Signaling effectors underlying pathologic growth and remodeling of the heart

Jop H. van Berlo, Marjorie Maillet, and Jeffery D. Molkentin

Department of Pediatrics, University of Cincinnati, Cincinnati Children's Hospital Medical Center, Howard Hughes Medical Institute, Cincinnati, Ohio, USA.

Cardiovascular disease is the number one cause of mortality in the Western world. The heart responds to many cardiopathological conditions with hypertrophic growth by enlarging individual myocytes to augment cardiac pump function and decrease ventricular wall tension. Initially, such cardiac hypertrophic growth is often compensatory, but as time progresses these changes become maladaptive. Cardiac hypertrophy is the strongest predictor for the development of heart failure, arrhythmia, and sudden death. Here we discuss therapeutic avenues emerging from molecular and genetic studies of cardiovascular disease in animal models. The majority of these are based on intracellular signaling pathways considered central to pathologic cardiac remodeling and hypertrophy, which then leads to heart failure. We focus our discussion on selected therapeutic targets that have more recently emerged and have a tangible translational potential given the available pharmacologic agents that could be readily evaluated in human clinical trials.

Cardiac hypertrophy and clinical considerations

The primary function of the heart is to contract and pump blood. When contractile performance is perturbed or reduced in response to diverse (patho-)physiologic stimuli, the heart typically remodels and hypertrophies, in association with increases in myocyte cell volume (1). Pathologic hypertrophy of the myocardium temporarily preserves pump function and reduces ventricular wall stress, but prolonged cardiac hypertrophy is a leading predictor for arrhythmias and sudden death as well as dilated cardiomyopathy and heart failure (2–5). The hypertrophic growth of the myocardium is typically initiated by signal transduction pathways in response to either neuroendocrine factors or an ill-defined mechanical stretch– or wall tension–sensing apparatus (6–10). Pathologic growth of the myocardium can induce concentric remodeling of the ventricle that results in myocyte growth in a cross-sectional area, such as with hypertension or from hypertrophic cardiomyopathy due to mutations in sarcomeric genes (Figure 1). Alternatively, select pathologic stimuli, or the transition to heart failure, can also elicit an eccentric or dilatory growth response in which the chamber effectively dilates with wall thinning, most likely through a predominate lengthening of individual myocytes (Figure 1).

In contrast to pathologic stimuli that elicit cardiac hypertrophy with poor patient prognosis, exercise and pregnancy induce a purely physiologic cardiac hypertrophy that is typically not associated with a predisposition toward future disease (11). In these cases, the myocardium grows more uniformly, with increases in chamber size, wall thickness, and myocyte length and width (Figure 1). Interestingly, Rockman and colleagues showed that the duration of the stimulus does not determine the difference between physiological and pathological hypertrophy, suggesting instead that the stimuli are inherently different (12). Accordingly, other studies have clearly shown activation of different signaling pathways in transducing either response. Physiological hypertrophy typically involves activation of IGF1/PI3K/AKT/PKB-dependent signaling, ERK1/2, or CEBPβ (13–19). These pathways or effectors have been shown to antagonize cell death in the heart or to stimulate myocyte renewal, suggesting that physiologic growth stimulation through such pathways can be cardioprotective despite causing mild heart enlargement.

Clinical management of pathologic cardiac remodeling is targeted to the underlying cause (e.g., hypertension) and typically involves a select array of pharmacologic agents that have shown efficacy in reducing hypertrophy and/or negative remodeling of the myocardium. In both humans and animal models, targeting the renin-angiotensin-aldosterone system can reverse cardiac hypertrophy or induce positive remodeling of the ventricles back to predisease states independent of effects on blood pressure, though blood pressure management is an additional protective aspect of these antagonists and is often the reason for initiating treatment (20, 21). Another highly employed agent that has antihypertrophic properties is the β-adrenergic receptor blocker (β-blocker), which can also positively influence the heart and regress negative ventricular remodeling and hypertrophy as well as extend life span in heart failure patients (21). Ca^{2+} channel blockers are also used to manage hypertension in patients, and work in animal models has suggested antihypertrophic effects of these agents that are independent of blood pressure lowering (22). All of these agents are thought to positively affect the heart and reduce hypertrophy and remodeling by limiting signaling through neuroendocrine circuitry and intracellular transduction pathways that underlie myocyte growth and are at the molecular basis of both cardiac hypertrophy and remodeling as well as heart failure (23, 24). However, additional agents are needed, as some patients are refractory to the beneficial effects of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, and Ca^{2+} channel blockers. Furthermore, the overall efficacy of these agents is somewhat limited, as cardiovascular disease still progresses even in responsive patients (25, 26). Here we discuss additional signaling pathways that have emerged more recently from mechanistic and genetic studies in animal models of cardiac remodeling that offer new treatment opportunities.

β-Adrenergic receptor signaling and associated kinases in cardiac hypertrophy and remodeling

Inhibition of β-adrenergic receptor signaling is perhaps the most effective and frequently employed therapy in addressing negative

Conflict of interest: The authors have declared that no conflict of interest exists.
Citation for this article: *J Clin Invest.* 2013;123(1):37–45. doi:10.1172/JCI62839.

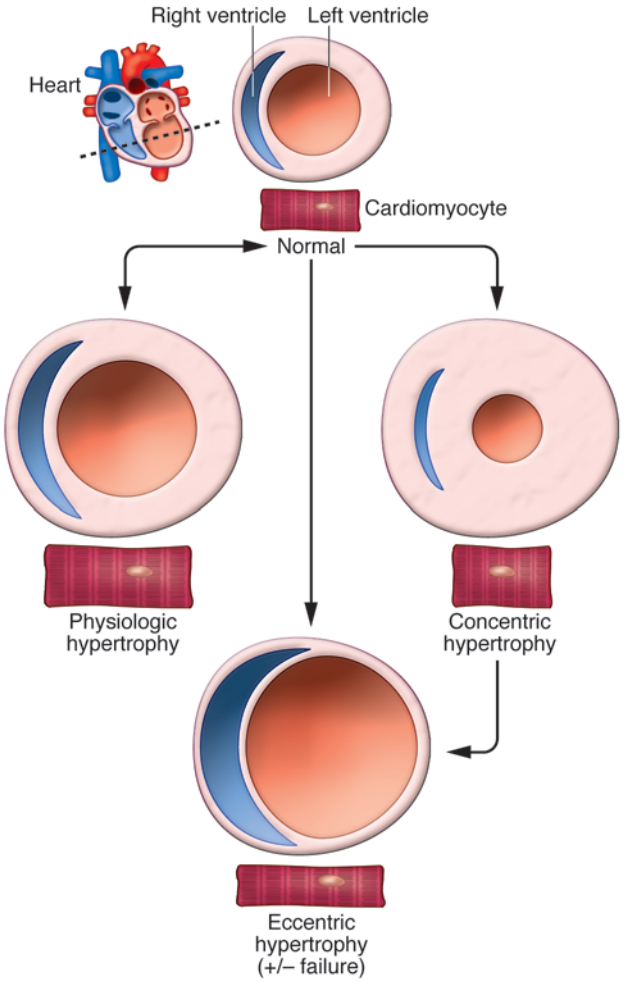

Figure 1
Overview of different types of cardiac hypertrophy. The normal heart can develop different types of hypertrophic remodeling depending on the stress. Exercise and pregnancy result in physiologic hypertrophy, in which individual cardiomyocytes increase in length and width and the heart undergoes a balanced type of eccentric hypertrophy (chambers, walls, and septum enlarge in unison). Pathologic stress or hypertrophic cardiomyopathy activates neuroendocrine factors that stimulate cardiac hypertrophy, often resulting in concentric remodeling, in which cardiomyocytes mostly increase in width compared with length, resulting in wall and septal thickening and a loss of chamber area. Over time, this state can deteriorate into dilated and eccentric hypertrophy, in which individual cardiomyocytes reduce in width and lengthening becomes excessive, leading to extreme chamber enlargement with loss of wall and septal thickness, along with large increases in wall tension. Some disease states can lead directly to dilated cardiomyopathy without a prior concentric remodeling phase.

