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Protective transcriptional mechanisms in cardiomyocytes and cardiac fibroblasts

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Abstract

Heart failure is the leading cause of morbidity and mortality worldwide. Several lines of evidence suggest that physical activity and exercise can pre-condition the heart to improve the response to acute cardiac injury such as myocardial infarction or ischemia/reperfusion injury, preventing the progression to heart failure. It is becoming more apparent that cardioprotection is a concerted effort between multiple cell types and converging signaling pathways. However, the molecular mechanisms of cardioprotection are not completely understood. What is clear is that the mechanisms underlying this protection involve acute activation of transcriptional activators and their corresponding gene expression programs. Here, we review the known stress-dependent transcriptional programs that are activated in cardiomyocytes and cardiac fibroblasts to preserve function in the adult heart after injury. Focus is given to prominent transcriptional pathways such as mechanical stress or reactive oxygen species (ROS)- dependent activation of myocardin-related transcription factors (MRTFs) and transforming growth factor beta (TGFβ), and gene expression that positively regulates protective PI3K/Akt signaling. Together, these pathways modulate both beneficial and pathological responses to cardiac injury in a cell-specific manner.

Keywords

Cardioprotection; cardiomyocyte; cardiac fibroblast; transcription; exercise

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1. Introduction

Physiological and pathological responses to stress are regulated by a variety of gene expression programs. In particular, the heart undergoes structural remodeling to compensate for external stresses that impede cardiac homeostasis. Cardiomyocytes (CMs) and cardiac fibroblasts (CFs) both respond to acute and chronic stress by secreting cytokines and increasing the synthesis of structural proteins. Although these changes provide temporary protection after injury, persistent stress can lead to CM death. The inability of adult CMs to proliferate partially underlies the development of heart failure following injury. As a compensatory response to preserve cardiac contractility, quiescent resident CFs transition to an activated myofibroblast state and secrete extracellular matrix (ECM). Unfortunately, this reparative mechanism of replacing lost myocardium with connective tissue ultimately hinders cardiac function and accelerates the progression to heart failure. Thus, it is of therapeutic interest to identify mechanisms that permit a hypertrophic response without triggering maladaptive changes, and that enhance cardioprotection by promoting CM survival and/or inhibiting myofibroblast activation. This review will highlight cardioprotective pathways activated in CMs and CFs based on studies of myocardial infarction (MI), ischemia/reperfusion (I/R) injury, exercise or cardiac regeneration using animal models of genetic deletion or gain-of-function.

2. Cardioprotection: insights from exercise and disease models

2.1 Evidence of cardioprotection

When subjected to oxygen deprivation or mechanical stress, cardiac cells respond by upregulating gene programs that promote survival from injury referred to as ‘cardioprotection’. This phenomenon enables the heart to defend against various stresses that impede cardiac function. A classic example of cardioprotection is through ischemic preconditioning, whereby sub-lethal episodes of ischemia can prime the heart to resist the damaging effects of subsequent MI and I/R injury. In animal models, ischemic preconditioning is achieved by temporarily occluding the coronary vasculature followed by reperfusion or reoxygenation over several episodes. Such preconditioning significantly reduces infarct size, myocardial necrosis and endothelial dysfunction in multiple animal models of permanent coronary occlusion [1–5]. The protective effects of ischemic preconditioning are orchestrated by a variety of signaling pathways. For instance, anti-apoptotic proteins such as Bcl-XL (*Bcl2l1*), Mcl-1 (*Mcl1*), and c-Flip (*Cflar*) are induced with ischemic preconditioning [6]. Overexpression of Bcl-XL is sufficient to protect cardiomyocytes against I/R injury [7]. Ischemic preconditioning also suppresses pro-apoptotic signals such as the activation of caspase 3 and subsequent cleavage of the DNA repair protein poly (ADP-ribose) polymerase-1 (PARP-1) [6]. While experimental study of ischemic preconditioning in humans is not feasible given obvious ethical concerns, the salutary effects of ‘preconditioning’ in humans has been observed in patients with unstable angina prior to myocardial infarction [8] or with coronary angioplasty-related preconditioning [9]. These observations suggest that the heart has an innate capacity to protect itself in response to environmental stress.

Anecdotal evidence from heart failure (HF) patients also suggests a link between exercise and improved or sustained cardiac performance [10]. Studies in rodents further gave credence to this link with the observation that rats exhibit reduced infarct size and increased vascularity with chronic exercise training [11]. The beneficial effect of exercise has prompted its use as a therapeutic for HF patients. In a multi-center, randomized study of HF patients (HF-ACTION), regular physical activity was found to be associated with reduced cardiovascular mortality and HF hospitalizations [12]. Likewise, exercise also improves myocardial perfusion in patients with stable coronary artery disease [13]. Thus, exercise serves as an example for a non-injury stimulus that affords cardioprotection.

2.2 PI3K/Akt survival signaling

To understand cardioprotective mechanisms, it is important to discuss physiological processes such as exercise, and pro-survival pathways such as the phosphoinositide-3 kinase (PI3K)/Akt pathway. Exercise induces a physiological response in the heart that promotes a protective, pro-proliferative state that extends to cells with minimal proliferative capacity such as adult CMs [14, 15]. Indeed, moderate exercise provides notable improvements in cardiac function and reduced fibrosis in mouse models of cardiomyopathy as well as human HF patients [16–18]. Traditionally, PI3K/Akt promotes cell survival by inhibiting caspase- or p53-dependent apoptosis via GSK3β and Bcl2-family members, and activating mammalian target of rapamycin (mTOR) signaling to promote cellular growth [19]. In the heart, PI3K/Akt is stimulated by exercise and participates in the canonical reperfusion injury salvage kinase (RISK) pathway of cardioprotective kinases [20]. In concert with insulin growth factor (IGF), PI3K activates Akt and triggers physiological cardiac hypertrophy and survival instead of a pathological transition to dilation and heart failure [21–24]. Transient activation of Akt via acute viral gene transfer similarly attenuates CM apoptosis and infarct size in rats subjected to I/R injury [25]. However, excessive Akt signaling can negatively impact the heart; chronic activation of Akt promotes pathological hypertrophy at baseline and exacerbates maladaptive remodeling after I/R injury [26, 27]. Thus, temporal but not persistent activation of Akt is necessary to harness its protective capacity.

