Targeting Cardiac Fibroblasts to Treat Fibrosis of the Heart: Focus on HDACs

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Abstract

Cardiac fibrosis is implicated in numerous physiologic and pathologic conditions, including scar formation, heart failure and cardiac arrhythmias. However the specific cells and signaling pathways mediating this process are poorly understood. Lysine acetylation of nucleosomal histone tails is an important mechanism for the regulation of gene expression. Additionally, proteomic studies have revealed that thousands of proteins in all cellular compartments are subject to reversible lysine acetylation, and thus it is becoming clear that this post-translational modification will rival phosphorylation in terms of biological import. Acetyl groups are conjugated to lysine by histone acetyltransferases (HATs) and removed from lysine by histone deacetylases (HDACs). Recent studies have shown that pharmacologic agents that alter lysine acetylation by targeting HDACs have the remarkable ability to block pathological fibrosis. Here, we review the current understanding of cardiac fibroblasts and the fibrogenic process with respect to the roles of lysine acetylation in the control of disease-related cardiac fibrosis. Potential for small molecule HDAC inhibitors as antifibrotic therapeutics that target cardiac fibroblasts is highlighted.

1. Introduction

Heart failure constitutes a major medical and financial burden in the U.S. and other developing countries, where it remains the leading hospital discharge diagnosis, and accounts for 400,000–700,000 deaths and $20–$40 billion in yearly healthcare costs in the US alone (www.cdc.gov/dhdsp). This situation exists despite impressive advances in our understanding of cardiac biology, disease pathophysiology, and medical therapy. These circumstances therefore justify and motivate continuing basic research in this field and highlight the need for ongoing exploration of novel therapeutic approaches.

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Whether originating from genetic abnormality, viral infection, toxic insult, atherosclerosis, long-standing hypertension, or diabetes, heart failure was historically viewed as a disease of the cardiac myocyte, where failure reflects a final common pathway of myocyte hypertrophy, pathological gene expression, and apoptosis [1]. This focus on the myocyte was understandable given the bottom-line inability of the failing heart to meet the metabolic needs of peripheral tissues. Moreover, experiments in genetically manipulated mouse models and also human heart failure have demonstrated that single gene defects in myocyte contractile proteins are sufficient to trigger cardiac hypertrophy and failure [2]. More recently, though, it has become clear that there are other significant "players" in addition to the cardiac myocyte that are involved in the myocardial response to injury, and to the progression and severity of heart failure [3]. In this context, the cardiac fibroblast represents a compelling and understudied contributor to cardiac remodeling in myocardial injury and failure.

While significant advancements have been made in our understanding of the pathologic structure and function of the cardiac myocyte in disease, it has only been recently that various groups have started to focus their investigations on what could arguably be viewed as "the elephant in the room" – cardiac fibrosis. Unlike myocyte or endothelial cell function, fibrosis is a biologic process implicated in virtually all forms of cardiovascular disease, ranging from hypertension and atherosclerosis, to hereditary and even toxin-related cardiomyopathies. Because of this broad scope, research into the molecular mechanisms underlying the development of cardiac fibrosis has the potential to drastically change our siloed view of cardiovascular disease processes and identify therapeutic targets for a wide variety of disease states.

Fibrosis and its relationship to cardiovascular disease is not a new discovery. As early as the 1850s when pathologist Rudolf Virchow first described how the extracellular space around what we now refer to as fibroblasts becomes "fibrillated", we have at least in a basic sense understood that there is a significant relationship between fibroblasts, fibrosis and disease [4]. This observation has been made in numerous organs and tissue types, including the lung parenchyma, bone marrow, kidneys and liver. Unfortunately, despite over a century of research, our understanding of the fibrogenic process remains very limited and there are still no FDA-approved medications for the prevention or treatment of fibrosis in any organ.

Why has there been such slow progress in the field of fibrotic diseases? Possibly the largest barrier has been our lack of understanding about what exactly a fibroblast is and the identification of reliable, distinct and defining characteristics capable of distinguishing fibroblasts from other cell types. In addition, since the increase in extracellular matrix (ECM) that characterizes fibrosis is involved in such a wide variety of both pathologic and physiologic processes, it has been difficult to clearly identify the mechanisms underlying its development in these distinctly different settings. In part this stems from the redundancy seen between pathways that lead to physiologic fibrosis ("repair") and those that lead to pathologic fibrosis.

Research into the molecular basis of cardiac fibrosis is now rapidly evolving, and several potential therapeutic targets have been identified. Such targets include regulators of matrix

components themselves (collagen, fibronectin, and elastin), enzymes involved in matrix degradation (matrix metalloproteinases and their inhibitors the TIMPs), and also cell surface receptors that promote cardiac fibroblast activation and differentiation. Here, we review the molecular mechanisms controlling cardiac fibrosis, with an emphasis on characteristics and origins of the cardiac fibroblasts. Furthermore, we highlight emerging data suggesting that enzymes that control reversible lysine acetylation are ideal drug targets for the treatment of pathological fibrosis of the heart.