cardiac remodeling associated with hypertension, postmyocardial infarction remodeling, or early stages of heart failure (27–30). One proposed benefit of β-receptor blockade is a myocyte-autonomous reduction in hypertrophy and cell death (31, 32). β-Adrenergic receptors are a subclass of GPCRs that elicit a number of downstream signaling events, including activation of adenylyl cyclase and elevation of cAMP as part of the acute fight-or-flight response that dramatically increases myocyte inotropy, chronotropy and lusitropy (Figure 2 and ref. 32). Increases in cAMP in the cardiomyocyte result in activation of PKA, which serves as a critical regulator of cardiac contractility by directly phosphorylating key Ca^{2+}-handling proteins and contractile proteins to augment function. However, when activated for extended periods of time, such as during hypertension, heart failure, or volume overload due to valve dysfunction, these pathways may lose their initial beneficial positive chronotropic and inotropic effects (33, 34), leading to hypertrophy and β-receptor desensitization (35).

β-Receptor desensitization and loss of dynamic and acute responsiveness may be the most detrimental effect associated with chronic receptor stimulation with augmented catecholamine load (33, 35). This process is directly regulated by GPCR receptor kinases (GRKs), some of which specifically phosphorylate the β-adrenergic receptor and modulate β-arrestin signaling, which together negatively affect the myocardium by reducing the dynamic range in β-receptor function (refs. 36, 37 and Figure 2).

For example, inhibition of GRK2 in genetically modified mouse models has been shown to abate heart failure and hypertrophic remodeling while maintaining optimal contractile performance (38). These results suggest that inhibiting this pathway may have clinical applications. Indeed, viral vector–mediated overexpression of a truncated dominant-negative protein that blocks GRK2 function toward the β-receptor is advancing into phase 1 and 2 clinical trials (39). Additionally, M119, a selective GRK2 small molecule inhibitor that antagonizes $G_{\beta\gamma}$ interaction with GRK2, enhances cardiomyocyte contractility in vitro and slows hypertrophy and heart failure progression in mice chronically treated with isoproterenol (Figure 2 and ref. 40).

Although various cardiac GPCRs have negative effects when stimulated chronically, β-arrestin has recently been recognized to mediate potentially beneficial downstream signaling (41). The currently available GPCR receptor blockers inhibit both G protein– and β-arrestin–mediated responses. However, select types of GPCR blockers known as biased receptor blockers can differentiate between the two components. Just such an antagonist specifically targeting the angiotensin receptor is currently being evaluated in phase 2 clinical trials (42, 43). TRV120027 inhibits the G protein–coupled response downstream of the angiotensin receptor but leaves the positive effects associated with β-arrestin signaling untouched (Figure 2). The hope is that such biased receptor inhibitors may have long-term benefits over simply blocking all downstream signaling of the receptor. Indeed, some of the existing β-receptor antagonists used in humans, such as carvedilol, function as biased agonists for β-arrestin and hence might provide additional benefit beyond simply blocking traditional coupled signaling through $G_{\alpha s}$ (44, 45).

PKCα inhibition

PKCα is activated by GPCR signaling in cardiomyocytes, elicited by most neuroendocrine effectors that function through $G_{\alpha q}$ and phospholipase C activation (46). Once activated, PKCα appears to function as a nodal regulator of contractility by affecting key intracellular Ca^{2+} and myofilament contractile proteins that alter signaling and myocyte function. For example, deletion of *Prkca* in the mouse, which encodes PKCα, results in markedly increased basal cardiac contractility, including increased sarcoplasmic reticulum Ca^{2+} levels and Ca^{2+} cycling efficiency that protects these mice from cardiac hypertrophy and heart failure induced by cardiac stress (47, 48). Transgenic mice with dominant-negative PKCα

