Review

Inhibitory Molecules in Signal Transduction Pathways of Cardiac Hypertrophy

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Cardiac hypertrophy is induced by a variety of diseases, such as hypertension, valvular diseases, myocardial infarction, and endocrine disorders. Although cardiac hypertrophy may initially be a beneficial response that normalizes wall stress and maintains normal cardiac function, prolonged hypertrophy is a leading cause of heart failure and sudden death. A number of studies have elucidated molecules responsible for the development of cardiac hypertrophy, including the mitogen-activated protein (MAP) kinases pathway, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, and calcium/calmodulin-dependent protein phosphatase calcineurin pathway. These molecules may be targets for therapies designed to prevent the progression of cardiac hypertrophy. Numerous studies have focused on characterization of the intracellular signal transduction molecules that promote cardiac hypertrophy in order to clarify the molecular mechanisms, but there have been only a few reports on the inhibitory regulators of hypertrophic response. Recently, several molecules have attracted much attention as endogenous inhibitory regulators of cardiac hypertrophy. Enhancement of these inhibitory regulators would also seem to be a potential approach for the pharmacological treatment of hypertrophy. In this review, we summarize the inhibitory molecules of cardiac hypertrophy. (Hypertens Res 2002; 25: 491–498)

Key Words: cardiac hypertrophy, heart failure, inhibitor, signal transduction

Introduction

Cardiac hypertrophy is recognized as an adaptive increase in heart size characterized by a growth of individual cardiomyocytes rather than an increase in cell number. Cardiac hypertrophy is induced by a variety of diseases, such as hypertension, valvular diseases, myocardial infarction, and endocrine disorders. Although cardiac hypertrophy may initially be a beneficial response that normalizes wall stress and maintains normal cardiac function, prolonged hypertrophy is a leading cause of heart failure and sudden death (1). Therefore, it is very important to understand the precise mechanisms and mediators of cardiac hypertrophy. Although numerous signal transduction pathways that promote cardiac hypertrophy have been characterized, only a few studies have focused on inhibitory regulators of hypertrophic response. The modification of antihypertrophic regulators is expected to be a novel therapeutic strategy for cardiac hypertrophy. This review focuses on the signaling molecules that negatively regulate cardiac hypertrophy. Descriptions of the detailed signal transduction pathways that promote cardiac hypertrophy have been reviewed in other articles (1–3).

MCIP

Calcineurin is a serine-threonine phosphatase that is activated by Ca\(^{2+}\)-calmodulin. Activated calcineurin dephosphorylates a family of transcription factors known as nuclear factor of activated T cells (NFAT), which subsequently translocates from the cytoplasm to nucleus. NFAT interacts with GATA4 or possibly with other transcription factors that are
involved in transcription of hypertrophic response genes in the nucleus. Molkentin et al. demonstrated that transgenic mice overexpressing calcineurin in the heart displayed significant cardiac hypertrophy and rapidly progressed to heart failure (4). Transgenic mice overexpressing constitutively active NFAT3 in the heart also showed pronounced cardiac hypertrophy. These results suggest that calcineurin-mediated activation of NFAT plays a crucial role in cardiac hypertrophy. In vitro, adenoviral-mediated gene transfer of activated calcineurin has also been shown to induce hypertrophy in cardiac myocytes (5). Further, the calcineurin inhibitory agents cyclosporin A and FK506 blocked hypertrophic responses in some animal models of pressure overload-induced or genetic cardiac hypertrophy (6–16). However, several other groups have reported the opposite i.e., that these agents failed to block hypertrophic responses in these animal models (17–20).

Rothermel et al. have recently identified a family of proteins known as the myocyte-enriched calcineurin-interacting protein (MCIP) family. The members of this family, which include MCIP1 and MCIP2, are highly expressed in striated muscles and inhibit calcineurin activity due to their binding to the catalytic subunit (CnA) of calcineurin (21). Expression of MCIP1, but not MCIP2, is induced by activated calcineurin in cardiac myocytes and skeletal myotubes. In contrast, expression of MCIP2 is upregulated by thyroid hormone. The region located between exons 3 and 4 of the MCIP1 gene includes 15 consensus binding sites for NFAT and mediates the transcriptional regulation of MCIP1 gene by calcineurin. MCIP1 induced by activation of calcineurin inhibits the enzymatic activity of calcineurin and functions as a negative feedback regulator (22). Transgenic mice overexpressing MCIP1 in the heart have been shown to be viable and to exhibit no obvious abnormalities (23). However, the heart weight/body weight ratio was slightly (5–10%) lower in the transgenic mice than in wild-type mice. Double transgenic mice (MCIP1 ΔCnA) displayed less cardiac hypertrophy and dilatation than transgenic mice overexpressing CnA alone (23). In double transgenic mice, cardiac function was restored to the same level as that in wild-type mice. Moreover, isoproterenol (ISO)- or exercise-induced hypertrophy was also less in double transgenic mice than in wild-type mice (23). Because MCIP1 is abundantly expressed in cardiac myocytes and its expression is induced by activated calcineurin, it is expected to function as an endogenous inhibitor for cardiac hypertrophy.

