# Chapter 30
# The ACE2/Ang-(1–7) Pathway in Cardiac Fibroblasts as a Potential Target for Cardiac Remodeling

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**Abstract** Despite advances in its treatment, heart failure prevalence continues to increase and this condition remains a major contributor to mortality and morbidity in both industrialized and developing nations throughout the world. Cardiac remodeling that is activated by injury to the myocardium and/or increased wall stress adversely affects cardiac function over time and is known to play a critical role in the progression of heart failure. Excessive accumulation and stiffening of collagen (i.e., fibrosis) is a well-recognized component of the remodeling process, and it contributes to progression of disease and worsening heart failure by perturbing cardiac contractility, relaxation, and electrical conduction. Cardiac fibroblasts are interstitial cells that are responsible for synthesis and turnover of collagen in the myocardium. Current experimental and clinical evidence suggests that cardiac fibroblasts are an important target when inhibitors of the renin–angiotensin system (RAS) are used to treat heart failure. An alternative pathway of the RAS, involving angiotensin-converting enzyme 2 (ACE2), angiotensin-(1–7) (Ang-(1–7)), and the Mas receptor, has been found recently to be cardioprotective. Evidence suggests that Ang-(1–7), acting predominantly through the Mas receptor, can regulate maladaptive growth-promoting effects of angiotensin ll and inhibit both cardiomyocyte hypertrophy and cardiac fibrosis. This chapter highlights the potential regulatory role of the ACE2/Ang-(1–7)/Mas pathway and how it relates to cardiac fibroblasts during the remodeling process.

**Keywords** Angiotensin-(1–7) • Fibroblast • Angiotensin-converting enzyme 2 • Fibrosis • Heart • Collagen

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## 30.1 Introduction

### 30.1.1 The Cardiac Fibroblast and Cardiac Remodeling

Almost 6 million people suffer from heart failure in the United States today [1]. Similar high rates of heart failure prevalence are seen in other industrialized countries, and there is evidence that heart failure is growing rapidly as a public health problem in developing nations [2–5]. Current medical and device therapies have reduced morbidity and mortality in patients with heart failure. However, the effects of available treatment strategies have plateaued, and novel targets are needed if improvements in outcome are to continue in the future. In patients with chronic heart failure, the condition develops as a consequence of progressive changes in the structure of the heart that impair its normal function [6]. Hypertrophy of cardiomyocytes is a hallmark of the remodeling process, and there is evidence that the structural changes in these cells are intimately associated with abnormalities in their function. Another change in the structure of the heart that occurs as part of the remodeling process and which plays a critical role in the progression to heart failure is the deposition of collagen within the heart (i.e., cardiac fibrosis). Cardiac fibrosis stiffens the heart wall and alters electrical contractility, leading to diminished diastolic and/or systolic function and increasing the probability of debilitating or even fatal arrhythmias. Activation of the mechanisms that cause fibrosis as well as the extent of fibrous tissue that has been deposited within the failing heart has been associated with worse outcomes in heart failure patients. Thus, minimizing the development of fibrosis is expected to improve cardiac function, slow the development of heart failure, and help reduce morbidity and mortality in the heart failure population.

### 30.1.2 ACE2/Ang-(1–7)/Mas Pathway

There are several current treatment strategies for heart failure that target the renin–angiotensin system (RAS). These involve inhibition of the generation of angiotensin II (Ang II) with angiotensin-converting enzyme (ACE) inhibitors or blocking activation of Ang II receptors with angiotensin receptor blockers (ARBs). The importance of the RAS in the progression of heart failure is apparent by the beneficial effects of both ACE inhibitors and ARBs in large-scale, well-designed clinical trials [7–11]. These agents have been shown to inhibit the remodeling process as well as reduce morbidity and mortality across a broad spectrum of patients ranging from those with asymptomatic left ventricular (LV) dysfunction, to MI survivors, to patients with clinically manifested heart failure including those with advanced disease.

