Title:

Fibroblast-mediated pathways in cardiac hypertrophy

Running title:

Fibroblast modulates cardiac hypertrophy

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Abstract

Under normal physiological conditions, cardiac fibroblasts are the primary producers of extracellular matrix and supply a mechanical scaffold for efficacious heart contractions induced by cardiomyocytes. In the hypertrophic heart, cardiac fibroblasts provide a pivotal contribution to cardiac remodeling. Many growth factors and extracellular matrix components secreted by cardiac fibroblasts induce and modify cardiomyocyte hypertrophy. Recent evidence revealed that cardiomyocyte–cardiac fibroblast communications are complex and multifactorial. Many growth factors and molecules contribute to cardiac hypertrophy via different roles that include induction of hypertrophy and the feedback hypertrophic response, fine-tuning of adaptive hypertrophy, limitation of left ventricular dilation, and modification of interstitial changes. This review focuses on recent work and topics and provides a mechanistic insight into cardiomyocyte–cardiac fibroblast communication in cardiac hypertrophy.

Key words: Cardiomyocyte, Cardiac fibroblast, Cardiac hypertrophy, Fibroblast growth factor signaling, Notch signaling, Thrombospondin-1 signaling, Ras-association domain family 1 isoform A/Mammalian sterile 20-like kinase 1 pathway, S100A4, and Mineralocorticoid receptor

Introduction

Cardiomyocytes are undoubtedly the primary cellular components responsible for cardiac contractility. However, cardiomyocytes in the normal mouse, rat, and human hearts make up only 56%, 30%, and 28%, respectively, of total number of heart cells[1, 2]. On the other hand, cardiac fibroblasts, in the mouse and rat, and nonmyocytes (mainly composed by cardiac fibroblast), in human, have been reported to make up 27%, 64%, and 72%, respectively, of total heart cells [1, 2]. Recently, investigators have primarily focused on the role of cell–cell interactions during heart failure and aimed to identify the precise mechanism(s) of heart failure at the cellular level[3]. The Cre-loxP system is a promising *in vivo* method used to analyze the contribution of specific cell types in the development of pathophysiological heart conditions[4]. Several established Cre recombinase-expressing transgenic or knockin mouse lines, such as the α -myosin heavy chain promoter-Cre line, enable cardiomyocyte-specific gene-knockout strategies[4]. Indeed, several scientific reports regarding cardiomyocyte-specific gene deletion have been published in recent years. In contrast, there is no mouse line available with a cardiac fibroblast-specific Cre recombinase. Although not organ specific, fibroblast-specific transgenic lines that express Cre and are driven by a *Postn* promoter (*Postn-Cre* mice)[5, 6], a *Sl100a4*

[Fibroblast Specific Protein 1 (*Fsp1*)] promoter[6, 7], and Transcription Factor 21 [*Tcf21*, also known as Podocyte-Expressed 1 (Pod1) combination, or a Capsulin or Class A Basic Helix-Loop-Helix 23 (*bHLHa23*)] promoter, have been reported[8]. However, a study of cardiac disease patients identified that recombinase was not specifically expressed in the heart tissue of *S100a4-Cre* mice[9]. Specifically, in a myocardial infarction or pressure overload model, *S100a4-Cre*-driven gene-deleted cells were not only cardiac fibroblasts, but also hematopoietic, endothelial, and vascular smooth muscle cells[9]. More recently, *Tcf21-Cre* mice were used to examine epicardium-derived cardiac fibroblasts[8, 10]. *Tcf21* is specifically expressed in the epicardium and a majority of epicardial cells migrate into the myocardium during the epicardial epithelial-to-mesenchymal transition. Furthermore, epicardium-derived mesenchymal cells form cardiac fibroblasts during development[10, 11]. Notably, *Tcf21* is required for the epicardial epithelial-to-mesenchymal transition and *Tcf21*-knockout mice lack cardiac fibroblasts[11]. Studies that evaluate fibroblast-specific gene deletion by *Tcf21-Cre* in cardiac disease models, such as myocardial infarction and pressure overload, are required. However, similar to *Periostin-Cre* mice, *Tcf21-Cre* mice are a useful model for *in vivo* studies that examine epicardium-derived cardiac fibroblasts. In fact, cardiac fibroblast-specific gene functions have been studied in *Tcf21-Cre* mouse models of cardiovascular disease[6, 12].

Although cardiac fibroblasts are difficult to study, publications regarding their role continue to

increase[3, 13]. In this review, we focus on the most recent and outstanding publications regarding the role of cardiac fibroblasts in cardiomyocyte hypertrophy, including fibroblast growth factor signaling, Notch signaling, thrombospondin signaling, the Ras-association domain family pathway, S100a4 signaling, and mineralocorticoid receptor signaling.

