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Review

# Cardiac fibrosis in heart failure

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The prevalence of heart failure is rising with poor prognosis and health costs. Cardiac remodeling is a cardinal process mediating the progression to heart failure, and cardiac fibroblasts play a critical role in the regulation of left ventricular remodeling mainly through the excessive extracellular matrix protein deposition and their differentiation to a myofibroblast phenotype with excessive proliferative and migratory properties. All this characteristics are known also as cardiac fibrosis. This process is crucial for the structural integrity of myocardial tissue. After myocardial infarction, are activated two important type of cardiac fibrosis: reparative and reactive cardiac fibrosis. Both of them are mediated by a huge number of inflammatory and hormonal mediators. In this review we will focus on the role of three important systems involved in cardiac fibrosis and described as the great contributors in heart failure: inflammatory, RAA and beta-adrenergic system. The deeper understanding of these great contributors is of interest in the development of new therapeutic strategies toward cardiac fibrosis and heart failure.

**Key words:** Cardiac fibrosis, Cardiac fibroblasts, Cytokines, RAA system, Beta-adrenergic receptors

## INTRODUCTION

The prevalence of Heart Failure (HF) is approximately 1-2% in adult population, rising to more than 10% of elderlies over 70 years of age [1 2]. Epidemiological studies show high incidence and prevalence of HF among elderlies. The prognosis of HF in this population is poor with rising cases of hospitalization, comorbidity and health costs [3-5]. Cardiac remodeling is a cardinal process mediating the progression to HF. Cardiac fibroblasts (CFs) play a critical role in the regulation of left ventricular remodeling following myocardial infarction [6 7], mainly through the excessive extracellular matrix (ECM) protein deposition and their differentiation to a myofibroblast phenotype [8] with excessive proliferatory and migratory properties. This process known as cardiac fibrosis is crucial for the structural integrity of the myocardial tissue. However in the advanced phases, cardiac fibrosis leads to ventricular stiffness, altered chamber compliance and contractile dysfunction, in addition cardiac fibrosis impairs the electrical coupling of cardiomycytes increasing the risk of developing potential arrythmias and fatal events [9-11].

Heart tissue is constituted by cardiomyocytes, CFs, endothelial and neuronal cells. Initially it was thought that CFs are the most prevalent cell type accounting for up to 70% of cells [12], recent studies report that the CFs population is not more than 20% of whole cell population in murine cardiac tissue [13]. Although the numerous CFs markers reported in the present literature, like vimentin, alpha-smooth muscle actin, collagen 1 alpha1, periostin, discoidin domain receptor-2, fibroblast specific protein-1, thymus cell antigen-1, the transcription factor 21, platelet derived growth factor receptor-alpha [14], a robust specific molecular biomarker of both resident and activated CFs is still lacking. The absence of a universal marker for the detection of CFs can be the explanation to the different interpretation of results on CFs cell number reported till now.

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CFs represent a structural scaffold for the myocardial tissue, responsible for the cardiac ECM homeostasis, distribution of mechanical forces and electric conduction in cardiac tissue. CFs seem to arise mainly from the embryonic epicardium, although other sources have been evaluated in different studies. Linkage tracking studies suggest that vascular endothelial cells contribute to the CFs population even if this contribution is not predominant [15 16].

Bone marrow-derived progenitor cells are considered an important source of CFs within the cardiac injury. Moreover it is believed that they account for up to 60% of CFs in the post- ischemic injury [17]. Under investigations are also other type of cells like fibrocytes and perivascular cells.