2.3 Exercise, C/EBPβ and CITED4

The beneficial effects of exercise in cardioprotection are likely attributed to multiple factors, one of which may be modulation of the immune response. Indeed, chronic exercise training improves cardiac function and attenuates pro-inflammatory cytokines in rats with isoproterenol-induced HF [28]. Chronic Akt activation has been linked to tumor necrosis factor (TNF)-mediated activation of the transcription factor NFκB, which subsequently regulates the inflammatory response [29, 30]. Stimulation of the NFκB pathway is also facilitated by the transcription factor CCAAT Enhancer Binding Protein Beta (C/EBPβ) [31]. C/EBPβ induces hypertrophic gene programs and is downregulated in response to exercise [31, 32]. Mice that are haploinsufficient for C/EBPβ are resistant to pathological remodeling with pressure overload injury [32]. Inhibition of C/EBP also modulates hypoxia-induced epicardial activation, and blunts neutrophil recruitment after MI or I/R injury [33]. Of note, the transcriptional activator CITED4 is expressed reciprocally to C/EBPβ in CMs after exercise [32, 34]. Exercise stimulates microRNA (miR)-222 expression in CMs, and overexpression of miR-222 is sufficient to block pathological remodeling by upregulating

the *Cited4* gene [35]. CM-specific expression of CITED4 improves cardiac function, and reduces CM death and ventricular fibrosis after I/R [36]. Treating mice with anti-neoplastic 5-fluorouracil blocks exercise-induced proliferation and CITED4 stimulation, reducing the ability of exercise to protect the myocardium from I/R injury [37]. Thus, inhibiting C/EBPβ or activating CITED4 may be potential approaches to mitigate pathological remodeling and promote cell survival.

2.4 Mitochondrial Dynamics and ROS

Mitochondria are fundamental gatekeepers of cellular metabolism. In order to maintain homeostasis, CMs must produce an uninterrupted supply of energy [38]. However, oxidative phosphorylation and ATP production contribute to the production of ROS, which can promote CM dysfunction if produced in excess. Mitochondria isolated from the hearts of rodents subjected to exercise training are more resistant to calcium-induced mitochondrial permeability transition pore (mPTP) opening [39] and ROS-induced cytochrome *c* release [39, 40]. Exercise not only activates PI3K/Akt signaling, which in turn enhances mitochondrial cardioprotection via hexokinase-mediated resistance to mPTP opening [41], but also elicits profound changes to the mitochondrial proteome [42].

Independent of PI3K/Akt, exercise also alters mitochondrial dynamics [43]. For instance, increasing energy demand can induce protective mitochondrial fission in the heart [44]. Consistent with this concept, cardiac-specific depletion of the mitochondrial fission protein DRP1 exacerbates fibrotic remodeling after I/R, and impairs cardiac function after pressure overload [45, 46]. The fragmentation of mitochondria by fission may facilitate cardioprotection by promoting mitophagy, and/or reducing CM apoptosis in response to mitochondrial dysfunction [45–49]. For example, RhoA signaling reduces translocation of pro-apoptotic proteins such as BAX to mitochondria [50] (Fig. 1) Modulating the mitochondrial transcriptome may also facilitate cardioprotection. Mitochondrial transcription factor A (TFAM) is a critical regulator of the transcription and replication of mitochondrial DNA (mtDNA) [51]. Elevated oxidative stress and reduced mitochondrial biogenesis is associated with reduced mtDNA content in end-stage heart failure patients [52] and in mice after MI [53]. Cardiac-restricted overexpression of *Tfam* attenuates CM hypertrophy and apoptosis, and improves mitochondrial respiration post-MI injury [54, 55]. Thus, maintaining mitochondrial function through regulation of either gene expression or physical dynamics can protect the heart when it is burdened by stress.

3. Cardiomyocyte Specific Transcriptional Pathways

Cardiac injury such as I/R causes CM mitochondrial dysfunction and extracellular inflammatory signaling that facilitate cell death. As a result, millions of CMs are lost that cannot be restored due to the refractoriness of CMs to re-enter the cell cycle. Limiting the loss of CMs to these stimuli is critical to maintain cardiac function. Non-ischemic stress can instead trigger a hypertrophic response in CMs. However, if the initial cardiac insult persists, this hypertrophy can transition from compensatory to maladaptive, and thus must be limited. Here, we will review gene expression pathways that occur specifically within the CM to limit both acute cell death and pathological remodeling.

3.1 Stress-sensitive RhoA and MRTF signaling

Small GTP (guanosine 5'-triphosphate)-binding proteins or "GTPases" serve as molecular switches to regulate cellular processes. The Rho subfamily is best known for its role in regulating cell motility, myosin-based contractility and actin polymerization [56]. Upon GTP activation, RhoA stimulates myosin phosphorylation and regulates actin dynamics via the Rho-associated protein kinases, ROCK1 and ROCK2 [57, 58] (Fig. 1). Several studies demonstrate that aberrant activation of RhoA in mice can lead to pathological hypertrophy and fibrosis, typically by processes that require ROCK1 [59–61]. In contrast, inducible expression of a constitutively active RhoA transgene in CMs affords protection from I/R injury independent of the ROCK isoforms [62]. RhoA activity is also regulated by ROS [63], which may be one mechanism utilized by CMs to sense oxidative stress. In other contexts, CM-specific knockout of RhoA reveals a role for RhoA signaling in the hypertrophic response to cardiac pressure overload, but is not necessary for the progression to heart failure [64]. The seemingly disparate results between acute and chronic RhoA activity indicate that temporal regulation dictates cardioprotection versus maladaptive dysfunction.

