

Novel Molecular Targets in the Treatment of Cardiac Hypertrophy

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Abstract: Left ventricular hypertrophy represents the heart's response to increased biomechanical stress such as arterial hypertension or valvular heart disease. Cardiac hypertrophy has traditionally been considered a compensatory mechanism required to normalize wall tension and to maintain cardiac output. However, recent clinical studies as well as several animal models have shown that sustained cardiac hypertrophy is rather a maladaptive process, ultimately leading to heart failure and sudden death independent of the underlying cause of hypertrophy. Throughout the past decade, much effort has thus been spent on deciphering the molecular signaling pathways mediating cardiac growth. Identification of novel molecules regulating cardiac hypertrophy could offer the basis for a new generation of cardiovascular drugs. In this review we focus on recent insights into hypertrophic signaling and consider current and emerging approaches to inhibit hypertrophy with the ultimate goal to prevent or delay the onset of heart failure and sudden death in patients.

Keywords: Hypertrophy, calcineurin, Cyclic GMP/PKG-1, phosphoinositide 3-kinase, Akt, glycogen synthase kinase-3, myocyte-enhancer factor 2, histone deacetylases, G_q/G_{11} , small G proteins, MAPK, GP130, Na/H exchanger.

INTRODUCTION

Though many drugs have improved the treatment of cardiovascular disorders during the past decades, heart failure remains one of the leading causes of death in all industrialized nations. The quest for novel treatment options has recently directed attention to cardiac hypertrophy as a promising target, since this approach might provide the opportunity to prevent disease progression rather than treating established cardiomyopathy and heart failure [1, 2]. Two forms of cardiac hypertrophy can be differentiated: While physiological hypertrophy occurs during growth or in response to exercise, pathological cardiac hypertrophy is a response to stress signals that arise from a variety of cardiovascular disorders, including pressure and volume overload due to valvular dysfunction, arterial hypertension, ischemic heart disease or intrinsic contractile abnormalities resulting from sarcomeric protein mutations.

For a long time the traditionally accepted opinion has considered cardiac hypertrophy evoked by pathologic stress as a homeostatic mechanism that is required to normalize left ventricular wall stress and restore cardiac output. However, data collected from numerous studies suggest, that long-lasting cardiac hypertrophy is independently associated with an increased risk of sudden death and heart failure [3, 4]. On the other hand, clinical studies investigating antihypertensive medications have taught us that left ventricular hypertrophy associated with hypertension is principally reversible [5]. Recent reports from the Losartan Intervention for Endpoint reduction in hypertension (LIFE) trial suggested that some types of antihypertensive drugs, such as Angiotensin receptor blockers, are more effective than others in reducing left ventricular mass and that a greater decrease in left

ventricular mass is associated with a favourable clinical outcome [6-8]. These important data, that were obtained using clinically available drugs, indirectly challenge the old dogma of "beneficial" or adaptive hypertrophy. Moreover, data from experimental animal studies also call into question the necessity of a normalization of wall stress that is achieved by hypertrophic growth (as outlined below). However, hypertension is only one of many stimuli that cause cardiac growth, thus a deep understanding of the multitude of signaling pathways, that trigger and modulate hypertrophy seems to be necessary to afford a broad clinical benefit. Particularly essential in this context is the identification and exploration of pathways that mediate maladaptive aspects of hypertrophy like arrhythmogenicity and transformation to heart failure. These pathways might represent rewarding targets for novel treatment strategies, in contrast to other features of hypertrophy that might be essential to maintain contractile function or to avoid cardiomyocyte apoptosis. Here, we thus focus on signaling pathways that hold promise as new and potentially "drugable" targets for antihypertrophic therapies. For an overview of hypertrophic signaling in general, we refer to several recent reviews [9-11].

CALCINEURIN/NUCLEAR FACTOR OF ACTIVATED T CELLS

Calcineurin [12, 13] is a calmodulin-dependent phosphatase that dephosphorylates transcription factors known as NFAT (nuclear factor of activated T cells) [14-16]. It is expressed in multiple tissues and consists of a catalytic A subunit and a regulatory B subunit. Three different calcineurin A subunits (CnA₁, CnA₂, CnA₃) with largely overlapping expression patterns have been described in vertebrates [17]. The role of calcineurin was first discovered in T cells, where calcium/calmodulin-triggered activation of calcineurin leads to dephosphorylation of NFAT, which in turn translocates to the nucleus and directly activate immune response genes (as reviewed by Crabtree and Olson [18]).

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The calcineurin inhibitors cyclosporine A and FK 506 are well established immunosuppressive agents mainly used in solid organ transplantation.

In addition, calcineurin has been shown to play a critical role in cardiomyocyte hypertrophy via that same principal pathway. Constitutive activation of calcineurin in mouse hearts by a transgenic strategy is sufficient to induce massive cardiac enlargement and heart failure [19]. By overexpression of a constitutively nuclear NFAT-3 mutant a similar, though somewhat milder response was observed, suggesting that NFAT is the primary target of calcineurin-dependent signaling in cardiomyocytes as well. While it is well established that calcineurin is sufficient to induce cardiac hypertrophy and heart failure, its necessity in this process has been controversial (reviewed in [9] and [20]). Given that cyclosporine A (CsA) and FK 506 are effective inhibitors of calcineurin, many groups utilized pharmacological approaches to evaluate if calcineurin is required for cardiac hypertrophy. In numerous rodent-based studies pharmacological calcineurin inhibition attenuated agonist-induced hypertrophy as well as hypertrophy due to pressure overload [21-24]. In contrast, other well-controlled studies concluded that calcineurin-inhibitors had no perceptible effect in blocking hypertrophy *in vivo* [25, 26]. These conflicting results might in part be explained by differences in experimental methodology including factors like effective drug dosage, surgical preparations, strain, age and sex of laboratory animals. Additionally, the observed disparities could be explained by a lack of tissue-specificity since calcineurin is widely expressed in tissues other than the heart. For example, recognized systemic side-effects of CsA include nephrotoxicity and hypertension, which would be expected to promote cardiac hypertrophy [27]. Finally, the usage of these immunosuppressives as antihypertrophic agents is further complicated by the fact that much higher dosages of CsA (or FK506) are required to suppress calcineurin activity in the heart compared to T cells [28].

While interpretation of some of these early studies might be difficult in respect to the antihypertrophic effect of pharmacologic calcineurin inhibition, they first challenged the long-held tenet that hypertrophy is an essentially required process in response biomechanical stress: Interestingly, in some studies calcineurin inhibition resulted in abolition of hypertrophy, yet no signs of cardiac compromise were observed [23, 29, 30]. Despite enduring chronically elevated wall stress for several months, ventricular size and function were preserved and no increase in mortality was observed. These findings suggest that hypertrophy is not required for a "compensatory" response to stress signals, and inhibition of hypertrophy might not necessarily be accompanied by adverse effects, such as contractile dysfunction.

A more specific approach to inhibit calcineurin was recently made possible by the discovery of endogenous calcineurin modulators such as AKAP 79, Cabin/Cain, DSCR/MCIP and calsarcin. Employment of these molecules facilitated the modulation of calcineurin activity by genetic means and has confirmed the earlier data implicating calcineurin signaling as a pivotal pathway in regulating hypertrophy:

The scaffolding protein AKAP79 (A kinase-anchoring Protein) [31] directly binds calcineurin which leads to its inactivation [32]. Targeted inhibition of calcineurin with an adenovirus expressing the calcineurin inhibitory domain of AKAP79 attenuated agonist-induced cardiomyocyte hypertrophy *in vitro* [33]. A similar effect was evoked by adenovirus-mediated overexpression of a truncated form of cabin/cain, whose C-terminal domain acts as a non-competitive inhibitor of calcineurin [34, 35]. De Windt *et al.* confirmed these findings by an *in vivo*-approach [36]. Transgene-mediated overexpression of the calcineurin-inhibitory domains of Cain or AKAP79 inhibited calcineurin activity, thereby attenuating both pressure-overload and isoproterenol-induced cardiac hypertrophy. Finally, calcineurin B homology protein (CHP), which shares a high degree of homology to the Ca²⁺-binding regulatory B subunit of calcineurin, has been identified as an inhibitor of calcineurin activity [37]. In contrast to CHP, Cain and AKAP79 are not expressed at significant levels in the heart. Thus, these proteins are most likely not involved in the physiological regulation of cardiac calcineurin activity [35].

In contrast, a family of proteins known as the myocyte-enriched calcineurin-interacting protein family (MCIP), which include MCIP-1 and MCIP-2, are capable of binding to and inhibiting the catalytic A subunit of calcineurin. Thus they may function as endogenous modulators of calcineurin activity in the heart [38-40]. Interestingly, expression of MCIP-1, but not MCIP-2 is induced by activated calcineurin. This interaction is mediated by multiple NFAT-binding sites in the MCIP-1 promoter [41], suggesting that MCIP-1 functions in a feedback inhibition loop to suppress calcineurin activity. Animal studies provide strong evidence for an antihypertrophic role of MCIP in the heart: Cardiac overexpression of MCIP-1 inhibited cardiac hypertrophy, reinduction of fetal gene expression and progression to dilated cardiomyopathy in MCIP-1/calcineurin double-transgenic mice [30]. Likewise, overexpression of MCIP-1 also repressed the hypertrophic response to pressure overload [30, 42]. The role of MCIP-mediated calcineurin inhibition in the heart was further underlined by a recent *in vitro*-study by Pedram *et al.* [43], who demonstrated that estradiol limits agonist-induced cardiac hypertrophy at least in part by upregulation of MCIP-1 and subsequent repression of calcineurin activity. However, investigation of genetic loss of MCIP-1 in the mouse has revealed a more complex functionality of MCIP in the heart [44]. While mice with targeted deletion of MCIP-1 displayed an exacerbated hypertrophic response to activated calcineurin expressed from a muscle-specific transgene, cardiac hypertrophy in response to pressure overload or chronic adrenergic stimulation was paradoxically blunted. In addition, another recent report confirmed that loss of MCIP-1 or -2 in mice significantly blunts hypertrophic growth due to pressure overload, suggesting MCIPs could also function as activators of calcineurin at low "physiological" levels [45]. The dual role of MCIP-1 in hypertrophy might thus depend on the nature of the stimulus as well as the level of MCIP-expression. In addition, a recent report [46] demonstrated that phosphorylation of MCIP by GSK-3 provides another means of switching from calcineurin inhibition to activation.