After myocardial infarction injury and cardiomyocytes apoptosis, there is an up-regulation of the inflammatory, paracrine-autocrine profibrotic factors in CFs leading to the increased proliferation and migration process. During this phase CFs differentiate to myofiboblasts, which are not present in the healthy cardiac tissue and represent characteristics of both CFs and smooth muscle cells. They are characterized by migratory, proliferation and contractile properties, thanks to alpha smooth muscle actin, a filament protein [18]. This process is defined as replacement or reparative fibrosis, which is compound by the inflammatory, proliferative and maturation response. Myocardial injury activates the secretion of IL-1, IL-6, TNF-alpha, and beta, CXC chemokines which recruit macrophages and neutrophils in the infarcted area which clear the dead cardiomyocites cells and trigger the migration of CFs within myocardial injury. CFs produce collagen type I and III, and enhance deposition of collagen in cardiac ECM. After the pro-inflammatory phase the involved inflammatory cells undergo apoptosis, and CFs cells begin the migratory and proliferative phase differentiating toward a myofibroblasts phenotype. Furthermore myofibroblasts produce large amounts of collagen contributing to the scar maturation. At the end of this phase myofibroblasts undergo apoptosis, however a portion of myofibroblasts survive and lead to cardiac remodelling. The activated myofibroblasts expand their collagen deposition, proliferative and migratory properties in the non-infarcted areas of myocardium. This process is called the reactive fibrosis. The exact explanation of this mechanism is not clarified yet. However the activation of myofibrablasts and their profibrotic factors within the infarcted area, might transvers to the remote areas and can lead to cardiac fibrosis activation [19]. The pathophysiological importance of this process is developing further studies in the understanding of profibrotic mechanisms and new potential therapies targeting cardiac fibrosis [20-22].

In this review we discuss the role of inflammatory, renin-angiotensin-aldosteron (RAA) axis, and beta-adrenergic system in the development of cardiac fibrosis in heart failure (Fig. 1).

## METHODOLOGY

For the purposes of this review we identified original research articles and other reviews published in English and available in PUBMED from 1997-2016.

## INFLAMMATORY PATHWAY

Inflammatory pathway is a crucial mediator of cardiac fibrosis. Initially its activity is important during the process of reparative fibrosis. The most studied inflammatory factors implicated in the mechanisms of cardiac fibrosis in the present literature are: tumor necrosis alpha, IL-1B, IL-6, IL-18, chemokines like CC, CXC subtype and INF-gamma [23]. Their profibrotic effect is complex and it is quite difficult to define it as a harmful or beneficial effect. After myocardial injury is observed an increase in cytokine expression by CFs like: IL-1B and IL-6. They induce the protein metabolism turnover in cardiac ECM and promote CFs migration and proliferation [24 25]. This inflammatory response seems to include the MAPK and NF-KB pathway. NF-KB is a transcriptional effector of inflammation that as a consequence of its nuclear translocation triggers cytokines, chemokines and adhesion molecules genes. NF-KB is composed of 5 different subunits, which induce different transcriptional response. For example p50 and p52 subunit repress genes transcription, whereas p65, c-Rel and Rel-B contributes to the activation of genes transcription. Importantly, inflammatory components exhibit a paracrine and autocrine response to myocardial tissue cells that express cytokine receptors and are localized in the remote areas. Furthermore, chemokines are responsible for the recruitment of leucocyte population within the infarcted area contributing this way to the basis of reparative cardiac fibrosis. IL-1 receptor type I deficient mice after surgical induced myocardial infarction and reperfusion techniques, present with better adverse left ventricular remodeling signs like: left ventricular chamber size and systolic function. However in this model, IL-1 receptor type I deficiency did not affect infarct size, suggesting that IL-1 modifies the synthesis of pro-fibrotic elements but does not influence cardiomyocyte injury [26]. Also, TNF$\alpha$ KO mice after ischemia/reperfusion surgery showed amelioration of LVEF, minor arrhythmia events and smaller infarct size. Similar results were demonstrated even after intra-coronary