The heart responds to hemodynamic stress by secreting peptide hormones and increasing overall size to accommodate elevated wall stress. Among the genes regulated, atrial natriuretic factor (ANF, also *Nppa*) is expressed and secreted in response to atrial stretch [65]. RhoA signaling is exquisitely sensitive to biophysical tension when neonatal ventricular myocytes (NVMs) are subjected to uniaxial stretching [66]. Early studies in NVMs identified the Rho effector Protein Kinase N1 (PKN) as a key regulator of ANF through Rho- and serum response factor (SRF)-dependent transcription [67]. The link between stress-activated RhoA and pro-survival pathways was further delineated by the observation that Rho kinases stimulate PI3K/Akt signaling through focal adhesion kinase (FAK) in NVMs [68]. Moreover, mice with inducible deletion of RhoA in CMs exhibit blunted FAK and Akt activation when challenged by transaortic constriction (TAC), along with a decline in cardiac function after injury [64]. Together, these findings demonstrate a RhoA-FAK-PI3K-Akt signaling circuit utilized by CMs in response to stress.

The ability of RhoA to transduce such protective signals from the plasma membrane to the nucleus requires regulators of transcription. Most notably, myocardin-related transcription factor (MRTF)-A and MRTF-B (collectively 'MRTFs') are Rho-stimulated activators of SRF- dependent transcription [69]. In a basal state, MRTFs are sequestered in the cytoplasm through direct interactions with globular (G)-actin, which sterically mask nuclear localization signals [70]. Upon stimulation, Rho-dependent actin polymerization promotes nuclear shuttling of MRTFs and target gene expression [71, 72] (Fig. 1). For instance, the RhoA-MRTF-SRF signaling axis drives the expression of the protective matricellular protein CCN1 *(Cyr61*) [73]. Secreted CCN1 signals back to CMs in an autocrine and/or paracrine manner to increase mitochondrial cardioprotection via PI3K/Akt signaling [74] (Fig. 1). CCN1 also promotes premature senescence of CMs to aid in recovery after MI [75]. Pro-survival signaling by MRTFs may also be mediated through indirect mechanisms. For instance, MRTF-A promotes PI3K/Akt signaling by repressing inhibitory PTEN and FoxO1a via upregulation of miR-486 [76] (Fig. 1).

The molecular mechanisms underlying MRTF-mediated cardioprotection are not completely understood. Genetic deletion studies in mice demonstrate a dispensable role for both RhoA and MRTFs in normal cardiac physiology [64, 77]. However, stress elicits a profound phenotype in the absence of both MRTF isoforms. CM-specific inactivation of MRTF-B in mice with a homozygous MRTF-A null background (MRTFcKO) results in rapid deterioration of cardiac function within one week after TAC [77]. Transcriptional profiling of MRTFcKO hearts revealed dysregulation of stress-dependent cytoskeletal pathways [77]. Of note, local pools of MRTFs are distributed at the intercalated discs of CMs [77]. Further investigation is necessary to elucidate if and how MRTFs translocate to CM nuclei from cytoskeletal signaling complexes. However, given the stress-dependent activation of RhoA signaling in CMs [68], tethering potent transcription factors to regions of mechanical tension may be one mechanism whereby CMs can “sense” stress.

3.2 Hippo and YAP

The adult mammalian heart has a limited regenerative capacity after birth [78, 79]. Identifying the mechanisms controlling CM proliferation could have profound impacts on heart regeneration and rejuvenation following MI. The Hippo/Yes-activated Protein (YAP) pathway has received a considerable amount of attention in this very endeavor; indeed, Hippo/YAP signaling modulates the expansion and migration of cardiac progenitor cells to sites of injury [80]. The Hippo kinase cascade restrains cell proliferation and growth by phosphorylating YAP and preventing its association with the TEAD family of transcription factors [81]. Cytoskeletal proteins, such as the alpha-catenins, may also inhibit YAP translocation to the nucleus [82]. Loss-of-function studies in the developing heart first demonstrated the pro-proliferative role of YAP in CMs. *Yap1*-deficient hearts display hypoplasia stemming from defects in CM proliferation, IGF1 signaling, and regulation of cell cycle genes [83–85]. Conversely, *Yap1* gain-of-function [84] or inactivation of the Hippo kinases (*Mst1/2, Sav1* or *Lats2*) [86] all stimulate CM expansion. These genetic studies support a critical role for Hippo signaling in regulating cardiac growth.

Although YAP is a potent activator of cell cycle genes and cardiac development, its role in the adult heart is less defined. Fetal heart growth occurs through cell proliferation, whereas postnatal heart growth primarily involves hypertrophy. Mice with homozygous, CM-specific loss of *Yap1* exhibit signs of cardiac dysfunction and hypertrophy at baseline, which is exacerbated in the presence of ischemic injury [87]. Paradoxically, YAP-mediated induction of miR-206 may promote compensatory hypertrophy in response to I/R [88]. In contrast to these observations, other groups have convincingly shown that YAP is not required nor is sufficient to induce CM hypertrophy *in vivo* using cell mosaic analysis or transgenic overexpression [83, 89]. The cardiac hypertrophy associated with *Yap1* deficiency may represent a secondary response to heart failure. Regardless of its role in cardiac hypertrophy, multiple studies support a cardioprotective role for YAP [89–92], or Hippo suppression that would increase YAP activity [86, 93, 94], following ischemic injury. The reverse has also been observed, as Hippo activation reduces cardioprotection [95]. Transgenic mice expressing constitutively active YAP in CMs show better preservation of heart function and approximately 2.5 fold more CM proliferation compared to control mice challenged by MI [89]. However, YAP activation may only be protective if negative feedback regulation is

present. For instance, CM-specific deletion of WW45, a scaffold protein that mediates YAP inhibition, results in increased cardiac dysfunction after injury presumably due to excessive CM de-differentiation [96].

The salutary effects of YAP activation may not be solely attributed to CM expansion. I/R injury damages the myocardium through excessive oxidative stress. Forced expression of YAP blunts hydrogen peroxide-induced cell death of CMs via Akt activation, suggesting that YAP regulates the antioxidant response [87] (Fig. 1). Interestingly, TEAD binding motifs share significant co-occurrence at fetal cardiac enhancers with *Pitx2*, a transcription factor that is responsive to ischemic injury [97]. Yap is also required for *Pitx2*-induced myocardial regeneration [97]. In addition to TEAD binding, YAP associates with FoxO1 to regulate catalase and superoxide dismutase gene expression following I/R injury [98]. Together, these studies indicate that Hippo/YAP signaling modulates cardioprotection and repair following ischemic injury through a series of elaborate pathways involving stress response, cell cycle re-entry and antioxidant signaling.