1. Early fibrotic activity before cardiac pathological hypertrophy

Physiological hypertrophy, such as athlete's heart caused by long-term training, is superficially similar to pathological hypertrophies, including hypertrophic cardiomyopathy. In patients with left ventricular hypertrophy, the differential diagnosis between physiological or pathological hypertrophy is usually performed and is based on a combination of findings[14] (Table 1).

Cardiovascular magnetic resonance imaging is one of the most reliable, unbiased, and assured methods used to detect cardiac fibrotic changes[14].

Cardiac fibrosis is a major feature of hypertrophic cardiomyopathy and contributes to ventricular dysfunction and life-threatening arrhythmia[15, 16]. Cardiac focal fibrosis detected by gadolinium-enhanced cardiovascular magnetic resonance imaging can predict clinical outcomes in hypertrophic cardiomyopathy patients[17-19]. The initiation of cardiac fibrosis in the hypertrophic heart remains unclear. However, cardiac fibrosis is triggered by cardiomyocyte death due to genetic mutations leading to abnormal sarcomere protein[20, 21], myocardial ischemia caused by coronary microvascular dysfunction, and intracavitary left ventricular obstruction[16, 22, 23]. Gene expression profiling in mice with a sarcomere gene mutation have early activation of cardiac profibrotic genes before cardiac hypertrophy develops[24]. These results indicate that abnormal profibrotic activity elevated serum C-terminal propeptide

of type I procollagen (PICP) levels. Interestingly, patients without cardiac hypertrophy who have a pathogenic sarcomere gene mutation known to cause cardiac hypertrophy have been identified[25]. This suggest that profibrotic activity precedes the development of cardiac hypertrophy, and that overt fibrosis as a result of sarcomere gene mutation and early activation of cardiac fibroblasts by cardiomyocytes, including abnormal sarcomere proteins, may contribute to the development of not only cardiac fibrosis, but also cardiac hypertrophy.

2. Fibroblast growth factor signaling

2.1 Fibroblast growth factor (FGF) -2

Fgf-2 is a major paracrine factor that can induce cardiac hypertrophy via mitogen-activated protein kinase (MAPK) activation[26, 27]. Global *Fgf2* knockout does not cause compensatory cardiac hypertrophy in an angiotensin II-induced cardiac hypertrophy mouse model[27]. The major source of Fgf-2 is from cardiac fibroblasts in the heart[28]. The function of Fgf-2 in cardiac hypertrophy development is well established in animal models and humans[29-32]. Fgf-2 consists of multiple low- (18 kDa) and high- (20–34 kDa) molecular-weight isoforms, which are translated from the same *Fgf2* mRNA but produced by alternative splicing[29, 32].

High-molecular-weight Fgf-2 induces cardiac hypertrophy *in vivo*[31]. Cardiac fibroblasts produce only high-molecular-weight Fgf-2 proteins (over 90%) because of hypertrophic stimuli, such as angiotensin II, endothelin-1, and isoproterenol, in neonatal rat heart-derived nonmyocytes[28]. In adrenal medullary cells, increased Fgf-2 protein levels are primarily regulated by increased *Fgf2* gene transcription[33], suggesting that the angiotensin II receptor type I may promote high-molecular-weight Fgf-2 protein translation during the cardiac hypertrophic process. Caspase-1 activity is required for high-molecular-weight Fgf-2 secretion from cardiac fibroblasts into the extracellular environment[28]. Therefore, high-molecular-weight Fgf-2, which is preferentially produced by angiotensin II stimulation in cardiac fibroblasts and secreted by caspase-1, may promote cardiac hypertrophy in a paracrine fashion. Moreover, caspase-1 is activated by inflammasomes and innate immune system signaling, and it induces the production of high-molecular-weight Fgf-2[34]. Collectively, these results suggest that cardiac hypertrophy caused by cardiac fibroblast Fgf-2 production is associated with general cellular responses to various stimuli.

In ischemia–reperfusion injury models with high- and low-molecular-weight *Fgf2*-knockout mice, Fgf-2 was the major isoform present in the heart[32]. This inconsistency can be explained by differences in mouse models, injury duration, and gene-targeting effects on cardiac anatomy.

