ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 6, 2007

DOI: 10.1089/ars.2007.1474

Comprehensive Invited Review

Cardiac Hypertrophy: Mechanisms and Therapeutic Opportunities

SUDHIRANJAN GUPTA, BISWAJIT DAS, and SUBHA SEN

Reviewing Editors: Nikolaos Frangogiannis and Manuel Vazquez–Carrera

Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic, Cleveland, Ohio.

ABSTRACT

Cardiac hypertrophy and heart failure are major causes of morbidity and mortality in Western societies. Many factors have been implicated in cardiac remodeling, including alterations in gene expression in myocytes, cardiomyocytes apoptosis, cytokines and growth factors that influence cardiac dynamics, and deficits in energy metabolism as well as alterations in cardiac extracellular matrix composition. Many therapeutic means have been shown to prevent or reverse cardiac hypertrophy. New concepts for characterizing the pathophysiology of cardiac hypertrophy have been drawn from various aspects, including medical therapy and gene therapy, or use of stem cells for tissue regeneration. In this review, we focus on various types of cardiac hypertrophy, defining the causes of hypertrophy, describing available animal models of hypertrophy, discussing the mechanisms for development of hypertrophy and its transition to heart failure, and presenting the potential use of novel promising therapeutic strategies derived from new advances in basic scientific research. *Antioxid. Redox Signal.* 9, 623–652.

I. INTRODUCTION

Cardiac hypertrophy, an increase in heart muscle mass, reflects a remodeling of the myocardium in response to mechanical stress and various stimuli. The primary molecular cause of enlargement of the heart is hypertrophy of myocytes (*i.e.*, enlargement of existing cells, without an increase in the number of cells). At a molecular level, it has been shown to be a dynamic process during the progression of long-standing hypertrophy to eventual heart failure. The important question is how the changes in molecular makeup of the heart occur throughout the process. An appropriate intervention can be designed only once the mechanisms are known to prevent such molecular events, which in turn have a significant impact on cardiac function. Although myocytes play an important role in the hypertrophying process, cells other than myocytes (*e.g.*, fibroblasts, endothelial cells, and smooth muscle cells) also are involved in this process, either directly or indirectly. The development of hypertrophy and its progression to heart failure are a result of multiple factors, including alteration in gene expression, humoral factors, apoptosis, deficits in energy metabolism, arrhythmia, vascular dysfunction, amount of collagen deposition, and levels of fibronectin, which in turn compromise both diastolic and systolic function, resulting in heart failure (Fig. 1). We divided this review into three major parts as follows: (a) definition and models for cardiac hypertrophy and heart failure available to date; (b) the underlying mechanisms for development of hypertrophy that transits to heart failure; and (c) available therapies, including pharmacologic agents, gene manipulation, and (most recently introduced) stem-cell therapy.

Advances in our understanding of the pathophysiology of cardiac hypertrophy, including the molecular mechanisms involved, are expected to help us design new therapeutic means to achieve regression of existing hypertrophy or to prevent cardiac remodeling and its advancement to heart failure.

Cardiac hypertrophy is an adaptive response of the heart; however, persistence of hypertrophy for longer periods can be detrimental, resulting in cardiac dysfunction and heart failure. Cardiac hypertrophy can occur as a result of a physiologic response (adaptive), in which hypertrophy is associated with increased myocardial compliance, whereas pathologic hypertrophy (concentric hypertrophy) is associated with maladaptive changes, including gene expressions. The response of the heart to meet the change in demands of the circulation is dependent on three different processes: first, either to fill or to empty the heart, to use chemical energy to perform the mechanical work, and to replace or change its molecular constituents. Each of these processes occurs to meet the demand over different time courses.

The physiologic mechanism is primarily filling and emptying the heart to provide beat-to-beat adjustment that enables the heart to meet short-term changes in hemodynamics and to equalize the output of the two ventricles. Physiologic hypertrophy can occur in response to adaptive changes (for example, swimming or running, in which hypertrophy occurs to meet the hemodynamic demand).

The second process depends on the biochemical changes that occur in the cardiac myocytes that enable the heart to meet the demand by modifying the biochemical composition of the myocytes.

The third and most complex of the mechanisms by which the heart adjusts to the changing demands involve growth abnormalities that modify gene expressions in the cardiac cells. These changes provide long-lasting adjustment in response to exogenous stimuli (cytokine growth factors, *etc*.).

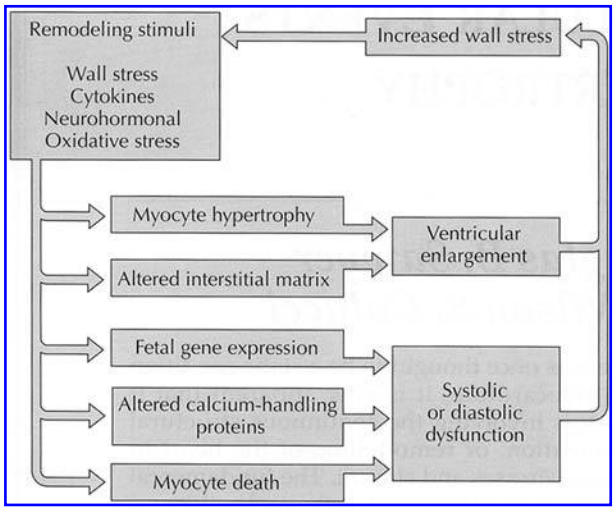

FIG. 1. Schematic presentation of cardiovascular remodeling. Chronic hemodynamic stimuli (*e.g.*, pressure or volume overloads) lead to ventricular remodeling through increase in wall stress, cytokines, signaling peptides, neuroendocrine signals, and oxidative stress. The net result of these changes ultimately leads to heart failure (Figure adapted from ref. 54a.)

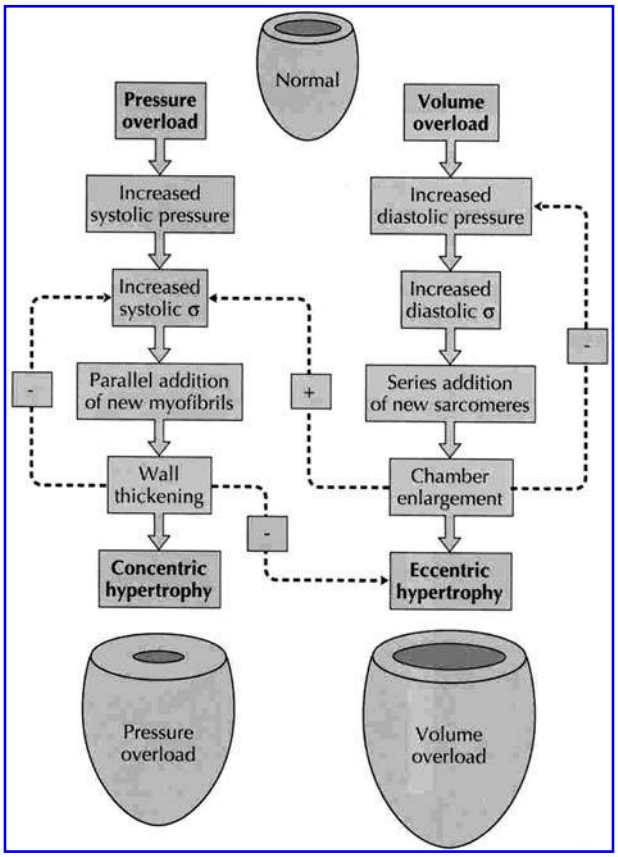

FIG. 2. Patterns of ventricular hypertrophy. A pattern of hypertrophic growth characterized as concentric, in which increased mass is out of proportion to chamber volume. In contrast, in volume-overload conditions, in which the major stimulus is diastolic loading, a predominant finding is a great increase in the cavity size or volume. (Adapted from ref. 105a.)

The mechanisms for physiologic hypertrophy versus pathologic hypertrophy are quite different. Physiologic hypertrophy is an adaptive process; therefore, no treatment is necessary for this type of myocardial growth. Conversely, pathologic hypertrophy, which is a maladaptive change as a result of volume or pressure overload or other hormonal or cytokine stimuli, ultimately results in cardiac dysfunction, which transits to heart failure. Because it is not practical to discuss both physiologic hypertrophy and pathologic hypertrophy in one review, this review discusses only the pathologic hypertrophy, with particular focus on the molecular changes, with special emphasis on the signaling mechanism and therapeutic means to prevent or treat the disease process (or both).

II. DEFINITION AND CLASSIFICATION OF CARDIAC HYPERTROPHY

Cardiac hypertrophy—the enlargement of cell size and mass of individual cardiomyocytes without an increase in cell number—is defined based on pressure or volume overload on the heart. One type, *eccentric hypertrophy*, in which the precipitating stress is volume overload, is characterized by increased heart-wall thickness and ventricular dilation but addition of sarcomere in series. Another type, *concentric hypertrophy*, in which the imposed stress is pressure overload, is characterized by an increase in wall thickness with the deposition of new sarcomeres, but the chamber radius may not change. The thickened ventricle is capable of generating greater forces and higher pressures, while the increased wall thickness maintains normal wall stress (Fig. 2).

Two types of cardiac enlargement are observed: hypertrophy and dilation. Hypertrophy involves an increase in the thickness of the heart muscle (myocardium), particularly in the ventricle, whereas dilation involves an increase in the size of the cavity of one or more chambers of the heart. The most common causes of hypertrophy are an increase in blood pressure (hypertension) associated with other stimuli. The extra workload of pumping blood against the increased pressure causes the ventricle to thicken over time. Another causal factor is stenosis (narrowing of the blood vessels) of the aortic valve, a condition in which the valve cannot open fully, for a variety of reasons. The most common causes of right ventricle hypertrophy (RVH) are associated with any form of right ventricular outflow obstruction or pulmonary hypertension diseases that damage the blood vessels in the lung, causing increased pressure in the remaining vessels. Conditions that decrease oxygen levels, such as chronic bronchitis and sleep apnea, also lead to RVH.

Other causes of dilation are conditions that directly damage the heart muscle, such as inflammation or long-term use of alcohol, the latter of which thins the muscle. Heart muscle

inflammation, or myocarditis, also is associated with a viral infection. Cardiac dilation may also be associated with valvular regurgitation (incomplete opening of the valve), causing the ventricle to dilate over time.

Hypertrophic cardiomyopathy, another class of hypertrophy, goes by many names: hypertrophic obstructive cardiomyopathy, idiopathic hypertrophic subaortic stenosis, and muscular subaortic stenosis (240). The etiology of hypertrophic cardiomyopathy is not yet known, but in the majority of cases, the condition is inherited and considered a genetic disease related to weakness of the individual muscle fibers of the heart. Hypertrophic cardiomyopathy occurs in 1 of 500 people and is the most common heart-related cause of sudden death in young athletes. Recently, mutations have been found in six genes, such as the genes for myosin, troponin T, α-tropomyosin, cardiac myosin-binding protein-C, or the essential and regulatory light chains causing hypertrophic cardiomyopathy; these are the most important and essential proteins for contraction of the heart muscle (180, 181).

The prevalence of different forms of hypertrophy varies considerably depending on the hemodynamic and demographic characteristics of the population. Eccentric hypertrophy is more common in young individuals, whereas concentric hypertrophy is correlated with increased hypertension in advancing age (256). Racial and gender differences also contribute to hypertrophy. The Framingham study showed that concentric hypertrophy is more common in women, and the eccentric, in men (114, 152, 186). Altogether, left ventricular hypertrophy (LVH) is considered to be a risk factor for coronary heart disease, congestive heart failure (CHF), ventricular arrhythmia, and sudden death (17, 20, 37, 92).

III. EXPERIMENTAL ANIMAL MODELS FOR CARDIAC HYPERTROPHY

A. Pressure overload/aortic banding

The most commonly used surgical intervention for pressure-overload–induced hypertrophy is aortic banding (*i.e.*, coarctation of the ascending aorta). In mice, transverse aortic constriction (TAC) is used to create mechanically induced cardiac pressure overload, ultimately leading to cardiac hypertrophy and heart failure (20, 84). TAC is generally induced by a traditional thoracotomy approach by minimally invasive aortic banding through a small incision in the proximal sternum. This TAC model is used to study the profiles of various genes expressed during the onset of hypertrophy, although it does not completely mimic human cardiac remodeling. Aortic banding is a good model system in which to evaluate the development of LVH in response to hemodynamic overload. After several months, a subset of animals progresses to heart failure.

B. Volume overload

CHF occurs when the heart can no longer meet the metabolic demands of the body at normal physiologic venous pressures. Ventricular volume overload occurs in intracardial shunting of blood or in valvular incompetence with backflow of blood. Volume overload, as observed in chronic aortic and/or mitral valvular regurgitant disease, shifts the entire diastolic pressure–volume curve to the right, indicating increased chamber stiffness, with concentric LVH (as occurs in aortic stenosis, hypertension, and hypertrophic cardiomyopathy) (36). In general, the volume-overload CHF model is induced by an aortocaval shunt. The vena cava and the abdominal aorta are dissected above the renal arteries, and the aorta is clamped proximal to the renal arteries; a 0.6-mm needle is used to puncture the aorta distally and is advanced into the vena cava to connect both vessels. The needle is removed afterward, and the wound is sealed. Cardiac hypertrophy develops within 4–5 weeks, with compromised LV contractility and increased end-diastolic pressure (257).

C. Coronary artery ligation

Coronary artery ligation is a procedure used to generate animal models of heart failure. Ligation of the rodent left anterior descending (LAD) artery occludes the supply of oxygen and nutrients to the heart muscle, thus causing a blockage similar to the kind that causes heart attacks in humans. Such occlusion results in myocyte death, which affects the overall structure of the heart, contributing to eventual cardiac dysfunction. Because these animal models closely mimic heart-failure disease progression in humans, they have proven to be valuable for investigating the mechanisms of heart failure (13).

D. Transgenic mouse models for cardiac hypertrophy

Several transgenic mouse models for cardiac hypertrophy and heart failure have been described over the past decades to study the molecular mechanisms underlying this deadly disease. It is not possible to give a comprehensive overview of all these models because of space limitations, but in this review, we describe one suitable transgenic mouse model in which hypertrophy developed and eventually progressed to heart failure. Table 1 describes the most established hypertrophy/heart failure mouse model we observed so far.

Table 1. Mouse Models for Heart Failure

Genetically engineered mouse models	*Metabolically altered models*	*ECM dysfunction transgenic models*
Myotrophin, TNFα, Gi, Gαq, PKCβ, PKA, β1 AR, Phospholamban, calsequestrin, calcineurin, L-type Ca^{2+} channel	Mitochondrial dysfunction Oxidative stress Impairment of fatty acid oxidation (FAO) pathway	MMP2 MMP9 TIMP1

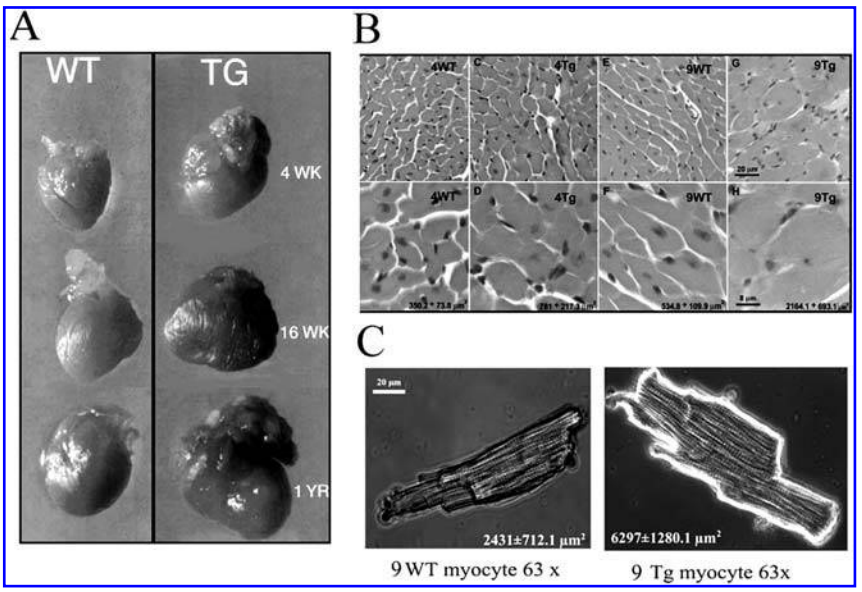

FIG. 3. (A) Hearts from WT and Tg mice during progression of cardiac hypertrophy. Quantitative estimation of HW/BW in WT and Tg mice during initiation of hypertrophy (4 weeks old) and transition from hypertrophy to heart failure (9 months old). (**B**) Quantitation of cross-sectional areas of myocytes in WT and Tg mice ($n = 5$). The *top panel* shows myocytes (stained with hematoxylin and eosin) from 4-week-old mice, WT (*extreme left*) (*A*), and Tg (*C*). *E* depicts 9-month-old WT cells, and *G* shows the myocytes from 9-month-old Tg animals at ×63 magnification. The *lower panel* represents a ×2.5 zoomed picture of the *upper panel.* (**B**) Myocytes from 4-week-old WT mice; *D*, myocytes from 4-week-old Tg mice; *F*, myocytes from 9-month-old WT mice; *H*, myocytes from 9-month-old Tg mice. A significant increase in the cross-sectional area was observed at as early as 4 weeks of age. The cross-sectional area of myocytes from Tg mice was significantly increased (2,431 ± 712 to 6,297 ± 280 μm^2; $p < 0.001$). (**C**) Myocytes isolated from 9-month-old WT (*left panel*) (×63 magnification) and 9-month-old Tg mice overexpressing myotrophin (*right panel*) (×63 magnification). (Figure adapted from ref. 255.)

1. Transgenic mouse model overexpressing myotrophin. We describe a transgenic mouse model in which myotrophin is overexpressed in the heart as a representative of a genetically engineered model to study the mechanism of cardiac hypertrophy and CHF. Myotrophin, a 12-kDa soluble protein, has been identified and characterized from spontaneously hypertensive rat (SHR) hearts and cardiomyopathic human hearts (277, 318). Myotrophin induces myocyte growth and enhances early proto-oncogenes and hypertrophic marker gene expression in neonatal myocytes (202). Myotrophin stimulates protein kinase C (PKC) activity (276) and interacts with NF-κB proteins (146, 279). A cardiac-specific overexpression of myotrophin, using α-myosin heavy chain as a promoter, causes development of hypertrophy that culminates in heart failure (255) (Fig. 3). This process is associated with mechanistic changes, including significant increases in expression of proto-oncogenes, hypertrophy marker genes, and growth factor genes, as well as increased collagen deposition (Fig. 4). The transgenic mice showed a gradual progression from hypertrophy to heart failure over a span of 9 months, exhibiting LVH, atrial dilation, myocyte necrosis, multiple focal fibrosis, pleural effusion, and compromised

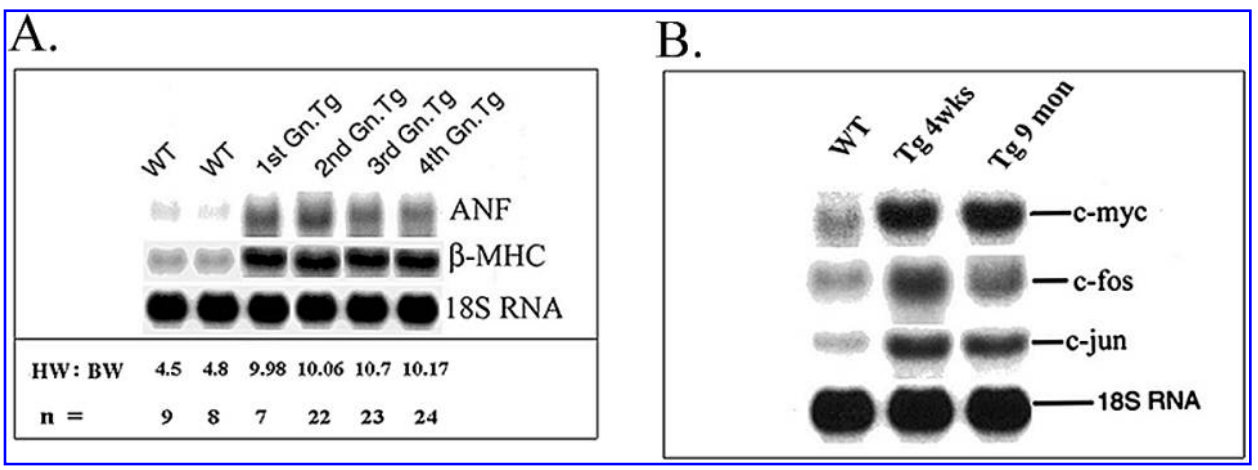

FIG. 4. (A) Tg mice show increased expression of ANF and β-MHC transcripts from four generations (Gn1-Gn4), representing all four Tg lines (24 weeks old), compared with age-matched WT mice. Increased expression of ANF and β-MHC transcripts in all four generations confirmed the presence of hypertrophy in Tg mice. (**B**) Tg mice show increased expression of proto-oncogenes in the hearts of young and old Tg mice compared with WT mice. (Figure adapted from ref. 255.)

