can be attenuated [92]. Downstream molecular events can be blocked by triplex-forming oligonucleotides to prevent collagen gene transcription and the accumulation of scar tissue [67, 68, 71, 93-95]. Such triplex-forming sequences are present in the promoter of Col1α1 gene and form efficient triplexes with the exogenously added triplex-forming oligonucleotides and inhibit type I collagen accumulation. Their efficiency in the control of cardiac fibrosis remains to be elucidated.

Limitations

One major caveat to these cardioprotective strategies is the fact collagen turnover is common to all tissues and must not be compromised. Cardiac-targeted strategies, therefore, must be tissue specific, delivered to precise cellular and subcellular locations. Moreover, scar tissue preserves the structural integrity of the heart. Should its formation be prevented at the site of myocyte necrosis, the myocardium would be weakened and subject to rupture. Limited proteolytic digestion of interstitial fibrosis, on the other hand, would be desirable if it releases atrophic myocytes ensnared by fibrillar collagen and regression of fibrous tissue would improve diastolic stiffness. Such has been attained in hypertensive heart disease with an ACE inhibitor or angiotensin-receptor antagonist [96-99].

Summary and Conclusions

When cardiomyocyte necrosis occurs, a population of collagen-expressing myofibroblasts appears to produce scar tissue and preserve the structural integrity of injured myocardium. Persistent myofibroblasts and their secretory phenotype, however, continue their ongoing deposition of stiff, cross-linked type I fibrillar collagen. This progressive fibrosis has

multiple adverse consequences on its structure and function. Indeed, fibrosis is fundamental to adverse structural remodeling of the failing heart, irrespective of its etiologic origins, and draws attention to the important role of myofibroblasts and the entity interstitial heart disease.

The myofibroblast secretome and its angiotensin II–AT_1 receptor–TGF-β_1-Smad autocrine signaling pathway to fibrosis, is common to sites of healing in the heart, as well as other tissues and organs. Both auto- and paracrine signaling involving myofibroblasts and myocytes, respectively, can be interrupted with an AT_1-receptor antagonist. By upregulating a counter-regulatory ACE2-angiotensin (1–7)-Mas receptor axis in degrading AngII, an alternate strategy in cardioprotection could be realized. Other cardioprotective strategies include: the use of mitochondria-targeted antioxidants to prevent cardiomyocyte necrosis and subsequent scarring; the use of therapies that modify myofibroblast survival; and targeting downstream pathways using microRNAs and triplex-forming oligonucleotides to prevent type I collagen transcription.

Expert Commentary

As is the case for any organ, fibrosis disrupts architecture and function of its parenchyma. In the heart, fibrosis can preserve the structure of this hollow muscular organ when injured, but at the expense of its diastolic and systolic function. When cardiomyocyte necrosis occurs, a population of collagen-expressing myofibroblasts appear at the site of injury to produce scar tissue and repair the wound. Myofibroblasts are persistent and have secretory phenotype which continues their deposition of stiff, cross-linked type I fibrillar collagen at and remote to the site of injury with this progressive fibrosis having multiple adverse consequences on ventricular structure and function. Indeed, fibrosis is a fundamental component of the adverse structural remodeling of the failing heart and the entity interstitial heart disease. Fibrosis therefore draws attention to the important role of myofibroblasts and their active participation in adverse cardiac remodeling.

The myofibroblast secretome and its angiotensin II–AT_1 receptor–TGF-β_1-Smad autocrine signaling pathway to fibrosis, is common to sites of healing in the heart irrespective of the etiologic origins of injury. Autocrine signaling involving myofibroblasts and paracrine heterocellular signaling with myocytes can each be interrupted with an AT_1-receptor antagonist. By upregulating a counter-regulatory ACE2-angiotensin (1–7)-Mas receptor axis in degrading AngII, an alternate strategy in cardioprotection could be realized. Other cardioprotective strategies include: the use of mitochondria-targeted antioxidants to prevent cardiomyocyte necrosis and subsequent scarring; the use of therapies that modify myofibroblast survival; and targeting downstream pathways using microRNAs and triplex-forming oligonucleotides to prevent type I collagen transcription are each under investigation. These are but several strategies that are under consideration in the prevention of cardiac fibrosis.