Recently, an alternative axis of the RAS, the ACE2/Ang-(1–7)/Mas pathway, has been receiving much attention as it is believed to function as a means of modulating the effects of Ang II and other growth-promoting factors during remodeling. Angiotensin-converting enzyme 2 (ACE2) was identified in 2000 by two independent

groups [12, 13]. ACE2 functions as a carboxymonopeptidase and has approximately 40% sequence similarity when compared to ACE. ACE2 can hydrolyze the carboxy-terminal phenylalanine from Ang II to form the heptapeptide angiotensin-(1–7) (Ang-(1–7)), or it can generate angiotensin-(1–9) from angiotensin I, at which point other peptidases can convert angiotensin-(1–9) to Ang-(1–7) [14]. Other substrates for ACE2 include des-Arg$^9$-bradykinin, apelin-13, dynorphin A (1–13), and β-caso-morphin [14]. When considering only the angiotensin peptides, ACE2 has two, potentially beneficial functions. (1) It can degrade Ang II and (2) it can generate Ang-(1–7). As opposed to Ang II, which is considered to activate cardiomyocyte hypertrophy and fibrosis and, thus, to be deleterious by promoting remodeling, Ang-(1–7) is considered to have potentially beneficial antigrowth and antifibrotic effects that would act to inhibit the remodeling process. In human myocardium, Ang II has been shown to be the preferred substrate for ACE2. Moreover, ACE2 effects appear to be impervious to the ACE inhibitors that are used in clinical practice.

Some investigators have reported that Ang-(1–7) can act through well-character-ized angiotensin receptors such as $AT_1$ or $AT_2$ [15–17]. However, Ang-(1–7) more frequently acts via its own dedicated receptor, which is widely considered to be the Mas receptor [18]. Mas is a G protein-coupled receptor that was originally described as a protooncogene [19, 20]. Mas becomes detectable after birth in the rat, increases its expression, and then plateaus at 4–6 months of age [21]. Mas exhibits a broad tissue expression profile, including the heart, kidney, testis, brain, retinal pigment epithelium, skeletal muscle, liver, and adipose tissue [22–24]. Within the heart, Mas is localized to the vasculature [25], cardiomyocytes [26, 27], and fibroblasts (detected by qRT-PCR, data not shown). Despite the passage of ten years since the initial characterization of Mas as the Ang-(1–7) receptor [18], the signaling pathways involved have not yet been fully delineated.

## 30.2 Responses of the Cardiac Fibroblast to Ang-(1–7)

### 30.2.1 Direct Responses to the Peptide

Direct activation of signaling pathways by Ang-(1–7) treatment of cardiac fibroblasts isolated from normal hearts has been reported. We have observed weak, transient phosphorylation of ERK1/ERK2 upon stimulation of adult rat cardiac fibroblasts with Ang-(1–7) (unpublished observations). We have also observed reduced endothe-lin-1 (ET-1) and leukemia inhibitory factor (LIF) mRNA levels between 1 and 2 h after stimulation of adult rat cardiac fibroblasts with Ang-(1–7), presumably via acti-vation of an undetermined signaling pathway [28]. We have observed modest inhibition of Ang II-stimulated increases in phosphorylation of MAPKs (e.g., ERK, JNK, and p38) in fibroblasts which had been pretreated with Ang-(1–7). McCollum et al. also observed that treatment of cardiac fibroblasts with Ang-(1–7) reduced the Ang II- or ET-1-stimulated increase in phospho-ERK1 and phospho-ERK2 [29]. They observed that Ang-(1–7) increased dual-specificity phosphatase DUSP1 immunoreactivity

and mRNA, suggesting that the heptapeptide hormone increases DUSP1 to reduce MAP kinase phosphorylation and activity. The mechanism through which Ang-(1–7) increased DUSP1, however, was not elucidated. Increased generation of second messengers by Ang-(1–7) stimulation has been reported, including nitric oxide in cardiomyocytes [26] and aortic endothelial cells [30], cAMP in glomerular mesangial cells [31], and arachidonic acid from Mas-transfected CHO cells [18]. However, we have failed to observe activation of these second messengers in response to Ang-(1–7) in cultured cardiac fibroblasts (data not shown).

## 30.2.2 Indirect Responses to the Peptide

As opposed to the direct stimulatory effects of Ang-(1–7) on cardiac fibroblasts, which tend to be mild, indirect effects of the peptide can produce a much greater response. These indirect responses include alteration of other signaling pathways and the influence of second messengers that have been generated by cardiac fibroblasts, their precursors, or other nearby cell types.