TABLE 2. EFFECT OF PHARMACOLOGIC INHIBITORS ON BLOOD PRESSURE AND CARDIAC HYPERTROPHY

Model	*Treatment*	*HW/BW (mg/g)*	*BP*	*Reference*
SHR	None	3.44 ± 0.15	196 ± 28	Sen S (268)
SHR	Captopril	2.64 ± 0.08	119 ± 23	Sen S (268)
SHR	Esidrix	3.30 ± 0.05	171 ± 60	Sen S (268)
SHR	Atenolol	3.00 ± 0.11	151 ± 50	Sen S (268)
SHR	Losartan	2.86 ± 0.04	146 ± 30	Alvarez BV (4)
SHR	Nifedipine	3.06 ± 0.05	140 ± 20	Alvarez BV (4)

Values are expressed as the mean ± SEM. SHR, spontaneously hypertensive rats; captopril, ACE inhibitor; esidrix, diuretic; atenolol, β-blocker; losartan, Ang II–receptor blocker; nifedipine, calcium channel blocker; HW/BW, heart weight–to–body weight ratio; BP, blood pressure.

cardiac function associated with significant reduction in ejection fraction and shortening (Fig. 5) (255). These changes contributed to substantial alterations in the expression and organization of sarcomeric and structural proteins. This model also documented the changes in gene expression during initiation of cardiac hypertrophy versus during its progression to heart failure. These changes very closely mimicked the symptoms associated with the human hypertensive heart and provided a unique opportunity to study molecular changes along with changes in growth factors and cytokines during the initiation of hypertrophy and its transition to heart failure. Identification of genes whose expression is altered during the initiation and transition phases might suggest novel strategies to limit hypertrophy and its progression to heart failure. We expect that this new mouse model will provide the key to elucidate further molecular mechanisms that occur during advancement of hypertrophy to heart failure and will facilitate the design of effective therapies.

2. Metabolic remodeling of the failing heart. The development of CHF is also associated with a plethora of intracellular changes, including alterations in sarcomeric protein function, in extracellular matrix composition, and in calcium flow and energy metabolism. Many experimental and clinical studies have shown that hypertrophied and failing myocardium are characterized by alterations in mitochondrial function, high-energy phosphate content, and the fatty acid oxidation (FAO) pathway. It is speculated that these metabolic alterations could reflect the high demand of adenosine triphosphate (ATP) for energy supply to cardiac muscles. However, the underlying mechanisms of "metabolic remodeling" in the context of cardiac failure are still open to discussion. In this review, we discuss how mitochondrial dysfunction and FAO lead to heart failure.

3. Mitochondrial dysfunction and cardiac failure. Mitochondria are considered to be the "powerhouse" of cells. Mitochondria are packed between the adjacent myofibrils and occupy up to 50% of the cytoplasmic volume of the heart muscle cell and contain several copies of the mitochondrial DNA (mtDNA) genome, ribosomes, transfer RNAs, and several enzymes that are required for the expression of the mitochondrial genes. Experimental and clinical studies have shown that an impaired respiratory function of cardiomyocyte mitochondria leads to CHF (140, 271). Mitochondria are considered important targets and releasers of various regulatory factors that play an important role in cardiac remodeling. Mitochondria have also been recognized as players in the apoptosis process, in which the opening of the "mitochondrial pore" plays a key role (130). The following features can address mitochondrial dysfunction.

a. Oxidative stress. Oxidative stress is characterized by the formation of reactive oxygen species (ROS) from the respiratory chain. Oxidative phosphorylation of mitochondria

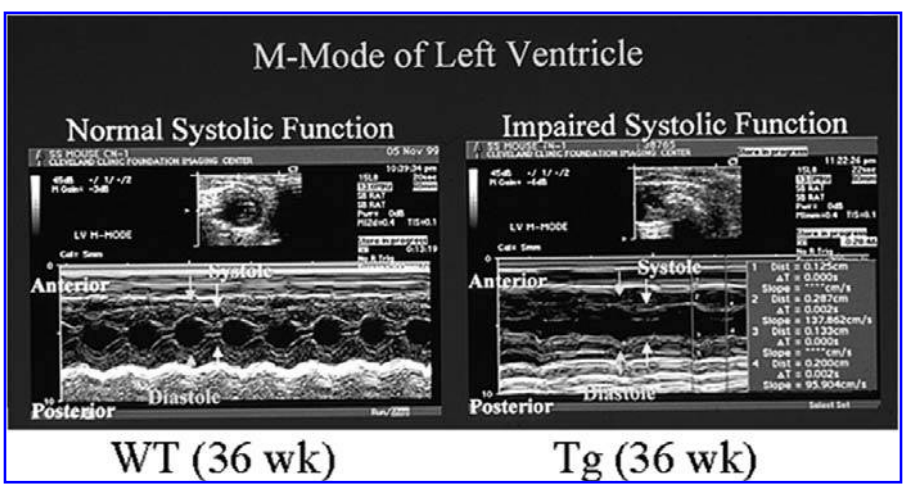

FIG. 5. A typical M-mode echocardiogram from 36-week-old WT and Tg mice. (Figure adapted from ref. 255.)

generates oxygen radicals, such as superoxide radicals, H_2O_2, and hydroxyl radicals (150). Another source of free radicals is lipid oxidation. It has been shown that because of the production of excessive free oxygen radicals, chronically ischemic hearts exhibit increased damage to mtDNA, which ultimately induces many genes responsible for oxidative phosphorylation (56). mtDNA is more susceptible to damage than is nuclear DNA because it lacks histones and also has an insufficient repair mechanism (49, 241, 242). mtDNA also exhibits 10- to 12-fold higher mutation rates, as it is continually exposed to free radicals because of generation of ATP by the respiratory chain. Mitochondrial enzymes are, therefore, the primary targets for inactivation by ROS. In the myocardium, oxygen radicals have been shown to alter Na^+/Ca^{2+} exchange, Na^+-K^+ ATPase, and Ca^{2+} ATPase activities. Mutant mice that lack manganese superoxide dismutase (a scavenging enzyme) show a severe reduction in complex II of the respiratory chain and rapidly develop dilated cardiomyopathy (167).

b. Acquired mutation. It has been shown that numerous defects in the mitochondrial respiratory chain lead to inherited CHF and cardiomyopathy (177, 323). It has also been reported that a deficiency in carnitine, a transporter molecule in mitochondria, showed familial cardiomyopathy (322). Although what role the mutation of mitochondrial proteins plays in the inherited cardiomyopathies is not fully understood (177), it is known that mtDNA and mitochondria are maternally inherited. Maternal inheritance has also been observed in point mutations of specific mitochondrial genes that cause massive defects in the respiratory chain, leading to CHF (347). Conversely, deletion of a larger segment of the mitochondrial genome results in cardiac conduction defects and cardiomyopathy, such as Kearns-Sayre syndrome (40). The deletion causes a major defect in the electron-transport system, and it is thought that deletion arises from spontaneous somatic mutations early in embryogenesis and may play a critical role in the development of the major cardiac disease (298).

c. Mouse models of mitochondrial dysfunction and heart failure. A number of transgenic mice models have been developed to study the relation between mitochondria and cardiac function. This has been done either by disrupting mitochondrial metabolism (loss of function) or by activating the mitochondrial oxidation pathway molecule (gain of function). Among the best-studied examples of mitochondrial metabolism is the adenine nucleotide translocator (ANT). ANT, a 32-kDa energy-transfer mitochondrial protein, encoded by three different genes (ANT1, 2, and 3) and coexpressed in a tissue-specific fashion, exchanges mitochondrial ATP for cytosolic adenosine diphosphate (ADP) across the inner mitochondrial membrane. ANT is a key molecule regulating ATP production and consumption in the mitochondrial matrix space. Graham *et al*. (102, 203) found that $Ant1^{-/-}$ mice exhibited mitochondrial abnormalities and a progressive cardiac hypertrophic phenotype. Another knockout model for disruption of mtDNA replication is Tfam. Tfam is a nuclear-coded mtDNA replication transcription factor. Using the *cre-lox* system, Larson's group (155, 165, 324) generated a series of tissue-specific Tfam knockout mice. Such knockout mice frequently die during the neonatal period, but in those that survive, cardiomyopathy develops by around age 2 months. These mice are characterized by cardiac hypertrophy, ventricular chamber dilation, and conduction defects, including atrioventricular blocks. In addition, mice null for the peroxisome proliferator–activated receptor-α (PPARα) gene exhibit a diminished myocardial fat catabolic pathway and a moderate cardiomyopathic phenotype (158, 328). It is also possible that the distinct profile of fatty acid intermediates that accumulate demands a specific form of cardiac disease.

Conversely, cardiac-specific overexpression of PPARα in certain transgenic mice is associated with the upregulation of genes for the mitochondrial FAO pathways and the downregulation of glucose transporter and glycolytic enzyme gene expression (88). These transgenic mice also exhibit cardiac hypertrophy, reduced fractional shortening, and ventricular wall thinning and chamber dilation. Another transgenic mouse model that has constitutive cardiac-specific overexpression of peroxisome proliferator–activated receptor-γ coactivator-1α (PGC-1α) develops dilated cardiomyopathy with ultrastructural abnormalities, including fibrosis, disruption of myofibrils, and an increased number of large mitochondria (160). Given the importance of mitochondrial dysfunction in these transgenic models, it is speculated that activation of PGC-1α, PPARα, or both, might play an important role in the development of myocardial hypertrophy and dysfunction.

d. Impairment of fatty acid oxidation (FAO) pathway. Mitochondrial FAO is the key metabolic pathway for ATP production in heart and skeletal muscle. Fatty acids are transported in the blood in the form of lipoprotein. Long-chain fatty acids are transported through the plasma membranes by fatty-acid transporters, such as fatty-acid transport protein (FATP) and CD36/FAT. On entry into the cell, fatty acids are converted to their acyl coenzyme A (CoA_ derivatives by fatty acyl CoA synthase (ACS1). The first step of the β-oxidation spiral is an oxidation reaction of acyl CoA that is catalyzed by a family of four very long-chain acyl CoA dehydrogenase (VLCAD) homologous acyl CoA dehydrogenase enzymes: long-chain acyl CoA dehydrogenase (LCAD), acyl CoA dehydrogenase, and short-chain acyl CoA dehydrogenase (SCAD) genes. It is observed that mutations in the VLCAD gene affect children and young adults, causing cardiomyopathy (183). Genetic disruption of VLCAD was associated with microvesicular lipid accumulation, mitochondrial proliferation, ventricular tachycardia, degenerative fibers, collagen deposition, and vacuolated myocytes in VLCD-deficient mice (86). This model may represent an interesting model system in which the pathophysiologic relations of intracellular fatty-acid homeostasis and myocardial dysfunction can be studied.

Another striking example of FAO impairment leading to cardiomyopathy is the *cre-lox*P-mediated cardiomyocyte-restricted deletion of PPARδ in mice (46). These mice have cardiac dysfunction, progressive myocardial lipid accumulation, cardiac hypertrophy and CHF with reduced survival. Chronic myocardial PPARδ deficiency leads to lipotoxic cardiomyopathy. Therefore, PPARδ is a crucial determinant

of myocardial FAO and is an important factor in maintaining energy balance and normal cardiac function.

4. Role of extracellular matrix in cardiac hypertrophy and heart failure. Extracellular matrix (ECM) is a ground network of collagen that is responsible for the integrity of myocardial structure. The ECM tethers myocytes and myofibrils together in proper alignment, provides an architectural support for the muscle cells, plays an important role in cardiac adaptation to diverse stresses, and promotes remodeling. The myocardial ECM is composed mainly of collagen type I (Col I) and collagen type III (Col III), which make a scaffolding support for the myocytes and maintain the integrity of the heart. Alteration of the Col I/Col III ratio therefore has an integral part in predicting both systolic and diastolic dysfunction. A differential increase in Col I and Col III and their ratio has been seen in patients with dilated cardiomyopathy and in hypertensive patients with CHF (174, 223). Loss of collagen fibrils leads to ventricular dilation, myocyte slippage, and contractile dysfunction. Therefore, modulation or balance between matrix protein synthesis and degradation is the crucial factor for cardiac remodeling and also in the pathophysiology of heart failure. In normal heart, collagen turnover is maintained by matrix metalloproteinases (MMPs) and their endogenous inhibitors [*i.e.*, tissue inhibitors of metalloproteinases (TIMPs)] (168). A fine balance between MMPs and TIMPs maintains the integrity of the matrix. Increased MMP activity leads to enhanced ECM degradation, which may cause ventricular dilation and CHF. MMPs are a family of zinc-dependent proteolytic enzymes that degrade ECM protein. More than 20 different types of MMPs have been identified and cloned and have a wide range of substrate specificity (58) Based on their structure and substrate recognition, MMPs are divided mainly into four classes: (a) collagenases, which include MMP-1, -8, -13, and -18; (b) the gelatinases, which include MMP-2 and -9; (c) the stromelysins, including MMP-3, -10, and -11; and (d) membrane-type MMP (MT-MMP), which includes MMP-14 to MMP-17 and MMP-24 and -25. MMPs are present in the heart (interstitium) and are enhanced during myocardial infarction (38). In this review, we focus on what is currently known about the MMP/TIMP system in human CHF, the novel mechanisms that regulate this system, and the movement toward clinical trials and applications with respect to this proteolytic pathway.

Several MMP species have been identified within the human myocardium and become altered in CHF. A number of studies suggest that an upregulation of MMP-1, -2, -3, and -9 occurs in human, rat, and porcine hearts during the remodeling process, suggesting that these MMPs play a role in disease states (53, 58, 300, 311). Importantly, increased myocardial MMP activity is observed both in clinical and experimental forms of dilated cardiomyopathy (287, 309, 310). MMPs that are expressed at very low levels in normal myocardium [such as collagenase-3 (MMP-13) and the membrane type-1 MMPs (MT1-MMP)] are substantially upregulated in CHF (281). However, MMP species are not uniformly increased in patients with end-stage CHF, suggesting that a specific "portfolio" of MMPs are expressed in the failing myocardium (281). With animal models of CHF, a mechanistic relation has been demonstrated with respect to myocardial MMP expression and the LV remodeling process. One of the most striking examples of constitutive overexpression of MMP-1 in a transgenic mouse model, demonstrating the first evidence of direct disruption of the ECM in the heart, mimics human CHF (144). Targeted deletion of MMP-2 in mice resulted in a better survival rate, lower incidence of LV rupture, less LV-cavity dilation, and improved fractional shortening than in WT mice after myocardial infarction (MI), suggesting a potentially useful therapeutic strategy to manage post-MI hearts (118). One MMP-9–deficient mouse model showed attenuation of ventricular enlargement and collagen deposition after MI, suggesting a candidate for a selective inhibitor of MI (76). Conversely, deletion of TIMP-1 leads to augmented adverse LV remodeling after MI (59). The aforementioned studies using both knockout and transgenic mice and MMP inhibitors have further established the significant contribution of these proteases in cardiac remodeling and have raised the possibilities of using MMP as a therapeutic target for the treatment of CHF. Pharmacologic intervention using MMP inhibition has been shown to reduce ventricular remodeling after MI in rat and mouse models, demonstrating the beneficial effect during the progression of CHF (226, 245). Interestingly, polymorphisms have been seen in the promoters of MMP-1, -3, -9, and -12 and have been linked with cardiovascular diseases such as coronary artery disease (CAD) and abdominal aortic aneurysms, indicating the variation in MMP gene transcription in MI patients that might influence the difference in LV remodeling and infarct healing (341, 348). Therefore, the use of genetic mice models and MMP inhibitors suggests the potential role of these proteases in cardiovascular diseases. Future studies exploring the possible correlation between MMP polymorphisms and MI are warranted.

IV. MECHANISMS INVOLVED IN CARDIAC HYPERTROPHY

The phenomenon of cardiac hypertrophy results from programmed and synchronized responses of cardiac cells at molecular and biochemical levels as an adaptive response to an altered cardiac environment, caused by a combination of factors such as increased hemodynamic pressure and hormonal imbalance. Hypertrophic responses begin as an adaptive mechanism to sustain the altered state. Irrespective of what triggers the process, an increase either in global protein synthesis or in the mass of cardiomyocytes, or both, leads to enlargement of the heart or cardiac hypertrophy. For the past several years, researchers have tried to find a single pivotal point through which the hypertrophic process is regulated. To date, such a confirmed converging point has yet to be discovered, although several mechanisms involved in the process have already been reported. In this review, we have tried to accumulate certain important reports, to define the mechanisms that are involved in cardiac hypertrophy.

A. G protein and cardiac hypertrophy

In the cardiovascular system, G protein–coupled receptors (GPCRs) play an important role with respect to its acute

hemodynamic and chronic hypertrophic effects (196). These receptors are coupled to three principal classes of heterotrimeric guanine triphosphate (GTP)-binding proteins, G_q, G_s, and G_i, and all of them have G_α and $G_{\beta\gamma}$ subunits (91). The binding of any ligand to the heptahelical GPCRs results in sequential conformational changes that lead to coupling of the receptor to a heterotrimeric G protein. On activation, because of a guanine diphosphate (GDP)-to-GTP exchange on the G_α subunit, the G protein dissociates into its component subunits, G_α and $G_{\beta\gamma}$, which independently activate intracellular signaling pathways through downstream effectors. $G_{\alpha s}$ and $G_{\alpha i}$ modulate the activity of adenylyl cyclase (AC), which generates cAMP and thereby activates cAMP-dependent protein kinase A (PKA). In contrast, $G_{\alpha q}$ activates phospholipase C (PLC) to generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) to activate protein kinase C (PKC) (Fig. 5). The $G_{\beta\gamma}$ subunit directly activates the mitogen-activated protein kinase (MAPK) signaling cascade, phosphatidyl inositol 3-kinase (PI3K) activity, and Ras signaling (Fig. 6).