2.2 FGF16

Fgf-16 expression is not high in the developing heart, but abundant in the adult heart. Three lines of *Fgf16*-knockout mice have been reported, but only 2 are viable[35-37]. Global *Fgf16* knockout decreases cardiomyocyte proliferation during embryogenesis in mice, although the cardiac function and cardiac morphology are normal in adult mice[35]. However, the pathophysiological role of Fgf-16 in heart diseases has not been analyzed. Cardiac hypertrophy is markedly increased in an angiotensin II infusion model with *Fgf16*-knockout mice (Figure 1)[38]. These results suggest that Fgf-16 prevents cardiac hypertrophy during hypertrophy-induced cardiac remodeling. In the same report, Fgf-2-induced cardiomyocyte FGF-16 levels and secretion following the angiotensin II-mediated induction of Fgf-2. In addition, Fgf-16 inhibits major hypertrophic and profibrotic signals, whereas Fgf-2 induces transforming growth factor (Tgf)- β 1 expression in both cardiomyocytes and cardiac fibroblasts[38]. In the heart, both Fgf-2 and Fgf-16 compete for binding sites on their primary receptor, the fibroblast growth factor receptor, in the heart. Therefore, Fgf-2 and Fgf-16 have opposite functions in cardiac hypertrophic remodeling[39] whereas Fgf-16 functions to antagonize Fgf-2-induced cardiac remodeling via the actions of TGF- β 1 in response to hypertrophic and profibrotic stimuli (Figure 1).

3. Notch signaling

Notch ligands (Jagged 1 and 2; Delta-like 1, 3, and 4) and their receptor (Notch 1, 2, 3, and 4) mediate various signaling between adjacent cells[40]. In the developmental heart, Notch signaling orchestrates the organization of cardiac morphology, including myocyte proliferation, trabeculation, myocardium compaction, and cardiac valve formation[41]. Genetic mutations of Notch and Notch ligands cause cardiac valve abnormalities, such as bicuspid aortic valve disease, aortic valve calcification, peripheral pulmonary stenosis, tetralogy of Fallot, and ventricular septal defects[42, 43]. Notch 1 signaling is predominantly activated in both cardiomyocytes and cardiac precursor cells (CPCs)[44-46] in the hypertrophic[47] and injured heart[48]. The lack of the Notch 1 expression, specifically in cardiomyocytes, results in markedly exacerbated cardiac hypertrophy in a pressure overload-stressed heart mouse model[47]. Mouse mortality rate increases because cardiomyocytes lacking Notch 1 fail to achieve an appropriate adaptive hypertrophic response following pressure overload[47]. These results suggest that Notch 1 signaling in the heart has protective effects against pathophysiological conditions. Notably, Notch 1 signaling was only analyzed in cardiomyocytes or CPCs and between mature cardiomyocytes and CPCs[47-49]. Indeed, the role of Notch signaling in cardiac fibroblasts is largely unknown. Transgenic Notch signaling reporter mice

were recently used to identify global Notch signaling events during cardiac hypertrophy development and cardiac repair in response to myocardial infarction[50]. Various cell types, including vascular endothelial cells, interstitial cells, and epicardial cells, are positive for Notch-activated indicators[50]. Epicardial cells multiply and increasingly experience fibroblast differentiation due to epithelial mesenchymal transition[50] (Figure 2). Meanwhile, Notch ligand-activated downstream genes are globally activated in epicardium-derived cardiac fibroblasts[50]. Notch signaling in the epicardium may contribute to cardiac-remodeling processes, including cardiac hypertrophy and fibrosis[50] (Figure 2). Notch signaling also occurs between the cardiomyocytes and cardiac fibroblasts of cardiomyocyte-specific Jagged 1 (a Notch 1 ligand)-overexpressing transgenic mice[51]. Forced Jagged-1 expression in cardiomyocytes activates Notch signaling in cardiac fibroblasts, CPCs, and several other immune cell and vascular interstitial cells. Neonatal Jagged-1 transgenic mice also exhibit sustained CPCs and cardiomyocyte proliferation. In the pressure overload model, cardiac fibroblast proliferation is limited, and cardiac hypertrophy, Tgfb/connecting tissue growth factor (Ctgf), and periostin-mediated fibrosis are significantly inhibited (Figure 2). When considering the relationship between Tgfb and Ctgf, Tgfb is secreted from cardiac fibroblasts and induces Ctgf secretion from cardiomyocytes. In addition, Ctgf from cardiomyocytes induces cardiomyocyte hypertrophy following pressure overload via the Akt pathway in a

cell-autonomous manner [52, 53]. Decreased *Ctgf* expression and Akt signaling causes the partial reduction of cardiac hypertrophy during the forced activation of stromal cells by Notch 1, including cardiac fibroblasts. On the other hand, the forced activation of Notch 1 results in the expansion of cardiac precursor cells[51]. These results suggest that Notch 1 signaling in cardiac fibroblasts limits the production of prohypertrophic factors and profibrotic factors in the heart's interstitial environment and contributes to the resolution of cardiac hypertrophy during healing and regeneration, but not during the induction of adaptive cardiac hypertrophy (Figure 2).