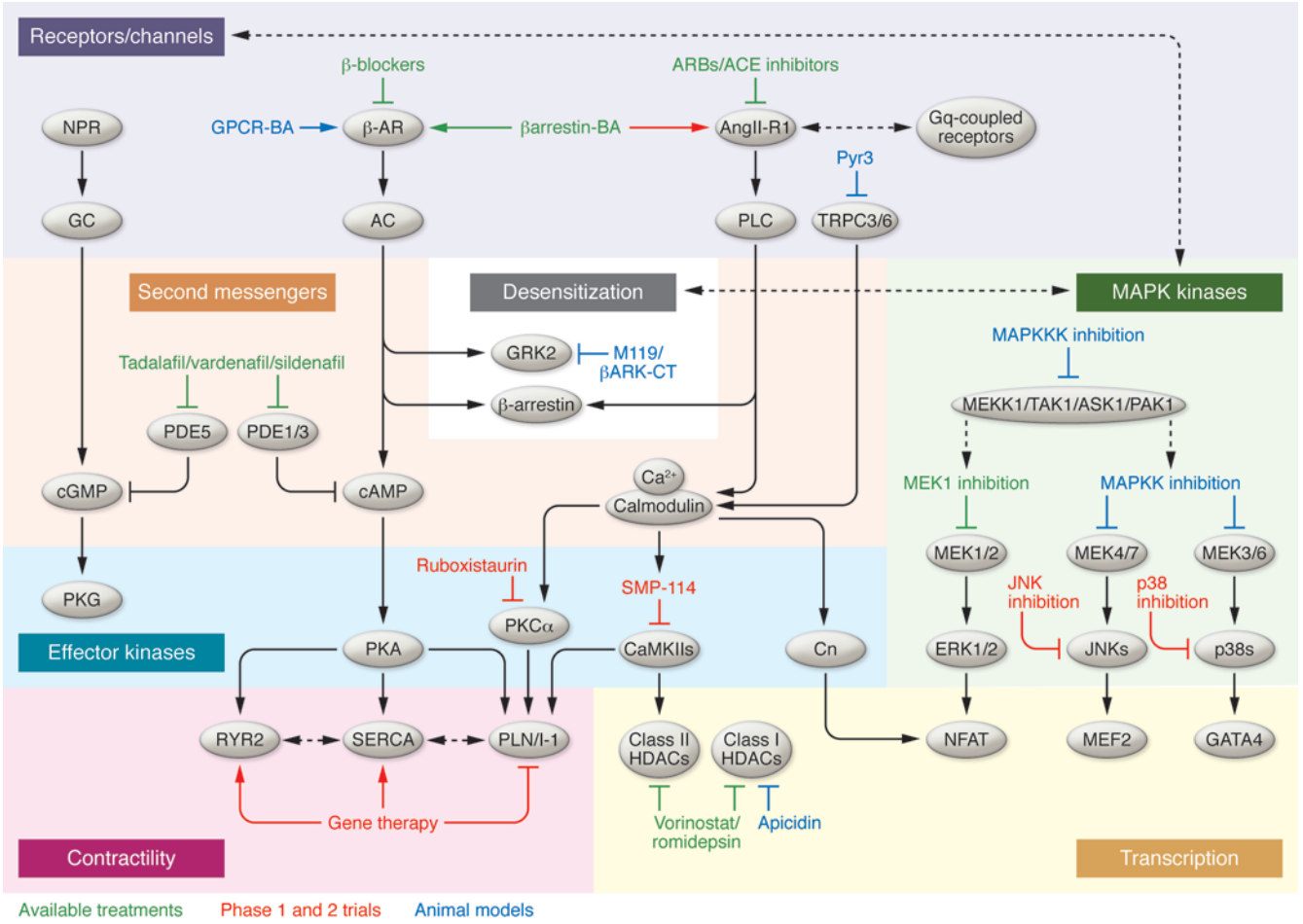

Figure 2

Signaling pathways underlying pathologic cardiac remodeling that have emerged as translational targets. Diagram of selected signaling effectors or signaling pathways that underlie cardiac hypertrophy or the transition to heart failure, with a special emphasis on immediate translational potential given the pharmacologic agents in development or clinical trials for other disorders. The diagram is also segregated into compartments in the cardiomyocyte, from receptors to second messengers to effector kinases. Some pathways lead to alterations in contractility and/or gene expression. The individual signaling mediators are discussed in the text. The diagram also depicts different therapeutic options based on known pharmacologic agents or gene therapy approaches. Green indicates drugs that are FDA approved, although not necessarily for cardiovascular indications. Red indicates treatments that are currently in phase 1 and 2 clinical trials, but again, not necessarily for cardiovascular indications. Blue indicates targets that have been identified in animal models and might be translated into phase 1 and 2 clinical trials with investigational compounds. NFAT, MEF2, and GATA4 are well-known cardiac-acting transcription factors that affect cardiovascular stress responsiveness. GPCR-BA, G-protein coupled receptor biased agonist, biased toward G-protein signaling; β-arrestin-BA, β-arrestin biased agonist. AC, adenylyl cyclase; ACE, angiotensin-converting enzyme; β-AR, β-adrenergic receptor; ARB, angiotensin receptor blocker; GC, guanylate cyclase; β-ARK-CT, β-adrenergic receptor kinase carboxyl terminus; NPR, natriuretic peptide receptor; PLC, phospholipase C; PLN, phospholamban; I-1, PP1 inhibitor 1; RYR2, ryanodine receptor 2; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase. Dotted lines indicate indirect pathways.

(dn-PKCα) overexpression specific to cardiomyocytes were also protected from myocardial infarction–induced heart failure, as were diseased mice treated with PKCα/β inhibitors such as Ro-31-8220 and ruboxistaurin (Figure 2 and refs. 48–50). Ruboxistaurin treatment also reduced cardiac remodeling, fibrosis, and heart failure in rat models of heart disease and improved cardiac function in pigs after myocardial infarction (51–54). By using gene-targeted mice for *Prkca*, *Prkcb*, and *Prkcg*, the protective effects of ruboxistaurin were shown to result exclusively from PKCα inhibition. (55). In addition to Ca^{2+} cycling effects, ruboxistaurin may also improve contractility by inhibiting PKCα phosphorylation of myofilament proteins (56–59). Hence, inhibition of PKCα could blunt the hypertrophic response by mildly augmenting contractile function at multiple levels, thereby antagonizing the need for enhanced neuroendocrine/catecholamine drive as disease begins and progresses. Similar effects may be induced by the myosin activator omecamtiv mecarbil (which is advancing to phase 3 clinical trials to evaluate its potential as a heart failure therapy), which may also lessen cardiac hypertrophy and pathologic remodeling (60–62). However, agents like omecamtiv might increase oxygen demand and thereby have serious adverse effects when dosed too high (60–62). Ruboxistaurin has been used in well over 1,000 patients in multiple phase 2/3 clinical trials for diabetic retinopathy, with some patients treated as long as 2 years, suggesting it

should be safe to use in cardiac patients as well (63–65). It is surprising that ruboxistaurin has yet to be evaluated in heart failure patients, even if just for mild inotropic support, since these agents appear safe in other human clinical trials and are overwhelmingly efficacious in animal models of heart disease (66).

Ca^{2+}/calmodulin-dependent kinase II signaling

In the last few years the importance of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) as a signaling regulator for cardiac remodeling and heart failure has become clear (67). Four genes encode the CaMKII isozymes α, β, γ, and δ, which are all activated by Ca^{2+}/calmodulin and other modifications (68). For example, when short-lasting activation through Ca^{2+}/calmodulin is sustained, CaMKII may become self-activated, thereby reinforcing its kinase activity (69, 70). Additionally, ROS can oxidize the kinase, which results in sustained activation (71). Like PKA and PKCα, CaMKII regulates the activity of key intracellular Ca^{2+} handling or regulatory proteins, thus affecting contractility and relaxation of the cardiomyocyte, but also affecting gene transcription by controlling nuclear shuttling of class II histone deacetylases (HDACs) (Figure 2 and refs. 67, 68). Overexpression of the CaMKIIδ isoform in the heart alters Ca^{2+} handling in several important ways and induces cardiac remodeling and disease (72, 73). Consistent with these results, mice lacking CaMKIIδ show a reduction in the cardiac hypertrophic response and/or less ventricular remodeling with cardiac pressure overload stimulation (74, 75).

Though CaMKII regulates multiple Ca^{2+}-handling proteins, genetic inhibition surprisingly does not result in negative side effects in animal models. Rather, these mice are protected from arrhythmia, suggesting an additional medical application if a specific small molecule inhibitor was developed (69, 76, 77). Furthermore, Anderson and colleagues showed a link between aldosterone antagonism and inhibition of CaMKII, suggesting that the beneficial effects of aldosterone antagonism are mediated by inhibition of CaMKII (78). However, aldosterone antagonists only block aldosterone/NADPH oxidase–mediated activation of CaMKII, leaving other modes of activation unaffected. Therefore, the challenge is to directly target the kinase itself and develop a safe inhibitor that is highly specific for CaMKII. One potential candidate is SMP-114, which has been evaluated in human clinical trials for rheumatoid arthritis (Figure 2 and ref. 79). However, this CaMKII inhibitory compound has not been extensively evaluated for selectivity, nor has it been applied to animal models of heart disease.

Phosphodiesterase 5 inhibition

Studies in genetically modified mice as well as pharmacological studies in animal models have suggested that inhibition of phosphodiesterase 5 (PDE5) could be a novel approach to ameliorate pathologic remodeling of the heart. PDE5 activity is dependent on cyclic GMP (cGMP) selectivity over cAMP, and enzyme inhibition with drugs such as sildenafil (Viagra) results in elevated cGMP, presumably within cardiomyocytes, which then has an antihypertrophic signaling effect (Figure 2 and ref. 80). Indeed, mice subjected to pressure overload stimulation show almost no cardiac hypertrophy when treated with sildenafil before (81, 82) and even after signs of heart failure have developed (83). Sildenafil has been evaluated in numerous human clinical trials of heart disease, including congestive heart failure, diabetic cardiomyopathy, and pulmonary hypertension, most of which showed improved endpoints and outcomes (84, 85). The molecular mechanism whereby sildenafil achieves this beneficial cardiovascular profile remains controversial, as it appears that PDE5 is not basally expressed in adult cardiomyocytes (86), and sildenafil may have some effect on PDE1 (80) and PDE3 (87) that could produce a mild increase in cAMP and an increase in inotropy (Figure 2). A mild increase in contractility might have cardioprotective effects on its own by reducing neuroendocrine drive, as discussed earlier and reviewed previously (88). An alternative mechanism for sildenafil action may be activation of PKG through an elevation in cGMP, which has been suggested to be antihypertrophic by signaling to downstream effectors such as calcineurin–nuclear factor of activated T cells (Cn-NFAT), regulator of G protein signaling, and transient receptor potential canonical 6 (TRPC6), as well as the RhoA-Rho kinase pathway, which by itself may be a potential therapeutic target against pathologic cardiac remodeling, especially since specific pharmacologic agents are already used in animal models as well as human patients (81, 82, 85, 89–93). Regardless of the downstream mechanisms, studies to date in animal models suggest that sildenafil, and possibly tadalafil and vardenafil, might be therapeutically efficacious in the treatment of pathologic cardiac remodeling prior to or coincident with the onset of heart failure, as results from early clinical trials already suggest some efficacy in patients with more advanced heart failure and/or right heart disease due to pulmonary arterial hypertension (Figure 2).

