Cardiac hypertrophy and fibrosis are two closely related adaptive response mechanisms of the myocardium to mechanical, metabolic, and genetic stress that finally contribute to the development of heart failure (HF). This relation is based on a dynamic interplay between many cell types including cardiomyocytes and fibroblasts during disease progression. Both cell types secrete a variety of growth factors, cytokines, and hormones that influence hypertrophic cardiomyocyte growth and fibrotic fibroblast activation in a paracrine and autocrine manner. It has become evident that, aside proteinous signals, microRNAs (miRNAs) and possible other RNA species such as long non-coding RNAs are potential players in such a cell-to-cell communication. By directly acting as paracrine signals or by modulating downstream intercellular signalling mediators, miRNAs can act as moderators of the intercellular crosstalk. These small regulators can potentially be secreted in a ‘micrine’ fashion, so that miRNAs can be assumed as the message itself. This review will summarize the recent findings about the paracrine crosstalk between cardiac fibroblasts and cardiomyocytes and addresses how miRNAs may be involved in this interplay. It also highlights therapeutic strategies targeting factors of pathological communication for the treatment of HF.

**Keywords**
Paracrine communication • Cardiomyocyte • Cardiac fibroblast • MicroRNAs • Cardiac remodelling • Therapeutics

**1. Introduction**

Heart failure (HF), or the impaired capacity of the heart to maintain haemodynamic demands, is a final manifestation of cardiovascular disease. This end-stage phase is preceded by chronic myocardial stress that results from cardiac injury and/or persistent elevation of ventricular pressure and/or volume. This leads to a series of initially compensatory morphological and functional changes. Persistent cardiac stress exacerbates maladaptive responses, including cardiac hypertrophy and fibrosis. Both pathologies are closely related and influence myocytes and non-myocyte cell types in the heart. Cardiomyocytes undergo phenotypical modifications, including increase of cell size, protein synthesis, and different sarcomeric assembly, accompanied by altered gene expression including re-induction of the foetal gene programme, abnormal Ca\textsuperscript{2+} handling, and cell death.\textsuperscript{1,2} Cardiac fibroblasts proliferate and differentiate to a myofibroblast phenotype, characterized by contractile smooth muscle markers and an elevated expression of extracellular matrix (ECM) components.\textsuperscript{3,4} A disproportional accumulation of ECM proteins alters the cardiac microstructure and forms a barrier that separates cardiomyocytes from each other. This impairs electrical coupling that provokes systolic dysfunctions and/or increases nutrient and oxygen diffusion distances with hypoxia of myocytes as consequence.\textsuperscript{5,6} Therefore, fibroblasts influence cardiomyocyte metabolism, performance, and hypertrophy, and conversely, cardiomyocytes can have an impact on fibroblast phenotype and function.\textsuperscript{6,7} The interplay between both cardiac cells requires an exchange of communicative signals. This occurs through indirect interactions via the ECM, direct cell–cell coupling through gap junctional channels (recently reviewed by Bowers et al.\textsuperscript{8} or through the secretion of various soluble molecules, including growth factors, cytokines, and hormones\textsuperscript{9} (summarized in Figure 1).

Recent findings suggest that, aside proteins, microRNAs (miRNAs or miRs) are additional players in cell-to-cell communication (reviewed by Chen et al.\textsuperscript{10,11}). These small regulators are single-stranded ribonucleotides and belong to the RNA interference (RNAi) pathway that leads to post-transcriptional gene silencing. This is mediated by an evolutionary conserved mechanism, where miRNAs, associated with protein aggregates (RNA-induced silencing complex), bind to partial complementary regions in transcripts, finally impeding protein synthesis or initiating the complete destruction of the target. Thus, individual miRNAs can modulate the expression of several different genes, or a single transcript can be targeted by multiple miRNAs.\textsuperscript{11,12} Therefore, miRNAs increase the
complexity of how gene expression is regulated affecting various biological processes. Genetic studies demonstrated that miRNAs play an essential role in the development and function of the heart. Further findings uncovered miRNA expression profiles in hypertrophic or fibrotic hearts and identified a subset of miRNAs that are deregulated under those pathological conditions. In contrast, little is known about their impact on intercellular communication. Among miRNAs targets are also those transcripts whose expression products are either communicated between fibroblasts and cardiomyocytes or that are part of signaling pathways downstream of paracrine factors. Therefore, miRNAs can be assumed as modulators of the intracellular crosstalk in the myocardium (summarized in Table 1). Since it has been shown that these small regulators are synthesized and released by various cells, including both cardiovascular cell types, miRNAs can also be supposed as the message between cells itself.

