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Transcriptional control of cardiac fibroblast plasticity

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Abstract

Cardiac fibroblasts help maintain the normal architecture of the healthy heart and are responsible for scar formation and the healing response to pathological insults. Various genetic, biomechanical, or humoral factors stimulate fibroblasts to become contractile smooth muscle-like cells called myofibroblasts that secrete large amounts of extracellular matrix. Unfortunately, unchecked myofibroblast activation in heart disease leads to pathological fibrosis, which is a major risk factor for the development of cardiac arrhythmias and heart failure. A better understanding of the molecular mechanisms that control fibroblast plasticity and myofibroblast activation is essential to develop novel strategies to specifically target pathological cardiac fibrosis without disrupting the adaptive healing response. This review highlights the major transcriptional mediators of fibroblast origin and function in development and disease. The contribution of the fetal epicardial gene program will be discussed in the context of fibroblast origin in development and following injury, primarily focusing on Tcf21 and C/EBP. We will also highlight the major transcriptional regulatory axes that control fibroblast plasticity in the adult heart, including transforming growth factor β (TGFβ)/Smad signaling, the Rho/myocardin-related transcription factor (MRTF)/serum response factor (SRF) axis, and Calcineurin/transient receptor potential channel (TRP)/nuclear factor of activated T-Cell (NFAT) signaling. Finally, we will discuss recent strategies to divert the fibroblast transcriptional program in an effort to promote cardiomyocyte regeneration. This article is a part of a Special Issue entitled “Fibrosis and Myocardial Remodeling”.

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Keywords

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1. Introduction

The heart is composed of three main cell types: contractile cardiomyocytes (CMCs), vascular cells, and fibroblasts. Fibroblasts contribute to ~10–30% of the total cardiac cell population, providing basic structural support via secretion of extracellular matrix (ECM) into the interstitial space [1–3]. In addition to generating an ECM scaffold that other cells adhere to, cardiac fibroblasts (CFs) play many underappreciated functions, including paracrine signaling, electrical coupling, and tissue repair [4]. Thus, CFs are emerging as a malleable cell type that is coaxed down various pathways based upon regional requirements and physiological conditions.

As the major source of ECM, fibroblasts play a stereotypical role in tissue replacement and repair following injury. The wound healing process is typified by the transformation of quiescent fibroblasts into a state of high contractility and ECM production, often referred to as a myofibroblast. However, the characteristics of myofibroblasts that allow for efficient wound repair are also responsible for the development of pathological fibrosis and scar formation when left unchecked. In the heart, aberrant scar formation disrupts electrical signaling and muscle contraction and leads to heart failure, the most common cause of death in the U.S. [5]. Thus, tight control of fibroblast plasticity is essential for the maintenance of normal cardiac function. This review highlights the transcriptional control of CF phenotype in the healthy heart and following injury or disease.

2. Fibroblast sources and plasticity

2.1. Fibroblast origins

The heart is lined by a single cell layer of mesothelium called the epicardium. The epicardium is a source of cardiovascular progenitor cells that undergo epithelial-to-mesenchymal-transition (EMT) and differentiate into various cardiac lineages including coronary vascular cells and CFs [6–10]. The CF population can be roughly grouped into three categories: ventricular CFs, atrial CFs, and CFs in specific structures within the heart such as around the sinoatrial node, valves, and annulus fibrosis. Compared to ventricular fibroblasts, atrial fibroblasts display a more robust response to congestive heart failure [11, 12]. Fibroblasts within the valves and annulus fibrosis share significant resemblance to ventricular and atrial fibroblasts, but are more densely packed and retain more specialized phenotypes that are possibly determined during EMT [13, 14]. Fibroblasts within these structures secrete high levels of ECM and create an electrically inert extension of the atrioventricular valves that separates the atria and ventricles to allow asynchronous contraction [15].

In the adult, new fibroblasts are hypothesized to derive from multiple sources including preexisting CFs, fibrocytes, circulating bone marrow stem cells, the epicardium and endothelium [10, 16–21] (Figure 1). Defining the source of fibroblasts remains difficult

however, largely due to their heterogenous nature and lack of a specific marker. Commonly used markers such as FSP1/S100A4, vimentin, discoidin domain receptor tyrosine kinase 2 (DDR2), periostin (*Postn*), and collagen 1a1 (*Col1a1*), and THY1/CD90 are also expressed by other cell types [22–25]. Although multiple groups have detected fibroblasts arising from circulating cells or EMT, a growing consensus is that resident fibroblasts are the primary source of myofibroblasts, at least in mouse models of pressure overload and ischemia-reperfusion (IR) induced remodeling [19–21].