2. Cardiac Fibroblasts

As Virchow alluded to in the 1800s, fibroblasts are a phenotypically distinct subset of cells generically defined as being from a mesenchymal origin and producing a variety of ECM components, such as collagen and fibronectin [4]. Over a century later, our definition of a fibroblast is only marginally more refined. Although several markers, including vimentin, fibronectin, periostin, and β1-integrin, have been found to be highly expressed in fibroblasts [5–7], none are truly fibroblast-specific [8–10]. Herein lays one of the largest barriers to the field of fibrosis research – the lack of a fibroblast-specific marker. As an approach to this problem, several groups have sought to find fibroblast markers that are not necessarily globally-specific for fibroblasts, but are at least organ-specific. In the heart, this work has resulted in the proposal of discoidin domain receptor (DDR) 2 [11, 12], cadherin-11 [5, 13, 14] and fibroblast-specific protein-1 [5, 15] as potential cardiac fibroblast-specific markers. Unfortunately, each of these markers has fallen short, as none are truly expressed exclusively in cardiac fibroblasts.

Fibroblasts, therefore, both generally and in the field of cardiac fibroblast research, are routinely classified solely based on morphology, culture characteristics and lack of specific markers for cells from other lineages (e.g., striated muscle). Collagen expression is also typically used as a fibroblast marker and, as will be described further below, α-smooth muscle actin (αSMA) defines a subset of fibroblasts known as myofibroblasts. Some groups are looking into other ways of identifying and tracking fibroblasts *in vivo* and *in vitro* by using reporter genes (e.g., green or red fluorescent protein) under the regulation of promoters for fibroblast markers such as collagen or αSMA [16, 17]. While this strategy represents a potentially exciting system to assess mechanisms of cardiac or other organ fibrosis, its use thus far has been limited to studies of liver and skin fibrosis [18–20].

Further complicating the issue is a large body of evidence that supports the existence of fibroblast heterogeneity. For example, Chang and co-workers found that distinct differences in fibroblast gene expression exist depending on the tissue from which the cells originated as well as the local microenvironment [21]. Additional evidence for tissue-specific fibroblast characteristics was provided by studies showing that, when fibroblasts from different tissues are cultured *in vitro*, they orient themselves in a tissue-specific manner reflective of the organ from which they were derived [22]. As a correlate to this, *in vitro* studies have revealed distinct profiles of cytokine signaling in fibroblasts derived from different tissues [23]. Finally, in a very recently published article, Driskell and co-workers were able to show with transplantation assays and lineage tracing in mice that within skin fibroblasts there are

different fibroblast subsets that in fact arise from distinct lineages, as opposed to merely differing in their differentiation patterns [24].

For the scope of this paper, however, we will focus on the cardiac fibroblast because in the heart, fibroblasts represent a particularly unique population of cells. Unlike cardiomyocytes, which have only minimal if any proliferative capacity beyond the neonatal stage, fibroblasts retain the ability to proliferate and differentiate throughout a human's lifespan. In this way, cardiac fibroblasts are a unique cellular component of the heart that functions in a dynamic way to adapt as the heart grows developmentally and as the adult heart responds to stress [11, 25]. The plasticity of fibroblasts is also particularly important in allowing the heart to respond to acute insults and chronic stressors such as myocardial infarctions, longstanding hypertension and heart failure [3, 26–29].

It is commonly accepted that cardiac fibroblasts are both phenotypically and functionally different than other fibroblasts. At a developmental level, cardiac fibroblasts differentiate from multi-potent progenitor cells or mesenchymal stem cells that are from a specific spatiotemporal locus; the cells ultimately undergo an epithelial-to-mesenchymal transition (EMT) to become mature cardiac fibroblasts [3, 27, 30]. Mature cardiac fibroblasts are more elongated and have higher cellular activity than immature cardiac fibroblasts, as evidenced by a highly elaborated endoplasmic reticulum [27, 31]. In adults, there are also differences between ventricular and atrial fibroblasts, which are constituents of the conduction system [11, 32, 33]. Additionally, there is evidence for unique subpopulations of fibroblasts that serve specialized functions in the control of electromechanical coupling [34, 35], autocrine and paracrine signaling, remodeling and angiogenesis [11, 36–41]. Finally, adult cardiac fibroblasts have been found to possess a functional intracellular renin-angiotensin aldosterone system (RAAS) that is of particular interest as a target for the treatment of cardiac fibrosis, as discussed below [42]. Collectively, research to date strongly supports the notion that cardiac fibroblasts are both different than other fibroblasts, and that within the heart, there exist subpopulations of fibroblasts with unique characteristics and functions.

