Article:
Turner, NA (2016) Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). Journal of Molecular and Cellular Cardiology, 94. pp. 189-200. ISSN 0022-2828

https://doi.org/10.1016/j.yjmcc.2015.11.002

Inflammatory and Fibrotic Responses of Cardiac Fibroblasts to Myocardial Damage Associated Molecular Patterns (DAMPs)

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Keywords: cardiac fibroblasts; damage-associated molecular patterns; inflammation; fibrosis; innate immune system;

Word count: 6168

Disclosures: None

ABSTRACT

Cardiac fibroblasts (CF) are well-established as key regulators of extracellular matrix (ECM) turnover in the context of myocardial remodelling and fibrosis. Recently, this cell type has also been shown to act as a sensor of myocardial damage by detecting and responding to damage-associated molecular patterns (DAMPs) upregulated with cardiac injury. CF express a range of innate immunity pattern recognition receptors (TLRs, NLRs, IL-1R1, RAGE) that are stimulated by a host of different DAMPs that are evident in the injured or remodelling myocardium. These include intracellular molecules released by necrotic cells (heat shock proteins, high mobility group box 1 protein, S100 proteins), proinflammatory cytokines (interleukin-1α), specific ECM molecules up-regulated in response to tissue injury (fibronectin-EDA, tenascin-C) or molecules modified by a pathological environment (advanced glycation end product-modified proteins observed with diabetes). DAMP receptor activation on fibroblasts is coupled to altered cellular function including changes in proliferation, migration, myofibroblast transdifferentiation, ECM turnover and production of fibrotic and inflammatory paracrine factors, which directly impact on the heart's ability to respond to injury. This review gives an overview of the important role played by CF in responding to myocardial DAMPs and how the DAMP/CF axis could be exploited experimentally and therapeutically.

1. Introduction to the Cardiac Fibroblast

Cardiac fibroblasts (CF) have wide-reaching functions that are fundamental to the development, physiology and pathophysiology of the heart (extensively reviewed in [1-6]). In response to specific biochemical and biophysical stimuli, fibroblasts can undergo proliferation, migration and transdifferentiation into myofibroblasts; an 'activated' secretory phenotype that is able to contract the extracellular matrix (ECM) to aid healing after injury. CF are the major producers of ECM proteins in the heart, particularly collagens I and III and fibronectin, thereby contributing to both reparative/replacement fibrosis (e.g. in the infarct region after myocardial infarction [MI]) and diffuse reactive fibrosis (e.g. in the non-infarcted myocardium after MI, or with hypertension, pressure/volume overload, diabetes and ageing). In addition, CF secrete a variety of ECM-degrading proteases (e.g. matrix metalloproteinases [MMPs]), as well as tissue inhibitors of metalloproteinases (TIMPs) [7], thus being able to fine-tune ECM turnover at multiple levels. An important form of communication between CF and other cardiac cell types is a paracrine system involving local production of soluble mediators (e.g. growth factors, cytokines) that can influence multiple aspects of function in adjacent cells [8,9]. The composition of the local biochemical micro-environment surrounding cardiac cell types is therefore a major determinant of acute and chronic cellular responses and hence cardiac function.

MI confers a rapid and catastrophic change on the structural and cellular composition of the heart. The healing response post-MI can be divided into three main phases; the inflammatory phase, the granulation phase and the maturation phase, all of which involve CF [10]. The inflammatory phase occurs rapidly after cardiac injury and is characterised by local up-regulation of proinflammatory cytokines (e.g. interleukin [IL]-1, IL-6, tumour necrosis factor α [TNFα]) by cardiac and inflammatory cells together with widespread neutrophil infiltration. The granulation phase takes place approximately 3 days post-MI and involves infiltration of the infarct area with macrophages and myofibroblasts, MMP-mediated ECM degradation and stimulation of angiogenesis. Once the infarct area has been cleared of necrotic cells and tissue, the maturation (healing) phase ensues, and this is characterised by upregulation of profibrotic and anti-inflammatory factors (e.g. transforming growth factor β [TGFβ]), IL-10), myofibroblast proliferation, collagen synthesis and scar contraction by myofibroblasts.

CF play important roles in all three phases of post-MI remodelling. Fibroblasts are more resistant to oxygen deprivation than cardiomyocytes [11,12] and can therefore act as important sensors of myocardial damage early after MI, resulting both in direct modification of CF function (e.g. proliferation, migration, myofibroblast transdifferentiation, ECM turnover) and indirect effects mediated through paracrine signalling, including production of

chemokines and cytokines that facilitate recruitment and activation of inflammatory cells in the damaged area of the heart [1-6,9].

The remarkable versatility of CF in normal physiology, together with their emerging role in the acute and chronic responses of the heart to stress, injury and fibrosis make them an attractive target for therapeutic manipulation.

2. Cardiac Fibroblasts as Sensors of Cell and Tissue Damage

2.1. DAMPs and the heart

The innate immune system plays a critical role in the initiation and progression of cardiac repair following MI [13-16]. The post-MI inflammatory response is a well-orchestrated process that is triggered by the injured myocardium and mediated by inflammatory cells including neutrophils and monocytes/macrophages. [17,18]. Molecular signals from the damaged myocardium modulate inflammatory cell function by activating cell surface or intracellular receptors of the innate immune system. These danger signals are collectively known as ‘damage-associated molecular patterns’ (DAMPs) or ‘alarmins’ and comprise a diverse range of molecules [13,14,19,20]. Known DAMPs that are relevant to the heart and wider cardiovascular system include intracellular molecules that are not normally accessible to the immune system (e.g. heat shock proteins [HSPs], high mobility group box 1 protein [HMGB1], histones, S100 proteins, RNA, mitochondrial DNA), cytokines released actively or passively from injured cells (e.g. IL-1α), ECM degradation products (e.g. hyaluronate fragments), specific ECM molecules that are up-regulated in response to tissue injury (e.g. EDA splice variant of fibronectin [FN-EDA], tenascin-C [TN-C]) or molecules modified by a pathological environment (e.g. advanced glycation end product [AGE]-modified proteins observed with diabetes) [13,14,19,20]; see **Figure 1**. Elevated levels of DAMPs are associated with several inflammatory and autoimmune diseases (e.g. rheumatoid arthritis, multiple sclerosis, type 1 diabetes), as well as with atherosclerosis, obesity, type 2 diabetes and cancer [19,21].

Leukocytes are the main cellular effectors of the innate immune response; however the nature of DAMPs and the widespread expression of DAMP receptors suggests that inflammatory cells are not necessarily essential for DAMP responses. Indeed, it is becoming increasingly apparent that DAMPs can also act to modulate the function of non-myeloid resident cardiac cells (e.g. cardiomyocytes, fibroblasts and endothelial cells) to directly regulate aspects of cardiac inflammation and remodelling. The prevalence of fibroblasts, their relative tolerance of ischaemic conditions, and their widespread localisation throughout the heart enables them to respond to myocyte injury as well as to ECM degradation and/or modification. Until recently, research on DAMP signalling in cardiac ischaemia had focused almost exclusively on leukocytes and cardiomyocytes, with little known about the direct

responses of fibroblasts to DAMPs [13,22].

2.2. DAMP receptors and cardiac remodelling

Individual DAMPs elicit their effects through activation of specific pattern recognition receptors (**Figure 1**), including Toll-like receptor (TLR) and NOD-like receptor (NLR) family members, the IL-1 receptor (IL-1R1) and the receptor for advanced glycation end products (RAGE); all of which are expressed at differing levels in various cardiac cell types. Activation of these receptors is often coupled to inflammatory responses driven by NFκB signalling [13,16], although proliferative, migratory and fibrotic outcomes have also been observed in response to different ligands [23-26].

TLRs comprise a family of at least 10 pattern-recognition receptors located on the cell surface (TLR1, 2, 4, 5, 6, 10) or intracellularly on lysosomal and endosomal membranes (TLR3, 7, 8, 9) that recognise a wide variety of microbial ligands and danger signals in the form of peptides, proteins and nucleotide fragments [14,27,28]. TLRs can form homodimeric and heterodimeric signalling complexes amongst themselves and with RAGE, and also interact with co-receptors (e.g. CD14, MD2); a complexity that contributes to the diversity of cellular responses to different DAMPs [29]. The IL-1R1 receptor is specifically activated by the two IL-1 isoforms, IL-1α and IL-1β. Ligand binding to IL-1R1 stimulates recruitment of the IL-1 receptor accessory protein, IL-1RAcP, which is necessary for IL-1 signalling. There is significant commonality between TLR and IL-1R1 signalling [30]. Upon activation, both TLR and IL-1R1 receptor complexes recruit specific adaptor proteins (e.g. MyD88) that facilitate activation of IL-1 receptor activated kinases (IRAKs), resulting in stimulation of stress-induced signalling pathways (e.g. NFκB, JNK, p38 MAPK) and transcription of pro-inflammatory cytokines (e.g. IL-1β, IL-6, IL-8, monocyte chemoattractant protein-1). An alternative MyD88-independent (TRIF-dependent) pathway couples TLR3 and 4 activation to interferon regulatory factor-3 and expression of interferon-inducible genes (e.g. interferon-β, CCL5, CXCL10).

NLRs are a large family of cytoplasmic receptors that recognise both microbial ligands and DAMPs and are coupled to inflammasome activation and inflammatory signalling pathways [31]. NOD1 and NOD2 are members of the NLRC subfamily that induce inflammatory signalling via kinase and caspase activation. Another major subfamily of NLRs comprises the NLRPs which are important activators of the inflammasome, a multi-protein complex comprising a NLRP, the ASC adaptor protein and caspase-1 that is important for proteolytic activation of specific proteins including IL-1β and IL-18. Endogenous NLRP3 ligands include ATP, S100A8/9, mitochondrial DNA, cholesterol crystals, serum amyloid A and hyaluronan [32].

RAGE is a transmembrane receptor that recognises not only AGE-modified proteins and lipids, but also a range of other non-AGE ligands including HMGB1 and S100 proteins. RAGE can form heterodimers with the β2-integrin Mac-1 and with some TLRs and signals via multiple pathways including NFκB, MAPKs and JAK/STAT [33].

A full description of the role of the innate immune system in cardiac remodelling lies beyond the scope of the present article, but the reader is directed towards several excellent reviews that explore this in more detail [13-16]. Knockout mouse studies have proven useful for identifying specific roles for DAMP receptors in modulating cardiac remodelling after injury or stress (summarised in **Table 1**). Global knockout or deficiency of TLR2 [34-39], TLR3 [40,41] and TLR4 [42-56] generally confers improvement of cardiac function and less adverse remodelling after injury, although detrimental effects have also been reported in TLR2 knockout mice [57]. In contrast to the perceived detrimental roles of TLRs 2, 3 and 4 in post-MI remodelling, a recent study suggested that TLR5 may play a beneficial role in protecting the heart from ischaemia/reperfusion injury [58]. However, it is not clear what the endogenous ligand for TLR5 is in the damaged heart as its only known ligand is bacterial flagellin. Studies on IL-1R1 knockout mice have consistently shown beneficial effects on post-MI remodelling [59-61]. Only a few studies have investigated the role of NLRs in cardiac remodelling and different effects were observed depending on the cardiac injury model used. In a permanent left anterior descending coronary artery ligation model of MI, NOD2 knockout mice had improved cardiac function and reduced inflammation [62]. However in an aortic banding model of pressure overload, NOD2 deletion exacerbated adverse myocardial remodelling [63]. Although results from NOD1 knockout mice have not been reported for the heart, NOD1 activation induced cardiac dysfunction with increased cardiac fibrosis and cardiomyocyte apoptosis [26], suggesting a detrimental role for this receptor in this context. RAGE knockout has beneficial effects following myocardial ischaemia/reperfusion injury [64,65], in keeping with other studies focused on RAGE activity in cardiac remodelling [66]. It is of course worth noting that knockout mouse studies have their limitations, however they can be very informative for determining the role of proteins particularly where other tools are lacking. It should also be noted that global knockout mice have genes ablated in all cell types, so ascribing distinct roles for DAMP signalling specifically to CF in these studies is difficult.

