Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart

Esther E. Creemers* and Yigal M. Pinto

Heart Failure Research Center, Academic Medical Center, Meibergdreef 15, Room L2-108-3, 1105 AZ Amsterdam, The Netherlands

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Abstract

When considering the pathological steps in the progression from cardiac overload towards the full clinical syndrome of heart failure, it is becoming increasingly clear that the extracellular matrix (ECM) is an important determinant in this process. Chronic pressure overload induces a number of structural alterations, not only hypertrophy of cardiomyocytes but also an increase in ECM proteins in the interstitium and perivascular regions of the myocardium. When this culminates in excessive fibrosis, myocardial compliance decreases and electrical conduction is affected. Altogether, fibrosis is associated with an increased risk of ventricular dysfunction and arrhythmias. Consequently, anti-fibrotic strategies are increasingly recognized as a promising approach in the prevention and treatment of heart failure. Thus, dissecting the molecular mechanisms underlying the development of cardiac fibrosis is of great scientific and therapeutic interest. In this review, we provide an overview of the available evidence supporting the general idea that fibrosis plays a causal role in deteriorating cardiac function. Next, we will delineate the signalling pathways importantly governed by transforming growth factor β (TGFβ) in the control of cardiac fibrosis. Finally, we will discuss the recent discovery that miRNAs importantly regulate cardiac fibrosis.

Keywords

Fibrosis • TGFβ • Heart Failure • miRNAs

1. Introduction

The extracellular matrix (ECM) forms the structural backbone of the heart and is composed of a complex of macromolecules, including collagens, elastic fibres, proteoglycans, and basement membranes.1 The most obvious function of the ECM is to provide a structural network for transmitting force generated by individual myocytes into organized systolic contraction of the heart. In addition, the ECM contributes to passive stiffness in diastole and prevents over-stretch, myocyte slippage, and tissue deformation during ventricular filling. Besides these structural functions, components of the ECM also serve as modulators of growth, tissue differentiation, and angiogenesis.2

An important hallmark of maladaptive hypertrophy and heart failure is cardiac fibrosis, which is characterized by an increase in collagens and other ECM components in the interstitium and perivascular regions of the myocardium.1 The most abundant collagen types in the heart are the fibrillar collagens, type I and III, accounting together for over 90% of the total collagen. Type I collagen molecules assemble into thick fibres which convey tensile strength and provide structural support. Type III collagens form a fine network of fibrils and relatively high levels are found in elastic tissue such as dermis, blood vessels, and lungs.2 Fibrosis is only observed in hypertrophic cardiomyopathy, but is also common in dilated and ischaemic cardiomyopathies. In this regard, fibrosis is multifactorial and can be caused by several processes including ischaemia, senescence, inflammation, and various hormones. Although two different types of fibrosis have been proposed, i.e. reparative fibrosis and reactive fibrosis, it is currently unclear whether they represent truly different entities. Reparative fibrosis refers to ECM deposition during scar formation as a result of tissue injury or cell death, a process which takes place in ischaemia or senescence. Reactive fibrosis refers to increased ECM deposition around vessels and in interstitium due to direct stimulation of fibroblasts without cell injury, as occurs in hypertension.3,4 Regulation of fibrosis in inflammation, ischaemia, and senescence is reviewed extensively by Swynghedauw5 and Spinale6 and will therefore not be our focus.

In this review, we delineate the signalling pathways that control reactive fibrosis in the pressure-overloaded myocardium, most commonly induced by hypertension or valvular disease. We will first review the available evidence supporting the general idea that fibrosis plays a causal role in deterioration of cardiac function in the progression to heart failure. In the subsequent sections, the molecular basis for the altered ECM turnover in the heart will be delineated. Finally, we will discuss the recent discovery that miRNAs critically

*Corresponding author. Tel: +31 205 668 544; fax: +31 206 976 177; Email: e.e.creemers@amc.uva.nl

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regulate cardiac fibrosis and open exciting opportunities for therapeutic interventions.