A new family of calcineurin-interacting proteins, calsarcons [47], was identified by conduction of a yeast two hybrid screen using the CnA subunit as a bait [48]. These proteins (also called FATZ and myozenin) are exclusively expressed in striated muscle and colocalize with calcineurin at the Z-disc, where they also interact with other sarcomeric proteins, including α -actinin, α -filamin and T-Cap [48-51]. Thereby calsarcons provide a potential linkage between the Z-disc, which has been proposed to be a critical point for signaling in striated muscle [52], and the central hypertrophic calcineurin/NFAT pathway. Calсарсin-1 is the only member of the calсарсin-family expressed in the adult heart and slow-twitch skeletal muscle, whereas calсарсin-2 and -3 are expressed in fast-twitch muscle. Calсарсin-1-deficient mice [53], showed no overt phenotype but a marked induction of the fetal gene program, which typically accompanies cardiac hypertrophy. This finding supports the notion that the expression of hypertrophic markers can be dissociated from cardiac growth. Several observations in this animal model suggest that calсарсin-1 derogates calcineurin-mediated hypertrophy *in vivo*: The relative NFAT-binding activity was enhanced in Calсарсin-1-deficient hearts as well as MCIP-1-expression, which is tightly controlled by calcineurin activity [41]. Heart-specific overexpression of calcineurin in calсарсin-1-deficient mice induced massive cardiac enlargement that exceeded hypertrophy induced by the calcineurin transgene alone. Furthermore, calсарсin-1-deficient mice displayed an exaggerated hypertrophic response to pressure overload compared to control animals. In contrast, cardiac adaptation to chronic beta-adrenergic stimulation and exercise was not affected. These findings suggest an important role of calсарсin-1 in modulating calcineurin signaling and cardiac growth in response to only a specific subset of prohypertrophic signals. Further elucidation of this pathway might thus offer a basis for novel antihypertrophic strategies, which target solely the maladaptive aspects of cardiac hypertrophy.

The impact of another Z-disc protein, muscle LIM protein (MLP) [48, 54], on calcineurin signaling was recently described as well [55, 56]. Calcineurin and calсарсin-1 are both dissociated from the Z-disc in heterozygous mutant MLP mice, indicating that MLP is essential for anchorage of the calсарсin-1/calcineurin-complex to the Z disc [56]. In contrast to calсарсin-1, MLP promotes calcineurin signaling, as shown by a blunted transcriptional NFAT-activation in cardiomyocytes of heterozygous mutant MLP-mice. Downregulation of MLP by a specific antisense oligonucleotide prevented the agonist- and stretch-induced increase in NFAT activity, suggesting that MLP might even be required for calcineurin activity in response to specific stress stimuli. The exact molecular nature of the calcineurin/calsарсin/MLP-interaction and its potential for therapeutic modulation, however, remains to be determined.

In a recent study, another mechanism of calcineurin modulation in the heart was identified by characterization of the atrogen-1/muscle atrophy F box (MAFbx) protein [57]. Atrogen-1 is a skeletal/cardiac muscle-specific component of the SCF complex with ubiquitin ligase activity, also consisting of the F-box containing ubiquitin ligases Skpl,

Cull and Roc1 [58, 59]. Ubiquitin ligases function as adaptors, that bind specific substrates to target them for ubiquitin-dependent degradation [57]. A critical role for atrogen-1 was delineated in the pathogenesis of skeletal muscle atrophy [58]. Li and coworkers [57] showed that atrogen-1 co-localizes with calcineurin at the Z-disc, and confirmed a direct atrogen-1/calcineurin interaction by a yeast two-hybrid experiment. Overexpression of atrogen-1 in neonatal cardiomyocytes reduced endogenous CnA levels and calcineurin phosphatase activity by promoting ubiquitination of CnA. Conversely, downregulation of atrogen-1 expression enhanced agonist-induced calcineurin activity and cardiomyocyte hypertrophy. In accordance with these *in vitro* findings, transgenic mice overexpressing atrogen-1 displayed decreased calcineurin protein levels and reduced cardiac hypertrophy in response to thoracic aortic banding [57]. Interestingly, in response to aortic banding atrogen-1-transgenic mice displayed enhanced ventricular dilatation and a dramatic reduction in ejection fraction compared to WT littermates, suggesting a potentially beneficial role of calcineurin/NFAT-signaling at least in this model. Taken together, these *in vivo* studies emphasize the prominent role of the calcineurin/NFAT signaling pathway in cardiac hypertrophy and strongly implicate that calcineurin is not only sufficient to induce cardiac growth but also required. The necessity of calcineurin activation for cardiac hypertrophy is emphasized by genetic studies targeting the calcineurin molecule itself: Cardiac overexpression of a mutated, catalytically inactive calcineurin molecule, which acts as a dominant-negative protein, averts hypertrophy and subsequent development of fibrosis after abdominal aortic constriction [60]. Finally, gene-targeted mice deficient in calcineurin A [61] display a 12% reduction in basal heart size and are largely resistant to diverse hypertrophic stimuli, such as pressure-overload and infusion of AngII or isoproterenol [62]. Moreover, calcineurin is closely related to other pro- and antihypertrophic pathways, such as those controlled by Nitric oxide/cGMP-dependent protein kinase [63, 64], mitogen activated protein (MAP) kinase signaling and glycogen synthase kinase (GSK)-3 [46, 65, 66].

The pivotal role of calcineurin/NFAT-signaling for cardiac growth underlines the attractiveness of modulating this pathway to prevent and eventually treat hypertrophy (Fig. 1). However, at present it is still unclear to what extent a basic activity of calcineurin has to be maintained to prevent potential adverse effects, such as atrophy of the heart. Furthermore the role of calcineurin in physiological vs. pathological hypertrophy needs to be further clarified. For example, exercise-induced hypertrophy was blunted in MCIP-transgenic hearts, suggesting a function of calcineurin in "physiological" hypertrophy [30]. In contrast, Wilkins *et al.* [67] showed a lack of calcineurin/NFAT activation in two models of exercise-induced hypertrophy, implying that calcineurin/NFAT-signaling serves a regulatory role specifically in maladaptive hypertrophy and heart failure.

Likewise, the data concerning the role of calcineurin in the process of stress-induced remodelling of the heart seem to be inconsistent: Calcineurin inhibition by either Atrogen-1- or MCIP-1-overexpression attenuates pressure-overload induced hypertrophy in mice [42, 57]. Yet, mice

