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Cardiac Fibroblasts: At the Heart of Myocardial Remodeling

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ABSTRACT

Cardiac fibroblasts are the most prevalent cell type in the heart and play a key role in regulating normal myocardial function and in the adverse myocardial remodeling that occurs with hypertension, myocardial infarction and heart failure. Many of the functional effects of cardiac fibroblasts are mediated through differentiation to a myofibroblast phenotype that expresses contractile proteins and exhibits increased migratory, proliferative and secretory properties. Cardiac myofibroblasts respond to proinflammatory cytokines (e.g. TNFα, IL-1, IL-6, TGF-β), vasoactive peptides (e.g. angiotensin II, endothelin-1, natriuretic peptides) and hormones (e.g. noradrenaline), the levels of which are increased in the remodeling heart. Their function is also modulated by mechanical stretch and changes in oxygen availability (e.g. ischaemia-reperfusion). Myofibroblast responses to such stimuli include changes in cell proliferation, cell migration, extracellular matrix metabolism and secretion of various bioactive molecules including cytokines, vasoactive peptides and growth factors. Several classes of commonly prescribed therapeutic agents for cardiovascular disease also exert pleiotropic effects on cardiac fibroblasts that may explain some of their beneficial outcomes on the remodeling heart. These include drugs for reducing hypertension (ACE inhibitors, angiotensin receptor blockers, beta-blockers), cholesterol levels (statins, fibrates) and insulin resistance (thiazolidinediones). In this review, we provide insight into the properties of cardiac fibroblasts that underscore their importance in the remodeling heart, including their origin, electrophysiological properties, role in matrix metabolism, functional responses to environmental stimuli and ability to secrete bioactive molecules. We also review the evidence suggesting that certain cardiovascular drugs can reduce myocardial remodeling specifically via modulatory effects on cardiac fibroblasts.

Key words: heart; fibroblast; myofibroblast; remodeling; therapeutic agents

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Abbreviations:

15d-PGJ2	15-deoxy-$\delta^{12,14}$-prostaglandin J2
α-SMA	Alpha smooth muscle actin
ACE	Angiotensin converting enzyme
Ang II	Angiotensin II
ANP	A-type (atrial) natriuretic peptide
ARB	Angiotensin receptor blocker
AT1R/AT2R	Angiotensin receptor type 1/2
β-AR	Beta adrenergic receptor
BM	Bone marrow
BNP	B-type (brain) natriuretic peptide
CF	Cardiac fibroblast
CNP	C-type natriuretic peptide
CTGF	Connective tissue growth factor
ECM	Extracellular matrix
EPDC	Epicardial-derived cells
ERK	Extracellular signal-regulated kinase
ET-1	Endothelin-1
ETA/ETB	Endothelin receptor type A/B
FGF	Fibroblast growth factor
FPP	Farnesyl pyrophosphate
GGPP	Geranylgeranyl pyrophosphate
HF	Heart failure
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
IL-1	Interleukin-1

IL-6	Interleukin-6
ISO	Isoproterenol
MAPK	Mitogen-activated protein kinase
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MT-MMP	Membrane-type matrix metalloproteinase
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
myoFb	Myofibroblast
NA	Noradrenaline
NF-κB	Nuclear factor kappa B
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositol-3-kinase
PPAR	Peroxisome proliferator-activated receptor
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
TGF-β	Transforming growth factor beta
TIMP	Tissue inhibitor of metalloproteinase
TNFα	Tumor necrosis factor alpha
TZD	Thiazolidinedione
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

In the mammalian heart normal cardiac function is regulated by co-coordinated and dynamic interactions of the two major cell types, cardiomyocytes and cardiac fibroblasts (CF), which together account for 90% of the cells in the myocardium. CF, which account for 60-70% of the cells in the human heart, are a key source of components of the extracellular matrix (ECM) that regulates the structure of the heart and hence mechanical, chemical and electrical signals between the cellular and non-cellular components. Cardiomyocytes, whilst fewer in number, occupy the bulk volume of the myocardium and are contractile cells that provide mechanical force, transmission of which is one of the principal functions of the ECM network. Collagen is the major stress-bearing element within the ECM and forms a three-dimensional network around bundles of myocytes to generate a stress-tolerant network. Hence the importance of CF extends far beyond being simple regulators of ECM production. These cells are critical not only to normal myocardial function, but also in the remodeling that occurs in response to pathological changes, such as hypertension, myocardial infarction (MI) and heart failure (HF). Many of the functional effects of CF are mediated through differentiation of CF to myofibroblasts (myoFb), cells that express contractile proteins, including alpha-smooth muscle actin (α-SMA), and exhibit increased migratory, proliferative and secretory properties. Cardiac myoFb are particularly responsive to proinflammatory cytokines (including tumor necrosis factor-alpha (TNFα), interleukin-1 (IL-1), interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β)), vasoactive peptides (including angiotensin II (Ang II), endothelin-1 (ET-1) and A- and B-type natriuretic peptides (ANP, BNP)) and hormones (including noradrenaline (NA)), the levels of which are increased in the remodeling heart. Cardiac myoFb function is also modulated by mechanical stretch and conditions of ischaemia and reperfusion. Cardiac myoFb respond to these stimuli by altering their proliferative and migratory properties, modifying ECM turnover through altered production of ECM proteins and matrix metalloproteinases (MMPs), and modulating secretion of bioactive molecules including cytokines (TNFα, IL-1, IL-6, TGF-β),

vasoactive peptides (Ang II, ET-1, ANP, BNP) and growth factors (e.g. vascular endothelial growth factor (VEGF)).

A number of widely prescribed therapeutic agents for treatment of cardiovascular disease have been shown to exert pleiotropic effects on CF that underlie some of their beneficial effects on the remodeling heart. These include anti-hypertensive agents (ACE inhibitors, angiotensin receptor blockers, beta-blockers), lipid-lowering drugs (statins, fibrates) and drugs for lowering insulin resistance (thiazolidinediones; TZDs). The success of many of these pharmacological agents in treatment of cardiovascular disease may therefore be due to additional off-target effects on CF.

The importance of selecting appropriate models is critical in interpreting the value of experimental data. Much of the data on the role of the CF has been obtained from non-human animal models (rat, mouse, rabbit and dog). Moreover, the vast majority of information on CF has been generated from in vitro cell culture models that do not take into account many of the complex interactions that regulate cell behaviour in vivo. Many studies have employed neonatal rat CF as a convenient model, however caution should be exercised in extrapolating results from these developmentally immature cells to those of the adult CF. Throughout this review we have referred to the species and developmental stage of CF to aid the reader in their interpretation. Nevertheless, in vitro studies remain at the forefront of approaches to delineate the role of individual molecules and their underlying mechanisms in the responses of specific cell types to defined stimuli.

In this review, we will give a detailed insight into the properties of CF that underscore their importance, including their origin, organization, distribution, electrophysiological properties and role in physiological and pathological myocardial remodeling. We will consider their key role in ECM metabolism, highlight the functional responses of CF to changes in their environment, and discuss their significance as a source of bioactive molecules. Finally, we will review evidence that proposes that CF are important targets for the pleiotropic effects of many cardiovascular drugs that reduce adverse myocardial remodeling.

2. CARDIAC FIBROBLASTS

The major cellular components of the heart are cardiomyocytes, fibroblasts and vascular cells (smooth muscle, endothelium). Fibroblast cells are phenotypically diverse and experimental evidence has indicated that considerable heterogeneity exists between fibroblasts from different tissues (Brown et al., 2005).

2.1. Origin of cardiac fibroblasts

Seminal studies performed over two decades ago revealed that following birth, the neonatal heart adapts to a sudden increase in systolic pressure by increasing ventricular wall thickness and tensile strength (Borg et al., 1984). This occurs as a result of a significant increase in numbers of CF together with synthesis and deposition of collagen fibrils that provides contact between myocytes and fibroblasts, the fibroblasts themselves or the ECM. Whilst there is considerable knowledge concerning the structure and function of cardiomyocytes, much less is known about CF.

There are reportedly two major sources of CF. Firstly, it has been shown that CF originate from a spatiotemporal locus in the developing embryo and are derived from mesenchymal cells originating in the proepicardial organ (Moorman and Christoffels, 2003; Norris et al., 2008). These cells migrate over the surface of the heart to form the epicardium which in turn give rise to epicardial-derived cells (EPDCs) that later in development invade the myocardial walls and establish a compact myocardium through interaction with cardiomyocytes (Manner et al., 2001). In vivo these EPDCs within the wall of the heart progressively differentiate into fibroblasts by undergoing a defined sequence of events that allows them to acquire their differentiated phenotype which is strictly regulated by a sequence of coordinated expression of a number of growth factors, including platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF)(Kalluri and Neilson, 2003; Wessels and Perez-Pomares, 2004). During embryonic development the EPDC-derived cells can alternate between the spindle-shaped fibroblast and the α-SMA-expressing myoFb

phenotype. Ultimately most EPDCs assume the fibroblast phenotype which in adult life can be reactivated to a myoFb during events that trigger pathological remodeling (Norris et al., 2008). In normal healthy myocardium however, myoFb are not typically resident.

In addition to EPDC-derived CF, it is thought that some fibroblasts may originate from progenitor stem cells present in the circulation or in the heart itself. Recent evidence has shown postnatal recruitment of circulating fibroblast progenitor cells into the ventricular myocardium (Visconti and Markwald, 2006). Irrespective of their origin, CF through the production of growth factors, cytokines, proteases and ECM components have been shown to be vital for tissue repair and fibrosis (Brown et al., 2005; Brown et al., 2007).

2.2. Organization of cardiac fibroblasts

At the cellular level, the normal adult human heart comprises 30% cardiomyocytes and 70% non-myocytes, of which the majority are CF (Jugdutt, 2003b). CF are arranged in sheets and strands that run in parallel with muscle fibres and maintain continuity between cells in the different layers of the myocardium (Kohl et al., 2005). CF are recognized primarily for their ability to maintain the structural integrity of the heart through controlled proliferation and ECM turnover (Brown et al., 2005; Camelliti et al., 2005; Weber, 2004). However, the organization of fibroblasts in the normal heart is such that, in addition to maintaining ECM integrity, they are also essential for other physiological functions that are determined by dynamic and coordinated cell-cell and cell-matrix interactions. In this role, fibroblasts respond to a variety of mechanical, electrical and chemical stimuli that are pivotal to maintaining normal cardiac function (Banerjee et al., 2006; Kohl et al., 2005). Thus, in addition to CF being fundamental to the normal structure and function of the heart, differentiated myoFb play a key role in adverse myocardial remodeling - a pathological process that can ultimately lead to HF. Therefore the key role of CF in both myocardial physiology and pathophysiology and their unique properties compared with fibroblasts from other tissues, both in

terms of developmental lineage and in their response to cytokines (Brown et al., 2005), makes them a potentially appealing therapeutic target for the treatment of the failing heart.

2.3. Fibroblast distribution in the heart

There are relatively few studies that have examined differences in fibroblast density in the heart although it is known that their number increases with aging and during disease (Camelliti et al., 2006). Indeed, other studies have also reported that fibroblast numbers fluctuate in response to physiological signals (Goldsmith et al., 2004; Kohl, 2004; Morales et al., 2005). During cardiac remodeling and repair the number of fibroblasts increases locally, for example increased density of fibroblasts is observed in the vicinity of healing scar tissue following MI (Holmes et al., 2005) and in mature infarct scars a persistence of myoFb has been documented (Sun and Weber, 2000).

2.4. Electrophysiology of cardiac fibroblasts

A key role of CF is that of maintaining the ECM, and connective tissue has been generally perceived as being an insulator as far as myocardial electrical signaling is concerned. The traditional view was that in disease states, alterations in cardiac structure due to increased CF proliferation would present more obstacles to conduction and hence lead to arrhythmias. Studies in recent years have now revealed data that challenge this concept.

Although CF are electrically unexcitable they can contribute to the electrophysiology of myocytes. It is well established that in cell cultures, single fibroblast cells are capable of synchronizing contraction between individual myocytes (Goshima and Tonomura, 1969), and observed membrane potential fluctuations in the interconnecting fibroblasts provide evidence of electrical communication. More recently, CF were shown to modify electrophysiological properties of myocytes in coculture, suggestive of electrical coupling between the two cell-types (Feld et al., 2002). CF respond to myocardial contraction (mechanical stretch) with changes in their resting potential, known as the mechanically-induced potential (Kamkin et al., 2003). Such changes may

contribute to increased risk of post-MI arrhythmias, a proposal supported by observations that the sensitivity of CF to mechanical stimulation is enhanced after MI and correlates with decreased heart rate. In vitro differentiated myoFb express a variety of K^+ channel subunits, and the inwardly rectifying K^+ current has been shown to modulate resting membrane potential, thus altering essential physiological functions, proliferation and collagen contraction (Chilton et al., 2005).

Intercellular transfer of electrical signals between CF and myocytes occurs via gap junctions that are characterized by expression of connexin (Cx) proteins. Co-cultured neonatal rat myocytes and CF readily form functional gap junctions comprising Cx43 and Cx45 (Gaudesius et al., 2003; Rook et al., 1992) that can synchronize electrical activity. The first in vivo studies of this nature were performed in the rabbit sinoatrial node, where fibroblasts preferentially expressed Cx40 in fibroblast-rich areas and Cx45 when intermingled with myocytes (Camelliti et al., 2004b). Temporal and spatially distinct expression of Cx43 and Cx45 have been demonstrated in a sheep MI model (Camelliti et al., 2004a) and were related to progression of infarction.

Fibroblasts can be genetically modified to produce excitable cells capable of electrical coupling (Kizana et al., 2005) supporting the prospect of developing gene-based strategies for repairing cardiac conduction defects. Mutant Cx43 variants have been shown to diminish intercellular coupling and normalise cardiac conductivity (Kizana et al., 2007). The functional relevance of Cx43 was recently highlighted in a study of murine CF whereby intercellular coupling was increased by Cx43 overexpression and decreased by genetic ablation; moreover proliferation was inversely associated with Cx43 expression levels (Zhang et al., 2008). In principle therefore, Cx expression has potential to alter CF function, ECM turnover and ventricular remodeling although how this might be tested in vivo presents considerable challenges.

2.5. Role of cardiac fibroblasts in adaptive remodeling and scar formation

CF are key components of the myocardial ECM due to their ability to synthesize and secrete fibrillar collagen types I and III (Eghbali et al., 1989). The regulation and turnover of the ECM and

fibroblasts contained within it are crucial to normal myocardial homeostasis as well as in the response to pathophysiological stimuli. The myocardial remodeling that accompanies HF progression is characterized by cardiomyocyte hypertrophy, apoptosis and necrosis (Cohn et al., 2000; Swynghedauw, 1999). Under these circumstances, normally quiescent CF undergo phenotypic modulation to myoFb (Brown et al., 2005; Camelliti et al., 2005; Cohn et al., 2000; Swynghedauw, 1999; Weber, 2004). Differentiating fibroblasts express α-SMA, indicating acquisition of a secretory, myoFb phenotype, a transition that correlates with increased secretion of profibrotic molecules such as collagen and fibronectin (Gabbiani, 1998; Petrov et al., 2002).

Activated myocardial fibroblast cells are responsive to specific hormones, growth factors and proinflammatory cytokines, the levels of which are increased in the remodeling heart. In response to such stimuli, cardiac myoFb become highly proliferative and invasive, and actively remodel the cardiac interstitium by increasing secretion of ECM-degrading metalloproteinases (MMPs) and increasing collagen turnover (Brown et al., 2005; Camelliti et al., 2005; Weber, 2004). The fibroblasts themselves secrete increased amounts of growth factors and cytokines (see Section 5), and a number of these cytokines such as IL-1β and TNFα have been co-localized in fibroblasts. In this situation they can activate MMPs and regulate TIMPs (tissue inhibitors of metalloproteinases) (Jugdutt, 2003a), thereby contributing to remodeling (Mann and Spinale, 1998). Although these changes serve initially as an important reparative wound healing response, in the longer term they become maladaptive leading to net accumulation of collagen, cardiac fibrosis and loss of cardiac function. Irrespective of the nature of an initial injury, wound repair in most tissues and organs follows a common sequence of events that culminates in remodeling during which the early inflammatory and repair processes decline and cease (Gurtner et al., 2008).