3.3 GPCR-stimulated pathways of gene expression

While several transcription factors mediate cardioprotection, their activity is dependent on upstream regulators such as G-protein coupled receptors (GPCRs), a large, diverse group of transmembrane signal receptors. The lysophospholipid S1P binds to the S1PR subset of GPCRs [73, 99]. The therapeutic promise of S1PR activation has been demonstrated with the FDA approved S1P analog FTY720 (Fingolimod), which sustains cardiac function after I/R injury [100–102]. Exogenous S1P reduces infarct size and cellular apoptosis after I/R injury without stimulating CM hypertrophy [103], and promotes the proliferative function of cardiac progenitor cells through the activation of RhoA and MRTF-A dependent gene expression [104]. Mechanistically, the S1P/RhoA/MRTF pathway promotes cardioprotection in part through the induction of CCN1 [73]. CCN1 expression can also be induced by S1P-mediated activation of YAP [105, 106]. In other contexts, S1P attenuates hypertrophic signaling through increased expression of the transcription factor KLF4 [107]. Taken together, S1P-mediated cardioprotection is likely orchestrated by multiple pathways in diverse cellular contexts [108].

While the S1P signaling activates RhoA in the heart to facilitate cardioprotection [50, 73, 103], cardiac GPCR signaling is certainly not restricted to RhoA-dependent pathways [109]. GPCRs coupled to other G-proteins can also facilitate protection. Signaling through the beta-2 adrenergic receptor (β2-AR) has protective effects in CMs, primarily dependent upon anti-apoptotic signaling through the PI3K/Akt pathway [110–112] (Fig. 1). Gene programs that facilitate the protective PI3K/Akt and nitric oxide/PKG pathways can be modulated by nuclear β-ARs [113, 114]. Adenoviral mediated delivery of β2-AR to CMs can increase the expression of the anti-inflammatory cytokine IL-10 following aortic constriction [115]. β2-AR stimulation also increases the expression of the soluble inhibitory binding protein of IL-18, limiting pathological hypertrophy of the myocardium [116]. In other contexts, isoproterenol stimulation of β-AR promotes transactivation of epidermal growth factor receptor (EGFR), which signals to inhibit transcription of pro-inflammatory cytokines and pro-apoptotic factors in CMs [117–119]. Opioid receptor agonists such as morphine also

facilitate cardioprotection through the delta-opioid receptor, increasing transcription of sarcomeric stress response proteins, repressing inflammation, and expressing miR-133b-5p to inhibit CM apoptosis [120–123]. Interestingly, β2-AR signaling may be responsible for delta-opioid receptor agonism and subsequent cardioprotection in CMs [124]. Common transcriptional activators could be targeted by β2-AR and delta-opioid GPCR signaling to promote downstream gene expression.

Certain members of the α-AR family are also important in transcriptional protection of CMs.Specifically, the α1a- and α1b-ARs decrease CM apoptosis, and CM-specific deletion of both α1-ARs increases apoptosis, reduces cardiac function, and reduces survival in response to TAC [125, 126]. Notably, α1-AR activation facilitates cardioprotection through ERK-dependent gene regulation and NFkB inhibition, limiting expression of pro-inflammatory stimuli such as TNFα [127]. Thus one effect of GPCR signaling at the transcriptional level, particularly stimulation of the β2-AR and α1-AR, appears to be limiting excessive pro-inflammatory expression from CMs.

4. Cardiac Fibroblast Specific Transcriptional Pathways

Cardiac fibroblasts (CFs) are a heterogeneous population of cells that have important roles in ECM secretion, paracrine signaling, electrical coupling, and the immune response. When subjected to injury such as MI or chronic pressure overload, CFs transition from a quiescent fibroblast into an activated myofibroblast, expressing contractile genes and secreting ECM as a protective mechanism to maintain myocardial wall integrity. However, excessive myofibroblast activation causes maladaptive remodeling that disrupts intracellular contacts, hinders contractility, and leads to cardiac fibrosis [128–130]. In contrast, physiological stimuli such as exercise and pregnancy do not result in fibroblast activation, indicating that CFs are important “first responders” in sensing and reacting to extracellular stress. Here, we will discuss the context-dependent mechanisms of fibroblast activation and how modulation of these pathways could promote cardioprotection or mitigate maladaptive remodeling.

4.1 Roles for fibroblasts in cardiac repair

While fibrosis in other organs can be resolved by inducing apoptosis in activated fibroblasts, subpopulations of activated CFs are less susceptible to apoptosis [131, 132]. Mechanisms to resolve cardiac fibrosis have thus proven to be challenging. Indeed, a recent study defined the matrifibrocyte, a specialized myofibroblast that has lost the ability to proliferate but persists after MI resolution to act as a scaffold [133]. Despite the repercussions of excessive CF activation, controlled activation is also required to preserve cardiac function. Conditional deletion of the ECM component periostin (*Postn*), or p38α (*Mapk14*) demonstrates that ECM production from activated fibroblasts is required to prevent cardiac rupture after MI [134–136]. Similarly, CF-specific deletion of Klf5 prevents pressure overload-induced fibrosis but is accompanied by increased mortality [137]. This may be attributed to the effects of IGF-1 on CFs; IGF-1 signaling is regulated by Klf5, and IGF-1 is enriched in CFs and can increase contraction of fetal and adult rat CFs in collagen gel assays [138]. The effects of IGF-1 on CFs are complex, as treatment of mouse CFs with IGF-1 and H_2O_2 (IGF/ROS) mimics exercise conditions in vitro by stimulating Akt phosphorylation without

stimulating transforming growth factor beta (TGFβ) signaling [139]. Furthermore, IGF/ROS treatment in human CFs significantly downregulates transcription of activated CF markers such as *POSTN*, *ACTA2*, and *COL3A1* compared to control or TGFβ-treated CFs, together suggesting that IGF signaling mitigates pathological remodeling in CFs [140].