Five-Year View

Heart failure continues to be a worldwide health problem of ever-increasing proportions. The appearance of the congestive heart failure (CHF) syndrome with its characteristic symptoms and signs is rooted in neurohormonal activation. Accordingly, today's management of CHF focuses on pharmacologic interference with effector hormones of the renin-angiotensin-aldosterone and adrenergic nervous systems. This is a palliative approach. It does not address the pathophysiologic basis of the heart's failure as a muscular pump. The adverse structural remodeling of myocardium by a progressive fibrosis imparted by a population of persistent, metabolically active myofibroblasts offers several potential cardioprotective strategies, which are under development and implementation. They include: inducing myofibroblast apoptosis through inactivation of antiapoptotic proteins; AT_1 receptor antagonist to interfere with auto-/paracrine myofibroblast signaling or to induce counterregulatory expression of ACE2; and attacking the TGF-β_1-Smad pathway by antibody or antisense or triplex-forming oligonucleotides. Other potential strategies under development are beyond the scope of this brief perspective. This notwithstanding, targeting the secretory phenotype of this important nonmyocyte cell and its active participation in the appearance of interstitial heart disease offers potential in the prevention of heart failure. The next five years will determine whether this potential can be realized.

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Reference annotations

* Of interest

** Of considerable interest

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Key Issues

- When cardiomyocyte necrosis occurs, a population of collagen-expressing myofibroblasts appear to produce scar tissue and preserve the structural integrity of injured myocardium.
- Myofibroblasts (myoFb) are phenotypically transformed, contractile fibroblast-like cells expressing α-smooth muscle actin microfilaments. They are integral to collagen fibrillogenesis with scar tissue formation at sites of repair irrespective of the etiologic origins of injury or tissue involved.
- MyoFb can persist long after healing is complete, where their ongoing turnover of collagen accounts for a progressive structural remodeling of an organ (a.k.a. fibrosis, sclerosis or cirrhosis). Such persistent metabolic activity is derived from their secretome consisting of requisite components in the *de novo* generation of angiotensin (Ang) II.
- Autocrine and paracrine signaling induced by tissue AngII is expressed via AT_1 receptor ligand binding to respectively promote: *i*) regulation of myoFb collagen synthesis via the fibrogenic cytokine TGF-β_1-Smad pathway; and *ii*) dedifferentiation and protein degradation of atrophic myocytes immobilized and ensnared by fibrillar collagen at sites of scarring.
- Several cardioprotective strategies in the prevention of fibrosis are considered. They include: inducing myoFb apoptosis through deactivation of antiapoptotic proteins; AT_1 receptor antagonist to interfere with auto-/paracrine myoFb signaling or to induce counterregulatory expression of ACE2; and attacking the TGF-β_1-Smad pathway by antibody or the use of antisense or triplex-forming oligonucleotides.