2.3. DAMP receptor expression in cardiac fibroblasts

The exact ligand/receptor systems that couple DAMPs to changes in fibroblast function in the injured heart are only just beginning to emerge, and recent evidence suggests the DAMPs/fibroblast axis is important in both inflammatory and fibrotic responses of the heart to damage [25,67,68]. Identification and characterisation of these systems is important for

designing novel therapies for reducing post-MI damage [14,69]. The remainder of this review will focus on our current knowledge about the interaction between DAMPs and CF.

CF express multiple DAMP receptors including TLRs, NLRs, IL-R1 and RAGE. All 10 TLRs are expressed at the mRNA level in human heart, with TLR4 being most abundant, followed by TLR2 and 3 [70]. At the cellular level, isolated rat and mouse cardiomyocytes express TLR2, 3, 4 and 6 [71,72], but TLR expression in fibroblasts is less well characterised. However, there is evidence for functional expression of TLR2 [57,73], TLR3 [73], TLR4 [68,73,74] and TLR9 [73,75] in CF. Of the known members of the NLR family, CF express NOD1 and NOD2 [26,62], as well as the inflammasome-associated NLRP3 [73,76] RAGE is expressed in several cell types within the heart [64] and in cultured human and rodent CF [23,77-79].

The range of different DAMP receptors expressed by CF, coupled with the wide variety of DAMP ligands that activate these receptors (**Figure 1 and 2**), suggests that fibroblasts are a particularly important cell type within the cardiac innate immune system.

2.4. Effects of DAMPs on cardiac fibroblast function

Several recent studies [25,67,68] have added significant credence to the hypothesis that CF can act as sensors of cell and tissue damage in the heart, triggering both inflammatory and fibrotic responses as a consequence of passively released DAMPs from damaged cells. Moreover, CF are also able to react to changes in ECM composition, for example in response to AGE-modified collagen [23] or FN-EDA [74]. **Table 2** provides a summary of specific studies relating to soluble DAMPs and CF activation. **Figure 2** summarises the known molecular mechanisms by which extracellular DAMPs can modulate CF function.

2.4.1. S100 proteins

The S100 proteins are a large family of dimeric calcium-binding proteins that are important for calcium buffering and intracellular signalling, but can also act as extracellular signalling molecules when released from cells actively or passively [80]. Indeed, several S100 proteins act as important DAMPs that are released from leukocytes and act on target cells primarily via TLR4 and RAGE activation [81].

S100A1 is the most abundantly expressed S100 isoform in cardiomyocytes and can be released from damaged cardiomyocytes after ischaemia/reperfusion injury, resulting in increased serum levels [68,82]. It was shown recently that cardiomyocyte-derived S100A1 is taken up by surrounding CF through endocytosis and activates MAPK/SAPK and NFκB signalling pathways in CF in a TLR4/MyD88-dependent (RAGE-independent) manner [68]. S100A1 stimulated an anti-fibrotic phenotype in CF (increased expression of MMP-9 and reduced expression of collagen I, α-smooth muscle actin and connective tissue growth

factor) and conferred a complex immunomodulatory phenotype involving changes in both pro- and anti-inflammatory factors [68]. In a mouse MI model, anti-S100A1 neutralising antibody increased infarct size, increased fibrotic markers and increased cardiac dysfunction [68] suggesting that S100A1 plays a beneficial role in the heart's response to injury.

S100 proteins released from other cell types present in the injured heart may also elicit some of their effects through CF stimulation. For example the S100A8/A9 heterodimer is abundantly expressed in neutrophils and monocytes and can induce neutrophil chemotaxis through TLR4 and RAGE activation, thereby amplifying the inflammatory response [83]. In a mouse model of cardiac damage and inflammation in response to angiotensin II infusion, S100A8/A9 was shown to be actively released from $CD11b^{+}Gr1^{+}$ neutrophils [79]. S100A8/A9 modulated expression of >200 genes in CF, including many related to chemokine and cytokine activity and chemokine receptor binding [79]. As well as inducing neutrophil migration, S100A8/A9 was also chemotactic for CF, an effect mediated via RAGE [79]. Administration of an anti-S100A9 neutralising antibody to Ang II-infused mice reduced cardiac myofibroblast accumulation and fibrosis without affecting hypertension, and suppressed cardiac inflammation, indicating that S100A9 contributes to myocardial injury and inflammation at least partly through effects on fibroblasts [79]. In another recent study, S100A8 and S100A9 were identified in supernatants released passively from necrotic cardiac tissue and were shown to stimulate fibroblast proliferation [25].

Together these studies reveal that S100 proteins, released either directly from injured cardiomyocytes or from infiltrating inflammatory cells, can induce CF to adopt an inflammatory anti-fibrotic phenotype as a result of TLR4 or RAGE activation.

2.4.2. Interleukin-1α

IL-1 comprises two distinct gene products (IL-1α and IL-1β) with seemingly indistinguishable biological activities that play a significant role in the pathogenesis of inflammatory heart disease [84-86]. IL-1α is an intracellular cytokine that is released when cells undergo necrosis and was originally shown to be a key trigger for sterile inflammation in the liver [87]. In contrast to IL-1α, IL-1β requires proteolytic processing by the inflammasome for activation and is secreted from cells in response to specific stimuli. Increased myocardial IL-1α/β levels are observed with several cardiovascular pathologies including MI, cardiomyopathy, hypertension and myocarditis [84-86]. In studies using knockout mice, IL-1R1 deletion led to smaller infarcts, less inflammation and reduced cardiac dysfunction following MI [59-61].

We have previously demonstrated that human CF are very responsive to IL-1α [88], which is expressed by cardiomyocytes [89] and CF [90] and can act as a DAMP when released from damaged or dying cells [67,87]. In response to IL-1α stimulation, human CF

secrete a host of proinflammatory cytokines (e.g. IL-1β, IL-6, TNF-α) and neutrophil-attracting chemokines (e.g. CXCL-1, 2, 5 and 8), and express specific neutrophil-binding adhesion molecules including ICAM-1 and E-selectin [88,90-93], as well as the matricellular protein TN-C [94]. Thus, CF may contribute to the inflammatory milieu that occurs in the myocardium early after MI [10,95]. In addition to this inflammatory response, we [7,88,93,94,96-98] and others [61,99,100] have demonstrated that CF alter the balance of cardiac ECM turnover in favour of degradation in response to IL-1; for example by increasing secretion of MMPs, decreasing collagen synthesis and decreasing expression of profibrotic factors (e.g. connective tissue growth factor).

It was reported recently that IL-1α was released from necrotic cardiac tissue [25] and necrotic cardiomyocytes [67] and could act as a danger signal to modulate CF function. Conditioned medium from necrotic neonatal mouse ventricular myocytes induced inflammatory signalling pathways (ERK, p38, JNK, NFκB) and IL-6 and monocyte chemoattractant protein-1 expression and secretion in mouse ventricular fibroblasts [67]. The inflammatory effect on CF was mediated in a Myd88-dependent but TLR-independent manner, suggesting a possible role for the IL-1 family of cytokines acting via IL-1R1/Myd88 [67]. Necrotic cardiomyocytes were found to release IL-1α (but not IL-1β) and the effect of conditioned medium was eliminated upon addition of an IL-1 receptor antagonist or an IL-1α neutralising antibody. Finally, it was shown that after experimental MI, plasma inflammatory markers and neutrophil infiltration were markedly reduced in IL-1$\alpha^{-/-}$ knockout mice compared with wild type mice, suggesting that IL-1α contributed to the post-MI inflammatory response [67]. IL-1α knockout did not influence infarct size or plasma markers of myocardial damage [67].

Thus, IL-1α is emerging as a potentially important therapeutic target for controlling both inflammatory and fibrotic responses after MI through effects on CF [88]. A similar role for IL-1α in atherosclerosis has also been proposed [101], making IL-1α-selective inhibitory strategies an attractive proposition for treating a range of cardiovascular diseases. A number of previous studies have focused exclusively on inhibiting IL-1β or the IL-1R1 receptor and clinical trials are currently underway to assess blanket IL-1 inhibition as a therapeutic strategy for inflammatory heart disease [102]. However, it remains to be established whether IL-1α-selective targeting can offer increased specificity and improved outcome after MI.

2.4.3. High mobility group box 1 protein

HMGB1 is a highly conserved nuclear DNA binding protein that acts as a transcriptional regulator as well as playing roles in maintaining genome stability and DNA repair. In addition, HMGB1 can act as a potent DAMP when released passively from necrotic

nucleated cells, or actively by stressed monocytes/macrophages. HMGB1 stimulates several DAMP receptors including TLR2, TLR4, TLR9 and RAGE [103]. Several studies have investigated the role of HMGB1 in cardiac remodelling after myocardial injury, and conclude that HMGB1 plays either favourable or unfavourable roles depending on the nature of the initiating injury [13]. In models of MI with no reperfusion (less inflammation), HMGB1 appears to play a beneficial role, whereas in ischaemia/reperfusion models (more inflammation), HMGB1 is generally detrimental [13]. This may relate to valuable profibrotic healing effects in non-reperfusion models, compared with amplification of the inflammatory response in reperfusion models; scenarios in which fibroblasts may be important effectors.

There are several studies that have investigated the effects of HMGB1 on in vitro CF function (summarised in **Table 2**). The consensus is that HMGB1 stimulates CF to adopt a migratory, proliferative, pro-fibrotic phenotype via activation of TLR2 and TLR4 receptors and ERK/AKT pathways [25,77,104-107]. However, opposing results have also been reported with HMGB1 inducing anti-fibrotic effects through inhibition of TGFβ/Smad pathways [108]. Some of the beneficial effects of HMGB1 injection on cardiac function after MI appear to be due to increased differentiation of cardiac progenitor cells to cardiomyocytes; a paracrine effect driven by HMGB1 acting on CF to upregulate growth factors, cytokines and chemokines [106].

In a recent study, supernatants from freeze/thawed myocardial tissue (a model for myocardial cell necrosis/lysis) were found to contain HMGB1, as well as several other DAMPs including galectin-3, S100A8/A9 and IL-1α [25]. This mixture of myocardial DAMPs induced functional changes in CF, including increased cell proliferation, α-smooth muscle actin expression (a marker of myofibroblast transdifferentiation) and collagen I and III expression. Similar responses were observed in the NIH/3T3 fibroblast cell line, in which subsequent mechanistic studies were explored. These identified HMGB1 (and galectin-3) as being the main components of the DAMPs extract that could directly induce fibroblast proliferation and collagen expression [25]. These profibrotic effects were mediated via TLR4 and RAGE receptors acting through ERK and Akt pathways. Importantly, injection of myocardial DAMPs into the LV apex of mouse hearts produced an inflammatory and fibrotic response that was not evident in $TLR4^{-/-}$ knockout mice, implicating TLR4 as being key for the response to myocardial DAMPs in vivo. Finally, it was shown that these effects of myocardial DAMPs, and the role of TLR4, could be mimicked by ventricular injection of HMGB1 in mice [25].