2.2. Myofibroblast activation

Fibroblasts proliferate and become myofibroblasts in response to various genetic, mechanical, and humoral cardiac insults [26–30]. The expression of a number of characteristic genes distinguishes myofibroblasts from quiescent fibroblasts, none of which is a particularly specific or defining feature in isolation. Myofibroblasts express high levels of genes encoding contractile proteins that are typically associated with smooth muscle cells (SMC), including smooth muscle α actin (*Acta2*, *Sma*) and Transgelin (*Tagln*, *Sm22*), although they generally lack smooth muscle myosin (*Myh11*) [31]. Indeed, ACTA2 incorporation into stress fibers is among the most accepted myofibroblast markers, albeit with the obvious limitations with regards to cell specificity. Myofibroblasts also possess mature focal adhesions consisting of vinculin, paxillin, integrin αvβ3, focal adhesion kinase, and actin [32, 33], allowing for a directed migration to the source of injury in MI. Finally, myofibroblasts express and secrete an abundance of ECM proteins, including collagen 1, collagen 3, fibronectin 1 (FN1), fibronectin splice variant ED-A, tenascin-C (TNC), POSTN, and MMPs [30, 34, 35]. This ECM provides temporary structural support for disrupted tissue. It can also act as an anchor for static myofibroblasts to adhere to, which allows for the contraction of surrounding tissue. ECM components can also trigger mechanical signals via activation of cell surface receptors such as integrins and TRPC6, inducing downstream signaling pathways that contribute to changes in fibroblast and CMC gene expression and phenotype.

Many organs, including the heart, share this stereotypical fibroblast response to injury or disease. Pathological stresses on the heart including high blood pressure, ischemic heart or coronary artery disease, and inherited cardiomyopathy mutations can lead to CMC apoptosis and replacement by CFs. Indeed, following a cardiac insult such as myocardial infarction (MI), myofibroblasts are essential for necrotic tissue replacement and prevention of cardiac wall rupture [28, 36, 37]. However, key differences distinguish the CF injury response from that of other organs. First, ECM deposition in tissues such as the skin and lung is often followed by proliferation and replacement by other specialized cell types, which ultimately leads to the repair of organ structure and function [38–40]. Unlike organisms such as zebrafish that retain the ability to regenerate the adult heart after resection [41], adult mammalian CMCs are postmitotic and do not support cardiac repair [42]. Therefore, damage resulting in CMC death is considered virtually irreparable. Cardiac fibrosis thus serves as a compensatory mechanism to prevent the disastrous loss of cardiac integrity. Second, the heart becomes more rigid upon accumulation of interstitial fibrosis during the healing process [43]. While this increased rigidity is an adaptive response to preserve tissue integrity, cardiac fibrosis reduces muscle contractility and is a risk factor for arrhythmia and

heart failure. Third, in the absence of continued pathological stress, myofibroblasts are eventually lost from most tissues, either by reverting back to quiescent fibroblasts or through apoptotic cell death [44–46]. For reasons that are not fully understood, clearance of myofibroblasts from the diseased heart appears to be an inefficient process, leading to persistent fibrosis and deterioration of cardiac function. Because increased cellular tension is a major mediator of myofibroblast activation [47–49] (Figure 2), the healing heart is an ideal substrate for persistent myofibroblast activation that may lead to a pathological feed-forward loop.

Thus, despite the short-term advantages of CF activation in adaptive remodeling, this process remains a double-edged sword that ultimately leads to pathological fibrosis, maladaptive remodeling, and heart failure. Novel therapeutic strategies that directly target the fibroblast are needed to limit fibrosis following injury and perhaps coax activated fibroblasts towards a cardiac fate. The transcriptional changes that underlie fibroblast origin and plasticity may form a scaffold for the development of such reprogramming strategies in the pursuit of cardiac regenerative medicine.

3. Transcriptional regulators of the cardiac fibroblast phenotype

The CF transcriptome is altered by various pathological signals including mechanical tension, activation of cell surface receptors, and alterations in calcium signaling (Figure 2). The culmination of transcriptional changes following a cardiac insult leads to dramatic changes in fibroblast function. The transcriptional mechanisms contributing to fibroblast phenotypic plasticity in development and disease are highlighted in the following sections.