2. Interstitial fibrosis in the progression to heart failure

Landmark studies by Weber and Janicki have demonstrated, using scanning electron microscopy, that the collagen weave thickens extensively in pressure-overloaded, hypertrophied myocardium. The severity of interstitial fibrosis closely correlates with the extent of LV hypertrophy and impaired ejection fraction. In this regard, autopsy studies in humans have consistently shown that the percentage of fibrosis is proportional to the ventricular mass, until the threshold of 250 g of heart weight is reached. Above this value, fibrosis no longer correlates with mass and reaches a plateau of 30% of the myocardial area. It seems that in the initial phases of pressure overload, enhanced collagen deposition is matched by myocyte hypertrophy, resulting in an enlarged ventricle with normal collagen composition. This increase in collagen deposition in the compensatory phase of cardiac hypertrophy may be beneficial as the collagen matrix grows to accommodate the increase in muscle mass. However, chronic or more severe pressure overload is associated with excessive forms of collagen deposition or fibrosis, and this affects myocardial compliance characteristics with a resulting increase in myocardial stiffness. Canine studies have shown that excessive fibrosis in the interstitial space impairs electrical coupling between cardiomyocytes and can lead to hypoxia of cardiomyocytes by reducing the capillary density and an increased oxygen diffusion distance. Altogether, fibrosis can profoundly affect myocyte metabolism and performance and ultimately ventricular function.

It has been suggested that the proportionate increase in ECM deposition and contractile components, as seen in hypertrophy, is induced by a common mechanism, i.e. the direct mechanical load on the heart. Indeed, cell culture experiments with neonatal rat cardiac fibroblasts, the collagen producing cells of the heart, revealed a robust increase in collagen synthesis within 24 h of cyclic mechanical strain. Fibroblasts are equipped with three key systems to sense and transduce the mechanical forces (strain) to a biochemical event such as proliferation and transcription of ECM components. These mechanisms involve the cytoskeleton of the cell, integrins, and stretch-activated channels. Although the precise mechanisms responsible for the transition from normal expansion of the collagen mass to excessive fibrosis in cardiac hypertrophy are not fully understood, there is evidence for a role of locally produced growth factors such as FGF-2, transforming growth factor β (TGFβ), platelet-derived growth factor, or plasma hormones such as AngII, endothelin-1, and catecholamines.

A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into an active fibroblast phenotype or myofibroblasts. Myofibroblasts are not present in the healthy heart (except in valve leaflets), but in the stressed myocardium fibroblasts adopt morphological and biochemical characteristics in between those of fibroblasts and smooth muscle cells. There is evidence that fibroblasts isolated from failing or infarcted myocardium show increased levels in migration, collagen synthesis, α-smooth muscle actin expression, and proinflammatory cytokines secretion, compared with those isolated from healthy hearts. Altogether, these hypersecretory activated fibroblasts are responsible for ECM production in the stressed myocardium.

3. Modulating fibrosis in experimental animal models

Identification of fibrosis as a cause of impaired function in the failing heart has led to attempts to prevent or reverse fibrosis by agents that inhibit collagen formation or disrupt collagen cross-linking. For example, β-aminopropionitrile (BAPN) is an agent which has been shown to inhibit the cross-linking of collagen. BAPN treatment decreased diastolic stiffness in pig myocardium and improved the function of LV papillary muscles from hypertensive rats with heart failure. Despite promising results in experimental models in the reversal of fibrosis, BAPN is a poor candidate for treatment of established heart failure in patients because it has adverse systemic effects. An interesting study on the function of collagen type I in the heart was reported by Miller and Tyagi. They described a genetic mouse model with a mutation in the type I collagen gene that rendered this procollagen resistant to collagenase digestion. This mutation in collagen led to enhanced fibrosis of the heart and elicited abnormalities in the remodelling, structure, and function of the heart. Strikingly, these mice also displayed alterations in composition of other ECM components (25% less elastin in LV), probably due to the dramatically disturbed expression or activity of matrix metalloproteinases (MMPs) and their inhibitors. Therefore, the development of cardiomyopathy in these collagen mutant mice may not have been the consequence of elevated tissue stiffness due to resistant degradation of collagen type I, but rather due to increased basal levels of ECM degradation. Nevertheless, this study provides strong evidence that the turnover of different ECM components is strongly interwoven and shows that primary changes in collagen composition suffice to provoke cardiac dysfunction. Analysis of the knockout mouse for α2 type I collagen has supported this concept, as the LV of these mice exhibited ~35% lower collagen area fraction and this was associated with a 20% reduction in LV stiffness.