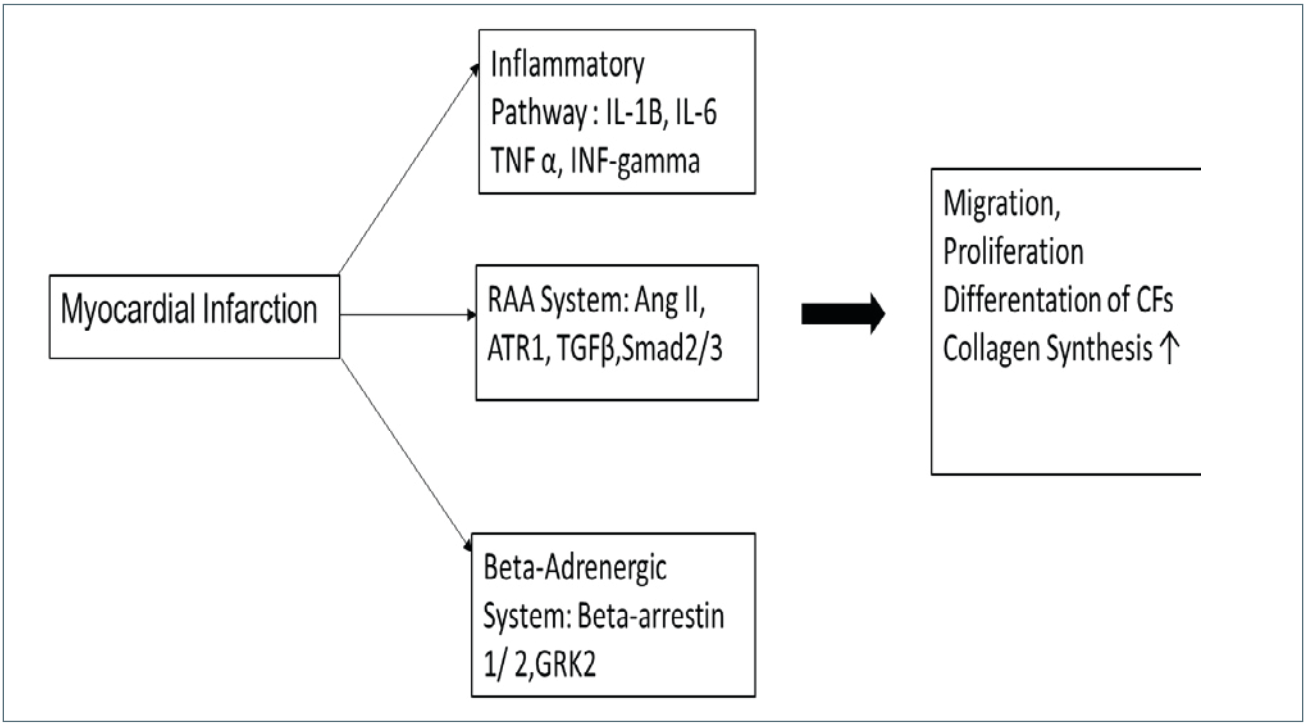


**Figure 1.** Schematic presentation of RAA system, beta-adrenergic system and inflammatory pathway influence on cardiac fibroblasts (CFs).

TNFα antibody use [27]. From the other side, mice deficient for TNF receptors report contrasting results in left ventricular remodeling following myocardial infarction. It seems that type 1 receptor induces the inflammatory response within the infarcted areas inducing left ventricular remodeling, whereas type-2 receptor ameliorates it. Also the circulating levels of IL-1B seem to induce the leukocyte proliferation within infarcted area and are also implicated in the proliferation of bone marrow stem cells after myocardial injury. These findings suggest a possible beneficial role of the inflammatory agents in myocardial tissue repair [28 29].

Interferon-gamma has an important role in the development of cardiac fibrosis. Mice, knock out for interferon-gamma, reduce the expression of alpha-smooth muscle actin indicating in this way the reduction of CFs differentiation to a myofibroblast phenotype. Importantly, null mice reduce cardiac fibrosis [30 31].

Recently in a mouse model of pressure overload, was observed that cytokine-like1 mRNA expression was highly elevated. Furthermore, in cytokine-like 1 KO mice, cardiac fibrosis together with other profibrotic proteins as TNF-α, TGF-β, and collagen 1 were significantly attenuated. In this study was also reported that this protein is able to induce the trans-differentiation of CFs in myofibrolast phenotype. From the data reported in this study it seems that cytokine-like 1 activity is not related to C-C chemokine receptor 2 and further research is needed to identify its receptor [32].

The role of inflammatory pathway in cardiac remodeling adds new perspectives in the therapeutic strategies in heart failure. However the modulation of inflammatory response in myocardial infarction, and the time point where potential modulators of inflammatory components can act by enhancing reparative processes without aggravating the healing process is still challenging.

## RENIN-ANGIOTENSIN-ALDOSTERON SYSTEM (RAA)

Inhibitors of RAA signaling, angiotensin receptor blockers and angiotensin converting enzyme inhibitors have a well-established role in the treatment of HF and in the attenuation of cardiac remodeling. This class of drugs have demonstrated significant efficacy in reducing cardiac fibrosis in either animal or human models of heart failure [33 34].