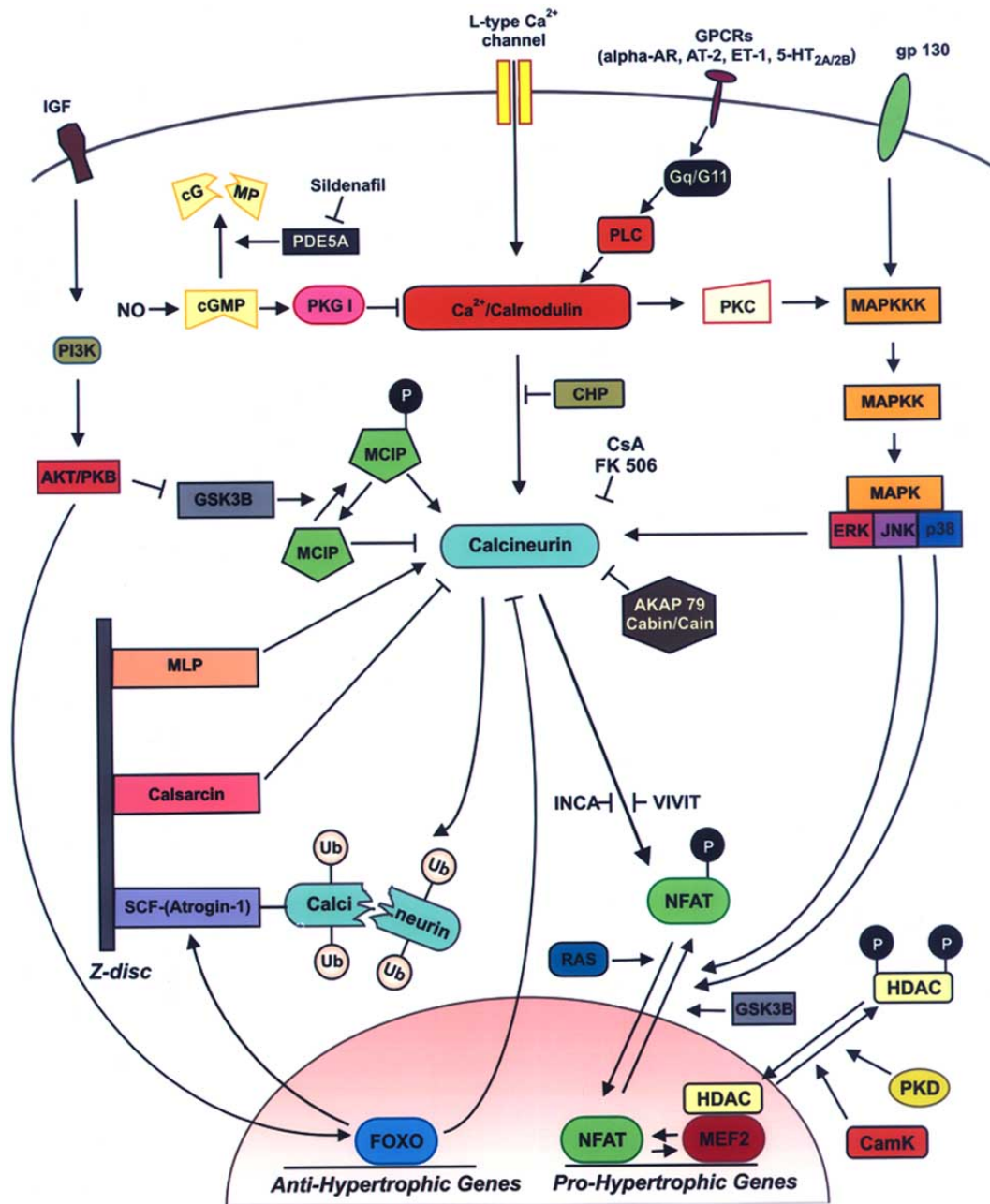


Fig. (1). Molecules and agents that interfere with the calcineurin signaling pathway, providing possible targets for modulating cardiac growth.

overexpressing atrogin-1 displayed a dramatic decrease in cardiac contractility when subjected to aortic banding, whereas mice overexpressing MCIP-1 are clinically healthy with normal cardiac performance as long as 3 months following aortic banding. These apparent discrepancies, however, could be resolved by the notion that atrogin-1 may have other targets in the heart, that might contribute to the observed maladaptive phenotype. In addition, calcineurin is present in several intracellular pools [48, 68], which opens the possibility that calcineurin's subcellular localization determines a differential regulation. Finally, calcineurin-mediated remodeling might also depend on the nature of the

hypertrophic stimulus, given that calcineurin inhibition in a model of experimental myocardial infarction was associated with attenuated hypertrophy yet even *improved* contractile performance [69]. These seemingly converse results point to the need for further studies to elucidate the specific circumstances of calcineurin activation in muscle cells. Clarification of muscle-specific allocation of calcineurin, with special regard to docking-molecules like calsarcin-1, could be a promising approach.

Another strategy to repress calcineurin activity without provoking adverse effects might be the delineation of

downstream targets other than NFAT and a more specific inhibition of calcineurin signaling tailored to particular targets. For example, the synthetic peptide MAGPHPVIVI TGPHEE (VIVIT), selectively inhibits NFAT activation by calcineurin [70]. Overexpression of VIVIT in cardiomyocytes prevents phenylephrin-induced hypertrophy, yet leads to increased cardiomyocyte apoptosis *in vitro* [71], further emphasizing that not all calcineurin-interfering strategies necessarily yield a beneficial response. Recently, Roehrl *et al.* conducted a screen for small molecules that specifically interfere with the calcineurin/NFAT interaction domain, yielding several new organic substances, termed INCAs (inhibitors of NFAT-calcineurin association) [72]. INCAs are potent inhibitors of calcineurin-mediated NFAT-activation, however, their role in the attenuation of cardiac hypertrophy remains to be determined.

The potential value of small molecule screens for calcineurin-modulating substances was also demonstrated by Bush and colleagues [73]. Conducting a screen for small molecules upregulating MCIP1 expression in muscle cells, a new 4-aminopyridine derivative named PAMH was identified. PAMH, which contains an entrenched structural motif of serotonin (5-hydroxytryptamine, 5-HT), was shown to markedly induce cardiomyocyte hypertrophy and the fetal gene program via activation of calcineurin/NFAT signaling. PAMH activity is competed for by antagonists of 5-HT_{2A/2B} receptors, indicating that PAMH activity is mediated by ligand-binding to this receptor family. This experiment is a nice proof of concept that small molecule screens are not only useful in the identification of lead substances for a desired therapeutic effect, but can also provide unexpected mechanistic insight.

CYCLIC GMP/ PKG-1

The cGMP/PKG-1 signaling pathway has recently emerged as another promising target for antihypertrophic interventions. Cyclic GMP (cGMP) is the second messenger for membrane-bound guanylate-cyclase receptors, the principal receptors for Atrial natriuretic peptide (ANP) or B-type natriuretic peptide (BNP) as well as the ubiquitous gas nitric oxide (NO) [64, 74]. ANP is a hormone synthesized and secreted by the heart. While vasorelaxing and thereby blood pressure-lowering properties of this molecule may also have indirect antihypertrophic effects, it has been shown that ANP directly inhibits cardiomyocyte hypertrophy *in vitro* [75]. Moreover, cardiomyocyte-restricted deficiency of guanylyl-cyclase-A (GC-A) in mice leads to cardiac hypertrophy already under resting conditions, which is exaggerated upon pressure overload due to aortic banding [76].

Similar to ANP, the messenger molecule NO stimulates cGMP formation via activation of soluble guanylyl cyclase. In experiments conducted in cultured cardiomyocytes, NO-dependent cGMP formation has demonstrated antihypertrophic effects [77]. Conversely, transgenic mice lacking the endothelial form of NO-Synthase display increased cardiac growth in response to pressure overload, suggesting an antihypertrophic effect of NO *in vivo* [78].

A major downstream effector of cGMP-signaling in cardiomyocytes is the cGMP dependent protein kinase type I

(PKG-I) [79]. PKG-1 mediates many of the effects of NO in different tissues, such as vascular smooth muscle cell dilatation [80]. So what are the mechanisms that mediate the antihypertrophic properties of PKG-1 in the heart? In this regard, recent studies demonstrated that cGMP-induced PKG-1 activation reduces NFAT nuclear translocation and transcriptional activity following phenylephrine (PE) stimulation of cardiomyocytes [79, 81]. This inhibitory effect on calcineurin/NFAT signaling seems to occur upstream of calcineurin, since NFAT-activation and cardiomyocyte hypertrophy evoked by adenoviral expression of a constitutively active calcineurin mutant were not affected by PKG-1 signaling [81]. However, the molecular target that mediates calcineurin-inhibition by PKG-1 remains to be revealed. In this regard, several calcium regulating proteins have been identified as substrates of PKG-1 in the heart, including the L-type calcium channel, the ryanodine receptor calcium release channel, and troponin I [74]. Studies conducted in vascular smooth muscle cells demonstrate that PKG-1 activates the regulator of G-protein signaling (RGS2), which interrupts cellular calcium signaling [82]. A similar mechanism in cardiomyocytes could contribute to antihypertrophic properties of PKG-1. Further exploration of cGMP/PKG-1 signaling could reveal new therapeutic strategies to modulate cardiac hypertrophy: For example, Takimoto *et al.* [83] recently showed that Sildenafil can suppress cardiac hypertrophy *in vivo*. Sildenafil, which is patented and in wide clinical use for the treatment of erectile dysfunction, raises intracellular cGMP levels by inhibiting PDE5A, a phosphodiesterase, that degrades cGMP [84-89]. Remarkably, mice treated with Sildenafil displayed not only a diminished hypertrophic response to pressure overload induced by transthoracic aortic banding (TAC), but also an improved contractile function compared to WT littermates. Moreover, Sildenafil treatment was also sufficient to reverse established hypertrophy induced by TAC [83]. The underlying molecular mechanisms include deactivation of calcineurin/NFAT-, PI3K/Akt-, as well as ERK1/2-signaling. Moreover, a very recent report also suggested inhibition of RhoA/rho kinase (ROCK, see below) as an additional mechanism by which prevention of cGMP catabolism by Sildenafil results in blunted cardiomyocyte hypertrophy [90]. At present, it is not clear if the beneficial effects of sildenafil on pressure overload-induced hypertrophy can be reproduced in humans, yet the promising data by Takimoto and colleagues certainly deserve further investigation.

PHOSPHOINOSITIDE 3-KINASE/ AKT/ GLYCOGEN SYNTHASE KINASE-3

Growing evidence suggests that normal cardiac growth and exercise-induced hypertrophy are in large part regulated by the PI3K/Akt pathway (reviewed in [91]). The Phosphoinositide 3-kinase (PI3K)-family of enzymes that display both protein and lipid kinase activity, have been linked to signaling in many cellular functions, such as growth, survival and proliferation [92]. PI3K is activated by receptor tyrosine kinases, such as the IGF-1 receptor, as well as G protein-coupled receptors (GPCRs), including α -[93] and β -adrenergic receptors [94, 95]. Overexpression of a constitutively active PI3K (p110) mutant led to cardiac hypertrophy in transgenic mice [96]. Conversely, expression

of a dominant-negative form of p110 ((DN)-p110) led to significantly reduced heart weight/body weight ratios in transgenic mice. Moreover, (DN)-p110 mice displayed significant hypertrophy in response to aortic banding but not to exercise training, suggesting a critical role of p110 – signaling in physiological but not pathological cardiac growth [97, 98]. Interestingly, banded (DN)-p110 mice showed substantial cardiac dysfunction, implying that p110 is also essential for the preservation of contractile function in the face of stress stimuli. Another study showed that cardiomyocyte-specific inactivation of the general PI3K antagonist PTEN [99, 100] results in cardiac hypertrophy, but unexpectedly a dramatic decrease in cardiac contractility was observed as well [101]. Further analysis of double mutant mice revealed that pathways for hypertrophic growth and contractile function can be dissociated *in vivo*: While both p110 and p110 mediate cardiac growth, p110 negatively modulates cardiac contractile function by inhibiting cAMP production [98, 101]. This maladaptive impact on cardiac contractility seems to outweigh possible positive effects of p110 on systolic/diastolic function in PTEN-deficient mice. Thus, an approach of general PI3K inactivation to inhibit hypertrophy appears less attractive. In contrast, Oudit and coworkers [98] could demonstrate, that PI3K is critical for the induction of myocardial hypertrophy, interstitial fibrosis and cardiac dysfunction in response to α -adrenergic stimulation. This link between α -adrenergic signaling and the PI3K/Akt Pathway could therefore represent a more promising therapeutic target for the treatment of the transition from hypertrophy to heart failure.