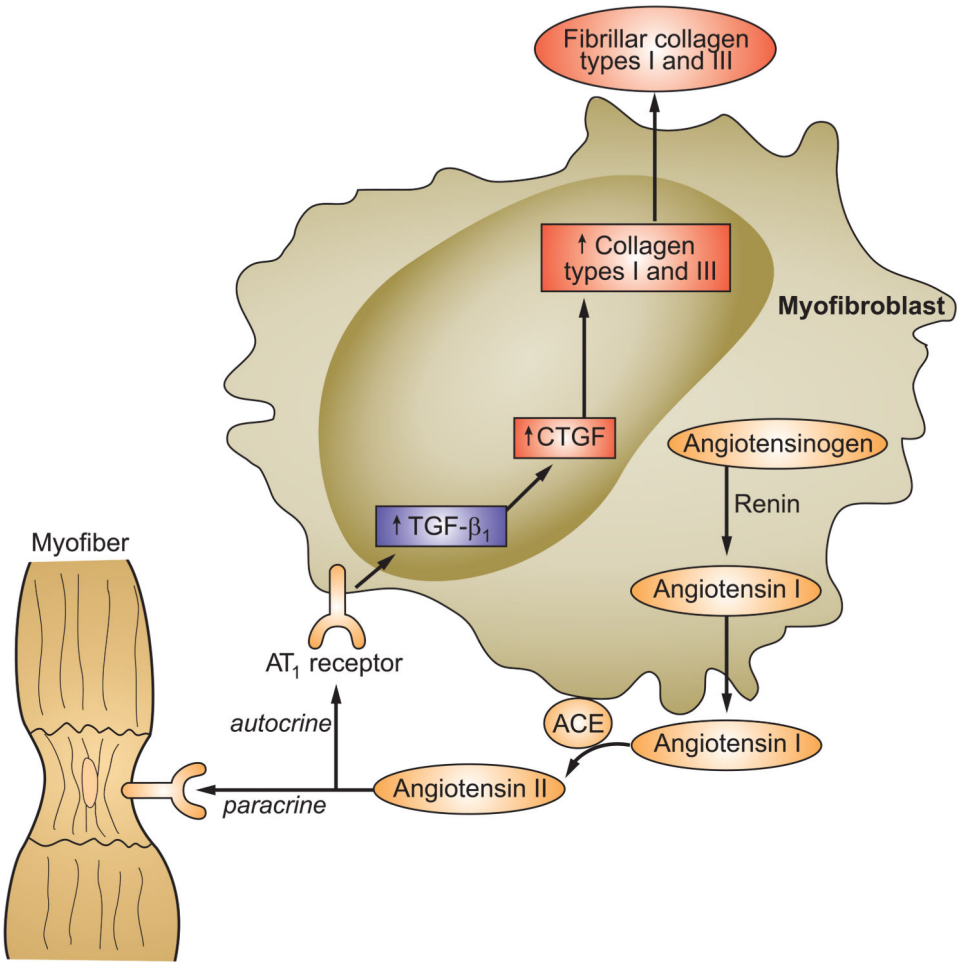

Figure 1.

The myofibroblast secretory phenotype found at the site of healing. This myofibroblast secretome includes the *de novo* generation of angiotensin II and subsequent induction of collagen synthesis by these cells. Included in the secretome is the expression of renin, ACE and AT_1 receptors. Autocrine actions of angiotensin II, mediated via AT_1 receptor binding, results in expression of fibrogenic TGF-β_1 and CTGF to stimulate myofibroblast production of fibronectin, which forms a provisional scaffold for type I and type III collagen fibrillogenesis. Abbreviations: ACE, angiotensin-converting enzyme; AT_1, angiotensin II type 1; CTGF, connective tissue growth factor; MMPs, matrix metalloproteinases; TGF-β_1, transforming growth factor β_1. Adapted from 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.

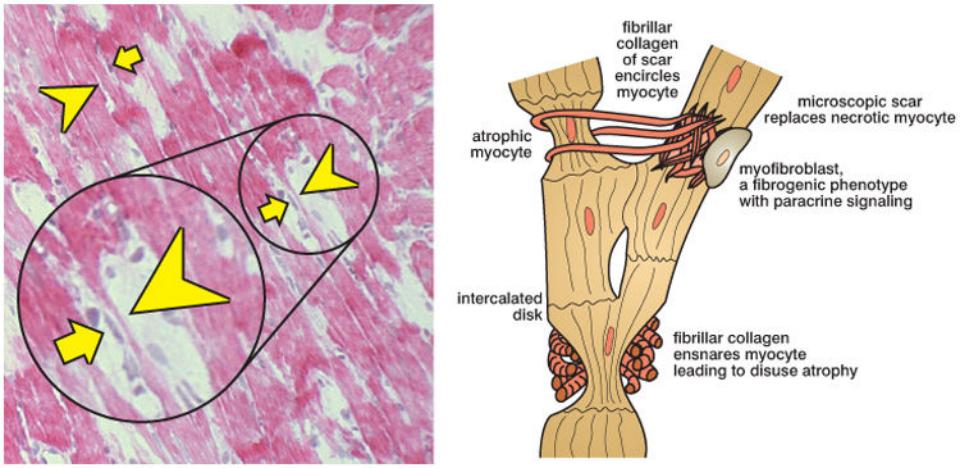

Figure 2.