HMGB1 is one of the most extensively studied DAMPs in many systems and has been shown to induce a plethora of downstream effects through its ability to activate a number of innate immune receptors. In CF, HMGB1 is coupled mostly to profibrotic effects

(e.g. migration, proliferation, collagen synthesis) and appears to play both beneficial and detrimental roles in cardiac repair and remodelling. Hence, if HMGB1 is to be considered as a realistic therapeutic target then great care will be needed to ensure its targeting is appropriate to both the type of pathology (ischaemic vs. reperfusion vs. non-ischaemic) and the stage of pathology being studied.

2.4.4. Heat shock proteins

HSPs comprise a highly conserved family of proteins that have diverse actions involved in protecting cells against various types of cellular stress. A number of HSPs are also known to act as DAMPs when released from necrotic cells including HSP60, HSP70 and HSP27. The inflammatory response to myocardial ischaemia/reperfusion is mediated in part via cellular release of cardiac HSP60 acting via TLR4 to induce cardiomyocyte apoptosis and cytokine release [51,109], although it is not clear whether CF play a role in this. HSP70 is also released into the circulation early after MI, and extracellular HSP70 stimulated inflammatory cytokine release from monocytes via TLR4 [110]. Extracellular HSP70 activated rat CF proliferation and proinflammatory cytokine expression via TLR2 activation and was important for the response to pressure overload in this model [57]. Finally, human myocardium has been shown to exude HSP27 after global ischaemia which can trigger a proinflammatory effect through TLR2 and TLR4 [111]. Thus, HSPs represent an important class of myocardial DAMPs that can elicit inflammatory effects in the myocardium. Specific roles for CF in these responses are beginning to emerge [57], but have yet to be fully defined.

2.4.5. Modified ECM components

Fibronectin-EDA

FN is an adhesive glycoprotein that is a major constituent of the ECM. The alternatively spliced FN-EDA isoform is upregulated in the infarct area after MI [112] and is important for myofibroblast transdifferentiation [6,113]. FN-EDA is a ligand for TLR2 [114] and TLR4 [115], stimulating proinflammatory signalling, myocardial inflammation and ECM turnover [112]. FN-EDA acting via TLR4 stimulates dermal fibroblasts to adopt a migratory myofibroblast phenotype with increased collagen synthesis as well as upregulated inflammatory responses such as IL-6 production [116]. FN-EDA has also been shown to upregulate cyclooxygenase-2 expression and down-regulate connective tissue growth factor expression in adult rat CF [74]. Despite having similar infarct sizes, FN-EDA knockout mice exhibit less LV dilatation and enhanced systolic performance compared with wild-type mice after experimental MI [112]. FN-EDA knockout mice also exhibited less post-MI inflammation, MMP2/9 activity, myofibroblast differentiation and remote fibrosis than wild type mice [112].

Tenascin-C

TN-C is a large hexameric matricellular glycoprotein that is normally expressed at low levels in the adult heart but is markedly upregulated in response to myocardial damage [117,118]. TN-C is a ligand for several integrin receptors [119], as well as for TLR4 [120]. In synovial fibroblasts, TN-C has been shown to couple to proinflammatory cytokine expression via TLR4 activation [120].

TN-C is produced by a wide variety of cardiovascular cell types [118], including CF [94,117]. TN-C expression is an independent predictor of mortality in patients with dilated cardiomyopathy [121] and serum TN-C levels are a potentially useful predictor of LV remodelling and prognosis after MI [122]. A number of preclinical murine studies have shown that TN-C knockout improves cardiac remodelling and fibrosis after MI [123] or Ang II infusion [124]. TN-C knockout mice also exhibit delayed recruitment of myofibroblasts after MI [125]. Indeed, TN-C can directly stimulate CF migration and myofibroblast transdifferentiation [125]. In addition to direct effects, TN-C may have indirect effects on CF for example by inducing IL-6 production from macrophages with resultant increased collagen production by CF [124].

AGE-modified collagen

Type 2 diabetes mellitus is a rapidly escalating health problem that has reached epidemic proportions. The main cause of death in individuals with type 2 diabetes is cardiovascular disease, and diabetes is a well-established independent risk factor for cardiovascular disease. One of the characteristics of a particular cardiomyopathy observed in diabetic patients is diastolic dysfunction in which increased ECM stiffness impairs the ability of the heart to relax [126,127]. Hyperglycaemia that occurs with both type 1 and type 2 diabetes leads to formation of AGEs; proteins and lipids that become non-enzymatically glycated and oxidized after prolonged exposure to glucose [128]. This is particularly problematic for long-lasting proteins with low turnover rates, such as collagens. AGEs mediate intermolecular and intramolecular collagen cross-links, leading to increased collagen stiffness and resistance to proteolytic digestion which manifests as increased ECM stiffness and diastolic dysfunction.

In addition to direct effects on collagen structure, AGEs can modulate cardiac function through RAGE activation [24,128]. For example, AGE-modified proteins can induce CF proliferation and collagen production in an angiotensin II-dependent manner [23]. Several recent studies on CF derived from type 2 diabetic patients or diabetic animal models have reported that fibroblasts from diabetic hearts possess an inherent pro-fibrotic phenotype, including elevated collagen synthesis, myofibroblast transdifferentiation and, in some cases, upregulation of RAGE expression [24,78,129,130].

It is clear that a number of components of the ECM that are modified under pathological conditions can act as DAMPs. Much of the evidence for this has come from knockout mouse models and in vitro cell culture studies, so it remains to be seen whether these modified ECM components can be targeted therapeutically. The commonality of DAMP receptor signalling by these ligands (predominantly via TLR4 and RAGE; see **Figure 2**) indicates that receptor inhibition may be one such approach. Strategies targeting RAGE activation may be particularly attractive for reducing myocardial fibrosis observed in diabetic patients.

2.4.6. NOD-like receptors and the inflammasome

Both NOD1 and NOD2 are expressed by CF and appear to play important roles in regulating cardiac remodelling [26,62]. A NOD1-specific ligand (C12-iE-DAP) induced NFκB and TGFβ pathway activation in CF, and promoted inflammatory signalling, apoptosis, fibrosis and cardiac dysfunction in mice [26]. NOD2 expression was elevated in the infarcted area in a mouse MI model, and NOD2 knockout mice had improved cardiac function, less inflammation and less remodelling after MI compared with wild-type animals [62]. An important role for fibroblasts was suggested in this model as NOD2 activation induced MAPK signalling, proinflammatory cytokine production and MMP-9 activation in CF; outcomes that were inhibited by NOD2 RNA interference [62]. Most known NOD1/2 ligands are pathogen-associated molecular patterns, rather than DAMPs, so the precise DAMP ligands that activate NOD1 and NOD2 receptors in the heart requires further analysis.

NLRPs are an essential component of the inflammasome and, in combination with the ASC adapter protein, are able to activate caspase-1 and stimulate IL-1β activation. CF express NLRP3 [73,76] and studies on ASC and caspase-1 knockout mice have revealed an essential role for the inflammasome in fibroblasts, but not cardiomyocytes, in the initial inflammatory response to myocardial ischaemia/reperfusion injury [131]. Furthermore, NLRP3 in CF has been shown to be important for producing IL-1β in response to extracellular ATP from damaged cardiomyocytes [73]. A pivotal role for the NLRP3 inflammasome in CF for induction of myocardial dysfunction in sepsis has also recently been reported [132]. Additionally, a novel inflammasome-independent role for NLRP3 in CF was recently described in which mitochondrial NLRP3 was found to be important for driving fibrotic responses after Ang II administration including myofibroblast transdifferentiation and fibrosis [76].

As well as fibroblasts, some studies have also highlighted the importance of the inflammasome in cardiomyocytes [133] and macrophages [134] in the response of the heart to ischaemic injury. Other reports concluded that NLRP3 is not important for the immediate inflammatory events post-MI but may play roles at later stages [135]. It is therefore likely that

inflammasome activation occurs in several different cardiac cell types at different times and in different locations following MI.

Interestingly, a novel small molecule inhibitor of NLRP3 inflammasome formation has been developed (16673-34-0) that can reduce caspase-1 activity in the heart by >90% in mice subjected to experimental MI with reperfusion [136]. This resulted in a marked decrease in cell damage (troponin I levels) and infarct size after 24 h [136] and reduced LV dysfunction after 7 days [137]. Additionally, the NLRP3 inhibitor reduced LV dysfunction and remodelling in mice 1 week after permanent LAD ligation without affecting infarct scar size [137]. 16673-34-0 was also found to be effective against non-ischaemic cardiac injury (doxorubicin-induced cardiomyopathy), with a resultant decrease in fibrosis and preservation of systolic function [137]. Hence, irrespective of the precise cellular context, it may be that NLRP3 represents a useful therapeutic target for reducing post-MI injury and remodelling.

3. Future Perspectives

3.1. Cardiac fibroblast-specific genetic models

Although global knockout models have been important for understanding the role of DAMPs and their receptors in cardiac inflammation and remodelling, their interpretation in the context of individual cell types is complicated and often difficult to resolve. To address this, cardiac cell-type specific genetic models have been developed that allow gene knockout, mutation or overexpression in individual cardiac cell types including cardiomyocytes, endothelial cells, inflammatory cells and smooth muscle cells. However, attempts to specifically manipulate CF function in vivo have been hampered by a lack of a fibroblast-specific targeting promoter; a complication likely related to the heterogeneous nature and origin of fibroblasts for which no truly specific marker has been identified. The three main genetic strategies that have been employed for targeting CF to date are based on enhancer elements from the rather misleadingly termed “fibroblast-specific protein” gene (FSP1, also known as S100A4), the periostin gene (Postn) and the proα2(I) collagen gene (Col1a2).

Despite the use of FSP1-driven approaches for fibroblast-specific targeting, a recent study with an FSP1-GFP reporter mouse raised serious questions about the validity of this approach for targeting fibroblasts in the heart [138]. Relatively few cells were found to express FSP1 in the normal heart and these were mostly endothelial cells and smooth muscle cells. The number of FSP1-positive cells increased markedly after MI, but more than half of FSP1-positive cells were of haematopoietic origin (mostly inflammatory leukocytes), with less than 2% of FSP-positive cells being myofibroblasts [138]. Hence, FSP1 promoter-driven approaches for fibroblast-selective gene deletion in the heart are inappropriate.

The matricellular protein periostin is not expressed in the normal heart but it is upregulated exclusively in activated fibroblasts after cardiac injury [139]. A 3.9 kb promoter

region of the mouse periostin gene (Postn) has been used to drive Cre expression in CF and facilitate targeted gene knockout [140], and offers much greater fibroblast-specificity than FSP1 mice when compared in a side-by-side manner [138]. A major value of Postn-Cre mice is for studying the role of myofibroblasts in cardiac remodelling after injury / stress as the promoter becomes active in this context. However, these mice are less useful for investigating normal CF function or immediate responses of fibroblasts to injury, conditions in which the Postn promoter is inactive.