Phenotypically transformed myoFb are not observed in the normal myocardium but are abundant at infarct sites where synthesis and deposition of collagen promotes scar formation and fibrosis (Sun and Weber, 1996). MyoFb are more motile than undifferentiated CF and can contract collagen gels and as such are believed to be important in maintaining structural integrity of healing

scars (Sun and Weber, 2003). They confer mechanical tension to the remodeling matrix by anchoring and contracting; hence closing the wound and limiting scarring (Gabbiani, 2003). Synthesis and deposition of collagen provides tensile strength. As the scar matures, myoFb undergo apoptosis and leave a healed scar composed predominantly of cross-linked collagen and other matrix proteins with a low cellular content (Gurtner et al., 2008). However, chronic or repeated injury in the heart overcomes these regulatory mechanisms and as such, fibrous tissue deposition may be a persistent process rather than a transient one. In contrast to skin, where myoFb undergo apoptosis once healing is complete (Desmouliere et al., 1995), it is still unclear why cardiac myoFb persist in mature infarct scars. MyoFb have been observed six months post-MI in a rat model (Sun and Weber, 2000), and in post-mortem human myocardium, they have been shown to persist for months (Willems et al., 1994) or years (Jugdutt, 2003a). Persistence of myoFb can thus facilitate hypertrophic scarring and fibrosis that directly influences pathological remodeling, compromises cardiac function and ultimately leads to HF (Frangogiannis, 2006; Sun and Weber, 2000).

2.6. Origin of cardiac fibroblasts in the remodeling heart

The classical model of myoFb being derived from CF resident in the myocardium has been re-evaluated in recent years following observations that a proportion of CF/myoFb in remodelling areas of the heart can also be derived from circulating hematopoietic bone marrow (BM)-derived cells, as well as from endothelial cells.

The contribution of BM-derived cells to the post-MI healing and remodeling process has been studied in murine experimental MI models in which BM is replaced with an enhanced green fluorescent protein (eGFP)-labeled stem cell pool (Möllmann et al., 2006; Zeisberg et al., 2007; van Amerongen et al., 2008). One study reported that as many as 57% of myoFb in the infarct zone co-expressed eGFP 7 days after MI, indicating these cells were of a BM-derived origin. CF and myoFb in the remote areas were found not to express eGFP (Möllmann et al., 2006). In a second study, up to 24% of myoFb in the infarct area were shown to be collagen I-producing BM-derived myoFb

(van Amerongen et al., 2008). These findings are in direct contrast to an earlier study in which eGFP-BM from transgenic mice was transplanted into immunodeficient nude rats (Yano et al., 2005). In that study, myocardial scar fibroblasts were found to be derived exclusively from an existing fibroblast population. However, the choice of recipient model (T cell-deficient nude rats) may itself have affected the ability of BM-derived cells to differentiate into myoFb (van Amerongen et al., 2008).

A role for hematopoietic cell-derived fibroblasts has also been observed in a murine model of fibrotic ischemic cardiomyopathy in the absence of cardiomyocyte death (Haudek et al., 2006; Haudek et al., 2008). A proportion of fibroblastic cells populating the fibrotic myocardium were found to be derived from monocytes. These spindle-shaped highly proliferative cells represented 3% of all live non-myocyte cells and co-expressed myoFb markers (collagen I and α-SMA), hematopoietic markers (CD45) and primitive cell markers (CD34) (Haudek et al., 2006). Monocyte-to-fibroblast transformation has also been reported in the infarcted myocardium of a mouse MI model (Fujita et al., 2007) and in fibrotic areas of a pressure overload model (Endo et al., 2007; Zeisberg et al., 2007). Recent data suggest that monocyte-to-fibroblast differentiation is dependent on transendothelial migration in response to monocyte chemoattractant protein-1 (Haudek et al., 2008).

Evidence for derivation of myocardial fibroblasts from endothelial precursor cells has come from a murine pressure overload model, in which endothelial-to-mesenchymal transition contributed to the total pool of CF in fibrotic areas of the heart (Zeisberg et al., 2007). This source of fibroblasts was entirely separate from BM-derived CF that were also observed in the fibrotic myocardium in the same study (Zeisberg et al., 2007).

Thus, it is apparent that resident myocardial CF are not the sole source of myoFb in the remodeling heart. Non-resident cells derived from other cell types (including monocytes and endothelial cells) account for a small, but functionally significant, proportion of these CF that infiltrate the heart in response to ischemia, MI or pressure overload.

2.7. Cardiac fibroblasts in vitro

During studies in our own laboratories conducted in recent years we have consistently observed that primary cells cultured from human atrial appendage are already differentiated into myoFb at the first passage independently of an additional stimulus to promote this phenotypic change (Porter et al., 2004b). Moreover, they maintain this phenotype through subsequent passages, evidenced by consistent and sustained expression of α-SMA (Mughal et al., 2009; Porter et al., 2004b). Our findings differ, however from those of another study in which primary rat ventricular CF retained a fibroblast-like phenotype up to third passage, before acquiring a myoFb phenotype characterized by increased α-SMA expression (Teunissen et al., 2007). Studies in cardiac and other tissues have frequently reported the requirement for a specific stimulus, notably TGF-β, to induce fibroblasts to differentiate to a myoFb phenotype (Hao et al., 2008; Leask, 2007; Lenga et al., 2008); it has also been demonstrated that ovine CF in culture adopt a myoFb phenotype in the presence or absence of cytokine stimulation (Jarvis et al., 2006).

In view of these markedly differing reports, a full characterization of the source of fibroblasts under investigation is paramount (animal or human, adult or neonatal, atrial or ventricular, in vivo or in vitro) and their relevance to the clinical scenario should be interpreted with caution. Interestingly, a recent study reported that human atrial CF cultured in Dulbecco's modified Eagles medium have a myoFb phenotype, but the same cells grown in endothelial growth medium were shown to maintain a fibroblast phenotype (Rossini et al., 2008), suggesting that culture conditions alone can affect phenotype. Another study suggested that TZDs can prevent the phenotypic conversion of fibroblasts to myoFb, an effect that would potentially confer beneficial anti-fibrotic effects (Burgess et al., 2005; Hao et al., 2008). However, in our own studies using spontaneously differentiated human atrial myoFb, such an effect would be impossible to determine, although we have confirmed that TZDs do not reverse this phenotype (Mughal et al., 2009).

It is clear therefore that the cardiac myoFb phenotype adopted in culture can be induced by a plethora of stimuli and/or culture conditions. Importantly, it has been shown that cultured myoFb characteristics can be markedly influenced by the site from which they are derived, for example, infarcted or non-infarcted myocardium (Jarvis et al., 2006).

The key aspects of CF function of relevance to myocardial remodeling therefore include proliferation, migration, differentiation, ECM turnover and secretion of growth factors and cytokines (summarized in Figure 1). These facets of fibroblast function will serve as the focus for discussion in subsequent sections of this review.

3. CARDIAC FIBROBLASTS ARE KEY REGULATORS OF ECM TURNOVER

In the normal heart CF are recognized chiefly as regulators of ECM metabolism, thereby maintaining myocardial structure. In this regard, these cells control ECM homeostasis and produce growth factors, cytokines and MMPs that maintain a balance between synthesis and degradation of ECM components. In order to maintain ECM homeostasis the synthetic and degradative aspects of ECM metabolism are continuously ongoing, but tightly regulated (Jugdutt, 2003a). However, in cardiovascular disease CF/myoFb perform pivotal roles in remodeling characterized by cardiomyocyte death or hypertrophy, migration and proliferation of fibroblasts and changes in the synthesis and deposition of ECM. Although such events serve initially as an important adaptive response that may augment cardiac function, in the long term this progresses to maladaptation and ultimately HF. The turnover of ECM and the fibroblasts contained within are therefore important not only to normal cardiac function but also in pathological states.

3.1. Main components of myocardial ECM

The non-cellular component of the heart, the ECM, forms an organized network surrounding and connecting the cellular components. It is a highly differentiated structure that comprises a 3-dimensional network that includes interstitial collagens, proteoglycans, glycoproteins and proteases (Eghbali and Weber, 1990; Corda et al., 2000; Holmes et al., 2005). The major fibrillar collagens are type I and type III that comprise approximately 80% and 10% of the ECM, respectively. Other less abundant matrix molecules are collagens type IV, V and VI, elastin and laminin (Bosman and Stamenkovic, 2003; Jugdutt, 2003a). In different regions of the heart the arrangement of collagen, although qualitatively similar, can differ quantitatively with respect to type I and III collagens, for example between the atria and ventricles (Bing et al., 1997).

The ECM network provides a support for the myocardial cellular fractions and in addition is responsible for distribution of mechanical forces and signal transduction to individual cells throughout the myocardium via cell surface ECM receptors. CF themselves form a highly organized network within which they are not only connected to other fibroblasts but also cardiomyocytes via cell-cell contacts (Goldsmith et al., 2004). Indeed, fibroblast-fibroblast and fibroblast-myocyte contacts are apparent, together with evidence of functional coupling in both normal and diseased myocardium (Camelliti et al., 2004a; Camelliti et al., 2004b). Cell junction proteins such as connexin-43 have been localized on both cardiomyocytes and fibroblasts (Goldsmith et al., 2004) and appear to connect adjacent fibroblasts to one another or to cardiomyocytes. Similarly, cadherins are likely to be important in cellular communications and play a role in physical connection between fibroblasts and other myocardial cells (Angst et al., 1997).

In the healthy heart, collagen serves to maintain normal cardiac architecture that assists in co-coordinating the contractile capacity of the cardiomyocytes. Fibrillar collagen plays a crucial role in maintaining cardiac shape, size and function due to its relatively rigid structure that is in close contact with all the other cellular and non-cellular components of the myocardium (Bishop and Laurent, 1995). Since myocardial collagen is not a static protein, ECM homeostasis therefore requires equilibrium between synthesis and degradation that is maintained by coordinated activity

of stimulators and inhibitors. However, in the failing heart activation of a number of humoral, autocrine and paracrine pathways determines how ECM metabolism is regulated and ultimately dictates the extent of myocardial remodeling. In this respect, changes in the balance of ECM synthesis and degradation may thus lead to disruption of the composition of the collagen network in the heart.

3.2. Role of cardiac fibroblasts in ECM degradation

Collagen degradation requires the expression and activity of proteolytic enzymes, the MMPs, that constitute a family of more than 20 zinc-dependent enzymes that collectively have the capacity to degrade all the components of the ECM (Raffetto and Khalil, 2008; Visse and Nagase, 2003). There are two principal types of MMPs; those that are secreted into the extracellular space as latent proenzymes, which constitute the majority of known MMPs. In addition are the more recently described membrane-bound (membrane-type (MT)-MMPs), of which there are six (Visse and Nagase, 2003). All are tightly regulated at three levels – transcription, activation of latent pro-enzymes and by their naturally-occurring inhibitors, TIMPs (Nagase and Woessner, Jr., 1999). Although MMPs undoubtedly play an important role in maintenance of normal cardiac architecture and function, increased expression and activation are associated with progression to pathological states. Prolonged activation therefore leads to excessive ECM degradation and impaired healing (Baudino et al., 2006).

Following a myocardial injury, CF responses are influenced by cytokines and/or growth factors released from inflammatory cells, cardiomyocytes or the CF themselves. Moreover, the ability of CF to respond to such factors is advocated by the expression of appropriate receptors for example TGF-β, Ang II, endothelin (Weber, 1997) and proinflammatory cytokines (Brown et al., 2007; Porter et al., 2004a; Turner et al., 2007b). Cytokine-induced CF migration is vital to wound healing and scar formation, and requires co-coordinated expression and activity of MMPs (Brown et

al., 2007). The mechanisms by which MMPs, TIMPs and bioactive molecules interact are complex, but their contribution to HF progression is undoubtedly substantial.

Activated cardiac myoFb play a pivotal role in the wound healing response by initiating a cascade of events in order to restore tissue integrity and homeostasis. This sequence of events is similar to that observed in other tissues and likewise, scar formation leads to similar tissue dysfunction irrespective of the nature of the injury or the affected organ (Gurtner et al., 2008). In response to initiating injury CF degrade fibrillar collagen by expression and sequential activity of MMPs thus permitting migration and proliferation to the zone of injury to repopulate areas of necrotic myocytes and cell debris (Deschamps and Spinale, 2006; Tao et al., 2004). The importance of bioactive molecules in the regulation of MMP/TIMP activity in the myocardium and progression to HF has been previously reviewed (Tsuruda et al., 2004). Not all MMPs are expressed by CF; this review will therefore focus exclusively on expression of MMPs specifically in this cell-type.

3.2.1. MMPs

CF from a variety of species have been shown to express a limited subset of MMPs, including collagenases (MMP-1, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3) and membrane-type MMPs (predominantly MT1-MMP). A variety of chemical, physical and environmental stimuli modulate MMPs in CF by inducing gene expression and/or activation of the latent proenzyme. Table 1 summarizes the modulatory effects of key stimuli detailed in this review on MMP expression by CF of both human and animal origin. MMP-1 (collagenase I) expression can be induced by IL-1, BNP, PDGF and anoxia-reoxygenation, but is consistently reduced by Ang II treatment. MMP-2 (gelatinase A) is constitutively secreted by CF in culture, although its expression can be further induced by cytokines (IL-1β, TNFα, TGF-β), BNP, oxidative stress and mechanical loading. In contrast, Ang II generally decreases MMP-2 secretion in mouse and rat CF. Cytokines, PDGF, collagen and oxidative stress can induce activation of secreted MMP-2, while Ang II and hypoxia decrease MMP-2 activation. MMP-3 (stromelysin-1) secretion from CF can be

induced by IL-1 and BNP. MMP-9 (gelatinase B) expression by CF is very low under basal conditions, but is markedly increased in response to proinflammatory cytokines and oxidative stress. Reports on the effects of Ang II on MMP-9 secretion are at variance, with increased expression observed in adult human CF (Pan et al., 2008), but decreased expression in adult murine CF (Stacy et al., 2007). CF exhibit increased expression and activity of MMP-13 (collagenase-3) in response to IL-1β and oxidative stress. Finally, cellular expression of MT1-MMP (MMP-14) is increased in CF in response to TGF-β, BNP, collagen and stretch. In summary, proinflammatory cytokines, BNP, mechanical stretch and oxidative stress generally increase expression and activity of multiple MMPs, whereas Ang II has opposing effects by reducing MMP expression and activity (Table 1).

3.2.2. TIMPs

The TIMPs are locally synthesized secreted proteins with multiple functions although their principal role is that of inhibiting MMPs. Four TIMPs have been identified that inhibit the activity of various MMPs by binding in a 1:1 stoichiometry with differential affinities (Li et al., 1998). In normal cardiac tissue MMPs and TIMPs are co-expressed and tightly regulated to maintain integrity of the cardiac interstitium (Tyagi et al., 1995b). As such the MMP/TIMP system is a key contributor to ECM turnover and its dysregulation plays a vital role in myocardial remodeling and progression to HF. Clinical studies have revealed that in the failing heart, whilst the activity of MMPs is increased, TIMPs are decreased (Li et al., 1998; Thomas et al., 1998; Tyagi et al., 1996); a scenario where MMP/TIMP balance is disturbed leads to changes in net proteolytic activity and a potentially deleterious outcome (Spinale et al., 2000).

CF are the key source of TIMPs in the myocardium (Tyagi et al., 1995a) and they can be modulated at the level of expression and/or activity by chemical, physical and environmental stimuli. Although there are four TIMP isoforms, TIMP-1 and TIMP-2 are predominant in CF. For example, Ang II increased TIMP-1 and TIMP-2 in adult rat CF (Jiang et al., 2007; Lijnen et al.,

2008), although in human CF Ang II reportedly decreased TIMP-1, TIMP-2 and TIMP-3 mRNA expression (Pan et al., 2008). In neonatal rat CF, TNFα stimulated an increase in TIMP-1 protein both directly and indirectly, via increased angiotensin receptor expression and subsequent stimulation with Ang II (Peng et al., 2002). In adult rat CF, IL-1β stimulated a robust increase in TIMP-1 secretion, but TIMP-2 was constitutively secreted and was not changed by any of a number of different cytokines (Brown et al., 2007). In these same studies, TIMP-3 and TIMP-4 were not detected. In neonatal rat CF, TIMP-1 expression was enhanced during reoxygenation after hypoxia (Makino et al., 2006) and in adult rat CF, mechanical stretch increased TIMP-2 expression (Husse et al., 2007). Our own recent data show that cultured human atrial myoFb express very high levels of TIMP-1 and TIMP-2 mRNA under basal conditions, but TIMP-3 mRNA was undetectable (Turner and Porter 2008, unpublished).