This review focuses on the crosstalk between cardiomyocytes and fibroblasts, mediated by paracrine signals, and addresses its impact on the hypertrophic and fibrotic remodelling in the adult myocardium. We emphasize the specific role of miRNAs as regulators and possible direct signals in the cellular interplay, and finally speculate on potential therapeutic benefits targeting pathological cell-to-cell communication.

2. Paracrine factors and their regulation by miRNAs

2.1 Angiotensin II

Angiotensin II (Ang II) is a vasoactive hormone that additionally acts as a strong inducer of hypertrophic and fibrotic response in vitro and in vivo, and is suggested to play a crucial role in the crosstalk between non-myocytes and myocytes. This peptide acts via Ang II receptors (Type 1: AT1 and Type 2: AT2) that are expressed by all cardiac cell types and is induced in several cardiac disease. Most of the physiological effects of Ang II are mediated by the AT1 receptor, while the AT2 receptor plays a functional but controversial role in cardiac hypertrophy and fibrosis.

One downstream effect of Ang II in cultured fibroblasts is the induction of proliferation and ECM protein expression. In neonatal cardiomyocytes, the hormone promotes cell growth. Ang II can act as a mediator, since mechanical stretch causes the release of Ang II from cardiomyocytes, presumably stimulating surrounding cardiomyocytes and/or fibroblasts. However, an indirect effect seems to be the most important mechanism by which Ang II influences the cardiac crosstalk, since the hormone induces the release of further autocrine and paracrine factors from cardiomyocytes and/or fibroblasts. These include transforming growth factor-β, endothelin-1 (ET-1), and interleukin-1/2 (IL-1/2).

Several miRNAs are involved in Ang II-mediated cardiac hypertrophy and fibrosis (Figure 2). Most of them are known to be implicated in HF development. Stimulation of cultured cardiac fibroblasts with Ang II deregulated several miRNAs that have been related to fibrosis. Among them are miR-132, miR-132, miR-212, miR-146, and miR-29b, as well as miR-21 and miR-101a/b. This seems to affect ECM synthesis and turnover as well as fibroblast proliferation. Consistently, Ang II-treated cardiomyocytes exhibit reduced levels of anti-hypertrophic miRNAs, such as miR-1, miR-133, and miR-30, while hypertrophy-promoting miRNAs, including miR-212/-132 and miR-22, are found to be up-regulated. Nevertheless, miRNAs that directly target Ang II or act upstream of this paracrine factor remain elusive.

2.2 Transforming growth factor β

TGF-β is a cytokine with pleiotropic effects regulating the response to myocardial injury and pressure overload, including cardiac hypertrophy and fibrosis. TGF-β exists in three structurally similar isoforms (TGF-β1, 2, and 3), encoded by distinct genes. Among them, TGF-β1 is the predominant and ubiquitous form. It is produced by many cell types, including myocytes and cardiac fibroblasts, and secreted as a latent complex. A group of extracellular proteases and enzymes...
can activate TGF-β that, in turn, binds to TGF-β type 1 and type 2 receptors on both cardiomyocytes and fibroblasts. This finally activates Smad-mediated transcriptional events and further signalling pathways.

TGF-β is a central mediator of cardiac fibrosis and accordingly, affects the phenotype and function of cardiac fibroblasts. It induces their transition to the myofibroblast state and enhances the production of matrix proteins, favouring ECM accumulation rather than degradation. TGF-β signalling induces pro-fibrotic miR-21 that is specifically elevated in fibroblasts upon cardiac remodelling (Figure 2). miR-21 promotes interstitial fibrosis at least in part by repression of sprouty homologue 1 (Spry1). This leads to a derepression of mitogen-activated kinase signalling that enhances fibroblast growth factor 2 (FGF-2) secretion from cardiac fibroblasts (FGF-2) and targeting TGF-β III (TGF-β RIII) is an additional target of miR-21. Decreased expression of TGF-β III after transfection of miR-21 into cardiac fibroblasts leads to an elevation of TGF-β.

miR-29 is a further TGF-β-dependent that, in contrast to miR-21, exhibits an anti-fibrotic function by reducing ECM formation and myofibroblast differentiation. Several genes coding for ECM components, including collagens, fibronectin, and elastin, are predicted targets of this

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CF, cardiac fibroblast; CM, cardiomyocyte; MyoCF, myofibroblast; 293T and COS7, cell lines.
miRNA, and miR-29 repression was shown to increase collagen expression. TGF-β is an upstream regulator of miR-29 (Figure 2), since the treatment of cultured cardiac fibroblasts with TGF-β decreases the levels of this miRNA. Therefore, down-regulation of miR-29 upon pathological remodelling is presumably mediated by TGF-β. Cardiac fibrosis is further regulated by miR-101a and miR-24 that repress the expression of TGF-β. miR-101a seems to impair Ang II-mediated induction of TGF-β by targeting the c-fos, a promoter of TGF-β expression. miR-24 has been shown to directly repress furin, a protease that controls the activation of TGF-β. And, miR-133a and miR-590 seem to act as direct repressors of TGF-β. TGF-β also contributes to cardiac fibrosis by miR-21-stimulated endothelial–mesenchymal transition.