Finally, a fibroblast-specific transcriptional enhancer located in the far upstream region of the pro-α2(I) collagen gene (Col1a2) has been identified that can direct expression in fibroblasts but not in other type I collagen-producing cells [141,142] and has been used to induce gene expression [143] or selective gene knockout [144] specifically within fibroblasts of various tissues. More recent studies in the heart have shown that the Col1a2 promoter drives expression specifically in fibroblasts, so these mice may represent a useful model for selective genetic manipulation of fibroblasts and myofibroblasts in the heart [145-147]. A drawback of the Col1a2 approach however is that it is not selective for fibroblasts in the heart over other sources of fibroblasts (e.g. skin, lungs) and therefore other organs are also likely to be affected.

3.2. MicroRNAs

Understanding the responses of individual myocardial cell types to different stimuli is an important consideration for designing effective therapies for cardiac pathologies. With the continued development and refinement of fibroblast-specific genetically modified mouse models, the roles of CF in physiological and pathological processes will become better defined. Future cell type-selective therapies may be possible, for example through methods targeting microRNAs, small non-coding RNA molecules that regulate multiple genes in a cell type-specific manner [148]. For example, several microRNAs have been identified that play key roles in a variety of fibroblast functions (myofibroblast transdifferentiation, survival, migration, fibrosis) but not in cardiomyocytes; including microRNA-21, -24, -29 and -30 [6,149].

A complex two-way interplay between microRNAs and DAMP signalling is evident in a variety of tissues. For example, TLR activation is coupled to altered expression of several different microRNAs, including some that play key roles in regulating CF function such as microRNA-21 [150]. On the other hand, a number of microRNAs are able to regulate expression of components of the TLR and IL-1R1 signalling pathways, with microRNA-146a being the best studied example [150,151]. Overexpression of microRNA-146a in the heart was shown to reduce post-MI myocardial injury and inflammation; effects caused by reduced expression of the IRAK1 and TRAF6 proteins, key components of the TLR and IL-1R1

signalling pathways [152]. It is therefore an exciting prospect that cardiac cell-specific therapies may be possible in the future through methods targeting individual microRNAs or microRNA subsets in the heart.

3.3. Therapeutic approaches to target DAMP pathways

Several preclinical studies have described successful attempts to inhibit DAMPs or DAMP receptors to improve cardiac function after MI, mostly in mouse models. For example OPN-301, a novel anti-TLR2 monoclonal antibody, was shown to reduce infarct size and preserve cardiac function and geometry when administered before reperfusion in a mouse MI model [34]. Importantly, the same group recently reported development of a clinical-grade humanised anti-TLR2 antibody (OPN-305) and showed similar benefit in pigs after myocardial ischemia/reperfusion injury [153]. These data suggest that OPN-305 may have potential as an adjunctive for reperfusion therapy in MI patients. Other attractive DAMP receptor targets include the inflammasome receptor NLRP3. A novel pharmacological NLRP3 inhibitor improved cardiac function in mouse models of ischaemic and non-ischaemic myocardial injury [136,137].

There is a relative wealth of pre-clinical data to suggest that inhibition of IL-1 signalling may be an effective therapeutic strategy for improving cardiac function and outcome following MI (reviewed in [85,102,154]). Small-scale clinical trials have also sought to evaluate the effect of IL-1 inhibition on inflammation and cardiac remodelling in stable-STEMI and non-STEMI patients [155,156], reporting that IL-1 inhibition reduced post-MI inflammation but had little effect on cardiac function in the longer term. This may relate to the relatively mild inflammatory phenotype in these patient cohorts compared with less stable STEMI patients, in which IL-1 blockade was more effective [157]. Further studies in patients with more severe MI are likely to be required to evaluate the true potential of IL-1 inhibition as an effective therapy for post-MI morbidity and mortality.

All of the above studies have been designed to target global DAMP production or DAMP receptors. However, it is clear that DAMPs can elicit markedly different effects on individual cell types depending on DAMP receptor expression, receptor crosstalk, expression of signalling pathway components and the functional nature of the particular cell type. Developing strategies to specifically target DAMP responses in individual cell types is a major challenge, but cell-specific approaches (e.g. CF-specific genetic mouse models and microRNA-targeted therapies) may allow us to understand more clearly the role that CF play in responding to DAMPs in the injured myocardium.

4. Conclusion

A complex array of DAMPs are produced and/or released from cells and their surroundings when the myocardium is damaged or stressed. In addition to effects on immune cells and cardiomyocytes, these danger signals can directly modulate CF function at multiple levels, including cell proliferation, migration, transdifferentiation and production of inflammatory and fibrotic mediators. The precise effect of DAMPs on CF function, and the relative influence that this may have, is dictated by the nature, location and extent of the initiating injury. Cardiac DAMPs and DAMP receptors on fibroblasts (as well as other cardiac cell types) represent attractive therapeutic targets for potentially controlling inflammatory and fibrotic aspects of the myocardial response to injury. Future studies in transgenic mice with fibroblast-targeted genetic manipulation will also be important for delineating the role of CF in these responses to cardiac injury.

Acknowledgements

Research on cardiac fibroblasts in the author's laboratory is funded by the British Heart Foundation (PG/11/110/29248, PG/11/80/29135, FS/15/48/31665).

References

[1] Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther 2009;123:255-78.

[2] Brown RD, Ambler SK, Mitchell MD and Long CS. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. Annu Rev Pharmacol Toxicol 2005;45:657-87.

[3] Souders CA, Bowers SL and Baudino TA. Cardiac fibroblast: the renaissance cell. Circ Res 2009;105:1164-76.

[4] van den Borne SW, Diez J, Blankesteijn WM, Verjans J, Hofstra L and Narula J. Myocardial remodeling after infarction: the role of myofibroblasts. Nat Rev Cardiol 2010;7:30-7.

[5] Krenning G, Zeisberg EM and Kalluri R. The origin of fibroblasts and mechanism of cardiac fibrosis. J Cell Physiol 2010;225:631-7.

[6] Turner NA, Porter KE. Function and fate of myofibroblasts after myocardial infarction. Fibrogenesis Tissue Repair 2013;6:5.

[7] Turner NA, Porter KE. Regulation of myocardial matrix metalloproteinase expression and activity by cardiac fibroblasts. IUBMB Life 2012;64:143-50.

[8] Martin ML, Blaxall BC. Cardiac intercellular communication: are myocytes and fibroblasts fair-weather friends? J Cardiovasc Transl Res 2012;5:768-82.

[9] Van Linthout S., Miteva K and Tschope C. Crosstalk between fibroblasts and inflammatory cells. Cardiovasc Res 2014;102:258-69.

[10] van Nieuwenhoven FA, Turner NA. The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction. Vascul Pharmacol 2013;58:182-8.

[11] Tanaka M, Ito H, Adachi S, Akimoto H, Nishikawa T, Kasajima T et al. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. Circ Res 1994;75:426-33.

[12] Zhang X, Azhar G, Nagano K and Wei JY. Differential vulnerability to oxidative stress in rat cardiac myocytes versus fibroblasts. J Am Coll Cardiol 2001;38:2055-62.

[13] Arslan F, de Kleijn DP and Pasterkamp G. Innate immune signaling in cardiac ischemia. Nat Rev Cardiol 2011;8:292-300.

[14] Mann DL. The emerging role of innate immunity in the heart and vascular system: for whom the cell tolls. Circ Res 2011;108:1133-45.

[15] Frangogiannis NG, Smith CW and Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002;53:31-47.

[16] Frangogiannis NG. The immune system and cardiac repair. Pharmacol Res 2008;58:88-111.
[17] Hulsmans M, Sam F and Nahrendorf M. Monocyte and macrophage contributions to cardiac remodeling. J Mol Cell Cardiol 2016;In press JMCC9602.
[18] Hartupee J, Mann DL. Role of inflammatory cells in fibroblast activation. J Mol Cell Cardiol 2016;In press, JMC9594.
[19] Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. Mediators Inflamm 2010;2010.
[20] de Haan JJ, Smeets MB, Pasterkamp G and Arslan F. Danger signals in the initiation of the inflammatory response after myocardial infarction. Mediators Inflamm 2013;2013:206039.
[21] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
[22] Lin L, Knowlton AA. Innate immunity and cardiomyocytes in ischemic heart disease. Life Sci 2014;100:1-8.
[23] Yamazaki KG, Gonzalez E and Zambon AC. Crosstalk between the renin-angiotensin system and the advance glycation end product axis in the heart: role of the cardiac fibroblast. J Cardiovasc Transl Res 2012;5:805-13.
[24] Zhao J, Randive R and Stewart JA. Molecular mechanisms of AGE/RAGE-mediated fibrosis in the diabetic heart. World J Diabetes 2014;5:860-7.
[25] Zhang W, Lavine KJ, Epelman S, Evans SA, Weinheimer CJ, Barger PM et al. Necrotic myocardial cells release damage-associated molecular patterns that provoke fibroblast activation in vitro and trigger myocardial inflammation and fibrosis in vivo. J Am Heart Assoc 2015;4:e001993.
[26] Fernandez-Velasco M, Prieto P, Terron V, Benito G, Flores JM, Delgado C et al. NOD1 activation induces cardiac dysfunction and modulates cardiac fibrosis and cardiomyocyte apoptosis. PLoS One 2012;7:e45260.
[27] Frantz S, Ertl G and Bauersachs J. Mechanisms of disease: Toll-like receptors in cardiovascular disease. Nat Clin Pract Cardiovasc Med 2007;4:444-54.
[28] Chao W. Toll-like receptor signaling: a critical modulator of cell survival and ischemic injury in the heart. Am J Physiol Heart Circ Physiol 2009;296:H1-12.
[29] Reuven EM, Fink A and Shai Y. Regulation of innate immune responses by transmembrane interactions: lessons from the TLR family. Biochim Biophys Acta 2014;1838:1586-93.
[30] Hacker H, Tseng PH and Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. Nat Rev Immunol 2011;11:457-68.
[31] Motta V, Soares F, Sun T and Philpott DJ. NOD-like receptors: versatile cytosolic sentinels. Physiol Rev 2015;95:149-78.

[32] Takahashi M. Role of the inflammasome in myocardial infarction. Trends Cardiovasc Med 2011;21:37-41.

[33] Xie J, Mendez JD, Mendez-Valenzuela V and Aguilar-Hernandez MM. Cellular signalling of the receptor for advanced glycation end products (RAGE). Cell Signal 2013;25:2185-97.

[34] Arslan F, Smeets MB, O'Neill LA, Keogh B, McGuirk P, Timmers L et al. Myocardial ischemia/reperfusion injury is mediated by leukocytic toll-like receptor-2 and reduced by systemic administration of a novel anti-toll-like receptor-2 antibody. Circulation 2010;121:80-90.

[35] Favre J, Musette P, Douin-Echinard V, Laude K, Henry JP, Arnal JF et al. Toll-like receptors 2-deficient mice are protected against postischemic coronary endothelial dysfunction. Arterioscler Thromb Vasc Biol 2007;27:1064-71.

[36] Mersmann J, Habeck K, Latsch K, Zimmermann R, Jacoby C, Fischer JW et al. Left ventricular dilation in toll-like receptor 2 deficient mice after myocardial ischemia/reperfusion through defective scar formation. Basic Res Cardiol 2011;106:89-98.

[37] Mersmann J, Tran N, Latsch K, Habeck K, Iskandar F, Zimmermann R et al. Akt or phosphoinositide-3-kinase inhibition reverses cardio-protection in Toll-like receptor 2 deficient mice. Resuscitation 2012;83:1404-10.

[38] Shishido T, Nozaki N, Yamaguchi S, Shibata Y, Nitobe J, Miyamoto T et al. Toll-like receptor-2 modulates ventricular remodeling after myocardial infarction. Circulation 2003;108:2905-10.