### 30.2.2.1 Attenuation/Augmentation of Other Receptor Signaling Pathways

Stimulation of cells with Ang-(1–7) can affect the subsequent activation of other signaling pathways. Stimulation of nitric oxide production by bradykinin in endothelial cells can be augmented by prior exposure of the cells to Ang-(1–7) [32]. There is also evidence that Ang-(1–7) stimulation can prove inhibitory to signaling pathways, such as those activated by Ang II [29, 33, 34], glucose [35], and ET-1 [29]. As noted in the previous section, preincubation with Ang-(1–7) has been reported to reduce subsequent activation of MAP kinases by Ang II and other ligands [29, 33–36]. The mechanism of these effects is not known, although activation of tyrosine phosphatases in proximal tubular cells [35] and upregulation of DUSP1 in neonatal rat cardiac fibroblasts [29] have been implicated. Although DUSP1 upregulation has been implicated in the inhibitory effect of Ang-(1–7) in neonatal rat cardiac fibroblasts [29], we were unable to detect this effect in the adult cells. In adult rat cardiac fibroblasts, we found that Ang-(1–7) preincubation inhibited subsequent Ang II-stimulated upregulation of ET-1 and LIF mRNA. We also found that Ang II-induced secretion by adult rat cardiac fibroblasts of unknown paracrine factors that induced hypertrophy in cultured cardiomyocytes could be significantly inhibited by preincubation of the fibroblasts with Ang-(1–7) (Fig. 30.1 and [28]).

### 30.2.2.2 Autocrine/Paracrine Second Messengers

Fibroblasts can originate from other sources in addition to the cardiac interstitium, especially when the heart is actively remodeling. Sources of nonresident fibroblasts
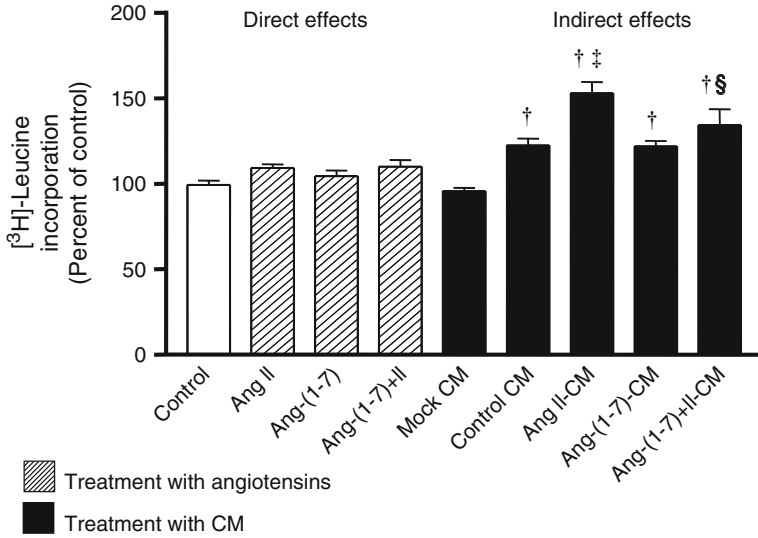
**Fig. 30.1** Effects of angiotensins and conditioned media (CM) from adult rat cardiac fibroblasts (ARCFs) treated with angiotensins on [³H]leucine incorporation in cardiomyocytes. Cardiomyocytes were stimulated for 24 h with Ang II, Ang-(1–7), both peptides after 1 h pretreatment with Ang-(1–7) or CM from ARCFs to assess cardiomyocyte hypertrophy measured by [³H] leucine incorporation. Ang II-CM, CM from Ang II-treated ARCFs; Ang-(1–7)-CM, CM from Ang-(1–7)-treated ARCFs; Ang-(1–7)+II-CM, CM from ARCFs treated with both peptides after 1 h pretreatment with Ang-(1–7). † $P < 0.001$ vs. mock CM; ‡ $P < 0.001$ vs. control CM; § $P < 0.05$ vs. Ang II-CM (Reproduced with permission [28])