3.1. Fetal gene program

Reactivation of the fetal cardiac gene program, which is thought to provide the basis of compensatory remodeling during heart disease, is commonly considered a CMC response. However, recent studies suggest that fibroblast biology may also be impacted by the induction of developmental programs in the damaged heart. In fact, adult CFs display heterogeneous expression of early cardiogenic and stem cell markers such as TBX20 and SCA1. SCA1-positive fibroblasts have reduced *Acta2* expression, suggesting they may reflect a more stem-like population that may be resistant to activation. Furthermore, conditional knockout of *Tbx20* in CFs results in an increase in BMP10 expression and myocardial hypoplasia, providing further evidence of CF – CMC crosstalk [50].

During embryonic development, epicardial cells are identified by the heterogeneous expression of various transcription factors, including Wilms Tumor 1 (Wt1), C/EBP, RALDH2/ALDH1A2, TBX18, TCF21 (also known as Capsulin or POD1), HAND2, and Myocardin-Related Transcription Factors (MRTF) which influence EMT and epicardial-derived progenitor cell (EPDC) differentiation [51–57]. This gene signature is silenced after birth, but is reactivated by disease or injury, potentially mobilizing EPDCs and leading to the generation of nascent fibroblasts. TCF21 is expressed in a population of EPDCs and appears to be essential for the formation of CFs during embryonic development at the expense of the SMC lineage [58]. Animals deficient in *Tcf21* fail to produce CFs, instead accumulating cells expressing SMC markers on the surface of the heart [58, 59]. RNA-

sequencing of *Tcf21*-deficient coronary SMC combined with Ingenuity Pathway Analysis revealed that TCF21 promotes a gene expression signature consistent with cell proliferation and migration while inhibiting SMC differentiation [60]. ChIP-sequencing identified 5' – CAGCTG – 3' as the canonical binding sequence for TCF21 and suggests shared genomic occupancy with other transcription factors, including AP-1, TEAD, C/EBP, and ATF. Currently, the direct transcriptional targets of TCF21 that mediate the differentiation of epicardial derived cells into fibroblasts are not clear. However, these findings hint at a potential cooperative regulation between TCF21 and AP-1, which was previously shown to bind to and activate the type 1 collagen promoter in fibroblasts and regulate CF migration [61, 62]. The Olson group recently defined a requisite early upstream role of C/EBP in the reactivation of *Raldh* and *Wt1* following MI or IR injury [52]. Animals that lack C/EBP in the epicardium have improved cardiac function following ischemic injury, at least partially stemming from reduced inflammatory cell recruitment. Although this study did not test the possibility that C/EBP may directly modulate the fibroblast phenotype, it is interesting to speculate that C/EBP and TCF21 may also coordinate the generation of epicardial-derived fibroblasts in the adult heart. Taken together, these studies highlight the transcriptional regulation of CF formation in the embryo and suggest a combinatorial transcriptional code in the epicardium that may contribute to the adult injury response.

3.2. TGF-β signaling

One of the best-characterized regulators of fibroblast activation in the adult is transforming growth factor (TGF)-β [63]. Most tissues harbor high levels of biologically inactive latent TGFβ that is cleaved into an active form by proteases, thrombospondin 1, integrins, and reactive oxygen species [64–66]. The MI injury response also leads to the accumulation of additional TGFβ, which is secreted from inflammatory cells or resident fibroblasts [63, 67]. TGFβ signaling is mediated through the stimulation of a heterodimer of the TGFβRI/ALK5 and TGFβRII receptors [68]. The canonical TGFβ pathway is defined by the subsequent phosphorylation and activation of the intracellular SMAD2/3 proteins. SMAD2/3 then interacts with SMAD4 and enters the nucleus, binding to and activating SMAD-binding elements (minimally 5' – GTCT – 3') in the promoters of target genes [69–71] (Figure 2). The inhibitory SMADs 6/7 prevent SMAD2/3/4 nuclear accumulation and the activation of TGFβ-SMAD targets, which includes the core myofibroblast gene program such as *Col1a*, *Acta2*, *Tagln* [72–77]. CFs isolated from *Smad3*-deficient animals secrete less collagen and have fewer ACTA2 positive stress fibers compared to fibroblasts from control animals [78]. Furthermore, loss of *Smad3* attenuates fibrotic remodeling in mouse models of MI, idiopathic pulmonary fibrosis, and diabetes mellitus [67, 79, 80]. Animals heterozygous for *Smad3* appear to be protected from diabetes-induced cardiac hypertrophy suggesting a dose-dependent role of SMAD3-regulated TGFβ signaling [79]. Expression of the inhibitory SMAD7 is reduced in the infarcted rat heart, which is thought to relieve the repression of the TGFβ-Smad axis and promote fibroblast activation *in vivo* [81]. Indeed, overexpression of *Smad7 in vivo* prevented angiotensin (Ang) II-induced fibrosis and loss of contractility while overexpression *in vitro* prevented ROS-induced expression of MMP and collagen [82, 83].