In another study, MacKenna et al. showed that intra-arteriolar administration of collagenase into the myocardium of rats disrupted the small and medium collagen fibrils and increased LV volume but did not change the pressure–strain relationship. They concluded that fibrillar collagens may be more important for maintaining LV geometry by constraining sarcomere length than by contributing to passive ventricular stiffness. A causal relation between fibrosis and stiffness in the setting of LV hypertrophy is hard to prove mainly because increased stiffness cannot solely be attributed to increased interstitial collagen content, as it is known that LV hypertrophy itself can also enhance tissue stiffness.

From a therapeutic perspective, there is no direct evidence showing an important causal role for fibrosis in the development of human heart failure. Strategies aimed to reduce fibrosis in cardiac pathology have focused on the effectiveness of ACE inhibitors, angiotensin II receptor antagonists, and aldosterone inhibitors, but these are complex effects and many mechanisms interact. Other promising approaches to target cardiac fibrosis include the use of TGFβ antagonists, which will be discussed in the next sections.
4. Control of interstitial fibrosis by TGFβ

4.1 Animal studies
TGFβ is the prototype of a large family of cytokines. In mammals, three isoforms of TGFβ (TGFβ1, TGFβ2, and TGFβ3) have been identified, and exhibit similar, but not identical, biological properties. Several lines of evidence indicate that TGFβ1 is a crucial regulator of ECM metabolism in a wide range of organ systems. Also in the heart, ECM production is importantly regulated by TGFβ, as has been consistently demonstrated by transgenic and knockout mouse models. For example, heterozygous TGFβ1 deficient mice reveal attenuated fibrosis of the aging heart. On the other hand, overexpression of TGFβ1 in transgenic mice results in both interstitial fibrosis and hypertrophic growth of cardiac myocytes. Tranilast is a non-specific drug that inhibits transcription of TGFβ1, and it has been shown that tranilast attenuates LV fibrosis in rats with renovascular hypertension and in a diabetic rat model. Finally, in the pressure-overloaded rat, blockade of TGFβ1 function using specific neutralizing antibodies prevented the induction of collagen mRNA, myocardin fibrosis, and diastolic dysfunction. Besides TGFβ1, also TGFβ2 and TGFβ3 are expressed in the adult heart. Although TGFβ1 levels increase in the course of pressure-overload induced hypertrophy, TGFβ3 protein levels gradually decrease, indicating a reciprocal expression of TGFβ1 and TGFβ3. In the same study, myocardin TGFβ2 protein levels remained unchanged in a period of 6 weeks after aortic constriction in the rat. More research is needed to unravel the functional interplay between the three isoforms during cardiac remodelling.

4.2 Human studies
Also in the pressure-overloaded human heart, TGFβ1 is significantly upregulated. Human variants and mutations in TGFβ have been related to various cardiovascular abnormalities, like Marfan syndrome, but none of them is related to cardiac dysfunction. Genetic studies have indicated that TGFβ polymorphisms may predispose individuals to ischaemic heart disease. Individuals with such polymorphisms exhibit higher levels of serum TGFβ. It is however currently unknown whether those individuals have enhanced interstitial fibrosis in their hearts, or whether cardiac function is impaired.

TGFβ expression is induced by angiotensin II, and this has been demonstrated in a number of studies in kidney, vascular smooth muscle cells, and in cardiac fibroblasts. The ameliorative effects of ACE inhibitors and angiotensin II receptor blockers on the heart in clinical trials are at least partially attributed to a reduction in ECM protein accumulation, most likely mediated through inhibition of TGFβ and its downstream signals. The beneficial effects of spironolactone (aldosterone receptor inhibitor) in clinical trials with heart failure patients were shown to be at least partly the result of reduced ECM turnover and could have been mediated by TGFβ signalling. Specific TGFβ inhibitors or TGFβ directed antibody therapy has not been tested in human heart disease, but the above studies indicate that the use of TGFβ antagonists as anti-fibrotic agents may be effective in combating pressure-loaded heart failure. However, inhibition of TGFβ does not come without risk, as TGFβ has a wide range of other functions in the body. Thus, potential adverse consequences of TGFβ inhibition must be further explored before its clinical use. In particular its role in cellular proliferation and in the immune system suggests there may be serious side effects. It may be more attractive to target a specific down-stream component of the TGFβ signalling cascade.