MAPK inhibition

The MAPK signaling cascade is classically initiated by activation of small G proteins in cardiomyocytes, followed by activation of successively acting protein kinases composed of three to five levels of phosphorylation-based amplification signaling (Figure 2). The MAPK cascade is subdivided into three main branches consisting of p38 kinases, JNKs, and ERK1/2. Additional side branches in this cascade include ERK5 and its upstream activator MEK5, as well as ERK3/4, although the upstream regulatory kinases for these effectors are not well characterized (94, 95). The JNKs and p38 kinases, which are activated by MEK4/7 and MEK3/6, respectively, generally serve as more specialized transducers of stress or injury responses, hence their classification as stress-activated protein kinases, while ERK1 and ERK2, which are activated by MEK1/2, are more specialized for mitogenic and growth factor transduction events and associated cellular processes.

Nearly all MAPK signaling components (upstream and downstream) are activated in end-stage human heart failure as well as in animal models of pathologic cardiac hypertrophy (96–98). Since this topic has been extensively reviewed previously (95), here we will only highlight the most medically relevant results that suggest therapeutic options. Constitutive activation of ERK1/2 signaling in the heart through expression of activated MEK1 produces concentric cardiac hypertrophy with thickening of individual myocytes, although it does not progress to failure and is protective against cell death (99, 100). Moreover, genetic inhibition of ERK1/2 signaling in the heart with either constitutive expression of an ERK1/2-specific dual-specificity phosphatase or by combinatorial deletion of *Erk1* and *Erk2* promotes cardiac dilation by enhancing growth in myocyte length (101–103). Thus, inhibition of MEK1/2 or ERK1/2 in patients with severe concentric remodeling and restrictive cardiomyopathy might be a therapeutic option. A number of different MEK1 inhibitors, such as PD-0325901, are being used in human clinical trials for cancer, all of which show safety and some of which have good oral bioavailability (Figure 2 and ref. 104).

Overexpression of MKK6 (p38 activation), MKK7 (JNK activation), or MEK5 (ERK5 activation) in the hearts of transgenic mice have each suggested a disease-causing effect of these kinases (105–107). Each appears to induce extreme cardiac dilation and decompensation with loss of contractile function, suggesting that inhibiting JNK, p38, or ERK5 with an appropriate pharmacologic agent might be therapeutic and antagonize the transition to dilated heart failure (Figure 2 and refs. 95, 108). However, cardiac-specific deletion of the gene encoding p38α predisposes mice to disease with worse cardiac function, and dn-p38α mice develop more cardiac hypertrophy with pressure overload stimulation (109, 110). Similarly, transgenic mice expressing dn-JNK1/2 in the heart as well as combinatorial deletion of the 3 and 4 alleles of *Jnk1* and *Jnk2* (deletion of all 4 allele result in embryonic lethality) produced more hypertrophy with pressure overload stimulation (111). Mice lacking cardiac MKK4 or MKK7 are also more prone to heart disease with pressure overload stimulation, suggesting a protective function for these pathways in addition to the previously described maladaptive aspects of their signaling (112, 113). Thus pharmacologic inhibition of JNK or p38 as a treatment for human heart disease would be more complicated if these animal studies directly translate (Figure 2). However, hamsters with muscular dystrophy and associated heart disease show less fibrosis and better cardiac function with systemic p38 MAPK inhibitor treatment, though a JNK inhibitor has no effect (114). Similar cardioprotection has also been observed in diabetic mice treated with a p38 MAPK inhibitor (115) and in mice following myocardial infarction injury (116). Therefore, pharmacological inhibition of p38 (or MKK6) and JNK (or MKK4/7) might have value to inhibit fibrosis or for select types of cardiac disease such as diabetic cardiomyopathy.

In addition to the terminal MAPK effectors and their upstream MAPKKs, the MAPKKKs have been suggested as important regulators of cardiac hypertrophy and/or transition to dilation. A number of these MAPKKKs modulate cardiac hypertrophy and heart failure, such as TAK1, PAK1, ASK1, and MEKK1 (117–123). However, these are less tangible targets since there are currently no drugs available with high specificity toward any of these. Indeed, the ability to achieve appropriate specificity toward just one individual kinase within a backdrop of some 500 serine-threonine kinases is a unique challenge for drug development, as even current investigational compounds that have been highly refined only achieve "fingerprints" of varying selectivity. Despite this note of caution, the translation of many of the kinase-inhibitory drugs into the cardiac disease realm is still potentially important toward the goal of achieving greater efficacy and life span extension than the current standard of care agents offer.

HDAC inhibitors

HDACs remove acetyl groups from lysine residues in target proteins and are key regulators of epigenetics through their activity toward histones in chromatin (124). Hence inhibition of HDACs might represent a novel therapeutic vantage point for pathologic cardiac hypertrophy/remodeling, given the known profile of gene expression changes that are commensurate with the disease (Figure 2). Genetically, mice lacking *Hdac9* or *Hdac5* genes show spontaneous cardiac hypertrophy with aging or exaggerated pathologic hypertrophy with pressure overload stimulation, suggesting that select class IIa HDACs are normally suppressors of disease (125). Thus, a pharmacologic compound that selectively inhibits class IIa HDACs would likely be deleterious. Indeed, a pharmacologic compound that selectively inhibits class IIa HDAC binding with MEF2 has been shown to impair myogenesis, which could be deleterious to cardiac function (126).

A more relevant therapeutic target are the class I HDACs, for which a number of clinical-grade pharmaceuticals have been developed (127). Indeed, pan-HDAC inhibitors reduce cardiac hypertrophy due to pressure overload stimulation, angiotensin II infusion, or isoproterenol in rodent models (128–130). This effect may be more specific to HDAC1, -2, or -3, as the more selective class I HDAC inhibitor apicidin suppresses cardiac hypertrophy and improves function in a mouse model of pressure overload (131). HDAC inhibitors also reduce fibrosis and negative remodeling of the heart after myocardial infarction injury in the rat, as well as in rat models of spontaneous and salt-sensitive hypertension (132–134). These results are consistent with genetic studies in *Hdac2*-null mice, which show less cardiac hypertrophy induced by various pathologic stimuli (135). However, conflicting results from another group that created heart-specific *Hdac1*- or *Hdac2*-null mice showed no attenuation of the hypertrophic response by either gene deletion following pressure overload stimulation or isoproterenol infusion (136). Thus, the genetics remain complicated by redundancy in this gene family, although pharmacologic studies in animal models of hypertrophy consistently support a therapeutic effect (Figure 2). The HDAC inhibitor romidepsin is FDA approved for the treatment of cutaneous T cell lymphoma, but arrhythmia and low white blood cell counts have been noted as significant concerns with this agent (137, 138). Although another FDA-approved HDAC inhibitor, vorinostat, has not shown such complications at this point, caution is warranted for its use in hypertrophy or heart failure because potential side effects cannot be ruled out at this point (139, 140).

Manipulation of Ca^{2+} dependent signaling effectors

Ca^{2+} channel blockers used for hypertension are also effective in reducing cardiac hypertrophy. While these drugs lower blood pressure, they have not shown benefit in heart failure and are generally avoided because of the potential risk of arrhythmias and negative inotropic effects (141). Recent evidence in genetically modified mice with cardiac-specific loss of the L-type Ca^{2+} channel, which is the primary mechanism for Ca^{2+} entry in a cardiomyocyte, showed that reduced activity of these channels leads to a compensatory leak from the ryanodine receptor and induction of cardiac hypertrophy (142). Thus, Ca^{2+} channel blockers might not be a straightforward therapeutic option for other forms of heart disease that are independent of hypertension. Additional coverage of alterations in Ca^{2+} handling through ryanodine receptor 2, sarcoplasmic reticulum Ca^{2+} ATPase, and phospholamban in heart failure are directly discussed in this Review series (Figure 2 and ref. 143).