Segmental myocyte atrophy along a myofiber composed of individual myocytes joined end-to-end to form an in-series syncytium. Left panel: longitudinal perspective of several myofiber syncytia as seen by light microscopy. Arrowheads indicate atrophied cells composing this syncytia while arrows identify myofibroblasts juxtaposed to these atrophied myocytes (hematoxylin and eosin, ×200). Right panel: a schematic representation of normal and atrophic myocytes of the myofiber syncytium and where collagen fibrils emanating from scar tissue encircle myocytes. Myocytes so ensnared are smaller and subject to disuse atrophy. An activated myofibroblast with a fibrogenic phenotype is seen in proximity to an atrophied myocyte. Reprinted with permission from Al Darazi F, Zhao W, Zhao T, Sun Y, Marion TN, Ahokas RA, Bhattacharya SK, Gerling IC, Weber KT. Small dedifferentiated cardiomyocytes bordering on microdomains of fibrosis: evidence for reverse remodeling with assisted recovery. *J Cardiovasc Pharmacol.* 2014;64:237-246.

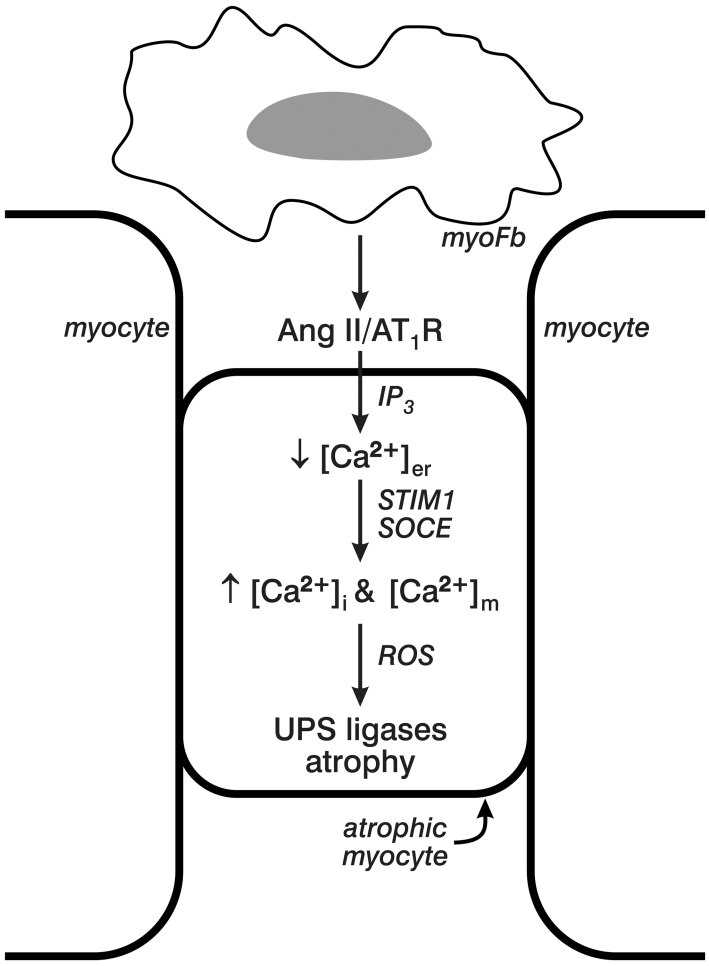

Figure 3.

Myofibroblast (myoFb) cross-talk with neighboring myocytes via paracrine signaling involving *de novo* angiotensin (Ang) II generation and AT_1 receptor binding. Ensuing IP_3 stimulation leads to the release from and subsequent fall in endoplasmic reticulum $[Ca^{2+}]_{er}$ whose Ca^{2+} sensor, STIM1, in turn, is then activated to promote store-operated Ca^{2+} channel entry (SOCE) to raise cytosolic $[Ca^{2+}]_i$ and mitochondrial $[Ca^{2+}]_m$. Ensuing oxidative stress and reactive oxygen species (ROS) activate proteolytic UPS ligases (MuRF1 and atrogin-1) leading to myocyte protein degradation with resultant atrophy.