**HDAC**

Histone acetyltransferase (HAT) and histone deacetylase (HDAC) play an important role in the regulation of transcription. Acetylation of histones and non-histone proteins is regulated by interaction of HATs and HDACs (24). HATs catalyze the acetylation of core histones of chromatin and relax nucleosomes, resulting in transcriptional activation. In contrast, HDACs deacetylate the N-terminal tails of core histones and repress transcription. HDACs are categorized into three classes, I, II, and III, based on size and sequence homology (24). Class I HDACs (HDAC1, 2, 3, and 8) are expressed ubiquitously, whereas class II HDACs (HDAC4, 5, 6, and 7) are expressed primarily in the heart, brain, and skeletal muscle. Class II HDACs interact with the MADS/myocyte enhancer factor-2 (MEF2) domain of MEF2 through a unique 18-amino acid motif and repress the transcriptional activity of MEF2. It has been reported that Ca²⁺/calmodulin-dependent protein kinases (CaMKs) activate MEF2 through a posttranslational mechanism in the hypertrophic heart, and that CaMKs and the calcineurin-signaling pathways cooperate to induce cardiac hypertrophy (25).

McKinsey et al. recently demonstrated that CaMKs phosphorylate HDAC5 at serine-259 and -498 and that HDAC5 is subsequently transported from the nucleus to cytoplasm through a C-terminal sequence (nuclear export sequence), resulting in dissociation of MEF2 from HDAC5 and activation of MEF2 (26). It was further demonstrated that 14-3-3 protein binds to phosphoserines of HDAC4/5 and masks the nuclear localization signal of HDAC4/5 (27). As a result, 14-3-3 protein-HDAC4/5 complex is exported to the cytoplasm (27, 28). It has been well known that the 14-3-3 protein family is expressed in all eukaryotic cells and is involved in numerous signal transduction pathways (29). Although 14-3-3 has been reported to exist in the cytoplasm, nucleus, and membranes, the significance of such differential localization is still unclear. Furthermore, whether HDACs inhibit cardiac hypertrophy in vivo remains to be shown.

**GSK3β**

Glycogen synthase kinase 3β (GSK3β) is a serine/threonine protein kinase which plays an important role in transduction of regulatory and proliferative signals. GSK3β phosphorylates many substrates, including glycogen synthase, β-catenin (30), translation initiation factor eIF2B (31), cyclin D1 (32), and NFAT (33). Unlike most protein kinases, GSK3β is active in the absence of external signals and becomes inactivated in response to a variety of mitogens or the Wnt/wingless pathway (34, 35). The activity of GSK3β is regulated by the phosphorylation status of serine-9, and phosphorylation of this site inactivates GSK3β (36, 37). It was recently demonstrated that Akt/protein kinase B phosphorylates and inactivates GSK3β (38).

Hypertrophic stimuli such as endothelin-1 (ET-1), ISO, and aortic banding also inhibit GSK3β activity through phosphorylation of the kinase in cardiac myocytes (39). Overexpression of GSK3β, but not of the Akt-insensitive mutant of GSK3β, has been shown to inhibit ET-1, phenylephrine (PE), or ISO-induced hypertrophic response. In contrast, kinase-inactive GSK3β was found to increase hypertrophic response (40). Antos et al. generated transgenic mice that overexpress a constitutively active form of GSK3β in
the heart. The transgenic mice appeared normal and exhibited normal cardiac function, but the heart weight/body weight ratio was slightly decreased in these mice compared to wild-type mice. While transgenic mice overexpressing activated calcineurin developed massive cardiac hypertrophy, double transgenic mice (calcineurin + GSK3β) displayed reduction in size of cardiac myocytes (41). Moreover, ISO- or pressure overload-induced cardiac hypertrophy was also inhibited in GSK3β transgenic mice compared to wild-type mice. Nuclear localization of NFAT was increased in calcineurin transgenic mice, but was partially reduced in double transgenic mice. These data suggest that activated GSK3β phosphorylates NFAT and translocates NFAT to the cytoplasm, resulting in inhibition of calcineurin induced-hypertrophic responses. In a recent report, activation of Akt and inhibition of GSK3β were recognized in the hearts of patients with advanced heart failure, but not in the hearts of those with hypertrophy (42). Further studies will be needed to elucidate whether GSK3β has inhibitory effects on the transition from hypertrophy to heart failure, as well as on compensated cardiac hypertrophy.