3. Myofibroblasts

Of the different cardiac fibroblast subpopulations, myofibroblasts have been the focus of most studies because of their purported role in pathological fibrosis. Myofibroblasts have a phenotype more closely resembling smooth muscle cells (SMCs), with expression of αSMA and other SMC markers not typically found in cardiac fibroblasts; this SMC-like phenotype enables myofibroblasts to promote wound contracture [5, 43]. Myofibroblasts are commonly referred to as "activated" fibroblasts because their differentiation from resident fibroblasts (and other precursor cell types discussed below) is triggered by various cellular stresses, including exposure to TGF-β1 [44], IL-18 [45], and platelet-derived growth factor (PDGF)-D [46]. In addition, mechanical stress can lead to differentiation of fibroblasts into myofibroblasts, both *in vivo*, as seen in pressure overload models of heart failure, and *in vitro*, with stretching or extended culture duration [47–50]. It should be noted as well that *in vitro* culture conditions alone rapidly stimulate the differentiation of fibroblasts to myofibroblasts, as evidenced by increased αSMA expression with each cell passage. As a result, virtually all *in vitro* studies done with "fibroblasts" are more accurately a mix of

fibroblasts and myofibroblasts, depending on cell passage number [51–53]. Although the vast majority of the pathways shown to mediate fibroblast to myofibroblast differentiation involve changes in TGF-β, there is growing evidence to support alternative pathways, such as those functioning through transient receptor potential (TRP) ion channels [54, 55].

Although myofibroblasts express αSMA, similar to SMCs, and contain an extensive rough endoplasmic reticulum, similar to fibroblasts, they are distinctly different from both cell types in terms of gene expression and phenotype. For example, work by Gan and co-workers showed that myofibroblasts and SMCs utilize distinctly different transcriptional control mechanisms to regulate αSMA expression [56]. Likewise, myofibroblasts also have been found to express the embryonic form of smooth muscle myosin heavy chain (SMemb) and focal adhesion components, which are not expressed in cardiac fibroblasts [43, 50, 57]. Phenotypically, in contrast to cardiac fibroblasts, myofibroblasts have a high level of exocytotic vesicles and can routinely be distinguished from fibroblasts using electron microscopy [31, 44]. Perhaps most importantly, however, there are distinct functional differences that set myofibroblasts apart from cardiac fibroblasts. *In vitro*, myofibroblasts have the ability to contract collagen gels and exhibit decreased motility [5, 50, 58], which *in vivo* translates to their ability to stabilize scar tissue at an infarct site as well as mediate normal valve function [59–62]. It is also important to note that myofibroblasts are never found in normal heart tissue, apart from in valve leaflets. Furthermore, myofibroblasts in the diseased heart persist for a longer period and are found at sites that are remote from the initial injury compared to myofibroblasts from other organs [5, 31, 63, 64].

4. Extra-Cardiac Sources of Cardiac Fibroblasts and Myofibroblasts

During development, cardiac fibroblasts originate from mesenchymal cells primarily derived from the embryologic epicardium. It was previously thought that this source of mesenchymal stem cells does not persist into adulthood [65, 66]. However, using an infarct mouse model, mesenchymal stem cells have been found to significantly contribute to wound healing post-MI by differentiating into cardiac fibroblasts and myofibroblasts [67, 68]. In addition, as depicted in Figure 1, there is evidence for other sources of cardiac fibroblasts, both in the developing and adult heart [65, 69, 70]. There is evidence to support bone marrow-derived fibrocytes [71–77], endothelial cells [7, 78, 79], epithelial cells [80], vascular smooth muscle cells [81], epicardial derived cells [82–86] and pericytes of the microvasculature [87] all as sources of activated fibroblasts and myofibroblasts that contribute to cardiac fibrosis. Nonetheless, although it is now more widely accepted that there are other sources of myofibroblasts beyond resident cardiac fibroblasts, there is still debate about the extent to which these cells contribute to cardiac fibrosis [88, 89].

5. Myofibroblasts as Therapeutic Targets

The observation that myofibroblasts persist in the heart for prolonged periods after acute injury, and in locations distant from the insult, has led to the hypothesis that this cell type is crucially involved in the pathogenesis of heart failure and arrhythmia. As such, there is intense interest in pharmacologically targeting cardiac myofibroblasts. Since myofibroblasts express renin and ACE (the molecules necessary for the formation of Ang II), as well as

AT1 receptors, which bind Ang II [90–92], effects of ACE-inhibition and AT1-receptor blockade on cardiac fibrosis have been studied extensively. Although most findings are promising, the delicate interactions between ACE and angiotensin subtypes, as well as the dynamic differences in their expression patterns, make this a challenging pharmacological approach [60, 93, 94]. For example, a large clinical trials revealed that the AT-1 receptor blocker, losartan, failed to reduce fibrosis in many patients [95], highlighting the need for alternative anti-fibrotic strategies for the heart.

6. Histone Deacetylases (HDACs)

With the multitude of mechanisms discovered that mediate the development of cardiac fibrosis, it has become clear that many of the signaling pathways, such as those mediated by TGF-β and Ang II, are highly redundant. This functional redundancy has prompted many groups to search for common downstream mediators of cardiac fibrosis, so-called 'nodal' regulators. Recent studies suggest that histone deacetylases (HDACs), which remove acetyl groups from lysine residues in a vast array of proteins [96, 97], may represent such targets. Indeed, small molecule HDAC inhibitors have been shown to block cardiac fibrosis in response to diverse upstream signaling cascades.