4. Thrombospondin (TSP)-1

Recently, the cardiac extracellular matrix has been recognized as a functional organelle in addition to its function in tissue support. Indeed, the extracellular matrix acts as a signal transducer for cell–cell communication and modulates other molecules in the interstitial space[54, 55]. Functional and nonstructural extracellular matrix components are called “matricellular proteins”[55]. Many matricellular proteins, including, but not limited to, thrombospondins, osteopontin, tenascins, and periostin, are expressed in the embryonic heart, whereas there is almost no expression in the adult heart. However, most of these proteins are produced in response to cardiac injury, such as myocardial infarction or pressure overload, and

usually play a protective role[54, 55]. Gene sequences and protein structures of TSP family members are similar. Tsp-1 is a 450-kDa homotrimeric protein that is induced by tissue injury in several organs[55]. Tsp-1 activates TGF β signaling by transforming the latent TGF β complex into its active form[56] (Figure 3). The contribution of Tsp-2 in the cardiovascular field has been previously reported[57]. Tsp-2 is significantly upregulated in the hypertrophic hearts of renin-overexpressing rats (Ren-2 rats) and in the myocardium of hypertrophic human hearts[57]. Adult *Tsp2*-knockout mice exhibit normal morphology and cardiac function. During angiotensin II stimulation, *Tsp2*-knockout mice demonstrate significant increases in mortality because of cardiac rupture or cardiac dysfunction[57] with no significant differences in cardiac hypertrophy. Recently, the contribution of the *Tsp1* gene during the development of cardiac hypertrophy was reported[58] (Figure 3). During left ventricular pressure overload, *Tsp1*-knockout mice exhibit increases in cardiac hypertrophy during the early stage (day 7 after left ventricular pressure overload) and late stage of left ventricular dilation (day 28 after pressure overload)[58]. As a result, pressure overload increases *Tsp1*-knockout mouse mortality[58]. The hearts from pressure overloaded *Tsp1*-knockout mice have marked increases in myofibroblast proliferation and cardiomyocyte damage. Despite the increases in myofibroblast number, pressure overload does not increase heart collagen content in *Tsp*-knockout mice compared with wild-type mice[58]. These results may be reflective of insufficient myofibroblast differentiation in

Tsp1-knockout compared with wild-type mice[58] (Figure 3). In *Tsp1*-knockout mouse fibroblasts, impaired myofibroblasts are associated with decreased TGFb signaling, such as SMAD2 phosphorylation[58] (Figure 3). Furthermore, matrix metalloproteinase 3 and 9 are upregulated in *Tsp1*-knockout mice. During adaptive pressure overload, Tsp-1 proteins activate TGFb and fine tune excessive cardiac hypertrophy to critically control fibroblast maturation and matrix remodeling, and preserve chamber dilatation[58] (Figure 3). Tsp-1 is specifically expressed in cardiomyocytes[59]. Tsp-1 directly suppresses cardiac hypertrophy in a cell-autonomous fashion. Tsp-1 is released from cardiomyocytes and induces cardiac fibroblast activation. Mature myofibroblasts and extracellular matrix components that are modified by an abnormal increase in matrix metalloproteinases suppress excessive cardiac hypertrophy and left ventricular chamber dilatation (Figure 3).

Other cellular functions performed by TSPs during cardiac remodeling have been a recent focus[60]. For example, *Tsp4*-knockout mice show exacerbated cardiac function after pressure overload[60]. In wild-type mouse cardiomyocytes, Tsp-4 is induced, binds to activating transcription factor 6a (*Atf-6a*) on the luminal membrane of the endoplasmic reticulum (ER), and activates a Tsp-4/Atf-6a ER response after cardiac hypertrophic stress[60]. The ER stress response provokes a cardiac adaptive response that contributes to cell survival. However, the contribution of cardiac fibroblast Tsp-4 to the ER stress response during cardiac hypertrophy is

unknown.

5. Wnt-Frizzled system

The Wnt-Frizzled G-protein-coupled receptor (Fzd) consists of 19 Wnt ligands and 10 Fzd receptors[61, 62]. Wnt-Fzd signaling during cardiac hypertrophy and fibrosis occurs through the canonical pathway disheveled-1 (Dvl-1)-dependent β -catenin-mediated nuclear translocation and transcription, the noncanonical phospholipase-C/calcium mobilization Dvl-1-mediated activation of small GTPase-like Rho/ROCK pathway, and the Rac/JNK pathways. In a myocardial infarction model, pressure overload model, or hypertrophic stimuli (catecholamines or angiotensin II infusion) model cardiomyocyte and cardiac fibroblast Wnt1[63-65], 2[66], 3A[67, 68], 4[64, 66], 5A[67], 7A[64], 10B[66], 11[69, 70], and Fzd1-2 have been analyzed, and reviewed[71].