1. Role of G_{aq}. Angiotensin II (Ang II), endothelin-1 (ET-1), and α-adrenergic receptors (αARs) remain coupled to $G_{\alpha q}$, and this pathway plays an important role in hypertrophic responses (182, 252, 274). Overexpression of these receptors and G_q protein has been shown to be a factor in the development of cardiac hypertrophy (61, 147, 219, 253). Myocardial overexpression of constitutively active α_1B-adrenergic receptor has been reported to develop cardiac hypertrophy and dilated cardiomyopathy (162, 191, 320). A reduction in hypertrophic responses to chronic pressure overload has been shown by inhibiting G_q-mediated signaling.

Reduction of hypertrophic responses to chronic pressure overload has been demonstrated by inhibiting G_q-mediated signaling or blocking of β-hydroxylase (the enzyme responsible for norepinephrine synthesis). This result indicates that some signaling event, perhaps G_q-mediated signaling, is involved in the hypertrophic process, which is no longer only an adaptive response (84, 264).

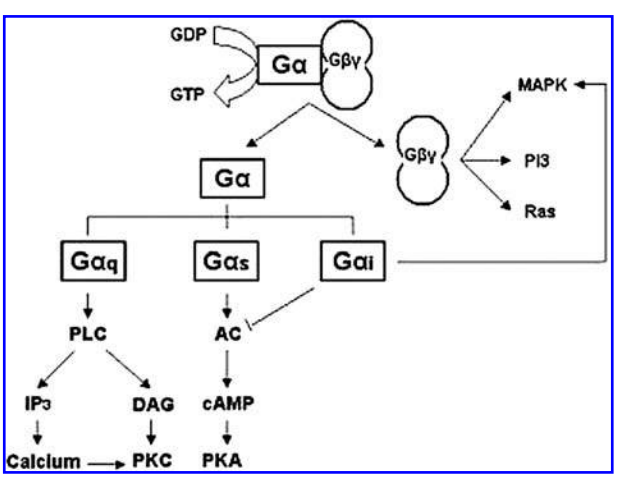

FIG. 6. G protein–mediated signaling pathway. PLC, Phospholipase C; IP_3, inositol 3-phosphate; DAG: diacylglycerol; PKC: protein kinase C; PKA: protein kinase A; AC, adenylyl cyclase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inositol 3-kinase. (Adapted and modified from ref. 196.)

2. Role of G_s. The β_1-adrenergic receptors is the most abundant of the adrenergic receptors that remain coupled to G_s, which in turn activates AC-mediated downstream signaling. The less abundant β_2-adrenergic receptor subtype couples to G_s and G_i, resulting in an additional β-adrenergic signaling event (62). The G_s protein is reported to be involved more in cardiac contractility than in cardiac growth (264). Overexpression of the β_1-adrenergic receptor in the hearts of transgenic mice has been implicated in the progressive deterioration of cardiac performance and in hypertrophy and fibrosis (18, 82). A similar phenomenon was observed as AC-independent responses in transgenic mice overexpressing G_s (95). In addition to an increase in myocardial contractility, delayed fibrotic cardiomyopathy has been observed in transgenic mice overexpressing the β-adrenergic receptor or the α-subunit of the G_s protein (74). Uncoupling protein 2 (UCP2, which controls mitochondrial membrane potential) and the four-and-a-half LIM domain protein (FHL1) have been reported as important candidate protein molecules that correlate with the development of βAR-induced cardiomyopathy in a different mouse model (96).

3. Role of G_i. Upregulation of G_i in human heart failure and impaired AC activity suggest that G_i-mediated signaling contributes to cardiomyopathy (83, 209). Conditional overexpression of the G_i-coupled receptor leads to delayed ventricular conduction and lethal cardiomyopathy (237). The coexistence of pressure overload and loss of contractile mass in the ischemic heart leads to the transition from cardiac hypertrophy to CHF through a G_i-mediated signaling cascade (149). Furthermore, involvement of extracellular signal–regulated kinase (ERK) activation in cardiomyocyte hypertrophy through G_i and G_s has been reported (357). cAMP-dependent PKA activation through G_s phosphorylates βAR, which in turn results in coupling of the receptor from G_s to G_i. ERKs become activated by G_i through $G_{\beta\gamma}$, the Src family of tyrosine kinases, Ras and Raf-1 kinase (357).

B. Small G protein and cardiac hypertrophy

Small G proteins are involved in activation of several downstream signaling pathways and are related to myocyte hypertrophy. The low-molecular-weight or small GTPase superfamily consists of more than 100 members, grouped based on their structural similarities. Five such subfamilies are known: (a) Ras, (b) Rho/Rac/cdc42, (c) Arf/Sar1, (d) Rab, and (e) Ran (29). All of them are 20- to 30-kDa monomeric G proteins. The small GTPase consists of only a single subunit and thus differs from heterotrimeric G proteins. Small GTPase resembles the G protein α-subunit but is about half its size and becomes active when GTP is exchanged for GDP (29). Only the Ras and Rho subfamilies have been studied extensively in the heart (51) and are discussed in this review.

Myocytes exposed to Ang II, ET-1, and PE showed activation of Ras isoforms (48, 235). Several signaling proteins

such as c-Raf (MAPK kinase or the ERK cascade), PI3K, and Ral are activated by binding with Ras. Ras-mediated activation of the c-*jun* NH_2-terminal kinase (JNK) cascade has also been reported (235). Rho and Rac-1 known to be activated by integrins, adhesion molecules, cytokines, and receptor tyrosine kinase (29). The effects of RhoA and Rac-1 on the actin cytoskeleton and cell morphology are mediated through stimulation of downstream effector kinases by the activated Rho proteins. The best-known effector is Rho kinase (ROCK). RhoA has the ability for transcriptional activation of AP-1 (178, 179), GATA-4 (39), nuclear factor κB (NF-κB) (6, 60, 299), and myocyte enhancer factor 2 (MEF2) pathways (39, 296). The Gq-RhoA-ROCK pathway can be activated by several neurohumoral factors and is believed to function as an important signaling pathway for cardiac hypertrophy in transition from LVH to CHF (198). Morel *et al*. (2005) (238) reported Epac as a genuine exchange factor (GEF) that is activated by cAMP and is involved in activation of prohypertrophic signaling such as Ca^{2+}-sensitive phosphatase, calcineurin, and downstream effector nuclear factor of activated T-cell (NFAT). Rac is found to be involved in Epac-induced NFAT-dependent cardiomyocyte hypertrophy (198). By using adenoviral-mediated gene transfer of wild-type and dominant-negative mutants of PKCα and PKCδ, Pan *et al*. (218) showed that stretch-induced activation of Rho GTPases and phosphorylation of the Rho-guanine nucleotide dissociation inhibitor (Rho-GDI) are regulated mainly by PKCα and PKCδ is involved in regulating the activation of Rac1. Clerk *et al.* (50) reported that Rac1 might stimulate the ERK cascade by promoting the phosphorylation of c-Raf by increasing MEK1 and/or 2 associations with cRaf to facilitate MEK1 and/or 2 activation.

C. Renin–angiotensin system (RAS) and transforming growth factor-β1 (TGF-β1) and cardiac hypertrophy

RAS and TGF-β_1 are found to be involved in cardiomyocyte hypertrophy after pressure overload. It has been postulated in several studies that angiotensin II (Ang II) and TGF-β_1 act together as key mediators for cardiac remodeling. Ang II, the effector molecule of the RAS, has direct growth-promoting activity on neonatal cardiac cells but not on adult cells. It promotes the growth activity of neonatal myocytes through the ERK pathway (2, 247). A locally generated cytokine, TGF-β_1, is particularly expressed in hypertrophic myocardium, and its expression is induced by Ang II, thus indicating that Ang II indirectly stimulates cardiomyocyte growth through TGF-β_1, which acts locally by autocrine or paracrine mechanisms (329). In pressure-overloaded human heart, the degree of fibrosis is correlated with the upregulation of angiotensin-converting enzyme (ACE) and TGF-β_1 during progression from compensated hypertrophy to CHF, indicating an interconnection between RAS and TGF-β_1 (329). Several *in vitro* studies have shown upregulation of TGF-β_1 mRNA and protein by Ang II in cardiac cells (199, 329).

Binding of Ang II to its receptor AT_1R promotes the synthesis of TGF-β_1 in cardiac fibroblasts and myocytes. TGF-β_1 causes the proliferation of fibroblasts by binding to Tβ receptors and results in fibrosis through fibroblast proliferation (116, 254, 307). TGF-β_1 also induces ECM components, such as collagen, fibronectin, and proteoglycans, and results in cardiac hypertrophy (78, 120, 321). Links between Ang II and TGF-β_1 are further supported in tissue-culture experiments: paracrine release of TGF-β_1 from fibroblasts mediates Ang-II–induced cardiac myocyte hypertrophy as blocking antibodies to TGF-β_1 inhibit myocyte growth caused by conditioned medium of Ang II–treated fibroblasts (247). In a rat model, blocking of Ang II action by an AT_1-receptor antagonist was found to reverse the process of TGF-β_1 expression and myocardial hypertrophy/fibrosis and thus further supports the link between Ang II and TGF-β_1 (306, 308, 329). Schultz *et al*. (261) also reported that TGF-β_1–deficient mice do not show any myocardial growth, even if treated with Ang II.

Ang II has also been found to cause an increase in Akt activity via activation of the AT_1-receptor. Hingten *et al*. (2006) (123) reported that increased formation of superoxide (O_2^-) radicals from an Rac1-regulated Nox2 containing NADPH oxidase is a key upstream mediator of Ang II–induced activation of Akt, and this cascade plays a crucial role in Ang II–dependent cardiomyocyte hypertrophy. Mice lacking ACE2 (the cleaving enzyme of Ang II) showed a marked increase in Ang II concentration and activity of MAPK in response to transverse aortic constriction. Administration of AT_1-receptor blocker attenuates the hypertrophic responses and suppresses the MAPK activity in these ACE2-deficient mice, indicating that ACE2 plays an important role in the hypertrophic responses to pressure overload. Zou *et al*. (356) reported that an Ang II-independent mechanism also activates the AT_1-receptor. A novel signaling mechanism driven by Ang II type 2 receptor was reported by Senbonmatsu *et al*. (269). They described that Ang II stimulation induces the cytosolic transcription factor promyelocytic zinc finger protein (PLZF) to drive AT2 from the cell membrane to the perinuclear region. PLZF then enters the nucleus and binds to the consensus sequence of p85 alpha PI3K, followed by enhanced expression of p70S6 kinase, which is essential for protein synthesis. In other studies, inhibition of calcineurin activity by use of a specific inhibitor has been shown to decrease [^{3}H] leucine incorporation in Ang II–treated cardiomyocytes, indicating that calcineurin also plays an important role in Ang II–induced cardiomyocyte hypertrophy (93). Everett *et al*. (85) reported that an Ang II–dependent increase in protein synthesis includes activation of eukaryotic elongation factor-2 (eEF-2) via dephosphorylation by protein phosphatase-2A by a process that involves both PI3K and MAPK. Therefore, elements of the RAS, particularly Ang II, induce cardiac hypertrophy by involving several pathways, but among them, the interconnection between Ang II and TGF-β1 is the most vital one.

D. PI3K/Akt and cardiac hypertrophy

Phosphoinositide 3-kinases (PI3K) and Akt play an important role in the heart through regulation of cardiomyocyte growth, survival, function, and metabolism (184). PI3Ks comprise a family of enzymes that has both protein and lipid kinase activity and can be activated by several receptor tyrosine kinases such as the IGF-1 receptor, as well as GPCRs, including the α- and β_2-adrenergic receptors (258). The activated

IGF receptor phosphorylates the insulin-receptor substrates (IRSs), and the IRS1 in particular interacts with the SH2 domain of PI3K for its activation (67, 196). Binding of growth factors like IGF-1 and insulin to their membrane tyrosine kinase receptors activates 110-kDa lipid kinase, PI3K subgroup Iα (also referred as p110α) (215). Activated PI3K leads to D3 phosphorylation of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PI-4,5-P_2), generating phosphatidylinositol 3,4,5-trisphosphate (PI-3,4,5-P_3) (75, 184). PI-4,5-P_2 is the main precursor for PI-3,4,5-P_3 biosynthesis by class I PI3K (90, 286, 319, 336). PI-3,4,5-P_3 is degraded by the lipid phosphatases, phosphatase, tensin homolog deleted on chromosome 10 (PTEN), and SH2 domain–containing inositol 5-phosphatase 2 (SHIP2) to generate PI-4,5-P_2 and PI-3,4-P_2, respectively (63, 215) (Fig. 7). PIP_2 and PIP_3 accumulate in the cell membrane and recruit serine/threonine kinase Akt (also known as protein kinase B or PKB) and its activator 3-phosphoinositide–dependent protein kinase 1 (PDK1) to the cell membrane via interactions between the kinase pleckstrin homology (PH) domain and the 3′-phosphorylated lipid (27). Furthermore, this enforced colocalization results in phosphorylation of PDK1, which activates Akt1. Of the three Akt genes, only Akt 1 and 2 are highly expressed in the heart (75). Once activated, Akt affects such diverse intracellular processes as translational regulation and cell survival.

PI3K also is activated in a $G_{\beta\gamma}$-dependent fashion in pressure-overload hypertrophy (206). It has been reported that the catalytic domain of the PI3Kγ isoform directly interacts with the $G_{\beta\gamma}$ subunit to activate the PI3K/Akt signaling cascade (163). Czupalla *et al.* (60) reported that autophosphorylation of p110γ in response to $G_{\beta\gamma}$ does not reduces its lipid kinase activity. Thus, GPCR stimulation in pressure-overload hypertrophy selectively activates p110γ without affecting p110α (206).

Two well-established downstream targets of Akt are glycogen synthase kinase-3 (GSK-3) and mammalian target of rapamycin (mTOR) (91, 184) (Fig. 7). GSK-3 is a serine/threonine kinase that phosphorylates and inactivates glycogen synthase (72). The two mammalian isoforms (GSK-3α and β) are both expressed in the heart (72, 105, 299, 305). Akt directly phosphorylates GSK-3β, which in turn phosphorylates a series of serine/threonine residues in the N-terminal regulatory region of NFAT proteins (354) and masks their nuclear import sequences, thereby promoting cytoplasmic translocation and transcriptional inactivation of NFAT proteins. Numerous other transcription factors reported to be phosphorylated by GSK-3β include c-*jun* (22), c-*myc* (263), STAT (98), and NF-κB (124); all of these have been found to be involved directly or indirectly in the development of cardiac hypertrophy. The activity of GSK-3β is regulated by the phosphorylation status of its serine-9 residue, and phosphorylation of this site creates an inhibitory pseudosubstrate for the enzyme (91). Inhibition of GSK-3 is considered to be both necessary and sufficient to induce cardiomyocyte hypertrophy in both *in vivo* and *in vitro* models (110, 200, 201).

Another downstream target of Akt is mTOR (also called FKBP-12 rapamycin–associated protein or FRAP). It is a 289-kDa evolutionarily conserved serine/threonine kinase that is inhibited by the drug rapamycin (215). Akt activation activates mTOR via both translational and phosphorylation mechanisms, leading to altered metabolism and increased cell growth, mediated by changes in gene transcription and translation (259, 288). More specifically, mTOR phosphorylates and therefore inactivates eukaryotic initiation factor 4E-binding protein (eIF-4E BP1), leading to increased protein translation (111, 113, 345). mTOR also phosphorylates and activates p70S6kinase (259), which is a short isoform of the ribosome S6 kinase 1 (S6K1); S6K1 and S6K2 are considered key regulators of cell growth through the control of protein translation (77, 273). The regulation of translation by p70S6kinase occurs for ~20–30% of the total mRNA, which includes many genes essential for the determination of cell size, growth, and proliferation (215, 224, 273, 349). It can be concluded that significant crosstalk occurs between PI3K/Akt/GSK-3 and other members of the hypertrophic

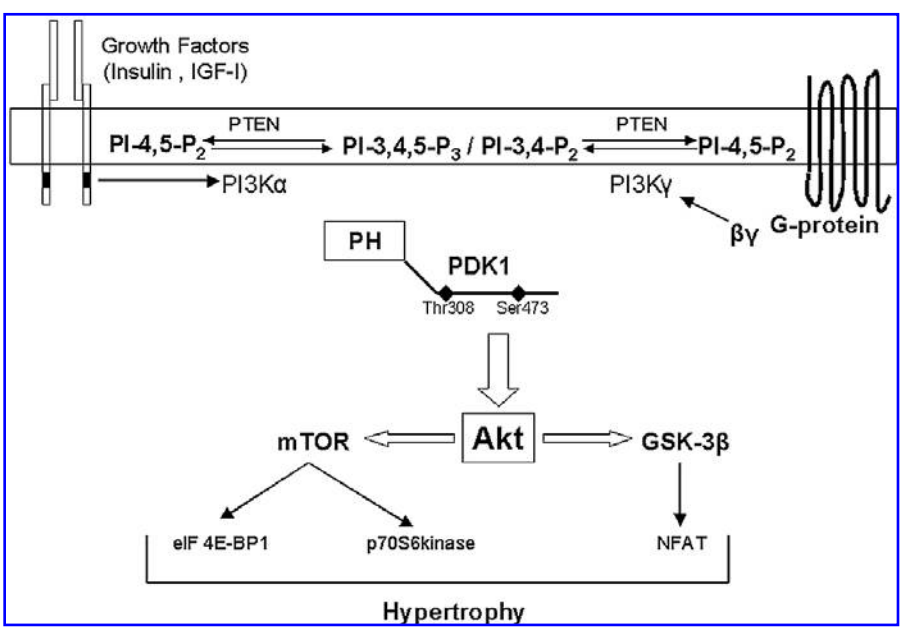

FIG. 7. Schematic representation of PI-3 kinases and Akt signaling pathways and their components involved in hypertrophy process. (Adapted and modified from ref. 184).

pathways that are involved in the cardiac growth-signaling cascade.

E. Calcineurin and cardiac hypertrophy

Calcineurin is a serine/threonine protein phosphatase that is involved in several physiologic processes, such as T-cell activation, skeletal myocyte differentiation, and cardiac hypertrophy. The calcineurin heterodimer consists of calcineurin A and B subunits. Subunit A contains the catalytic domain, the calmodulin-binding domain, and an autoinhibitory domain; subunit B is a member of the "EF hand" family of calcium-binding proteins. On calcium/calmodulin binding, calcineurin undergoes a conformational change and the release of the autoinhibitory domain (73, 104) (Fig. 8). Activation of the T-cell receptor increases the concentration of intracellular calcium and results in the activation of calcineurin. Activated calcineurin directly dephosphorylates members of the NFAT family in the cytoplasm, resulting in their translocation to the nucleus and in the activation of immune-response genes. The role of activated calcineurin and the calcineurin-dependent pathway in cardiac hypertrophy has been demonstrated in transgenic mice overexpressing calcineurin (197) and calmodulin.(212) The role of NFAT in the development of cardiac hypertrophy during pressure overload (153) and the involvement of calcineurin B in controlling calcineurin activity in compensated LVH (103) have also been documented. More specifically, calcineurin has been shown to operate through NFAT3 in cardiac hypertrophy (171, 197). Although calcineurin-NFAT signaling is considered to play an important role in the hypertrophic growth of the myocardium, controversy exists regarding its role in maintaining versus initiating hypertrophy, as well as its role in pathologic versus physiologic hypertrophy and in CHF.