Pharmacological modulation of TIMPs appears impracticable due to their short half life in vivo, and although MMP inhibition has been more extensively investigated, clear therapeutic strategies remain to be defined (Spinale, 2002).

3.3. Role of cardiac fibroblasts in ECM protein synthesis

In the normal heart collagen deposition is low, but is markedly increased in disease states, for example in hypertrophy, MI and HF (Weber, 1989). Whilst ECM degradation due to increased MMP activity dominates the early, adaptive wound-healing response after MI, enhanced collagen synthesis is a feature of the later stages of healing and results in increased ECM deposition (Brown et al., 2005; Eghbali and Weber, 1990). Early after an injury such as MI, a series of cellular responses are activated to promote tissue repair and scar formation in the infarct zone. However, in some cases the repair process involves myocardial tissue remote from the infarct, resulting in superfluous fibrous tissue being deposited in non-infarcted myocardium i.e. fibrosis (Sun and Weber, 2000). Fibrosis appears to underlie most cardiac pathologies where overproduction of ECM can alter the structure and architecture of the heart with deleterious effects on cardiac function

(Jugdutt, 2003b; Khan and Sheppard, 2006). The formation of myocardial scar tissue is believed to progress to congestive HF and/or myocardial arrhythmias that account for significant morbidity and mortality (Frangogiannis, 2006). Healed myocardial infarcts were originally thought to be inert structures of fibrillar cross-linked collagen but key studies that systematically examined remodeling in the canine heart revealed new perspectives on the dynamic nature of myocardial scar tissue (Jugdutt and Amy, 1986; Jugdutt et al., 1992).

3.3.1. Interaction of Ang II and TGF-β systems in myocardial fibrosis

At the molecular level, myocardial ECM remodeling is mediated by the activation of a number of neurohumoral systems, specifically the renin-angiotensin system (RAS), TGF-β and beta-adrenergic systems (Rosenkranz, 2004). How these bioactive molecules participate in and/or contribute to a broad range of CF functions key to remodeling are discussed in detail in Sections 4 and 5.

Evidence for a pivotal role of TGF-β in inducing cardiac fibrosis has been provided by overexpression and knockout studies (Nakajima et al., 2000; Rosenkranz et al., 2002; Schultz et al., 2002; Tomita et al., 1998). Moreover, TGF-β inhibition has been shown to ameliorate the pro-fibrotic effects of this cytokine in animal models (Kuwahara et al., 2002). A recent study using tissue from failing and non-failing human hearts demonstrated a significant increase in the transcript levels of TGF-β in the pathological heart tissue (Sivakumar et al., 2008), that were accompanied by significantly elevated levels of type I and III collagens. The key role of TGF-β in myocardial tissue remodeling and fibrosis has been extensively reviewed elsewhere (Bujak and Frangogiannis, 2007; Lijnen et al., 2000).

3.3.2. Profibrotic effects of Ang II and TGF-β on cardiac myofibroblasts

Ang II and TGF-β secreted by myoFb enhance collagen synthesis at both the infarct site and beyond. Moreover it has been shown that activated myoFb are capable of producing significantly

larger quantities of collagen than their fibroblast counterparts (Petrov et al., 2002). The resultant fibrosis in the myocardium is believed to be a major contributor to adverse remodeling observed in HF progression. Increasing evidence has defined a role for Ang II in influencing both synthesis and degradation of the myocardial ECM, and in vitro studies have demonstrated that Ang II stimulates cardiac myoFb fibrillar collagen expression via activation of the type 1 angiotensin receptor (AT1R)(Hafizi et al., 1998; Weber et al., 1994; Zhou et al., 1996). See also Section 4.3.2. Furthermore, in vivo studies have shown that Ang II, via the AT1R, stimulates fibrosis in infarcted hearts by promoting TGF-β synthesis (Sun et al., 1998).

CF are the primary source of TGF-β in the heart (Bujak and Frangogiannis, 2007) and several studies have provided clear evidence that Ang II indirectly regulates collagen synthesis in CF by upregulation of other growth factors, principally TGF-β (Dostal, 2001). There is now extensive evidence supporting a direct functional association between the RAS and the TGF-β pathways indicating that Ang II stimulation induces TGF-β expression by CF and myoFb (Campbell and Katwa, 1997; Crabos et al., 1994; Gray et al., 1998; Lee et al., 1995; Zhou et al., 1996), clearly demonstrating that TGF-β acts downstream of Ang II. Another study reported that both β-adrenergic stimulation (norepinephrine) and Ang II stimulated secretion of TGF-β in neonatal rat CF in vitro (Fisher and Absher, 1995). The classical signaling cascade from stimulation of the TGF-β receptor involves Smad proteins which are phosphorylated and translocate to the nucleus where they act as transcription factors (Moustakas et al., 2001). In cultured rat CF, Ang II stimulation directly initiated phosphorylation and translocation of Smad proteins, an effect dependent on the AT1R (Hao et al., 2000). This study thus provides further evidence of cross-talk between Ang II and TGF-β downstream of receptor activation at the level of Smad signaling.

It is clear that the RAS and TGF-β play essential independent and overlapping roles in the progression of myocardial remodeling (Rosenkranz, 2004). Although multiple systems and mechanisms are involved, networking between Ang II and TGF-β at the level of the CF is evident. Ang II induces expression of TGF-β in CF which in turn increases expression of ECM proteins.

However, due to its ability to regulate a multitude of cellular responses with often opposing effects (see Section 4.4), a greater understanding of the underlying signaling mechanisms will be required before therapeutic strategies targeted at modulation of TGF-β can be addressed.

4. HOW CARDIAC FIBROBLASTS RESPOND TO ENVIRONMENTAL STIMULI

CF respond to a diverse array of environmental stimuli that occur in the remodeling heart, including changes in mechanical stretch, oxygen levels, hormones and cytokines. Many of these stimuli serve as important activators of CF that facilitate early adaptive remodeling to aid myocardial repair or compensation. However, if these stimuli are prolonged beyond the duration of cardiac repair, then they also serve to drive pathological remodeling that leads to loss of cardiac function and HF progression.

4.1. Mechanical stretch

CF are exposed to cyclic mechanical stretch with every heart beat (frequency of ~1 Hz). Under pathological conditions, the frequency and force of stretch is altered and this results in compensatory effects at the level of ECM metabolism and CF function. The effects of mechanical stretch on CF have been reviewed in depth elsewhere (Gupta and Grande-Allen, 2006; MacKenna et al., 2000).

Mechanical stretch (both cyclic and static) decreased proliferation of neonatal rat CF, with a role for up-regulated $p21^{Waf1}$ and down-regulated cyclin B1 expression being proposed (Atance et al., 2004; Liao et al., 2004). Mechanical loading may also modulate the myoFb phenotype. For example, static stretch increased SMemb expression in cultured neonatal rat CF (Shiojima et al., 1999) and 1-day cultures of neonatal rat CF in which α-SMA expression was low (Wang et al., 2003). However, in mouse and rat cells with already high expression of α-SMA (i.e. myoFb), static stretch decreased α-SMA expression (Wang et al., 2000; Wang et al., 2001; Wang et al., 2003).

Mechanical loading exerts predominantly profibrotic effects on CF. In fetal, neonatal and adult rat CF, cyclic stretch induced expression of type I collagen (Butt and Bishop, 1997; Lindahl et al., 2002; Papakrivopoulou et al., 2004; Atance et al., 2004; Husse et al., 2007), and type III collagen (Carver et al., 1991; Husse et al., 2007). Cyclic loading also induced MMP expression, including MT-MMP in cultured human ventricular CF (Tyagi et al., 1998) and MMP-2 and TIMP-2 in adult rat CF (Husse et al., 2007).

Cytokine and growth factor expression by CF is also responsive to mechanical stretch. For example, stretch increased TNFα (Yokoyama et al., 1999), TGF-β (Ruwhof et al., 2000; van Wamel et al., 2001; Lindahl et al., 2002) and ET-1 (van Wamel et al., 2001; Pikkarainen et al., 2006) gene expression in rat CF. Static stretch (Lal et al., 2008), but not cyclic stretch (Ruwhof et al., 2000; van Wamel et al., 2001), also increased angiotensinogen gene expression in neonatal rat CF.

4.2. Ischaemia-reperfusion

The cessation of blood flow to the myocardium caused by blockage of the coronary arteries results in a reduced supply of oxygen and nutrients (ischaemia) to the heart tissue i.e. MI. This leads to necrotic death of the affected regions of heart muscle within several hours. Rapid reperfusion of the infarcted myocardium is therefore essential for restoring function, but this can result in further damage to the heart most notably through increased production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide. Compared with CF, cardiomyocytes are particularly sensitive to hypoxia and ROS production and undergo necrotic and apoptotic cell death under the same conditions that stimulate CF proliferation (Li et al., 1999). Thus, CF are able to adjust to altered oxygen tension and modify their activity appropriately. Indeed, “normoxia” for CF is an adjustable variable, and restoration of oxygen supply to hypoxia-adjusted cells results in “perceived hyperoxia” which stimulates myoFb transformation and inhibition of cell cycle progression in mouse CF (Roy et al., 2003b; Roy et al., 2003a; Roy et al., 2007).

Exposure of CF to hypoxia stimulates myoFb differentiation (Clancy et al., 2007), reduces cell proliferation (Agocha et al., 1997a; Griffin et al., 2000; Zhao and Eghbali-Webb, 2002) and MMP-dependent invasion (Morley et al., 2007), and increases collagen synthesis (Agocha et al., 1997a; Tamamori et al., 1997). Recently we have shown that hypoxia reduces MMP-2 activity without affecting expression levels in human atrial myoFb (Morley et al., 2007), which contrasts with a prior report showing hypoxia-induced increases in MMP-2 expression in neonatal rat CF (Bergman et al., 2003). Hypoxia can also stimulate CF to secrete cytokines and growth factors, including TNFα and VEGF (Shivakumar et al., 2008; Clancy et al., 2007). Indeed, myocardial ischaemia promotes increased TNFα expression and secretion specifically from CF in rat hearts (Shames et al., 2002).

Reoxygenation of anoxic adult CF leads to increased cell proliferation (Chen et al., 2004b). However, perceived hyperoxia in mouse CF inhibits cell cycle progression through a mechanism involving increased expression of $p21^{Cip1}$ (Roy et al., 2003b; Roy et al., 2003a; Roy et al., 2007). Reoxygenation is also a potent stimulus for increased ECM turnover, resulting in synthesis of collagen I and III, MMPs and TIMPs by CF (Chen et al., 2004b; Makino et al., 2006; Grobe et al., 2007). Hypoxia/reoxygenation also stimulates TGF-β and BNP synthesis in neonatal rat CF (Grobe et al., 2007; Makino et al., 2006). The effects of ROS (superoxide and hydrogen peroxide) on neonatal rat CF have been investigated and shown to induce cell proliferation (Li et al., 1999), decrease collagen synthesis (Siwik et al., 2001) and increase expression and activity of multiple MMPs (Siwik et al., 2001).

4.3. Neurohormonal stimuli, vasoactive peptides and adenosine

4.3.1. Noradrenaline

Myocardial and circulating levels of the catecholamines adrenaline and NA are elevated in HF patients and are associated with worse outcome (Swynghedauw, 1999). Increased catecholamine levels increase adrenergic drive and the force and rate of contraction of the heart to compensate for

reduced pumping capacity. In addition to these cardiomyocyte-directed effects, catecholamines can directly modulate CF function.

Catecholamines elicit cellular effects via activation of specific adrenergic receptors (AR) located on the cell surface of target cells. Adrenaline and NA can both activate members of the α-AR and β-AR families of receptors. CF express β2-AR, but not β1-AR, β3-AR or members of the α-AR family (Meszaros et al., 2000; Gustafsson and Brunton, 2000; Turner et al., 2003; Yin et al., 2003b). See Section 6.2.1 for more detail on β-AR expression in CF. Thus, the effects of catecholamines on CF are mediated predominantly via activation of the β2-AR.

In vivo stimulation of β-AR has been reported to induce myoFb activation and myocardial remodeling. In a rat model of myocardial injury induced by isoproterenol (ISO; a non-selective β-AR agonist), α-SMA-expressing cardiac myoFb were observed at the border of the injured area 3-7 days after ISO injection, with peak myoFb proliferation observed after 3 days (Nakatsuji et al., 1997). In a similar model, proliferation of CF in ISO-treated hearts was purported to be a direct response to ISO rather than a result of myocyte loss (Benjamin et al., 1989).

There is good evidence that β2-AR stimulation induces proliferation of CF in culture (Table 2). NA, ISO and clenbuterol (a selective β2-AR agonist) all induce proliferation of CF from multiple species (human, rat, rabbit) and developmental stages (adult, neonatal), both at the level of DNA synthesis and increased cell number. However there is variation in the proposed mechanism of cell proliferation, specifically whether β2-AR stimulation is itself mitogenic, or whether it stimulates proliferation via up-regulation of secreted autocrine growth factors, as we and others have observed (Turner et al., 2003; Turner et al., 2004; Leicht et al., 2003).

The effects of β-AR stimulation on differentiation of CF to myoFb have not been thoroughly investigated. Although one study found that NA had no modulatory effects on TGF-β-induced differentiation of adult rabbit CF (Eghbali et al., 1991b), it has been reported more recently that ISO reduces TGF-β-induced differentiation of rat CF into myoFb (Liu et al., 2006).

NA was shown to induce ECM protein expression (collagen I, fibronectin) in one study of neonatal rat CF (Akiyama-Uchida et al., 2002), although earlier studies reported no such effects (Bhambi and Eghbali, 1991; Iwami et al., 1996). Moreover, β-AR activation by ISO appears to reduce collagen synthesis in adult rat CF (Ostrom et al., 2003; Liu et al., 2006; Liu et al., 2008). Thus, the ability of NA and β-AR stimulation to influence ECM protein expression by CF remains somewhat unclear. There are no reports on the ability of β2-AR stimulation to influence MMP or TIMP activity in CF.

NA acting via the β2-AR can stimulate cytokine and growth factor secretion by CF. There are many reports that the pro-inflammatory cytokine IL-6 is secreted by rat and mouse CF in response to both NA and ISO (Bürger et al., 2001; Briest et al., 2003; Leicht et al., 2003; Jaffre et al., 2004; Yin et al., 2003a; Yin et al., 2006; Du et al., 2007). The ability of β-AR stimulation to modulate CF expression of other important pro-inflammatory cytokines, such as TNFα and IL-1β, is less clear. For example, NA had no effect on IL-1 or TNFα mRNA expression in rat CF (Bürger et al., 2001), whereas ISO increased IL-1 and TNFα secretion in mouse CF (Jaffre et al., 2004). The situation is further confused by the observation that ISO inhibited TNFα mRNA expression and protein secretion in neonatal rat CF (Yokoyama et al., 1999). The reasons for these discrepancies may relate to the species and developmental stage of the CF studied, although this was not directly addressed in any of the studies. The ability of β-AR stimulation to influence expression of the pro-fibrotic cytokine TGF-β1 by CF has been studied by a number of groups. The majority of studies found no effect of NA or ISO treatment on TGF-β1 levels (Bhambi and Eghbali, 1991; Takahashi et al., 1994; Colombo et al., 2001), although there is a report suggesting that NA can stimulate TGF-β expression in neonatal rat CF (Fisher and Absher, 1995).