The consensus SMAD binding element (SBE) consists of only four bases and is found in nearly every promoter. Thus, interactions between Smads and other transcriptional

activators or repressors, such as AP-1, SP1, TFE3, KLF15 and P300, confer the magnitude and specificity of target gene expression. Various points of intersection mediate a coordinated response to the TGF β-Smad axis and other signal transduction pathways. For example, AngII signaling induces the expression of the Kruppel-like transcription factor KLF5, which subsequently activates the expression of TGFβ, linking these signaling axes [84, 85]. Conversely, TGFβ activation in myofibroblasts attenuates the expression of KLF15, an inhibitor of SMAD3-dependent expression of connective tissue growth factor (CTGF) (Figure 2). Consistent with this finding, *Klf15*-null mice exhibit increased CTGF levels and fibrosis in response to pressure overload induced cardiac remodeling [86, 87]. Finally, a unique interaction exists between SMAD3 and the basic helix-loop-helix transcription factor scleraxis, which is induced by TGFβ-Smad activation and subsequently synergizes with SMAD3 to activate *Col1a* expression [88].

TGFβ activity is also mediated by the non-canonical pathway via TGFβ-activated kinase (TAK1) stimulation of mitogen activated protein kinases (MAPKs) including ERK1/2, c-Jun N-terminal kinase (JNK), and p38 (MAPK14) [89, 90] (Figure 2). TAK1/p38α has been specifically implicated in promoting myofibroblast activation; pharmacological inhibition of p38 blunts TGFβ-dependent *Acta2* expression and the development of fibrosis in multiple organs, including the heart [91–94]. TGFβ stimulation of human dermal fibroblasts also triggers ERK phosphorylation and CTGF expression, which contributes to myofibroblast activation and cytoskeletal rearrangements [95, 96]. ERK also transduces mechanical tension through focal adhesion kinase (FAK) in fibroblasts [97, 98]. Finally, non-canonical TGFβ intersects with canonical TGFβ signaling to induce expression of TIMP-3 in human gingival fibroblasts in a synergistic manner [94].

3.3. MRTF/SRF/RhoA

Serum response factor (SRF) is an ubiquitously expressed and highly conserved transcription factor that is essential for life. SRF binds to and activates promoters harboring a DNA element called a CArG box ($CC(A/T)_6GG$) [99, 100]. More than 8000 evolutionarily conserved CArG elements exist [101, 102] that are predicted to regulate the expression of thousands of protein coding genes [103]. SRF target gene selection and the magnitude of transcriptional activation depends upon interactions with various tissue-restricted or signal responsive co-factors.

The expression of genes encoding SMC contractile proteins, which are nearly always regulated by a CArG element, is potently stimulated by interactions between SRF and members of the myocardin family of transcriptional co-activators [104, 105]. The founding member of this family, *myocardin* is restricted to SMC and CMCs and constitutively induces the SMC gene program in vascular and visceral smooth muscle [106–110]. In contrast, myocardin-related transcription factor (MRTF)-A (also called MAL/MKL1/BSAC) and MRTF-B (MKL2) are broadly expressed, signal responsive transcription factors [111, 112]. Under basal conditions, MRTFs interact with monomeric (G)-actin through an N-terminal RPEL domain, masking a nuclear localization signal [113–115]. Polymerization of filamentous (F)-actin reduces the pool of G-actin, allowing MRTFs to enter the nucleus and bind to SRF, activating components of the SMC gene program such as *Acta2* [116–120]

(Figure 2). Conversely, inhibiting F-actin polymerization with latrunculin B or other means blocks MRTF-dependent *Acta2* expression [114]. Thus, MRTFs control fibroblast phenotypic plasticity by linking changes in the actin cytoskeleton to regulation of the SMC gene program [105, 121].