in the remodeling heart include circulating fibrocytes and mesenchymal cells generated from epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndMT). It is still not clear whether these additional cells serve some unique purpose or simply elevate the pool of fibroblasts during increased need. However, the possibilities exist that (1) these cells may be affected by Ang-(1–7) differently than the resident fibroblasts and (2) the recruitment and/or differentiation of these cells could be altered by Ang-(1–7). Indeed, Ang-(1–7) has been shown to reduce the number, proliferative capacity, and collagen secretion of human circulating fibrocytes, possibly by increasing nitric oxide production [37]. Also, cGMP-dependent protein kinase (PKG) has been shown previously to disrupt transforming growth factor-β1 (TGF-β1)-induced nuclear translocation of pSmad3 [38]. Considering (1) the involvement of TGF-β1 in EMT (or EndMT) and myofibroblast differentiation and (2) the activation of guanylate cyclase and subsequent cGMP generation after nitric oxide exposure, there is a strong possibility that Ang-(1–7)-induced nitric oxide synthesis could inhibit fibroblast functions in a remodeling tissue. Ang-(1–7) has been shown to directly induce the release of nitric oxide in platelets, cardiomyocytes, and aortic endothelial cells [26, 30, 39], a process which involves activation of Akt and eNos in cardiomyocytes. As noted above, Ang-(1–7) has also been shown to augment the release of nitric oxide from endothelial cells

that have been stimulated with bradykinin [32]. Many of these indirect effects can theoretically influence cardiac fibroblasts during cardiac remodeling, but their effects in animal models or human patients have not been studied.

## 30.3    Effects of the ACE2/Ang-(1–7)/Mas Pathway on Cardiac Remodeling

Germline ablation of ACE2 results in a phenotype of cardiac dilatation and dysfunction in some [40], but not all [41], mouse models, and it predisposes to cardiac failure when the heart is stressed [42]. We have shown that pharmacologic inhibition of ACE2 in the post-MI rat heart using the selective inhibitor C16 increases infarct size and adversely affects contractile function [43]. Treatment with C16 had no significant effect on the increased level of apoptosis in the infarct and border zones nor did it significantly affect capillary density surrounding the MI. It did, however, significantly reduce the number of c-kit(+) cells in the border region, a finding consistent with the possibility that Ang-(1–7) or some other product of ACE2 helped reduce infarct size by enhancing the viability of cardiomyocytes in the border zone either directly or via paracrine effects of mediators released from the c-kit(+) cells. The observation that C16 inhibited increases in wall thickness and fibrosis in non-infarcted LV, however, raises the possibility that ACE2 activity has diverse (and perhaps even some adverse) effects on post-MI remodeling. Others have reported that loss of ACE2 accelerates maladaptive post-MI LV remodeling [44], while increasing ACE2 levels by gene transfer therapy inhibits post-MI remodeling [45].

In addition to degrading Ang ll, many favorable effects of ACE2 appear to be due to increased Ang-(1–7) levels. Elevated Ang-(1–7) levels are predicted to be inhibitory to both cardiomyocyte hypertrophy and fibrosis. In rats, post-MI remodeling can be inhibited by infusing Ang-(1–7) [46] or ingestion of either AVE 0991 (a nonpeptide Ang-(1–7) analogue) [47], an Ang-(1–7) hydroxypropyl β-cyclodextrin preparation [48], or a stabilized Ang-(1–7) analogue [49]. Angiotensin II-stimulated cardiac hypertrophy in mice is inhibited by cardiac-specific Ang-(1–7) overexpression [50, 51]. Whether Ang-(1–7) cardioprotection is due to effects of the peptide within the heart or effects of the peptide on extracardiac cells/tissues, however, is uncertain as some evidence suggests that circulating rather than cardiac Ang-(1–7) produces the beneficial effects of the peptide on post-MI remodeling [52]. We have shown that ACE2 inhibition increases post-MI infarct expansion [43] so that there is also uncertainty whether Ang-(1–7) inhibits remodeling due to an early reduction in infarct size or due to later effects on the remodeling process. Among strategies for using Ang-(1–7) to treat post-MI remodeling, gene transfer therapy is one of the most appealing [53–55]. Evidence from experiments in which a lentiviral vector encoding an engineered Ang-(1–7) fusion protein was injected directly into the LV wall several weeks prior to coronary ligation supports this possibility [56].