β2-AR stimulation is also coupled to increased secretion of vasoactive peptides, including Ang II, ET-1, ANP and BNP. For example, ISO stimulates Ang II production in neonatal rat CF (Dostal et al., 2000; Singh et al., 2008). Human cardiac myoFb in culture secrete ET-1, a vasoactive

peptide of import to myocardial remodeling, and we demonstrated that this was necessary for β2-AR-induced cardiac myoFb proliferation, although β2-AR stimulation did not alter expression of ET-1 per se (Turner et al., 2004). Adult rat myoFb cultured from the scar region of a rat model of MI were shown to express ANP and BNP, and BNP (but not ANP) levels were increased by ISO treatment (Calderone et al., 2006). In contrast, ISO reduced BNP levels in neonatal rat CF (Calderone et al., 2006). Although this may suggest differences between fibroblasts and myoFb, it cannot be ruled out that these differences reflect the use of adult versus neonatal cells. NA and ISO also increase generation of extracellular adenosine in cultured adult rat CF (Dubey et al., 2001a).

4.3.2. Angiotensin II

Myocardial levels of Ang II are increased in a number of pathologies characterized by myocardial remodeling (Swynghedauw, 1999). Although the cellular effects of Ang II can be mediated via two different receptor subtypes (AT1R and AT2R), cultured CF express predominantly the AT1R (see Section 6.1.1 for more detail).

It is generally perceived that Ang II is mitogenic for CF and detailed analysis of the supporting evidence has confirmed this to be the case for neonatal CF, in which Ang II directly stimulates DNA synthesis via AT1R activation (Bouzegrhane and Thibault, 2002). However, reports on the ability of Ang II to induce proliferation in adult rat CF are much less consistent, and when effects have been observed they are suggestive of an autocrine/paracrine mechanism whereby Ang II stimulates CF to secrete growth-promoting substances that feed back on the cells (Bouzegrhane and Thibault, 2002). Reports on the mitogenic effects of Ang II on human CF are also somewhat at variance (Bouzegrhane and Thibault, 2002; Hafizi et al., 2004a). Interestingly, Ang II could induce proliferation of cardiac myoFb from sham hearts or the non-infarcted zone of MI rat hearts, whereas a hypertrophic response was observed in myoFb obtained from the infarct zone (Staufenberger et al., 2001)

Ang II (acting via AT1R) can induce differentiation of neonatal or adult rat CF into myoFb, as evidenced by increased α-SMA expression and stress fibre organization (Klett et al., 1995; Thibault et al., 2001; Olson et al., 2005; Samuel et al., 2004). As Ang II is a potent stimulus for expression of TGF-β (Rosenkranz, 2004), itself a strong inducer of the myoFb phenotype, it is not clear from these studies whether Ang II can directly induce the myoFb phenotype or whether it does so via increased TGF-β expression.

Ang II acting on CF elicits pro-fibrotic effects on the heart through multiple mechanisms, including increased ECM protein synthesis, decreased MMP activity and increased TIMP activity. Specifically, Ang II stimulated collagen I, collagen III and fibronectin synthesis through AT1R activation in adult rodent and human CF (Agocha et al., 1997b; Crabos et al., 1994; Hafizi et al., 1998; Staufenberger et al., 2001; Zhou et al., 1996; Lijnen et al., 2001). A gene array study has also recently demonstrated that AT2R activation may stimulate collagen I synthesis in adult rat CF (Jiang et al., 2007), although how this fits with the perceived lack of expression of the AT2R in CF (see Section 6.1.1) is not clear. Ang II reduced expression of collagenases (e.g. MMP-1) in rat (Brilla et al., 1995; Chen et al., 2004a; Lijnen et al., 2008), mouse (Stacy et al., 2007) and human (Pan et al., 2008) CF. Ang II also down-regulated expression of MMP-2 in rodent and human CF (Stewart, Jr. et al., 2006; Stacy et al., 2007; Pan et al., 2008). The effects of Ang II on MMP-9 expression are less consistent, with inhibitory (Stacy et al., 2007), neutral (Lijnen et al., 2008), and even stimulatory (Pan et al., 2008) effects all being reported. The ability of Ang II to modulate CF expression of other members of the MMP family has not been reported. The inhibitory effects of Ang II on MMP activity are exacerbated by a coincident increase in activity of TIMPs, the endogenous inhibitors of MMPs. For example, Ang II increased TIMP-1 and TIMP-2 in adult rat CF (Jiang et al., 2007; Lijnen et al., 2008). This may not be the case in human CF however, as Ang II was recently shown to decrease TIMP-1, TIMP-2 and TIMP-3 mRNA expression (Pan et al., 2008).

Ang II can also induce secretion of several other important bioactive molecules from CF, thereby regulating cellular function in an autocrine/paracrine manner. For example, Ang II (acting predominantly via the AT1R) induced expression and secretion of TGF-β (Campbell and Katwa, 1997; Rosenkranz, 2004), TNFα (Yokoyama et al., 1999; Sato et al., 2003), IL-6 (Sano et al., 2001), ET-1 (Fujisaki et al., 1995; Gray et al., 1998; Chao et al., 2005), natriuretic peptides (Makino et al., 2006; Wang et al., 2007; Calderone et al., 2006) and VEGF (Chintalgattu et al., 2003) in a number of CF and myoFb models.

It is noteworthy that Ang-(1-7), a product of Ang II cleavage by the ACE homologue ACE-2, appears to oppose the actions of Ang II on collagenThe profibrotic and

4.3.3. Endothelin-1

Myocardial and circulating levels of ET-1 are elevated in HF patients and in experimental models of HF (Motte et al., 2006). ET-1 elicits effects via two distinct cell surface receptors, ETA and ETB. Both these receptors are expressed by adult human and rat CF and myoFb, with ETB being predominant (Modesti et al., 1999; Asano et al., 2002; Katwa et al., 1993; Fareh et al., 1996; Katwa, 2003). This is in marked contrast to cardiomyocytes that express predominantly the ETA subtype (Fareh et al., 1996; Modesti et al., 1999).

ET-1 can stimulate proliferation of neonatal and adult rat CF via activation of the ETA receptor (Piacentini et al., 2000; Ogata et al., 2004; Kuruvilla et al., 2007), although this has been challenged (van Kesteren et al., 1997). In human cells, ET-1 did not induce proliferation of human atrial myoFb (Turner et al., 2004) and actually decreased DNA synthesis in adult human CF (Hafizi et al., 2004b). ET-1 was recently shown to induce neonatal rat CF to express markers of the myoFb phenotype, including α-SMA (Nishida et al., 2007). ET-1 has predominantly pro-fibrotic effects on the heart by stimulating CF from different species, including human, to increase collagen synthesis (Guarda et al., 1993; Katwa, 2003; Chintalgattu and Katwa, 2004; Hafizi et al., 2004b; Nishida et al., 2007; Kuruvilla et al., 2007). Both the ETA (Hafizi et al., 2004b; Kuruvilla et al., 2007) and

ETB (Guarda et al., 1993) receptors have been implicated in this process. Further pro-fibrotic effects of ET-1 occur at the level of MMPs, with evidence that ET-1 acting via the ETA receptor can reduce collagenase activity (Guarda et al., 1993).

4.3.4. Natriuretic peptides

ANP and BNP are cardiac-derived cyclic polypeptides that are upregulated in a number of cardiac pathologies and exhibit important autocrine and paracrine effects on the heart (D'Souza et al., 2004). Natriuretic peptides act predominantly via two membrane-associated guanylyl cyclase receptors (NPR-A and NPR-B), both of which are expressed by CF (Cao and Gardner, 1995; Lin et al., 1995). Both ANP and BNP exert anti-proliferative effects on CF, as determined by reduced DNA synthesis in human, mouse and rat CF (Cao and Gardner, 1995; Fujisaki et al., 1995; Calderone et al., 1998; Huntley et al., 2006; Li et al., 2008b; Kapoun et al., 2004). Natriuretic peptides also inhibit phenotypic transformation of CF to myoFb (Kapoun et al., 2004; Li et al., 2008b). Both ANP and BNP reduced collagen synthesis in human, rat, mouse and canine CF (Tamamori et al., 1997; Redondo et al., 1998; Maki et al., 2000; Tsuruda et al., 2002a; Kapoun et al., 2004; Li et al., 2008b) and BNP increased expression of MMPs in adult canine CF (Tsuruda et al., 2002a). Natriuretic peptides inhibited ET-1 synthesis in neonatal rat CF (Fujisaki et al., 1995), while ANP increased renin and angiotensinogen mRNA and protein levels in neonatal rat CF (Sanghi et al., 2005). A third member or the natriuretic peptide family, C-type natriuretic peptide (CNP), signals via NPR-B and reduces proliferation and collagen synthesis in adult rat CF (Horio et al., 2003). Thus, CF undergo reduced proliferation, myoFb differentiation and ECM synthesis in response to natriuretic peptides; a pattern of responses that appears favorable to reducing pathological myocardial remodeling.

4.3.5. Adenosine

The nucleoside adenosine is produced by all cardiovascular cell types including smooth muscle cells, endothelial cells, cardiomyocytes and CF (Villarreal et al., 2003). Adenosine is synthesized both intracellularly and extracellularly, with the latter being regulated by ecto-nucleotidases that convert cAMP to AMP and then adenosine (Villarreal et al., 2003). The extracellular cAMP-adenosine pathway has been studied in adult rat CF and shown to be coupled to inhibition of CF proliferation (Dubey et al., 2000).

There is good evidence, both clinical and experimental, that adenosine can reduce adverse myocardial remodeling, likely due to direct effects on CF function (Villarreal et al., 2003). Extracellular adenosine elicits cellular effects by stimulating cell membrane adenosine receptors, of which there are four known subtypes; A1R, A2aR, A2bR and A3R. Analysis of mRNA expression has concluded that adult rat CF express all four adenosine receptor subtypes, although there is a lack of agreement on their relative expression levels (Chen et al., 2004d; Grden et al., 2006; Epperson et al., 2009). The functional effects of adenosine on CF include reduced cell proliferation, reduced collagen synthesis and reduced TNFα secretion, most (if not all) of which are coupled specifically to A2bR activation (Dubey et al., 1997; Dubey et al., 1998; Dubey et al., 2001b; Villarreal et al., 2003; Chen et al., 2004d). All of these studies utilized adult rat CF cultures, but similar anti-proliferative effects have been noted in canine and murine CF (Villarreal et al., 2003). The signaling pathway mediating the functional effects of A2bR stimulation in CF involves stimulatory G-protein (Gs) and adenylyl cyclase-induced generation of cAMP (Villarreal et al., 2003; Epperson et al., 2009). The anti-proliferative, anti-fibrotic and anti-inflammatory effects of A2bR stimulation on CF likely contribute to the in vivo observation that A2bR (but not A1R, A2aR or A3R) stimulation can reduce adverse post-MI myocardial remodeling (Wakeno et al., 2006).

4.4. Cytokines

Both pro-inflammatory (TNFα, IL-1, IL-6) and pro-fibrotic (TGF-β) cytokines are expressed at high levels in MI and HF patients (Nian et al., 2004). Individually, these cytokines are capable of

eliciting diverse effects on CF function, some of which are comparable and some of which oppose each other. Delineating the in vivo response of CF to cytokines is extremely difficult given the complex array of cytokines prevalent in the remodeling myocardium, many of which exert synergistic effects with one another.

TNFα modulates cellular function through binding to two distinct cell surface receptors, TNF-RI and TNF-RII, both of which are expressed by human cardiac myoFb (Porter et al., 2004a). We have demonstrated that TNFα exerts wide-ranging effects on human atrial myoFb, including increased migration/invasion, proliferation and expression of MMPs and pro-inflammatory cytokines (Porter et al., 2004a; Turner et al., 2007a; Turner et al., 2007b). TNFα also stimulates proliferation (Jacobs et al., 1999; Hellkvist et al., 2002) and migration (Mitchell et al., 2007) of rat CF. Moreover, TNFα stimulated MMP expression and decreased collagen synthesis in both neonatal and adult rat CF (Siwik et al., 2000; Peng et al., 2002). TNFα may also influence the myocardial RAS by inducing AT1R expression in neonatal rat CF via increased NF-κB-dependent transcription (Gurantz et al., 1999; Cowling et al., 2002; Peng et al., 2002; Gurantz et al., 2005; Cowling et al., 2005).

IL-1β acting at the IL-1 receptor (IL-1R) stimulates CF migration (Brown et al., 2007; Mitchell et al., 2007; Mughal et al., 2009) but inhibits CF proliferation (Palmer et al., 1995; Koudssi et al., 1998; Piacentini et al., 2000; Xiao et al., 2008). IL-1β stimulates net ECM degradation through reduced collagen I and III synthesis (Siwik et al., 2000) and increased secretion of MMPs, including MMP-2, -3, -9 and -13 (Siwik et al., 2000; Brown et al., 2007; Xie et al., 2004; Xiao et al., 2008; Mughal et al., 2009). IL-1β can also induce AT1R expression in neonatal rat CF via increased gene transcription and translation of AT1R splice variants (Cowling et al., 2002; Gurantz et al., 2005; Cowling et al., 2005).

IL-6 modulates cellular function by signaling through heterodimeric gp130/IL-6R cell surface receptors, which are expressed by CF in culture (Tsuruda et al., 2002b; Fredj et al., 2005). IL-6 can induce proliferation of adult rodent CF and shifts the balance of ECM turnover in favour

of net degradation through decreased collagen synthesis and increased MMP expression (Siwik et al., 2000). IL-6 does not act as a chemotactic stimulus for neonatal rat CF (Mitchell et al., 2007).

The pro-fibrotic cytokine TGF-β1 exerts its effects through type I, II and III cell surface serine/threonine kinase receptors, all of which are expressed by CF (Sigel et al., 1996; Chen et al., 2004c). The most well established effects of TGF-β on CF function include increased fibrillar collagen, fibronectin and proteoglycan synthesis (Eghbali et al., 1991a; Heimer et al., 1995; Villarreal et al., 1996) and phenotypic conversion to myoFb (Hao et al., 2008; Tomasek et al., 2002; Leask, 2007) - see Section 3.3. In addition, TGF-β is reported to have anti-proliferative effects on adult CF and myoFb cultured from human, rabbit and rat (Sigel et al., 1996; Agocha et al., 1997b; Drobic et al., 2007). However, TGF-β appears to be pro-proliferative for adult mouse CF (Li et al., 2008b; Squires et al., 2005). TGF-β is not chemotactic for CF (Stawowy et al., 2004), but is reported to modulate migration in response to other chemotactic stimuli (Stawowy et al., 2004; Brown et al., 2007). The effects of TGF-β on MMP expression by CF are not well described, and opposing effects have been reported (Chua et al., 1991; Stawowy et al., 2004; Brown et al., 2007).

5. CARDIAC FIBROBLASTS ARE A KEY SOURCE OF BIOACTIVE MOLECULES

CF can synthesize an array of bioactive molecules and secrete them into the surrounding interstitium, thereby delivering highly localized changes in the cellular environment. These bioactive molecules can exert autocrine/paracrine effects by not only acting on other cell types of the heart (cardiomyocytes, vascular cells, inflammatory cells), but also on the fibroblasts themselves. The particular molecules secreted by CF depends largely on the initiating stimuli, which may be as diverse as proinflammatory cytokines, mechanical stretch and altered oxygen levels (discussed in Section 4). The action of secreted factors on specific cells is determined by regulated expression of specific receptors on the surface of target cells. In this section, we will focus on particular bioactive molecules that are relevant to current therapeutic strategies aimed at

reducing myocardial remodeling. The complex nature of the intact heart largely precludes the identification of the cell types responsible for secretion of particular biomolecules in vivo, and therefore much of the data discussed below has been obtained using in vitro CF cultures.

5.1. Cytokines

5.1.1. TNFα

CF are the major cellular source of TNFα in the myocardium (Yokoyama et al., 1999; Shames et al., 2002). CF isolated from rats one week after experimental MI exhibit increased levels of TNFα mRNA (Yue et al., 1998). Neonatal rat CF and adult human CF, but not myoFb, have been reported to spontaneously secrete TNFα in culture (LaFramboise et al., 2007; Rossini et al., 2008). TNFα expression is increased under hypoxic conditions in adult rat CF (Shivakumar et al., 2008), an observation that likely contributes to the increase in myocardial TNFα observed in non-myocytes of ischaemic rat hearts (Shames et al., 2002). TNFα expression by CF can also be induced by Ang II (Yokoyama et al., 1999; Sato et al., 2003), β-AR stimulation (Jaffre et al., 2004), serotonin (Jaffre et al., 2004), cytokines (Rossini et al., 2008) and mechanical stretch (Yokoyama et al., 1999). Binding of the ATF-2/c-jun transcription factor complex to the cyclic AMP response element within the human TNFα gene promoter mediates Ang II-induced TNFα expression in neonatal rat CF (Sato et al., 2003). In contrast to the above molecules that induce TNFα expression, adenosine reduces TNFα secretion in adult rat CF (Villarreal et al., 2003), likely explaining some of the beneficial effects of adenosine on myocardial remodeling.