Recent studies have uncovered a dominant role for SRF / MRTF-dependent transcriptional activation in regulating the myofibroblast phenotype [103, 122]. Exogenous expression of MRTF-A in fibroblasts or epithelial cells is sufficient to induce phenotypic transformation into migratory and contractile myofibroblasts [123–126]. Tomasek et al. first demonstrated in a dermal wound healing model that expression of *Acta2* in granulation tissue fibroblasts requires binding sites for both SRF and Smads [74]. Induction of *Acta2* in myofibroblasts was later shown to depend upon Rho/Rho kinase (ROCK1) signaling [127]. Consistent with this, myofibroblasts express higher levels of RhoA than quiescent fibroblasts [128]. Further evidence linking Rho to MRTF target genes comes from pharmacological inhibition of ROCK1 with fasudil or Y-27632, which prevents remodeling and fibrosis *in vitro* and *in vivo* [129–131]. We and others have since demonstrated overlapping functions of TGFβ and Rho-ROCK1 signaling in mediating F-actin polymerization and MRTF activation in myofibroblasts of various sources [120, 124, 130, 132–134]. Indeed, myocardin family members and SMAD3 synergistically activate SBE/CArG element-containing promoters in SMCs and during EMT or myofibroblast activation [73, 135, 136].

It is important to note the context-dependent role of Rho-ROCK1 activation in regulating MRTF activity and myofibroblast differentiation. Although the fibroblast phenotype is directly affected by substrate stiffness and growth factors, external factors such as cell density and contact inhibition are also important modulators of fibroblast activation. In cell culture, confluent monolayers of fibroblasts and epithelial cells form adherens junctions consisting of cadherins and β-catenin [137–140]. Disruption of adherens junctions leads to the release of β-catenin from the cell membrane, which is typically rapidly degraded. TGFβ stimulation is not only sufficient to prevent degradation of β-catenin, but stabilization of β-catenin in cells with reduced intercellular contacts is required for TGFβ-induced *Acta2* expression [141–144]. Cytoplasmic β-catenin may indirectly promote nuclear localization of MRTFs by competing for GSK3β-mediated ubiquitination [145, 146]. Furthermore, stabilized β-catenin can function as a transcriptional activator of Wnt signaling, which promotes expression of ECM in epithelial cells and mouse embryonic fibroblasts (MEFs), pointing to a potential role of WNTs in modifying the fibroblast phenotype [147, 148] (Figure 2).

MRTF stability and activity is also influenced by post-translational modifications, including phosphorylation, sumoylation and ubiquitination [149, 150], and proteasome inhibitors lead to MRTF-A accumulation [124]. In line with these findings, the four-and-a-half LIM-only protein 2 (FHL2) protein, which is thought to inhibit MURF3-dependent ubiquitination, prevents proteosomal degradation of MRTF-A [151]. In contrast, FHL2 also competes with MRTF-A for SRF binding and inhibits expression of SRF target genes [151, 152]. Consistent with the latter data, FHL2-knockout animals treated with bleomycin had increased pulmonary fibrosis and expression of TNC [153]. It is interesting to note that SRF

induces *Fhl2* expression in embryonic stem cells, suggesting the possibility of a negative feedback loop that limits myofibroblast activation [152].

The importance of regulating MRTFs in fibroblast activation is becoming increasingly clear. Global knockout of *Mrtfb* or *Srf* results in embryonic lethality [154, 155]. In contrast, deletion of *Mrtfa* results in viable and fertile adults although female dams fail to nurse their young due to a defective mammary myoepithelial cell differentiation [156]. The contribution of MRTF-A in cardiac fibrosis was determined when MRTF-A-deficient animals were subjected to myocardial infarction; MRTF-A-null animals had reduced scar formation after MI [130]. Similarly, bleomycin-induced pulmonary fibrosis is reduced in MRTF-A-deficient animals [129, 157]. Taken together, this suggests that MRTF-A is central in promoting the myofibroblast phenotype.

Although therapeutic strategies typically target receptor-ligand interactions or intercellular kinases, manipulation of upstream signaling molecules can potentially have a wide range of off-target effects. Many of the studies mentioned previously demonstrate the efficacy and specificity of controlling MRTF expression and function *in vitro* and *in vivo*. In the adult, MRTFs are generally inactive and tethered in the cytoplasm, and can become precociously activated in response to pathological signals that lead to alterations in the actin cytoskeleton. In an attempt to harness the therapeutic potential of MRTF activity, recent studies have identified small molecules that specifically inhibit MRTFs. In an *Acta2* promoter-based luciferase screen, Velasquez et al. identified N-cyclopropyl-5-(thiophen-2-yl)-isoxazole-3-carboxamide (isoxazole/ISX) as a stimulator of fibroblast activation in a CArG-box and MRTF-dependent manner. These results were confirmed in human foreskin fibroblasts and in cutaneous wound healing experiments where isoxazole promoted more rapid wound healing compared to control-treated animals [132]. A similar screen for modulators of RhoA-mediated signaling led to the identification of CCG-1432 [158]. Compounds related to CCG-1432 bind the nuclear localization signal within the RPEL domain of MRTFs, inhibiting importin-dependent nuclear translocation [159]. Subsequent studies have revealed that inhibition of MRTF activity with this class of compound blocks dermal, colonic, and lung fibrosis *in vivo* [160–162]. Together, these studies demonstrate the potential of targeting signal responsive transcription factors, such as MRTF-A, to regulate the fibroblast response.