In contrast to pathological stress, physiological stimuli induce cardiac hypertrophy in the absence of fibrosis, highlighting CF plasticity and indicating an inherent difference in the CF response despite similar increases in cardiac demand and mechanical stress. Notably, the malleability of CFs has stimulated a series of elegant reprogramming studies that have generated functional CMs from CFs both *in vitro* and *in vivo* using cocktails of transcription factors including *Gata4*, *Hand2*, *Mef2a*, and *Tbx5* [141, 142]. The addition of microRNAs and chemical enhancers to reprogramming cocktails further enhances CM differentiation efficiency [143–145]. Thus, resident CFs may serve as an endogenous source of cells that could rejuvenate the diseased myocardium if redirected to a CM cell fate. Although promising, this strategy may first require modulation of pro-fibrotic responses in disease [143].

4.2 TGFβ regulation of fibroblast plasticity and proliferation

TGFβ-induced activation of canonical (SMAD) and non-canonical (TAK1/P38/MAPK) signaling pathways is critical for transcriptional regulation of myofibroblast differentiation [146–148] (Fig. 2). For instance, inhibition of SMAD3 by gene dosage or via expression of the SMAD3-inhibiting protein KLF15 decreases maladaptive diabetes-induced hypertrophy and fibrosis [149, 150]. SMAD2/3-dependent transcription can also be modulated by SMAD7, an endogenous repressor of TGFβ signaling. Reduced *Smad7* levels are associated with elevated fibrosis in MI, whereas overexpression of *Smad7* suppresses collagen expression [151] and attenuates angiotensin II (Ang-II) induced cardiac remodeling [152, 153]. One approach to mitigating the fibrotic response following injury is to target excessive CF proliferation. Recently, small proline rich protein 2B (*Sprr2b*) was shown to be induced by TGFβ/ROS treatment in CFs, and elevated in failing hearts from humans and mice [140]. In the absence of its canonical keratinocyte-binding partners, non-receptor tyrosine phosphorylation of SPRR2B promotes complex formation with USP7 and MDM2, two components of the P53 ubiquitination complex. This complex stimulates ubiquitination and degradation of P53, thereby promoting cell cycle progression and proliferation of CFs [140]. While TGFβ signaling is essential to initiate CF activation, a recent study demonstrated that ablation of TGFβR2 in activated *Postn*+ CFs reduced fibrosis, indicating that sustained TGFβ signaling is required to maintain CF activation [154].

The P38 family consists of P38α/β/δ/γ that are all expressed to varying degrees in the heart and cooperate with TAK1 or TRPC6 to induce pathological remodeling [155, 156]. Inhibition of P38 signaling in the heart relieves pathological remodeling [157, 158] and knockout of tumor necrosis factor receptor associated death domain (TRADD) can blunt TAK1/P38 mediated hypertrophy and fibrosis [159]. Moreover, bleomycin-induced fibroblast activation can be blocked by the potassium ionophore, salinomycin, in a P38-dependent manner [160]. Although these data collectively suggest that the P38/MAPK signaling axis is an attractive therapeutic target, inhibition of P38 can also induce

pathological remodeling [161]. The opposing effects of P38/MAPK inhibition may stem from differences in the activity of specific P38 isoforms. For instance, *in vitro* models of cardiac ischemia reveal activation of P38α but deactivation of P38β, suggesting that P38α may play a central role in the progression to heart failure [162]. Furthermore, P38α is stimulated by the pro-inflammatory cytokines IL1α and TNFα, whereas P38γ promotes adaptation of skeletal muscle to exercise by upregulating PGC1α expression [163, 164]. Selective inhibition of specific P38 isoforms may be required to promote protection versus dysfunction downstream of TGFβ.

4.3 RhoA and MRTFs

While RhoA and MRTFs appear to be necessary for CM integrity and survival when mice are challenged by pressure overload, these pathways are also responsible for driving the pathological CF response to injury [128–130]. Myofibroblast differentiation requires MRTF-dependent activation of smooth muscle contractile genes (*Acta2, Tagln, Cnn1*) and ECM (*Col1a2*) in CFs [165]. In serum-depleted conditions, forced overexpression of MRTF-A is sufficient to upregulate cytoskeletal genes associated with the myofibroblast phenotype [12, 166] (Fig. 2). Conversely, MRTF-A null mice exhibit decreased scar formation induced by MI or Ang-II infusion [167]. While MRTFs appear to have dichotomous roles and maladaptive remodeling has toxic effects on the heart, wound healing is a natural defense mechanism to prevent myocardial rupture following acute cardiac injury. MRTF-dependent fibroblast activation is essential for physiological processes including cell migration and skin wound healing [71, 168, 169]. Thus, a fine balance of MRTF activity may be required in CFs, which could be accomplished by modulating upstream regulators such as RhoA in a temporal manner. Like MRTFs, RhoA/ROCK signaling also promotes pro-fibrotic pathways when CFs are stimulated with Ang-II [170]. Pharmacological inhibition of ROCK with Fasudil or Y-27632 prevents cardiac remodeling and fibrosis *in vitro* and *in vivo* [167, 169, 171, 172] (Fig. 2). More recently, screens have also identified CCG-1432 and its chemical derivatives as effective inhibitors of fibroblast activation by preventing nuclear translocation of MRTFs in fibroblasts from the lung and skin [173, 174]. An alternative strategy to regulate MRTF signaling may be through modulation of interacting proteins such as four-and-a-half LIM-only protein 2 (FHL2), as overexpression of FHL2 can also disrupt activation of a subset of MRTF/SRF target genes including *Acta2* [175]. The relationship between FHL2 and MRTF/SRF is complex, as FHL2 can prevent degradation and stabilize expression of MRTF-A [176] and loss of FHL2 impairs SRF-dependent migration [177]. However, the evidence collectively suggests that targeting the RhoA/MRTF/SRF axis may attenuate maladaptive remodeling in situations of persistent stress.

RhoA can also be regulated through the G-protein coupled receptor kinase (GRK2). The function of GRK2 has been best characterized in the CMs where it promotes GPCR recycling to maintain contractility [178]. Increased GRK2 activity is commonly associated with the progression to HF, and genetic deletion of GRK2 is sufficient to prevent development of HF following MI [179, 180]. More recently, GRK2 has been shown to form a direct complex with the catalytic domain of RhoA to activate ERK in an EGFR-dependent manner [181] (Fig. 2). Consistent with this finding, ablation of GRK2 in *Col1a2*-positive cells attenuates CF activation, pathological remodeling, and neutrophil recruitment 48 hours

after I/R injury [128, 182]. Multiple studies suggest beneficial roles for GRK2 in this inflammatory cell recruitment to initiate the reparative process and inhibition of p38/MAPK signaling *in vitro* [182–184]. Together, these data suggest that regulation of CF-specific GRK2 activity contributes to the reparative resolution phase following cardiac injury during which there is activation of RhoA-dependent pathways.