Germline deletion of Mas in mice has been reported to cause decreased cardiac performance, which is believed to be the result of increased levels of

extracellular matrix proteins in the right ventricles and AV valves [57]. Pharmacologic stimulation of the Mas receptor has shown promise in animal models of cardiac dysfunction. The nonpeptide Mas agonist, AVE 0991, improves cardiac function in rats with diabetes [58, 59], with isoproterenol treatment [60] and following experimentally induced MI [61]. AVE 0991 has also been shown to ameliorate progression of atherosclerosis in apoE-null mice [62]. Another Mas agonist peptide, CGEN-856S, which has been reported to be more stable than Ang-(1–7), demonstrated anti-arrhythmogenic effects in isolated rat hearts [63], but this peptide has not been extensively studied. To our knowledge, no human data exist regarding these Mas agonists, but their clinical potential as cardioprotective agents is recognized. Based on these considerations, further exploration of the effects of stable Ang-(1–7) analogues or Mas receptor agonists in cardiovascular disease is clearly warranted.

## 30.4   Future Considerations

There are many questions that still need to be answered about the relevance of this relatively new axis of the RAS and its potential role in regulating cardiac remodeling. Ang-(1–7)-induced signaling pathways (whether direct or indirect) remain unclear and require further study. Although Mas is widely regarded as the Ang-(1–7) receptor, there are still indications that other receptors are utilized in certain contexts [15–17]. Regarding cardiac fibroblasts, very little study has been devoted to understanding how ACE2/Ang-(1–7)/Mas can influence the recruitment and/or production of other cell types that contribute to the interstitial cell pool in the remodeling heart. Positive outcomes have been obtained when perturbing the ACE2/Ang-(1–7)/Mas system in animal models of disease. However, to our knowledge, no clinical use of such strategies has yet been reported. Since ACE2/Ang-(1–7)/Mas is considered cardioprotective, stimulation of this pathway, rather than inhibition, is expected to be beneficial. Ang-(1–7) infusion would be clinically difficult due to its short half-life. However, synthetic genes have been engineered to enhance Ang-(1–7) secretion from transfected or transduced cells [64]. Theoretically, increased expression of Ang-(1–7) would act cooperatively with existing ACE inhibitor or ARB pharmacologic treatment regimes, but this has yet to be tested. Enhanced ACE2 activity alone could demonstrate considerable improvements over existing therapies since it would simultaneously degrade Ang II and generate the cardioprotective Ang-(1–7). Stimulation of ACE2 enzymatic activity may prove challenging using pharmacology, but overexpression of ACE2 is always possible by transfection or viral transduction. We must remain cautious, however, since ACE2 overexpression by such methods has proven to be detrimental in at least one published work [65]. Also, discrepancies as to whether cardioprotective effects are due to activation of this pathway in the myocardium or in extracardiac tissues demand further studies [52, 56].

## 30.5   Conclusions

Although the past several decades have witnessed substantial improvements in heart failure outcomes, morbidity and mortality still remain unacceptably high. This, along with the relentless increase in new cases of heart failure throughout the world, makes it clear that novel treatment strategies are still needed. The RAS has been a pharmacologic target for heart failure treatment for many years. A more recently discovered branch of the RAS, namely, the ACE2/Ang-(1–7)/Mas system, has demonstrated cardioprotection when activated during remodeling in animal models of heart failure. The ACE2/Ang-(1–7)/Mas system can affect many cell types, but the cardiac fibroblast has been shown to be an important target of its action. Effects of Ang-(1–7) on cardiac fibroblasts can involve direct activation of signaling pathways or indirect effects on their function that involve either the paracrine release of second messengers, alterations in the differentiation/recruitment of fibroblast "precursors," or crosstalk with other signaling pathways. Ultimately, activation of the ACE2/Ang-(1–7)/Mas system is predicted to produce an "antifibrotic" phenotype and should improve cardiac remodeling. Despite favorable results in animal models, however, adaptation of the promising strategies for enhancing the effects of this alternate pathway and proof of efficacy in well-designed human trials are still needed.

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