5.1.2. IL-1β

CF are the principal source of IL-1β in the post-MI myocardium (Long, 2001; Yue et al., 1998). Increased mRNA levels of IL-1β were observed in CF isolated from rats as early as one day after experimental MI, and levels remained elevated for at least one week thereafter (Yue et al., 1998). Constitutive IL-1β secretion has been observed in unstimulated rat and mouse CF cultures

(Nagamatsu et al., 2006; Jaffre et al., 2004), whereas cultured human CF or myoFb do not appear to secrete IL-1β under basal conditions (Ancey et al., 2002; Turner et al., 2007b). IL-1β expression can be induced by β-AR stimulation (Jaffre et al., 2004), hypoxia (Long, 2001) or serotonin (Jaffre et al., 2004). Overexpression of constitutively active Gα13 also induced IL-1β expression via a ROS/NF-κB pathway in neonatal rat CF (Nagamatsu et al., 2006). In addition, IL-1β expression can be induced by other cytokines. For example, we have demonstrated that TNFα stimulated IL-1β mRNA expression in human atrial myoFb via a mechanism involving activation of the p38 MAPK, PI3K/Akt and NF-κB pathways, but not the ERK pathway (Turner et al., 2007b). HMGB1, a cytokine released by necrotic cells and activated macrophages, also enhanced IL-1β secretion from human CF in culture (Rossini et al., 2008).

5.1.3. IL-6

Myocardial IL-6 is synthesized by both CF and cardiomyocytes (Ancey et al., 2002). CF isolated from rats one week after experimental MI exhibited increased levels of IL-6 mRNA (Yue et al., 1998). Human and rat CF/myoFb spontaneously secrete IL-6 in culture (Ancey et al., 2002; Turner et al., 2007b; LaFramboise et al., 2007). Moreover, IL-6 production can be further induced by β-AR stimulation (Bürger et al., 2001; Briest et al., 2003; Leicht et al., 2003; Jaffre et al., 2004; Yin et al., 2003a; Yin et al., 2006; Du et al., 2007), Ang II (Sano et al., 2001), TNFα (Turner et al., 2007b), serotonin (Jaffre et al., 2004) and adiponectin (Liao et al., 2008) in CF from a variety of sources. The signal transduction pathways that mediate changes in IL-6 gene expression include ERK, p38 MAPK, NF-κB and PI3K/Akt (Vanden Berghe et al., 2000; Du et al., 2005; Yin et al., 2006; Sano et al., 2001; Nagamatsu et al., 2006; Turner et al., 2007b).

5.1.4. TGF-β

The primary source of TGF-β in the myocardium is the CF (Bujak and Frangogiannis, 2007). Increased mRNA levels of TGF-β1 were observed in CF isolated from rats one week after

experimental MI (Yue et al., 1998). Human CF secrete TGF-β1 in culture (Zhao and Eghbali-Webb, 2001), as do neonatal rat CF (LaFramboise et al., 2007). Ang II is a particularly potent stimulus for expression of TGF-β by CF and myoFb (Campbell and Katwa, 1997; Rosenkranz, 2004). Other stimuli reported to induce CF TGF-β expression include NA (Fisher and Absher, 1995), cyclic stretch (Ruwhof et al., 2000; van Wamel et al., 2001; Lindahl et al., 2002), superoxide (Li et al., 1999) and altered oxygen tension (Roy et al., 2003a).

5.2. Vasoactive peptides and growth factors

5.2.1. Angiotensin II

Ang II is the effector molecule of the RAS that plays a key role in regulating blood pressure and volume. In the systemic RAS, renin (produced in the kidneys) cleaves angiotensinogen (produced in the liver) into Ang I which is further cleaved into the octapeptide Ang II by angiotensin-converting enzyme, ACE-1. In addition to the systemic RAS, a local RAS exists in the myocardium and plays an important role in the regulation of cardiac function (Dostal and Baker, 1999). CF, in common with cardiomyocytes, express all the components of the RAS including angiotensinogen, renin and ACE-1, allowing CF to synthesize extracellular Ang II (Katwa et al., 1997; Sano et al., 1998; Sanghi et al., 2005). Moreover, CF cultured from adult spontaneously hypertensive rats had higher angiotensinogen mRNA levels than CF from control Wistar-Kyoto rats (Klett et al., 1995; Sano et al., 1998). Adult human atrial myoFb have also been shown to express the ACE-1 homologue ACE-2 (Guy et al., 2008), although ACE-2 activity appears to be undetectable in neonatal rat CF (Grobe et al., 2007; Gallagher et al., 2008). ACE-2 cleaves Ang II to the heptapeptide Ang-(1-7), a molecule that can also modulate CF function by opposing the effects of Ang II on collagen and growth factor synthesis (Iwata et al., 2005). Recent studies have described a novel intracellular RAS in rat neonatal ventricular CF (Singh et al., 2008), which may have important implications for the role of the RAS in myocardial remodeling.

Studies using neonatal rat CF have shown that β-AR stimulation (Dostal et al., 2000; Singh et al., 2008) or ANP, acting via a protein kinase A-dependent mechanism (Sanghi et al., 2005), can increase angiotensinogen mRNA and protein secretion. Angiotensinogen gene expression in neonatal rat CF was also increased by static stretch through a p38 MAPK-dependent pathway (Lal et al., 2008).

5.2.2. Endothelin-1

Myocardial levels of ET-1 are elevated in clinical and experimental HF through increased ET-1 synthesis by cardiomyocytes, vascular endothelial cells and CF (Motte et al., 2006). MyoFb cultured from adult rat infarcts express prepro-ET-1 mRNA and ET-converting enzyme-1, allowing them to synthesize and secrete mature ET-1 (Katwa, 2003). Cultured human atrial myoFb also express prepro-ET-1 mRNA and secrete mature ET-1 protein in culture (Turner et al., 2004), as do neonatal rat CF (Suzuki et al., 1997). ET-1 expression in neonatal rat CF can be induced by ET-1 itself (Fujisaki et al., 1995; Cheng et al., 2003a; Cheng et al., 2003b) and by Ang II (Fujisaki et al., 1995; Gray et al., 1998; Chao et al., 2005). The mechanism of ET-1-induced ET-1 gene expression in neonatal rat CF involves a ROS/ERK/AP-1-dependent pathway (Cheng et al., 2003a; Cheng et al., 2003b).

5.2.3. Natriuretic peptides

ANP and BNP are synthesized predominantly in the myocardium and act in an endocrine manner to regulate blood pressure, as well as having important autocrine/paracrine effects on the heart (D'Souza et al., 2004). Production of ANP and BNP is increased under pathological conditions including MI, cardiac hypertrophy and HF. Although classically thought to be synthesized and secreted by cardiomyocytes, there is evidence that natriuretic peptides can also be produced by CF. In a sheep MI model, elevated ANP (not BNP) mRNA expression was observed in the infarct region, and this was localized to the invading cardiac myoFb (Cameron et al., 2000). Moreover,

myoFb cultured from MI scar of adult rats expressed ANP and BNP mRNA and protein, and protein levels (but not mRNA) were increased in response to ISO or Ang II (Calderone et al., 2006). IL-1β increased mRNA and protein expression of both ANP and BNP in cultured neonatal rat non-myocytes, i.e. predominantly CF (Harada et al., 1999). Furthermore, BNP mRNA and secreted protein were observed in adult canine ventricular myoFb in response to TNFα (Tsuruda et al., 2002a) and in neonatal rat CF in response to hypoxia/reoxygenation or Ang II (Makino et al., 2006; Wang et al., 2007). The third member of the natriuretic peptide family, CNP, is also expressed by adult rat CF in culture (Horio et al., 2003). Thus there is significant evidence that natriuretic peptides can be synthesized by CF, in addition to cardiomyocytes.

5.2.4. VEGF

VEGF is a dimeric glycoprotein synthesized and secreted by many cell types and is associated with the ECM through interactions with heparan sulphate proteoglycans, thus regulating its bioavailability. VEGF acts primarily on vascular endothelial cells and is important for stimulating angiogenesis and coronary collateral formation for restoring the blood supply to the infarcted myocardium. Both cardiomyocytes and CF are able to secrete VEGF in response to hypoxic or inflammatory stimuli (Ladoux and Frelin, 1993; Maruyama et al., 1999; Chintalgattu et al., 2003). VEGF mRNA expression and secretion of mature protein has been reported in CF of human (Zhao and Eghbali-Webb, 2001; Weiss et al., 2004; Rossini et al., 2008) and rat (Ladoux and Frelin, 1993; Chintalgattu et al., 2003; Kelly et al., 2003) origin. Indeed, multianalyte profiling of conditioned medium from neonatal rat CF recently identified VEGF as one of the most prevalent growth factors produced by these cells in culture (LaFramboise et al., 2007). Similarly, VEGF was present in high levels in conditioned media from adult human CF and myoFb (Rossini et al., 2008). Ang II (Chintalgattu et al., 2003) and hypoxia (Kelly et al., 2003) have been shown to increase VEGF expression in rat CF.

6. THERAPEUTIC MODULATION OF CARDIAC FIBROBLAST FUNCTION

A number of therapeutic agents prescribed for specific cardiovascular disorders have been shown to exert beneficial effects on CF, which may underlie some of their potential benefits on myocardial remodeling and HF progression. These unexpected "off-target" properties are often referred to as "pleiotropic" effects. Classes of drugs that exhibit pleiotropic effects on CF include anti-hypertensive agents (ACE inhibitors, angiotensin receptor blockers, beta-blockers), lipid-lowering drugs (statins, fibrates) and pharmacological agents prescribed for lowering insulin resistance in diabetic patients (TZDs). In the following section we will explore in detail the reported pleiotropic effects of these classes of drugs on CF function. The majority of data is drawn from in vitro CF cultures, as the complexity of the in vivo scenario makes it difficult to delineate direct effects of agents on CF from indirect effects caused by changes in other factors involved in the myocardial remodeling process.

6.1. ACE inhibitors and angiotensin receptor blockers

The primary use of ACE inhibitors and angiotensin receptor blockers (ARBs) is for treatment of hypertension. However, there are numerous studies describing beneficial effects of these agents on adverse myocardial remodeling in animal models (Rosenkranz, 2004; Anavekar and Solomon, 2005), and they are now prescribed to HF patients for this purpose (Hunt et al., 2005). Ang II acts as a mitogen for a range of cardiovascular cell types, and myocardial angiotensin receptor activation can induce cardiac remodeling independently of changes in blood pressure (Ainscough et al., 2009). ACE inhibitors and ARBs both act to antagonize the effects of Ang II, albeit via quite different mechanisms. ACE inhibitors prevent Ang II synthesis by inhibiting ACE-mediated cleavage of Ang I. ARBs inhibit the AT1R and offer a potentially more targeted therapeutic tool than blanket ACE inhibition. The ability of ACE inhibitors and ARBs to reduce cardiovascular remodeling is well documented and has been reviewed at length elsewhere (Rosenkranz, 2004; Anavekar and

Solomon, 2005). In this review we will concentrate on the modulatory effects of Ang II specifically on CF function and the potential benefits that ACE inhibitors and ARBs have at the level of the CF.

6.1.1. Angiotensin receptor and ACE expression in cardiac fibroblasts

Ang II elicits its cellular effects by binding to and activating the specific cell surface receptors AT1R and AT2R. The majority of the known effects of Ang II are mediated via AT1R activation. Both AT1R and AT2R subtypes are expressed in the human heart, and a relative increase in AT2R occurs in the failing human heart (Anavekar and Solomon, 2005).

Cultured CF and myoFb from several species have been shown to express the AT1R, but not the AT2R (Crabos et al., 1994; Ohkubo et al., 1997; Hafizi et al., 1998; Regitz-Zagrosek et al., 1998; Staufenberger et al., 2001). However, both human (Tsutsumi et al., 1998) and hamster (Ohkubo et al., 1997) studies provided evidence that the increase in AT2R expression in failing hearts was localized specifically to CF. Inter-species differences are apparent in AT1R levels, with cultured rat CF expressing significantly higher levels than rabbit or human CF (Gallagher et al., 1998). AT1R expression in CF can be up-regulated by various growth factors and cytokines that are present in the remodeling heart, including TNFα and IL-1 (Gurantz et al., 1999; Cowling et al., 2002; Peng et al., 2002; Gurantz et al., 2005; Cowling et al., 2005).

CF express all the key components of the RAS including angiotensinogen, renin and ACE, allowing CF to synthesize extracellular Ang II (Katwa et al., 1997; Sano et al., 1998; Sanghi et al., 2005). Recent studies have also characterized an intracellular RAS in CF, which represents a novel form of angiotensin signaling (Singh et al., 2008). TGF-β-induced differentiation of adult rat CF to myoFb is accompanied by increased ACE expression (Lijnen et al., 2004), consistent with ACE activity being localized to myoFb following MI (Sun and Weber, 1996).

6.1.2. Cell proliferation

Ang II can stimulate CF proliferation (discussed in Section 4.3.2) and therefore one method by which ACE inhibitors or ARBs could target CF function would be to antagonize Ang II-induced CF proliferation. Interestingly, the ARB telmisartan was found to exhibit direct anti-proliferative effects on neonatal rat CF, independently of angiotensin receptor stimulation (Benson et al., 2008). This property of telmisartan was not apparent with other ARBs, including candesartan, eprosartan and irbesartan. These data are therefore indicative of cardioprotective effects of telmisartan beyond simple AT1R inhibition.

6.1.3. Myofibroblast differentiation

Ang II (acting via AT1R) can induce differentiation of CF into myoFb (see Section 4.3.2 for more detail). ACE inhibition or AT1R blockade could therefore exert beneficial effects by reducing the number of cardiac myoFb in the remodeling heart, as has been observed in vivo (Yu et al., 2001).

6.1.4. ECM turnover

Ang II elicits pro-fibrotic effects on the heart through stimulating CF to increase ECM protein synthesis, decrease MMP activity and increase TIMP activity (discussed in Section 4.3.2). In addition to direct effects of ACE inhibitors and ARBs on these Ang II-mediated effects, ACE inhibitors may also modulate collagen synthesis independently of angiotensin. For example, the ACE inhibitor lisinopril reduced basal and TGF-β-induced collagen synthesis in adult rat CF in the absence of Ang II, conditions in which ARBs were without effect (Lijnen et al., 2004). Conversely, another ACE inhibitor (imidaprilat) was recently shown to reduce IL-1-induced MMP-2 expression in human CF (Guo et al., 2008).

6.1.5. Cytokines and growth factors

Ang II induces expression of cytokines (e.g. TGF-β, TNFα, IL-6), peptides (e.g. ET-1, ANP, BNP) and growth factors (e.g. VEGF) from CF (discussed in Section 4.3.2). ACE inhibitors and ARBs

therefore have the potential to reduce local concentrations of these bioactive molecules in the myocardium, with resultant modulatory effects on myocardial remodeling.

6.1.6. Summary

Ang II, acting predominantly via the AT1R, can stimulate CF to adopt a myoFb phenotype and undergo increased proliferation, ECM turnover and secretion of proinflammatory cytokines and growth factors, all of which contribute to myocardial remodeling. The ability of ACE inhibitors and ARBs to prevent these detrimental effects of Ang II, together with emerging evidence of direct Ang II-independent effects, likely underlies the ability of these drugs to reduce adverse myocardial remodeling at the level of the CF.