Many pro-hypertrophic stimuli have been shown to activate the serine/threonine kinase Akt, (also known as protein kinase B (PKB)), and PI3K-activation is an important common step in this process [91, 102, 103]. Of the 3 Akt genes, only Akt-1 and -2 are expressed at substantial levels in the heart. It is well established that transgenic overexpression of Akt/PKB is sufficient to induce significant cardiac hypertrophy in mice with [104] or without [105, 106] affecting systolic function. A recent study provided deeper insights into Akt-dependent hypertrophy and the transition from compensated hypertrophy to heart failure. Shiojima *et al.* [107] demonstrated that short-term cardiac-specific inducible overexpression of Akt-1 leads to a compensated state of hypertrophy with preserved contractility and regression of hypertrophy after suspension of the stimulus. This phenotype mimics features of physiologic hypertrophy. In contrast, long-term overexpression of an activated Akt-1 gene induced contractile dysfunction and dilated cardiomyopathy. Interestingly, coronary angiogenesis was enhanced during the acute phase of heart growth but impaired in the prolonged phase, which led to decreased capillary density and worsened cardiomyopathy. The enhanced angiogenesis in the acute phase was associated with mTOR-dependent (s. below) induction of vascular endothelial growth factor (VEGF) and angiotensin-2 expression, while VEGF expression was suppressed upon decompensated hypertrophy. On the one hand these findings thus reveal an important role for Akt-signaling in cardiac angiogenesis, on the other hand they support the notion that ineffective angiogenesis might contribute to the transition

from hypertrophy to heart failure. Factors like mTOR and VEGF, that promote angiogenesis, could therefore be interesting targets for the prevention and/or treatment of maladaptive hypertrophy.

What are the mediators of PI3K/Akt signaling? At least two direct target proteins participate in cardiac growth: mammalian target of Rapamycin (mTOR) and Glycogen synthase kinase 3 (GSK-3) [108, 109]. Rapamycin, an immunosuppressive macrolide, binds to FKBP12, thereby forming a complex that subsequently binds to and inhibits mTOR, a large serine/threonine kinase (290 kDa) [110]. Although the detailed mechanism underlying mTOR activation by AKT/PKB remains the subject of controversy, the involvement of the tuberous sclerosis genes TSC-1 and TSC-2 in this process was demonstrated in numerous studies (reviewed in [111]). Overexpression of TSC-1/2 impairs the regulation of downstream mTOR-targets [112]. TSC-2 is phosphorylated at at least two sites by Akt and its phosphorylation apparently alleviates the inhibitory effects of TSC-1/2 on mTOR signaling. The influence of TSC-1/2 on cardiac hypertrophy is not sufficiently explored yet, but a negative regulation of cell size has been attributed to these proteins in different cell types [113], suggesting a similar role in cardiomyocytes. The significance of mTOR in regulating cardiac growth is established by its ability to inhibit protein synthesis via the translational effectors 4E-BP1 and p70 ribosomal S6 kinases (S6K1) [114], (reviewed in [91, 115]). In isolated cardiomyocytes, Rapamycin inhibited hypertrophy-associated protein synthesis but not activation of hypertrophy-associated genes [116, 117]. *In vivo*, Rapamycin blocked mTOR/S6K1-dependent phosphorylation of S6K and blunted the development of pressure overload hypertrophy induced by aortic banding. Moreover, Rapamycin also regressed established hypertrophy [114, 118]. However, Rapamycin was more effective in reverting pressure-overload-induced hypertrophy in compensated hearts than in hearts with significant contractile dysfunction and advanced adverse remodeling [114]. These findings suggest that the mTOR-S6K1-pathway may be activated during compensated hypertrophy, but alternative pathways are recruited when transition from compensated to decompensated hypertrophy occurs. In accordance with this notion, one study demonstrated that genetic deletion of S6K1 in mice alone had no impact on development of hypertrophy, directing the view to alternative targets of mTOR-dependent signaling [114]. Further studies will be necessary to determine if treatment with Rapamycin, which is already in clinical use as a therapy for transplant rejection, could be a therapeutic tool to regress myocardial hypertrophy.

In addition to mTOR, another downstream target of PI3K signaling is GSK-3, a widely expressed kinase that phosphorylates transcription factors of the NFAT family, thereby promoting their translocation to the cytoplasm where they are inactive [119]. The activity of GSK-3 is controlled by the phosphorylation status of serine-9. Several protein kinases, including Akt, inactivate GSK-3 by phosphorylation of serine-9. Various *in vitro*- and *in vivo*-studies have indicated that GSK-3 is a central endogenous negative regulator of cardiac hypertrophy in response to many stress stimuli, like pressure overload, ET-1, PE, Isoproterenol and Fas-signaling [120-124]. In addition,

GSK-3 has also been found to be a regulator of physiological cardiac growth [125]. Moreover, Sanbe and colleagues [126] could recently demonstrate, that GSK-3 is also sufficient to partially revert established pressure-overload-induced hypertrophy.

In contrast to other kinases, GSK-3 is highly active in unstimulated cells and is inactivated by hypertrophic agonists. GSK-3 negatively regulates most of its targets, therefore inhibition of GSK-3 by growth stimuli abrogates the tonic inhibition of its substrates. In addition to the NFAT family of transcription factors, several other transcription factors involved in the hypertrophic response are phosphorylated by GSK-3, including GATA-4 [124], eukaryotic initiation factor (EIF) [127] and β -catenin [128]. Given that GSK-3 phosphorylates and inactivates NFAT, it is tempting to refer to the protein kinase GSK-3 as the opponent of the central phosphatase that positively regulates cardiac growth, calcineurin. In fact, overexpression of GSK-3 in calcineurin-transgenic mice significantly diminished cardiac hypertrophy [120]. Interestingly, despite inhibition of cardiac hypertrophy, these mice displayed increased levels of ANF and B-type natriuretic peptide expression compared to mice solely overexpressing calcineurin. These surprising findings differ from *in vitro* experiments in neonatal cardiomyocytes, in which ET-1-induced expression of ANF could be inhibited by activated GSK-3 [122]. Similarly, a dominant-negative PI3K-mutation, which would be expected to result in enhanced GSK-3 activity, also upregulated ANF expression *in vivo*, while suppressing cardiac growth [104]. ANF and its receptor have been demonstrated to possess antihypertrophic activity [77]. It is thus interesting to consider whether its up-regulation by GSK-3 contributes to the anti-hypertrophic effects mediated by this kinase.

The intimate connection of the GSK-3- and calcineurin/NFAT signaling pathways is further demonstrated by the fact that GSK-3 directly phosphorylates the calcineurin interacting protein (MCIP-1) [46, 129]. GSK-3's important role in the process of stress-induced remodeling is also underlined by a recent study from Lembo's group:

Melusin, a muscle-specific protein that interacts with the integrin α -cytoplasmic domain, is a necessary element of the hypertrophic response to chronic pressure overload [130]. Melusin-dependent signaling is likely to prevent maladaptive remodeling in this context, which is demonstrated by contractile dysfunction and dilated cardiomyopathy in melusin-null-mice subjected to aortic banding. Phosphorylation of GSK-3 was specifically blunted in melusin-null hearts. Conversely, transgenic mice overexpressing melusin in the heart showed an increased phosphorylation of Akt and GSK-3 and a better contractile function after 12 weeks of pressure overload compared to WT mice [131]. Moreover, left ventricles of melusin-transgenic mice displayed a very low level of cardiomyocyte apoptosis as well as an increased capillary density compared to WT animals. These results suggest that melusin-dependent signaling protects from the transition to cardiac failure in response to long-term pressure overload. Akt activation/GSK-3 inactivation could thus be beneficial at least in this context. One explanation for these findings could

be the positive impact of Akt/mTor-signaling on cardiac angiogenesis, that was shown to be advantageous in the setting of pressure-induced hypertrophy [107]. Another issue to be addressed is the direct effect of GSK-3 on cardiac function. A recent study demonstrated, that GSK-3 negatively regulates contractile function by downregulation of the sarcoplasmic reticulum calcium ATPase (SERCA2a), which leads to abnormal calcium handling and impaired diastolic relaxation [125]. These findings have to be taken into consideration when new therapeutic strategies that modulate GSK-3-signaling are developed. Further explorations of the effects of GSK-3 on cardiac function could help to identify ways to inhibit cardiac hypertrophy without negatively affecting remodeling of the heart and systolic or diastolic function.

Very recent observations by Ni *et al.* [132] revealed the Forkhead box O (FOXO) transcription factors 1 and 3a as targets of PI3K/Akt signaling. FOXO-1 and -3a are inactivated by hemodynamic stress in hearts undergoing hypertrophic growth through a mechanism requiring the PI3K/Akt pathway. Accordingly, overexpression of Foxo-1 proteins in cardiomyocytes blocked both angiotensin II-induced growth and calcineurin activation. This mechanism could involve the activation of the atrogen-1 promoter [133, 134]. FOXO transcription factors could therefore represent new interesting targets to modulate cardiac hypertrophy *in vivo*.