As shown in Figure 2A, there are four classes of mammalian HDACs encompassing 18 different isoforms, each encoded by distinct genes. Class I (1, 2, 3 and 8), class II (4, 5, 6, 7, 9 and 10) and class IV (HDAC11) HDACs are all zinc-dependent enzymes. These HDACs are distinct from the class III HDACs (SirT 1–7), referred to as sirtuins, that require nicotinamide adenine dinucleotide (NAD+) for catalytic activity [98]. Although sirtuins clearly regulate cardiac fibrosis [99], the remainder of this review will be focused on zinc-dependent HDACs, since these are the targets of the small molecule inhibitors that have been shown to block fibrosis in the heart (Fig. 2B).

7. Suppression of Cardiac Fibrosis by HDAC Inhibitors

HDAC inhibitors have been shown to block fibrosis in diverse organ systems in response to a variety of stress stimuli. In the heart, HDAC inhibitors have been shown to reduce pressure overload-driven interstitial cardiac fibrosis and to reverse pre-established atrial fibrosis and arrhythmic inducibility in Hop transgenic mice [100–103]; these early studies were performed primarily with trichostatin A (TSA), a 'pan' inhibitor of all zinc-dependent HDACs (Fig.2B) Despite these promising findings, however, the molecular basis for the anti-fibrotic actions of HDAC inhibitors remains incompletely understood. It seems likely that HDAC inhibitors block cardiac fibrosis by multiple mechanisms, including inhibition of cardiac fibroblast proliferation and/or migration, induction of genes that suppress extracellular matrix production from fibroblasts, suppression of pro-inflammatory cues for fibrosis, and blockade of endothelial-to-mesenchymal transition (EndoMT). In addition, our recent work has demonstrated that HDAC inhibitors have remarkable ability to block differentiation of monocytic precursors into mature, collagen-producing fibrocytes (see below).

Endo-MT defines the process of pathological de-differentiation of vascular endothelial cells into matrix-producing mesenchymal cells. This process has emerged as another mechanism

for production of excessive numbers of cardiac fibroblasts in adult hearts in response to stress [7, 104]. Cardiac Endo-MT is stimulated by transforming growth factor-beta (TGF-β) and suppressed by Bone Morphogenic Protein-7 (BMP-7), which is known to block fibrosis [105]. Endothelin-1, a potent vasoconstrictor with pro-mitogenic properties, was also shown to stimulate cardiac fibrosis by promoting EndoMT [79]. The anti-oncogenic action of HDAC inhibitors has been attributed, in part, to blockade of a related process, epithelial-to-mesenchymal transition (EMT) [106]. As such, future studies should address whether HDAC inhibition alters EndoMT in the heart.

HDAC inhibitors also likely have direct effects on cardiac fibroblasts. TSA blocks TGF-β-mediated induction of collagen synthesis in cultured rat ventricular fibroblasts [102]. HDAC inhibitors do not, however, affect TGF-β-mediated phosphorylation or nuclear translocation of SMAD transcription factors, which control collagen gene expression, but do appear to suppress other signaling mediators (e.g., ERK, AKT and PI3K) that impact collagen synthesis [107]. Studies in models of renal fibrosis have suggested that HDAC inhibitors also suppress TGF-β protein expression [108].

Recently, we showed that selective class I HDAC inhibition potently blocks Ang II-mediated cardiac fibrosis [109], in part by suppressing cardiac fibroblast proliferation. MGCD0103, a selective small molecule inhibitor of class I HDACs [110], blocked cultured neonatal and adult rat cardiac fibroblasts in the G_0/G_1 phase of the cell cycle via inhibition of Rb phosphorylation, which is mediated by cyclin-dependent kinases (CDKs) and is required to stimulate downstream expression of E2F target genes that drive the G_1-to-S transition. A major mechanism for inhibition of cancer cell proliferation by HDAC inhibitors involves induction of expression of the p21 CDK inhibitor [111–115]. Surprisingly, class I HDAC inhibition failed to stimulate expression of p21 in cardiac fibroblasts. Instead, a survey of expression of the six other endogenous CDK inhibitors revealed that class I HDAC inhibition selectively upregulates p15 and p57, suggesting a previously unrecognized role for these genes in cardiac fibrosis. The results suggest that one mechanism by which class I HDACs stimulate fibrosis in the heart is by repressing expression of anti-proliferative genes in cardiac fibroblasts, resulting in expansion of the pool of ECM-producing cells in the myocardium in response to stress.