4.3 Signal transduction in TGFβ-induced fibrosis: Smads and Rho/ROCK signalling

4.3.1 Smads
TGFβ elicits its biological responses through a heteromeric receptor complex comprising two serine-threonine kinase receptors, termed TGFβ receptor types 1 and 2 (TβR1 and TβR2). Both TGFβ ligand and receptors are present in the heart, and all are expressed in cardiac myocytes as well as fibroblasts. Canonical signalling through TGFβ receptors activates Smad proteins, which are phosphorylated upon receptor activation, associate with Co-Smad, and subsequently translocate to the nucleus where they act as transcription factors. In hypertension-induced heart failure, Smad2 was found to be increasingly phosphorylated. The consensus DNA binding site of Smads comprises only four bases ‘GGCT’ to which Smads bind with a low affinity. This sequence can be found in nearly every promoter region and is therefore not sufficient to confer promoter selectivity. Because Smads are weak transcriptional activators, transcriptional control by Smads relies on interaction with transcription factors and coactivators such as AP-1, Sp1, TFE3, and p300 to activate promoters. Depending on their binding partners, Smads may either activate or inhibit gene transcription. Evidence from mesangial cell lines indicates that the promoter of collagen type I (both α1 and α2 chains) is a primary site for Smad binding. Besides the Smad binding site, the α2 collagen type I gene also contains functional sites for Sp1 and AP-1 as putative coactivators.

4.3.2 Rho/ROCK signalling
Recently, Small et al. demonstrated a role for myocardin-related transcription factor A (MRTF-A) in the direct regulation of fibrosis-associated genes, including α2 collagen type I (Figure 1). Myocardin, which is the founding member of the MRTF family and a potent transcriptional activator of serum response factor (SRF), is mainly expressed in the myocytes of the heart and not in fibroblasts. MRTF-A on the other hand, also a potent activator of SRF-dependent transcription is expressed in cardiac fibroblasts. The study of Small et al. showed that TGFβ promotes the nuclear translocation of MRTF-A through Rho/ROCK signalling in cardiac fibroblasts and this induces a subset of genes consistent with a myofibroblast-like cell type. Moreover, genetic deletion of MRTF-A in mice abrogated fibrosis in response to myocardial infarction and this was attributed to decreased expression of multiple ECM markers including Col1a1, Col1a2, Col3a1, and elastin. Interestingly, the Col1a2 promoter contains an evolutionarily conserved CArG box and chromatin IP and gel-shift assays provided evidence that Col1a2 is a direct transcriptional target of SRF/MRTF-A in fibroblasts. It is well known that Rho-ROCK signalling plays an important role in sensing the environment and generating a cellular response to stress by assembly and stabilization of the actin cytoskeleton. There are also other lines of evidence that demonstrate the involvement of Rho kinase signalling in the development of cardiac fibrosis. In this regard, Kagiya et al. demonstrated that Rho kinase activity is increased in cardiac fibrosis, and that a specific Rho kinase inhibitor (fasudil) prevented cardiac fibrosis in aldosterone treated mice. Besides TGFβ receptors, also mechanical force has been shown to stimulate Rho signalling and
the subsequent nuclear translocation of MRTF-A to activate a smooth muscle-like myofibroblast phenotype.54

4.3.3 Kruppel-like transcription factors
Two of the Kruppel-like transcription factors, KLF15 and KLF5 have also been implicated in cardiac fibrosis. Both KLF5 and KLF15 knock-out models display reduced fibrosis in response to pressure overload after aortic constriction.55,56 The group of Mukesh Jain showed that KLF15 inhibits Smad3 activity on the connective tissue growth factor (CTGF) promoter (Figure 1), by direct association with p300/CBP associated factor (a potent transcriptional activator of Smad3). Interestingly, we and others have shown that KLF15 is down-regulated by TGFβ in cardiac fibroblasts and myocytes.55,57 This loss of KLF15 will therefore relieve its transcriptional repression and thereby stimulate fibrotic signalling. KLF5 expression is regulated differently, as it is strongly induced by AngII stimulation. Increased KLF5 protein in turn activates TGFβ expression and may therefore act as the transcription factor that connects AngII and TGFβ signalling. It will be of major interest to find out whether there is cooperativity among the Smads, Rho/ROCK pathway, and KLFs in TGFβ-induced fibrosis.