TRANSCRIPTIONAL CONTROL OF CARDIAC HYPERTROPHY BY MEF2/HDAC

An emerging body of evidence suggests that the transcription factor Myocyte Enhancer Factor 2 (MEF2) [135], which integrates multiple Ca^{2+} /calmodulin-dependent signaling pathways in muscle cells as well as neurons, is a common downstream target of redundant hypertrophic stimuli in the heart (reviewed in [136-138]). There are four MEF2 genes encoding distinct MEF2 proteins (MEF2A-D) in vertebrates. Of these, only two isoforms, MEF2A and MEF2D are expressed at significant levels in the adult myocardium. On the transcriptional level, MEF2 displays only low basal activity, which might be required for the maintenance of energy metabolism and contractile protein gene expression [139]. Rapid activation, however, is induced upon upstream stimulation [140]. In the context of myocardial hypertrophy, MEF2 is regulated by a plethora of signaling pathways including MAPKs and calcineurin [137]. The transcriptional activity of MEF2 is enhanced by interaction with other transcription factors like GATA-4 and NFAT, which synergize with MEF2 to recruit the cofactor p300 [141].

Recent studies demonstrated that MEF2 activity is controlled by a direct association with histone deacetylases (HDACs) [142-149]. HDACs deacetylate nucleosomal histones, thereby promoting chromatin condensation and transcriptional repression when recruited to target genes via binding of specific transcription factors such as MEF2. As a counterpoise to HDAC activity, histone acetyltransferases (HATs) relax chromatin and thereby activate target genes. HDACs fall into three different classes, based on their homology with three distinct yeast HDACs. In the heart, class II HDACs play a prominent role, as four members of

this group (4,5,7,9) are enriched in this organ. The N-terminal region of these proteins contains two conserved CaMK/PKC/PKD (s. below) phosphorylation sites [137]. Phosphorylation of these sites induces HDAC nuclear export, resulting in disruption of MEF-2-HDAC-complexes and derepression of HDAC target genes [150]. Transgenic overexpression of CaMK stimulates MEF2 activity and is sufficient to induce cardiac hypertrophy *in vivo*, [151, 152] implicating the HDAC-MEF2 axis as a potential therapeutic target to control pathological cardiac signaling [153].

Consistent with this notion, mutant forms of HDAC5 or HDAC9, that lack the regulatory serine residues, render cardiomyocytes resistant to serum- or PE-induced fetal gene expression and cardiomyocyte hypertrophy. Mice lacking HDAC9 or HDAC5 display normal cardiac size and function at early age, but develop spontaneous cardiac hypertrophy at advanced age [154, 155]. Importantly, these animals respond to either thoracic aortic banding or chronic calcineurin activation with exaggerated hypertrophy and superinduction of fetal genes.

Recently, Vega and coworkers [156] demonstrated that Protein kinase D (PKD) [157], a downstream effector of Protein kinase C (PKC), directly phosphorylates HDAC5 and stimulates its nuclear export, thereby inducing cardiac hypertrophy. This process seems to be mediated by the calcium-independent PKC-isoforms PKC δ and PKC ζ , since overexpression of these isoforms induces nuclear export of HDAC5, whereas calcium-dependent PKC isoforms and have no effect on its subcellular localization. Phosphorylation of HDAC5 is likely to be required for PKC-signaling in cardiomyocyte hypertrophy, since expression of a nonphosphorylatable HDAC5 mutant prevents hypertrophy in response to pharmaceutical activators of PKC. Intriguingly, PKC-signaling leads to phosphorylation of the same sites of HDAC5 that were shown to be phosphorylated by CaMK [158]. Thus HDAC5/9 might represent a point of convergence for miscellaneous calcium-dependent and -independent hypertrophic signaling pathways. By testing the effect of different PKC-inhibitors on HDAC5 nuclear export, Vega *et al.* [156] could further show that ET-1- and serum-induced HDAC-phosphorylation was not effected by the general PKC inhibitors Bis-I and Gö-6983, but efficiently blocked by the direct PKD-inhibitor Gö-6976, implying that alternative pathways of PKD-activation must exist. In this regard, PKD can also be activated by the G β subunits of the heterotrimeric G proteins [159].

Taken together, these data support the notion that class II HDACs constitute the key integrators of hypertrophic stimuli and that small molecular inhibitors of HDAC phosphorylation could be a powerful therapeutic tool to prevent cardiac growth [153]. The therapeutic attractiveness of modulating HDAC activity is further highlighted by the fact that inactivation of class II HDACs is likely to be specifically involved in the regulation of pathological cardiac growth while physiological growth of the myocardium is not affected [153]. However, in order to specifically target HDAC activity, the complex network of HDAC signaling, in particular the role of class I HDACs, needs further investigation. A recent study demonstrated that unspecific chemical inhibitors of both class I and II HDACs, such as

hydroxamic acid trichostatin A (TSA) repress the increase in cell size induced by hypertrophic agonists [160]. How can this seemingly paradoxical result be reconciled with the ability of class II HDACs to inhibit cardiac growth? It is conceivable that one or several class I HDACs might counteract class II HDACs by repressing anti-hypertrophic genes. If these gene products function dominantly over the genes suppressed by class II HDACs, general HDAC blockage would result in attenuated hypertrophy [136, 161]. However, the components of this potential anti-hypertrophic network remain to be determined. Identification of novel isoform-selective class I HDAC inhibitors [162, 163] could provide appropriate tools to investigate the role of class I HDACs in cardiac hypertrophy and to explore new therapeutic options to modulate cardiac hypertrophy.

G-PROTEIN COUPLED RECEPTORS: G $_q$ /G $_{11}$ -SIGNALING

Myocardial G-protein-coupled receptors (GPCRs) [164] represent a group of seven-transmembrane-spanning-domain-receptors, which play an important role in the regulation of acute hemodynamic and chronic myotrophic effects within the cardiovascular system (reviewed in [11, 91, 165]). Three functional classes of GPCRs principally correspond to three major classes of heterotrimeric GTP-binding proteins: α -adrenergic receptors signal primarily to G $_s$, cholinergic receptors are typically connected to G $_i$, while members of the third class, including angiotensin II, endothelin, and β -adrenergic receptors, are linked to G $_q$ and the functionally redundant G $_{11}$. All heterotrimeric G proteins consist of the subunits G α and G β , which dissociate upon receptor activation and independently activate intracellular signaling pathways. The role of G $_q$ /G $_{11}$ in cardiac growth has been explored intensely (reviewed in [166]).

Activation of G $_q$ -coupled receptors induces hypertrophy *in vitro* [167]. Subsequent studies utilizing *in vivo* approaches, including transgenic overexpression and conditional knockouts, have confirmed that G $_q$ /G $_{11}$ signaling is both required for pressure overload hypertrophy [168, 169] as well as sufficient [168, 169] to provoke hypertrophy in the absence of hemodynamic stress [167, 170]. Thus, G $_q$ signaling seems to be particularly involved in maladaptive cardiac growth. In contrast, G $_q$ -ablated mice displayed significantly attenuated deterioration of ventricular function compared to WT littermates, despite continuously elevated wall stress due to the absence of hypertrophy [171]. These results once again support the notion that hypertrophy is not a necessary compensatory response to stress stimuli.

What are the downstream effectors of G $_q$ signaling in the heart? A well-established target triggered by G $_q$ /G $_{11}$ is activation of Phospholipase C (PLC), which leads to generation of diacylglycerols via an IP $_3$ -mediated increase in cytosolic [Ca $^{2+}$] [11]. Prolonged elevation of intracellular [Ca $^{2+}$]-levels activates the central mediator of hypertrophy, calcineurin. Another mediator of G $_q$ -signaling, that has become focus of recent investigations, is the protein kinase C (PKC) family, which is comprised of at least 12 members, that share structural homology and induce a great variety of intracellular responses when activated by lipid products of phospholipase C or D activity. PKC activation results in translocation of these enzymes to isoform-specific

intracellular locations [165] and binding to isoform-specific anchoring-proteins, labeled as receptors for activated C kinases (RACKs) [172]. Although multiple studies implicate the various PKC isoforms in the pathogenesis of cardiac hypertrophy, the definite role of each isoform in cardiac growth has not yet been fully elucidated: Phorbol esters, such as PMA, activate PKC and mimic the prohypertrophic effects of PE-mediated PKC-activation on cultured cardiomyocytes. While Braz *et al.* [173] showed that PKC is both required and sufficient for cardiomyocyte hypertrophy *in vitro*, other studies demonstrated that PKC seems to be more important as a regulator of myocardial contractility than cardiac growth [174, 175]. Similarly, animal models overexpressing PKC yielded conflicting results regarding its sufficiency and necessity to induce and mediate hypertrophy (reviewed in [91] with references therein), further complicating the issue of the relative importance of PKC isoforms in hypertrophic signaling.