In a normal adult heart the Wnt/Fzd signal is not activated[72]. However, the Wnt/Fzd pathway is activated during the development of cardiac hypertrophy. For example, the cardiomyocyte Fzd2 receptor and Dvl-1 is upregulated by pressure overload[73, 74]. In mice, *Dvl-1* knockout does not cause cardiomyocyte hypertrophy in response to catecholamine[74]. Catecholamine also increases glycogen synthase kinase 3 β (Gsk-3 β) activity and augments the degradation of

beta-catenin resulting in a decreased hypertrophic response to pressure overload in *Dvl1*-knockout mice[75].

GSK-3 β suppresses beta-catenin activity under normal heart physiological conditions. Gsk-3 β is primarily inactivated by serine-9 phosphorylation in response to hypertrophic stimuli (adrenergic β signaling[76], endothelin-1 and phenylephrine[77], and phosphorylated Gsk-3 β suppression of beta-catenin signaling) and results in increased hypertrophy[78].

Beta-catenin is a major effector in the Wnt pathway. The *in vitro* and *in vivo* force stabilization of beta-catenin induces cardiomyocyte hypertrophy[79]. In contrast, cardiomyocyte-specific beta-catenin deletion in mice with pressure overload attenuates hypertrophy[80]. Moreover, angiotensin II stimulation has also been reported to stabilize the beta-catenin attenuated hypertrophic response in mice, resulting in impaired cardiac function[81]. These ambiguous results suggest that different hypertrophic stimuli may differentially affect the beta-catenin pathway. Furthermore, the temporal downregulation of beta-catenin after pressure overload might be required for an adaptive response. Noncanonical Wnt pathways (JNK, Calcineurin and CaMKII activation) are also involved in cardiac hypertrophy[74, 82]. *Dvl-1* overexpression in mice induces cardiac hypertrophy through a canonical Wnt pathway as well as noncanonical pathways[74].

Several reports demonstrate that the Wnt pathway in cardiac fibroblasts contribute to

cardiovascular remodeling. Wnt1, Wnt10b, and Dkk-1 are upregulated after myocardial infarction[64, 66, 83, 84] and cardiac fibroblasts Wnt1 overexpression markedly produce extracellular matrix[64]. In a failing heart model, cardiac fibroblasts express abundant Fzd2 and Fzd4[85]. In cardiac fibroblasts Wnt ligand-receptor effects are very complex. For example, Wnt3a activates Fzd1, but inactivates collagen synthesis, myofibroblast differentiation, and Tgf- β expression in cardiac fibroblast cell culture. In contrast, Wnt3a and Wnt5a have the opposite effect on Fzd2 function[67]. Recent efforts have focused on cardiac fibroblast paracrine signaling to cardiomyocytes during cardiac hypertrophy development. However, the mechanistic role of Wnt in this pathway remains unclear.

6. The Ras-association domain family 1 isoform A (*Rassf1A*)/Mammalian sterile 20-like kinase 1 (*Mst1*) pathway

Hippo signaling controls organ-size by mediating cell proliferation and apoptosis in *Drosophila*[86]. In mice, Hippo signaling inhibits cardiomyocyte proliferation and controls the size of the heart by negative Wnt target gene regulation[87]. The Hippo effector gene Yes-associated protein (*Yap*) interacts with beta-catenin on its target genes[87]. In addition, Yap activation of insulin-like growth factor signaling and glycogen synthase 3 β in

cardiomyocytes increases beta-catenin, which leads to cardiomyocyte proliferation during development[88] and the maintenance of cardiomyocyte populations and cardiac function[89].

Mst1, the human homolog of Hippo, is a serine/threonine kinase and a key regulator of cell death and proliferation[86]. *Mst1* is activated in heart disease models of cellular stress, such as the ischemic reperfusion or myocardial infarction models[90, 91]. *Mst1* modulates cardiac hypertrophy and apoptosis in cardiomyocytes and maintains cardiac function. In *Drosophila*, Hippo forms a signaling complex with Warts. In mice, *Mst1* also activates the kinase *Lats2*, a *Warts* homolog, and inhibits compensatory cardiac hypertrophy-induced heart failure[92].