**SOCS**

Cardiotrophin-1 (CT-1), which is a member of the interleukin (IL)-6 family, was originally identified as a novel factor capable of inducing cardiac hypertrophy (43). CT-1 is substantially expressed in the heart but is also found in a variety of other tissues. Its expression has been shown to be upregulated in a variety of pathological states that include hypertension, acute myocardial infarction, dilated cardiomyopathy, myocarditis, and heart failure (44–51). In addition to CT-1, this family includes leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), IL-6, and IL-11. It has been reported that gp130 is a common receptor component of the IL-6 family and that CT-1, LIF, and OSM signaling require the heterodimerization of gp130 and LIF receptor (52). Many reports have demonstrated that gp130-dependent signaling plays an important role in the development of cardiac hypertrophy (53–55). Interestingly, the hypertrophic response induced by the signaling through gp130 is morphologically distinct from that through G protein-coupled receptors. Angiotensin II (Ang II), ET-1, and PE induce a rather uniform increase in cardiomyocyte size, while CT-1 and LIF induce an increase in cell length with addition of new sarcomeric units in series (52).

Stimulation of gp130 by this family leads to activation of two types of signaling cascades, one involving the MAP kinase pathway (56–59) and one involving the JAK/STAT pathway (57–59). Hirota et al. have demonstrated that ventricle-restricted gp130 knockout mice display a rapid-onset dilated cardiomyopathy and massive myocyte apoptosis by pressure overload (53). Recently, we have demonstrated that gp130 plays a critical role in pressure overload-induced cardiac hypertrophy by using transgenic mice overexpressing the dominant negative mutant of gp130 in the heart. While pressure overload activated extracellular signal-regulated kinases (ERKs) and STAT3 in the heart of WT mice, the activation of STAT3, but not of ERKs, was suppressed in the transgenic mice (55). Although these data suggest that the gp130-JAK/STAT signaling pathway is involved in CT-1- and pressure overload-induced cardiac hypertrophy, the mechanism of inactivation has remained unknown. Three groups independently identified a family of proteins known as the cytokine-inducible SH2-containing protein (CIS)/suppressor of cytokine signaling (SOCS)/STAT-induced STAT inhibitor (SSI), which inhibits the JAK/STAT pathway by a negative feedback mechanism (60–62). These proteins have a variable N-terminal region, a central SH2 domain, and a conserved C-terminal region known as the CIS homology domain. Cytokines rapidly induce SOCS expression, and SOCS proteins in turn regulate STAT activation negatively by direct binding to the kinase domain of JAKs or the receptor complex (63, 64). Recently, Hamanaka et al. have demonstrated that CT-1 transiently induces STAT3 activation in various tissues, including heart and lung, and subsequently increases the expression of SOCS-1 and SOCS-3 (65). In their study, forced expression of SOCS-1 or SOCS-3 directly blocked CT-1-induced STAT activation (65). Yasukawa et al. have reported that SOCS-3 expression is induced by pressure overload in mice and is closely correlated with STAT phosphorylation. Adenovirus-mediated gene transfer of SOCS-3 to ventricular myocytes completely suppressed the LIF-induced activation of STAT3, ERKs and Akt, as well as cardiac hypertrophy (66). These results suggest that the expressions of SOCS-1 and SOCS-3 are induced by a gp130-mediated signaling pathway that includes mechanical stress and cytokines, and that these proteins may act as negative regulators of cardiac hypertrophy.