The role of TGF-β in cardiac hypertrophy seems to be ambiguous. Although this growth factor has been considered as a pro-hypertrophic actor downstream of Ang II, recent studies implicate that endogenous cardiomyocyte TGF-β signalling involving Smads preserves cardiomyocytes from hypertrophic growth. The ambiguous role of TGF-β in cardiac hypertrophy is also reflected on the miRNA level. Wang et al. found that mechanical stretch enhances the expression of pro-hypertrophic miR-208a in cultured cardiomyocytes and suggest that this effect is mediated by an autocrine release of TGF-β1. In contrary, TGF-β1 impaired the expression of pro-hypertrophic miR-27b and prevented cardiomyocyte growth.

2.3 Further growth factors

Connective tissue growth factor (CTGF) is a downstream target of TGF-β and is involved in both fibrotic and hypertrophic response in the heart (Figure 2). Cardiac fibroblasts predominantly express CTGF; however, upon cardiac remodelling, this growth factor is also released by cardiac myocytes. CTGF enhances fibroblast proliferation, migration, and ECM accumulation during progression of fibrosis. The expression and secretion of CTGF is regulated by two miRNAs that directly target the transcript. miR-30c is expressed in both cardiac cell types, whereas miR-133a is myocyte-specific. Both miRNAs repress CTGF expression and fine-tune protein levels of this growth factor in a cell type-specific manner.

FGF-2 is another mediator of cardiac hypertrophy and fibrosis. Mice lacking FGF-2 showed a less developed phenotype in both pathologies, while cardiac-specific overexpression exacerbated hypertrophy. FGF-2 can be detected inside and outside the cell and is synthesized by cardiomyocytes and fibroblasts. Since this protein lacks a signal sequence, its release mechanism remains unknown. Secreted by fibroblasts, FGF-2 acts in a paracrine manner and promotes hypertrophic response in cardiomyocytes via initiation of MAPK signalling (Figure 2). An autocrine mechanism induces fibroblast proliferation and the secretion of further pro-hypertrophic factors. Secretion of FGF-2 is influenced by miR-21. As described above, the inhibition of
this miRNA enhances mitogen-activated kinase signalling that elevates FGF-2 release from cardiac fibroblasts.40

Insulin-like growth factor (IGF-1) is an essential actor of cardiac hypertrophy and adaptive response in the heart.30 This protein is produced and released by fibroblasts, but not found in myocytes.31 Endogenous IGF-1 promotes collagen synthesis in fibroblasts.91,92 Exogenous IGF-1, induced in a frequency- and time-dependent manner upon cyclic stretch,93 seems to mediate cardioprotective function of fibroblasts in vivo.90 The IGF-1 transcript is directly targeted by miR-1.38

Under hypertrophic conditions, miR-1 is repressed leading to an up-regulation of IGF-1.39 Protemic analysis of secreted proteins and in vitro approaches revealed that IGF-1 expression is further effected by miR-29b and miR-30c.36,37

Evidence for the role of platelet-derived growth factor (PDGF) in cardiac fibrosis is mainly derived from gain- and loss-of-function studies in animals.94-96 Its expression is elevated in cardiac hypertrophy and heart failure.97 Interstitial cells, including fibroblasts, are the main source of PDGF and its isoforms.98 and PDGF signalling drives fibroblast proliferation and migration as well as ECM deposition.99 Accinno et al.100 suggested that PDGF has not a direct effect on cardiomyocytes during pressure overload, but induces fibroblast proliferation and promotes cardiac hypertrophy through the secretion of further growth factors and cytokines. As for IGF-1, miR-29 is involved in the regulation of PDGF expression.37

Although ET is a paracrine growth factor that is mainly derived from endothelial cells, its isoform (ET-1) and the two corresponding receptors are also found in myocytes and non-myocytes.54,62 It is induced by TGF-β1 and seems to act as a mediator of the TGF-β system to promote fibroblast activation and fibrosis as well as cardiac hypertrophy.62,101,102 In vivo, ET-1 seems to initiate cardiac hypertrophy through a miR-23a-dependent pathway41 and Ang II-mediated ET receptor activation, increased miR-132 and miR-212 levels.42 miR-1 has a repressive effect on ET-1 expression.40

2.4 Tumour necrosis factor α

Cytokines, like tumour necrosis factor α (TNF-α), are well-known messengers in inflammatory signalling and in regulation of cell behaviour. They are involved in cardiac remodelling and wound healing,103 and released upon cardiac injury.104 Short-term activation of pro-inflammatory cytokines is suggested to be cardioprotective, while long-term exposure might induce maladaptive effects.