Ras-association domain family (*Rassf*) proteins are tumor suppressor genes, and *Rassf1a*-knockout mice exhibit tumorigenesis[93]. *Raaf1a* is a *Mst1* activator in both cardiomyocytes and cardiac fibroblasts[94]. However, the *Raaf1a*-*Mst1* signaling pathway is cell type dependent and promotes cardiomyocyte apoptosis. The same pathway in cardiac fibroblasts inhibits *Tnfa* production via NF- κ B inhibition, suppresses cardiac hypertrophy and fibrosis, and improves cardiac function[94]. The *Raaf1a*-*Mst1* cardiac fibroblast-signaling

pathway may serve a detrimental function in cardiomyocytes and a protective role in cardiac fibroblasts. Mechanistic insights into various cell types in the heart, such as cardiomyocytes, cardiac fibroblasts, and immune cells will provide clinicians toward a deeper understanding of heart disease pathophysiology.

7. S100A4

S100a4 (also known as fibroblast-specific protein 1, *Fsp1*; metastatic cell protein 1, *Mts1*; metastasin 1) is an S100 family member and calcium-binding protein expressed in the spleen, while blood cells, bone marrow cells, lymph nodes,[95] and fibroblasts[96, 97]. S100a4 is involved in the development of fibrosis, tumor metastasis,[98] and tissue remodeling in various diseases, including cancer[98], pulmonary hypertension[99], atherosclerosis/neointimal formation of arteries[100], and kidney disease[101]. S100a4 physically interacts with cytoskeletal proteins associated with cell motility, such as nonmuscle myosin II[98]. Although S100a4 is secreted into the interstitium and functions as a secreted signaling molecule, S100a4 also modulates the DNA binding of the tumor suppressor gene P53 in the nucleus[102]. S100a4 is also upregulated in the hypertrophied heart[103]. In addition, recombinant S100a4 induces marked cardiomyocyte hypertrophy *in vitro*, and increases cell survival by inhibiting apoptosis via ERK1/2 activation[103]. However, the *in vivo* functions of S100a4 in cardiac hypertrophy have not been analyzed. Recently, the contribution of S100a4 in the development of cardiac hypertrophy was reported using *S100a4*-knockout mice[104]. *S100a4*-knockout mice do not exhibit differences in baseline compared with wild-type mice. Specifically, no significant

differences in the degree of cardiac hypertrophy were detected between wild-type and *S100a4*-knockout mice during transverse aortic constriction induced pressure overload. *S100a4*-knockout mice exhibit a reduction in cardiac fibrosis and cardiac myofibroblast cell numbers during pressure overload. S100a4 proteins are also increased in various cardiac cell types, including cardiomyocytes, cardiac fibroblasts, and immune cells residing in the cardiac interstitial space. Interestingly and particularly in cardiac fibroblasts, S100a4 colocalizes with P53 and regulates P53 target genes that are associated with cell proliferation and collagen expression[104]. S100a4 and P53 may promote pressure overload induced cardiac fibrosis. However, the role of S100a4 during cardiac fibrosis in noncardiac fibroblast cells is not known.

Therefore, it is likely that S100a4 plays a major role in the profibrotic proliferation and maturation of cardiac fibroblasts. In mice, S100a4 is not involved in pressure overload-induced cardiac hypertrophy. Cardiac hypertrophy/injury and cardiac fibrosis often simultaneously develop in genetically modified mice. A causative relationship between cardiomyocytes injury and fibrosis is envisaged. However, in mice, *S100a4*-knockout decreases cardiac fibrosis, but not cardiac hypertrophy, suggesting that both pathological pathways act independently. A similar phenomenon is observed in a kidney injury model associated with S100a8 and S100a9 signaling[105].

8. Mineralocorticoid receptor (MR)

The introduction of mineralocorticoid receptor (MR) antagonists, such as spironolactone or eplerenone, has improved the prognosis of heart failure patients[106, 107]. MR antagonists decrease ventricular remodeling, including cardiac perivascular fibrosis and coronary artery interstitial fibrosis[108], following myocardial infarction and inhibit the development of heart failure and tissue inflammation under pressure overload[109]. Although MR antagonists produce favorable results in clinical and basic research, the MR antagonist target cell type and mechanism of action (MOA) remains unclear. Recently, in attempts to identify MR antagonist MOA, several cell-specific MR-knockout mice were created. Cardiomyocyte-specific MR-knockout mice exhibit decreased levels of left ventricular dysfunction and dilatation in a pressure overload model without improved cardiac hypertrophy or fibrosis[110]. In angiotensin II infusion[111] and deoxycorticosterone/salt-induced cardiac fibrosis models, macrophage-specific MR-knockout mice exhibit decreased macrophage infiltration and classical activation, cardiac hypertrophy, cardiac fibrosis, vascular damage, and blood pressure[112]. The macrophage-specific MR deletion closely mimics the effects of MR antagonists[111, 112]. In addition, smooth muscle cell-specific MR deletion decreases systolic blood pressure, suggesting that MR located in vascular walls directly controls blood pressure[113]. Surprisingly, there are

no differences in cardiac hypertrophy, cardiac fibrosis or the cardiac function in fibroblast-specific **MR-knockout mice** compared with wild-type mice[110]. These results indicate that MR in cardiomyocytes and cardiac fibroblasts does not contribute to cardiac hypertrophy.