Studies with NFAT-luciferase reporter transgenic mice have led to the hypothesis that separate signaling pathways regulate pathologic versus physiologic hypertrophy and that calcineurin-NFAT signaling plays a regulatory role for maladaptive hypertrophy (331). It has been reported that active mitogen-activated protein/ERK kinase kinase 3 (MEKK3) is capable of stimulating calcineurin-NFAT signaling in cardiac myocytes. MEK5 and ERK5 (also called big MAP kinase-1) function as downstream targets of MEKK3 in a signaling cascade that induces calcineurin activity through phosphorylation of modulatory calcineurin-interacting protein 1 (MCIP1). Once phosphorylated, MCIP1 dissociates from calcineurin and binds with 14-3-3, thereby relieving its inhibitory activity on calcineurin activity (1). The role of several intermediate molecules and causative molecules in calcineurin-NFAT signaling with respect to cardiac hypertrophy has been reported. Prostaglandin F (2α) is known to be involved in the hypertrophic process through the calcineurin signal transduction pathway in monocrotalin (MCT)-injected rat (134). It has been found that GTPase activation occurs downstream of calcineurin in cardiac hypertrophy (239). Furthermore, the calcineurin-NFAT and MAPK signaling pathways are interdependent and together orchestrate the cardiac hypertrophic responses (195). The cAMP-binding protein Epac is able to activate a pro-hypertrophic signaling pathway involving Ca^{2+}-sensitive phosphatase, calcineurin, and its primary downstream effector NFAT. Rac is involved in Epac-induced NFAT-dependent cardiomyocyte hypertrophy (198). Calcineurin A remains associated with muscle A kinase–anchoring protein (mAKAP), a scaffold protein, and the complex is required for the full activation of the prohypertrophic transcription factor NFATc. A novel function of the mAKAP complex involves integration of cAMP and Ca^{2+} signals to promote myocyte hypertrophy (220).

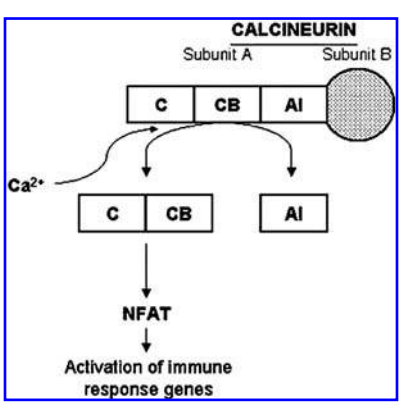

FIG. 8. Activation of calcineurin and nuclear translocation of NFAT (nuclear factor of activated T-cell) for calcineurin-mediated signaling. C, catalytic domain; CB, calmodulin-binding domain; AI, autoinhibitory domain. (Adapted and modified from ref. 196.)

F. MAPKs (ERK/JNK/p38 MAPK) and cardiac hypertrophy

Mitogen-activated protein kinases (MAPKs) play a significant role in hypertrophic signaling (91). MAPKs are serine/threonine kinases that become activated on tyrosine/threonine phosphorylation. Several nuclear substrates, such as c-*myc,* c-*jun*, ATF-2, and $p62^{TCF}$ and other kinases like $p90^{RSK}$ MAPKAPkinase2, are phosphorylated and activated by MAPKs (65). The MAPK pathway is a three-module cascade of phosphorylating kinases: MEKK, MEK, and MAPK. The MEKKs are serine/threonine kinases that activate MEKs by dual phosphorylation of a serine and serine/threonine residue (353). Then MEK activates MAPKs by dual phosphorylation of a tyrosine and a threonine residue (54). MAPKs preferentially phosphorylate the substrate on serine/threonine-proline (249). Based on the type of amino acid present in the "threonine xxx tyrosine" phosphorylation motif, the MAPK superfamily is grouped into the following three categories: (a) the ERKs that contain Glu at the earlier-mentioned xxx, (b) the JNKs having Pro at xxx, and (c) the p38 MAPKs containing Gly at xxx (249, 293). JNKs and p38 MAPKs belong to the stress-activated protein kinases (SAPKs).

The MEKs in the ERK pathway are MEK1 and MEK2, and the MEKKs are Raf kinase and MEKK1 (126, 154) (Fig. 9). Mechanical stretch, GPCR agonist, or stimulation of receptors with intrinsic tyrosine kinase activity principally activates the ERK cascade. Stretching of cardiomyocytes causing activation of ERKs and subsequent increase in expression of c-*fos* and skeletal α-actin has been reported by Sadoshima *et al.* (101) and Yazaki and Komaro (340), indicating that

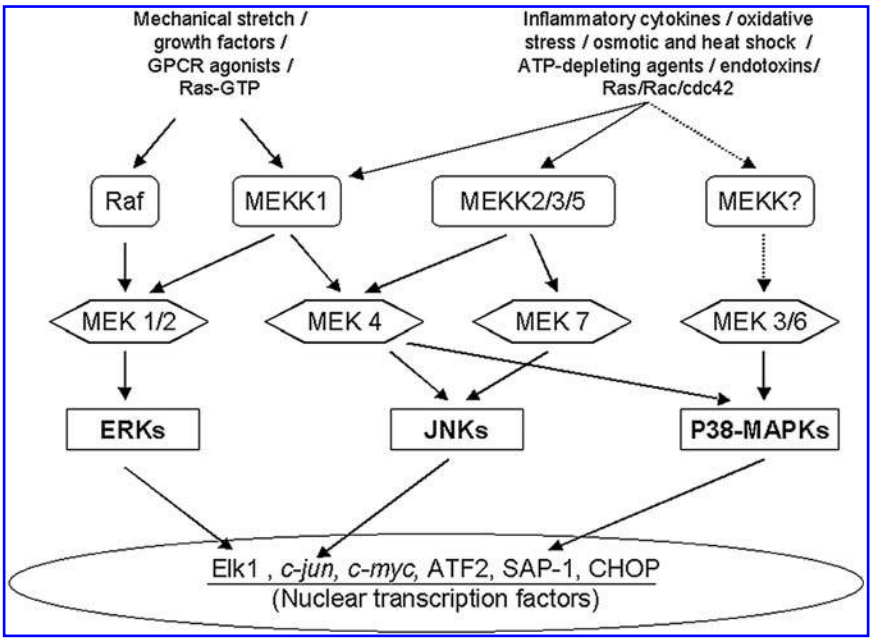

FIG. 9. Three module cascades of mitogen-activated protein kinase and its component subfamilies ERK, JNK and p38MAPK pathways, showing their upstream activators and downstream targets involved in cardiac hypertrophy. (Adapted and modified from refs. 145 and 249.)

activation of the ERK cascade is linked with mechanical stretch and thereby stress-induced hypertrophy. Activation of the ERK pathway also is mediated through the binding of GPCR agonist (Ang II, ET-1) (19, 251) and on occupation of receptors with intrinsic tyrosine kinase activity by binding of growth factors such as IGF-I (89). On phosphorylation of Raf by tyrosine kinases after the recruitment of Raf and cofactor 14-3-3 conjugates at the membrane by Ras-GTP, activated Raf can initiate the ERK cascade (176). ERKs increase the enzymatic activity of cytoplasmic phospholipase A_2 , resulting in an increase in the release of arachidonic acid and the formation of lysophospholipids (249). ERKs also phosphorylate Elk-1, which forms a complex with serum-responsive factor and together binds to the promoter of several serum response elements containing genes such as c-*fos* that leads to the increased c-*fos* mRNA induction (142). c-*Fos* protein is considered an important component of transcription factor activator protein-1 (AP-1), and thus activation of ERKs is thought to cause AP-1 activation. In this regard, ERKs have been reported to be responsible for the development of hypertrophy (100).

Unlike ERKs, JNKs and p38 MAPKs are activated mainly by cellular stresses such as inflammatory cytokines, ischemia, oxidative stress, osmotic shock, heat shock, ATP-depleting agents, and endotoxins (108, 121, 145). Initially, both the JNK and p38 MAPK pathways were considered SAPKs. Based on differences in their dual phosphorylation motif (mentioned earlier in this section) and their upstream activators and differences in their downstream targets, they are now considered two different pathways (291). Although little research has been done on the upstream activators of JNKs in heart tissue, MEK4 and MEK7 are considered upstream kinases of the JNK pathway (290). MEKK1 phosphorylates MEK4, which in turn phosphorylates and activates JNKs (338) (Fig. 9). Therefore, MEKK1 also can be considered an upstream activator of JNKs. Wang *et al.* (325) pointed out the probability of MEKK5 as an upstream activator of MEK4. MEKK2 and -3 also are found to activate JNKs (244). Whatever the upstream activator is, the JNK pathway is supposed to be triggered by heterotrimeric G proteins coupled to membrane receptors and by rho family small G proteins such as Ras, Rac, and cdc42 (57, 192, 249). As downstream targets, JNKs are able to phosphorylate c-*jun* (52, 121). JNK also activates the N-terminal transcriptional activation domain of activating transcription factor-2 (ATF-2) and thus leads to increased transcriptional activating activity (52, 330). Being a potent activator of AP-1 (142), the JNK/AP-1 cascade is considered to be involved in the increased expression of ANF, skeletal α-actin, TGF-β_1, and collagen type I in cardiac hypertrophy (145).

p38 MAPK, a member of the MAPK subfamily, has a potential role in cardiomyocyte hypertrophy in response to stress stimuli (99, 208, 326). MKK3 and MKK6 directly phosphorylate the dual site of p38 MAPK and act as upstream activators of p38MAPKs (Fig. 9). MEK4 also is considered a probable upstream activator (210, 346). Among the p38 isoforms, it has been reported that p38α and p38β are most important for hypertrophic responses (196). Activated p38 MAPK phosphorylates serine-threonine residues of several cytoplasmic proteins and transcription factors, such as ATF-2, serum response factor accessory protein-1 (SAP-1), and CHOP (330), to mediate hypertrophic signaling. Activated p38 MAPKs also can induce cardiac hypertrophy through the integrin-FAK-Src-Ras pathway (3). Inactivation of p38 MAPK is essential in the calcineurin-mediated hypertrophic process in which MKP-1 expression increases (172) and reduced p38 signaling causes myocyte growth through calcineurin-NFAT signaling (26). In contrast, it also has been reported that p38 is not involved in calcineurin-mediated hypertrophy (66). Norepinephrin-induced hypertrophy associated with p38 MAPK activation (64) and norepinephrine can activate p38 MAPK via the βAR in mouse cardiomyocytes, but this activation alone is not sufficient to induce hypertrophy (250). Epidermal growth factor (EGF) has the

ability to promote hypertrophic responses by activating Stat5 in conjunction with p38 MAPK stimulation (236). p38 MAPK stimulation resulted in brain natriuretic protein (BNP) promoter activation, which is a marker of cardiac hypertrophy (169, 170). It can be said that the subfamilies of MAPK (*i.e.*, ERKs, JNKs, and p38 MAPKs) play a role in the transduction of mechanical stress into a hypertrophic response (249).

G. PKC and cardiac hypertrophy

The protein kinase C (PKC) family consists of several serine/threonine kinases that are ubiquitously expressed and act downstream of almost all of the membrane-associated signal-transduction pathway (211). Activated PKC isoforms translocated to different subcellular sites, depending on the types of isoforms. Differential subcellular compartmentalization of PKC isoforms includes translocation of PKCβ1 from cytosol to nucleus on αAR stimulation (196), and of PKCβII from fibrillar structures to perinucleus and sarcolemma. PKCϵ, translocates from nucleus and cytosol to myofibrils, whereas PKCδ translocates to the perinuclear region (70, 71). These unique differential subcellular localizations of PKC isoforms indicate that they have distinct substrate preferences and specific functions for each isoform (129, 285). Unstimulated PKC remains in a folded conformation, keeping the substrate-binding site occupied by a pseudo-substrate domain (Fig. 10). PKC isoforms, such as PKCδ and PKCϵ, detect the increase in membrane diacylglycerol (DAG), translocate to the membrane, and initiate the PKC activation (292). Once stimulated by DAG or calcium, the PKC protein unfolds and exposes the substrate and RACK (receptor for activated C kinases) binding site. PKC then binds to the anchoring protein RACK (Fig. 10), resulting in the specific subcellular translocation of PKC isoforms, and coordinates the PKC-mediated hypertrophic signaling (70, 194, 196, 221, 222).

Among the isoforms, PKCα is the most abundant representative isoform in the heart (264). Several supportive as well as contradictory reports are available regarding which isoform of PKC is most important in causing cardiac hypertrophy. PKCβ overexpression is reported to be sufficient to elicit cardiac hypertrophy and sudden death (21), whereas other findings failed to support the role of PKCβ in cardiac hypertrophy (128, 246, 302). Pressure overload induced by aortocaval shunt (AVS) resulted in an increase in calcium-dependent and -independent PKC activities after 2 and 4 weeks, but after 8 and 16 weeks, the activity and content remained unchanged in the failing left ventricle. In the hypertrophied right ventricle, no increase occurred in PKC isoforms, even after 16 weeks of AVS. All these data indicated that increases in PKC isoform content do not play an important role during the development of cardiac hypertrophy as well as in volume-overload heart failure (270). In another study, it was postulated that PKCβ is not required for hypertrophic responses to pressure overload (246). On the contrary, PKCϵ has been reported to participate in compensated hypertrophy (297) and during progression to heart failure (222). The requirement of PKCα form in cardiomyocytes hypertrophy has been proved *in vitro* by using a dominant negative adenoviral expression construct (25). The involvement of PKC in TGF-β1–induced cardiac hypertrophic response also has been demonstrated (173). Differential activation of PKCα, δ, and ϵ isoforms was observed in the spontaneously hypertensive heart failure rat heart (136). A similar observation regarding differential activation of PKC isoforms in pressure-overload hypertrophy (24) and in uremic cardiomyopathy has been reported (332). PKCα and δ are considered to be important regulators in mediating the activation of Rho GTPase and MAPKs in cyclic stretch-induced hypertrophy (295). In the cardiac hypertrophic state, the expression of PKCα, β, and δ increased and remained elevated at the heart-failure stage, whereas increased levels of PKCϵ declined at the CHF stage, suggesting that two different groups of PKC (PKCα, -β, -δ, and PKCϵ) have different functions at the CHF stage (148). Studies in hypertension and gradually developing LVH induced by suprarenal abdominal aortic coarctation in Sprague–Dawley rats showed that PKCδ is involved in the induction of pressure-overload hypertrophy, whereas PKCα along with PKCδ is involved in the transition to heart failure (14). A similar observation was reported in MI induced by ligation of the left anterior coronary artery in rats, in which PKCα upregulation found to be involved in the development of hypertrophy, and PKCδ outlasts the process of developing hypertrophy and persists in the failing heart (278). PKCβII is found to be involved in cardiac hypertrophy and fibrosis through increased activity of angiotensin-converting enzyme (ACE) in the myocardium (351). PKC isoforms has been shown to play differential functional roles in cardiac hypertrophy and heart failure. PKCα, -β, and -δ are found to be involved in the development of cardiac hypertrophy and heart failure, whereas PKCϵ plays a role in the physiologic hypertrophic responses and in cardioprotection (43, 148, 297).

H. Myotrophin and cardiac hypertrophy

Myotrophin, a soluble protein, has been demonstrated to play an integral role in initiating cardiac hypertrophy (267). Studies from our laboratory demonstrated that mechanisms involved in the initiation or regression of hypertrophy in

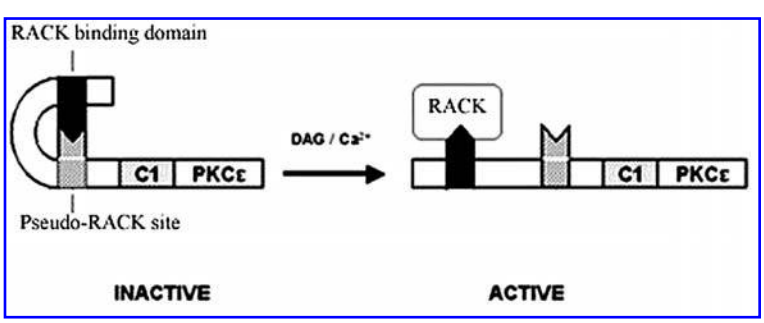

FIG. 10. Mechanism of protein kinase C activation. DAG, diacylglycerol; RACK, receptor for activated C kinase. (Adapted and modified from ref. 96.)

spontaneously hypertensive rats (SHRs) cannot be fully explained as a response to blood pressure control alone. We hypothesized that the development of hypertrophy is initiated by a humoral or mechanical signal to the myocardium, which in turn produces a soluble factor that triggers protein synthesis and initiates myocardial growth. In exploring the factor that initiates cardiac hypertrophy, our laboratory has identified a factor, myotrophin, from SHRs and DCM human hearts (267, 277) that stimulates myocyte growth. Myotrophin has been shown to stimulate (a) incorporation of [^{3}H] leucine as well as [^{14}C] phenylalanine into myocyte protein *in vitro*, (b) cell growth, and (c) the increase in cell-surface area in a dose-dependent fashion compared with controls (318). Neonatal myocytes treated with myotrophin displayed an accelerated myofibrillar growth and an organization into sarcomeres (318). Myotrophin also has been shown to be specific for myocytes only, because it has no effect on fibroblasts, endothelial cells, or smooth muscle cells. It was observed that myotrophin selectively increased the transcript level of the proto-oncogenes c-*myc*, c-*fos*, and c-*jun*, along with connexin (gap-junction protein), atrial natriuretic factor (ANF), and β-myosin heavy chain (β-MHC) gene in cultured myocytes (202). The protein synthesis stimulatory activity of myotrophin action was mediated through the PKC signaling pathway (276). The myotrophin gene was mapped, for the first time, to human chromosome 7q33 (193). The myotrophin gene was upregulated in different hypertensive models including renal hypertension, DOCA-salt, and aortic coarctation when myotrophin levels were elevated (275), suggesting its potential role in cardiac hypertrophy. Myotrophin has been shown to interact with NF-κB proteins (146, 279), and the signaling mechanism of myotrophin-induced myocyte growth is shown to be mediated through NF-κB (106). Altogether, it is suggested that myotrophin may play an important role in the pathogenesis of cardiac hypertrophy as well as in the normal development of cardiac myocytes.

I. NF-κB and cardiac hypertrophy

Intensive investigation has also focused on characterizing intracellular regulatory pathways that are linked with hypertrophy and heart failure in an attempt to develop novel therapeutic antagonists that may alleviate these responses. Injury to the myocardium transmits multiple signaling pathways that ultimately lead to changes in gene expression associated with the activation of various transcription factors, including AP-1, NF-κB, Egr-1, and Stat3. Among them, the role of NF-κB is becoming apparent in the pathophysiology of myocardial injury, ischemia preconditioning, DCM, and unstable angina (41). The significance of NF-κB activation in various cardiac pathologic processes [*e.g.*, ischemia preconditioning (185), CHF (334), and unstable angina (243)] is now emerging.

NF-κB is a redox-sensitive transcription factor that regulates a variety of genes involved in inflammation, immune response, progression of pathogenesis of atherosclerosis, autoimmune arthritis, septic shock, and apoptosis (8-11, 41, 97, 313). NF-κB was first identified as a B-cell nuclear factor and was given its name on the basis of its ability to bind to a decameric motif of an enhancer of κ-light chain immunoglobin gene (266). NF-κB is activated by a wide range of stimuli, including growth factors, cytokines, UV irradiation, reactive oxygen species, hypoxia/anoxia, and bacterial or viral products such as double-stranded RNA (dsRNA) and lipopolysaccharide (LPS) (9, 97, 301, 313). In resting cells, NF-κB resides in the cytoplasm in an inactive form bound to an inhibitory protein called IκBα. On external stimulation, IκB is phosphorylated and proteolytically degraded through the 26S proteasome–mediated pathway. This proteolytic process activates free NF-κB by unmasking its nuclear localization signal, which then proceeds or translocates into the nucleus, binds to the promoter or enhancer regions (κB motif) of specific genes, and regulates or initiates gene transcription (10, 301) (Fig. 11). A crucial step in the activation of NF-κB is the phosphorylation of IκBs by a multimeric complex, called IκB kinase (IKK) complex. (68) The IKK complex consists of two catalytic subunits (IKK1/IKKα and IKK2/IKKβ) (68, 343, 344), the NF-κB essential modulator (NEMO) (337), IKKγ (248), and IKK-associated protein (189). A general paradigm for the activation of NF-κB by the IKK complex supports the idea that signal-induced activation of IκB kinases phosphorylates IκBα, which triggers subsequent IκBα degradation and NF-κB activation/translocation.