**PTEN**

We have reported that mechanical stretch induces cardiac hypertrophy (3). However, the mechanism by which mechanical stress induces hypertrophic responses in cardiac myocytes remains unknown. Because mechanical stretch activates focal adhesion kinase (FAK) in cardiac myocytes, integrins, which are receptors of the extracellular matrix (ECM), have been considered candidates for potential receptors of mechanical stretch. The tumor suppressor gene PTEN (phosphatase and tensin homologue on chromosome 10) is a protein tyrosine phosphatase associated with the actin cytoskeleton at focal adhesion and inhibits outside-in signals but not inside-out signals of integrins through dephosphorylation of FAK, an integrin-associated kinase (67–70). Tamura et al. have reported that PTEN is involved in the regulation of several different cellular processes, such as cell growth, ECM interactions, and cell migrations (70). Mutations of PTEN are frequently detected in human brain, breast, and prostate cancer (67, 68).
Schwartzbauer et al. have recently demonstrated that the protein level, but not the mRNA level, of PTEN is increased in ISO-treated mouse hearts (71). Overexpression of wild-type PTEN caused apoptosis in cultured neonatal rat cardiomyocytes and inhibited insulin growth factor-1-induced activation of Akt. In contrast, overexpression of a catalytically inactive PTEN mutant induced an increase in Akt activity and cardiomyocyte hypertrophy (71). Mechanical stretch activates various hypertrophic responses, such as MAP kinases. Because mechanical stretch-induced activations of c-Jun NH2-terminal kinase and p38 MAP kinase are independent of humoral growth factors, we recently elucidated the role of integrin in mechanical stretch-induced hypertrophic responses. Our results showed that overexpression of PTEN significantly inhibits stretch-induced activation of p38 MAP kinase (72). Although PTEN has been reported to inhibit the PI3-kinase/Akt pathway (73), pretreatment with wortmannin, a PI3-kinase inhibitor, did not have any effects on stretch-induced activation of p38 MAP kinase. These data suggest that PTEN suppresses stretch-induced activation of p38 MAP kinase and cardiac hypertrophy by inhibiting FAK but not PI3-kinase. Further studies will be needed to elucidate how PTEN expression is controlled in cardiac myocytes and whether expression of PTEN is decreased in hypertrophic hearts of various genetic models.

CHAMP

A number of specific regulatory functions of MEF2 have been identified in immune, skeletal muscle, and cardiac muscle cells (74). MEF2 knockout mice have altered cardiac gene expression and die during embryonic development (75). And MEF2 has been shown to be activated by Ca2+ (76, 77), calcineurin (78), and p38 MAP kinase (79). It is suggested that MEF2 plays important roles in both developmental and pathophysiological responses of cardiac myocytes and is a critical regulator of cardiac gene expression (80, 81). Liu et al. have performed differential array analysis using subtractive hybridization to identify the genes regulated by MEF2C2 in the developing heart (82). They cloned a novel MEF2C-dependent gene, CHAMP (cardiac helicase activated by MEF2 protein), that contains seven conserved motifs characteristic of RNA helicases (82). Members of the RNA helicase superfamily play various roles in RNA metabolism, including regulation of transcription, ribosome biogenesis, pre-mRNA splicing, RNA export to the cytoplasm, translation initiation and termination, and RNA degradation (83). The expression of CHAMP commences in the linear heart tube at embryonic day (E) 8.0 and is highest in the trabecular region, where the proliferative rate is diminished relative to that of the adjacent compact zone at E 15.5 (82). These results suggest that CHAMP may function downstream of MEF2C in a cardiac-specific regulatory pathway and negatively regulate the proliferation and growth of cardiac myocytes. The same group has recently demonstrated that overexpression of CHAMP blocks serum- or PE-induced cardiac hypertrophy, but not ERKs activation in vitro (84). This inhibitory effect was accompanied by upregulation of cyclin-dependent kinase (CDK) inhibitor p21 CIP1 and required the conserved ATPase domain of CHAMP (84). Interestingly, CHAMP mRNA and protein levels were downregulated in the hypertrophic hearts from mice overexpressing calcineurin. Because it has been reported that G1 CDK activation is involved in cardiac hypertrophy and that the CDK inhibitors p16 INK4a and p21 CIP1 prevent the development of cardiac hypertrophy (85, 86), there is the possibility that CHAMP exerts an inhibitory effect through p21 CIP1 in cardiac myocytes. However, the precise mechanisms by which upregulation of p21 CIP1 is induced by CHAMP and inhibits cardiac hypertrophy are still not fully elucidated.