Pro-inflammatory cytokines activate cardiac fibroblasts to produce extracellular matrix [27]. At least part of the anti-fibrotic action of HDAC inhibitors may be due to anti-inflammatory actions of the compounds. In spontaneously hypertensive rats (SHR), treatment with valproic acid, a weak inhibitor of class I HDACs [116], for 20 weeks led to reduced LV expression of the pro-inflammatory cytokines IL-1β and TNFα, which correlated with inhibition of cardiac fibrosis and improved cardiac function [117]. In a related study, HDAC inhibitors were shown to reduce plasma cytokine levels in a rat deoxycorticosterone acetate (DOCA)-salt model of hypertensive cardiomyopathy [118]. Four weeks of treatment, SAHA, which potently inhibits class I and IIb HDACs [119], significantly reduced circulating levels of multiple pro-inflammatory cytokines, including IL-1β, IL-6 and TNFα, and these decreases correlated with reduced cardiac hypertrophy and suppression of interstitial fibrosis in the LV. Together, these data support a significant role for HDACs in cytokine-mediated development of cardiac fibrosis.

8. Targeting Bone Marrow-Derived Fibrocytes with HDAC Inhibitors

Cardiac fibroblasts have long been viewed as the major producers of ECM during the fibrotic process. However, recent studies have revealed an important role for a population of bone marrow-derived cells, termed fibrocytes, in the control of cardiac fibrosis. Fibrocytes have features of both monocytes and fibroblasts, and are able to adopt a mesenchymal phenotype and contribute to tissue remodeling in response to pathological stress [120–122]. Studies by the Entman lab have demonstrated that age-related cardiac fibrosis and diastolic dysfunction coincide with accumulation of fibrocytes in ventricular interstitial space [123]. Additional studies in mice have demonstrated roles for fibrocytes in Ang II-meditated cardiac fibrosis [73, 124, 125], and in fibrosis due to intermittent ischemia [72]. More recently, patients with hypertensive heart disease were found to have elevated levels of circulating, activated fibrocytes, and fibrocyte numbers correlated with disease severity [126].

We recently described results of flow cytometry studies focused on elucidating effects of HDAC inhibitors on cellular infiltration into the heart in response to chronic Ang II signaling in mice [109]. Interestingly, despite having known anti-inflammatory properties, HDAC inhibitors did not reduce Ang II-mediated leukocyte infiltration in the heart. Remarkably, however, class I HDAC-selective inhibition completely blocked Ang II-mediated increases in fibrocyte numbers in the heart, and also decreased circulating levels of fibrocytes; fibrocytes were defined by co-expression of CD34 (stem cell marker), CD45 (hematopoietic cell marker), a monocyte markers (e.g., CD11), and either collagen or α-smooth muscle actin (mesenchymal markers). HDAC inhibitor-mediated suppression of fibrocytes did not appear to be due to blockade of recruitment to the heart, since monocytic fibrocyte precursors were equally abundant in hearts of mice treated with Ang II in the absence or presence of HDAC inhibitor. Consistent with this, cardiac expression of monocyte chemoattractant protein-1 (MCP-1), which is critical for fibrocyte recruitment to the heart [73], was induced by Ang II despite class I HDAC inhibition.

Using an *in vitro* assay, we found that class I HDAC inhibition blocks fibrocyte differentiation as efficiently as serum amyloid P, an Fcγ receptor antagonist that is currently in clinical development for idiopathic pulmonary fibrosis [127]. Compared to cardiac fibroblasts, little is known about the molecular mechanisms that control fibrocyte differentiation and growth. Several receptor agonists have been shown to stimulate fibrocyte differentiation, including TGF-β, ET-1, IL-4 and IL-13 [120–122]; TNFα signaling also appears to be involved [128]. Recently, ERK1/2 was also found to be a critical downstream effector of fibrocyte differentiation [129]. Rho kinase [130], p38 kinase [131] and STAT transcription factors [132] have also been implicated in the control of fibrocyte differentiation. A survey of pathways known to control fibrocyte differentiation demonstrated that class I HDAC inhibition selectively blocked activation of ERK1/2 [109]. The mechanism by which class I HDAC inhibition blocks ERK activation in fibrocytes remains unknown, but could be related to our recent finding that class I HDAC inhibitors suppress ERK signaling in cardiac myocytes by derepressing expression of an ERK-specific phosphatase, termed DUSP5 [133]. Together, our findings establish class I HDACs as key regulators of cardiac fibrosis that serve dual fibrogenic functions by promoting cardiac

fibroblast activation and controlling differentiation of bone marrow-derived fibrocytes (Fig. 3).

9. Summary

As a consequence of recent efforts to define regulatory mechanisms controlling fibroblast function and differentiation, there is an expanding list of potential targets for the treatment of cardiac fibrosis. Among the list of targets, HDACs are particularly promising because these enzymes serve as a nexus for multiple pro-fibrotic signaling networks. It is now recognized that thousands of proteins are subject to reversible lysine acetylation [96, 97], and thus there is no doubt that effects of HDAC inhibitors on cardiac fibroblasts will be mediated by genomic (histone targets) and non-genomic (non-histone targets) mechanisms. The development of isoform-selective HDAC inhibitors will facilitate efforts to more specifically define the mechanisms by which HDACs control fibrosis, and should establish HDAC inhibition as a realistic and viable therapeutic option for cardiac fibrosis. Given the absence of FDA-approved drugs to treat fibrosis in any organ, rapid pre-clinical and clinical assessment of isoform-selective HDAC inhibitors for the treatment of pathological fibrosis is paramount.