NF-κB signaling is critically involved in atherosclerosis, myocarditis, ischemia, cardiac hypertrophy and dilated cardiomyopathy (137, 231, 313). Recent studies have suggested that the activation of NF-κB play an important role in the pathogenesis of cardiac remodeling and heart failure (139, 243, 334). A major focus of attention is also directed toward cardiac hypertrophy, both *in vitro* (106, 232) and *in vivo* (166) systems (232). Studies from different experimental models and *in vitro* cell culture systems suggest that the NF-κB activation pathway is also involved in "cell-death" (apoptosis) pathways. However, it has become apparent that NF-κB can protect cells from death (314). The paradoxical "survival" action of NF-κB is due to an induction of antiapoptotic factors (15). It is assumed that the role of NF-κB in the regulation of cell viability is multidimensional. Much more future work will be needed for a complete understanding of these mechanisms. Both systemic and cardiac activation of NF-κB has been found in unstable coronary syndromes, although the functional consequences have yet to be determined. Thus, a possibility may exist of a new therapeutic target for increased myocardial protection.

V. THERAPEUTIC APPROACH

Hypertensive heart disease is characterized mainly by LVH associated with altered cardiac function and coronary-flow abnormalities. It also is considered an independent risk factor related to cardiovascular complications with hypertension. Attenuating the left ventricular mass has been one of the main therapeutic targets over the past few decades. Antihypertrensive therapy has been used effectively in regression of cardiac hypertrophy in animal models as well as in humans with hypertension. Treatment with various classes of antihypertensive therapy demonstrated for the first time that factor(s) other than blood pressure control are responsible for regression or development of cardiac hypertrophy (262). To date, many pharmaceutic agents, such as ACE inhibitors,

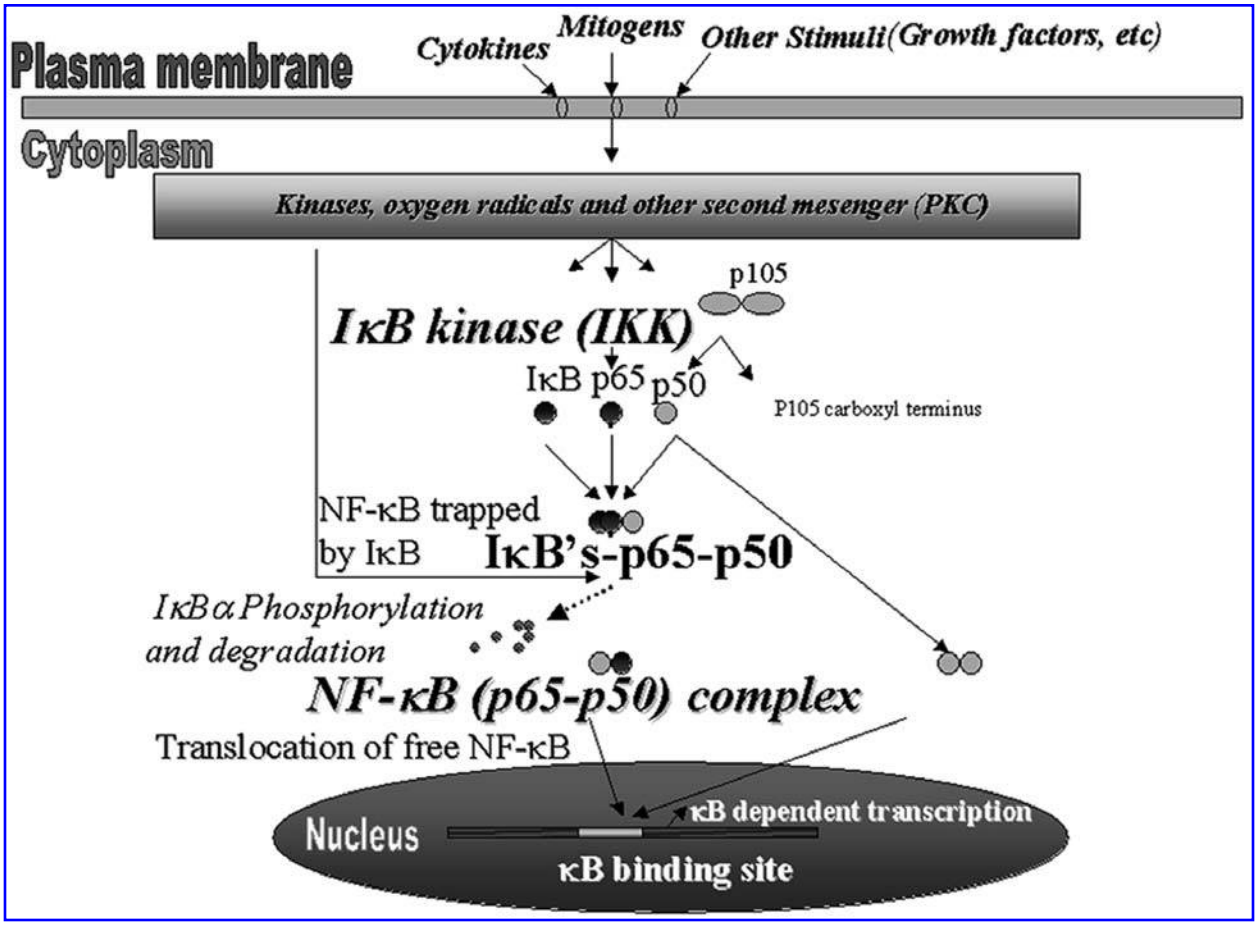

FIG. 11. NF-κB activation cascade. NF-κB is activated by various types of stimuli including growth factor, mitogens, and cytokines. These stimuli then activate various second-messenger molecules, which in turn may activate IκB kinase, which ultimately triggers the phosphorylation and degradation of IκBα and facilitates NF-κB to translocate into the nucleus. The activated NF-κB binds to the κB motifs and regulates various gene expressions.

β-blockers, angiotensin-receptor blockers, calcium ion channel blockers, and diuretics, have been commonly used to treat myocardial hypertrophy and hypertension in humans. Gene-based therapy also is considered to be a promising means of treating cardiovascular diseases. In this review, we summarize various classes of antihypertensive drugs used to treat hypertrophy and heart failure.

A. Antihypertensive therapy

Five different types of antihypertensive drugs are commonly used to treat humans with hypertension with heart failure:

1. ACE inhibitors
2. AT_1-receptor blockers
3. β-Blockers
4. Ca^{2+} channel blocker
5. Diuretics

1. Angiotensin converting enzyme (ACE) inhibitors. Renin, an enzyme released from the kidney, cleaves angiotensinogen and converts it to angiotensin I, which is further converted to angiotensin II by ACE. The final active messenger of the renin–angiotensin system (RAS) pathway is angiotensin II, which binds to AT_1 receptors to cause vasoconstriction and fluid retention, both of which lead to an increase in blood pressure. ACE inhibitors inhibit ACE and prevent the activation of angiotensin, dilating the blood vessels and thus lowering blood pressure. ACE is not the only enzyme capable of this conversion; trypsin, cathepsin, and heart chymase can also convert Ang I into Ang II. ACE inhibitors (*e.g.*, captopril, accupril) partially block this process by inhibiting ACE. It has been shown that ACE inhibitors cannot completely block Ang II formation, so an alternative to the Ang II pathway, an angiotensin receptor (AT_1) antagonist, has been shown to be effective for the treatment of hypertension.

2. Angiotensin (AT_1)-receptor blockers. AT_1 blockers prevent the binding of Ang II to the AT_1 receptor. Ang II–receptor blockers (ARBs; also known as Ang II–receptor antagonists) are a new class of antihypertensive medications. These medications selectively bind to the AT_1 receptor, preventing the effects of Ang II. Losartan, an ARB, is a good alternative to ACE inhibitors for heart failure (229). The side effects reported so far after using ARBs include tachycardia, bradycardia,

dizziness, and ataxia. The underlying mechanisms of action of both ARBs and ACE are mediated through the RAS. The RAS is integral in the pathophysiology of hypertension by regulating fluid and electrolyte balance. Renin is produced primarily by the juxtaglomerular cells in the kidneys and catalyzes the conversion of angiotensinogen to angiotensin I (Ang I). Ang I also can be generated by the non-renin enzymes tonin and cathepsin (31). Ang I is then converted into Ang II, primarily by ACE. Ang II binds to AT_1 and AT_2 receptor sites. AT_1-receptor sites are located in the kidney, heart, brain, vascular smooth muscle cells, placenta, and platelets, as well as in fat cells (303). AT_2-receptor sites are important in fetal development but are not well understood.

3. b-Blockers. β-Blockers are a class of drugs that block β-adrenergic substances such as adrenaline (epinephrine), a key agent in the "sympathetic" portion of the autonomic (involuntary) nervous system and activation of heart muscle. By blocking the action of the involuntary nervous system on the heart, β-blockers relieve stress on the heart. They slow the heartbeat, lessen the force with which the heart muscle contracts, and reduce blood vessel contraction in the heart, brain, and throughout the body. β-Blockers lower blood pressure and are an effective treatment for hypertension. β-Blocker therapy is commonly used in patients with NYHA class II or class III symptoms resulting from left ventricular systolic dysfunction. Carvedilol, a commonly used drug, is a novel agent with antagonist activity against $alpha_1$, $beta_1$ and $beta_2$ receptors, as well as some antioxidant activity (28, 216), which has been shown to regress cardiac hypertrophy (216).

4. Ca^{2+} channel blockers. Ca^{2+} is the most important contractile ion and plays a key role in cardiac function. Changes in intracellular Ca^{2+} regulate cardiac contraction through different mechanisms in cardiac and smooth muscle. In cardiac muscle, the binding of Ca^{2+} to troponin C relieves the troponin-induced inhibition of actin–myosin interactions. In smooth muscle, the binding of Ca^{2+} to calmodulin activates myosin light-chain kinase, which in turn phosphorylates the P-light chain of myosin. This step triggers contraction (*i.e.,* actin–myosin interactions), but additional Ca^{2+} regulatory mechanisms appear to be present (79, 117, 304). The World Health Organization identified two types of calcium channel blocker (CCB) that are used in patients: those that are selective for L-type (long-lasting, large-current, or slow), voltage-dependent calcium channels, and those that are nonselective. In general, CCBs bind to L-type calcium channels located on the vascular smooth muscle, cardiac myocytes, and cardiac nodal tissue [sinoatrial (SA) and atrioventricular (AV) nodes]. These channels are responsible for regulating the influx of calcium into muscle cells, which in turn stimulates smooth muscle contraction and cardiac myocyte contraction (79). In cardiac nodal tissue, L-type calcium channels play an important role in pacemaker current and in phase 0 of the action potentials. Therefore, by blocking calcium entry into the cell, CCBs cause vascular smooth muscle relaxation (vasodilation), decreased myocardial force generation (negative inotropy), decreased heart rate (negative chronotropy), and decreased conduction velocity within the heart, particularly at the AV node (159, 304, 228).

5. Diuretics. Diuretics, also called water pills, eliminate sodium and water from the body. The primary therapeutic goal of diuretic use is to reduce edema by reducing the extracellular fluid volume (151). Three types of diuretics are used to treat hypertension and heart failure: thiazide, loop, and potassium sparing. They work in different parts of the kidneys and have different uses, side effects, and precautions associated with them. Thiazide diuretics, the most commonly used, inhibit the sodium-chloride transporter in the cortical thick ascending limb and early distal convoluted tubule in the kidney. Thiazides are used to treat hypertension, but their mechanisms of action are not completely understood, although it is known that they depend on renal prostaglandin production (87). One recent report suggests that thiazides cause vasoconstriction by calcium desensitization in smooth muscle cells linked to the Rho-Rho kinase pathway (355). Loop diuretics act on the ascending loop of Henle in the kidney and are used primarily to treat hypertension and edema but also to treat CHF and renal insufficiency (23, 30, 35, 87, 125, 181, 282, 315).

B. Gene therapy and tissue regeneration

With the advancement of gene-transfer technology, treatment using delivery of the gene to the heart is another state-of-the-art means of treating cardiac hypertrophy/heart failure. Gene transfer is transfection of the common gene into the cells to study the mechanisms and consequences of gene expression. They are transfected into the cells using vectors, which can be lentivirus, adenovirus, liposomes, or polymers. The most commonly used method is by using lentivirus or adenovirus. *In vitro,* the gene is transferred by the cell-mediated transfer method. Usually, this is a combination technique, using small interfering RNA (siRNA) and direct delivery of the gene. The siRNA against the candidate molecule that is suspected of initiating hypertrophy is directly constructed and conjugated with adenovirus or lentivirus, and then directly delivered into the heart, where it has been successful in treating cardiac hypertrophy and heart failure. Gene transfer is a useful approach by which to introduce a nucleic acid into the somatic cells of animals to define gene function.

Gene therapy works by introducing a gene into individual cells or tissues to treat diseases. It replaces defective genes with functional ones. In certain patients, conventional therapies such as surgical procedures or pharmacologic treatment sometimes are not effective; this is the reason for the emergence of gene therapy as the newest alternative means of treating disease. Although gene-therapy technology is still in its infancy, some success has been reported. The first gene therapy in the United States was done in 1990, treating a 4-year-old girl with a rare genetic disease called severe combined immune deficiency (SCID). Although the procedure was temporarily successful, it was not a complete cure, because the genetically engineered white blood cells worked for a few months only, and the process had to be repeated every few months.

The basic principle of gene therapy is to replace the "defective" gene with a "normal" gene inserted into the genome, and a vehicle/carrier molecule is needed to carry the "normal" gene for this therapy. Adenovirus-mediated gene transfer is a powerful technique and shows great potential to improve existing drug treatments. It allows transient expression of gene products (proteins) in targeted tissue and has the potential to be a highly specific treatment. Adenovirus-mediated gene therapy has recently shown its limitations because patients have exhibited harmful inflammatory responses to the high doses of adenovirus vectors (161). It is likely that existing vectors must be improved for successful application in humans; such efforts are currently under way (335). Over the past few decades, several transgenic mouse models of heart failure have increased our knowledge of the underlying molecular mechanisms of heart failure and have given us experience in manipulating the various cardiac target genes. Several recent animal studies provide compelling evidence that gene therapy will be of great benefit as a treatment for cardiovascular diseases. The use of gene therapy in the cardiovascular system has increased the number of potential therapeutic modalilities for the better treatment of cardiac dysfunction. To date, several attractive therapeutic options have become available to treat various cardiovascular disorders. The limitations of gene therapy also must be considered. The efficiency of gene delivery and its controlled expression are the most significant challenges to the use of this therapy. In this review, we discuss a few instances of significant progress using both RNA interference (RNAi) technologies and gene therapies.

1. Treatment with RNAi and microRNA (miRNA). RNAi is a general mechanism for silencing the transcript of an active gene via dsRNA. RNAi has recently been applied to mammalian cells with the use of siRNA (80, 112, 272). The endogenous mediators of sequence-specific mRNA degradation are 21- and 22-nt siRNA generated from longer double-stranded RNAs by the ribonuclease III activity of the evolutionary conserved dicer enzyme (16, 80). RNAi is mediated by RNA-induced protein complex (RISC), guided by siRNA to achieve the specific recognition of homologous mRNA sequences and subsequent degradation by nucleases (Fig. 12) (109, 187). RNAi-mediated gene silencing also is obtained in cultured mammalian cells by delivery of chemically synthesized short (<30 nt) double-stranded siRNA molecules (80) or by endogenous expression of short hairpin RNAs (shRNAs) bearing a foldback stem–loop structure (81, 217, 294). The common feature of the RNAi process is its sole reliance on siRNA oligonucleotides whose sequence targets the specific genes to be inhibited. A number of studies have examined the extent of gene inhibition as a selective manner but not as an absolute selectivity (47, 141, 265, 280). Sometimes, it is difficult to determine the "off-target" effect of a wrong gene and the outcome of RNAi-silenced genes, but in most cases, a substantial RNAi effect is observed (132).

The use of siRNA to characterize the gene function and exploring the possibilities for therapeutic use is widespread in all biologic fields including the cardiovascular arena. Recently, several reports also addressed the use of RNAi technology in studying cardiovascular diseases, suggesting its potential use for therapeutic purposes (107, 227, 316, 327, 350). The success of *in vitro* transfection of siRNA (either by virus mediated or synthetic short oligonucleotides) into cells extends those studies to *in vivo* systems; however, the *in vivo* delivery of siRNA into a specific organ or tissue is more complicated. The key to the success of *in vivo* application is the method of delivery system that carries the siRNA or shRNA into the target organ and, most important, their efficacy in inhibiting the target gene's function (175).

Another major role of RNAi is the regulation of cellular activity by altering endogenous gene expression via a noncoding imperfectly palindromic sequence of RNA, called miRNA. The majority of miRNAs are imperfectly complementary to their mRNA targets and inhibit translation through unknown mechanisms (5, 12, 119). miRNAs comprise a group of small RNAs of 21–25 nt that bind imperfectly to the 3' UTR sequences of mRNA and inhibit protein synthesis. Recent studies show that miRNAs are involved in many aspects of cell-signaling pathways (164, 230, 233). One significant case of involvement of miRNA is insulin signaling. For the possible role of miRNAs in pancreatic cells, islet-specific miRNA (mir-375) has been determined from a library of small RNAs cloned from the pancreatic beta-cell line MIN6 and alpha-cell line TC1 (230). In addition, cell-culture–based assay has shown that myotrophin (mtpn) regulation is controlled by mir-375, and siRNA-silencing experiments further support that myotrophin is a target for mir-375 (230). This new regulator of insulin signaling, mir-375, has become a potential therapeutic target for diabetes treatment.

Other recent studies also revealed that miRNAs are involved in cancer and other diseases (33, 34, 135, 190). In the context of cardiovascular diseases, few reports are found. microRNA-1–1 and miR-1–2 are specifically expressed in cardiac and skeletal muscle precursor cells and target muscle differentiation regulators (*e.g.*, serum response factor, MyoD, and Mef2) (352). This study further reveals that excess miR-1 in the developing heart leads to a decreased pool of proliferating ventricular cardiomyocytes and controls the balance between differentiation and proliferation during cardiogenesis (352). The other study explores the distinct role of miRNA-1 and miRNA-133 in skeletal muscle differentiation and proliferation (42). This study shows that miRNA-1 and miRNA-133, which are clustered on the same chromosomal loci, are transcribed together in a tissue-specific manner during development, but they have distinct roles in modulating skeletal muscle proliferation and differentiation in cultured myoblasts *in vitro* and in *Xenopus laevis* embryos *in vivo*. The miR-1 promotes myogenesis by targeting histone deacetylase 4 (HDAC4), a transcriptional repressor of muscle gene expression, and miR-133 enhances myoblast proliferation by repressing serum response factor (SRF).