6.2. Beta blockers

Although originally prescribed for treating hypertension and cardiac arrhythmia, beta-blockers are now recommended therapeutic agents for patients with mild to severe (NYHA class II-IV) HF (Swedberg et al., 2005; Hunt et al., 2005). These recommendations are based on substantial clinical and experimental evidence demonstrating that beta-blockers can reduce adverse myocardial remodeling and improve HF mortality (Bristow, 2000; Lechat et al., 1998). Beta-blockers act by inhibiting β-AR and thereby reducing the detrimental effects of elevated catecholamine levels in the heart. The efficacy of beta-blockers is often ascribed solely to their action on cardiomyocytes, with no acknowledgment of their prospective effects on CF. Here we identify the potential benefits that beta-blockade has at the level of the CF that may ultimately contribute to reduced adverse myocardial remodeling.

6.2.1. β-AR expression in cardiac fibroblasts

Data from several independent studies support the concept that cultured CF express the β2-AR subtype, but not the β1-AR. Reverse transcription PCR analysis revealed that β2-AR mRNA is

highly expressed in adult rat CF and adult human myoFb, with little or no β1-AR mRNA detectable (Gustafsson and Brunton, 2000; Turner et al., 2003), data confirmed by radioligand binding studies in neonatal rat CF (Yin et al., 2003b). In both adult rat CF and adult human myoFb, adrenaline (a non-selective β-AR agonist) was a more potent inducer of cyclic AMP than NA (a more β1-AR-selective agonist), indicating a β2-AR-mediated response (Meszaros et al., 2000; Turner et al., 2003). Moreover, cyclic AMP accumulation in response to the non-selective β-agonist ISO was inhibited by a β2-selective antagonist, but not a β1-selective antagonist, in adult rat CF and adult human myoFb (Gustafsson and Brunton, 2000; Turner et al., 2003). Taken together these data clearly establish that CF from different species express predominantly the β2-AR subtype.

This is in marked contrast to cardiomyocytes, which exhibit high expression levels of the β1-AR subtype (Brodde and Michel, 1999). Thus, the recent trend towards the use of β1-selective beta-blockers (e.g. metoprolol) for treatment of HF patients ignores the potential benefits of inhibiting the β2-AR on CF, which may be a previously overlooked explanation for the greater benefit observed with carvedilol (non-selective α/β-antagonist) compared with the β1-selective antagonist metoprolol in the COMET (Carvedilol Or Metoprolol European Trial) clinical trial (Kveiborg et al., 2007).

6.2.2. Cell proliferation

There is strong evidence that β2-AR stimulation (by NA or ISO) is coupled to proliferation of CF derived from several species (human, rat, rabbit) at different developmental stages (adult, neonatal) (discussed in Section 4.3.1). These mitogenic effects of NA and ISO were inhibited by non-selective β-antagonists or β2-selective antagonists, but not by β1-selective antagonists or α-AR antagonists (Calderone et al., 1998; Leicht et al., 2000; Turner et al., 2003; Yin et al., 2003b). Moreover, the effects of ISO could be mimicked by the β2-selective agonist clenbuterol (Colombo et al., 2003).

All the above studies described inhibitory effects of beta-blockers on βAR-mediated proliferation (i.e. in response to catecholamines). However, there is evidence that the non-selective beta-blocker carvedilol can inhibit CF proliferation in the absence of β-AR stimulation (Grimm et al., 2001; Lotze et al., 2002). In adult rat CF, carvedilol (but not metoprolol) inhibited serum-induced DNA synthesis and proliferating cell nuclear antigen expression (Grimm et al., 2001). Carvedilol (but not metoprolol or propranalol) also inhibited PDGF-induced DNA synthesis in human ventricular CF by a mechanism involving reduced PDGF receptor auto-phosphorylation (Lotze et al., 2002). Both these reports describe effects of carvedilol that are likely to be β-AR-independent, and may reflect the α1 antagonist or antioxidant properties of this particular beta-blocker.

Taken together, these data strongly suggest that a major benefit of beta-blockade (non-selective or β2-selective) would be to inhibit catecholamine-induced CF proliferation and subsequent adverse remodeling.

6.2.3. Myofibroblast differentiation

The ability of β-AR stimulation, and thereby beta-blockers, to modulate differentiation of CF to myoFb (discussed in Section 4.3.1) has not been highly researched and the results are far from conclusive (Eghbali et al., 1991b; Liu et al., 2006). It therefore remains to be determined whether beta-blockers can directly modulate the phenotypic transformation of CF to myoFb.

6.2.4. ECM turnover

The ability of beta-blockers to reduce fibrosis in HF models (Asai et al., 1999; Kobayashi et al., 2004) implies that stimulation of the βAR on CF would increase ECM protein synthesis. However, in vitro evidence (discussed in Section 4.3.1) does not appear to support this. Thus, the ability of β-AR stimulation, and hence beta-blockers, to influence ECM protein expression by CF remains somewhat unclear. Interestingly, carvedilol reduced collagen (type I and III) and fibronectin

expression in adult rat CF in the absence of exogenous β-AR stimulation, but metoprolol had no effect (Grimm et al., 2001). Thus, carvedilol may exert anti-fibrotic effects on CF independently of β-AR blockade.

6.2.5. Cytokines and growth factors

Some of the beneficial in vivo actions of beta-blockers on CF may be due to modulation of β-AR-stimulated cytokine, growth factor and vasoactive peptide secretion, all of which impact on the myocardial remodeling process. However, with the marked exception of IL-6, there is a lack of consistency in reports regarding the ability of β-AR stimulation to modulate expression of other cytokines and vasoactive peptides (reviewed in Section 4.3.1). Therefore the ability of beta-blockers to inhibit NA-induced IL-6 secretion remains the only firmly established mechanism by which these agents could affect CF-induced cytokine and growth factor expression. Indeed, NA and ISO-induced IL-6 expression in adult rat and neonatal mouse CF can be blocked by non-selective β-antagonists and β2-selective antagonists, but not β1-selective antagonists (Bürger et al., 2001; Yin et al., 2006).

6.2.6. Summary

It is now well established that CF express β2-AR (but not β1-AR), and that β2-AR stimulation is coupled to CF proliferation and IL-6 secretion. The effects of β2-AR activation on ECM turnover, myoFb differentiation and secretion of other cytokines and growth factors are less clear. The beneficial effects of beta-blockers on adverse myocardial remodeling are therefore likely to be due to a combination of effects on cardiomyocyte and CF function, the latter being due specifically to β2-AR blockade. Thus, the use of β1-AR-selective antagonists (e.g. metoprolol) for HF patients appears to overlook the potential beneficial effects to be gained by inhibiting the β2-AR on CF.

6.3. Statins

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol synthesis pathway, and are well established agents for the treatment of hypercholesterolaemia. There have been a number of landmark clinical trials such as 4S (Scandinavian Simvastatin Survival Study Group, 1994), CARE (Sacks et al., 1996), LIPID (The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, 1998), WOSCOPS (West of Scotland Coronary Prevention Study Group, 1998) and the Heart Protection Study (Heart Protection Study Collaborative Group, 2002) that have demonstrated unequivocally the beneficial effects of statin therapy for primary and secondary prevention of cardiovascular disease. Most of the large statin trials have also demonstrated a positive effect of statins in HF patients, the most important of which have been recently and extensively reviewed (Tsouli et al., 2008).

Importantly, emerging evidence strongly suggests that statins possess beneficial anti-remodeling effects in the chronic HF setting and that these may be additional to those observed with standard therapy, such as ACE inhibitors and beta-blockers. These are collectively referred to as "pleiotropic" effects. In experimental studies, the effects of statins have largely focused on global myocardial remodeling in vivo or cardiomyocytes in vitro (Dechend et al., 2001; Patel et al., 2001; Senthil et al., 2005; Tsai et al., 2008). There are considerably less data relating to statins and the CF; our current knowledge and understanding will therefore be discussed here.

6.3.1. HMG-CoA reductase

The activity of statins is generally accepted to be a class effect of these drugs that can be explained by the inhibition of HMG-CoA reductase. Moreover, statins can exert cholesterol-independent pleiotropic effects. By inhibiting the conversion of HMG-CoA to mevalonate, statins also inhibit the synthesis of important isoprenoids that are intermediates in the cholesterol-synthetic pathway, including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). FPP and GGPP are important lipids necessary for post-translational prenylation of small G-proteins such as

Ras, Rho and Rac thus permitting correct cellular localization and signaling of intracellular proteins (Van Aelst and Souza-Schorey, 1997). Prenylated proteins control a variety of cellular functions and hence through this mechanism the diversity of direct cellular effects attributable to statins independently of cholesterol-lowering might be explained.

6.3.2. Cell proliferation and migration

Statins have consistently been shown to inhibit CF/myoFb proliferation irrespective of species, growth stimulus or the method utilized to quantify proliferation. Using [^{3}H]-thymidine uptake as a marker of DNA synthesis, several studies have reported anti-proliferative effects of statins on neonatal rat CF (Martin et al., 2005; Tian et al., 2003; Xu et al., 2006), adult rat CF (He et al., 2008), adult mouse CF (Chen and Mehta, 2006) and canine atrial CF (Shiroshita-Takeshita et al., 2007). In our own studies simvastatin dose-dependently inhibited human atrial myoFb proliferation in response to a serum stimulus, as determined by direct cell counting and the inhibition of cyclin A expression (Porter et al., 2004b). Subsequently we also demonstrated that simvastatin reduced myoFb proliferation induced by the proinflammatory cytokine TNF-α (Porter et al., 2004a).

In human atrial myoFb we demonstrated that simvastatin inhibited TNF-α-induced migration and invasion (Porter et al., 2004a; Turner et al., 2007a) by two distinct mechanisms. Statin treatment reduced MMP-9 secretion post-transcriptionally, a mechanism distinct from those previously reported in other cell types. Inhibition of migration, however, was attributable to cytoskeletal disruption via inhibition of Rho kinase (Turner et al., 2007a). It is conceivable that the actin-destabilizing properties of simvastatin might also explain the reduced MMP-9 secretion, although this was not investigated.

6.3.3. Myofibroblast differentiation

In canine atrial fibroblasts, TGF-β increased expression of α-SMA that was attenuated in the presence of simvastatin, suggesting that simvastatin reversed the myoFb phenotype (Shiroshita-

Takeshita et al., 2007). Similarly, pravastatin was shown to suppress phenotypic transformation of CF to myoFb (Moiseeva et al., 2007).

6.3.4. ECM turnover

In Ang II-treated mouse CF, pro-collagen expression was mildly inhibited by low dose (0.1 μM) pravastatin, an effect that was markedly enhanced when combined with a TZD, pioglitazone (Chen and Mehta, 2006). In rat neonatal CF, stimulation with Ang II or TGF-β increased pro-collagen mRNA and collagen deposition ([^{3}H]-proline incorporation), both of which were dose-dependently inhibited by atorvastatin (Martin et al., 2005). Similar effects were observed in human CF in the same study. Collagen synthesis in neonatal rat CF ([^{3}H]-proline) was decreased by atorvastatin in a concentration-dependent manner (Tian et al., 2003).

In human cardiac myoFb, simvastatin inhibited TNFα-induced MMP-9 secretion, leading to reduced invasive capacity (Porter et al., 2004a). Ang II was shown to upregulate MMP-3 and MMP-9 expression in mouse CF and pravastatin inhibited MMP activity but only when combined with pioglitazone (Chen and Mehta, 2006).

6.3.5. Cytokines and growth factors

HF patients undergoing statin therapy exhibit reduced plasma levels of proinflammatory cytokines (Tousoulis et al., 2005; Sola et al., 2006), and in vivo and clinical studies suggest that statins can reduce local expression of proinflammatory cytokines in the myocardium (Zhang et al., 2005; Wallace et al., 2005). As CF are an important source of myocardial cytokines, we investigated whether statins could affect proinflammatory cytokine expression in human cardiac myoFb (Turner et al., 2007b). TNFα induced expression of IL-1α, IL-1β and IL-6, but this was not modulated by statin therapy, indicating that CF are not the cellular targets for the anti-inflammatory effects of statins on the heart. In a recent study, the pleiotropic effects of pravastatin on CF proliferation were claimed to be attributable to inhibition of TGF-β expression (Moiseeva et al., 2007).

6.3.6. Signaling and transcription

In adult rat CF, simvastatin was reported to inhibit activation of the Akt and ERK pathways (He et al., 2008) and in mouse CF, a combination of pravastatin and pioglitazone inhibited Ang II-activated p38 MAPK and ERK signaling, together with a potent inhibitory effect on the activation of AP-1 and NF-κB (Chen and Mehta, 2006). However, in our study on human atrial myoFb, although TNFα activated the ERK, p38 MAPK, Akt and NF-κB signaling pathways, none of these were influenced by simvastatin (Turner et al., 2007a). In the same study we determined that the modulatory effects of simvastatin on myoFb invasion were attributable to two distinct mechanisms. Firstly, that Rho kinase inhibition and subsequent cytoskeletal disruption led to attenuated cell migration and secondly, that MMP-9 secretion was inhibited via a post-transcriptional mechanism (Turner et al., 2007a). In a separate study, we established that the anti-proliferative effect of simvastatin on human cardiac myoFb was also mediated by Rho kinase inhibition and subsequent cell cycle arrest (Porter et al., 2004b).

6.3.7. Alternative lipid-lowering drugs

Fibrates have been in clinical use for over 30 years and although there have been fewer intervention studies than with statins, the majority of evidence suggests long-term beneficial effects in high-risk patients (Goldenberg et al., 2008). The lipid-lowering effects of fibrates have been recognized since the 1970s but it was some two decades later that the mechanism of their effects was shown to be via activation of peroxisome proliferator-activated receptor alpha (PPARα) (Staels and Fruchart, 2005)□. Fibrates lower plasma triglycerides and VLDL particles and increase HDL-cholesterol, effects that are associated with cardiovascular benefit. PPARs play key roles in the regulation of energy homeostasis and inflammation, and agonists of PPARα are currently used therapeutically not only for lipid-lowering effects but for their reported pleiotropic effects (Chinetti-Gbaguidi et al., 2005), such as in the prevention of HF (Perrone et al., 2005). In general, most current evidence

suggests PPARα activation acts as an anti-atherogenic factor that regulates various targets that should decrease atherosclerosis and its complications, although there is considerable debate.

Expression of PPARα has been demonstrated in neonatal rat CF and its activity was increased when cells were treated with adiponectin (Fujita et al., 2008). The authors suggested that in Ang II-induced cardiac fibrosis the protective effects of adiponectin were mediated via a PPARα-dependent mechanism. Another study in neonatal and adult rat CF and myoFb revealed expression of PPARα, although PPARδ was the most abundant isoform (Teunissen et al., 2007). On the contrary, an earlier study reported that PPARα was the predominant isotype expressed in both rat cardiomyocytes and CF (Wayman et al., 2002).

There are few reports on the effects of fibrates on CF. One study demonstrated that treatment of neonatal rat CF with ET-1 increased DNA synthesis, and that this was attenuated by fenofibrate, paralleled by a decrease in expression of c-jun (Ogata et al., 2004). However, a different study noted that in dog atrial fibroblasts, fenofibrate at clinically relevant concentrations had no observable effect on proliferation in response to a serum stimulus (Shiroshita-Takeshita et al., 2007).

6.3.8. Summary

It is now well accepted that statins, in addition to their primary function of lowering cholesterol, undoubtedly possess a variety of beneficial effects on myocardial remodeling and in patients with HF. In clinical trials statins have conclusively been shown to confer protective effects in primary and secondary prevention trials and in HF patients. Although most experimental evidence has been provided from studies in animal models or cultured cardiomyocytes, it is also likely that modulation of CF function contributes to their wide-ranging pleiotropic effects. Evidence to date indicates that statins can directly inhibit CF proliferation and migration, myoFb differentiation and ECM turnover, all of which would confer beneficial effects in the myocardial remodeling process.

The pleiotropic effects of statins and fibrates exhibit remarkable similarity and suggest a mechanistic link between the two classes of drugs, as has been reviewed recently (Paumelle and Staels, 2008). The impact of fibrates on improving cardiovascular outcomes remains contentious (Zandbergen and Plutzky, 2007). Given the current paucity of information with respect to the effects of fibrates on CF function, it would seem that fully elucidating their effects and associated mechanisms in the laboratory remains a worthwhile aim. Further studies are needed to compare the cellular benefits of statins, fibrates or the combination of both.