Another potential cutting-edge therapeutic angle that affects Ca^{2+} signaling and pathologic hypertrophy is the inhibition of TRPC channels (Figure 2). TRPC channels permeate Ca^{2+} and Na^{+} in specific microdomains to initiate and/or maintain cardiac hypertrophy in association with GPCR signaling, which generates diacylglycerol to activate these channels (144, 145). Studies in mice have suggested that increased activity of either TRPC3 or TRPC6 in the heart, both of which are normally induced with hypertrophy, is sufficient to cause ventricular remodeling, dilation, hypertrophy, and disease (146, 147). Cardiac-specific expression of dn-TRPC3, dn-TRPC4, or dn-TRPC6 each antagonized the

degree of cardiac hypertrophy after pressure overload stimulation or infusion of phenylephrine/angiotensin II (148). Similarly, mice deficient in *Trpc1* showed reduced cardiac hypertrophy and disease after pressure overload or neuroendocrine agonist stimulation (149). These results suggest that an appropriately designed inhibitor that blocks one or more of these TRPC channels might be an effective therapeutic for cardiac hypertrophy or the transition to heart failure. Indeed, the presumed TRPC3/6 selective inhibitor Pyr3 was shown to attenuate pressure overload cardiac hypertrophy in mice (150). Thus, TRPC channels might represent novel targets for cardiac hypertrophy and failure, especially given the numerous compounds emerging from the pharmaceutical industry with specificity for the TRPM and TRPV subfamilies (151).

An important downstream effector that mediates the pro-hypertrophic effects of TRPC channels in the heart is the Cn-NFAT signaling circuit. Cn is a Ca^{2+}-activated serine-threonine protein phosphatase that dephosphorylates NFAT in the cytoplasm, resulting in its nuclear translocation and activation of hypertrophic gene expression (Figure 2). Nearly 15 years ago, Cn-NFAT signaling was proposed to be both necessary and sufficient for cardiac hypertrophy (152, 153). Since then, a large number of genetic studies in the mouse and pharmacologic-based studies in mice and rats have proven the absolute centrality of this Ca^{2+}-activated signaling circuit in mediating cardiac hypertrophy (154, 155), suggesting that Cn inhibitors could be used to treat associated cardiac disease states. However, the immunosuppressive effects of Cn inhibitors, together with other more severe side effects associated with the higher dosages that are needed to treat hypertrophy, likely preclude such an approach in humans.

Conclusion

Although other potential targets exist, such as metabolism and redox regulation, mTOR inhibition by rapamycin, or even treatment with peptides such as adiponectin, the pathways discussed here fit a central theme of emerging inibitors that affect signaling pathways (156, 157). Indeed, the current standard-of-care pharmacologic agents that are used to treat hypertension and heart failure also reduce cardiac hypertrophy by targeting signaling effectors. Despite these current agents, the incidence and prevalence of heart failure is still increasing, underscoring the need for agents that act earlier or are more effective (26, 158, 159). Because many of the targets highlighted here have existing or newly developed pharmacologic agents with reasonable specificity and safety, the hope is that additional clinical trials can be instituted quickly for patients in early-stage heart failure, patients with hypertrophy that is nonresponsive to the standard-of-care agents, or in patients with hypertrophic cardiomyopathy due to mutations in sarcomeric genes. While presentation of cardiac hypertrophy is not typically an endpoint in clinical trials, diastolic dysfunction and early-stage heart failure might be. Many of the signaling pathways and effectors that induce pathologic hypertrophy in the first place likely lead to diastolic dysfunction and heart failure, so many of the pathways outlined here might be effective early and late in the disease process that culminates in heart failure. Therefore, as the use of animal models continues to uncover additional signaling effectors of cardiac hypertrophy and heart failure, agents against these targets might be translated into clinical trials to maintain a seamless pipeline of therapeutic options until a more efficacious treatment is uncovered.

Acknowledgments

This work was supported by an American Heart Association postdoctoral fellowship (to J.H. van Berlo) and by grants from the NIH (to J.D. Molkentin and M. Maillet) and the Howard Hughes Medical Institute (to J.D. Molkentin).

Address correspondence to: Jeffery D. Molkentin, Howard Hughes Medical Institute, Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, 240 Albert Sabin Way, MLC 7020, Cincinnati, Ohio 45229-3039, USA. Phone: 513.636.3557; Fax: 513.535.5958; E-mail: Jeff.molkentin@cchmc.org.

1. Karsner HT, Saphir O, Todd TW. The state of the cardiac muscle in hypertrophy and atrophy. *Am J Pathol.* 1925;1(4):351–372.
2. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med.* 1990; 322(22):1561–1566.
3. Levy D, Anderson KM, Savage DD, Balkus SA, Kannel WB, Castelli WP. Risk of ventricular arrhythmias in left ventricular hypertrophy: the Framingham Heart Study. *Am J Cardiol.* 1987;60(7):560–565.
4. Drazner MH, et al. Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years: the Cardiovascular Health Study. *J Am Coll Cardiol.* 2004;43(12):2207–2215.
5. de Simone G, Gottdiener JS, Chinali M, Maurer MS. Left ventricular mass predicts heart failure not related to previous myocardial infarction: the Cardiovascular Health Study. *Eur Heart J.* 2008; 29(6):741–747.
6. Grossman W, Jones D, McLaurin LP. Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest.* 1975;56(1):56–64.
7. Lee WC, Shideman FE. Role of myocardial catecholamines in cardiac contractility. *Science.* 1959; 129(3354):967–968.
8. Taylor RR, Covell JW, Ross J Jr. Left ventricular function in experimental aorto-caval fistula with circulatory congestion and fluid retention. *J Clin Invest.* 1968;47(6):1333–1342.
9. Hood WP Jr, Rackley CE, Rolett EL. Wall stress in the normal and hypertrophied human left ventricle. *Am J Cardiol.* 1968;22(4):550–558.
10. Sharif-Naeini R, et al. Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels. *J Mol Cell Cardiol.* 2010;48(1):83–89.
11. Weeks KL, McMullen JR. The athlete's heart vs. the failing heart: can signaling explain the two distinct outcomes? *Physiology (Bethesda).* 2011;26(2):97–105.
12. Perrino C, et al. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest.* 2006; 116(6):1547–1560.
13. Tanaka N, et al. Effects of growth hormone and IGF-I on cardiac hypertrophy and gene expression in mice. *Am J Physiol.* 1998;275(2 pt 2):H393–H399.
14. McMullen JR, et al. The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase(p110alpha) pathway. *J Biol Chem.* 2004;279(6):4782–4793.
15. Shiojima I, et al. Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. *J Biol Chem.* 2002;277(40):37670–37677.
16. Pallafacchina G, Calabria E, Serrano AL, Kalhovde JM, Schiaffino S. A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification. *Proc Natl Acad Sci U S A.* 2002;99(14):9213–9218.
17. Rommel C, et al. Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. *Science.* 1999;286(5445):1738–1741.
18. Bostrom P, et al. C/EBPbeta controls exercise-induced cardiac growth and protects against pathological cardiac remodeling. *Cell.* 2010;143(7):1072–1083.
19. Sussman MA, et al. Myocardial AKT: the omnipresent nexus. *Physiol Rev.* 2011;91(3):1023–1070.
20. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens.* 2003;21(11):1983–1992.
21. Chobanian AV, et al. The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA.* 2003;289(19):2560–2572.
22. Feron O, Salomone S, Godfraind T. Action of the calcium channel blocker lacidipine on cardiac hypertrophy and endothelin-1 gene expression in stroke-prone hypertensive rats. *Br J Pharmacol.* 1996; 118(3):659–664.
23. Sekiguchi K, et al. Cross-regulation between the renin-angiotensin system and inflammatory mediators in cardiac hypertrophy and failure. *Cardiovasc Res.* 2004;63(3):433–442.
24. Feldman DS, Carnes CA, Abraham WT, Bristow MR. Mechanisms of disease: beta-adrenergic receptors—alterations in signal transduction and pharmacogenomics in heart failure. *Nat Clin Pract Cardiovasc Med.* 2005;2(9):475–483.
25. Calhoun DA, et al. Resistant hypertension: diagnosis, evaluation, and treatment. A scientific statement from the American Heart Association Pro-