Another interesting study was performed to assess the role of miRNAs in the pathogenesis of hypertension in Dahl salt-sensitive rats (DS). A cDNA library was constructed from kidney, and more than 100 miRNAs were assessed in the kidneys of DS and Lewis rats (LW) fed a normal and a high-salt diet. No significant differences in the miRNA profiles were observed in the kidneys and heart (207). Although the investigators identified some new miRNAs in the kidneys, the miRNAs were not differentially expressed in all four groups. This

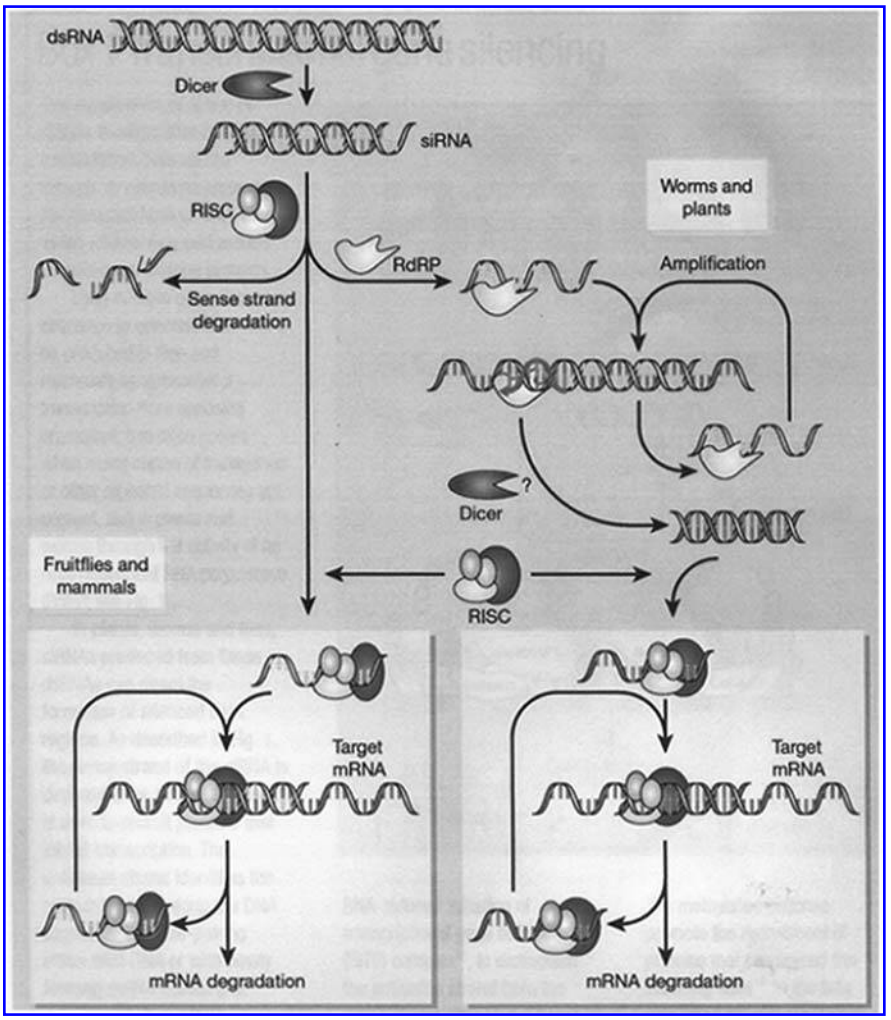

FIG. 12. Schematic presentation of the RNAi system. RNAi is triggered when a cell encounters a long double-stranded RNA (dsRNA). An enzyme called Dicer cleaves the long dsRNA into siRNAs. An RNA-induced silencing complex (RISC) then distinguishes between the different strands of the siRNA. The sense strand is degraded. The antisense strand is used to target genes for silencing and has one of several fates, depending on the organism. In fruitflies and mammals, the antisense strand is incorporated directly into RISC to target a complementary mRNA for destruction. In the absence of siRNAs, the RISC lacks sequence-specific mRNA-binding properties. But when bound to the antisense strand, the now-activated RISC can participate in repeated cycles of degradation of specific mRNAs, such that no protein is made, effectively silencing the gene from which the mRNAs are produced. In worms and plants, the antisense strand of the siRNA might first be used in an amplification process. The antisense strand, bound by an RNA-dependent RNA polymerase (RdRP) enzyme, can pair with a complementary mRNA and act as a start point for the synthesis of a new long dsRNA. Dicer is then required to generate new siRNAs, which are specific to different sequences on the same mRNA. Again, the target mRNA is destroyed. (Adapted from ref. 211a.)

could be due to a low level of expression in the kidney, as it comprises different cell types, and some cell-specific miRNA expression could be diluted in the total RNA extracted from the kidney. This limitation could be overcome by using a more sensitive detection method (*e.g.*, a microarray system, TaqMan-based RT-PCR, or RNase mapping). Most recently, the role of miRNA in regulating myocardial hypertrophy has been determined (317). It has been shown that more than 12 miRNAs are up- or down-regulated in cardiac tissue from mice in response to TAC or expression of activated calcineurin, stimuli that induce pathologic cardiac remodeling. Importantly, many of these miRNAs were similarly regulated in failing human hearts. It has been observed that overexpression of stress-inducible miRNAs is sufficient to induce hypertrophy in cultured cardiomyocytes. Moreover, cardiac overexpression of miR-195, which was upregulated during cardiac hypertrophy, resulted in pathologic cardiac growth and heart failure in transgenic mice (317). It would also be interesting to determine whether forced overexpression of miR-133 and other miRNAs that are downregulated during cardiac hypertrophy suppress cardiac growth *in vivo*. Future study should be directed toward the identification of mRNA targets of the miRNAs responsible for adverse cardiac remodeling for therapeutic benefit.

2. Tissue regeneration using stem-cell therapy. For the past few years, stem-cell therapy seemed to hold promising new insights into the mechanisms of cardiac repair, but still many questions remain to be answered (*e.g.*, the types of stem cells, the route of delivery, the efficiency of transforming into new totipotent cells, the ability to differentiate, and most important, defining the mechanisms of such transformation and how much functional significance can be achieved).

Stem cells are undifferentiated cells that can divide, differentiate into various cell types, and replace cells in a damaged region of the body. The first stem-cell research was attributed to the Canadian scientists Ernest McCulloch and James Till (131).

Several stem-cell types are known: totipotent (*i.e*., cells that can differentiate into embryonic and extraembryonic cell types); unipotent (*i.e.,* cells that evolve into only one cell type); multipotent [*i.e*., cells that produce only cells of a closely related family of cells (*e.g*., hematopoeietic stem cells differentiate into red blood cells, white blood cells, platelets]; and pluripotent (*i.e.,* cells that are the descendants of totipotent cells) (55, 133, 157, 188, 225). Three different stages were used for treatment in the harvested stem cells: prenatal (embryonic and fetal), postnatal (umbilical cord and placenta), and adult. Stem-cell therapy has recently generated much interest because new insights into the mechanisms of cardiac repair have been documented (55, 127, 188). The use of stem-cell–based therapies in the heart has recently appeared in the forefront of research (133, 157, 225, 333). In this review, we briefly discuss the feasibility and "pros and cons" of the application of stem cells to cardiac remodeling.

Scientific opinion holds that the heart is an organ composed of terminally differentiated myocytes. Heart failure is characterized mostly by a loss of function of contractile properties of myocytes. Therefore, heart transplantation is currently the treatment of ultimate choice for end-stage heart failure, but the lack of availability of donor hearts is a major limitation of the treatment. Therefore, the new therapeutic paradigm for treating heart failure is the replacement of new cardiac cells into the infarcted area of myocardium to restore cardiac function. Conceptually, a variety of cell-based therapies could be used for cardiac repair. These include bone marrow–derived cells (BMCs), endothelial progenitor cells (EPCs), embryonic cells (ECs), and mesechymal stem cells (MSCs). BMCs have been used as cell-based therapy to treat chronic heart failure (CHF) and have also been tested in clinical trials (283, 284, 289). In one uncontrolled pilot study, patients with CD 1333^{+} BMCs injected into the infarcted border zone showed improvement in left ventricular ejection fraction and shrinkage of the infarcted area (283, 284). Other studies also showed a similar trend, suggesting the efficacy and safety of using BMCs (7, 156). MSCs, another type of widely used pluripotent progenitor cells with the ability to generate cardiomyocytes, are capable of renewal and differentiation into various lineages of mesenchymal tissues. These features of MSCs have attracted considerable attention from investigators in the context of cell-based therapies for several human diseases, including heart failure. Although bone marrow represents the main available source of MSCs, the use of bone-marrow–derived cells is not always acceptable because of the high degree of viral infection and the significant decrease in cell number and differentiation capacity with age. The effectiveness of MSCs in treating MI was recently assessed in humans and showed favorable left ventricular remodeling (*e.g.,* higher ejection fraction and higher movement velocity in the infarcted area) (45). Clinical applications of stem-cell therapy for acute myocardial infarction (AMI) have also been documented and have shown a better survival in AMI patients, but no "absolutes" have yet come to light about the underlying mechanisms of the cardiac remodeling process (*e.g*., changes to the vasculature in the infarcted area or the repair of cardiomyocytes) (143, 204, 205, 214, 333, 342).

Although significant progress has been made in revascularization techniques, some patients with coronary artery disease and myocardial ischemia have no options for revascularization because of the diffuse nature of their disease. In that scenario, stem-cell–based therapy could be an alternative. It has been reported that transendocardial injection of bone marrow cells or progenitor endothelial cells enhances capillary density, collateral flow, and contractility in pigs with acute myocardial ischemia (94, 138, 143, 260). Iwanagawa *et al*. (131) showed that G-CSF prevents cardiac dysfunction and remodeling after MI in large animals. Further support of this observation came from a recent report by Hasewgawa *et al*. (115), who showed that delivering G-CSF improved contractile function in pig myocardium, inferring the alternative route of less-invasive therapeutic approach. In contrast, a recent study showing that no evidence existed of improved regional wall motion or enhanced myocardial perfusion raised a "red flag" about the safety of using G-CSF in this treatment (122).

Recently, much attention has been directed toward the characterization of cardiac progenitor cells (CPCs). They were characterized as stem cells that have been programmed to form heart muscle during fetal growth (32, 156). The cells are capable of differentiation into fully mature heart tissue, called *isl1*+ cells, able to form beating cardiac muscle tissue. It is thought that throughout the myocardium, continuous replenishment of dying myocytes with so-called "sleeping cells" occurs, the CPCs maintained at a very low level. These cells generally do not express all cardiac marker proteins, but they can self-proliferate. It is claimed that other progenitor cells, such as c-*kit* and Sca-1, can be transformed into cardiomyocytes *in vitro,* as they express cardiac marker genes and have been shown to repair the infarcted area (213, 234). Moreover, low levels of CPCs are the major limitation in this therapy, but attempts to mobilize and expand these cells by recruiting growth factors hold the promise of benefit but still remain controversial (312).

Clinical experience suggests that stem-cell therapy (with the appropriate cell types) can be safely performed and has the potential to enhance myocardial contractile performance in patients with MI, CAD, or CHF. Further research should be focused on the understanding of cell types with true cardiac transdifferentiation capacity for the benefit of heart-failure patients.

Finally, recent advances in chemical library and high-throughput screening of small molecules will serve as useful chemical tools to control stem-cell fate and could contribute a new and effective medical paradigm for cardiac tissue repair and regeneration (44, 69, 339).

VI. CONCLUDING REMARKS

Cardiac hypertrophy and heart failure have been shown to be major causes of morbidity and mortality in the world. Heart failure is a complex clinical syndrome that results from any structural or functional disorders that reduce the ability of the ventricles to fill with or eject blood. One of the major causes for this defect is long-standing hypertrophy, which remodels the heart in response to multiple stimuli. Cardiac hypertrophy that leads to heart failure is a major public health

problem in this country. Approximately 5 million patients have heart failure, and 500,000 patients are diagnosed with heart failure for the first time each year. Deaths due to heart failure have increased steadily despite advances in medical treatment.

The principal manifestation is cardiac remodeling, that is, change in the geometry and structure of the left ventricle as a result of cardiac hypertrophy and/or dilation. Many factors are thought to be responsible for left ventricle remodeling, including activation and circulation of cytokines or growth factors, which influence human dynamics by increasing stresses on the ventricles directly. One reason is that the causes of long-standing hypertrophy or transition to heart failure are complex, and the actual mechanisms of action are not known. Therefore, prevention of the development of hypertrophy is an important goal in preventing mortality. Prevention or reversal of target-organ damage has been shown to improve the long-term prognosis of LVH and has a strong independent association with cardiovascular morbidity/mortality. Therefore, effective reversal of LVH is a priority in treating patients. Many drugs have been designed to prevent or reverse hypertrophy, thereby improving cardiac function. A recent study has confirmed that antihypertensive therapy can be effective in reversing target organ damage, thereby improving the long-term prognosis, particularly of hypertensive patients with LVH. It has also been proven that multidrug therapy appears to be better than monotherapy in the treatment of cardiac hypertrophy. The selection of drugs appears to vary from one group of patients to another; therefore, the selection of hypertensive drugs or other inhibitors should be designed depending on the etiology of LVH. One of the most promising treatments appears to be gene therapy, arising from stem-cell research, which may be effective in preventing or causing the regression of LVH. However, translation of data obtained from animal studies to human beings is still an open question.

ACKNOWLEDGMENT

This report was supported by grant 47794 from the NIH (to Dr. S. Sen.) and American Heart Association (Ohio Valley Affiliate) Beginning Grant-in-aid 0565226B (to S. Gupta).

REFERENCES

1. Abbasi S, Lee JD, Su B, Chen X, Alcon JL, Yang J, Kellems RE, and Xia Y. Protein kinase-mediated regulation of calcineurin through the phosphorylation of modulatory calcineurin-interacting protein 1. *J Biol Chem* 281: 7717–7726, 2006.
2. Aikawa R, Komuro I, Nagai R, and Yazaki Y. Rho plays an important role in angiotensin II-induced hypertrophic responses in cardiac myocytes. *Mol Cell Biochem* 212: 177–182, 2000.
3. Aikawa R, Nagai T, Kudoh S, Zou Y, Tanaka M, Tamura M, Akazawa H, Takano H, Nagai R, and Komuro I. Integrins play a critical role in mechanical stress-induced p38 MAPK activation. *Hypertension* 39: 233–238, 2002.
4. Alvarez BV, Ennis IL, De Hurtado MC, and Cingolani HE. Effects of antihypertensive therapy on cardiac sodium/hydrogen ion exchanger activity and hypertrophy in spontaneously hypertensive rats. *Can J Cardiol* 18: 667–672, 2002.
5. Ambros V. The functions of animal microRNAs. *Nature* 431: 350–355, 2004.
6. Anwar KN, Fazal F, Malik AB, and Rahman A. RhoA/Rho-associated kinase pathway selectively regulates thrombin-induced intercellular adhesion molecule-1 expression in endothelial cells via activation of I kappa B kinase beta and phosphorylation of RelA/p65. *J Immunol* 173: 6965–6972, 2004.
7. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, and Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 106: 3009–3017, 2002.
8. Baeuerle PA and Baltimore D. NF-kappa B: ten years after. *Cell* 87: 13–20, 1996.
9. Baeuerle PA and Henkel T. Function and activation of NF-kB in the immune system. *Annu Rev Immunol* 12: 141–179, 1994.
10. Baldwin AS Jr. The NF-κB and I-κB proteins: new discoveries and insights. *Annu Rev Immunol* 14: 649–683, 1996.
11. Barnes PJ and Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066–1071, 1997.
12. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281–297, 2004.
13. Bayat H, Swaney JS, Ander AN, Dalton N, Kennedy BP, Hammond HK, and Roth DM. Progressive heart failure after myocardial infarction in mice. *Basic Res Cardiol* 97: 206–213, 2002.
14. Bayer AL, Heidkamp MC, Patel N, Porter M, Engman S, and Samarel AM. Alterations in protein kinase C isoenzyme expression and autophosphorylation during the progression of pressure overload-induced left ventricular hypertrophy. *Mol Cell Biochem* 242: 145–152, 2003.
15. Beg AA and Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 274: 782–784, 1996.
16. Bernstein E, Caudy AA, Hammond SM, and Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409: 363–366, 2001.
17. Bikkina M, Larson MG, and Levy D. Asymptomatic ventricular arrhythmias and mortality risk in subjects with left ventricular hypertrophy. *J Am Coll Cardiol* 22: 1111–1116, 1993.
18. Bisognano JD, Weinberger HD, Bohlmeyer TJ, Pende A, Raynolds MV, Sastravaha A, Roden R, Asano K, Blaxall BC, Wu SC, Communal C, Singh K, Colucci W, Bristow MR, and Port DJ. Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. *J Mol Cell Cardiol* 32: 817–830, 2000.
19. Bogoyevitch MA, Glennon PE, Andersson MB, Clerk A, Lazou A, Marshall CJ, Parker PJ, and Sugden PH. Endothelin-1 and fibroblast growth factors stimulate the mitogen-activated protein kinase signaling cascade in cardiac myocytes: the potential role of the cascade in the integration of two signaling pathways leading to myocyte hypertrophy. *J Biol Chem* 269: 1110–1119, 1994.
20. Boluyt MO, Robinson KG, Meredith AL, Sen S, Lakatta EG, Crow MT, Brooks WW, Conrad CH, and Bing OH. Heart failure after long-term supravalvular aortic constriction in rats. *Am J Hypertens* 18: 202–212, 2005.
21. Bowman JC, Steinberg SF, Jiang T, Geenen DL, Fishman GI, and Buttrick PM. Expression of protein kinase C beta in the heart causes hypertrophy in adult mice and sudden death in neonates. *J Clin Invest* 100: 2189–2195, 1997.
22. Boyle WJ, Smeal T, Defize LH, Angel P, Woodgett JR, Karin M, and Hunter T. Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA-binding activity. *Cell* 64: 573–584, 1991.
23. Brater DC. Pharmacology of diuretics. *Am J Med Sci* 319: 38–50, 2000.
24. Braun MU, LaRosee P, Schon S, Borst MM, and Strasser RH. Differential regulation of cardiac protein kinase C isozyme expression after aortic banding in rat. *Cardiovasc Res* 56: 52–63, 2002.
25. Braz JC, Bueno OF, De Windt LJ, and Molkentin JD. PKC alpha regulates the hypertrophic growth of cardiomyocytes through extracellular signal-regulated kinase1/2 (ERK1/2). *J Cell Biol* 156: 905–919, 2002.