4.3.4 TAK/p38 signalling
As an alternative pathway, signalling through TGFβ-activated kinase (TAK1) to the transcription factor ATF2 has been described in the regulation of cardiac fibrosis.58 Zhang et al.59 found that overexpression of TAK1 activity in transgenic mice induced cardiac hypertrophy, interstitial fibrosis, and severe myocardiast dysfunction. Since a cardiomyocyte-specific promoter was used to generate TAK1 transgenic mice, the observed fibrotic response of the heart may have been a secondary effect mediated by released growth factors of the hypertrophic heart. In conclusion, in vitro studies clearly demonstrate the involvement of the TAK/p38/ATF2 signalling pathways in ECM synthesis, however insights in the in vivo function of TGFβ in the cardiac fibroblast are importantly hampered by the fact that fibroblast-specific promoters have not been used in the conditional mouse models.

The lack of a reliable and specific fibroblast marker is a major limiting factor in the study of fibroblasts in vivo. Although there are several established markers of fibroblast phenotype (i.e. vimentin, discoidin domain receptor (Ddr2), fibroblast-specific protein (Fsp1), periositin, and several key ECM proteins),60 none is exclusive to fibroblasts or is present in all fibroblasts.60 One of the reasons is the heterogeneity of these cells and the observation that fibroblasts from different anatomical sites, such as atrium vs. ventricles61, have distinct characteristics and phenotypes.

Recently, the 3.9-kb periositin promoter was successfully used to induce cardiac fibroblast-specific deletion of KLF5 in a mouse line.56 Periositin expression is restricted to the non-cardiomyocyte lineage and is minimally expressed in the adult ventricles unless a pathologic stress or injury occurs.61,62 It will be interesting to use this promoter for fibroblast-specific deletion of Smads, Rho/Rock, or TAK1 to unravel the specific signalling mechanisms of ECM turnover in the stressed or injured myocardium.

4.4 CTGF as a downstream target of TGFβ
A critical profibrotic target gene of TGFβ signalling is CTGF. CTGF is a member of the CCN family of secreted proteins.63 The CCN family comprises of secreted cysteine-rich growth regulators and its name is an abbreviation derived from the main members: Cysteine-rich 61 (Cyr61/CCN1), CTGF (CCN2), and Nephroblastoma (Nov/CCN3). The importance of CTGF as a regulator of tissue fibrosis has been described in different organs, including the heart.64–66 In cultured kidney and foreskin fibroblasts, it was recently demonstrated that CTGF is essential for the TGFβ-induced collagen synthesis. In this regard, Duncan et al.67 demonstrated that TGFβ-induced collagen synthesis was effectively blocked after knockdown with siRNA against CTGF or treatment with anti-CTGF antibodies. CTGF induction in response to TGFβ does not occur in Smad3−/− mouse embryonic fibroblasts or in the presence of an MEK inhibitor.
Expression of CTGF is induced by TGFβ through signalling cascades involving Smads, Ets-1, and ras/MEK/ERK.63 It is currently unknown whether all these factors converge directly on the promoter regions of CTGF, or whether the induced expression is indirect.

The CTGF protein contains growth factor binding domains, functional motifs for integrin recognition, proteoglycan binding, and dimerization motifs. CTGF is believed to promote its effects through interactions with integrins, proteoglycans, and receptors, however a specific CTGF receptor has yet to be identified. The current hypothesis is that CTGF acts by modulating the activity of specific receptor–ligand interaction and in this manner, it plays a broad role in matrix organization and signal transduction by promoting cell adhesion and/or by acting as a bridge between extracellular and intracellular signalling networks.63 Lee68 and colleagues recently demonstrated that CTGF is sufficient to induce mesenchymal stem cells to differentiate into fibroblasts. Interestingly, CTGF-treated stem cells were αSMA- and differentiated into αSMA+ cells only when stimulated subsequently with TGFβ. This suggests that a step-wise process of fibroblast commitment, differentiation and fibrosis, and that CTGF is critically involved.