9. Regression of pathological hypertrophy and cardiac fibrosis

Physiological cardiac hypertrophy is defined as a reversible condition caused by pregnancy or physical training in athletes, whereas pathological hypertrophy is an irreversible condition[114].

However, recent studies demonstrate the cardioprotective effects in patients with a left ventricular assist device (LVAD) implant and provide evidence for the regression of

pathological hypertrophy in humans. LVAD patients show decreases in the left ventricular mass

3 months after implantation, with a 60% decrease in the hypertrophied cardiomyocyte size[115].

Furthermore, LVAD implant normalized the b-adrenergic receptor myocardial density and

recovered the b-adrenergic response to isoproterenol[116, 117].

Angiogenesis is accompanied by the development of cardiac hypertrophy, and it is well

balanced with cardiac hypertrophy physiological hypertrophy[118]. Microvessel density also

increases during cardiac hypertrophy initiation, but decreases at later stages of pathological

hypertrophy[118]. Angiogenesis inhibition by antivasular endothelial growth factor (VEGF) signaling can completely suppress both the angiogenesis and cardiac hypertrophy[119]. On the other hand, angiogenesis is also required for cardiac pathological hypertrophy regression in an experimental mouse model[120]. VEGF signaling through VEGF receptor 1 is a key pathway in both development and regression of hypertrophy[121-123]. In cardiomyocytes, VEGF expression is regulated by hypoxia-inducible factor-1 (*Hif1*) transcription factor that requires sufficient copper[120, 124]. During pathological hypertrophy, myocardium VEGF production and copper concentrations decrease. In a pressure overload mouse model copper supplementation induces VEGF production causing increased microvessel density and pathological hypertrophy regression[120, 123]. Moreover, inhibition of cyclic GMP phosphodiesterase 5A (*Pde5a*) by sildenafil[125] results in VEGF and angiopoietin-1 production[126, 127], cGMP-dependent protein kinase-1 (*Pkg-1*) activation[128], and deactivation of multiple hypertrophic signals (calcineurin/NFAT, phosphoinositide-3 kinase/Akt, ERK1/2 signaling[125]), and regression of pre-established pathological hypertrophy in a pressure overload mice model. In these studies, the analyzed cell types were mainly cardiomyocytes and vascular endothelial cells, but not cardiac fibroblasts. The results of our literature review of cardiac fibrosis during regression of cardiac hypertrophy or recovery of cardiac contractile function after LVAD support were too confusing and controversial. Reports

regarding the impact of LVAD support on cardiac fibrosis are ambiguous and range from no change[129] to regression of fibrosis[130] to increase in fibrosis[131]. Although, results from human studies have suggested that cardiac fibroblasts contribute to the regression of established hypertrophy[132], further clinical and experimental studies are required.

Conclusion

Studies using cardiomyocyte-specific knockout mice to examine cardiomyocytes greatly outnumber studies of cardiac fibroblasts using fibroblast-specific gene deletion. However, there are several recent reports examining the role of cardiac fibroblasts and/or cardiac fibroblast–cardiomyocyte communication. Analyzing cell–cell communication between different cell types is difficult and challenging. Furthermore, results are often unclear because of methodological limitations. Cardiac remodeling is accomplished by the coordinated efforts of several cell types, including cardiomyocytes, cardiac fibroblasts, immune cells, and vascular cells. However, the mechanisms of different cell types must be considered in order to comprehend the complex mechanisms associated with cardiac remodeling and the development of heart disease.

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Figure legends

Figure 1 Cardiomyocyte and cardiac fibroblast communication affects cardiac hypertrophy via fibroblast growth factor signaling

Cardiac fibroblasts secrete Fgf2 that induces cardiomyocyte hypertrophy via the secretion of Tgfb from cardiomyocytes. Tgfb is a strong inducer of cardiac hypertrophy. Fgf2 also induces the proliferation of cardiac fibroblasts and promotes fibrosis via Tgfb1 secretion from cardiac fibroblasts in an autocrine manner. Fgf16 is induced in cardiomyocytes by Fgf2 secreted primarily from cardiac fibroblasts and also by cardiomyocytes. Secreted Fgf16 inhibits Fgf2-induced Tgfb1 secretion from cardiomyocytes and cardiac fibroblasts, cardiac hypertrophy, and fibrosis. Fgf2 secretion and Fgf16 signaling function as a negative feedback system in the development of cardiac hypertrophy and fibrosis.