fessional Education Committee of the Council for High Blood Pressure Research. *Hypertension*. 2008; 51(6):1403–1419.
26. Roger VL, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation*. 2012;125(1):e2–e220.
27. Packer M, et al. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *N Engl J Med*. 1996;334(21):1349–1355.
28. Lindholm LH, Carlberg B, Samuelsson O. Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. *Lancet*. 2005;366(9496):1545–1553.
29. Snow PJ. Effect of propranolol in myocardial infarction. *Lancet*. 1965;2(7412):551–553.
30. Freemantle N, Cleland J, Young P, Mason J, Harrison J. beta Blockade after myocardial infarction: systematic review and meta regression analysis. *BMJ*. 1999;318(7200):1730–1737.
31. Remme WJ. Pharmacological modulation of cardiovascular remodeling: a guide to heart failure therapy. *Cardiovasc Drugs Ther*. 2003;17(4):349–360.
32. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? *Circ Res*. 2003;93(10):896–906.
33. Rockman HA, Choi DJ, Rahman NU, Akhter SA, Lefkowitz RJ, Koch WJ. Receptor-specific in vivo desensitization by the G protein-coupled receptor kinase-5 in transgenic mice. *Proc Natl Acad Sci U S A*. 1996;93(18):9954–9959.
34. Rockman HA, Koch WJ, Milano CA, Lefkowitz RJ. Myocardial beta-adrenergic receptor signaling in vivo: insights from transgenic mice. *J Mol Med (Berl)*. 1996;74(9):489–495.
35. Strulovici B, Cerione RA, Kilpatrick BF, Caron MG, Lefkowitz RJ. Direct demonstration of impaired functionality of a purified desensitized beta-adrenergic receptor in a reconstituted system. *Science*. 1984;225(4664):837–840.
36. Benovic JL, Strasser RH, Caron MG, Lefkowitz RJ. Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc Natl Acad Sci U S A*. 1986;83(9):2797–2801.
37. Rockman HA, et al. Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc Natl Acad Sci U S A*. 1998;95(12):7000–7005.
38. Raake PW, et al. G protein-coupled receptor kinase 2 ablation in cardiac myocytes before or after myocardial infarction prevents heart failure. *Circ Res*. 2008;103(4):413–422.
39. Shah AS, et al. In vivo ventricular gene delivery of a beta-adrenergic receptor kinase inhibitor to the failing heart reverses cardiac dysfunction. *Circulation*. 2001;103(9):1311–1316.
40. Casey LM, et al. Small molecule disruption of G beta gamma signaling inhibits the progression of heart failure. *Circ Res*. 2010;107(4):532–539.
41. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discov*. 2010;9(5):373–386.
42. Rajagopal K, et al. Beta-arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. *Proc Natl Acad Sci U S A*. 2006;103(44):16284–16289.
43. Wei H, et al. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci U S A*. 2003;100(19):10782–10787.
44. Whalen EJ, Rajagopal S, Lefkowitz RJ. Therapeutic potential of beta-arrestin- and G protein-biased agonists. *Trends Mol Med*. 2011;17(3):126–139.
45. Poole-Wilson PA, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*. 2003;362(9377):7–13.
46. Dorn GW 2nd, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest*. 2005;115(3):527–537.
47. Braz JC, et al. PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nat Med*. 2004;10(3):248–254.
48. Hambleton M, et al. Inducible and myocyte-specific inhibition of PKCalpha enhances cardiac contractility and protects against infarction-induced heart failure. *Am J Physiol Heart Circ Physiol*. 2007; 293(6):H3768–H3771.
49. Hambleton M, et al. Pharmacological- and gene therapy-based inhibition of protein kinase Calpha/beta enhances cardiac contractility and attenuates heart failure. *Circulation*. 2006;114(6):574–582.
50. Wang GS, Kuyumcu-Martinez MN, Sarma S, Mathur N, Wehrens XH, Cooper TA. PKC inhibition ameliorates the cardiac phenotype in a mouse model of myotonic dystrophy type 1. *J Clin Invest*. 2009;119(12):3797–3806.
51. Boyle AJ, et al. Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction. *J Mol Cell Cardiol*. 2005; 39(2):213–221.
52. Connelly KA, et al. Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. *Circ Heart Fail*. 2009; 2(2):129–137.
53. Kong L, et al. PKCbeta modulates ischemia-reperfusion injury in the heart. *Am J Physiol Heart Circ Physiol*. 2008;294(4):H1862–H1870.
54. Ladage D, et al. Inhibition of PKCalpha/beta with ruboxistaurin antagonizes heart failure in pigs after myocardial infarction injury. *Circ Res*. 2011; 109(12):1396–1400.
55. Liu Q, et al. Protein kinase C{alpha}, but not PKC{beta} or PKC{gamma}, regulates contractility and heart failure susceptibility: implications for ruboxistaurin as a novel therapeutic approach. *Circ Res*. 2009;105(2):194–200.
56. Belin RJ, et al. Augmented protein kinase C-alpha-induced myofilament protein phosphorylation contributes to myofilament dysfunction in experimental congestive heart failure. *Circ Res*. 2007; 101(2):195–204.
57. Hidalgo C, et al. PKC phosphorylation of titin's PEVK element: a novel and conserved pathway for modulating myocardial stiffness. *Circ Res*. 2009; 105(7):631–638.
58. Kooij V, et al. Protein kinase C alpha and epsilon phosphorylation of troponin and myosin binding protein C reduce Ca2+ sensitivity in human myocardium. *Basic Res Cardiol*. 2010;105(2):289–300.
59. Sumandea MP, Pyle WG, Kobayashi T, de Tombe PP, Solaro RJ. Identification of a functionally critical protein kinase C phosphorylation residue of cardiac troponin T. *J Biol Chem*. 2003;278(37):35135–35144.
60. Cleland JG, et al. The effects of the cardiac myosin activator, omecamtiv mecarbil, on cardiac function in systolic heart failure: a double-blind, placebo-controlled, crossover, dose-ranging phase 2 trial. *Lancet*. 2011;378(9792):676–683.
61. Malik FI, et al. Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science*. 2011;331(6023):1439–1443.
62. Teerlink JR, et al. Dose-dependent augmentation of cardiac systolic function with the selective cardiac myosin activator, omecamtiv mecarbil: a first-in-man study. *Lancet*. 2011;378(9792):667–675.
63. Davis MD, et al. Effect of ruboxistaurin on the visual acuity decline associated with long-standing diabetic macular edema. *Invest Ophthalmol Vis Sci*. 2009;50(1):1–4.
64. Aiello LP, et al. Oral protein kinase c beta inhibition using ruboxistaurin: efficacy, safety, and causes of vision loss among 813 patients (1,392 eyes) with diabetic retinopathy in the Protein Kinase C beta Inhibitor-Diabetic Retinopathy Study and the Protein Kinase C beta Inhibitor-Diabetic Retinopathy Study 2. *Retina*. 2011;31(10):2084–2094.
65. Sheetz MJ, Aiello LP, Shahri N, Davis MD, Kles KA, Danis RP. Effect of ruboxistaurin (RBX) On visual acuity decline over a 6-year period with cessation and reinstitution of therapy: results of an open-label extension of the Protein Kinase C Diabetic Retinopathy Study 2 (PKC-DRS2). *Retina*. 2011; 31(6):1053–1059.
66. Liu Q, Molkentin JD. Protein kinase Calpha as a heart failure therapeutic target. *J Mol Cell Cardiol*. 2011;51(4):474–478.
67. Anderson ME, Brown JH, Bers DM. CaMKII in myocardial hypertrophy and heart failure. *J Mol Cell Cardiol*. 2011;51(4):468–473.
68. Erickson JR, He BJ, Grumbach IM, Anderson ME. CaMKII in the cardiovascular system: sensing redox states. *Physiol Rev*. 2011;91(3):889–915.
69. Zhang R, et al. Calmodulin kinase II inhibition protects against structural heart disease. *Nat Med*. 2005;11(4):409–417.
70. De Koninck P, Schulman H. Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations. *Science*. 1998;279(5348):227–230.
71. Erickson JR, et al. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell*. 2008;133(3):462–474.
72. Zhang T, et al. The cardiac-specific nuclear delta(B) isoform of Ca2+/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. *J Biol Chem*. 2002; 277(2):1261–1267.
73. Zhang T, et al. The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. *Circ Res*. 2003; 92(8):912–919.
74. Backs J, et al. The delta isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. *Proc Natl Acad Sci U S A*. 2009;106(7):2342–2347.
75. Ling H, et al. Requirement for Ca2+/calmodulin-dependent kinase II in the transition from pressure overload-induced cardiac hypertrophy to heart failure in mice. *J Clin Invest*. 2009;119(5):1230–1240.
76. Tsuji Y, et al. Ca(2+)-related signaling and protein phosphorylation abnormalities play central roles in a new experimental model of electrical storm. *Circulation*. 2011;123(20):2192–2203.
77. van Oort RJ, et al. Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. *Circulation*. 2010;122(25):2669–2679.
78. He BJ, et al. Oxidation of CaMKII determines the cardiotoxic effects of aldosterone. *Nat Med*. 2011; 17(12):1610–1618.
79. Westra J, et al. Role for CaMKII inhibition in rheumatoid arthritis: effects on HIF-1-induced VEGF production by rheumatoid synovial fibroblasts. *Ann N Y Acad Sci*. 2009;1173:706–711.
80. Vandeput F, et al. cGMP-hydrolytic activity and its inhibition by sildenafil in normal and failing human and mouse myocardium. *J Pharmacol Exp Ther*. 2009;330(3):884–891.
81. Takimoto E, et al. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med*. 2005;11(2):214–222.
82. Chau VQ, Salloum FN, Hoke NN, Abbate A, Kukreja RC. Mitigation of the progression of heart failure with sildenafil involves inhibition of RhoA/Rho-kinase pathway. *Am J Physiol Heart Circ Physiol*. 2011;300(6):H2272–H2279.
83. Nagayama T, et al. Sildenafil stops progressive chamber, cellular, and molecular remodeling and improves calcium handling and function in hearts with pre-