[39] Wang L, Li YL, Zhang CC, Cui W, Wang X, Xia Y et al. Inhibition of Toll-like receptor 2 reduces cardiac fibrosis by attenuating macrophage-mediated inflammation. Cardiovasc Res 2014;101:383-92.

[40] Chen C, Feng Y, Zou L, Wang L, Chen HH, Cai JY et al. Role of extracellular RNA and TLR3-Trif signaling in myocardial ischemia-reperfusion injury. J Am Heart Assoc 2014;3:e000683.

[41] Lu C, Ren D, Wang X, Ha T, Liu L, Lee EJ et al. Toll-like receptor 3 plays a role in myocardial infarction and ischemia/reperfusion injury. Biochim Biophys Acta 2014;1842:22-31.

[42] Avlas O, Fallach R, Shainberg A, Porat E and Hochhauser E. Toll-like receptor 4 stimulation initiates an inflammatory response that decreases cardiomyocyte contractility. Antioxid Redox Signal 2011;15:1895-909.

[43] Chong AJ, Shimamoto A, Hampton CR, Takayama H, Spring DJ, Rothnie CL et al. Toll-like receptor 4 mediates ischemia/reperfusion injury of the heart. J Thorac Cardiovasc Surg 2004;128:170-9.

[44] Ding HS, Yang J, Gong FL, Yang J, Ding JW, Li S et al. High mobility group box 1 mediates neutrophil recruitment in myocardial ischemia-reperfusion injury through toll like receptor 4-related pathway. Gene 2012;509:149-53.

[45] Ding HS, Yang J, Chen P, Yang J, Bo SQ, Ding JW et al. The HMGB1-TLR4 axis contributes to myocardial ischemia/reperfusion injury via regulation of cardiomyocyte apoptosis. Gene 2013;527:389-93.

[46] Dong RQ, Wang ZF, Zhao C, Gu HR, Hu ZW, Xie J et al. Toll-like receptor 4 knockout protects against isoproterenol-induced cardiac fibrosis: the role of autophagy. J Cardiovasc Pharmacol Ther 2015;20:84-92.

[47] Fallach R, Shainberg A, Avlas O, Fainblut M, Chepurko Y, Porat E et al. Cardiomyocyte Toll-like receptor 4 is involved in heart dysfunction following septic shock or myocardial ischemia. J Mol Cell Cardiol 2010;48:1236-44.

[48] Ha T, Li Y, Hua F, Ma J, Gao X, Kelley J et al. Reduced cardiac hypertrophy in toll-like receptor 4-deficient mice following pressure overload. Cardiovasc Res 2005;68:224-34.

[49] Hua F, Ha T, Ma J, Li Y, Kelley J, Gao X et al. Protection against myocardial ischemia/reperfusion injury in TLR4-deficient mice is mediated through a phosphoinositide 3-kinase-dependent mechanism. J Immunol 2007;178:7317-24.

[50] Kim SC, Ghanem A, Stapel H, Tiemann K, Knuefermann P, Hoeft A et al. Toll-like receptor 4 deficiency: smaller infarcts, but no gain in function. BMC Physiol 2007;7:5.

[51] Li Y, Si R, Feng Y, Chen HH, Zou L, Wang E et al. Myocardial ischemia activates an injurious innate immune signaling via cardiac heat shock protein 60 and Toll-like receptor 4. J Biol Chem 2011;286:31308-19.

[52] Matsuda S, Umemoto S, Yoshimura K, Itoh S, Murata T, Fukai T et al. Angiotensin Activates MCP-1 and Induces Cardiac Hypertrophy and Dysfunction via Toll-like Receptor 4. J Atheroscler Thromb 2015.

[53] Oyama J, Blais C, Jr., Liu X, Pu M, Kobzik L, Kelly RA et al. Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. Circulation 2004;109:784-9.

[54] Riad A, Jager S, Sobirey M, Escher F, Yaulema-Riss A, Westermann D et al. Toll-like receptor-4 modulates survival by induction of left ventricular remodeling after myocardial infarction in mice. J Immunol 2008;180:6954-61.

[55] Stapel H, Kim SC, Osterkamp S, Knuefermann P, Hoeft A, Meyer R et al. Toll-like receptor 4 modulates myocardial ischaemia-reperfusion injury: Role of matrix metalloproteinases. Eur J Heart Fail 2006;8:665-72.

[56] Timmers L, Sluijter JP, van Keulen JK, Hoefer IE, Nederhoff MG, Goumans MJ et al. Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction. Circ Res 2008;102:257-64.

[57] Higashikuni Y, Tanaka K, Kato M, Nureki O, Hirata Y, Nagai R et al. Toll-like receptor-2 mediates adaptive cardiac hypertrophy in response to pressure overload through interleukin-1beta upregulation via nuclear factor kappaB activation. J Am Heart Assoc 2013;2:e000267.

[58] Parapanov R, Lugrin J, Rosenblatt-Velin N, Feihl F, Waeber B, Milano G et al. Toll-like receptor 5 deficiency exacerbates cardiac injury and inflammation induced by myocardial ischaemia-reperfusion in the mouse. Clin Sci (Lond) 2015;129:187-98.

[59] Abbate A, Salloum FN, Van Tassell BW, Vecile E, Toldo S, Seropian I et al. Alterations in the interleukin-1/interleukin-1 receptor antagonist balance modulate cardiac remodeling following myocardial infarction in the mouse. PLoS One 2011;6:e27923.

[60] Bujak M, Dobaczewski M, Chatila K, Mendoza LH, Li N, Reddy A et al. Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling. Am J Pathol 2008;173:57-67.

[61] Saxena A, Chen W, Su Y, Rai V, Uche OU, Li N et al. IL-1 induces proinflammatory leukocyte infiltration and regulates fibroblast phenotype in the infarcted myocardium. J Immunol 2013;191:4838-48.

[62] Li X, Li F, Chu Y, Wang X, Zhang H, Hu Y et al. NOD2 deficiency protects against cardiac remodeling after myocardial infarction in mice. Cell Physiol Biochem 2013;32:1857-66.

[63] Zong J, Salim M, Zhou H, Bian ZY, Dai J, Yuan Y et al. NOD2 deletion promotes cardiac hypertrophy and fibrosis induced by pressure overload. Lab Invest 2013;93:1128-36.

[64] Aleshin A, Ananthakrishnan R, Li Q, Rosario R, Lu Y, Qu W et al. RAGE modulates myocardial injury consequent to LAD infarction via impact on JNK and STAT signaling in a murine model. Am J Physiol Heart Circ Physiol 2008;294:H1823-H1832.

[65] Andrassy M, Volz HC, Igwe JC, Funke B, Eichberger SN, Kaya Z et al. High-mobility group box-1 in ischemia-reperfusion injury of the heart. Circulation 2008;117:3216-26.

[66] Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and implications for the pathophysiology of heart failure. Curr Heart Fail Rep 2012;9:107-16.

[67] Lugrin J, Parapanov R, Rosenblatt-Velin N, Rignault-Clerc S, Feihl F, Waeber B et al. Cutting Edge: IL-1α is a crucial danger signal triggering acute myocardial inflammation during myocardial infarction. J Immunol 2015;194:499-503.

[68] Rohde D, Schon C, Boerries M, Didrihsone I, Ritterhoff J, Kubatzky KF et al. S100A1 is released from ischemic cardiomyocytes and signals myocardial damage via Toll-like receptor 4. EMBO Mol Med 2014;6:778-94.

[69] Topkara VK, Evans S, Zhang W, Epelman S, Staloch L, Barger PM et al. Therapeutic targeting of innate immunity in the failing heart. J Mol Cell Cardiol 2011;51:594-9.

[70] Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. Biol Pharm Bull 2005;28:886-92.

[71] Boyd JH, Mathur S, Wang Y, Bateman RM and Walley KR. Toll-like receptor stimulation in cardiomyoctes decreases contractility and initiates an NF-kappaB dependent inflammatory response. Cardiovasc Res 2006;72:384-93.

[72] Frantz S, Kelly RA and Bourcier T. Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. J Biol Chem 2001;276:5197-203.

[73] Sandanger O, Ranheim T, Vinge LE, Bliksoen M, Alfsnes K, Finsen AV et al. The NLRP3 inflammasome is up-regulated in cardiac fibroblasts and mediates myocardial ischaemia-reperfusion injury. Cardiovasc Res 2013;99:164-74.

[74] Mesquita RF, Paul MA, Valmaseda A, Francois A, Jabr R, Anjum S et al. Protein kinase Cε-calcineurin cosignaling downstream of toll-like receptor 4 downregulates fibrosis and induces wound healing gene expression in cardiac myofibroblasts. Mol Cell Biol 2014;34:574-94.

[75] Ohm IK, Alfsnes K, Belland OM, Ranheim T, Sandanger O, Dahl TB et al. Toll-like receptor 9 mediated responses in cardiac fibroblasts. PLoS One 2014;9:e104398.

[76] Bracey NA, Gershkovich B, Chun J, Vilaysane A, Meijndert HC, Wright JR, Jr. et al. Mitochondrial NLRP3 protein induces reactive oxygen species to promote Smad protein signaling and fibrosis independent from the inflammasome. J Biol Chem 2014;289:19571-84.

[77] Rossini A, Zacheo A, Mocini D, Totta P, Facchiano A, Castoldi R et al. HMGB1-stimulated human primary cardiac fibroblasts exert a paracrine action on human and murine cardiac stem cells. J Mol Cell Cardiol 2008;44:683-93.

[78] Hutchinson KR, Lord CK, West TA and Stewart JA, Jr. Cardiac fibroblast-dependent extracellular matrix accumulation is associated with diastolic stiffness in type 2 diabetes. PLoS One 2013;8:e72080.

[79] Wu Y, Li Y, Zhang C, A X, Wang Y, Cui W et al. S100a8/a9 released by CD11b+Gr1+ neutrophils activates cardiac fibroblasts to initiate angiotensin II-Induced cardiac inflammation and injury. Hypertension 2014;63:1241-50.

[80] Marenholz I, Heizmann CW and Fritz G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). Biochem Biophys Res Commun 2004;322:1111-22.

[81] Foell D, Wittkowski H, Vogl T and Roth J. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol 2007;81:28-37.

[82] Bi H, Yang Y, Huang J, Li Y, Ma C and Cong B. Immunohistochemical detection of S100A1 in the postmortem diagnosis of acute myocardial infarction. Diagn Pathol 2013;8:84.

[83] Ryckman C, Vandal K, Rouleau P, Talbot M and Tessier PA. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol 2003;170:3233-42.

[84] Long CS. The role of interleukin-1 in the failing heart. Heart Fail Rev 2001;6:81-94.

[85] Bujak M, Frangogiannis NG. The role of IL-1 in the pathogenesis of heart disease. Arch Immunol Ther Exp (Warsz) 2009;57:165-76.

[86] Frangogiannis NG. Interleukin-1 in cardiac injury, repair, and remodeling: pathophysiologic and translational concepts. Discoveries (Craiova) 2015;3.

[87] Chen CJ, Kono H, Golenbock D, Reed G, Akira S and Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. Nat Med 2007;13:851-6.

[88] Turner NA. Effects of interleukin-1 on cardiac fibroblast function: Relevance to post-myocardial infarction remodelling. Vascul Pharmacol 2014;60:1-7.