Studies employing genetic ablation of single PKC-isoforms are likely to be limited by isoform crosstalk and overlapping functions, whereas effects of genetic overexpression of individual isoforms may be perturbed by altered intracellular locations compared to endogenous proteins. Thus targeting of intracellular PKC-docking sites seems to be a promising approach for the investigation of specific PKC-isoforms. In this regard, Arya and Coworkers recently introduced a new anti-hypertrophic pathway regulated by muscle ring finger protein-1 (MURF-1) [176]. This protein co-localizes and interacts with the scaffolding protein RACK-1. The PKC-isoform PKC δ is known to be a mediator of G_q -induced cardiac hypertrophy [170], at least in part by its membrane anchoring protein RACK-1 [177]. By binding to RACK-1, MURF-1 blocks PKC translocation to focal adhesions and inhibits PE-induced hypertrophy of cardiomyocytes *in vitro* [176]. In addition, MURF-1 inhibits the tyrosine phosphorylation of Focal adhesion kinase (FAK), a primary mediator of integrin signaling, that is induced by PKC [178] and plays a role in PE- and ET1-induced hypertrophy [179, 180]. Consistent with this finding, overexpression of MURF-1, as well as dominant-negative inhibition of FAK, blunt adrenergic ERK1/2 activation and ERK-dependent hypertrophy. Another downstream target of PKC is protein kinase D1 (PKD-1) [181]. Upon stimulation by GPCR agonists PKD-1 is activated by PKC and translocates to the Z-disc. This process was shown to be essential for GPCR-agonist induced hypertrophy in neonatal cardiomyocytes [181].

Besides modifying PKC-signaling, G_q/G_{11} proteins also modulate the PI3K/Akt signaling pathway via interaction with the catalytic PI3K subunit p110 [182]. In contrast to class p110 (as outlined above), p110 contains two binding sites for subunits of G_q/G_{11} . Association of G_q -subunits to p110 following GPCR stimulation leads to translocation from cytosol to the cell membrane and subsequent kinase activation [101]. P110 was shown to participate in two distinct signaling pathways. (1) a kinase-dependent pathway that controls PKB/AKT as well as MAPK phosphorylation, and seems to be beneficial in remodeling after pressure overload-induced hypertrophy [183], and (2) a kinase-independent activity that negatively modulates cardiac contractility. Specific inhibition of this maladaptive

consequence of P110 activation could be a promising goal of future therapeutic strategies to prevent the transition from hypertrophy to heart failure. In summary, these data demonstrate a crucial role of G_q/G_{11} in mediating maladaptive cardiac growth in response to pathological stress stimuli.

SMALL G PROTEINS

Multiple studies have demonstrated that the family of small G proteins functions as an important link between cell membrane receptors and numerous signaling pathways (reviewed in [184]). Thus, the involvement of small G proteins in cardiac hypertrophy has become an area of growing interest. This class of proteins comprises several members with a relative molecular mass of 21 kDa, which are activated by binding of GTP. The GTPase activity of small G proteins hydrolyzes GTP to GDP, thereby returning the molecules to their inactive state. This process is regulated by guanine nucleotide exchange factors (GEF's) and GTPase activating proteins (GAP's). Five subfamilies of small G proteins have been described (Rho, Ras, ARFs, Rab, Ran). Of these families, the role of Ras and Rho in cardiac hypertrophy has been examined in most detail.

Ras is activated by a variety of G_q/G_{11} linked agonists, like angiotensin II, ET-1 and PE, each of which is sufficient to induce cardiac hypertrophy [115]. The mechanism by which GPCRs regulate ras is not fully understood, probably this process involves PKC-activation [184]. Expression of a constitutively active Ras mutation in cardiomyocytes results in hypertrophic gene expression [185], likewise transgenic animals overexpressing this mutation display a significant increase in cardiac mass [186]. In contrast, dominant-negative Ras mutants avert PE-induced hypertrophy in cultured cardiomyocytes [187]. Ras-signaling is mediated by potent pro-hypertrophic downstream effectors, including c-Raf [188-191] (MAPK kinase kinase of the ERK cascade) [192], PI3K, the RAL-GDP-dissociation stimulator (Ral-GDS/rac) [193], and the calcineurin/NFAT-pathway [194, 195][Proud 2004, Ichida 2001 *et al.*]. Ras was also reported to activate the c-Jun N-terminal kinases (JNKs) in cardiomyocytes [196]. Recent studies revealed further insight into the mechanisms and pathways by which Ras-dependent cardiac growth is mediated: using a dominant-negative Ras mutant and inhibitors of Ras isoprenylation, Wang and Proud [197] demonstrated, that Ras is important for PE-induced activation of protein synthesis in adult cardiomyocytes, most likely via induction of the raf/MEK/ERK pathway. A second downstream clue for Ras-induced hypertrophic growth was introduced by Sano *et al.* [198]: This mechanism entails a global increase in RNA content per cell, which is regulated by RNA polymerase II (pol II). Phosphorylation of pol II in its carboxy-terminal domain (CTD) is a critical step of messenger RNA production [199]. Ras-dependent signals for hypertrophy were demonstrated to augment phosphorylation of the CTD [200] by the cyclin-dependent kinases Cdk7 and Cdk9 [201]. This process occurs independently of the fetal gene program that is typical of maladaptive growth [199]. The recently uncovered dual role of Cdk9 [202, 203] as a promoter of hypertrophic growth as well as a negative regulator of mitochondrial function [199] underline the potential therapeutic benefit of novel molecules that inhibit

Cdk9 to modulate cardiac hypertrophy and subsequent heart failure (Table 1).

The Rho family of small G proteins, consisting of Rho, Rac, and Cdc42 subfamilies, has been shown to both regulate the cytoskeletal organization of cardiomyocytes [204] as well as to modulate cardiac growth via augmentation of hypertrophic gene expression [205, 206]. The specific rho-dependent signaling pathways that induce cardiomyocyte hypertrophy are not fully understood. Rho activates several protein kinases, in particular Rho-associated kinase (ROCK) [207, 208], and facilitates GATA-4-transcriptional activity to provoke hypertrophy in neonatal rat cardiomyocytes [205]. ROCK-selective inhibitors and dominant negative ROCK-mutations blunt PE-, ET-1, or RhoA-induced hypertrophy [204, 209, 210]. Moreover, fasudil, a ROCK inhibitor, was also shown to inhibit angiotensin II-induced hypertrophy in apolipoprotein E-deficient mice [211]. In contrast, transgenic mouse hearts overexpressing RhoA displayed no change in left ventricular size [210].

Rac, another member of the Rho family of small G proteins participates in cardiac growth as well [212]: Constitutive activation of Rac-1 in cardiomyocytes *in vitro* [212] and *in vivo* [213] results in hypertrophy, while a dominant-negative Rac mutant (N17rac1) prevents PE-induced increases in protein synthesis as well as cardiomyocyte size. Potential mechanisms for these findings include activation of the ERK and/or JNK pathway [214] and alterations in focal adhesions [213]. In this regard, overexpression of a dominant-negative focal adhesions kinase in cultured cardiomyocytes was sufficient to attenuate the ET-1-induced [179] and PE-induced [180] increase of cardiomyocyte size as well as the induction of ANF.

While the importance of the Ras and Rho subfamilies of small g proteins in cardiac growth has been confirmed in numerous studies, a definite role of the Rab family in common causes of hypertrophy, such as pressure overload, remains to be established. Wu *et al.* [215] demonstrated that overexpression of Rab-1a (a small G protein that regulates vesicle transport from the endoplasmic reticulum to Golgi) in transgenic mice was sufficient to induce cardiac hypertrophy *in vivo*. This process involved upregulated expression and altered subcellular localization of several PKC isoforms, and ultimately led to cardiac dysfunction and heart failure, implying a maladaptive nature of Rab-mediated hypertrophy.

A novel interesting approach to inhibit small G protein activity in cardiac hypertrophy was recently proposed. activation of small G proteins necessitates covalent binding of isoprenoid intermediates (isoprenylation). This step is inhibited by the cholesterol lowering drug class of statins (HMG-CoA-reductase inhibitors). Adoption of these drugs into treatment of vascular events is well established. Beyond this, therapeutic properties of statins that exceed cholesterol lowering effects, have recently come into the focus of attention. Numerous studies revealed that statins are able to inhibit hypertrophy. In cardiomyocytes, statin treatment blunts both angiotensin II-induced [216] and PE-induced hypertrophy [217]. *In vivo*, Simvastatin substantially depletes the hypertrophic response to pressure overload due to transthoracic aortic banding [218]. Another HMG-

CoA-reductase inhibitor, cerivastatin, partly rescues the hypertrophic and cardiomyopathic phenotype of a double transgenic rat overexpressing renin and angiotensinogen [219]. Thus, in addition to antihypertrophic properties, statins also seem to withhold beneficial effects in the context of myocardial remodeling. This notion is supported by studies which demonstrate, that Fluvostatin augments survival in a murine model of myocardial infarction [220] and that Simvastatin derogates both cardiac hypertrophy and fibrosis in transgenic rabbits that overexpress a -MHC mutation, implying therapeutic benefits of statins that exceed the prevention of vascular events. Interestingly, a recent study in patients with diastolic heart failure demonstrated a beneficial influence of statin therapy on mortality, that was independent of cholesterol-lowering effects [221]. Although the authors did not directly correlate clinical outcomes to alterations of left ventricular mass, it might be tempting to speculate that antihypertrophic effects of statins contribute to the improved survival of the treated patients.

MAPK PATHWAYS

Numerous substances and molecules have been patented recently that inhibit the kinase activity of members of the mitogen-activated protein kinases (MAPKs) superfamily. Growing evidence suggests that modulation of the complex network of these signaling cascades could be a rewarding approach to treat cardiomyocyte hypertrophy and heart failure. The MAPKs are elements of three-tiered protein kinase cascades and comprise basically three subfamilies, the extracellularly responsive kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 MAPKs. While the ERKs are particularly implicated in growth-associated responses, the latter two groups are generally activated by cytotoxic stress factors [214].