In the healthy heart, CTGF is predominantly expressed in fibroblasts, however in the process of cardiac remodelling CTGF is also secreted by cardiac myocytes.64,69 Nevertheless, since fibroblasts, and in particular myofibroblasts are hyper-secretory cells, these cells are more likely to be the main source of secreted CTGF.70 CTGF expression is increased in the hypertrophic and failing myocardium of experimental animal models, such as the transverse aortic constriction model in rat. Together with ANF, BNP, and α-actin, CTGF is considered one of the marker genes of hypertrophy and heart failure.65,71 In vitro studies have demonstrated that CTGF is one of the earliest growth factors, transcriptionally induced by hypertrophic stimuli in cardiac myocytes.72 Upregulation of CTGF in fibrotic tissue, including the human heart, appears to correlate well with the severity of fibrosis.73,74

A knockout mutation for CTGF is lethal in mice.75 In these mice, abrogation of CTGF induced major skeletal defects due to impaired chondrocyte proliferation and matrix remodelling. Unfortunately, the hearts of these mice were not examined. Strikingly, a cardiomyocyte-specific overexpression model of CTGF in mice lacks a fibrotic phenotype.76 The cell type used to overexpress CTGF in this study may explain the absent effect on fibrosis. In this regard, a cardiomyocyte-specific promoter was used, while CTGF is mostly expressed in fibroblasts. Nevertheless, this is still an important finding as this may indicate that the increased CTGF expression, which is observed in cardiomyocytes in response to stress signals does not contribute to fibrosis. This is a possible explanation but may be too premature, since other obligate interaction partners of CTGF, or its putative receptor may have been a limiting factor in the induction of fibrosis in these transgenic mice. Further studies, preferably loss-of-function studies using fibroblast-specific promoters, are needed to determine the exact role of CTGF in the heart and to assess the therapeutic potential of CTGF-based therapies.

5. Control of cardiac fibrosis by miRNAs

In the last few years, it has become clear that miRNAs are important players in different aspects of cardiac remodelling, including cardiac fibrosis. Four cardiac miRNAs have so far been implicated in the control of cardiac fibrosis, namely miR-21, miR-29, miR-30, and miR-133. The molecular pathways targeted by those individual miRNAs are depicted in Figure 2.
5.1 miR-133 and miR-30

As discussed in the previous section, CTGF is considered an important profibrotic growth factor which expression in strongly induced in the hypertrophic and failing heart. Our lab has shown that this CTGF upregulation is partly mediated by two major cardiac miRNAs: miR-133 and miR-30. In rodent models and samples from patients with LVH, a reduction was shown in the expression of miR-133 and miR-30 and this inversely correlated with CTGF, collagen, and fibrosis levels. Furthermore, culture experiments in which the expression of these miRNAs was manipulated indicate that both miRNAs can effectively repress CTGF production by direct interaction with the 3’UTR of CTGF mRNA. Whereas miR-133 is expressed specifically in cardiomyocytes, miR-30 is expressed in cardiac fibroblasts as well as in cardiomyocytes. Both myocytes and fibroblasts are well-established sources of CTGF production and secretion in the heart. Together these findings show that fine-tuning of CTGF protein levels is regulated by miRNAs in a cell type-specific manner. Whereas cardiac myocytes express miR-133 and miR-30c to regulate CTGF protein levels, fibroblasts mainly possess miR-30 to inhibit CTGF expression. The existence of cell type-specific miRNAs regulating CTGF levels emphasizes the importance of tight regulation of CTGF protein levels in the heart. The potential importance of miR133 in the regulation of cardiac fibrosis was underscored by Liu et al. who showed that miR-133 knockout mice develop severe fibrosis and heart failure. Interestingly, mechanistic studies using antagoniRs against miR-133 in cultured atrial fibroblasts have shown that the inhibitory action of miR-133 on ECM production may have been mediated through targeting of TGFβ and TGFβ receptor II. However, to our knowledge miRNA-133 is muscle-specific and is not, or barely expressed in the cardiac fibroblasts. It thus remains to be seen whether this interaction is of any in vivo relevance.

5.2 miR-21

A recent study by Thum et al. has identified miR-21 as a disease target in heart failure. MiR-21 is among the most strongly upregulated miRNAs in response to cardiac stress, and Thum et al. showed by in situ hybridization that the expression of this miRNA is restricted to cardiac fibroblasts. Upregulation of miR-21 in response to cardiac hypertrophy was shown to enhance ERK-MAP kinase signalling, leading to fibroblast proliferation and fibrosis. In vivo silencing of miR-21 using antagoniRs in a mouse model of pressure overload, reduced cardiac ERK-MAP kinase activity, inhibited interstitial fibrosis, and attenuated cardiac dysfunction. One of the genes directly targeted by miR-21 was shown to be a negative regulator of the ERK-MAP kinase pathway: Sprouty homolog 1 (Spry1). Remarkably, miR-21 inhibition in fibroblasts not only inhibited interstitial fibrosis but also prevented cardiomyocyte hypertrophy in response to thoracic aortic banding. This supports a new paradigm which has recently been reported in that it assigns a primary role to cardiac fibroblast activation in myocardial disease. In another study, Roy et al. showed that increased expression of miR-21 in the ischaemia-reperfused heart induces MMP2 expression, by direct targeting of phosphatase and tension homolog (PTEN), a negative regulator of the phospho-inositol 3-kinase (PI3K)-Akt signalling pathway.