[89] Westphal E, Li C, Pilowski C, Koch S, Ebelt H, Muller-Werdan U et al. Endotoxin-activated cultured neonatal rat cardiomyocytes express functional surface-associated interleukin-1α. J Endotoxin Res 2007;13:25-34.

[90] Turner NA, Mughal RS, Warburton P, O'Regan DJ, Ball SG and Porter KE. Mechanism of TNFα-induced IL-1α, IL-1β and IL-6 expression in human cardiac fibroblasts: Effects of statins and thiazolidinediones. Cardiovasc Res 2007;76:81-90.

[91] Turner NA, Das A, Warburton P, O'Regan DJ, Ball SG and Porter KE. Interleukin-1α stimulates pro-inflammatory cytokine expression in human cardiac myofibroblasts. Am J Physiol Heart Circ Physiol 2009;297:H1117-H1127.

[92] Turner NA, Das A, O'Regan DJ, Ball SG and Porter KE. Human cardiac fibroblasts express ICAM-1, E-selectin and CXC chemokines in response to proinflammatory cytokine stimulation. Int J Biochem Cell Biol 2011;43:1450-8.

[93] Sinfield JK, Das A, O'Regan DJ, Ball SG, Porter KE and Turner NA. p38 MAPK alpha mediates cytokine-induced IL-6 and MMP-3 expression in human cardiac fibroblasts. Biochem Biophys Res Commun 2013;430:419-24.

[94] Maqbool A, Hemmings KE, O'Regan DJ, Ball SG, Porter KE and Turner NA. Interleukin-1 has opposing effects on connective tissue growth factor and tenascin-C expression in human cardiac fibroblasts. Matrix Biol 2013;32:208-14.

[95] Chen W, Frangogiannis NG. Fibroblasts in post-infarction inflammation and cardiac repair. Biochim Biophys Acta 2013;1833:945-53.

[96] van Nieuwenhoven FA, Hemmings KE, Porter KE and Turner NA. Combined effects of interleukin-1α and transforming growth factor-β1 on modulation of human cardiac fibroblast function. Matrix Biol 2013;32:399-406.

[97] Turner NA, Warburton P, O'Regan DJ, Ball SG and Porter KE. Modulatory effect of interleukin-1α on expression of structural matrix proteins, MMPs and TIMPs in human cardiac myofibroblasts: role of p38 MAP kinase. Matrix Biol 2010;29:613-20.

[98] Mughal RS, Warburton P, O'Regan DJ, Ball SG, Turner NA and Porter KE. Peroxisome proliferator-activated receptor γ-independent effects of thiazolidinediones on human cardiac myofibroblast function. Clin Exp Pharmacol Physiol 2009;36:478-86.

[99] Brønnum H, Eskildsen T, Andersen DC, Schneider M and Sheikh SP. IL-1β suppresses TGF-β-mediated myofibroblast differentiation in cardiac fibroblasts. Growth Factors 2013;31:81-9.

[100] Brown RD, Jones GM, Laird RE, Hudson P and Long CS. Cytokines regulate matrix metalloproteinases and migration in cardiac fibroblasts. Biochem Biophys Res Commun 2007;362:200-5.

[101] Sheedy FJ, Moore KJ. IL-1 signaling in atherosclerosis: sibling rivalry. Nat Immunol 2013;14:1030-2.

[102] Van Tassell BW, Raleigh JM and Abbate A. Targeting interleukin-1 in heart failure and inflammatory heart disease. Curr Heart Fail Rep 2015;12:33-41.

[103] Sims GP, Rowe DC, Rietdijk ST, Herbst R and Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol 2010;28:367-88.

[104] Wang WK, Wang B, Lu QH, Zhang W, Qin WD, Liu XJ et al. Inhibition of high-mobility group box 1 improves myocardial fibrosis and dysfunction in diabetic cardiomyopathy. Int J Cardiol 2014;172:202-12.

[105] Su Z, Yin J, Wang T, Sun Y, Ni P, Ma R et al. Up-regulated HMGB1 in EAM directly led to collagen deposition by a PKCbeta/Erk1/2-dependent pathway: cardiac fibroblast/myofibroblast might be another source of HMGB1. J Cell Mol Med 2014;18:1740-51.

[106] Limana F, Esposito G, D'Arcangelo D, Di CA, Romani S, Melillo G et al. HMGB1 attenuates cardiac remodelling in the failing heart via enhanced cardiac regeneration and miR-206-mediated inhibition of TIMP-3. PLoS One 2011;6:e19845.

[107] Tao A, Song J, Lan T, Xu X, Kvietys P, Kao R et al. Cardiomyocyte-fibroblast interaction contributes to diabetic cardiomyopathy in mice: Role of HMGB1/TLR4/IL-33 axis. Biochim Biophys Acta 2015;1852:2075-85.

[108] He Y, Zhou X, Zheng X and Jiang X. Exogenous high-mobility group box 1 protein prevents postinfarction adverse myocardial remodeling through TGF-beta/Smad signaling pathway. J Cell Biochem 2013;114:1634-41.

[109] Kim SC, Stice JP, Chen L, Jung JS, Gupta S, Wang Y et al. Extracellular heat shock protein 60, cardiac myocytes, and apoptosis. Circ Res 2009;105:1186-95.

[110] Satoh M, Shimoda Y, Akatsu T, Ishikawa Y, Minami Y and Nakamura M. Elevated circulating levels of heat shock protein 70 are related to systemic inflammatory reaction through monocyte Toll signal in patients with heart failure after acute myocardial infarction. Eur J Heart Fail 2006;8:810-5.

[111] Jin C, Cleveland JC, Ao L, Li J, Zeng Q, Fullerton DA et al. Human myocardium releases heat shock protein 27 (HSP27) after global ischemia: the proinflammatory effect of extracellular HSP27 through toll-like receptor (TLR)-2 and TLR4. Mol Med 2014;20:280-9.

[112] Arslan F, Smeets MB, Riem Vis PW, Karper JC, Quax PH, Bongartz LG et al. Lack of fibronectin-EDA promotes survival and prevents adverse remodeling and heart function deterioration after myocardial infarction. Circ Res 2011;108:582-92.

[113] Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C and Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002;3:349-63.

[114] Schoneveld AH, Hoefer I, Sluijter JP, Laman JD, de Kleijn DP and Pasterkamp G. Atherosclerotic lesion development and Toll like receptor 2 and 4 responsiveness. Atherosclerosis 2008;197:95-104.

[115] Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J et al. The extra domain A of fibronectin activates Toll-like receptor 4. J Biol Chem 2001;276:10229-33.

[116] Bhattacharyya S, Tamaki Z, Wang W, Hinchcliff M, Hoover P, Getsios S et al. FibronectinEDA promotes chronic cutaneous fibrosis through Toll-like receptor signaling. Sci Transl Med 2014;6:232ra50.

[117] Imanaka-Yoshida K, Hiroe M, Nishikawa T, Ishiyama S, Shimojo T, Ohta Y et al. Tenascin-C modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction. Lab Invest 2001;81:1015-24.

[118] Golledge J, Clancy P, Maguire J, Lincz L and Koblar S. The role of tenascin C in cardiovascular disease. Cardiovasc Res 2011;92:19-28.

[119] Tucker RP, Chiquet-Ehrismann R. Tenascin-C: Its functions as an integrin ligand. Int J Biochem Cell Biol 2015;65:165-8.

[120] Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E et al. Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. Nat Med 2009;15:774-80.

[121] Sarli B, Topsakal R, Kaya EG, Akpek M, Lam YY and Kaya MG. Tenascin-C as predictor of left ventricular remodeling and mortality in patients with dilated cardiomyopathy. J Investig Med 2013;61:728-32.

[122] Sato A, Aonuma K, Imanaka-Yoshida K, Yoshida T, Isobe M, Kawase D et al. Serum tenascin-C might be a novel predictor of left ventricular remodeling and prognosis after acute myocardial infarction. J Am Coll Cardiol 2006;47:2319-25.

[123] Nishioka T, Onishi K, Shimojo N, Nagano Y, Matsusaka H, Ikeuchi M et al. Tenascin-C may aggravate left ventricular remodeling and function after myocardial infarction in mice. Am J Physiol Heart Circ Physiol 2010;298:H1072-H1078.

[124] Shimojo N, Hashizume R, Kanayama K, Hara M, Suzuki Y, Nishioka T et al. Tenascin-C May Accelerate Cardiac Fibrosis by Activating Macrophages via the Integrin alphaVbeta3/Nuclear Factor-kappaB/Interleukin-6 Axis. Hypertension 2015.

[125] Tamaoki M, Imanaka-Yoshida K, Yokoyama K, Nishioka T, Inada H, Hiroe M et al. Tenascin-C regulates recruitment of myofibroblasts during tissue repair after myocardial injury. Am J Pathol 2005;167:71-80.

[126] Seferovic PM, Paulus WJ. Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes. Eur Heart J 2015;36:1718-27.

[127] Russo I, Frangogiannis NG. Diabetes-associated cardiac fibrosis: cellular effectors, molecular mechanisms and therapeutic opportunities. J Mol Cell Cardiol 2016;In press, JMC9592.

[128] Bodiga VL, Eda SR and Bodiga S. Advanced glycation end products: role in pathology of diabetic cardiomyopathy. Heart Fail Rev 2014;19:49-63.

[129] Fowlkes V, Clark J, Fix C, Law BA, Morales MO, Qiao X et al. Type II diabetes promotes a myofibroblast phenotype in cardiac fibroblasts. Life Sci 2013;92:669-76.

[130] Sedgwick B, Riches K, Bageghni SA, O'Regan DJ, Porter KE and Turner NA. Investigating inherent functional differences between human cardiac fibroblasts cultured from nondiabetic and Type 2 diabetic donors. Cardiovasc Pathol 2014;23:204-10.

[131] Kawaguchi M, Takahashi M, Hata T, Kashima Y, Usui F, Morimoto H et al. Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. Circulation 2011;123:594-604.

[132] Zhang W, Xu X, Kao R, Mele T, Kvietys P, Martin CM et al. Cardiac fibroblasts contribute to myocardial dysfunction in mice with sepsis: the role of NLRP3 inflammasome activation. PLoS One 2014;9:e107639.

[133] Mezzaroma E, Toldo S, Farkas D, Seropian IM, Van Tassell BW, Salloum FN et al. The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. Proc Natl Acad Sci U S A 2011;108:19725-30.

[134] Liu W, Zhang X, Zhao M, Zhang X, Chi J, Liu Y et al. Activation in M1 but not M2 macrophages contributes to cardiac remodeling after myocardial infarction in rats: a critical role of the calcium sensing receptor/NRLP3 inflammasome. Cell Physiol Biochem 2015;35:2483-500.

[135] Jong WM, Leemans JC, Weber NC, Juffermans NP, Schultz MJ, Hollmann MW et al. Nlrp3 plays no role in acute cardiac infarction due to low cardiac expression. Int J Cardiol 2014;177:41-3.

[136] Marchetti C, Chojnacki J, Toldo S, Mezzaroma E, Tranchida N, Rose SW et al. A novel pharmacologic inhibitor of the NLRP3 inflammasome limits myocardial injury after ischemia-reperfusion in the mouse. J Cardiovasc Pharmacol 2014;63:316-22.

[137] Marchetti C, Toldo S, Chojnacki J, Mezzaroma E, Liu K, Salloum FN et al. Pharmacologic inhibition of the NLRP3 inflammasome preserves cardiac function after ischemic and nonischemic injury in the mouse. J Cardiovasc Pharmacol 2015;66:1-8.