Infiltrating immune cells are not the only source for TNF-α, but this cytokine is expressed and secreted by cardiomyocytes as well as fibroblasts after certain forms of stress105-109 and found as an active component in fibroblast- or myocyte-conditioned media.110,111 Interaction of both cardiac cell types seems to be an important requirement for differential cytokine expression.112 These findings suggest that elevated levels have an impact on HF progression, mediated by various effects on myocytes and non-myocytes. In cultured cardiomyocytes, TNF-α induces hypertrophic as well as apoptotic responses110,113 and impairs contractility.114 While transgenic mice overexpressing TNF-α develop concentric hypertrophy that finally leads to dilated cardiomyopathy,115,116 Furthermore, this cytokine promotes fibroblast proliferation, secretion of metalloproteinases (MMPs), collagen up-regulation, and the release of further pro-inflammatory cytokines.111,117,118

Regarding TNF-α, miR-146a seems to mediate inflammatory signalling and cardiomyocyte response. Both mechanisms provoke an induction of this miRNA. miR-146a seems to prevent pathological signalling by inhibiting TNF-α expression among further factors.3,105

2.5 Interleukins

Among TGF-β1 and TNF-α, ILs are further markers detectable in the circulation of HF patients, contribute to cardiac remodelling.119 and presumably influence the crosstalk between myocytes and non-myocytes in a pro-inflammatory as well as cardioprotective manner. Several miRNAs are involved in inflammatory processes in the failing heart.105

IL-1β is a pro-inflammatory cytokine that exhibits largely overlapping characteristics with TNF-α.105 It is induced upon cardiac injury and affects pathological remodelling.120 Although IL-1β is expressed by cardiomyocytes in response to heart injury or stress,121,122 fibroblasts seem to be the main source.123 This cytokine acts through its corresponding receptor leading to elevated cell migration,124 impaired fibroblast proliferation,125 and ECM remodelling through reduced collagen expression126 and decreased secretion of MMPs.127 In cardiomyocytes, IL-1β provokes a hypertrophic response125 and cardiac-specific overexpression of IL-1α in mice induces ventricular hypertrophy.128 miRNAs directly affecting IL-1 expression are still not known, but they are involved in the signalling pathways downstream of this IL. Therefore, lentinival open expression of miR-146a was shown to be cardioprotective by targeting IL-1 receptor-associated kinase 1 (IRAK1), a putative signalling molecule, that becomes associated with the IL-1 receptor upon stimulation.129

IL-33 is related to IL-1 and is suggested to harbour a novel paracrine mechanism between cardiac myocytes and fibroblasts during mechanical overload.127 This cytokine seems to be primarily expressed by cardiac fibroblasts upon cyclic strain,129 but is also released by both fibroblasts and myocytes during cardiac necrosis.130 TNF-α and IL-1β promote IL-33 expression.130 IL-33 binds to the interleukin 1 receptor-like 1 (ST2) receptor, which is basally expressed by cardiomyocytes131 and seems to oppose the effect of pro-hypertrophic stimuli in vitro. In mice, IL-33 reduces cardiac hypertrophy and fibrosis induced by cardiovascular load. These data suggest a cardioprotective effect of this cytokine.129 Furthermore, the treatment of cultured primary fibroblasts with IL-33 impairs cell migration and activates the expression of chemokines and cytokines including IL-6, but seems to have no effect on collagen expression.132 The effect of hypertrophy- and fibrosis-associated miRNAs on components of the IL-33/ST2 system remains elusive.