After myocardial injury, angiotensin II levels are dramatically increased and the angiotensin II type I receptor (AT1R) expressed in CFs, mediates their proliferation, migration and ECM protein deposition [32]. Form one side it is the release of angiotensin II from stressed cardiomyocytes that activates the AT1R ù, but also as it has

**Table I.** The main factors implicated in the development of cardiac fibrosis. The table summarizes the main factors implicated in the development of cardiac fibrosis, their main mechanism, experimental models where antagonist of these factors were applied and their main results in cardiac remodeling.

| Factor | Mechanism | Application | Cardiac remodeling effects | References |
|---|---|---|---|---|
| IL-1 | MAPK and NFKB activation | IL1-R $I^{-/-}$ mouse, Ischemia/reperfusion model | LVEDD ↓, LVEDV ↓, FS ↓, collagen expression ↓ Infarct size: no changes | Bujak M et al. [25] |
| TNFα | Proinflammatory cytokine cascade: IL1, IL 6, NFKB activation | TNFα KO mouse, TNFα antibody Ischemia/reperfusion model | Peak LVSP ↑, dP/Dt max ↑, infarct size: ↓, arrhythmia ↓ | Maekawa N et al. [26] |
| INF gamma | MAPK kinase, PKC, jak/ stat,mda-9 | INF gamma KO mouse Ischemic model | Cardiac Fibrosis ↓, α-SMA ↓ | Levick SP et al. [30], Marko L et al. [32], Han YL et al. [29] |
| TGFβ | Activation of ATR1, ERK1/2, Smad 2/3,4. | Oral TGFβ1 receptor antagonist, Rat ischemic model | LVEDD ↓, LVESD ↓, FS ↓, LVEF ↑ | Tan SM et al. [40] |
| Angiotensin | RAAS, TGFβ activation | ACE-inhibitors, Sartans Human and animal models of HFrEF or HFpEF | Cardiac Fibrosis ↓ IVST ↓, Dec time ↑ LVEF ↑, Collagen ↓ | Diez J et al. [33], Pfieffer JM et al. [34], Villareal FJ et al. [35], Von Lueder TJ et al. [42] |
| Beta-arrestin 1, 2 | Beta-adrenergic System, G-protein | In vitro Beta-arrestin knock down, CF s isolated from human HF samples | Proliferation ↓, Migration ↓, αSMA ↓, Collagen ↓ | Li J et al. [54] |
| GRK2 | Beta-adrenergic System, G-protein | Barkct inhibitory peptide, rat model of HF Fibroblasts specific GRK2 KO mouse HF model | Cardiac Fibrosis ↓ Collagen ↓ Infarct size ↓ LVEF ↑ TNFα ↓ | Raake et al. [56] Rengo G. et al. [57] Woodall Mc et al. [58] |

LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter; FS: fractional shortening; IVST: intra ventricular septal thickness; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction.

been observed the mechanical stress due to hemodynamic impairment itself directly stimulates the AT1R in CFs [35 36].

Indeed, candesartan demonstrated to have antifibrotic role. It is reasonable to think that the reduction of pressure overload reduces the mechanical stretch and as a consequence the profibrotic RAA axis. However, the exact mechanism of this class of drugs on CFs behavior has not been clarified and it is a field of interest [37 38].

From the other hand, it has been reported that losartan reduces cardiac fibrosis blocking the AT1RTGF-beta induced phosphorylation of ERK1/2. As a matter of fact, the activation of AT1R induces the expression of TGF-beta1, which has been reported as the most important profibrotic mediator.

TGF-beta1 binds to its activin receptor-like kinase (ALK 5), induces the phosphorylation of Smad2/3, which binds to Smad 4 and translocate to nucleus. As result, many profibrotic genes are activated. In addition the activation of TGF-beta II receptor, the activation of TAK1 kinase and p38 protein showed to have an important role in collagen synthesis, cardiac hypertrophy and cardiac fibrosis [39-41].

Recently a new drug class, angiotensin receptor neprilysin inhibitors have been developed. In an experimental model of myocardial infarction was observed that neprylsin inhibitor component of LCZ696 did not reduce cardiac fibrosis but together with valsartan showed a synergic effect in the reduction of perivascular and interstitial cardiac fibrosis [42].

Interestingly a recent in vivo and in vitro study revealed that metformin potentiated the inhibitory effects of spironolactone in cardiac fibrosis without effecting systolic blood pressure, restored AMPK activation, and inhibited the migration and proliferation of CFs [43].