[138] Kong P, Christia P, Saxena A, Su Y and Frangogiannis NG. Lack of specificity of fibroblast-specific protein 1 in cardiac remodeling and fibrosis. Am J Physiol Heart Circ Physiol 2013;305:H1363-H1372.

[139] Snider P, Standley KN, Wang J, Azhar M, Doetschman T and Conway SJ. Origin of cardiac fibroblasts and the role of periostin. Circ Res 2009;105:934-47.

[140] Takeda N, Manabe I, Uchino Y, Eguchi K, Matsumoto S, Nishimura S et al. Cardiac fibroblasts are essential for the adaptive response of the murine heart to pressure overload. J Clin Invest 2010;120:254-65.

[141] Bou-Gharios G, Garrett LA, Rossert J, Niederreither K, Eberspaecher H, Smith C et al. A potent far-upstream enhancer in the mouse proα2(I) collagen gene regulates expression of reporter genes in transgenic mice. J Cell Biol 1996;134:1333-44.

[142] Denton CP, Zheng B, Evans LA, Shi-wen X, Ong VH, Fisher I et al. Fibroblast-specific expression of a kinase-deficient type II transforming growth factor β (TGFβ) receptor leads to paradoxical activation of TGFβ signaling pathways with fibrosis in transgenic mice. J Biol Chem 2003;278:25109-19.

[143] Zheng B, Zhang Z, Black CM, de Crombrugghe B and Denton CP. Ligand-dependent genetic recombination in fibroblasts : a potentially powerful technique for investigating gene function in fibrosis. Am J Pathol 2002;160:1609-17.

[144] Denton CP, Khan K, Hoyles RK, Shiwen X, Leoni P, Chen Y et al. Inducible lineage-specific deletion of TβRII in fibroblasts defines a pivotal regulatory role during adult skin wound healing. J Invest Dermatol 2009;129:194-204.

[145] Lal H, Ahmad F, Zhou J, Yu JE, Vagnozzi RJ, Guo Y et al. Cardiac fibroblast glycogen synthase kinase-3β regulates ventricular remodeling and dysfunction in ischemic heart. Circulation 2014;130:419-30.

[146] Ubil E, Duan J, Pillai IC, Rosa-Garrido M, Wu Y, Bargiacchi F et al. Mesenchymal-endothelial transition contributes to cardiac neovascularization. Nature 2014;514:585-90.

[147] Hemmings KE, Bageghni SA, Porter KE, Drinkhill MJ, Ainscough JF and Turner NA. A transgenic approach to study the effect of cardiac fibroblast-specific ablation of IL-1 signalling on myocardial remodelling. Heart 2014;100:A19-A20 (abstract).

[148] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215-33.

[149] Thum T. miRNA modulation of cardiac fibroblast phenotype. J Mol Cell Cardiol 2016;In press.

[150] Nahid MA, Satoh M and Chan EK. MicroRNA in TLR signaling and endotoxin tolerance. Cell Mol Immunol 2011;8:388-403.

[151] Saba R, Sorensen DL and Booth SA. MicroRNA-146a: a dominant, negative regulator of the innate immune response. Front Immunol 2014;5:578.

[152] Wang X, Ha T, Liu L, Zou J, Zhang X, Kalbfleisch J et al. Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. Cardiovasc Res 2013;97:432-42.

[153] Arslan F, Houtgraaf JH, Keogh B, Kazemi K, de JR, McCormack WJ et al. Treatment with OPN-305, a humanized anti-Toll-Like receptor-2 antibody, reduces myocardial ischemia/reperfusion injury in pigs. Circ Cardiovasc Interv 2012;5:279-87.

[154] Dinarello CA, Simon A and van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 2012;11:633-52.

[155] Abbate A, Van Tassell BW, Biondi-Zoccai G, Kontos MC, Grizzard JD, Spillman DW et al. Effects of interleukin-1 blockade with Anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial 2 (VCU-ART2) Pilot Study]. Am J Cardiol 2013.

[156] Morton AC, Rothman AM, Greenwood JP, Gunn J, Chase A, Clarke B et al. The effect of interleukin-1 receptor antagonist therapy on markers of inflammation in non-ST elevation acute coronary syndromes: the MRC-ILA Heart Study. Eur Heart J 2015;36:377-84.

[157] Abbate A, Kontos MC, Grizzard JD, Biondi-Zoccai GG, Van Tassell BW, Robati R et al. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). Am J Cardiol 2010;105:1371-7.

DAMP Receptor	Injury Model	Knockout / Deficiency	Responses	Reference
IL-1R1	ISC/REP	KO	↔ infarct size ↓ inflammatory cells ↓ inflammatory cytokines ↓ fibrosis	60,61
	ISC	KO	↓ infarct size ↑ cardiac function ↓ apoptosis	59
NOD2	ISC	KO	↑ cardiac function ↓ inflammatory cells ↓ inflammatory cytokines ↓ MMP9	62
	Pressure overload	KO	↑ hypertrophy ↑ fibrosis	63
RAGE	ISC/REP	KO	↓ infarct size ↓ ischaemic damage ↑ contractile function ↓ JNK STAT5	64,65
TLR2	ISC/REP	KO	↓ infarct size ↓ necrosis ↓ inflammation ↓ inflammatory cells ↑ LV dilatation ↓ scar collagen ↓ ROS	34-37
	ISC	KO	↔ infarct size ↑ survival ↓ reactive fibrosis ↓ LV diastolic dimensions ↑ fractional shortening	38
	Pressure overload	KO	↓ hypertrophy ↓ fibrosis ↑ LV dilatation ↓ systolic function	57
	Ang II-induced fibrosis	KO	↓ fibrosis ↓ inflammatory cells ↓ inflammatory cytokines	39
TLR3	ISC/REP	KO	↓ infarct size ↑ cardiac function ↓ inflammatory cells ↔ ↓ inflammatory cytokines ↑ apoptosis	40,41
	ISC	KO	↑ cardiac function	41
TLR4	ISC/REP	deficient strain	↓ infarct size ↔ cardiac function ↓ inflammatory cells ↓ inflammatory cytokines ↓ apoptosis ↓ JNK ↓ HMGB1	43,45,50,53

		KO	↓ infarct size ↓ ischaemic damage ↓ inflammatory cells ↓ inflammatory cytokines ↓ apoptosis	44,49,51,55
	ISC	deficient strain	↔ infarct size ↑ cardiac function ↓ LV remodelling ↓ fibrosis ↓ hypertrophy ↑ scar collagen ↓ inflammatory cells ↓ inflammatory cytokines ↓ MMP2 MMP9	56
		KO	↓ infarct size ↑ cardiac function ↓ hypertrophy ↓ fibrosis ↑ survival	42,47,54
	Pressure overload	deficient strain	↓ hypertrophy	48
	Ang II-induced fibrosis	deficient strain	↓ hypertrophy ↓ fibrosis ↓ inflammatory cells ↑ cardiac function ↑ ROS, cytokines	52
	ISO-induced fibrosis	KO	↑ cardiac function	46
TLR5	ISC/REP	KO	↑ infarct size ↓ cardiac function ↑ oxidative stress ↑ p38 AKT ↑ inflammatory cytokines	58

Table 1. Mouse models of DAMP receptor deficiency and their influence on cardiac remodelling after injury. Abbreviations: ISC, ischaemia; REP, reperfusion; KO, knockout; MMP, matrix metalloproteinase; JNK, c-Jun N-terminal kinase; STAT, signal transducer and activator of transcription; LV, left ventricle; ROS, reactive oxygen species; Ang II, angiotensin II; HMGB1, high mobility group box 1 protein; ISO, isoproterenol.

DAMP	Species	Age	Receptor & Signalling Pathway	Response	Reference
S100A1	Rat	Adult	**TLR4** MyD88 ERK p38 JNK NF-κB	↓ collagen I ↓ CTGF ↓ αSMA ↑ MMP9 ↑ ICAM, IL-10 ↑ SDF1,TSP2	68
S100A8/9	Mouse	Adult	**RAGE** NF-κB	↑ cell migration ↔ cell proliferation ↔ myofibroblast differentiation ↑ cytokines ↑ chemokines	79
		NIH/3T3*		↑ cell proliferation ↔ collagen I, III	25
IL-1α	Human	Adult	p38 JNK NF-κB PI3K/AKT	↑ cell migration ↓ myofibroblast differentiation ↓ αSMA ↑ IL-1β, IL-6, TNFα ↑ CXCL1, 2, 5, 8 ↑ MMP1, 3, 9, 10 ↑ ICAM, VCAM ↑ E-Selectin ↓ ADAMTS1 ↑ TNC ↓ CTGF ↓ collagen I ↑ collagen III	91-94,96-98,130
	Mouse	Neonatal	**IL-1R1**	↑ IL-6, MCP-1	67
		NIH/3T3*		↔ cell proliferation ↔ collagen I, III	25
HMGB1	Human	Adult		↑ cell migration ↔ cell proliferation ↔ myofibroblast differentiation ↑ cytokines ↑ chemokines ↑ growth factors	77
	Rat	Adult		↓ collagen I ↑ Smad7	108
		Neonatal	ERK JNK PI3K/AKT	↑ cell proliferation ↑ cell migration ↑ collagen I, III ↑ TGFβ ↑ MMP2, 9	104
	Mouse	Adult	**TLR4**	↑ collagen I ↓ TIMP3 ↑ miR-206	106,107

		Neonatal	**TLR2** **TLR4** PKC-β ERK	↑ cell proliferation ↑ cell migration ↑ collagen I, III ↑ OPN ↑ MMP1, 2 ↑ TIMP1	105
		NIH/3T3*		↑ cell proliferation ↑ collagen I, III	25
HSP70	Rat	Neonatal	**TLR2**	↑ cell proliferation ↑ cytokines	57

Table 2. Effects of secreted DAMPs on cardiac fibroblast function. DAMP and receptor (if determined) are shown in bold. Abbreviations for signalling pathways: MyD88, myeloid differentiation primary response gene 88; ERK, extracellular signal-regulated kinase; p38, p38 MAP kinase; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor kappa B; PI3K/AKT, phosphatidylinositol 3-kinase / AKT; PKC, protein kinase C. Abbreviations for genes/proteins regulated as part of response: CTGF, connective tissue growth factor (CCN2); αSMA, α-smooth muscle actin; MMP, matrix metalloproteinase; ICAM, intercellular cell adhesion molecule; IL, interleukin; TSP, thrombospondin; TNF, tumour necrosis factor; ADAMTS, a disintegrin and metalloproteinase domain with thrombospondin motifs; VCAM, vascular cell adhesion molecule; CXCL, C-X-C motif ligand; TNC, tenascin C; MCP, monocyte chemoattractant protein; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; OPN, osteopontin; HSP, heat shock protein.