Acknowledgments

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Highlights

- Cardiac fibroblasts are a unique cell population.

- Fibroblasts and myofibroblasts are involved in pathologic fibrosis of the heart.

- HDAC inhibition block cardiac fibrosis by targeting fibroblasts and myofibroblasts.
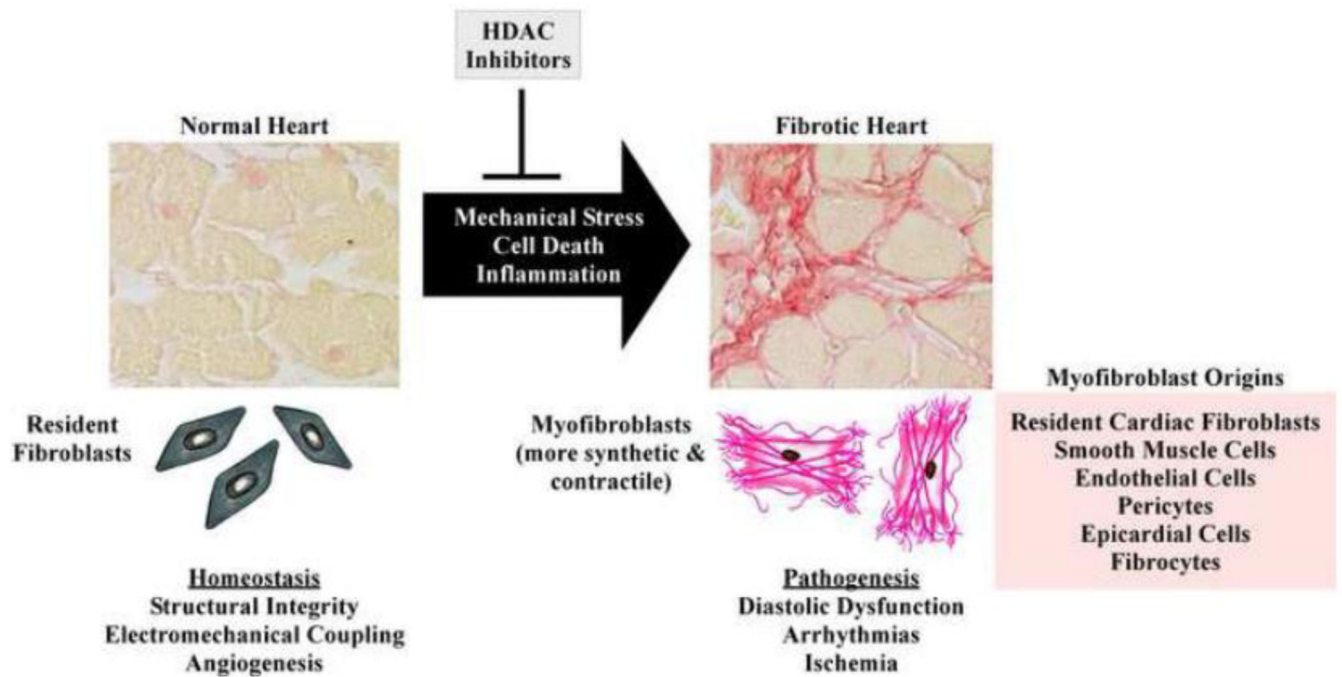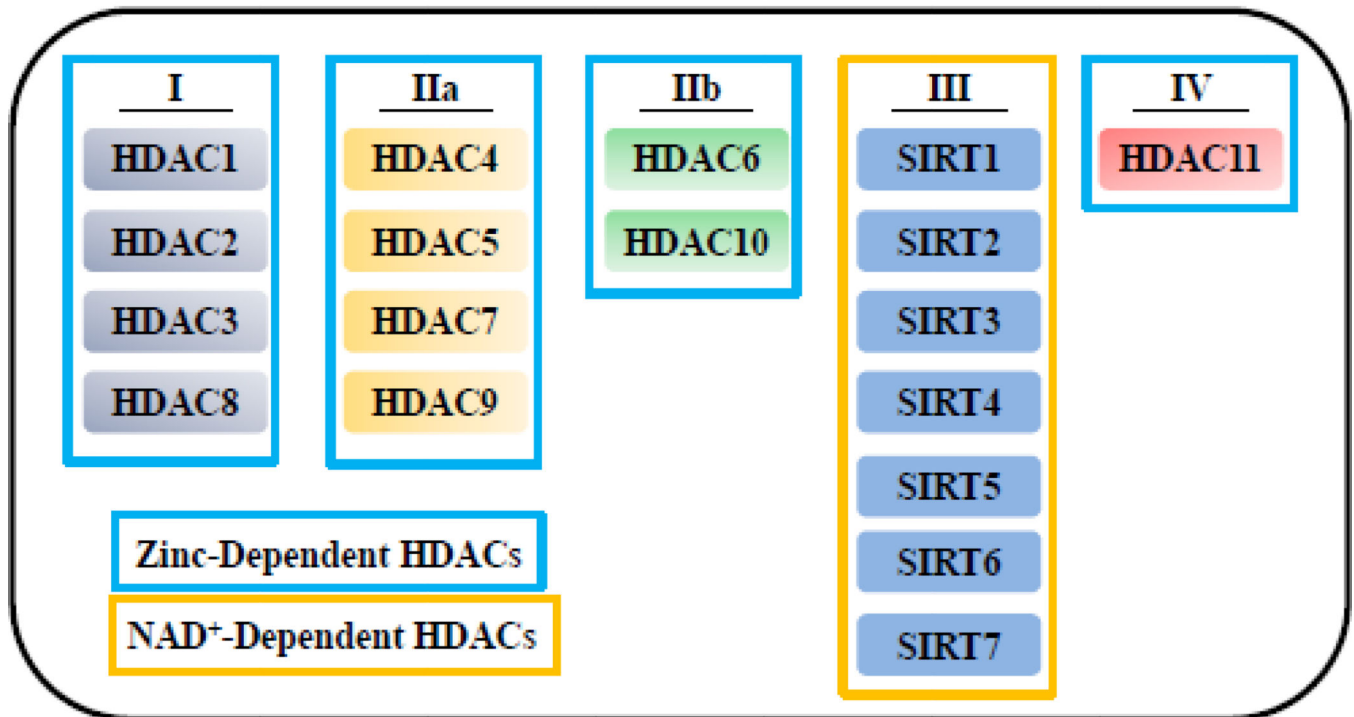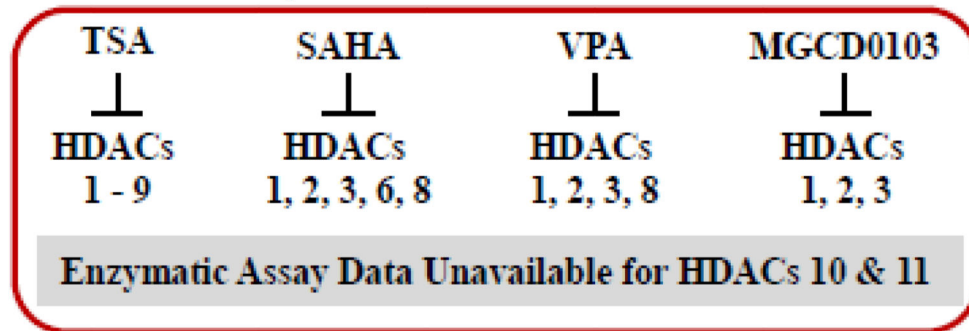
Figure 1. Processes mediating the development of cardiac fibrosis