Another component of RAA pro-fibrotic signalling seems to be also prorenin receptor. In this case the activation of this receptor induces the activation of angiotensin II,

intracellular second messengers and finally the activation of pro-fibrotic genes. Then there is an increased expression of collagen fibronectin-1, plasminogen and I activator inhibitor-1 [44]. However further studies are necessary to establish the role of RAA axis not only on the overall cardiac fibrosis but also on CFs activation and their phenotypic differentiation. Only in this way it would be possible to explore a more direct therapeutic targeting on CFs activation.

## BETA-ADRENERGIC SIGNALING

The alteration of beta-adrenergic signaling in HF is a central problem associated with important clinical and prognostic implications [45]. The chronic overstimulation of BARs by cathecolamines induces their downregulation/internalization. This mechanism is well described, and of great importance is the role of G-protein coupled receptor kinese (GRK2). The agonist-bound state of BARs induces the dissociation of G-protein into beta-gamma and alpha subunit, which triggers the activation of c-AMP and PKA. Consequently GRK2 changes its localization from intracellular to transmembranic, phosphorylating the BARs, which become the target for binding of B-Arrestin proteins that prevent BARs further coupling to G-protein. The final result is the internalization of BARs, their decreased density and desensitization [46-48]. Anyway in cardiomycytes it has been reported that is present in particular B1AR down-regulation and the HF impaired adrenergic responsiveness is related to GRK2 and Gi up regulation [49-52]. Differently, it has been reported that the predominant B-AR subtype expressed in CFs is the B2AR. Indeed CFs isolated from human atrial tissue expressed only B2AR subtype. It has been showed that a chronic stimulation with isoproterenol in human CFs culture induced their proliferation, migration and collagen synthesis. From the other side the acute stimulation of these receptors increased the levels of c-AMP and reported modulation of either proliferation or migration in *in vitro* CFs culture. In these experiments was noticed a significant reduction of proliferation, migration and collagen synthesis [53-55].

Beta-arrestins are important signaling molecules involved in BARs desensitization. Beta-arrestin 1 and 2 are significantly up-regulated in CFs isolated from human HF cardiac samples and their knock down led to inhibition of collagen synthesis [56].

Furthermore, in human failing CFs is reported an up-regulation of GRK2 and un-coupling of BARs signaling. The knock down of GRK2 restored the B-ARs stimulated inhibition of collagen synthesis and reduced the proliferative properties of failing CFs [15]. Indeed the inhibition of GRK2 by using BARKct peptide in animal models of HF has demonstrated to reduce cardiac fibrosis and to attenuate left ventricular remodeling [57-60]. Recently, fibroblasts-specific GRK2 knock out mice after myocardial ischemia/reperfusion injury showed decreased infarct size, increased LVEF, and decreased infiltration of neutrophils to injured area together with reduction of TNF-$\alpha$ expression [61].

## OTHER PROMISING MOLECULAR SIGNALING: DAMPS

Damaged myocardial cells and ECM release substances with danger signal properties recently defined as Danger Associated Molecular Patterns (DAMPs). DAMPs include heat shock proteins, S100 protein, HMGB1 protein, hyalunorate fragments, fibronectin, Tenascin C, mitochondrial DNA, IL-1 alfa and advanced glycation end product. Despite the main effector of these proteins are white blood cells, they are able to modulate the function of cardiomyocytes, endothelial cells and CFs. DAMPs activate different receptors like TLR, NLR, IL-1R and RAGE. These receptors even if not well defined are expressed in CFs and it seems that their activation have a pro-fibrotic effect. Recent emerging studies report that CFs are sensitive to ECM protein changes and are a significant sensor of DAMPs contributing in this way to the pathogenesis of cardiac fibrosis. From one side DAMPs-CFs interaction would trigger the proinflammatory pathway via the NFkB, p38 and JNK activation, resulting in ECM degradation, myofibroblasts differentiation. From the other side ERK, AKT, PKC-e pathway would be induced by CFs proliferation, migration and ECM synthesis. However, little is known about the direct CFs-DAMPs interaction and this could be of great interest for further studies [62].

## CONCLUSIONS

Cardiac fibrosis is a cardinal process mediating the progression to heart failure. A better understanding of CFs behavior after a myocardial injury, the physiological and pathophysiological pathways of natural reparative processes, the implication of inflammatory response, RAA and Beta-Adrenergic system would be of great interest in the therapeutic interventional strategies targeting cardiac fibrosis as the heart of left ventricular remodeling.

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