IL-6 is another cytokine family that can alter cell growth, apoptosis, and survival in the heart. Both leukaemia inhibitor factor (LIF) and cardiopoietin 1 (CT-1) belong to this family. IL-6 family members are secreted by cardiac myocytes as well as fibroblasts in response to cardiac injury and exhibit synergistic effects, such as IL-1β and TNF-α.105,131 Interaction between cultured cardiac cells induces IL-6 release.110,111 Derived from fibroblasts, LIF and CT-1 mediate pro-hypertrophic effects of Ang II.134,135 Furthermore, LIF and CT-1 promote fibroblast proliferation, while LIF impairs myocardial transition and collagen accumulation.135-137 Long-term delivery of LIF in vivo seems not to favour maladaptive remodelling, but also slightly induces the expression of miR-17, miR-21, and miR-199,43 while miR-29b seems to be a direct regulator of this IL.138 IL-17 family members are mainly associated with T-cell-dependent immune response, but also influence several cardiovascular diseases, including fibrosis,139 hypertension,139 and dilated cardiomyopathy.140 The finding that cardiac fibroblasts secrete IL-17 and express the corresponding receptor130,141 indicates the potential role of this IL in cellular communication between cardiac cells. Paracrine communication
triggers cardiomyocyte apoptosis,142 impairs contractility, and induces hypertrophy.110 In an autocrine fashion, IL-17A stimulates accumulation of collagen141 and MMP expression.143 Furthermore, IL-17A induces miR-101 and related signalling pathways, leading to migration and proliferation of fibroblasts.45

The anti-inflammatory cytokine IL-10 inhibits the production of various pro-hypertrophic cytokines144 and is notably augmented in response to TNF-α.145 IL-10 is induced in the failing heart,146 but limits pressure overload-induced cardiac remodelling.147 Although cardiomyocytes are a potential additional source for IL-10, non-myocytes seem to be the major source.148 Among IL-6 and TNF-α, fibroblasts release IL-10,110 which then protects cardiomyocytes by opposing apoptosis promoted by TNF-α.149 In vivo treatment of infarcted mice with IL-10 reduces both MMP-9 activity and fibrosis.150 The pro-fibrotic miR-27a was shown to target the IL-10 transcript and down-regulates protein levels abrogating the anti-fibrotic effect of IL-10.46

2.6 Natriuretic peptides
Apart from acting as circulating hormones in the plasma, which are widely used as biomarkers for cardiovascular risk determination, atrial and brain natriuretic peptides (ANP and BNP) act also locally at sites of their synthesis.151 Cardiac wall stretch and paracrine agents like ET-1 elevate natriuretic peptide expression and exocytosis in cardiac and ventricular myocytes.152,153 Expressing the appropriate natriuretic receptors, cardiomyocytes and fibroblasts are capable to sense both hormones.154 ANP inhibits collagen synthesis in fibroblasts and, together with BNP, blocks proliferation of these cell types.155,156 Furthermore, ANP and BNP exert anti-fibrotic effects in vivo and locally regulate ventricular remodelling.157,158 Secreted by fibroblasts, C-type natriuretic peptide decreases cardiac fibrosis on fibroblasts themselves159 and blocks ET-1-induced hypertrophy in myocytes.160

Most findings relating miRNAs and natriuretic peptides are derived from the biomarker research field.161 Less is known about miRNAs that regulate the expression of these hormones. Arora et al.49 identified miR-425 as a direct repressor of ANP production. Furthermore, the repression of miR-34 family members seems to protect the heart from pathological cardiac remodelling by regulating ANP among other hypertrophy-associated factors.48 When overexpressed, miR-26b opposes the effect of pro-hypertrophic proteins and elevates ANP levels.47

3. miRNAs as potential ‘mircrine’ factors
In the last decade, miRNAs have evolved to act as intracellular regulators of gene expression in a variety of cardiovascular disease.27,28 Since miRNAs are found to be present outside the cell in several body fluids such as blood, urine, and breast milk,162 they have emerged as novel intercellular communicators and paracrine signalling mediators in different biological processes.163–165 In the circulation, secreted miRNAs are protected from ribonuclease-dependent degradation either by the association with RNA-binding proteins such as Argonaute 2 (Ago2), the association with lipoproteins such as HDL or the packaging into small vesicles such as exosomes, microvesicles, or apoptotic bodies.163,164,166–171 Upon release to the extracellular space, miRNAs can mediate messages/signals between cells and affect gene expression of target mRNAs in the recipient cells.163,164,172,173 Circulating miRNAs have also been identified as prognostic and diagnostic markers in a number of heart diseases, including coronary artery disease or myocardial infarction.174,175