6.4. Thiazolidinediones

TZDs are agonists of the nuclear hormone receptor peroxisome proliferator activated receptor-gamma (PPARγ), a transcription factor expressed at high levels in adipose tissue and vascular cells (Touyz and Schiffrin, 2006). TZDs are used therapeutically to reduce insulin resistance and can significantly improve cardiovascular risk factors in patients with Type 2 diabetes mellitus (Irons et al., 2006). In addition to effects on diabetic patients, there is evidence that TZDs may exert beneficial cardiovascular effects in non-diabetic patients, independently of glycaemic control (Marx et al., 2005; Panunti and Fonseca, 2006). Moreover, there are also reports that TZDs can act via PPARγ-independent mechanisms (Gardner et al., 2005; Huang et al., 2005).

In addition to the well-established vascular benefits of TZDs (Touyz and Schiffrin, 2006), it is apparent that these drugs can exert direct actions on the heart. Early animal studies suggested that TZDs could reduce infarct size and cardiac hypertrophy (Asakawa et al., 2002; Sakai et al., 2002; Abdelrahman et al., 2005). However, more recently it has emerged that TZDs may be detrimental, and cause cardiac hypertrophy via PPARγ-independent mechanisms (Duan et al., 2005). This is now reinforced by clinical data (PROactive study) that indicated an increased frequency of serious HF in Type 2 diabetic patients receiving pioglitazone, despite a significant reduction in coronary events (Ryden et al., 2007; Erdmann et al., 2007). Furthermore, adverse cardiovascular effects of

rosiglitazone have been revealed following meta-analysis of clinical data (Nissen and Wolski, 2007).

Here we discuss the ability of TZDs and the endogenous PPAR-γ agonist, 15-deoxy-$\delta^{12,14}$-prostaglandin J2 (15d-PGJ2), to modulate CF function in an attempt to explain some of the effects of these drugs, both beneficial and detrimental, on myocardial remodeling.

6.4.1. PPARγ expression in cardiac fibroblasts

PPARγ is highly expressed in adipose tissue (Dumasia et al., 2005) and to a lesser extent in cells of the cardiovascular system, including vascular smooth muscle, endothelium and the heart (Ricote et al., 1998; Marx et al., 2004; Mendez and LaPointe, 2003). PPARγ is also expressed at both the mRNA and protein level in cultured CF and myoFb from different species (Wayman et al., 2002; Chintalgattu et al., 2007; Zhang et al., 2007; Teunissen et al., 2007; Hao et al., 2008; Mughal et al., 2009). TZDs and the endogenous PPARγ ligand 15d-PGJ2 have been reported to increase PPARγ activity and expression in cultured CF and myoFb (Chintalgattu et al., 2007; Hao et al., 2008).

In the normal in vivo rat heart, expression of PPARγ appears to be preferentially localized to the cardiomyocytes, rather than the CF (Fliegner et al., 2008). However, PPARγ expression was increased in both myocytes and CF in the infarct area 3 weeks after experimental MI (Fliegner et al., 2008). In a similar in vivo model, PPARγ expression was colocalised with α-SMA expression in myoFb in the infarct region 28 days after MI (Chintalgattu et al., 2007). Thus, PPARγ expression may be upregulated with differentiation of CF into myoFb in vivo, although this does not appear to be the case for myoFb differentiation in vitro (Teunissen et al., 2007).

6.4.2. In vivo effects

In a rat model of LV hypertrophy (stroke-prone spontaneously hypertensive rates), pioglitazone reduced the number of α-SMA-expressing CF (i.e. myoFb) and expression of collagen I, BNP,

connective tissue growth factor (CTGF) and MMP-2, but did not reduce the increase in collagen III, TGF-β, MMP-9 or ROS production (Shinzato et al., 2007).

6.4.3. Cell proliferation and migration

There are contrasting reports on the effects of TZDs on Ang II-induced CF proliferation. For example, pioglitazone had no effect on Ang II-induced proliferation of mouse CF, as determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Chen and Mehta, 2006). However, rosiglitazone and 15d-PGJ2 inhibited Ang II-induced proliferation (MTT assay) of neonatal rat CF via a PPARγ-dependent mechanism (Hao et al., 2008). There are several differences between these two reports that may account for the disparate results, including choice of TZD (pioglitazone vs. rosiglitazone), species (mouse vs. rat) and age/development (adult vs. neonatal). Pioglitazone reduced anoxia/reoxygenation-induced proliferation (MTT assay) of adult rat CF (Chen et al., 2004b), but rosiglitazone did not affect rat CF/myoFb proliferation as assessed by BrdU labeling (Teunissen et al., 2007). Rosiglitazone inhibited proliferation (MTT and cell cycle analysis) of neonatal rat CF induced by advanced glycation end products (Li et al., 2008a). Our own study demonstrated that three different TZDs and 15d-PGJ2 inhibited proliferation (cell counts, proliferating cell nuclear antigen expression) of human cardiac myoFb in response to fetal calf serum or TNFα, effects that were not reversed by PPARγ antagonists, suggesting a PPARγ-independent mechanism (Mughal et al., 2009).

Only a single study has sought to determine the effects of TZDs on CF migration. Using cultured human atrial myoFb, we showed that TZDs did not modulate cell migration towards an IL-1 chemotactic stimulus, either in the presence or absence of an ECM barrier (Mughal et al., 2009).

6.4.4. Myofibroblast differentiation

Several groups have investigated the effects of TZDs on myoFb differentiation. Rosiglitazone did not induce CF to differentiate into myoFb, as determined by a lack of effects on α-SMA expression

and stress fibre formation (Teunissen et al., 2007). Moreover, Ang II-induced α-SMA expression was not modulated by TZDs in neonatal rat CF (Hao et al., 2008). TZDs did not affect α-SMA expression or actin stress fibre organization in human cells with an already established myoFb phenotype (Mughal et al., 2009). Thus, TZDs have no effect on myoFb differentiation, either in terms of induction, modulation or reversal.

6.4.5. ECM turnover

TZDs generally have anti-fibrotic effects on CF. In rodent CF, TZDs or 15d-PGJ2 reduced collagen I expression induced by hypoxia-reoxygenation (Chen et al., 2004b; Makino et al., 2006) or Ang II (Chen et al., 2004a; Chen and Mehta, 2006; Hao et al., 2008). In neonatal rat CF, collagen III expression was reduced by rosiglitazone (Hao et al., 2008), but not by pioglitazone or 15d-PGJ2 (Makino et al., 2006). Rosiglitazone and 15d-PGJ2 have also been shown to inhibit Ang II-induced fibronectin expression (Hao et al., 2008). In contrast to the majority of reports, rosiglitazone increased collagen synthesis (as determined by [^{3}H]-proline incorporation) in both CF and myoFb (Teunissen et al., 2007).

The effects of TZDs and PPARγ agonists on MMP expression appear to depend on the initiating stimulus, which may suggest that they are acting via inhibition of specific signal transduction pathways. For example, hypoxia/reoxygenation-induced expression of MMP-1, MMP-2, MMP-9 and TIMP-1 was inhibited by TZDs (Chen et al., 2004b; Makino et al., 2006), as was phorbol ester-induced MMP-9 expression in human cardiac myoFb (Mughal et al., 2009). In contrast, MMP-2, MMP-3 and MMP-9 expression induced by Ang II (Chen and Mehta, 2006) or IL-1 (Mughal et al., 2009) was not affected by TZDs or 15d-PGJ2. In adult rat CF, Ang II reduced MMP-1 expression, and this was reversed by pioglitazone (Chen et al., 2004a).

6.4.6. Cytokines and growth factors

TZDs and 15d-PGJ2 can stimulate expression of VEGF and its receptors (VEGFR1 and VEGFR2) in adult rat cardiac myoFb (Chintalgattu et al., 2007). In vivo studies have demonstrated that increased VEGF production after MI can reduce infarct size and subsequent remodeling (Yoon et al., 2004). The ability of TZDs to stimulate VEGF secretion from myoFb may therefore help to explain the beneficial effects of TZDs on reducing infarct size in animal models of MI (Abdelrahman et al., 2005).

TZDs and PPARγ agonists have been shown to decrease expression of BNP, CTGF and to a lesser extent TGF-β1 in neonatal rat CF (Makino et al., 2006; Li et al., 2008a; Hao et al., 2008), all of which could confer potentially beneficial effects on post-MI remodeling of the heart.

On a cautionary note, our own work showed that TZDs increased TNFα-induced IL-6, IL-1α and IL-1β expression in human myoFb (Turner et al., 2007b), suggesting that TZDs may exert pro-inflammatory effects on cardiac myoFb that could exacerbate adverse myocardial remodeling.

6.4.7. Signaling and transcription

In cultured rat CF, TZDs reduced NF-κB activation induced by hypoxia/reoxygenation (Chen et al., 2004b; Makino et al., 2006) or Ang II (Chen et al., 2004a), and 15d-PGJ2 reduced NF-κB activity in rat cardiac myoFb (Chintalgattu et al., 2007). TZDs do not appear to modulate AP-1 activity in CF (Chen et al., 2004b; Chen et al., 2004a), despite their ability to disrupt MAP kinase (ERK, p38 and JNK) signaling in this cell type (Chen and Mehta, 2006; Hao et al., 2008).

6.4.8. Summary

The effects of TZDs on CF function are beginning to emerge, and may help to explain some of the effects of this class of drugs on myocardial remodeling, both beneficial and detrimental. TZDs exhibit anti-proliferative effects on CF and reduce ECM protein synthesis, but do not affect myoFb differentiation or CF migration. They also stimulate CF to secrete “beneficial” proteins, such as VEGF, while also reducing expression of “harmful” proteins, such as BNP and TGF-β. However,

the ability of TZDs to increase pro-inflammatory cytokine secretion may underlie some of the detrimental effects of these drugs that have come to light in recent clinical studies (Ryden et al., 2007; Erdmann et al., 2007; Nissen and Wolski, 2007). The insulin-sensitizing properties of TZDs are undoubtedly of immense benefit to Type 2 diabetic patients; however it remains to be established whether TZDs should be prescribed more widely for their pleiotropic effects.

7. SUMMARY AND FUTURE DIRECTIONS

CF perform key roles in cardiac development and contribute significantly to cardiac structure and function. In this review we have described the origin and distribution of CF and discussed their pivotal and numerous roles that support and maintain myocardial integrity. CF are both sources and targets of environmental stimuli and bioactive molecules, co-coordinating mechanical, chemical and electrical signals between the cellular and non-cellular compartments of the myocardium. As such, an in depth understanding of the mechanisms underlying their multiple functions in health and disease is fundamental.

A number of significant issues relating to the study of CF must be highlighted. Firstly, the choice of experimental model is extremely important for extrapolating laboratory results to clinical effects in the adult human. Many studies on CF use non-human animal cells, with the majority using neonatal cells due to their ready availability and ease of culture. However, the behaviour of neonatal cells can be very different from adult cells, and inter-species differences are also evident (Agocha et al., 1997b; Bouzegrhane and Thibault, 2002). Secondly, a single stimulus is often employed in culture models in order to simplify characterization of downstream signaling and functional end points. However, this is clearly not the case in vivo where individual CF are exposed to a complex array of physical and chemical stimuli that may be widespread, but are often highly localised, and the net effect on CF is determined by the combination of effects of all these stimuli acting together.

The unique features of CF compared with other myocardial cells and with fibroblasts from other tissues, make the CF an attractive therapeutic target for reducing detrimental remodeling (Brown et al., 2005). There is good evidence that several widely-prescribed pharmacological agents, including ACE inhibitors, ARBs, beta-blockers, statins and TZDs, can reduce adverse myocardial remodeling at least in part via effects on CF (summarized in Figure 2). By inhibiting CF proliferation, migration, differentiation, ECM turnover and growth factor/cytokine secretion, these agents elicit beneficial effects on pathological myocardial remodeling.

Cell therapy has received much attention as a means of regeneration or repair of damaged myocardium. The most popular approach has been direct injection of isolated cells into damaged hearts and studies using animal models have revealed improvement of heart function after the transplantation of various cell types (reviewed in Ramos and Hare, 2007; Zhang and Pasumarthi, 2008). Myoblasts or BM cells have been transplanted into patients suffering from ischemic heart disease and significant efficacies of these treatments have been reported (Hagege et al., 2006; Meyer et al., 2006). A considerable drawback to direct cell injection has been an inability to retain cells in the required location due to loss of viability and/or "washout" (Suzuki et al., 2004; Zhang et al., 2001). To date, cell therapies have primarily focussed on myocyte regeneration but a recent study used a different approach. Using co-cultures of dermal fibroblasts and endothelial progenitor cells, cell "sheets" were preformed and then transplanted into infarcted rat myocardium (Kobayashi et al., 2008). The study claimed that use of such a bicellular graft provided a significant improvement over previous reports that used fibroblast grafts alone (Kobayashi et al., 2008).

In addition to their derivation from resident CF, myoFb can also originate from differentiation of monocytes and endothelial cells (discussed in Section 2.6). Thus, targeting monocyte- or endothelial cell-to-CF transition may offer therapeutic potential. Serum amyloid P (SAP) has been shown to block differentiation of monocytes to CF in a murine fibrotic cardiomyopathy model through inhibition of the Fc receptor on circulating monocytes (Haudek et al., 2006; Haudek et al., 2008). Mice treated with SAP did not develop cardiomyopathy following

ischemia/reperfusion injury (Haudek et al., 2006; Haudek et al., 2008). Similarly, less fibrosis was evident if myoFb transition from monocytes or endothelial cells was blocked following pressure overload (Endo et al., 2007; Zeisberg et al., 2007). In the setting of MI however, increasing monocyte-to-myoFb transition appears to be beneficial in promoting infarct healing (Fujita et al., 2007).

Knowledge of cardiac biology and function has improved dramatically over the last decade. Understanding the function of both resident and progenitor cells is vital for successful implementation of these cells in the treatment of the diseased human heart. Nevertheless, cardiac injury and repair is a multifactorial and complex phenomenon and there is still much to be learned before cell therapy in cardiac repair is an achievable goal.

Gene therapy aimed at the CF is also in its early stages. Genetic approaches aimed at the cardiomyocyte have been facilitated through systemic or myocardial introduction of viral particles expressing genes under the control of cardiomyocyte-specific promoters such as that for alpha-myosin heavy chain (Heine et al., 2005). However, specific targeting of the CF in this way has been hampered by the lack of identification of a CF-specific promoter element that is absent in fibroblasts from other tissues, and therefore alternative strategies are warranted. A novel transplantation technique was recently employed to deliver genetic engineered CF to the infarct area of rats following experimental MI (Ruvinov et al., 2008). Neonatal rat CF were virally infected with a vector encoding recombinant human erythropoietin, and these cells were injected into the infarcted myocardium of adult rats seven days after MI. Disappointingly, despite encouraging in vitro results, there was no evidence of improved myocardial remodeling in rats injected with erythropoietin-expressing CF, indeed there was evidence of worse remodeling (Ruvinov et al., 2008). Thus, although CF represent an attractive gene carrier for targeting the infarcted heart due to their clinical accessibility and ease of expansion in culture, the finer details of delivering on the promise of CF-targeted gene therapy (including use of adult cells, timing of transplantation and selection of appropriate genes to target) have yet to be resolved.

Interest in small non-coding microRNAs (miRNAs) has been a focus of recent attention with respect to their dysregulation in various disease states, including cardiac disease (Sayed et al., 2007). MiRNAs are short regulatory RNAs that act as negative regulators of gene expression by inhibiting mRNA translation or by promoting mRNA degradation. The first report of an effect of miRNA-21 (miR-21) in CF was a demonstration of its ability to regulate the MAPK pathway, thereby modulating cell proliferation in the remodeling heart (Thum et al., 2008). Fibrosis is traditionally viewed as a phenomenon secondary to myocyte death and/or hypertrophy in reponse to injurious stimuli, but this study clearly demonstrated a direct primary role of miR-21 in CF that regulated their survival and contributed to myocardial dysfunction. MiR-21 was shown to be selectively increased in CF from failing hearts, thus augmenting growth factor release that subsequently enhanced cardiomyocyte hypertrophy (Thum et al., 2008). MiRNA expression profiling also recently identified MiR-21 as being increased in the mouse myocardium following experimental MI (Roy et al., 2009). In that study, miR-21 negatively regulated PTEN expression in CF leading to up-regulated AKT signaling and increased MMP-2 expression (Roy et al., 2009). These novel findings reveal that miRNAs expressed by CF may represent future therapeutic targets for treatment of adverse myocardial remodeling.