4.4 Exercise-induced pathways in CFs

In contrast to the cardiac response to disease, the hypertrophic response to exercise or pregnancy is not accompanied by fibrosis. Stimulation of the Akt pathway in the context of physiological cues such as microRNAs may be an important distinguishing factor between pathological and physiological hypertrophy [23]. MiR-33 is one such factor expressed by CFs that is required for CF proliferation, survival after oxidative stress, and stimulation of Akt signaling [185]. Loss of miR-33a also impairs systolic function in mouse models after TAC, consistent with the observation that miR-33 levels are decreased in human heart failure patients [185]. Another example is miR-29 which is expressed in both CFs and CMs [186, 187]. In CFs, miR-29 is upregulated by exercise and decreased after MI, and knockdown of miR-29 increases expression of collagen [186, 188]. Thus, exercise-induced miR expression may be a predictive factor for physiological rather than pathological CF responses.

The accumulation of ROS is often considered pathological and has been demonstrated to activate fibroblasts [189]. Clearance of ROS is an essential process that occurs through redundant pathways, but includes upregulation of antioxidants such as glutathiones and metallothioneins (MT) [190]. N-acetylcysteine, a glutathione precursor, has been successfully used to resolve fibrosis in a rabbit model of cardiomyopathy and reverse hypertrophy in human patients [191, 192]. Regulation of the antioxidant response program is controlled by Nuclear factor (erythroid-derived 2)-like 2 (*Nfe2l2*, or *Nrf2*) and MRTF can induce the expression of *Nrf2* in vitro [193] (Fig. 2). Although acute lethality was observed when *Nrf2*-deficient animals were subjected to TAC, *Nrf2*−/− animals that survived to 14 days post TAC exhibited decreased cardiac function [194]. Consistent with these findings, transcriptional differences in CFs after exercise or disease demonstrated that an NRF-mediated ROS detoxification program is maintained in healthy CFs but is lost in CFs after pressure overload [195]. Metallothionein 1 (*Mt1*) was among the most highly differentially expressed genes between exercise and disease and belongs to the MT family of ROS and toxin scavengers. Animals lacking both *Mt1* and *Mt2* exhibited signs of exercise intolerance and cardiac dysfunction when subjected to swim training as a model of physiological remodeling [195]. These findings suggest that clearance of excessive oxidative stress in CFs is an important step in preventing pathological remodeling.

4.5 Epigenetic regulation of cardiac fibrosis

In addition to studying transcription factors and their upstream regulators, groups have recently targeted epigenetic modifiers to investigate the role of epigenetics on the fibrotic response (Fig. 2). Indeed, class I histone deacetylases (HDACs) have been implicated in Ang-II mediated fibrosis, but the class I HDAC inhibitors TSA, MGCD0103, and apicidin can block cell- cycle progression of CFs [196, 197]. While HDAC inhibitors provide an exciting therapeutic avenue for cardiac fibrosis, MGCD0103 paradoxically induces

expression of pro-thrombotic plasminogen activator inhibitor 1 (PAI-1) in CFs. These findings warrant additional investigation into the ramifications of modulating epigenetics for therapeutic benefit. Epigenetic readers, such as the bromodomain-containing protein 4 (BRD4), have also been studied in the fibrotic response. Inhibition of BRD4 with miR-9 or the small molecule JQ1 potently attenuated MI or TAC-induced remodeling without affecting the ability of the heart to respond to physiological stimuli such as exercise [198, 199]. These findings suggest the possibility of blocking progression of CF-dependent pathological remodeling through epigenetic regulation, rather than targeting of transcription factors.

5. Intercellular communication

This review has highlighted multiple cell-autonomous mechanisms of cardioprotection in CMs and CFs. However, it is becoming increasingly clear that extracellular context and cell-cell communication influences how the heart responds to stress. Intricate dialogue is exchanged between CMs and CFs through direct cell contact, cytokine release, or secretion of miRNAs and other signaling molecules via exosomes [200–204]. In addition to CMs and CFs, immune and endothelial cells (ECs) also react to paracrine signals. Here we highlight examples of cellular crosstalk to describe how interactions between various cell types influence cardioprotective mechanisms.

The immune system, including cells such as neutrophils, dendritic cells, and macrophages, plays a critical role in modulating the heart's response to ischemic injury and regeneration [205]. A recent study demonstrated that CCR2+, and not CCR2-, resident macrophages promote monocyte infiltration; suggesting that heterogeneity within sub- populations of macrophages helps to direct monocyte recruitment [206]. Depletion of CCR2+ resident macrophages also improves cardiac outcome 28 days after I/R injury [206]. Similarly, cardiac remodeling may depend on differential stimulation of ventricular CFs with cytokines such as IFNγ or IL-10 that direct recruitment of distinct subpopulations of monocytes [207]. A recent analysis of CFs isolated 0–7 days post-MI revealed dynamic shifts in the transcriptional and secretion profiles of CFs, implicating CFs as mediators of the acute inflammatory and angiogenic injury response [208].