In the heart, miRNAs have been found to be actively released by multiple cardiac cells including cardiomyocytes and endothelial cells, suggesting a promising role of miRNAs to act as paracrine signalling mediators in several cardiovascular disease conditions.172,173 Hergenreider et al.172 implicated a miRNA/microvesicle-mediated cell–cell communication mechanism during atherosclerotic protection. Upon atherosclerotic stimuli, endothelial cells released microvesicles enriched with miR-143/145, which were taken up by smooth muscle cells regulating gene expression of miRNA downstream effectors. Injection of miR-143/145-enriched microvesicles in a mouse model of atherosclerosis diminished atherosclerotic lesion formation, suggesting a promising therapeutic potential of miRNAs in cardiovascular disease conditions. In the context of atherosclerosis, Zernecke et al. showed that circulating miR-126 can be taken up by distal endothelial cells and exert specific cellular function in atherosclerotic lesions, suggesting the involvement of miR-126 in cell–cell communication. Endothelial-derived apoptotic bodies enriched with miR-126 were transported to recipient cells in atherosclerotic lesions, leading to the secretion of paracrine protection signals via the activation of the chemokine CXCL12.168 Injecting miR-126-enriched apoptotic bodies in a mouse model of atherosclerosis initiated the recruitment of progenitor cells and reduced atherosclerotic lesions. Another study by Halkein et al.173 proposed a role for miRNAs as paracrine signalling mediators between endothelial cells and cardiomyocytes during the development of peripartum cardiomyopathy (PPCM). PPCM is characterized by sudden onset of HF in pregnant women and is triggered by the N-terminal prolactin fragment (16K PRL). In this study, 16K PRL was found to induce miR-146a expression in endothelial cells. Furthermore, 16K PRL provoked the secretion of miR-146a containing exosomes from endothelial cells, which were taken up by cardiomyocytes, resulting in reduced expression of the target genes Erbb4, Notch1, and Ikrk1, and subsequently impaired metabolic activity. Pharmacological inhibition of miR-146a in a mouse model of PPCM attenuated the development of PPCM. In plasma of patients with PPCM, miR-146a was found to be elevated, suggesting a role for miR-146a to function as a novel biomarker and therapeutic target in PPCM.

miRNAs can be involved in communication between cardiac cells either by affecting the secretion of cytokines and growth factors or by exerting a direct signalling function. A first indication that miRNAs are involved in the cardiomyocyte–fibroblast crosstalk was depicted in a study by Duisters et al., showing that cardiomyocyte-specific miR-133a affects fibroblast function. In this report, miR-133a was found to regulate the expression and secretion of the pro-fibrotic growth factor CTGF in cardiomyocytes, which upon secretion induces cardiac fibrosis.35 Cardiomyocyte-specific overexpression of miR-133 in mice led to reduced apoptosis and fibrosis after transverse aortic constriction.176 In contrast, miR-133 knockout in mice resulted in severe fibrosis and HF, while knockdown of miR-133 also led to cardiac hypertrophy with impaired cardiac function.177–179 The findings strongly indicate that inhibition of miR-133 derepresses the pro-hypertrophic response in cardiomyocytes and supports the involvement of a miR-133a-mediated paracrine crosstalk between cardiomyocytes and cardiac fibroblasts, leading to the development of fibrosis in the heart. In addition to the control of CTGF, miR-133a has been shown to regulate the expression and secretion of TGF-β (Figure 2).33,35 The expression and secretion of CTGF is also controlled by miR-30, which is highly expressed in cardiac
Another recent report implicated a role of miR-29b and miR-30c in the communication between cardiac fibroblasts and cardiomyocytes and their involvement in myocardial hypertrophy (Figure 2). The study demonstrated that miR-29b affected ECM protein deposition in cardiac fibroblasts, but also mediated the development of cardiomyocyte hypertrophy by regulating the secretion of cytokines and growth factors, such as IGF-1, LIF, and pentraxin-3. In contrast, miR-30c had only little effects on the secretion of ECM. Cardiomyocyte phenotype was affected indirectly by both miRNAs, as conditioned medium of miR-29b-transfected cardiac fibroblasts induced atrophy in cardiomyocytes, whereas medium derived of miR-30c-transfected cardiac fibroblasts induced a hypertrophic phenotype. The findings revealed that miR-29b and miR-30c had opposite effects on the secretion of ECM proteins from cardiac fibroblasts and thereby had opposite effects on cardiomyocyte phenotype. Consistently, Castoldi et al. showed that miR-133a which is highly expressed in cardiomyocytes also targeted collagen 1a1 (Col1a1), a target of miR-29b. This report proposed that cardiac fibrosis is not only regulated by miRNAs in cardiac fibroblasts, but also by cardiomyocyte-specific miRNAs, such as miR-133a, by synergistically affecting Col1a1 mRNA expression.