26. Braz JC, Bueno OF, Liang Q, Wilkins BJ, Dai YS, Parsons S, Braunwart J, Glascock BJ, Klevitsky R, Kimball TF, Hewett TE, and Molkentin JD. Targeted inhibition of p38 MAPK promotes hypertrophic cardiomyopathy through upregulation of calcineurin-NFAT signaling. *J Clin Invest* 111: 1475–1486, 2003.
27. Brazil DP, Yang ZZ, and Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 29: 233–242, 2004.
28. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB, Hershberger RE, Kubo SH, Narahara KA, Ingersoll H, Krueger S, Young S, and Shusterman N. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure: MOCHA Investigators. *Circulation* 94: 2807–2816, 1996.
29. Brown JH, Del Re DP, and Sussman MA. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res* 98: 730–742, 2006.
30. Brown MJ, Palmer CR, Castaigne A, de Leeuw PW, Mancia G, Rosenthal T, and Ruilope LM. Morbidity and mortality in patients randomised to double-blind treatment with a long-acting calcium-channel blocker or diuretic in the International Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment (INSIGHT). *Lancet* 356: 366–372, 2000.
31. Burnier M and Maillard M. Angiotensin II receptor antagonists: where do we stand? *IDrugs* 3: 304–309, 2000.
32. Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, and Evans S. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 5: 877–889, 2003.
33. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, and Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 99: 15524–15529, 2002.
34. Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, and Croce CM. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci U S A* 101: 11755–11760, 2004.
35. Capasso G, Unwin R, Rizzo M, Pica A, and Giebisch G. Bicarbonate transport along the loop of Henle: molecular mechanisms and regulation. *J Nephrol* 15(suppl 5): S88–S96, 2002.
36. Carabello BA. Models of volume overload hypertrophy. *J Cardiac Fail* 2: 55–64, 1996.
37. Casale PN, Devereux RB, Milner M, Zullo G, Harshfield GA, Pickering TG, and Laragh JH. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med* 105: 173–178, 1986.
38. Chakraborty A and Eghbali M. Collagenase activity in the normal rat myocardium: an immunohistochemical method. *Histochemistry* 92: 391–396, 1989.
39. Chang S, Bezprozvannaya S, Li S, and Olson EN. An expression screen reveals modulators of class II histone deacetylase phosphorylation. *Proc Natl Acad Sci U S A* 102: 8120–8125, 2005.
40. Channer KS, Channer JL, Campbell MJ, and Rees JR. Cardiomyopathy in the Kearns-Sayre syndrome. *Br Heart J* 59: 486–490, 1988.
41. Chen F, Castranova V, Shi X, and Demers LMA. New insights into the role of nuclear factor-κB, a ubiquitous transcription factor in the initiation of diseases. *Clin Chem* 45: 7–17, 1999.
42. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, and Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38: 228–233, 2006.
43. Chen L, Hahn H, Wu G, Chen CH, Liron T, Schechtman D, Cavallaro G, Banci L, Guo Y, Bolli R, Dorn GW, and Mochly-Rosen D. Opposing cardioprotective actions and parallel hypertrophic effects of delta PKC and epsilon PKC. *Proc Natl Acad Sci U S A* 98: 11114–11119, 2001.
44. Chen S, Hilcove S, and Ding S. Exploring stem cell biology with small molecules. *Mol Biosyst* 2: 18–24, 2006.
45. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, and Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 94: 92–95, 2004.
46. Cheng L, Ding G, Qin Q, Huang Y, Lewis W, He N, Evans RM, Schneider MD, Brako FA, Xiao Y, Chen YE, and Yang Q. Cardiomyocyte-restricted peroxisome proliferator-activated receptor-delta deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy. *Nat Med* 10: 1245–1250, 2004.
47. Chi JT, Chang HY, Wang NN, Chang DS, Dunphy N, and Brown PO. Genomewide view of gene silencing by small interfering RNAs. *Proc Natl Acad Sci U S A* 100: 6343–6346, 2003.
48. Chiloeches A, Paterson HF, Marais R, Clerk A, Marshall CJ, and Sugden PH. Regulation of Ras.GTP loading and Ras-Raf association in neonatal rat ventricular myocytes by G protein-coupled receptor agonists and phorbol ester: activation of the extracellular signal-regulated kinase cascade by phorbol ester is mediated by Ras. *J Biol Chem* 274: 19762–19770, 1999.
49. Clayton DA, Doda JN, and Friedberg EC. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc Natl Acad Sci U S A* 71: 2777–2781, 1974.
50. Clerk A, Pham FH, Fuller SJ, Sahai E, Aktories K, Marais R, Marshall C, and Sugden PH. Regulation of mitogen-activated protein kinases in cardiac myocytes through the small G protein Rac1. *Mol Cell Biol* 21: 1173–1184, 2001.
51. Clerk A and Sugden PH. Small guanine nucleotide-binding proteins and myocardial hypertrophy. *Circ Res* 86: 1019–1023, 2000.
52. Clerk A and Sugden PH. Cell stress-induced phosphorylation of ATF2 and c-Jun transcription factors in rat ventricular myocytes. *Biochem J* 325: 801–810, 1997.
53. Cleutjens JP, Kandala JC, Guarda E, Guntaka RV, and Weber KT. Regulation of collagen degradation in the rat myocardium after infarction. *J Mol Cell Cardiol* 27: 1281–1292, 1995.
54. Cobb MH, Robbins DJ, and Boulton TG. ERKs, extracellular signal-regulated MAP-2 kinases. *Curr Opin Cell Biol* 3: 1025–1032, 1991.

54a. Colluci WS (Ed). *Atlas of Heart Failure*; Series editor, Braunwald E. Philadelphia, PA: Current Medicine, Inc.

55. Condorelli G and Peschle C. Stem cells for cardiac repair: state of the art. *Front Biosci* 10: 3143–3150, 2005.
56. Corral-Debrinski M, Stepien G, Shoffner JM, Lott MT, Kanter K, and Wallace DC. Hypoxemia is associated with mitochondrial DNA damage and gene induction: implications for cardiac disease. *JAMA* 266: 1812–1816, 1991.
57. Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T, and Gutkind JS. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81: 1137–1146, 1995.
58. Creemers EE, Cleutjens JP, Smits JF, and Daemen MJ. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? *Circ Res* 89: 201–210, 2001.
59. Creemers EE, Davis JN, Parkhurst AM, Leenders P, Dowdy KB, Hapke E, Hauet AM, Escobar PG, Cleutjens JP, Smits JF, Daemen MJ, Zile MR, and Spinale FG. Deficiency of TIMP-1 exacerbates LV remodeling after myocardial infarction in mice. *Am J Physiol Heart Circ Physiol* 284: H364–H371, 2003.
60. Czupalla C, Culo M, Muller EC, Brock C, Reusch HP, Spicher K, Krause E, and Nurnberg B. Identification and characterization of the autophosphorylation sites of phosphoinositide 3-kinase isoforms beta and gamma. *J Biol Chem* 278: 11536–11545, 2003.
61. D'Angelo DD, Sakata Y, Lorenz JN, Boivin GP, Walsh RA, Liggett SB, and Dorn GW. Transgenic Galphaq overexpression induces cardiac contractile failure in mice. *Proc Natl Acad Sci U S A* 94: 8121–8126, 1997.
62. Daaka Y, Luttrell LM, and Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 390: 88–91, 1997.
63. Damen JE, Liu L, Rosten P, Humphries RK, Jefferson AB, Majerus PW, and Krystal G. The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. *Proc Natl Acad Sci U S A* 93: 1689–1693, 1996.

64. Dash R, Schmidt AG, Pathak A, Gerst MJ, Biniakiewicz D, Kadambi VJ, Hoit BD, Abraham WT, and Kranias EG. Differential regulation of p38 mitogen-activated protein kinase mediates gender-dependent catecholamine-induced hypertrophy. *Cardiovasc Res* 57: 704–714, 2003.
65. Davis RJ. The mitogen-activated protein kinase signal transduction pathway. *J Biol Chem* 268: 14553–14556, 1993.
66. De Windt LJ, Lim HW, Haq S, Force T, and Molkentin JD. Calcineurin promotes protein kinase C and c-Jun NH2-terminal kinase activation in the heart: cross-talk between cardiac hypertrophic signaling pathways. *J Biol Chem* 275: 13571–13579, 2000.
67. Dhand R, Hara K, Hiles I, Bax B, Gout I, Panayotou G, Fry MJ, Yonezawa K, Kasuga M, and Waterfield MD. PI 3-kinase: structural and functional analysis of intersubunit interactions. *EMBO J* 13: 511–521, 1994.
68. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, and Karin M. A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. *Nature* 388: 548–554, 1997.
69. Ding S and Schultz PG. A role for chemistry in stem cell biology. *Nat Biotechnol* 22: 833–840, 2004.
70. Disatnik MH, Buraggi G, and Mochly-Rosen D. Localization of protein kinase C isozymes in cardiac myocytes. *Exp Cell Res* 210: 287–297, 1994.
71. Disatnik MH, Jones SN, and Mochly-Rosen D. Stimulus-dependent subcellular localization of activated protein kinase C; a study with acidic fibroblast growth factor and transforming growth factor-beta 1 in cardiac myocytes. *J Mol Cell Cardiol* 27: 2473–2481, 1995.
72. Doble BW and Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci* 116: 1175–1186, 2003.
73. Dodge KL and Scott JD. Calcineurin anchoring and cell signaling. *Biochem Biophys Res Commun* 311: 1111–1115, 2003.
74. Dorn GW. Adrenergic pathways and left ventricular remodeling. *J Card Fail* 8: S370–S373, 2002.
75. Dorn GW and Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest* 115: 527–537, 2005.
76. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, and Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest* 106: 55–62, 2000.
77. Dufner A and Thomas G. Ribosomal S6 kinase signaling and the control of translation. *Exp Cell Res* 253: 100–109, 1999.
78. Eghbali M, Tomek R, Sukhatme VP, Woods C, and Bhambi B. Differential effects of transforming growth factor-beta 1 and phorbol myristate acetate on cardiac fibroblasts: regulation of fibrillar collagen mRNAs and expression of early transcription factors. *Circ Res* 69: 483–490, 1991.
79. Eisenberg MJ, Brox A, and Bestawros AN. Calcium channel blockers: an update. *Am J Med* 116: 35–43, 2004.
80. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, and Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411: 494–498, 2001.
81. Elbashir SM, Harborth J, Weber K, and Tuschl T. Analysis of gene function in somatic mammalian cells using small interfering RNAs. *Methods* 26: 199–213, 2002.
82. Engelhardt S, Hein L, Keller U, Klambt K, and Lohse MJ. Inhibition of Na(+)-H(+) exchange prevents hypertrophy, fibrosis, and heart failure in beta(1)-adrenergic receptor transgenic mice. *Circ Res* 90: 814–819, 2002.
83. Eschenhagen T, Mende U, Nose M, Schmitz W, Scholz H, Haverich A, Hirt S, Doring V, Kalmar P, and Hoppner W Increased messenger RNA level of the inhibitory G protein alpha subunit Gi alpha-2 in human end-stage heart failure. *Circ Res* 70: 688–696, 1992.
84. Esposito G, Rapacciuolo A, Naga Prasad SV, Takaoka H, Thomas SA, Koch WJ, and Rockman HA. Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. *Circulation* 105: 85–92, 2002.
85. Everett AD, Stoops TD, Nairn AC, and Brautigan D. Angiotensin II regulates phosphorylation of translation elongation factor-2 in cardiac myocytes. *Am J Physiol Heart Circ Physiol* 281: H161–H167, 2001.
86. Exil VJ, Roberts RL, Sims H, McLaughlin JE, Malkin RA, Gardner CD, Ni G, Rottman JN, and Strauss AW. Very-long-chain acyl-coenzyme a dehydrogenase deficiency in mice. *Circ Res* 93: 448–455, 2003.
87. Feig PU. Cellular mechanism of action of loop diuretics: implications for drug effectiveness and adverse effects. *Am J Cardiol* 57: 14A–19A, 1986.
88. Finck BN, Han X, Courtois M, Aimond F, Nerbonne JM, Kovacs A, Gross RW, and Kelly DP. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci U S A* 100: 1226–1231, 2003.
89. Foncea R, Andersson M, Ketterman A, Blakesley V, Sapag-Hagar M, Sugden PH, LeRoith D, and Lavandero S. Insulin-like growth factor-I rapidly activates multiple signal transduction pathways in cultured rat cardiac myocytes. *J Biol Chem* 272: 19115–19124, 1997.
90. Foster FM, Traer CJ, Abraham SM, and Fry MJ. The phosphoinositide (PI) 3-kinase family. *J Cell Sci* 116: 3037–3040, 2003.
91. Frey N and Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 65: 45–79, 2003.
92. Frohlich ED. Risk mechanisms in hypertensive heart disease. *Hypertension* 34: 782–789, 1999.
93. Fu M, Zhang J, Xu S, Pang Y, Liu N, and Tang C. Role of calcineurin in angiotensin II-induced cardiac myocyte hypertrophy of rats. *Chin Med Sci J* 16: 1–4, 2001.
94. Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE, and Kornowski R. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 37: 1726–1732, 2001.
95. Gaudin C, Ishikawa Y, Wight DC, Mahdavi V, Nadal-Ginard B, Wagner TE, Vatner DE, and Homcy CJ. Overexpression of Gs alpha protein in the hearts of transgenic mice. *J Clin Invest* 95: 1676–1683, 1995.
96. Gaussin V, Tomlinson JE, Depre C, Engelhardt S, Antos CL, Takagi G, Hein L, Topper JN, Liggett SB, Olson EN, Lohse MJ, Vatner SF, and Vatner DE. Common genomic response in different mouse models of beta-adrenergic-induced cardiomyopathy. *Circulation* 108: 2926–2933, 2003.
97. Ghosh S, May MJ, and Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 16: 225–260, 1998.
98. Ginger RS, Dalton EC, Ryves WJ, Fukuzawa M, Williams JG, and Harwood AJ. Glycogen synthase kinase-3 enhances nuclear export of a Dictyostelium STAT protein. *EMBO J* 19: 5483–5491, 2000.
99. Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ, and Sugden PH. Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. *Circ Res* 78: 954–961, 1996.
100. Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ, and Sugden PH. Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. *Circ Res* 78: 954–961, 1996.
101. Gossen M and Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci* 89: 5547–5551, 1992.
102. Graham BH, Waymire KG, Cottrell B, Trounce IA, MacGregor GR, and Wallace DC. A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat Genet* 16: 226–234, 1997.
103. Grammer JB, Bleiziffer S, Monticelli F, Lange R, and Bauernschmitt R. Calcineurin and matrix protein expression in cardiac hypertrophy: evidence for calcineurin B to control excessive hypertrophic signaling. *Basic Res Cardiol* 101: 292–300, 2006.
104. Griffith JP, Kim JL, Kim EE, Sintchak MD, Thomson JA, Fitzgibbon MJ, Fleming MA, Caron PR, Hsiao K, and Navia

MA. X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. *Cell* 82: 507–522, 1995.
105. Gross ER, Hsu AK, and Gross GJ. Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. *Circ Res* 94: 960–966, 2004.
105a. Grossman W, Carabello BA, Gunther S, Fifer MA. Ventricular wall stress and the development of cardiac hypertrophy and failure. In: Katz AM, Alpert NR, eds. *Perspectives in Cardiovascular Research: Myocardial Hypertrophy and Failure*. New York, NY: Raven Press Publishers, 7: 1–18, 1993.
106. Gupta S, Purcell NH, Lin A, and Sen S. Activation of nuclear factor-kappaB is necessary for myotrophin-induced cardiac hypertrophy. *J Cell Biol* 159: 1019–1028, 2002.
107. Hamada K, Sasaki T, Koni PA, Natsui M, Kishimoto H, Sasaki J, Yajima N, Horie Y, Hasegawa G, Naito M, Miyazaki J, Suda T, Itoh H, Nakao K, Mak TW, Nakano T, and Suzuki A. The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis. *Genes Dev* 19: 2054–2065, 2005.
108. Han J, Lee JD, Bibbs L, and Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265: 808–811, 1994.
109. Hannon GJ. RNA interference. *Nature* 418: 244–251, 2002.
110. Haq S, Choukroun G, Kang ZB, Ranu H, Matsui T, Rosenzweig A, Molkentin JD, Alessandrini A, Woodgett J, Hajjar R, Michael A, and Force T. Glycogen synthase kinase-3beta is a negative regulator of cardiomyocyte hypertrophy. *J Cell Biol* 151: 117–130, 2000.
111. Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng QP, Kasuga M, Nishimoto I, and Avruch J. Regulation of eIF-4E BP1 phosphorylation by mTOR. *J Biol Chem* 272: 26457–26463, 1997.
112. Harborth J, Elbashir SM, Bechert K, Tuschl T, and Weber K. Identification of essential genes in cultured mammalian cells using small interfering RNAs. *J Cell Sci* 114: 4557–4565, 2001.
113. Harris TE and Lawrence JC Jr. TOR signaling. *Sci STKE* 2003: re15, 2003.
114. Harshfield GA, Alpert BS, Willey ES, Somes GW, Murphy JK, and Dupaul LM. Race and gender influence ambulatory blood pressure patterns of adolescents. *Hypertension* 14: 598–603, 1989.
115. Hasegawa H, Takano H, Iwanaga K, Ohtsuka M, Qin Y, Niitsuma Y, Ueda K, Toyoda T, Tadokoro H, and Komuro I. Cardioprotective effects of granulocyte colony-stimulating factor in swine with chronic myocardial ischemia. *J Am Coll Cardiol* 47: 842–849, 2006.
116. Hautmann MB, Adam PJ, and Owens GK. Similarities and differences in smooth muscle alpha-actin induction by TGF-beta in smooth muscle versus non-smooth muscle cells. *Arterioscler Thromb Vasc Biol* 19: 2049–2058, 1999.
117. Hayashi K, Wakino S, Homma K, Sugano N, and Saruta T. Pathophysiological significance of T-type Ca2+ channels: role of T-type Ca2+ channels in renal microcirculation. *J Pharmacol Sci* 99: 221–227, 2005.
118. Hayashidani S, Tsutsui H, Ikeuchi M, Shiomi T, Matsusaka H, Kubota T, Imanaka-Yoshida K, Itoh T, and Takeshita A. Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am J Physiol Heart Circ Physiol* 285: H1229–H1235, 2003.
119. He L and Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 5: 522–531, 2004.
120. Heimer R, Bashey RI, Kyle J, and Jimenez SA. TGF-beta modulates the synthesis of proteoglycans by myocardial fibroblasts in culture. *J Mol Cell Cardiol* 27: 2191–2198, 1995.
121. Hibi M, Lin A, Smeal T, Minden A, and Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7: 2135–2148, 1993.
122. Hill JM, Syed MA, Arai AE, Powell TM, Paul JD, Zalos G, Read EJ, Khuu HM, Leitman SF, Horne M, Csako G, Dunbar CE, Waclawiw MA, and Cannon RO, III. Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J Am Coll Cardiol* 46: 1643–1648, 2005.
123. Hingtgen SD, Tian X, Yang J, Dunlay SM, Peek AS, Wu Y, Sharma RV, Engelhardt JF, and Davisson RL. Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. *Physiol Genomics* 26: 180–191, 2006.
124. Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, and Woodgett JR. Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature* 406: 86–90, 2000.
125. Hoes AW, Grobbee DE, Peet TM, and Lubsen J. Do non-potassium-sparing diuretics increase the risk of sudden cardiac death in hypertensive patients? Recent evidence. *Drugs* 47: 711–733, 1994.
126. Howe LR, Leevers SJ, Gomez N, Nakielny S, Cohen P, and Marshall CJ. Activation of the MAP kinase pathway by the protein kinase raf. *Cell* 71: 335–342, 1992.
127. Hristov M and Weber C. The therapeutic potential of progenitor cells in ischemic heart disease: past, present and future. *Basic Res Cardiol* 101: 1–7, 2006.
128. Huang L, Wolska BM, Montgomery DE, Burkart EM, Buttrick PM, and Solaro RJ. Increased contractility and altered Ca(2+) transients of mouse heart myocytes conditionally expressing PKCbeta. *Am J Physiol Cell Physiol* 280: C1114–C1120, 2001.
129. Hug H and Sarre TF. Protein kinase C isoenzymes: divergence in signal transduction? *Biochem J* 291: 329–343, 1993.
130. Ichas F and Mazat JP. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore: switching from low- to high-conductance state. *Biochim Biophys Acta* 1366: 33–50, 1998.
131. Iwanaga K, Takano H, Ohtsuka M, Hasegawa H, Zou Y, Qin Y, Odaka K, Hiroshima K, Tadokoro H, and Komuro I. Effects of G-CSF on cardiac remodeling after acute myocardial infarction in swine. *Biochem Biophys Res Commun* 325: 1353–1359, 2004.
132. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, and Linsley PS. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol* 21: 635–637, 2003.
133. Jain M, Pfister O, Hajjar RJ, and Liao R. Mesenchymal stem cells in the infarcted heart. *Coron Artery Dis* 16: 93–97, 2005.
134. Jiang QS, Huang XN, Yang GZ, Dai ZK, Zhou QX, Shi JS, and Wu Q. Cardiac hypertrophy induced by prostaglandin F(2alpha) may be mediated by calcineurin signal transduction pathway in rats. *Sheng Li Xue Bao* 57: 742–748, 2005.
135. Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, Nelson DL, Moses K, and Warren ST. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat Neurosci* 7: 113–117, 2004.
136. Johnsen DD, Kacimi R, Anderson BE, Thomas TA, Said S, and Gerdes AM. Protein kinase C isozymes in hypertension and hypertrophy: insight from SHHF rat hearts. *Mol Cell Biochem* 270: 63–69, 2005.
137. Jones WK, Brown M, Ren X, He S, and McGuinness M. NF-kappaB as an integrator of diverse signaling pathways: the heart of myocardial signaling? *Cardiovasc Toxicol* 3: 229–254, 2003.
138. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, and Asahara T. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 97: 3422–3427, 2000.
139. Kalra D, Baumgarten G, Dibbs Z, Seta Y, Sivasubramanian N, and Mann DL. Nitric oxide provokes tumor necrosis factor-alpha expression in adult feline myocardium through a cGMP-dependent pathway. *Circulation* 102: 1302–1307, 2000.
140. Kapelko VI, Veksler VI, Popovich MI, and Ventura-Clapier R. Energy-linked functional alterations in experimental cardiomyopathies. *Am J Physiol* 261: 39–44, 1991.
141. Kariko K, Bhuyan P, Capodici J, and Weissman D. Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. *J Immunol* 172: 6545–6549, 2004.
142. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *Philos Trans R Soc Lond B Biol Sci* 351: 127–134, 1996.
143. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, Uchida S, Masuo O, Iwaguro H, Ma H, Hanley A, Silver M, Kearney M, Losordo DW, Isner JM, and Asahara T. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation* 107: 461–468, 2003.