3.5. TRPC/Calcineurin/NFAT

Calcium influx into the CMC is critical for maintaining cardiac function in part by regulating nuclear factor of activated T-cells (NFAT)-dependent target genes implicated in cardiac hypertrophy. High intracellular levels of Ca2+ permits binding of a calcineurin (Cn) A/B heterodimer to calmodulin to induce a conformational change. This conformational change exposes the active site of CnA, leading to NFAT dephosphorylation and nuclear translocation, where it induces gene expression [163]. A number of factors, including mechanical tension, increase intracellular calcium levels and thus activate NFAT in fibroblasts. Activated Cn/NFAT signaling can then trigger the expression of the hypertrophic gene response in CMCs, *Col3* and *Mrtfa* in fibroblasts, or *Acta2* in SMCs [164, 165] (Figure 2). Not only is CnA overexpression sufficient to induce myofibroblast

differentiation both *in vivo* and *in vitro* in an NFAT-dependent manner, but this activity can be blocked by Cn inhibitors [163, 166–168].

Recent work has focused on the transient receptor potential (TRP) family of proteins as mediators of myofibroblast differentiation [169]. TRP channels form heterotrimeric channels *in vivo* and control Ca2+ influx levels in response to various stimuli including mechanical signals and oxidative stress. Formation of the TRPC channel depends on the expression of TRPC1, which is strongly expressed in rat CMCs and transcriptionally upregulated in CFs in response to TGFβ stimulation [166, 170]. Several TRPC channels are upregulated in models of heart failure such as TRPC1, 3, 5, and 6 [171–173]. TRPC3 is sufficient to drive myofibroblast differentiation in atrial and renal fibroblasts in an NFAT- and ERK1/2-dependent manner, respectively, and may play a more important role in atrial function or fibrosis of other tissues [174, 175]. The most notable of the TRPC proteins in fibroblast plasticity is TRPC6, was identified using an *in vitro* overexpression screen in MEFs. *Trpc6* expression is induced by non-canonical TGFβ signaling and SRF; SRF overexpression was sufficient to increase *Trpc6* transcription, but this increase was blocked with a p38-specific inhibitor [166]. Overexpression of TRPC6 is specifically required to promote TGFβ- or AngII-dependent myofibroblast differentiation in cell culture and is required to prevent cardiac wall rupture after myocardial infarction [166].

4. Fibroblast resolution and reprogramming

Resolution of fibrosis typically culminates in fibroblast apoptosis, however, a subset of CFs are resistant to apoptosis and remain within the scar [176–178]. A recent study revealed that P53+COL1A2+ cells express the endothelial cell (EC) marker VE-cadherin 3 days after IR injury [21]. Loss of p53 in CFs is correlated with decreased cardiac function due to reduced mesenchymal – endothelial transformation and capillary density. This not only suggests that there are inherent transcriptional differences between the types of CFs that become activated in disease, but that CFs that escape P53-dependent gene regulation may transdifferentiate into ECs [21] (Figure 1). CF-EC transdifferentiation in disease may improve cardiovascular function by both promoting neovascularization within a fibrotic infarct and reducing the number of activated fibroblasts. These studies may have further implications as a recent study demonstrated that ECs comprise up to 63% of cardiac cells and suggested that the role of ECs in cardiac physiology may be underappreciated [3]. Further support of this hypothesis demonstrates that some CFs can spontaneously adopt a proliferative myofibroblast phenotype *in vitro* whereas others adopt a non-proliferative TGFβ-induced myofibroblast phenotype [177]. Cells that retain a proliferative phenotype regress to a more quiescent fibroblast transcriptional profile after removing the TGFβ stimulus, including a decrease in *Mrtfa* transcription and susceptibility to apoptosis. Conversely, non-proliferative myofibroblasts remain activated after removal of TGFβ stimulation [177]. Further support of this comes from recent data from D'Souza et al, who demonstrate reduced activation in CFs isolated from rats treated with ACE inhibitors. One proposed mechanism is apoptosis of activation-prone CFs and survival of more quiescent CFs [179, 180]. These data suggest that a subpopulation of CFs may retain the ability to revert back into a quiescent state or undergo apoptosis. Taken together, these studies challenge the previously held notion that CFs are

terminally differentiated and further emphasize the need to identify novel methods of manipulating transcriptional regulators of the myofibroblast state.