However, a paracrine miRNA crosstalk between cardiomyocytes and cardiac fibroblasts or vice versa during cardiovascular disease conditions has not been shown so far. Although several studies revealed that miRNAs can act as moderators in the paracrine cardiomyocyte–cardiac fibroblast crosstalk, this action was mostly mediated by regulating the secretion of cytokines and growth factors, which upon secretion affected surrounding cardiac cells. The notion that cardiac cells such as cardiomyocytes and endothelial cells actively secrete miRNAs, and that miRNAs can be transferred to mouse embryonic fibroblasts and H9C2 cardiomyocytes, strongly supports the concept that miRNAs can also act as cell–cell communicators between cardiomyocyte and fibroblast crosstalk. Moreover, recent findings from our group indicate a possible miRNA-vesicle-mediated crosstalk between cardiac fibroblasts and cardiomyocytes during the development of cardiomyocyte hypertrophy.

Of note, a study by Waldenstrom et al. revealed that next to miRNAs also other nucleic acids can be involved in the paracrine crosstalk between cardiomyocytes and cardiac fibroblasts. Thus, DNA/RNA-containing cardiomyocyte-derived exosomes were transferred to fibroblasts, where they controlled downstream targets exerting a direct signalling function.

4. Cardiac myocyte–fibroblast communication in therapeutics

Cardiac fibroblasts were once seen only as providers of the extracellular environment for cardiomyocytes, but their role extends by large beyond matrix production. These cells govern many aspects of cardiac function, such as cardiac electrophysiology and contractility. A better understanding of the dynamic intercellular communication between fibroblasts and cardiomyocytes is necessary to develop new therapeutic strategies to diminish the effects of cardiac remodelling.

To date, no definite therapeutic strategies that target this interaction have been proposed. Some of the former approaches were based on targeting the secreted soluble molecules, including growth factors, which play a role in the cell–cell communication. For example, post-infarction gene therapies suppressing TGF-β signalling proved to significantly mitigate cardiac remodelling, improved survival, reduced remote, and infarct zone fibrotic lesions, and attenuated cardiac dysfunction in mice after 4 weeks of infarction. Also, the administration of flavonoids reduced interstitial fibrosis and cardiac dysfunction of infarct rats by inhibiting TGF-β1 expression. Peroxisome proliferator-activated receptor (PPARγ) agonists like rosiglitazone also seem to attenuate cardiac fibrosis through the interruption of Ang II-induced TGF-β1 expression.

Since the expression of Ang II receptors exceed that in cardiomyocytes, it might have a stronger effect on fibroblasts and consequently on the progression of fibrosis. In contrast, some other findings suggest that the interactions between cardiomyocytes and fibroblasts might have a beneficial effect by preventing cardiac remodelling. Kapoun et al. suggest that BNP secreted by cardiomyocytes inhibits fibrotic response by attenuating TGF-β signalling in cardiac fibroblasts, but the therapeutic advantage has still not been addressed.

Although all these approaches are promising strategies for targeting cardiac fibrosis and thus attenuating remodelling, there are several limiting facts that need to be taken into account. Cardiac fibroblasts display phenotypic heterogeneity within the heart itself and also differ from fibroblasts isolated from other tissues or organs. In addition, the mechanisms underlying the transition from initially compensatory wound healing, that aims to counteract cardiac injury, to maladaptive myocardial remodelling remain partly unresolved. As mentioned before, the role of TGF-β in cardiac hypertrophy seems to be ambiguous, since several studies implicate that TGF-β signalling might prevent cardiomyocyte hypertrophy.

Regardless of these facts, other therapeutic approaches have been proposed. As previously mentioned, miRNAs can be assumed as modulators of the cellular crosstalk in the myocardium, and thus, as emerging therapeutic targets in the treatment of HF. Recently, several in vivo studies have demonstrated that pharmacological modulation of miRNAs, either by overexpression or miRNA inhibition, influences myocardial remodelling.

Previous investigations by our research group reported the role of miR-21 in cardiac disease by affecting extracellular signal-regulated kinases–MAP kinase signalling in cardiac fibroblasts and suggested that miR-21 levels are increased selectively in fibroblasts of failing hearts. Silencing of miR-21 with a specific antagomir revealed improved cardiac function and reduced fibrotic response in a hypertrophy mouse model. The treatment prevented left ventricular dilatation, improved fractional shortening, and reduced the expression of collagens and ECM proteins. Probably by modulating paracrine fibroblast/ cardiomyocyte crosstalk, this intervention also indirectly reduced the development of cardiac hypertrophy.

Another group determined that the overexpression of miR-29 in fibroblasts was capable of reducing collagen expression. The level of miR-29b expression in fibroblast cultures increased by as much as 400-fold after 3 days of exposure to miR-29b mimic. These results suggest that the overexpression of miR-29 prevents cardiac fibrosis, and that strategies to maintain this expression may be beneficial in the settings of fibrotic diseases. This strategy may also be useful in vivo.