Cardiac fibrosis is triggered by diverse cues, including mechanical stress, myocyte death and inflammation. Shown are representative images of left ventricles stained with picrosirius red dye to assess interstitial fibrosis from an untreated mouse (left image) and a mouse treated with angiotensin II (Ang II) for two weeks. Pharmacologic inhibition of class I HDACs blocks development of Ang II-mediated cardiac fibrosis. Under normal conditions, resident cardiac fibroblasts contribute to various homeostatic mechanisms in the heart, including maintenance of structural integrity, electromechanical coupling and angiogenesis. Development of pathological cardiac fibrosis is dependent on differentiation of resident cardiac fibroblasts, as well as other cell types listed, into phenotypically and functionally distinct myofibroblasts, which contribute to arrhythmias, ischemia and diastolic dysfunction.

A

Histone Deacetylase (HDAC) Classes

I	IIa	IIb	III	IV
HDAC1	HDAC4	HDAC6	SIRT1	HDAC11
HDAC2	HDAC5	HDAC10	SIRT2	
HDAC3	HDAC7		SIRT3	
HDAC8	HDAC9		SIRT4	
			SIRT5	
			SIRT6	
			SIRT7	

Zinc-Dependent HDACs

NAD+-Dependent HDACs

B

Selectivity of Anti-Fibrotic HDAC Inhibitors

TSA	SAHA	VPA	MGCD0103
⊥	⊥	⊥	⊥
HDACs	HDACs	HDACs	HDACs
1 - 9	1, 2, 3, 6, 8	1, 2, 3, 8	1, 2, 3

Enzymatic Assay Data Unavailable for HDACs 10 & 11

Figure 2. HDAC classification and selectivity of inhibitors

(A) Class I HDACs include HDAC1, -2, -3 and -8. Class II HDACs are divided into IIa, HDAC4, -5, -7, -9, and IIb, consisting of HDAC6 and -10. Class III HDACs are also referred to as sirtuins (SIRT) and include SIRT1-7. HDAC11 represents the only class IV HDAC. Class III HDACs require NAD+ for catalytic activity while the all other HDACs are zinc-dependent (outlined in blue). (B) Routinely used and available HDAC inhibitors target class I, IIb and IV HDACs by chelating zinc in the active sites of the enzymes. Shown are the selectivity profiles for the anti-fibrotic HDAC inhibitors highlighted in this review: trichostatin A (TSA), SAHA, valproic acid (VPA) and MGCD0103.
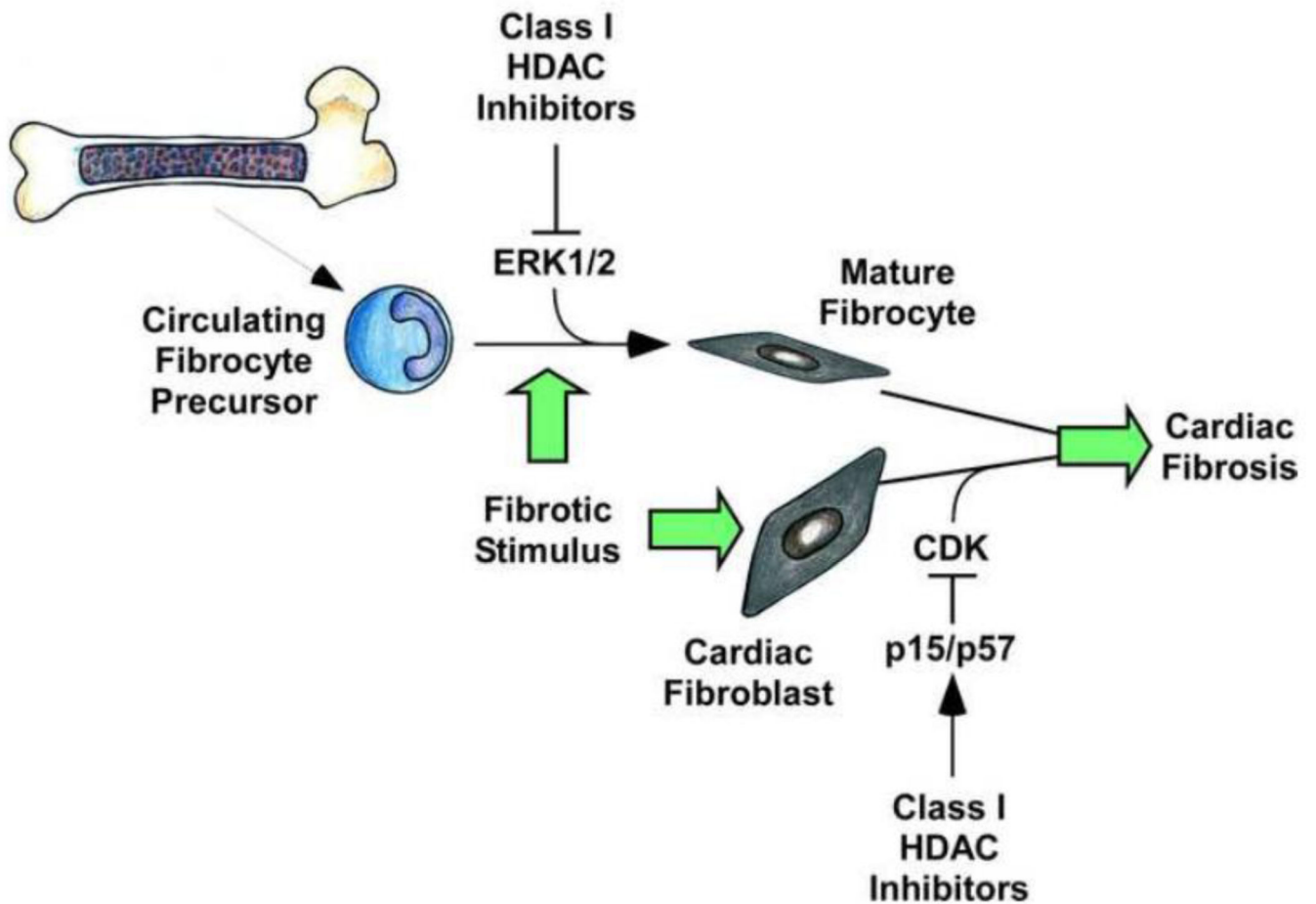
Figure 3. Mechanisms by which class I HDAC inhibition blocks cardiac fibrosis
Class I HDAC inhibition prevents the differentiation of bone-marrow derived fibrocytes into active fibrocytes and fibroblasts through inhibition of ERK1/2 activation. Class I HDAC inhibition arrests cardiac fibroblasts in G_0/G_1 of the cell cycle via upregulation of the cyclin-dependent kinase (CDK) inhibitors p15 and p57. Both of these processes result in decreased numbers of activated ECM-producing fibroblasts and myofibroblasts in the myocardium, leading to decreased fibrosis.