Indeed, CFs may function as important modulatory cells to balance pro-fibrotic mechanisms. CF-derived expression of CCN1 can mediate integrin dependent CM proliferation as part of recovery from injury [73, 209]. Similarly, RhoA-dependent induction of CCN1 in CMs can provide integrin-dependent, Akt-mediated protection [73]. Overexpression of CCN1 promotes CF senescence and reduces fibrosis [210, 211]. Although conditional deletion of CCN2 results in acute lethality within 2 weeks of TAC surgery, surviving animals appeared to be protected from pathological remodeling [212]. Consistent with these findings, recent work has demonstrated that CM-secreted CCN2 had no effect on fibrosis while CF-secreted CCN2 promotes fibroblast activation and proliferation [213]. Thus, CCN2 appears to act in an autocrine manner whereas CCN1 may function in both autocrine and paracrine mechanisms. The pro-fibrotic effects of CCN2 in CFs can be mitigated by overexpression of CCN5 and miR- 133 or miR-30, which can counteract PE-induced cardiac remodeling and reduce CCN2 transcripts, respectively [214–217]. CCN proteins also have pleiotropic effects

on other cardiac cell types; for instance, CCN1/2 promotes neovascularization in hibernating pig myocardium and ischemic hindlimb mouse models [218]. Thus, context and cell type are important considerations when modulating ECM-associated proteins such as the CCN family. Due to their contractile nature, CMs are subjected to a great deal of mechanical stress, and chronic pathological stress can trigger cell death. Mechanical tension is not only transmitted by direct contact between similar cell types; different cell types can form functional physical interactions. For instance, electrical current delivered to functional myocardial tissue in a transmural cryoinjury model can be detected in the scar, but depolarizations are reduced in animals lacking Cx43 in fibroblast-specific-protein-1 (FSP1) expressing cells [219]. Models of I/R injury demonstrate that necrotic CMs can elicit fibrosis and inflammation through the release of damage associated molecular patterns (DAMPs) and cytokines [220, 221]. ATP is one such DAMP secreted by CMs, which binds to the ATP-sensitive P2Y11 receptor (P2Y11R) on CFs to promote CF activation [222]. In other contexts, purinergic receptor activation can promote chemotaxis of neutrophils [223]. Although prolonged neutrophil recruitment is detrimental, neutrophils are required to initiate the reparative process that occurs 4–14 days post MI [184, 205]. Of note, P2Y11R stimulation in CFs may be beneficial; conditioned medium from human CFs treated with the P2Y11R agonist NF546 promotes pro-survival signaling in CMs subjected to hypoxia/reoxygenation (H/R) injury by activating the RISK pathway [224]. Furthermore, the secretome of P2Y11R stimulated CFs suppresses dendritic cell activation by LPS [224]. H/R injury likely promotes CF-mediated protection of CMs through multiple factors. For instance, CFs secrete tissue inhibitor of metalloproteinases-1 (TIMP-1) which promotes CM survival, in part, by stimulating PI3K/Akt signaling [225]. Altogether, these and other studies demonstrate that hypertrophic and cardioprotective responses are regulated by a complex interplay of communication between various cardiac cell types.

6. Conclusion

Numerous signaling pathways promote cardiac healing after injury through expression of protective factors in CMs and CFs; however, many of these factors also promote the progression of fibrosis and heart failure under chronic activation. For example, it remains unclear how chronic stimulation of CMs and CFs induces the RhoA/MRTF/SRF transcription pathway to sabotage cardiac function, yet interaction with other signaling pathways provide mechanisms for acute cardioprotection. Although a detailed understanding of how specific signaling pathways interact within individual cell types is critical to identifying cardioprotective factors, a number of questions remain unanswered that may resolve this paradox. How does modulating the behavior of one cell type impact the behavior of neighboring cell types? In an era of precision medicine and pharmacogenomics, are some of these consequences of greater significance to specific patient populations? Looking forward, an approach that considers intercellular communication combined with cell type heterogeneity in signaling responses will more comprehensively explain how the heart rapidly responds to cardiac injury.

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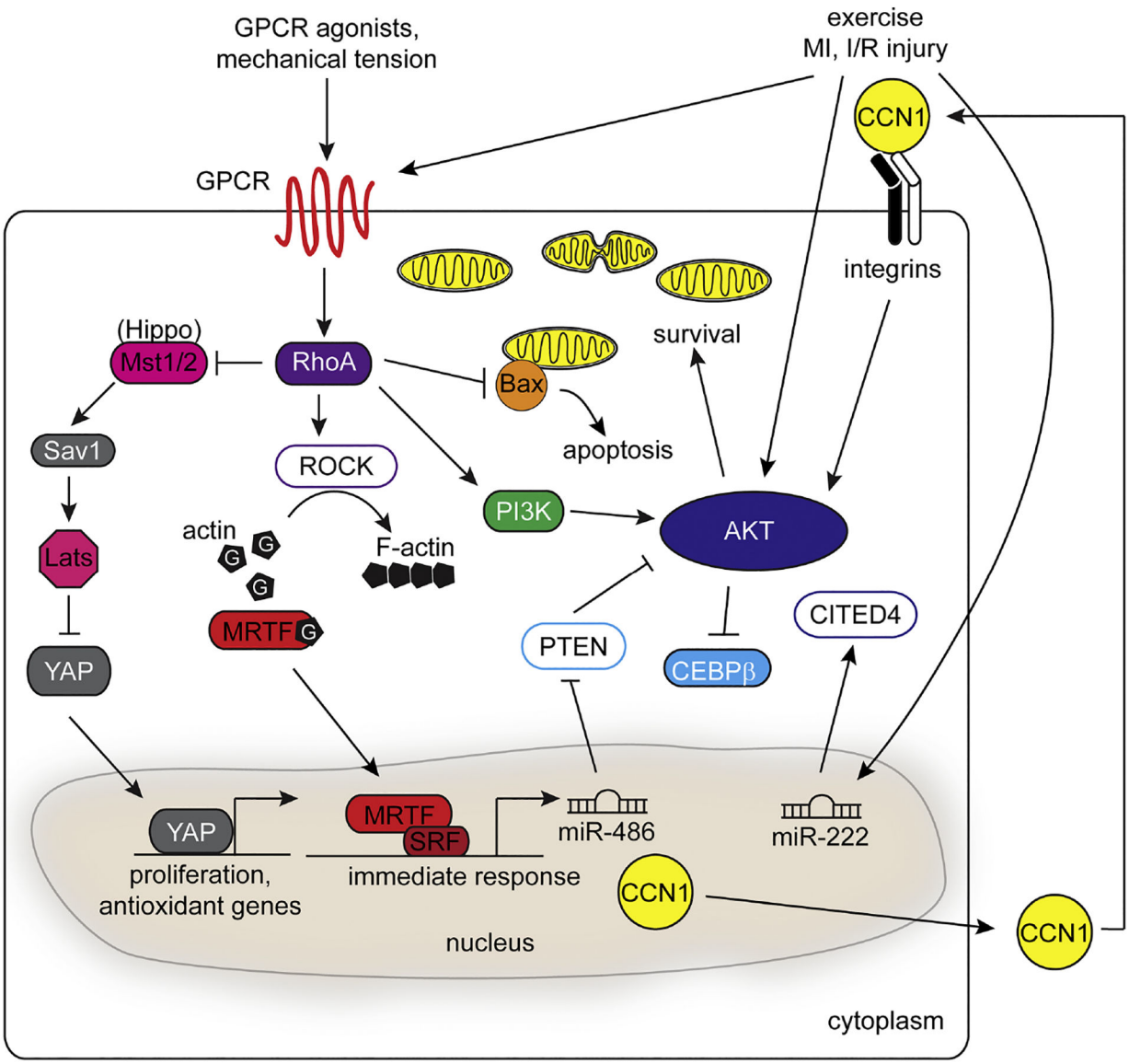

Figure 1.