PPAR

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor (RXR) and bind to specific response elements termed PPAR responsive elements (PPREs) in target gene promoters (87, 88). The PPREs are direct repeats of the hexameric consensus sequence AGGTCA, separated by one nucleotide. These nuclear receptors are ligand-dependent transcription factors, and activation of target gene transcription depends on the binding of the ligand to the receptor. PPARs have 3 isoforms, α, β, and γ. PPARα regulates genes involved in the β-oxidative degradation of fatty acids, whereas PPARγ promotes adipocyte differentiation and glucose homeostasis. The roles of PPARδ have yet to be ascertained because of the lack of any selective agonists or antagonists. PPARα is present in the liver, kidney, and muscle, whereas PPARγ is expressed predominantly in adipose tissue. PPARδ is almost ubiquitously expressed. Recently, it has become apparent that PPARs are present in a variety of different cell types. PPARγ has also been shown to be expressed in many other cell types in addition to adipocytes. The prostaglandin D2 (PGD2) metabolite, 15-deoxy-D12,14-prostaglandin J2, is the first endogenous ligand for PPARγ (89, 90). Although 15d-PGJ2 is the most potent natural ligand of PPARγ, the extent to which its effects are mediated through PPARγ in vivo remains to be determined. The antidiabetic thiazolidinediones (TZDs: troglitazone, pioglitazone, ciglitazone, and rosiglitazone) are pharmacological ligands of PPARγ (91, 92). They bind to PPARγ with various affinities, and it is conceivable that their insulin-sensitizing and hypoglycemic effects are exerted by activating PPARγ. Following reports that activation of PPARγ suppresses the production of inflammatory cytokines in activated macrophages (93, 94), medical interest in PPARγ has grown enormously, and a vast number of researches have been conducted on this receptor.

Many reports have demonstrated that activation of PPARγ...
has beneficial effects in modulating inflammatory responses in atherosclerosis (88, 95), while the effects of PPARγ on the heart have not been fully elucidated. We previously demonstrated that PPARγ ligands inhibit cardiac expression of tumor necrosis factor-α (TNF-α) at the transcriptional level in part by antagonizing nuclear factor-κB (NF-κB) activity (96). Because TNF-α and NF-κB are involved in cardiac hypertrophy and heart failure (97–101), the treatment with PPARγ ligands may prevent the development of cardiac hypertrophy and heart failure. We and other groups recently examined that the role of PPARγ on cardiac hypertrophy. Our results showed that TZDs, including troglitazone, pioglitazone, and rosiglitazone, inhibited Ang II-, PE-, and mechanical strain-induced hypertrophy in cultured neonatal rat cardiomyocytes (102, 103). In vivo, pioglitazone inhibited pressure overload-induced cardiac hypertrophy in mice (103). In contrast, pressure overload-induced cardiac hypertrophy was more prominent in heterozygous PPARγ-deficient mice than in wild-type mice (103). These results suggest that a PPARγ-dependent pathway inhibits the development of cardiac hypertrophy. Although it is conceivable that PPARγ may suppress the development of cardiac hypertrophy by antagonizing the activities of other transcription factors such as AP-1, STAT3, and GATA4 (94), the molecular mechanisms by which PPARγ suppresses cardiac hypertrophy remain to be determined. Because cardiac hypertrophy can be seen even in normotensive diabetic patients and diabetic cardiomyopathy is a major complication of diabetes, the antidiabetic agents known as TZDs would appear to be beneficial for treatment of cardiac hypertrophy and dysfunction in patients with diabetes mellitus.

In conclusion, it has been hypothesized that cardiac hypertrophy in response to pressure or volume overload is a necessary compensatory mechanism to normalize wall stress and maintain normal cardiac function. However, the results from the Framingham Heart Study demonstrated that left ventricular hypertrophy is associated with increased cardiac mortality and is an independent risk factor for cardiac morbidity and mortality (104). Therefore, whether hypertrophic response to an altered mechanical loading condition is adaptive or maladaptive remains uncertain. Recently, Hill et al. reported that the calcineurin inhibitor cyclosporin A maintains normal ventricular size and cardiac function despite inhibition of pressure overload-induced hypertrophy (105). In addition, Esposito et al. demonstrated that prevention of the development of cardiac hypertrophy after aortic banding is not associated with deterioration in cardiac function using two genetically engineered mouse models that have blunted hypertrophic responses to pressure overload (106). Indeed, cardiac function was better maintained in the genetic mice without hypertrophy than in the wild-type mice with hypertrophy. These results suggest that the development of cardiac hypertrophy and normalization of wall stress may not be necessary to preserve cardiac function. Therefore, it will become more and more important to elucidate the mechanisms of cardiac hypertrophy, and to develop novel therapeutic strategies for this disease.

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