existing advanced hypertrophy caused by pressure overload. *J Am Coll Cardiol*. 2009;53(2):207–215.
84. Schwartz BG, Levine LA, Comstock G, Stecher VJ, Kloner RA. Cardiac uses of phosphodiesterase-5 inhibitors. *J Am Coll Cardiol*. 2012;59(1):9–15.
85. Giannetta E, et al. Chronic Inhibition of cGMP phosphodiesterase 5A improves diabetic cardiomyopathy: a randomized, controlled clinical trial using magnetic resonance imaging with myocardial tagging. *Circulation*. 2012;125(19):2323–2333.
86. Movsesian M, Stehlik J, Vandeput F, Bristow MR. Phosphodiesterase inhibition in heart failure. *Heart Fail Rev*. 2009;14(4):255–263.
87. Nagendran J, et al. Phosphodiesterase type 5 is highly expressed in the hypertrophied human right ventricle, and acute inhibition of phosphodiesterase type 5 improves contractility. *Circulation*. 2007; 116(3):238–248.
88. Dorn GW 2nd, Molkentin JD. Manipulating cardiac contractility in heart failure: data from mice and men. *Circulation*. 2004;109(2):150–158.
89. Koitabashi N, et al. Cyclic GMP/PKG-dependent inhibition of TRPC6 channel activity and expression negatively regulates cardiomyocyte NFAT activation novel mechanism of cardiac stress modulation by PDE5 inhibition. *J Mol Cell Cardiol*. 2010; 48(4):713–724.
90. Nishida M, et al. Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition. *J Biol Chem*. 2010;285(17):13244–13253.
91. Takimoto E, et al. Regulator of G protein signaling 2 mediates cardiac compensation to pressure overload and antihypertrophic effects of PDE5 inhibition in mice. *J Clin Invest*. 2009;119(2):408–420.
92. Fukui S, et al. Long-term inhibition of Rho-kinase ameliorates diastolic heart failure in hypertensive rats. *J Cardiovasc Pharmacol*. 2008;51(3):317–326.
93. Shi J, Zhang L, Wei L. Rho-kinase in development and heart failure: insights from genetic models. *Pediatr Cardiol*. 2011;32(3):297–304.
94. Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. *Oncogene*. 2007; 26(22):3100–3112.
95. Rose BA, Force T, Wang Y. Mitogen-activated protein kinase signaling in the heart: angels versus demons in a heart-breaking tale. *Physiol Rev*. 2010; 90(4):1507–1546.
96. Gutkind JS, Offermanns S. A new G(q)-initiated MAPK signaling pathway in the heart. *Dev Cell*. 2009;16(2):163–164.
97. Haq S, et al. Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure. *Circulation*. 2001; 103(5):670–677.
98. Toischer K, et al. Differential cardiac remodeling in preload versus afterload. *Circulation*. 2010; 122(10):993–1003.
99. Bueno OF, et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J*. 2000;19(23):6341–6350.
100. Lips DJ, et al. MEK1-ERK2 signaling pathway protects myocardium from ischemic injury in vivo. *Circulation*. 2004;109(16):1938–1941.
101. Bueno OF, et al. The dual-specificity phosphatase MKP-1 limits the cardiac hypertrophic response in vitro and in vivo. *Circ Res*. 2001;88(1):88–96.
102. Purcell NH, et al. Genetic inhibition of cardiac ERK1/2 promotes stress-induced apoptosis and heart failure but has no effect on hypertrophy in vivo. *Proc Natl Acad Sci U S A*. 2007;104(35):14074–14079.
103. Kehat I, et al. Extracellular signal-regulated kinases 1 and 2 regulate the balance between eccentric and concentric cardiac growth. *Circ Res*. 2011;108(2):176–183.
104. Haura EB, et al. A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. *Clin Cancer Res*. 2010;16(8):2450–2457.
105. Liao P, et al. The in vivo role of p38 MAP kinases in cardiac remodeling and restrictive cardiomyopathy. *Proc Natl Acad Sci U S A*. 2001;98(21):12283–12288.
106. Wang Y, Su B, Sah VP, Brown JH, Han J, Chien KR. Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells. *J Biol Chem*. 1998;273(10):5423–5426.
107. Nicol RL, Frey N, Pearson G, Cobb M, Richardson J, Olson EN. Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. *EMBO J*. 2001;20(11):2757–2767.
108. Marber MS, Rose B, Wang Y. The p38 mitogen-activated protein kinase pathway--a potential target for intervention in infarction, hypertrophy, and heart failure. *J Mol Cell Cardiol*. 2011;51(4):485–490.
109. Nishida K, et al. p38alpha mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol Cell Biol*. 2004;24(24):10611–10620.
110. Braz JC, et al. Targeted inhibition of p38 MAPK promotes hypertrophic cardiomyopathy through upregulation of calcineurin-NFAT signaling. *J Clin Invest*. 2003;111(10):1475–1486.
111. Liang Q, Bueno OF, Wilkins BJ, Kuan CY, Xia Y, Molkentin JD. c-Jun N-terminal kinases (JNK) antagonize cardiac growth through cross-talk with calcineurin-NFAT signaling. *EMBO J*. 2003; 22(19):5079–5089.
112. Liu W, et al. Cardiac-specific deletion of mkk4 reveals its role in pathological hypertrophic remodeling but not in physiological cardiac growth. *Circ Res*. 2009;104(7):905–914.
113. Liu W, et al. Deprivation of MKK7 in cardiomyocytes provokes heart failure in mice when exposed to pressure overload. *J Mol Cell Cardiol*. 2011; 50(4):702–711.
114. Kyoi S, et al. Opposing effect of p38 MAP kinase and JNK inhibitors on the development of heart failure in the cardiomyopathic hamster. *Cardiovasc Res*. 2006;69(4):888–898.
115. Westermann D, et al. Inhibition of p38 mitogen-activated protein kinase attenuates left ventricular dysfunction by mediating pro-inflammatory cardiac cytokine levels in a mouse model of diabetes mellitus. *Diabetologia*. 2006;49(10):2507–2513.
116. Liu YH, et al. Inhibition of p38 mitogen-activated protein kinase protects the heart against cardiac remodeling in mice with heart failure resulting from myocardial infarction. *J Card Fail*. 2005; 11(1):74–81.
117. Liu W, et al. Pak1 as a novel therapeutic target for antihypertrophic treatment in the heart. *Circulation*. 2011;124(24):2702–2715.
118. Minamino T, et al. MEKK1 is essential for cardiac hypertrophy and dysfunction induced by Gq. *Proc Natl Acad Sci U S A*. 2002;99(6):3866–3871.
119. Taglieri DM, et al. Ablation of p21-activated kinase-1 in mice promotes isoproterenol-induced cardiac hypertrophy in association with activation of Erk1/2 and inhibition of protein phosphatase 2A. *J Mol Cell Cardiol*. 2011;51(6):988–996.
120. Taniike M, et al. Apoptosis signal-regulating kinase 1/p38 signaling pathway negatively regulates physiological hypertrophy. *Circulation*. 2008;117(4):545–552.
121. Yamaguchi O, et al. Targeted deletion of apoptosis signal-regulating kinase 1 attenuates left ventricular remodeling. *Proc Natl Acad Sci U S A*. 2003; 100(26):15883–15888.
122. Yamaguchi O, et al. Cardiac-specific disruption of the c-raf-1 gene induces cardiac dysfunction and apoptosis. *J Clin Invest*. 2004;114(7):937–943.
123. Zhang D, et al. TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. *Nat Med*. 2000;6(5):556–563.
124. Bush EW, McKinsey TA. Protein acetylation in the cardiorenal axis: the promise of histone deacetylase inhibitors. *Circ Res*. 2010;106(2):272–284.
125. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell*. 2002;110(4):479–488.
126. Nebbioso A, et al. Selective class II HDAC inhibitors impair myogenesis by modulating the stability and activity of HDAC-MEF2 complexes. *EMBO Rep*. 2009;10(7):776–782.
127. Kim HJ, Bae SC. Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *Am J Transl Res*. 2011;3(2):166–179.
128. Kee HJ, et al. Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation*. 2006; 113(1):51–59.
129. Kook H, et al. Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. *J Clin Invest*. 2003;112(6):863–871.
130. Kong Y, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation*. 2006;113(22):2579–2588.
131. Gallo P, et al. Inhibition of class I histone deacetylase with an apicidin derivative prevents cardiac hypertrophy and failure. *Cardiovasc Res*. 2008;80(3):416–424.
132. Lee TM, Lin MS, Chang NC. Inhibition of histone deacetylase on ventricular remodeling in infarcted rats. *Am J Physiol Heart Circ Physiol*. 2007; 293(2):H968–H977.
133. Cardinale JP, et al. HDAC inhibition attenuates inflammatory, hypertrophic, and hypertensive responses in spontaneously hypertensive rats. *Hypertension*. 2010;56(3):437–444.
134. Iyer A, et al. Antifibrotic activity of an inhibitor of histone deacetylases in DOCA-salt hypertensive rats. *Br J Pharmacol*. 2010;159(7):1408–1417.
135. Trivedi CM, et al. Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3 beta activity. *Nat Med*. 2007;13(3):324–331.
136. Montgomery RL, et al. Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes Dev*. 2007;21(14):1790–1802.
137. Shah MH, et al. Cardiotoxicity of histone deacetylase inhibitor depsipeptide in patients with metastatic neuroendocrine tumors. *Clin Cancer Res*. 2006; 12(13):3997–4003.
138. Molife R, Fong P, Scurr M, Judson I, Kaye S, de Bono J. HDAC inhibitors and cardiac safety. *Clin Cancer Res*. 2007;13(3):1068.
139. Duvic M, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood*. 2007;109(1):31–39.
140. Olsen EA, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol*. 2007;25(21):3109–3115.
141. Elkayam U. Calcium channel blockers in heart failure. *Cardiology*. 1998;89(suppl 1):38–46.
142. Goonasekera SA, et al. Decreased cardiac L-type Ca(2) channel activity induces hypertrophy and heart failure in mice. *J Clin Invest*. 2012;122(1):280–290.
143. Marks AR. Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J Clin Invest*. 2013;123(1):46–52.
144. Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. *Physiol Rev*. 2007;87(1):165–217.
145. Eder P, Molkentin JD. TRPC channels as effectors of cardiac hypertrophy. *Circ Res*. 2011;108(2):265–272.
146. Kuwahara K, et al. TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest*. 2006;116(12):3114–3126.
147. Nakayama H, Wilkin BJ, Bodi I, Molkentin JD. Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. *FASEB J*.