FGF2, fibroblast growth factor 2; FGF16, fibroblast growth factor 16; TGFb1, transforming growth factor 1; FGFR, fibroblast growth factor receptor

Figure 2 Jagged 1-Notch 1 signaling between cardiomyocytes and cardiac fibroblasts in the

development of cardiac hypertrophy

Under pressure overload, Jagged 1 on the surface of cardiomyocytes stimulates Notch 1 on cardiomyocytes[#] and cardiac fibroblasts^{*}. Notch signaling^{**} from cardiomyocyte to cardiac fibroblast inhibits cardiac hypertrophy and cardiac fibrosis via the inhibition of TGFb2 secretion from cardiac fibroblasts as a feedback system against cardiac hypertrophy^{***}.

Notch 1 on cardiomyocytes[#] does not contribute to the antihypertrophic effect (feedback system) of Jagged 1-Notch 1 signaling, but only to the prohypertrophic effect.

The reduction of TGFb2 decreases the production of CTGF from cardiomyocytes and suppresses Akt signaling. Akt signaling is involved in the downstream signaling pathway of CTGF in cardiomyocytes and associated with pro cardiac hypertrophy.

Notch signaling is also activated in epicardial epithelial cells during cardiac remodeling. The Notch signaling-activated epicardial epithelial cells are trans-differentiated into fibroblasts via the epithelial–mesenchymal transition and contribute to cardiac remodeling.

TGFb2, transforming growth factor beta 2; CTGF, connecting tissue growth factor; Akt,

Figure 3 Thrombospondin 1 exhibits a finely tuned cardiac adaptive response against pressure overload

Cardiomyocytes produce Tsp1 during the development of cardiac hypertrophy and secrete Tsp1 into the interstitial space. Tsp1 activates the latent Tgfb complex into the active Tgfb. Activated Tgfb affects cardiomyocytes and induces hypertrophy. In addition, Tgfb activates cardiac fibroblasts into myofibroblasts via Smad-signaling activation. Tgfb induced mature cardiomyofibroblasts fine tune adoptive cardiac hypertrophy and suppress pathological hypertrophy and left ventricular chamber dilatation.

TSP1, thrombospondin 1; TGF, transforming growth factor

Table 1

	physiological hypertrophy	pathophysiological hypertrophy
contrast enhanced	normal	late gadolinium enhancement
CMR		(LGE)+[19]
serum levels of		elevated (before development
PICP		of hypertrophy)[25]
regression of hypertrophy	Yes[133, 134]	No[14]
after deconditioning		
type of LV hypertrophy	symmetric[135]	asymmetric[136]
	homogenous[135]	
LV cavity dimension	enlarged[136]	normal or small
(end-diastolic diameter)	≥ 55 mm[135]	≤ 50 mm[136]
LV shape	ellipsoid[136]	distorted[136]

enlargement of	associated with	associated with
left atrium	with enlarged LV[137]	impaired LV diastolic function,
		not enlarged LV[14]
family history	-	+
gene mutation of	-	+(if detected)[138]
cardiac sarcomere protein		
diastolic function	normal[139]	normal or dysfunction+[140-142]
Exercise capacity	>50[143]	<40–45[143]
Peak oxygen consumption		
max VO ₂ (ml/kg/min)		
electrocardiogram	variety of abnormal	marked left axis deviation,
	patterns[144]	left atrial enlargement,
		deep Q wave, ST depression,
		diffuse T-wave inversion[145]
Histology		apoptosis+, necrosis+,
		Collagen I, III deposition+
		increased MMPs, TIMPs

Metabolism	fatty acid oxidation	fatty acid oxidation
	glucose oxidation	glucose oxidation
		glucose oxidation
		phosphocreatine
Gene expression		ANP , BNP , bMHC ,
		αskeletal actin
		Serca2a , αMHC ,

LV, left ventricle CMR, cardiovascular magnetic resonance; PIPC, C-terminal propeptide of type I procollagen; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; MHC, myosin heavy chain; SERCA, sarco/endoplasmic reticulum Ca^{2+} -ATPase

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Figure 1

development of cardiac hypertrophy and feedback

Figure 2

development of cardiac hypertrophy and feedback

Figure 3