Other findings indicate that some miRNAs such as miR-24 and miR-92 are highly expressed in cardiac endothelial cells. miR-24 was found to be up-regulated in endothelial cells after cardiac ischaemia in mice and acts as a major regulator of apoptosis and angiogenesis. By blocking this miRNA in vivo, cardiac function and survival were preserved. Another mouse model of limb ischaemia and myocardial infarction suggested that the administration of an antagonir for miR-92a enhanced blood vessel...
growth and the remodelling of the damaged tissue.\textsuperscript{189} A recent study used a pig model to treat acute ischaemia—reperfusion injury after myocardial infarction targeting the same miR-92. In this case, the treatment consisted in an infusion of an locked nucleic acid (LNA)-based miR-92a inhibitor (LNA-92a), thus reducing infarct size and post-ischaemic loss of function.\textsuperscript{190}

These findings in small and large animal models represent a novel therapeutic tool to preserve cardiac function after ischaemia. Reflecting further research fields aside the cardiovascular system, the importance of such strategies becomes apparent since miRNA therapeutics recently entered clinical trials. The miR-34 family (a, b, and c) has been described as potential tumour suppressors in a variety of cancers.\textsuperscript{191} In April 2013, the treatment with the first miRNA mimics reached Phase 1 studies (MRX34). The strategy consisted on restoring lost suppressor function of endogenous miR-34 using a synthetic miRNA mimic in patients with liver cancer, thereby regulating at least 24 known oncogenes.\textsuperscript{192}

Another example is miR-122. This miRNA has been implicated in viral infections like HCV. Sanzari's Pharma conducted a human phase II trial that demonstrated the safety and antiviral function of miravirsen (a locked nucleic acid-modified miR-122 antagonist). The treatment of patients suffering from chronic HCV infections with miravirsen resulted in a reduction of HCV RNA levels without viral resistance.\textsuperscript{191,193,194}

Taken together, these findings suggest that the modulation of miRNA levels represent a promising therapeutic strategy, also to abrogate pathological cell-to-cell communication finally preventing cardiac fibrosis and hypertrophy. This approach might not only be restricted to miRNAs that are involved in the endogenous control of fibrotic or hypertrophic gene expression, but could also target miRNAs that are secreted by cardiac cells and influence the response of the recipient cells. Finally, larger long non-coding RNAs may function as direct and indirect modulators of cell—cell communication in the heart.\textsuperscript{16}

5. Conclusions and further directions

Cardiomyocytes and fibroblasts play a critical role in the progression of myocardial remodelling towards HF. Since cardiac fibrosis and hypertrophy depend on their relationship and dynamic interplay, it will be important in future to consider them no separately from each other. Paracrine stimuli elicit multiple signalling pathways in both cardiomyocytes and fibroblasts, influencing genetic and even epigenetic regulation mechanisms.\textsuperscript{195} Such signal molecules activate fibroblasts to produce and release ECM proteins and enzymes, provoke hypertrophic cardiomyocyte growth, and stimulate the release of further autocrine and paracrine factors. Insights into such intracellular signalling networks will be required for an integrated understanding of determinants in the normal and diseased heart. It is likely that the crosstalk between cardiomyocytes and fibroblasts influences also further non-myocyte cells in the heart, and that such communicative networks influence therapeutic strategies and their effects.

In the recent years, miRNAs have become of interest for targeted therapies, since they regulate essential molecules and pathways in cardiac fibrosis and hypertrophy. The fact that miRNAs are frequently deregulated in a disease-specific manner, but play a minor role under normal conditions, raises their attractiveness as potential targets for new drugs. Thereby, it is not only of interest, whether miRNAs regulate paracrine factors communicated between cardiac cells but also of which effects are mediated by miRNAs when they are released as ‘mirocrine’ signals itself and which therapeutic benefits could be achieved from that. In regenerative approaches, it has been shown that ‘mirocrine’ mechanisms can improve the functionality and survival of intracardiac grafts. The heart-specific miR-499 translocates from cardiomyocytes to spatially coupled cardiac stem cells, favouring cardiomyogenesis.\textsuperscript{196} And, transplanted mesenchymal stem cells secrete miR-210 that seems to have a protective and pro-survival effect on cardiomyocytes.\textsuperscript{197} The elucidation of this novel transfer of ‘mirocrine’ signals will be important to understand various biological and pathological processes, including cardiac hypertrophy and fibrosis.

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References