2006;20(10):1660–1670.
148. Wu X, Eder P, Chang B, Molkentin JD. TRPC channels are necessary mediators of pathologic cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2010; 107(15):7000–7005.
149. Seth M, et al. TRPC1 channels are critical for hypertrophic signaling in the heart. *Circ Res*. 2009; 105(10):1023–1030.
150. Kiyonaka S, et al. Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. *Proc Natl Acad Sci U S A*. 2009;106(13):5400–5405.
151. Moran MM, McAlexander MA, Biro T, Szallasi A. Transient receptor potential channels as therapeutic targets. *Nat Rev Drug Discov*. 2011;10(8):601–620.
152. Molkentin JD, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell*. 1998;93(2):215–228.
153. Sussman MA, et al. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. *Science*. 1998;281(5383):1690–1693.
154. Wilkins BJ, Molkentin JD. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun*. 2004;322(4):1178–1191.
155. Fiedler B, Wollert KC. Targeting calcineurin and associated pathways in cardiac hypertrophy and failure. *Expert Opin Ther Targets*. 2005;9(5):963–973.
156. Shiojima I, Walsh K. Regulation of cardiac growth and coronary angiogenesis by the Akt/PKB signaling pathway. *Genes Dev*. 2006;20(24):3347–3365.
157. Smith CC, Yellon DM. Adipocytokines, cardiovascular pathophysiology and myocardial protection. *Pharmacol Ther*. 2011;129(2):206–219.
158. McCullough PA, Philbin EF, Spertus JA, Kaatz S, Sandberg KR, Weaver WD. Confirmation of a heart failure epidemic: findings from the Resource Utilization Among Congestive Heart Failure (REACH) study. *J Am Coll Cardiol*. 2002;39(1):60–69.
159. Bleumink GS, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. *Eur Heart J*. 2004;25(18):1614–1619.