In summary, it is clear that CF can regulate global myocardial function at a number of levels. Until recently, this cell-type has been somewhat overlooked, but through intensive research our understanding of the role of CF in pathological states has been progressively improved. As such, these multifunctional cells represent attractive but challenging therapeutic targets for the future.

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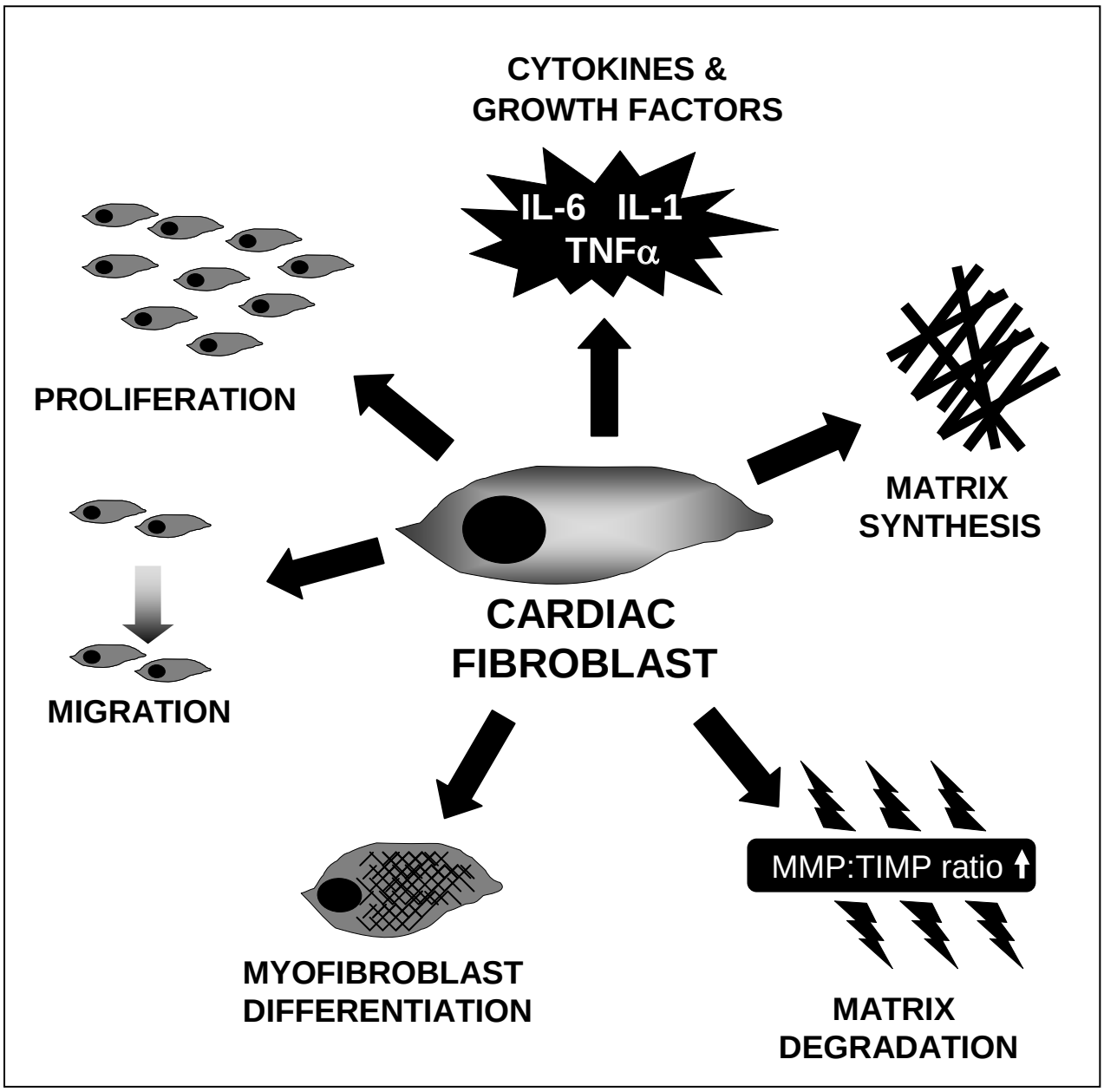

Figure 1. Modulation of cardiac fibroblast function associated with myocardial remodeling. The cardiac fibroblast responds to environmental stimuli in multiple ways, including transformation to a myofibroblast phenotype, proliferation, migration, secretion of cytokines and growth factors, and altering extracellular matrix turnover through changes in matrix protein synthesis and matrix degradation (increase in MMP:TIMP ratio). While these changes in fibroblast function are an important adaptive response to altered environment that can aid myocardial recovery, they can become maladaptive leading to pathological remodeling, fibrosis and heart failure.

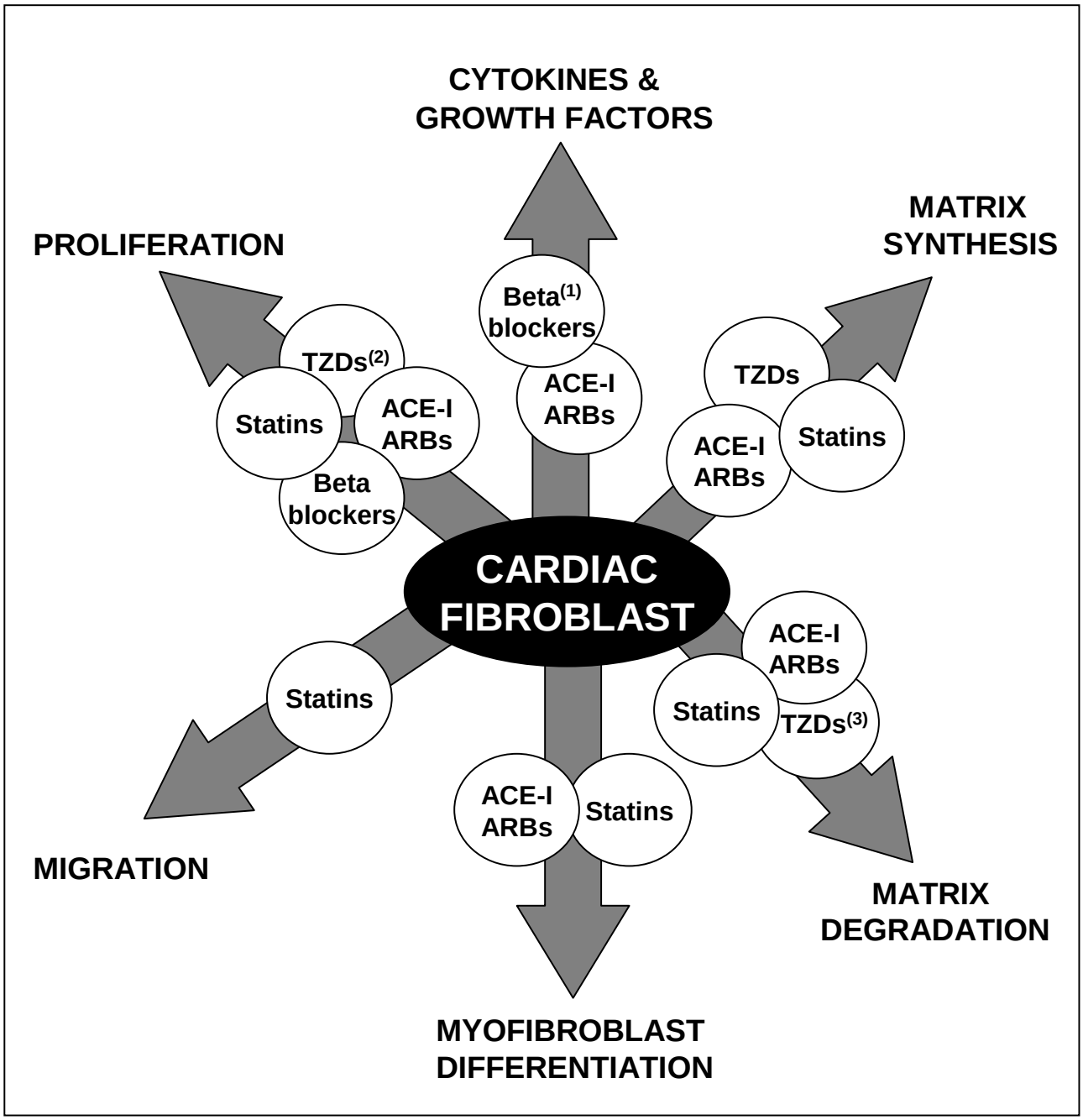

Figure 2. Overview of the modulation of cardiac fibroblast function by different classes of pharmacological agents.
ACE inhibitors (ACE-I), angiotensin receptor blockers (ARBs), beta blockers, statins and thiazolidinediones (TZDs) can inhibit specific aspects of CF function which likely contribute to the beneficial effects of these drugs on adverse myocardial remodeling. See Section 6 of the main text for detail. Notes: (1)β2-AR activation is most notably coupled to IL-6 production in CF and therefore beta blockers specifically reduce catecholamine-induced IL-6 production. (2)There is evidence for and against TZDs reducing CF proliferation. (3)The ability of TZDs to modulate MMP expression appears to be largely dependent on the initiating stimulus.

Table 1. Members of the MMP family expressed by cardiac (myo)fibroblasts.

Abbreviations of stimuli: Ang II = angiotensin II, Anox-Reox = anoxia + reoxygenation, BNP = B-type natriuretic peptide, Hypox-Reox = hypoxia + reoxygenation, IL-1 = interleukin-1, PDGF = platelet-derived growth factor, ROS = reactive oxygen species, TGF-β = transforming growth factor beta, TNFα = tumour necrosis factor alpha. Age: Ad = Adult, Neo = neonatal. *Asterisk indicates cells characterized as cardiac fibroblasts (α-SMA negative). **Double asterisk indicates cells characterized as cardiac myofibroblasts (α-SMA positive). No asterisk indicates phenotype of cells not precisely determined/reported.

MMP	Effect	Stimulus	Species	Age	References
MMP-1 (collagenase-1)	↑ Expression	IL-1	Human	Ad**	(Turner and Porter 2008, unpub)
		BNP	Canine	Ad**	(Tsuruda et al., 2002a)
		PDGF	Rat	Neo	(Yang et al., 2006)
		Anox-Reox	Rat	Ad*	(Chen et al., 2004b)
	↓ Expression	Ang II	Human	Ad	(Pan et al., 2008)
		Ang II	Rat	Ad*	(Brilla et al., 1994)
		Ang II	Rat	Ad*	(Chen et al., 2004a)
		Ang II	Rat	Ad*	(Chen et al., 2004c)
		Ang II	Rat	Ad	(Lijnen et al., 2008)
		Ang II	Mouse	Ad	(Stacy et al., 2007)
MMP-2 (gelatinase A)	↑ Expression	IL-1	Human	Ad	(Guo et al., 2008)
		IL-1	Rat	Ad	(Brown et al., 2007)
		TNFα	Rat	Neo	(Peng et al., 2002)
		TGF-β	Rat	Ad*	(Stawowy et al., 2004)
		TGF-β	Rat	Neo*	(Mookerjee et al., 2005)
		Ang II	Rat	Neo*	(Mookerjee et al., 2005)
		BNP	Canine	Ad**	(Tsuruda et al., 2002a)
		ROS	Rat	Neo	(Siwik et al., 2001)
		Hypox-Reox	Rat	Neo	(Makino et al., 2006)
		Hypoxia	Rat	Neo	(Bergman et al., 2003)
		Stretch	Rat	Ad*	(Husse et al., 2007)
	↑ Activity	IL-1	Human	Ad	(Guo et al., 2008)
		IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		IL-1	Rat	Neo	(Xiao et al., 2008)
		TNFα	Rat	Ad + Neo	(Siwik et al., 2000)
		TNFα	Rat	Neo	(Peng et al., 2002)
		TGF-β	Rat	Ad	(Stawowy et al., 2004)
		PDGF	Rat	Neo	(Yang et al., 2006)
		Collagen	Human	Ad**	(Morley et al., 2007)
		Collagen	Rat	Ad	(Guo and Piacentini, 2003)
		ROS	Rat	Neo	(Siwik et al., 2001)
	↓ Expression	Ang II	Human	Ad	(Pan et al., 2008)
		Ang II	Rat	Neo	(Stewart, Jr. et al., 2006)

		Ang II	Rat	Neo	(Peng et al., 2002)
		Ang II	Mouse	Ad	(Stacy et al., 2007)
	↓ Activity	Ang II	Rat	Neo	(Peng et al., 2002)
		Hypoxia	Human	Ad**	(Morley et al., 2007)
MMP-3 (stromelysin-1)	↑ Expression	IL-1	Human	Ad**	(Turner and Porter 2008, unpub)
		IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		IL-1	Rat	Ad	(Brown et al., 2007)
		BNP	Canine	Ad**	(Tsuruda et al., 2002a)
MMP-9 (gelatinase B)	↑ Expression	IL-1	Human	Ad**	(Turner and Porter 2008, unpub)
		IL-1	Rat	Ad	(Brown et al., 2007)
		IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		IL-1	Rat	Neo	(Xiao et al., 2008)
		TNFα	Human	Ad**	(Porter et al., 2004a)
		Ang II	Human	Ad	(Pan et al., 2008)
		ROS	Rat	Neo	(Siwik et al., 2001)
	↑ Activity	IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		IL-1	Rat	Ad	(Xie et al., 2003)
		PDGF	Rat	Neo	(Yang et al., 2006)
		ROS	Rat	Neo	(Siwik et al., 2001)
	↓ Activity	Ang II	Mouse	Ad	(Stacy et al., 2007)
MMP-13 (collagenase-3)	↑ Expression	IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		ROS	Rat	Neo	(Siwik et al., 2001)
	↑ Activity	IL-1	Rat	Ad + Neo	(Siwik et al., 2000)
		ROS	Rat	Neo	(Siwik et al., 2001)
MMP-14 (MT1-MMP)	↑ Expression	TGF-β	Rat	Ad	(Stawowy et al., 2004)
		BNP	Canine	Ad**	(Tsuruda et al., 2002a)
		Collagen	Human	Ad**	(Morley et al., 2007)
		Collagen	Rat	Ad	(Guo and Piacentini, 2003)
		Stretch	Human	Ad*	(Tyagi et al., 1998)

Table 2. Effects of β-AR stimulation on cardiac fibroblast proliferation.

Age: Ad = Adult, Neo = neonatal. Stimulus: ISO = isoproterenol, NA = noradrenaline, CLEN = clenbuterol (β2-AR agonist). Method: Cell counts = haemocytometer or Coulter counter, Thymidine = [^{3}H]-thymidine incorporation (DNA synthesis), WST = water soluble tetrazolium assay. Inhibition: β2 = β2AR-selective antagonist, β1 = β1AR-selective antagonist, β1/2 = non selective βAR antagonist, - = not investigated.

Species	Age	Stimulus	Method	Inhibition	References
Human	Ad	ISO	Cell counts	β2 (not β1)	(Turner et al., 2003)
	Ad	ISO	Cell counts	-	(Turner et al., 2004)
Rat	Ad	NA	Cell counts	β1/2, β2 (not β1)	(Leicht et al., 2000)
	Ad	NA	Cell counts	-	(Leicht et al., 2003)
	Ad	NA, ISO	Thymidine	-	(Dubey et al., 2001)
	Ad	ISO	Thymidine	-	(Kim et al., 2002)
	Neo	NA	Cell counts	-	(Fisher and Absher, 1995)
	Neo	NA	Thymidine	-	(Zheng et al., 1998)
	Neo	NA	Thymidine	β1/2	(Calderone et al., 1998)
	Neo	ISO, CLEN	Thymidine	-	(Colombo et al., 2003)
	Neo	ISO	Thymidine	β2 (not β1)	(Yin et al., 2003b)
	Neo	NA	WST	-	(Akiyama-Uchida et al., 2002)
Rabbit	Ad	NA	Thymidine	-	(Bhambi and Eghbali, 1991)