In line with the intrinsic plasticity of CFs, reprogramming strategies might be utilized as a means to repopulate lost myocardial tissue with functioning CMCs. Recent studies have defined transcription factor cocktails that can coax CFs into a beating CMC-like cell. Albeit a small percentage of cells, mouse and human CFs can be transformed into CMCs with viral overexpression of the core set of transcription factors: *Gata4*, *Mef2c*, and *Tbx5* (called GMT) [181]. Other early cardiac transcription factors, such as *Nkx2.5*, *Mesp1*, and *myocardin* are less critical, or even inhibit reprogramming whereas *Hand2* can increase the percentage of cells transformed into atrial, ventricular, and pacemaker CMCs [182–184]. Similar reports have used small molecules in combination with the pluripotency factor OCT4 to produce CMC-like cells from fibroblasts [185, 186]. Importantly, cellular reprogramming strategies have proven efficacious in blunting cardiac dysfunction and remodeling in rodent models of MI [182, 187, 188]. It is interesting to speculate that forced expression of reprogramming factors may lead to improved cardiac performance by diverting CFs away from the pro-fibrotic phenotype in addition to stimulating CMC production [189]. Of note, suppression of pro-fibrotic signaling with ROCK or TGFβ inhibitors dramatically improves CF transdifferentiation into CMCs *in vitro*, implying a potential mutual antagonism between reprogramming factors and pro-fibrotic signaling [184]. This study also suggests an intriguing similarity between embryonic stem cell-derived CMCs and CF reprogramming strategies, which are both inhibited by TGFβ signaling, and adds further support to the concept of fibroblast multipotency. Indeed, fibroblasts seem uniquely capable of re-programming, given current reports that ECs or other non-myocyte cell types do not efficiently transdifferentiate into CMCs [188]. Defining the genomic occupancy of GMT during fibroblast reprogramming or in response to inhibitors of reprogramming such as pro-fibrotic signals or Nkx2-5 might provide clues as to the combinatorial interactions that control fibroblast plasticity. Altogether, these studies provide a foundation for developing potential therapeutic strategies to promote cardiac repair.

5. Conclusion

Fibroblasts are no longer relegated to merely structural and supportive roles and are now appreciated as a highly plastic cell type that contributes to maintaining tissue homeostasis and wound repair. Interactions between CFs and CMCs, inflammatory cells, and other cell types promote a balanced environment that can quickly respond to the changing needs of a healthy heart. However, it has become increasingly clear that CFs also play a central role in the progression of heart failure. Resolution of activated myofibroblasts at the culmination of the cardiac injury response is an inefficient process that often leads unchecked fibrosis. Myofibroblast activation is rapidly induced by a growing number of signaling pathways that converge on a limited number of transcription factors. Targeting TGFβ/Smad/scleraxis or Rho-ROCK/MRTF/SRF pathways has already proven efficacious in blocking the progression of fibrosis in animal models of disease. Recently, fibroblasts were coaxed into beating CMC-like cells with exogenous expression of select transcription factors, both *in vitro* and *in vivo*. Additional therapeutic strategies that harness fibroblast phenotypic plasticity may stem from studies that better define CF origin and heterogeneity. While major

hurdles include the development of better markers of fibroblast identity and improved tools that specifically target the fibroblast, manipulating the CF phenotype in disease is certainly a challenging yet attainable goal.

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Highlights

- Cardiac fibroblasts are a uniquely plastic cell type.
- The myofibroblast is a primary source of extracellular matrix during cardiac repair.
- Excessive stimulation of the myofibroblast phenotype leads to cardiac fibrosis.
- Transcriptional regulators of fibroblast plasticity are reviewed.
- Diverting the fibrotic gene program may contribute to the benefits of reprogramming.