5.3 miR-29

Van Rooij et al. have shown that the miR-29 family directly targets mRNA encoding a multitude of ECM-related proteins involved in fibrosis including collagens, fibrillins, and elastin. In response to myocardial infarction, members of the miR-29 family are down-regulated in the spared myocardium, adjacent to the infarct, suggesting they contribute to the development of interstitial fibrosis in cardiac remodelling. Knockdown of miR-29 with antagoniRs induced expression of collagens, whereas overexpression of miR-29 in fibroblasts reduced expression of collagens. Together these data indicate that miR-29 acts as a regulator of cardiac fibrosis.

Given the importance of TGFβ signalling in cardiac fibrosis, it will be important to determine whether miR-21 expression is a downstream target of TGFβ. The identification of miRNAs as regulators of cardiac fibrosis also has clinical implications. Especially important in this regard are the findings that modulation of a single miRNA can disrupt processes of cardiac fibrosis and prevent functional deterioration in a mouse model of heart failure. In conclusion, miR-21, miR-29, miR-30, and miR-133 represent potential therapeutic approaches to combat fibrosis in the heart and other organs.

6. Conclusions

Interstitial fibrosis is a hallmark of most cardiac pathologies. The excessive production of ECM proteins increases myocardial stiffness and alters the mechanics of the heart, which contributes to the pathophysiology of heart failure. As a consequence, anti-fibrotic strategies are increasingly recognized as a promising approach in the prevention and treatment of heart failure. Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone receptor inhibitors are among the most effective therapies aimed at preventing adverse cardiac remodelling. The beneficial effects of these interventions are at least partly attributed to a reduction in ECM protein accumulation and this is believed to be mediated by TGFβ signalling. Animals studies have shown TGFβ antagonists are effective in combating pressure-loaded heart failure. In this regard blockade of TGFβ using neutralizing antibodies prevented the induction of collagen mRNA, myocardial fibrosis, and diastolic dysfunction. However, inhibition of TGFβ does not come without risk, since TGFβ has a wide range of other functions in the body. Thus, potential adverse consequences of TGFβ inhibition must be further explored before it can be clinically used. In particular, its role in cellular proliferation and in the immune system suggests there may be serious side effects. It may therefore be more attractive to target a specific cardiac down-stream component of the TGFβ signalling cascade. In this regard, CTGF may regulate the fibrosis pathway more specifically. However, to date it is still unknown whether CTGF blockade in vivo has beneficial effects on cardiac fibrosis. The Rho-ROCK signalling pathways is another putative interesting therapeutic target. A specific Rho kinase inhibitor, fasudil, seems efficacious in the prevention of cardiac fibrosis in aldosterone treated mice, without affecting blood pressure. Signal transduction pathways initiated by AngII or TGFβ converge in the nucleus, where specific transcription factors such as Smads, ATF2, MRTFs, and kruppel-like factors activate transcription of collagens and other ECM genes. Characterizing the precise mechanisms of action of those transcription factors will enhance our understanding of fibrotic signalling and may lead to the development of specific inhibitors that may prove useful for the treatment of fibrosis.
The concept of in vivo miRNA manipulation is becoming a feasible future therapeutic approach for a wide range of diseases. In this regard, silencing of the liver-specific miR-122 in monkeys recently resulted in a long-lasting lowering of plasma cholesterol levels. A specific inhibitor of miR-122 is the first miRNA-targeted drug that has entered a human clinical trial, in patients infected with Hepatitis C. Although there is still progress to be made on the optimal chemistry and delivery systems to silence miRNAs, the recent discovery that inhibition of a single miRNA (miR-21) disrupts processes of cardiac fibrosis and prevents functional deterioration in a mouse model of heart failure, indicates that it may be timely to explore these novel therapeutics also in patients with heart failure.

**Conflict of interest:** none declared

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