All three major branches of MAPK signaling can be blocked by overexpression of MAPK phosphatase-1 (MKP-1), which was shown to prevent agonist-induced cardiomyocyte hypertrophy *in vitro* and pressure overload-associated hypertrophy *in vivo* [222], suggesting a significant role of MAPK-signaling in cardiac growth. In this context, the ERK1/2 signaling cascade deserves special interest due to its pro-hypertrophic effects in cardiomyocytes as well as recent molecular approaches to modulate this pathway.

Several studies have shown that hypertrophic agonists, such as ET-1 or PE, activate ERKs through PKC mediated Ras-activation, which subsequently leads to cardiac myocyte hypertrophy [223-225]. Moreover, diminution of ERK1/2 *via* pharmacological inhibition of MEK1/2 MAPK kinases, which specifically activate ERK1/2 but not JNKs or p38 MAPKs) decreases the hypertrophic response to agonist-stimulation in cardiac myocytes [226]. Consequently, transgenic overexpression of MEK1 in mice, provokes substantial cardiac hypertrophy *in vivo* [227]. Remarkably, these mice exhibit supernormal systolic function but impaired diastolic function, resembling the phenotype of patients with hypertrophic obstructive cardiomyopathy. Other recent studies confirmed that ERK-activation leads to maladaptive cardiac remodeling: One study by Shibata *et al.*

Table 1. Recent Patents on Molecular Targets that Modulate Cardiac Growth

Target	Patents [Patent reference]	Genetic models	Hypertrophy challenge	References
Calcineurin	*WO03006619A3 [12] *US6875581 [13]	CNA (-/-) CNA(DN)-TG CNA-TG	Aortic banding Angiotensin II isoproterenol aortic banding CNA-TG	[62] [60] [19]
Nuclear Factor of Activated T Cells	US6780597 [14] AU0748334B2 [15] EP1486507A1 [16]	NFAT3-TG	NFAT3-TG	[19]
A kinase-anchoring Protein 79	US5807693 [31]	AKAP79-TG	aortic banding isoproterenol	[36]
Calsarcin	*US20040186275A1 [47]	Cs (-/-)xCNA-TG Cs (-/-)xMCIP-TG	aortic banding isoproterenol	[53]
cGMP-specific Phosphodiesterase-Inhibitors Sildenafil	US20050085486A1 [84] WO04037183A3 [85] US6338862 [86] WO0200658A1 [87] US6825197 [88] US6204383 [89]		Aortic banding	[83]
Myocyte enhancer factor-2	*US20020100069A1 [135]	MEF2a (-/-)		[139]
Histone Deacetylases	*EP1297851B1 [142] *WO04076386A2 [143] *US6706686 [144] *US20040186049A1 [145] *WO0114581C2 [147] *JP2003238445A2 [148]	HDAC5 (-/-) HDAC9 (-/-)	aortic banding CNA-TG aortic banding CNA-TG MEK5-TG	[153-155]
Protein kinase-D	*WO04112763A2 [157]			[156]
PTEN	US6020199 [99] US6284538 [100]	CRE-PTEN (-/-) (x DN-p110)	CRE-PTEN (-/-)	[101]
Glycogen synthase kinase -3b]	US6780625 [108] US6489344 [109]	GSK-3β-Tg	CNA-TG isoproterenol	[120]
G-Protein Coupled Receptors	US6887683 [164]	(DN)-Gq-Tg Gq/G11 (-/-)	Aortic banding	[168] [142]
Raf-kinase	US20040209883A1 [188] WO03022833A1 [189] US20040038964A1 [190] WO03022838A1 [191]	(DN)-RAF-TG	Aortic banding	[187]
Cyclin-dependent kinase-9	WO05027902A1 [202] US20040106647A1 [203]	Cyclin T1-TG	CNA-TG Gq-TG aortic banding	[198,199]
Rho-kinase	US20050014783A1 [207] WO04105757A2 [208]	ApoE (-/-)	Angiotensin II	[211]
MAPK/ERK kinase-5	CA2384907AA [231] US20030144176A1 [232] JP2003055266A2 [233]	MEK5-TG	MEK5-TG	[230]
Cardiotrophin-1	US6472585 [245]	(DN)-gp 130-TG	Aortic banding	[247-251] [258]
Leukemia inhibitory Factor	US6653287 [246]	(DN)-gp130-TG	Aortic banding	[251], [258]
Na/H-Exchanger	US6686384 [259]	1-AR-Tg	1-AR-Tg aortic banding	[264-266]

[228] demonstrated that mice deficient for adiponectin, an adipocytokine, which is downregulated in patients with obesity-linked diseases, exhibited enhanced concentric hypertrophy and increased mortality compared to WT littermates. Further experiments in cultures of cardiac myocytes showed that the antihypertrophic effects of adiponectin are, at least in part, mediated by inhibition of ERK activation. Hence, in this model, ERK activation seemed to promote maladaptive remodeling, suggesting that adiponectin might be useful in the treatment of obesity-related cardiac hypertrophy. Another protein that was recently shown to modulate ERK1/2-signaling, is the peptide hormone relaxin. Spontaneous hypertensive rats (SHR) display left heart hypertrophy and an elevation of left ventricular relaxin levels, which inversely correlate with the degree of hypertrophy [229]. In adult cardiomyocytes relaxin was shown to blunt PE-induced hypertrophy by inhibiting activated ERK1/2 kinases [229]. Interestingly, current findings in relaxin knockout mice suggest an antifibrotic and positive lusitropic effect of relaxin on the myocardium [230] implying a therapeutic potential in cardiac hypertrophy and failure.

Another MAPK-module, MEK5/ERK5 [231-233], induces eccentric cardiomyocyte hypertrophy, which is typically observed in volume overload-induced myocardial growth *in vivo* [234]. Constitutive activation of MEK5 leads to serial assembly of sarcomeres *in vitro*, whereas MEK5 overexpression in transgenic mouse hearts results in a severe form of dilated cardiomyopathy and sudden death.

While ERKs are firmly identified as important regulators of cardiac hypertrophy, the role of the c-Jun N-terminal kinases (JNKs) and p38 kinases remains a matter of debate. These pathways function as specific transducers of stress response, thus they are categorized as stress-activated protein kinases (SAPKs). A recent report demonstrated that JNK activity is specifically upregulated in response to pressure overload, while p38 activity is markedly induced in hearts subjected to volume overload [235]. MAPKs of the JNK class are directly phosphorylated by either MKK-4 or MKK-7, which in turn are regulated by MEKK1-phosphorylation. Experiments conducted in cell culture implicated a pro-hypertrophic role of JNKs in the heart: In cardiomyocytes, stimulation by either ET-1, PE, or AngII results in phosphorylation of JNK (reviewed in [9]). Expression of a dominant-negative MKK-4 mutant attenuated the hypertrophic response of neonatal rat cardiomyocytes to endothelin-1 in culture [236]. In addition, overexpression of a constitutively active mutant of MKK-7 was sufficient to induce a significant increase in cell size [237]. Notably, in this model combined activation of p38 and JNK led to induction of a pro-apoptotic response instead of hypertrophy, suggesting a complex interaction between different SAPKs. Observations in transgenic mice show inconsistent results: Adenoviral gene transfer of a dominant-negative MKK-4 protein to the adult rat heart blocked JNK activation and reduced cardiac hypertrophy by pressure overload [238], while a more recent study in JNK-1/2 gene-targeted mice and transgenic mice expressing dominant negative JNK-1/2 demonstrated that JNK signaling antagonizes myocardial growth through direct crosstalk with the calcineurin-NFAT pathway [239]. Similarly, one study in MEKK-1 gene-

targeted mice showed no positive correlation with the cardiac hypertrophic response *in vivo* [240].

The most important activators of p38 MAPKs are MKK-3 and MKK-6, both of which are sufficient to induce cardiomyocyte hypertrophy and ANF-induction in cultured cardiomyocytes [241]. p38 Activity is particularly induced in volume overload [235]. Moreover, TAK-1, which is located upstream of MKK-3/6, is upregulated and activated in mice subjected to aortic banding [242]. Similarly to JNK signaling, the role of p38 MAPK in cardiac hypertrophy is ambiguous. While expression of constitutively active TAK-1-mutant results in cardiac hypertrophy and subsequently heart failure in transgenic mice [242], a more recent study by Braz *et al.* [65] revealed an *inhibitory* function of p38 signaling on cardiac growth *in vivo*. Like in JNK signaling these antihypertrophic effects are mediated, at least in part, by an inhibition of calcineurin/NFAT signaling by direct phosphorylation of NFAT transcription factors [65, 243].

In summary, the role of JNK and p38 MAPK-signaling in cardiac hypertrophy is not fully clarified, however, small molecules that modify these pathways could prove valuable in the setting of heart failure: Growing evidence suggests that JNK and p38 MAPK are important mediators of apoptosis in the heart [244], thus the "magic moment" of SAPK inhibition could be the prevention of the transition from cardiac hypertrophy to heart failure.