Signaling pathways that modulate cardioprotection in CMs. Mechanical and oxidative stress produced in disease or exercise can induce a complex anti-apoptotic and hypertrophic response in CMs that converges on the RhoA and PI3K/Akt signaling pathways. Stimulation of GPCRs through ligands triggers RhoA-dependent actin polymerization and nuclear localization of MRTFs to activate transcription of MRTF/SRF target genes. Expression of targets such as miR-486 and CCNs, as well as exercise-induced mechanical tension can activate the Akt response to contribute to cardioprotection. RhoA can also modulate the Hippo/YAP signaling pathway to regulate proliferation and stress responsive genes during both development and disease.

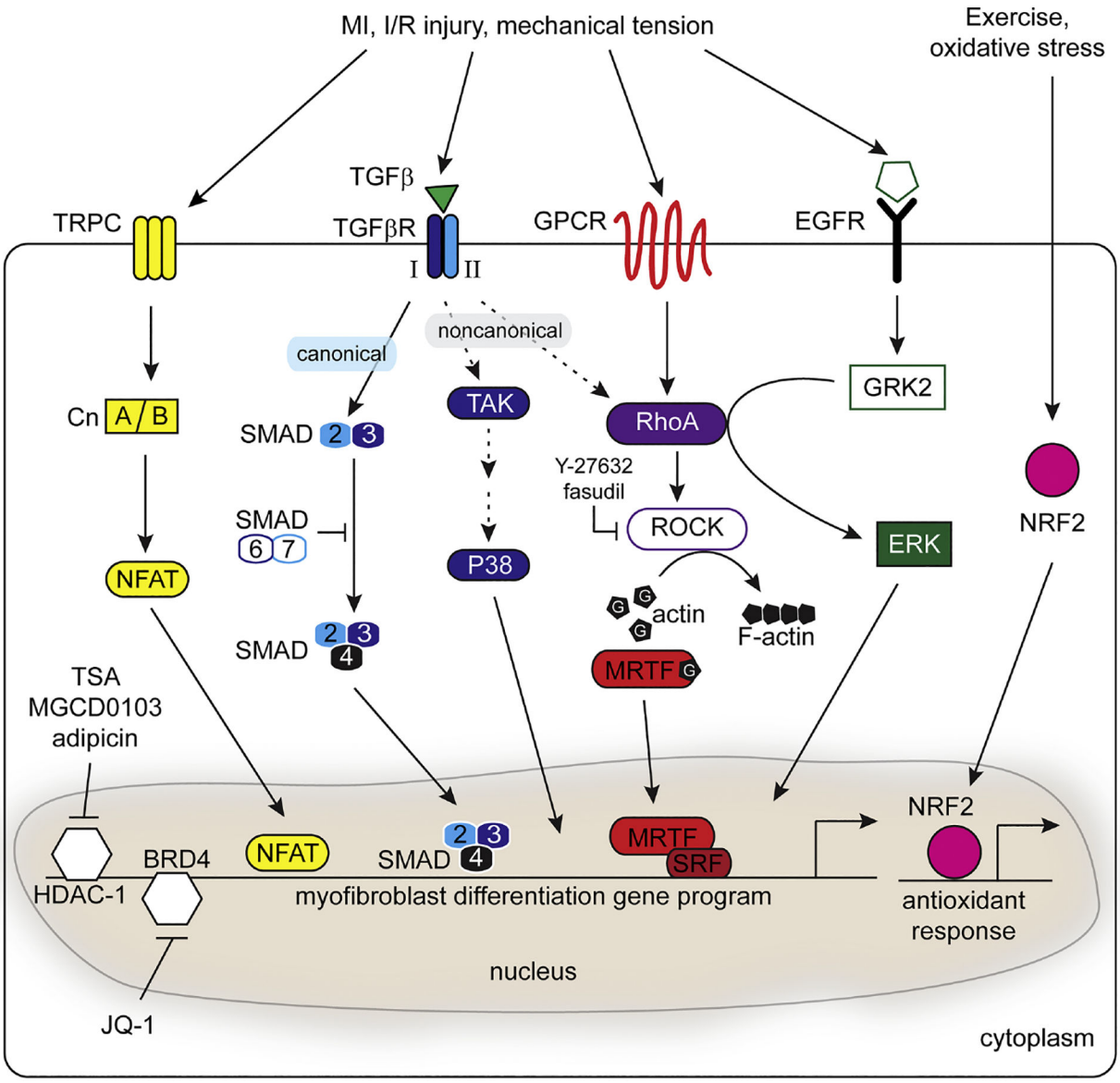

Figure 2.

Signaling pathways that contribute to CF activation and stress response. Disease or mechanical stress can stimulate multiple pathways including calcineurin (Cn)/NFAT, TGFβ, GPCR, and EGFR signaling. The TGFβ pathway provides an established mechanism to stimulate SMAD-dependent CF activation and fibrosis. RhoA/MRTF-dependent signaling plays a central role in CF activation through the non-canonical TGFβ pathway, GPCR stimulation, or EGFR activation. Inhibition of TGFβ signaling by Y-27632 or Fasudil treatment, or by repressive SMAD6/7 signaling, provide nodes to control CF activation. CF activation may also be modulated through epigenetic modulation such as pharmacological inhibition of HDAC-1 or BRD4. Finally, exercise can increase mechanical load in the absence of fibrosis, potentially by maintaining an antioxidant response regulated by NRF2.