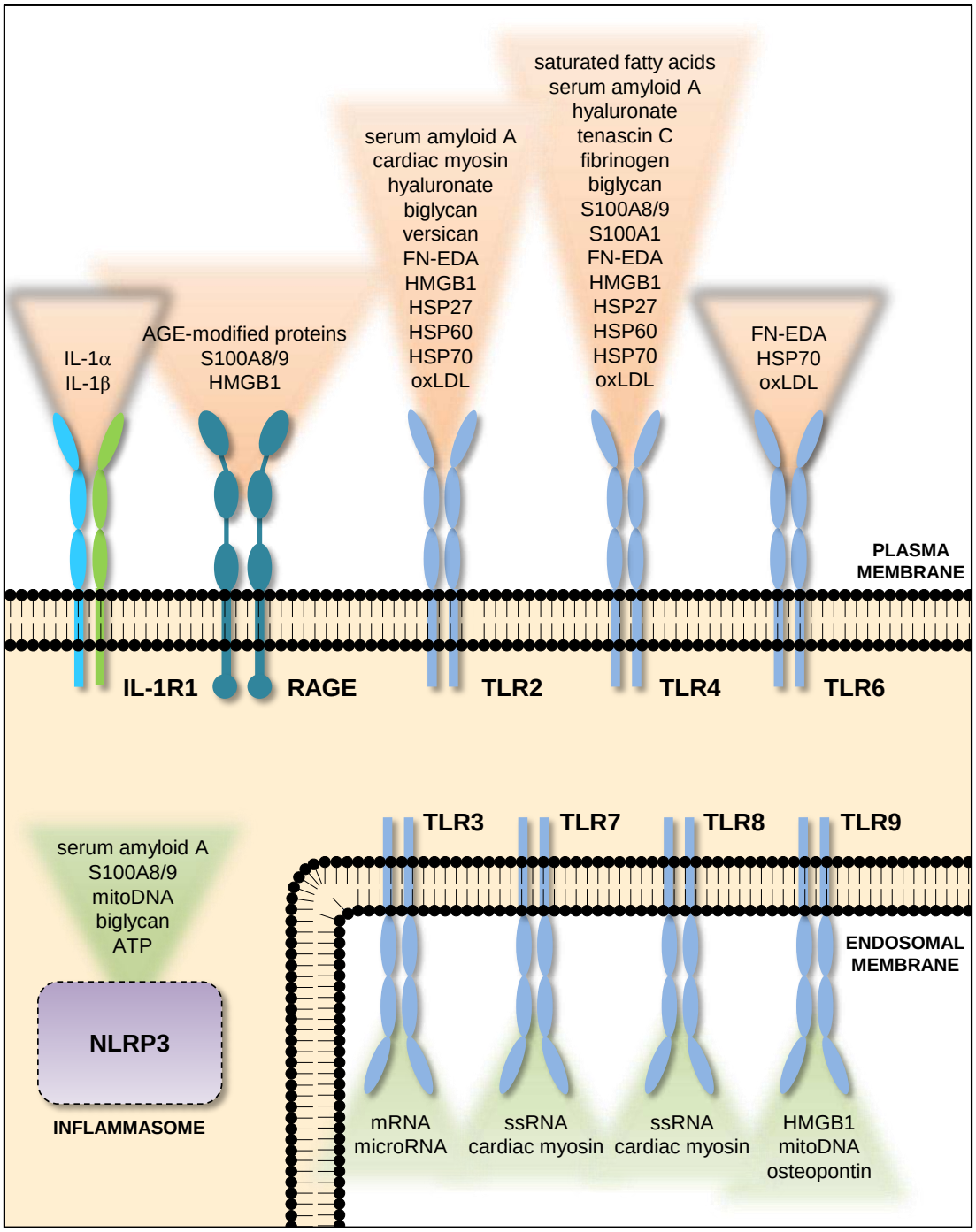
*Note that although NIH/3T3 cells are a murine embryonic fibroblast cell line, they are included as the DAMP responses were reportedly similar to those of adult mouse CF [25].

FIGURE LEGENDS

Figure 1. Summary of cardiovascular DAMPs and their receptors. The interleukin-1 receptor (IL-1R1), the receptor for advanced glycation end products (RAGE) and the Toll-like receptors (TLRs) 2, 4 and 6 are all located on the plasma membrane and respond to extracellular damage-associated molecular patterns (DAMPs). In contrast, TLRs 3, 7, 8 and 9 are localised on endosomal and lysosomal membranes and recognise DAMP ligands taken into the cell by endocytosis. The identity of DAMPs that can activate TLRs 1, 5 and 10, and the cytosolic NOD-like receptors (NOD1 and NOD2) are not known, so these receptors are not included in the diagram. The list of DAMPs represents those of particular relevance to the heart and wider cardiovascular system. Abbreviations: IL-1, interleukin-1; AGE, advanced glycation end product; HMGB1, high mobility group box 1 protein; FN-EDA, fibronectin with extra type III domain A repeat; HSP, heat shock protein; oxLDL, oxidised low density lipoprotein; mitoDNA, mitochondrial DNA.

Figure 2. Summary of main DAMP signalling pathways identified in cardiac fibroblasts. Myocardial damage (e.g. following myocardial infarction) results in passive release of damage-associated molecular patterns (DAMPs) from necrotic cardiac cells (e.g. cardiomyocytes, fibroblasts) and infiltrating leukocytes (e.g. neutrophils). Pathological modification of the extracellular matrix (ECM) can also produce DAMPs, such as fibronectin-EDA (FN-EDA), tenascin-C (TN-C) and advanced glycation end product (AGE)-modified collagen. These various DAMPs can activate pattern recognition receptors on cardiac fibroblasts (CF) to induce functional changes in CF activity that contribute to myocardial remodelling. Recent studies have identified a number of DAMPs and DAMP receptors that are particularly important for fibroblast responses. Interleukin (IL)-1α stimulates the IL-1R1/Myd88 pathway resulting in proinflammatory and ECM-degrading responses. Similar responses have been noted for S100A8/9 released from neutrophils which acts via the AGE receptor (RAGE). In contrast, RAGE activation can also be coupled to profibrotic responses (e.g. myofibroblast differentiation and collagen expression in response to AGE-modified collagen). S100A1 and high mobility group box 1 protein (HMGB1) both act via the Toll-like receptor 4 (TLR4)/Myd88 pathway but appear to have opposing effects, with S100A1 stimulating anti-fibrotic effects (reduced ECM synthesis, increased ECM degradation, reduced myofibroblast differentiation) and HMGB1 being pro-fibrotic (increased CF proliferation, increased ECM synthesis). See main text for further description.

Figure 1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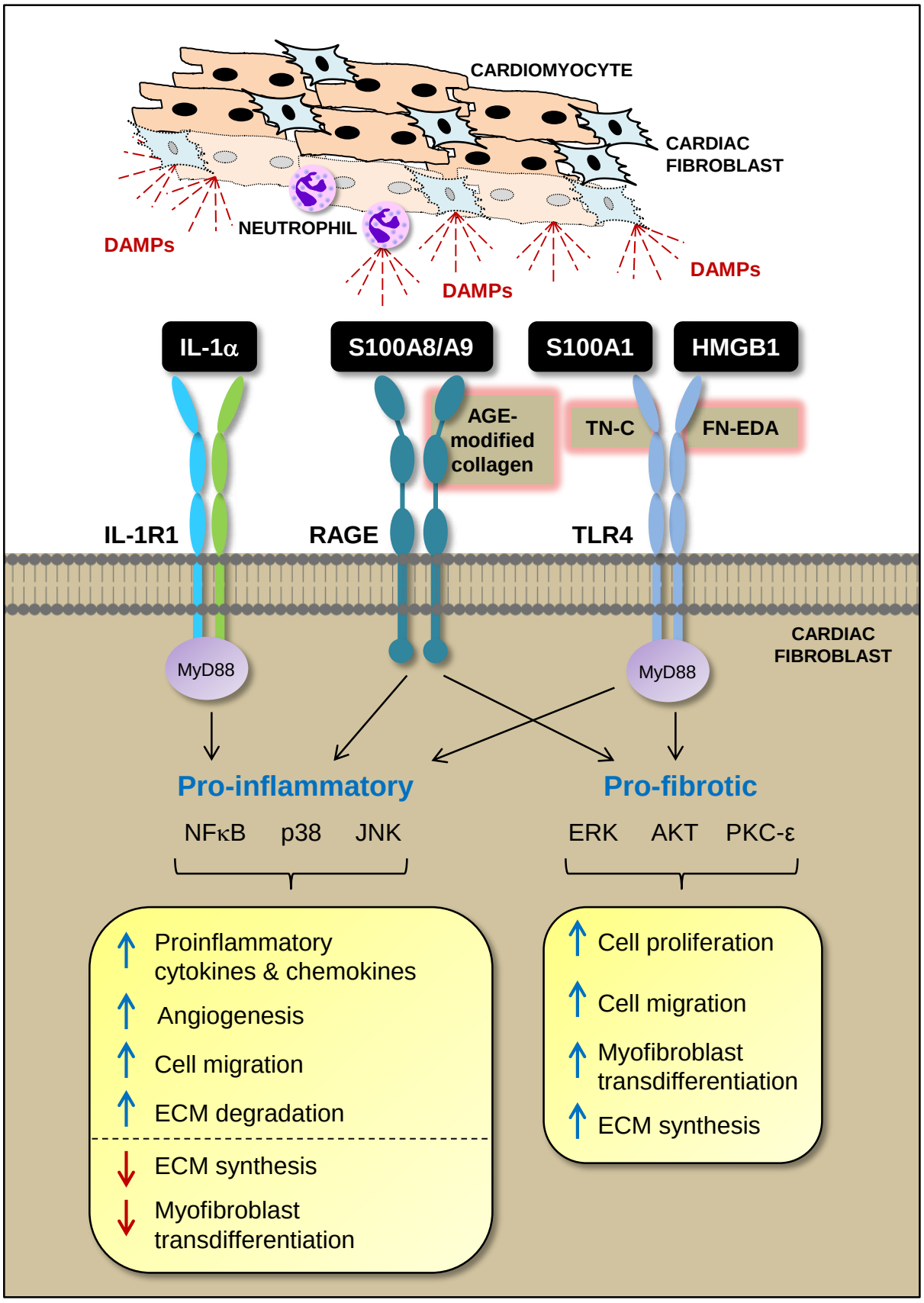
hyaluronate
tenascin C
fibrinogen
biglycan
S100A8/9
S100A1
FN-EDA
HMGB1
HSP27
HSP60
HSP70
oxLDL
serum amyloid A
cardiac myosin
hyaluronate
biglycan
versican
FN-EDA
HMGB1
HSP27
HSP60
HSP70
oxLDL
FN-EDA
HSP70
oxLDL
IL-1α
IL-1β
AGE-modified proteins
S100A8/9
HMGB1
PLASMA MEMBRANE
IL-1R1
RAGE
TLR2
TLR4
TLR6
TLR3
TLR7
TLR8
TLR9
serum amyloid A
S100A8/9
mitoDNA
biglycan
ATP
NLRP3
INFLAMMASOME
ENDOSOMAL MEMBRANE
mRNA
microRNA
ssRNA
cardiac myosin
ssRNA
cardiac myosin
HMGB1
mitoDNA
osteopontin

Figure 2


CARDIOMYOCYTE
CARDIAC FIBROBLAST
NEUTROPHIL
DAMPs
DAMPs
DAMPs
IL-1α
S100A8/A9
S100A1
HMGB1
AGE-modified collagen
TN-C
FN-EDA
IL-1R1
RAGE
TLR4
CARDIAC FIBROBLAST
MyD88
MyD88
Pro-inflammatory
Pro-fibrotic
NFκB p38 JNK
ERK AKT PKC-ε
Proinflammatory cytokines & chemokines
Angiogenesis
Cell migration
ECM degradation
ECM synthesis
Myofibroblast transdifferentiation
Cell proliferation
Cell migration
Myofibroblast transdifferentiation
ECM synthesis