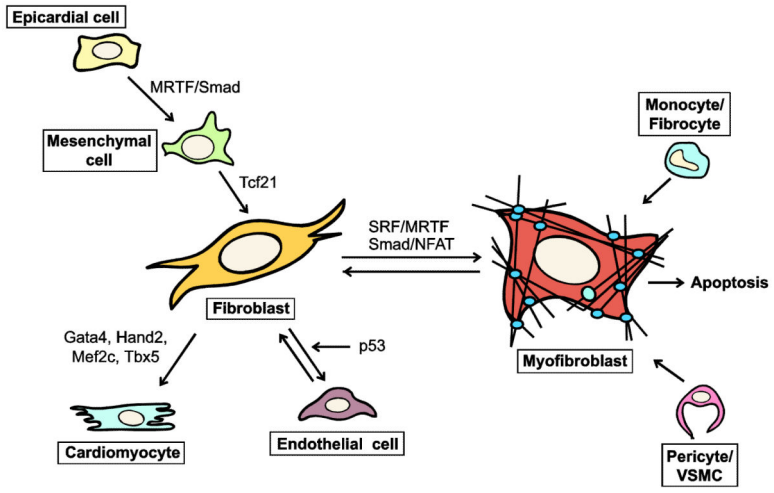

Figure 1. Fibroblast and myofibroblast origins

Multiple cell types have been hypothesized to differentiate or transdifferentiate into myofibroblasts including pericytes/vascular smooth muscle cells (VSMC), circulating monocytes and fibrocytes, endothelial cells, epicardial and mesenchymal cells, and quiescent fibroblasts. In development, quiescent fibroblasts primarily differentiate from epicardial and mesenchymal cells in a mechanism known as epithelial-to-mesenchymal transition (EMT). EMT is largely governed by TGFβ signaling through SMAD proteins and the MRTF and TCF21 transcription factors. Disease can cause fibroblasts to differentiate into myofibroblasts and cause tissue fibrosis. Resolution of fibrosis can occur through two mechanisms: apoptosis or dedifferentiation. Myofibroblasts that dedifferentiate into quiescent fibroblasts may also further transdifferentiate into endothelial cells in a p53-dependent manner. Chronic disease often results in cardiomyocyte death, and reprogramming fibroblasts into cardiomyocytes may offer potential therapies to reduce fibrosis.

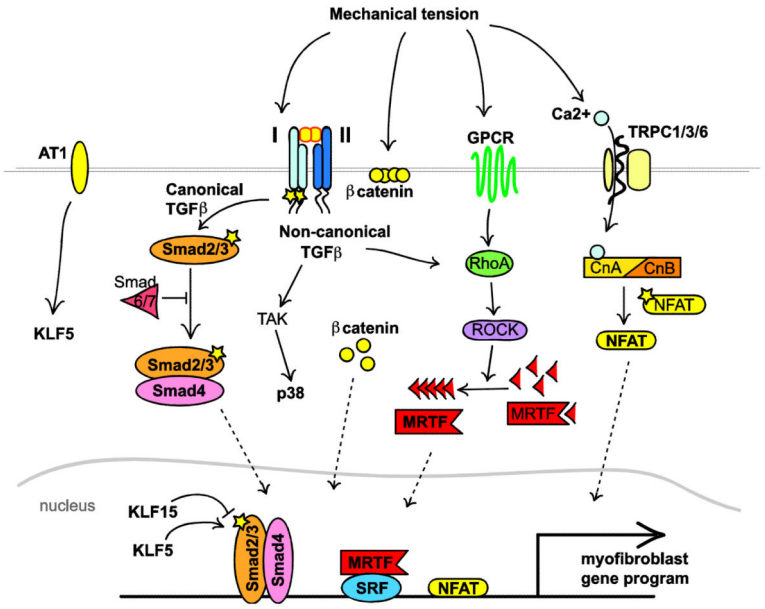

Figure 2. Major signaling pathways that promote fibroblast activation

Multiple pathways converge on the myofibroblast phenotype. Mechanical stress or ligand mediated receptor activation can induce TGFβ signaling, GPCR activation, or calcium influx. TGFβ signaling plays a central role where the canonical arm results in nuclear localization of SMAD2/3/4. Inhibitory SMADs 6/7 or KLF15 can block SMAD2/3/4-dependent transcription whereas angiotensin/KLF5 signaling can enhance canonical TGFβ signaling. Non-canonical TGFβ signaling not only promotes MAPK/p38/JNK/ERK-dependent transcription, but can feed into the Rho/ROCK signaling pathway to promote MRTF nuclear localization. Rho/ROCK can also be activated by G-protein coupled receptors (GPCRs). Finally, mechanical tension can also disrupt β-catenin localization in the adherens junctions to prime the cell for further stimulation or cause calcium influx which activates calcineurin/NFAT signaling.