GP130/STAT3 SIGNALING

GP 130 receptor signaling has been identified to mediate both hypertrophic stimuli and to promote cardiomyocyte survival. The gp 130 receptors bind to several ligands that share similar structural properties, including interleukin-6/-11, cardiotrophin-1 (CT-1) [245] and leukemia inhibitory factor (LIF) [246]. While gp130 and the LIF receptor are expressed in virtually every tissue in rodents and humans, CT-1 is expressed at high levels particularly in the heart and skeletal muscle [247]. CT-1 and LIF promote cardiac growth *in vitro* [247, 248], however, the specific downstream targets that mediate CT-1/gp130 –signaling are not fully understood. Several studies suggest that activation of the Janus kinase (JAK) and signal transducers and activators of transcription pathways (STAT), especially STAT3, are central mediators of CT-1/gp130 and LIF signaling [249]. STAT3 translocates to the nucleus in response to gp130-activation, which results in the induction of genes involved in hypertrophy and survival pathways [250, 251]. Overexpression of STAT3 in transgenic mice leads to cardiomyocyte hypertrophy *in vitro* [252] and *in vivo* [253]. In this regard, one report by Fukuzawa and coworkers demonstrated that upregulation of angiotensinogen mRNA via STAT3 contributes to CT-1 induced hypertrophy [248]. Angiotensin II is known to induce cardiac myocyte hypertrophic growth by activating the G-protein-coupled angiotensin II type 1 receptor. Moreover, angiotensin II also leads to upregulation of LIF, CT-1 and interleukin 6 [254]. Consequently, Losartan, an angiotensin II type 1 receptor antagonist, attenuated CT-1 induced hypertrophy [248]. In contrast to this observation, a recent investigation [255] revealed that adenoviral overexpression of dominant negative STAT3 mutants was not sufficient to inhibit CT-1-induced hypertrophy in cardiac myocytes. On the other hand, overexpression of the

suppressor of cytokine signaling (SOCS)-1 and-3, which act as endogenous inhibitors of JAK-mediated gp130 signaling, both inhibit CT-1 induced STAT3-phosphorylation and cardiomyocyte hypertrophy. Further experiments utilizing dominant negative MEK mutants suggested a central role of the MEK5-ERK5 signaling pathway in gp130-induced hypertrophy [255]. Interestingly, SOCS-3 has recently been shown to coimmunoprecipitate with calcineurin in T-cells, thus averting calcineurin/NFAT-dependent signaling [256]. Accordingly, a connection between these pathways may exist in (anti-)hypertrophic signaling within cardiomyocytes. However, therapeutic strategies to inhibit or revert cardiac growth by targeting the Gp130 pathway are challenged by the antiapoptotic properties of this signaling cascade [257] and its potential to prevent maladaptive remodeling: Unstressed mice with cardiac-specific ablation of the gp130 receptor display no overt phenotype. Yet, when subjected to pressure overload due to aortic banding, these animals display dilated cardiomyopathy allied with substantial cardiac myocyte apoptosis [258]. Hence, a specific quantity of gp130 mediated signaling seems to be required to maintain the heart's ability to adequately adapt to biomechanical stress.

Na/H-EXCHANGER

The activity of the cardiac Na/H-exchanger (NHE) [259] is increased in cardiomyocytes subjected to mechanical stress [260] as well as in several *in vivo*-models of cardiac hypertrophy, such as pressure overload [261] and postinfarction remodeling [262]. Enhanced NHE activity reduces the transmembrane Na⁺-gradient, which leads to increased intracellular Ca²⁺-levels via the Na-Ca-exchanger (reviewed in [263]). Increased calcium concentration triggers cardiomyocyte hypertrophy via numerous pathways, including CaMK- and MAPK-dependent pathways (reviewed in [9]). Accordingly, the usage of NHE-inhibitor Cariporide is able to prevent hypertrophy in several animal models. Interestingly, this effect is associated with favourable remodeling effects: Cariporide prevents hypertrophy and significantly improves cardiac contractility after myocardial infarction in rats [264]. Likewise, NHE-inhibition alleviates the cardiomyopathic phenotype of 1-adrenergic receptor transgenic mice, particularly the progression of cardiac fibrosis and the development of contractile dysfunction [265]. Finally, a recent study by Baartscheer and colleagues [266] demonstrated that chronic Cariporide treatment markedly attenuated development of hypertrophy and entirely abolished heart failure in rabbits that were subjected to combined volume and pressure overload. Taken together, these data imply that NHE inhibition might be a promising approach to modulate cardiac hypertrophy, particularly because of its positive "side-effects" on cardiac remodeling in response to different stimuli.

CURRENT & FUTURE DEVELOPMENTS

Numerous studies have found that cardiac hypertrophy is a risk factor for cardiovascular morbidity and mortality in humans. Concomitantly, the adaptive nature of hypertrophy in response to stress factors has been called into question [2, 201]. These findings have driven the interest to the quest for targets to modulate hypertrophic signaling pathways. In this

regard, many patents on antiproliferative agents in cancer therapy and immunomodulation have gained potential value in the treatment of heart failure (Table 1). What evidence exists about the maladaptive role of cardiac hypertrophy? A patient cohort that has been extensively analyzed in this regard are patients which display cardiac hypertrophy due to hypertension. In a significant part of these patients sensitive measures of systolic performance reveal contractile dysfunction (reviewed in [1]): Recent trials of antihypertensive therapy display that regression of hypertrophy is likely to be associated with improvements in systolic and diastolic performance and decreased incidence of clinical heart failure [8]. For example, in the Heart Outcomes Prevention evaluation (HOPE) trial, the ACE inhibitor Ramipril decreased the development (and caused regression) of hypertrophy independently of its blood-pressure lowering effects [267]. Similarly, the Losartan Intervention for Endpoint reduction in hypertension (LIFE) trial revealed a direct correlation between regression of left ventricular mass and lower rates of clinical endpoints [8]. These findings may comfort the notion that modulation of hypertrophic signaling pathways is likely to result in beneficial effects in many different clinical settings. However, some retentions pertain: Reports from animal models that display benefit from blockage of hypertrophy despite of perseverance of the initial stimulus [171] typically cover only a few months of follow-up, which cannot necessarily be extrapolated to years or even decades of treatment in human patients. Apart from the well known pitfalls of transferring results from animal models to human conditions, long term observations are thus obviously required to preclude that longtime inhibition of hypertrophy might ultimately lead to heart failure.

Additionally, most of the innumerable input signals that modulate hypertrophic growth also affect contractile function and apoptosis. In fact, some hypertrophic signaling cascades (e.g. PI3K/Akt/GSK3) may even be required to prevent maladaptive adaption to overload and heart failure [130]. In other instances, such as in athletes, development and pregnancy, cardiac hypertrophy is accepted as being physiological and not associated with adverse outcomes. This phenotype differs significantly from pathological hypertrophy by the absence of collagen accumulation, opposite β -MHC isoform expression and a lack of upregulation of "hypertrophic markers genes" [268]. Furthermore, the pattern of energy substrate utilization (or metabolic phenotype) and expression of metabolic genes differs dramatically between pathologic and physiologic cardiac hypertrophy with opposite changes in the oxidation of fatty acids and regulation of glycolysis [269, 270]. Moreover, an imbalance between increased energy demand and an insufficient mitochondrial proliferation seems to be a particular feature of maladaptive hypertrophy (reviewed in [270]). Little is known so far about the distinct signaling pathways that mediate physiological hypertrophy, however growing evidence suggests a central role of PI3K/Akt signaling in this background [97]. Additionally, the Akt pathway was demonstrated to counteract apoptotic signaling [271]. Recent results by Shiojima *et al.* [107] demonstrate an important role of AKT/m-TOR signaling in cardiac angiogenesis, whose disruption contributes to the transition

from hypertrophy to heart failure (as outlined above). A decreased capillary density of hypertrophied hearts has been known for a long time [272]. New insights into pathways mediating angiogenesis during hypertrophic growth could re-initiate therapeutic strategies, that support cardiac angiogenesis to prevent the transition from hypertrophy to heart failure. In addition, identification and modulation of genes, that promote mitochondrial proliferation, might help to keep pace with the rising energy demand of hypertrophied hearts.

In contrast to Akt-dependent signaling, the G_q -associated signaling cascades represent an example of clearly maladaptive pathways, as they promote decline of systolic function and conversion to heart failure [171], suggesting an evolving importance of patents on G_q -modulating agents. Similarly, the transcriptional Repressor NAB-1, which inhibits EGR-1-dependent transcription, was recently demonstrated to potently inhibit cardiac hypertrophy in response to pathological stimuli without affecting physiological growth [273], providing yet another potential target for therapeutic intervention

Numerous studies have established the pivotal role of calcineurin/NFAT signaling in cardiac hypertrophy. This pathway is connected to and modified by numerous other hypertrophic signaling cascades (Fig. 1) and appears to be involved in virtually every transductional sequence from prohypertrophic stress stimuli to the nucleus. Hence, agents that modify calcineurin signaling might prove especially valuable for future therapeutic strategies to prevent pathologic cardiac growth. This notion is supported by a recent report which has demonstrated that calcineurin is activated in pathological, but not in physiological hypertrophy [274]. Another current study by Rothermel and colleagues [275] showed that calcineurin is both upregulated in “compensated” hypertrophy in response to moderate pressure overload as well as in a decompensated stage of hypertrophy in mice subjected to severe pressure overload. Off note, calcineurin inhibition by MCIP-1 overexpression blunted the hypertrophic response in both models while not negatively affecting survival.

Taken together, the data presented in this review suggest, that agents and “small molecules”, that are patented to modulate hypertrophic signaling pathways, could be the basis of novel therapeutic strategies to prevent cardiac dysfunction and heart failure. However, a great deal of work is still needed to dissect the mechanisms underlying the maladaptive features of hypertrophy. It will be a demanding task to find approaches that modulate the complex network of hypertrophic signaling cascades without negatively affecting the precisely aligned balance of factors that influence contractile function and apoptosis. Moreover, many of the signaling cascades outlined above are not tissue-specific, thus it might be necessary to identify cardiac-specific components of these pathways and/or to explore tissue-specific means of drug-delivery in order to avoid systemic side-effects. But beyond doubt these efforts appear to be worthwhile, since they hold the promise to provide new tools in the prevention and treatment of heart failure, one of the main medical challenges in the 21st century.

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