144. Kim HE, Dalal SS, Young E, Legato MJ, Weisfeldt ML, and D'Armiento J. Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. *J Clin Invest* 106: 857–866, 2000.
145. Kim S and Iwao H. Activation of mitogen-activated protein kinases in cardiovascular hypertrophy and remodeling. *Jpn J Pharmacol* 80: 97–102, 1999.
146. Knuefermann P, Chen P, Misra A, Shi SP, Abdellatif M, and Sivasubramanian N. Myotrophin/V-1, a protein up-regulated in the failing human heart and in postnatal cerebellum, converts NFkappa B p50–p65 heterodimers to p50–p50 and p65–p65 homodimers. *J Biol Chem* 277: 23888–23897, 2002.
147. Koch WJ, Lefkowitz RJ, and Rockman HA. Functional consequences of altering myocardial adrenergic receptor signaling. *Annu Rev Physiol* 62: 237–260, 2000.
148. Koide Y, Tamura K, Suzuki A, Kitamura K, Yokoyama K, Hashimoto T, Hirawa N, Kihara M, Ohno S, and Umemura S. Differential induction of protein kinase C isoforms at the cardiac hypertrophy stage and congestive heart failure stage in Dahl salt-sensitive rats. *Hypertens Res* 26: 421–426, 2003.
149. Kouchi I, Zolk O, Jockenhovel F, Itter G, Linz W, Cremers B, and Bohm M. Increase in G(i alpha) protein accompanies progression of post-infarction remodeling in hypertensive cardiomyopathy. *Hypertension* 36: 42–47, 2000.
150. Kowaltowski AJ and Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med* 26: 463–471, 1999.
151. Krakoff LR. Diuretics for hypertension. *Circulation* 112: e127–e129, 2005.
152. Krumholz HM, Larson M, and Levy D. Sex differences in cardiac adaptation to isolated systolic hypertension. *Am J Cardiol* 72: 310–313, 1993.
153. Kuriyama M, Matsushita M, Tateishi A, Moriwaki A, Tomizawa K, Ishino K, Sano S, and Matsui H. A cell-permeable NFAT inhibitor peptide prevents pressure-overload cardiac hypertrophy. *Chem Biol Drug Des* 67: 238–243, 2006.
154. Kyriakis JM, App H, Zhang XF, Banerjee P, Brautigan DL, Rapp UR, and Avruch J. Raf-1 activates MAP kinase-kinase. *Nature* 358: 417–421, 1992.
155. Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, and Clayton DA. Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat Genet* 18: 231–236, 1998.
156. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, and Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature* 433: 647–653, 2005.
157. Lee N, Thorne T, Losordo DW, and Yoon YS. Repair of ischemic heart disease with novel bone marrow-derived multipotent stem cells. *Cell Cycle* 4: 861–864, 2005.
158. Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, and Gonzalez FJ. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 15: 3012–3022, 1995.
159. Leenen FH, Ruzicka M, and Huang BS. Central sympathoinhibitory effects of calcium channel blockers. *Curr Hypertens Rep* 3: 314–321, 2001.
160. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, and Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 106: 847–856, 2000.
161. Leiden JM. Human gene therapy: the good, the bad, and the ugly. *Circ Res* 86: 923–925, 2000.
162. Lemire I, Ducharme A, Tardif JC, Poulin F, Jones LR, Allen BG, Hebert TE, and Rindt H. Cardiac-directed overexpression of wild-type alpha1B-adrenergic receptor induces dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 281: H931–H938, 2001.
163. Leopoldt D, Hanck T, Exner T, Maier U, Wetzker R, and Nurnberg B. Gbetagamma stimulates phosphoinositide 3-kinase-gamma by direct interaction with two domains of the catalytic p110 subunit. *J Biol Chem* 273: 7024–7029, 1998.
164. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, and Burge CB. Prediction of mammalian microRNA targets. *Cell* 115: 787–798, 2003.
165. Li H, Wang J, Wilhelmsson H, Hansson A, Thoren P, Duffy J, Rustin P, and Larsson NG. Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. *Proc Natl Acad Sci U S A* 97: 3467–3472, 2000.
166. Li Y, Ha T, Gao X, Kelley J, Williams DL, Browder IW, Kao RL, and Li C. NF-κB activation is required for the development of cardiac hypertrophy in vivo. *Am J Physiol Heart Circ Physiol* 287: H1712–H1720, 2004.
167. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, and Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 11: 376–381, 1995.
168. Li YY, McTiernan CF, and Feldman AM. Interplay of matrix metalloproteinases, tissue inhibitors of metalloproteinases and their regulators in cardiac matrix remodeling. *Cardiovasc Res* 46: 214–224, 2000.
169. Liang F and Gardner DG. Mechanical strain activates BNP gene transcription through a p38/NF-kappaB-dependent mechanism. *J Clin Invest* 104: 1603–1612, 1999.
170. Liang F, Lu S, and Gardner DG. Endothelin-dependent and independent components of strain-activated brain natriuretic peptide gene transcription require extracellular signal regulated kinase and p38 mitogen activated protein kinase. *Hypertension* 35: 188–192, 2000.
171. Lim HW, De Windt LJ, Mante J, Kimball TR, Witt SA, Sussman MA, and Molkentin JD. Reversal of cardiac hypertrophy in transgenic disease models by calcineurin inhibition. *J Mol Cell Cardiol* 32: 697–709, 2000.
172. Lim HW, New L, Han J, and Molkentin JD. Calcineurin enhances MAPK phosphatase-1 expression and p38 MAPK inactivation in cardiac myocytes. *J Biol Chem* 276: 15913–15919, 2001.
173. Lim JY, Park SJ, Hwang HY, Park EJ, Nam JH, Kim J, and Park SI. TGF-beta1 induces cardiac hypertrophic responses via PKC-dependent ATF-2 activation. *J Mol Cell Cardiol* 39: 627–636, 2005.
174. Lopez B, Gonzalez A, Querejeta R, Larman M, and Diez J. Alterations in the pattern of collagen deposition may contribute to the deterioration of systolic function in hypertensive patients with heart failure. *J Am Coll Cardiol* 48: 89–96, 2006.
175. Lu PY, Xie FY, and Woodle MC. siRNA-mediated antitumorigenesis for drug target validation and therapeutics. *Curr Opin Mol Ther* 5: 225–234, 2003.
176. Marais R, Light Y, Paterson HF, and Marshall CJ. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO J* 14: 3136–3145, 1995.
177. Marin-Garcia J and Goldenthal MJ. Cardiomyopathy and abnormal mitochondrial function. *Cardiovasc Res* 28: 456–463, 1994.
178. Marinissen MJ, Chiariello M, and Gutkind JS. Regulation of gene expression by the small GTPase Rho through the ERK6 (p38 gamma) MAP kinase pathway. *Genes Dev* 15: 535–553, 2001.
179. Marinissen MJ, Chiariello M, Tanos T, Bernard O, Narumiya S, and Gutkind JS. The small GTP-binding protein RhoA regulates c-jun by a ROCK-JNK signaling axis. *Mol Cell* 14: 29–41, 2004.
180. Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA* 287: 1308–1320, 2002.
181. Maron B. Hypertrophic cardiomyopathy. *Circulation* 106: 2419–2421, 2002.
182. Maruyama Y, Nishida M, Sugimoto Y, Tanabe S, Turner JH, Kozasa T, Wada T, Nagao T, and Kurose H. Galpha(12/13) mediates alpha(1)-adrenergic receptor-induced cardiac hypertrophy. *Circ Res* 91: 961–969, 2002.
183. Mathur A, Sims HF, Gopalakrishnan D, Gibson B, Rinaldo P, Vockley J, Hug G, and Strauss AW. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. *Circulation* 99: 1337–1343, 1999.

184. Matsui T, Nagoshi T, and Rosenzweig A. Akt and PI 3-kinase signaling in cardiomyocyte hypertrophy and survival. *Cell Cycle* 2: 220–223, 2003.
185. Maulik N, Sato M, Price BD, and Das DK. An essential role of NFkappaB in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia. *FEBS Lett* 429: 365–369, 1998.
186. Mayet J, Shahi M, Foale RA, Poulter NR, Sever PS, and McGom SA. Racial differences in cardiac structure and function in essential hypertension. *BMJ* 308: 1011–1014, 1994.
187. McManus MT and Sharp PA. Gene silencing in mammals by small interfering RNAs. *Nat Rev Genet* 3: 737–747, 2002.
188. Menasche P. The potential of embryonic stem cells to treat heart disease. *Curr Opin Mol Ther* 7: 293–299, 2005.
189. Mercurio F, Murray BW, Shevchenko A, Bennett BL, Young DB, Li JW, Pascual G, Motiwala A, Zhu H, Mann M, and Manning AM. IκB kinase (IKK)-associated protein 1, a common component of the heterogeneous IKK complex. *Mol Cell Biol* 19: 1526–1538, 1999.
190. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, and James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 1: 882–891, 2003.
191. Milano CA, Dolber PC, Rockman HA, Bond RA, Venable ME, Allen LF, and Lefkowitz RJ. Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc Natl Acad Sci U S A* 91: 10109–10113, 1994.
192. Minden A, Lin A, Claret FX, Abo A, and Karin M. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81: 1147–1157, 1995.
193. Mitra S, Timor A, Gupta S, Wang Q, and Sen S. Assignment of myotrophin to human chromosome band 7q33–q35 by in situ hybridization. *Cytogenet Cell Genet* 93: 151–152, 2001.
194. Mochly-Rosen D. Localization of protein kinases by anchoring proteins: a theme in signal transduction. *Science* 268: 247–251, 1995.
195. Molkentin JD. Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. *Cardiovasc Res* 63: 467–475, 2004.
196. Molkentin JD and Dorn II GW. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu Rev Physiol* 63: 391–426, 2001.
197. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, and Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215–228, 1998.
198. Morel E, Marcantoni A, Gastineau M, Birkedal R, Rochais F, Garnier A, Lompre AM, Vandecasteele G, and Lezoualc'h F. cAMP-binding protein Epac induces cardiomyocyte hypertrophy. *Circ Res* 97: 1296–1304, 2005.
199. Moriguchi Y, Matsubara H, Mori Y, Murasawa S, Masaki H, Maruyama K, Tsutsumi Y, Shibasaki Y, Tanaka Y, Nakajima T, Oda K, and Iwasaka T. Angiotensin II-induced transactivation of epidermal growth factor receptor regulates fibronectin and transforming growth factor-beta synthesis via transcriptional and posttranscriptional mechanisms. *Circ Res* 84: 1073–1084, 1999.
200. Morisco C, Seta K, Hardt SE, Lee Y, Vatner SF, and Sadoshima J. Glycogen synthase kinase 3beta regulates GATA4 in cardiac myocytes. *J Biol Chem* 276: 28586–28597, 2001.
201. Morisco C, Zebrowski D, Condorelli G, Tsichlis P, Vatner SF, and Sadoshima J. The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. *J Biol Chem* 275: 14466–14475, 2000.
202. Mukherjee DP, McTiernan CF, and Sen S. Myotrophin induces early response genes and enhances cardiac gene expression. *Hypertension* 21: 142–148, 1993.
203. Murdock DG, Boone BE, Esposito LA, and Wallace DC. Up-regulation of nuclear and mitochondrial genes in the skeletal muscle of mice lacking the heart/muscle isoform of the adenine nucleotide translocator. *J Biol Chem* 274: 14429–14433, 1999.
204. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, and Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 428: 664–668, 2004.
205. Nadal-Ginard B, Kajstura J, Leri A, and Anversa P. Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. *Circ Res* 92: 139–150, 2003.
206. Naga Prasad SV, Esposito G, Mao L, Koch WJ, and Rockman HA. Gbetagamma-dependent phosphoinositide 3-kinase activation in hearts with in vivo pressure overload hypertrophy. *J Biol Chem* 275: 4693–4698, 2000.
207. Naraba H and Iwai N. Assessment of the microRNA system in salt-sensitive hypertension. *Hypertens Res* 28: 819–826, 2005.
208. Nemoto S, Sheng Z, and Lin A. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. *Mol Cell Biol* 18: 3518–3526, 1998.
209. Neumann J, Schmitz W, Scholz H, von ML, Doring V, and Kalmar P. Increase in myocardial Gi-proteins in heart failure. *Lancet* 2: 936–937, 1988.
210. New L and Han J. The p38 MAP kinase pathway and its biological function. *Trends Cardiovasc Med* 8: 220–228, 1998.
211. Nishizuka Y. Studies and perspectives of protein kinase C. *Science* 233: 305–312, 1986.
211a. Novina CD and Sharp PA. The RNAi; revolution. *Nature* 430:161–164, 2004.
212. Obata K, Nagata K, Iwase M, Odashima M, Nagasaka T, Izawa H, Murohara T, Yamada Y, and Yokota M. Overexpression of calmodulin induces cardiac hypertrophy by a calcineurin-dependent pathway. *Biochem Biophys Res Commun* 338: 1299–1305, 2005.
213. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, and Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A* 100: 12313–12318, 2003.
214. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, and Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature* 410: 701–705, 2001.
215. Oudit GY, Sun H, Kerfant BG, Crackower MA, Penninger JM, and Backx PH. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J Mol Cell Cardiol* 37: 449–471, 2004.
216. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, and Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure: U.S. Carvedilol Heart Failure Study Group. *N Engl J Med* 334: 1349–1355, 1996.
217. Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, and Conklin DS. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. *Genes Dev* 16: 948–958, 2002.
218. Pan J, Singh US, Takahashi T, Oka Y, Palm-Leis A, Herbelin BS, and Baker KM. PKC mediates cyclic stretch-induced cardiac hypertrophy through Rho family GTPases and mitogen-activated protein kinases in cardiomyocytes. *J Cell Physiol* 202: 536–553, 2005.
219. Paradis P, Li-Youcef N, Paradis FW, Thibault G, and Nemer M. Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proc Natl Acad Sci U S A* 97: 931–936, 2000.
220. Pare GC, Bauman AL, McHenry M, Michel JJ, Dodge-Kafka KL, and Kapiloff MS. The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling. *J Cell Sci* 118: 5637–5646, 2005.
221. Pass JM, Gao J, Jones WK, Wead WB, Wu X, Zhang J, Baines CP, Bolli R, Zheng YT, Joshua IG, and Ping P. Enhanced PKC beta II translocation and PKC beta II-RACK1 interactions in PKC epsilon-induced heart failure: a role for RACK1. *Am J Physiol Heart Circ Physiol* 281: H2500–H2510, 2001.
222. Pass JM, Zheng Y, Wead WB, Zhang J, Li RC, Bolli R, and Ping P. PKCepsilon activation induces dichotomous cardiac phenotypes and modulates PKCepsilon-RACK interactions and RACK expression. *Am J Physiol Heart Circ Physiol* 280: H946–H955, 2001.

223. Pauschinger M, Doerner A, Remppis A, Tannhauser R, Kuhl U, and Schultheiss HP. Differential myocardial abundance of collagen type I and type III mRNA in dilated cardiomyopathy: effects of myocardial inflammation. *Cardiovasc Res* 37: 123–129, 1998.
224. Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, Klumperman J, Thorens B, and Thomas G. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature* 408: 994–997, 2000.
225. Perin EC and Silva GV. Stem cell therapy for cardiac diseases. *Curr Opin Hematol* 11: 399–403, 2004.
226. Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, Bocan TMA, Coker ML, Etoh T, and Spinale FG. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circulation* 103: 2303–2309, 2001.
227. Pillai JB, Isbatan A, Imai S, and Gupta MP. Poly(ADP-ribose) polymerase-1-dependent cardiac myocyte cell death during heart failure is mediated by NAD+ depletion and reduced Sir2alpha deacetylase activity. *J Biol Chem* 280: 43121–43130, 2005.
228. Pitt B. Diversity of calcium antagonists. *Clin Ther* 19(suppl A): 3–17, 1997.
229. Pitt B, Segal R, Martinez FA, Meurers G, Cowley AJ, Thomas I, Deedwania PC, Ney DE, Snavely DB, and Chang PI. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study, ELITE). *Lancet* 349: 747–752, 1997.
230. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, and Stoffel M. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432: 226–230, 2004.
231. Purcell NH and Molkentin JD. Is nuclear factor kappaB an attractive therapeutic target for treating cardiac hypertrophy? *Circulation* 108: 638–640, 2003.
232. Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, and Lin A. Activation of NF-kappa B is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. *Proc Natl Acad Sci U S A* 98: 6668–6673, 2001.
233. Rajewsky N and Socci ND. Computational identification of microRNA targets. *Dev Biol* 267: 529–535, 2004.
234. Raju VS, Imai N, and Liang CS. Chamber-specific regulation of heme oxygenase-1 (heat shock protein 32) in right-sided congestive heart failure. *J Mol Cell Cardiol* 31: 1581–1589, 1999.
235. Ramirez MT, Sah VP, Zhao XL, Hunter JJ, Chien KR, and Brown JH. The MEKK-JNK pathway is stimulated by alpha1-adrenergic receptor and ras activation and is associated with in vitro and in vivo cardiac hypertrophy. *J Biol Chem* 272: 14057–14061, 1997.
236. Rebsamen MC, Arrighi JF, Juge-Aubry CE, Vallotton MB, and Lang U. Epidermal growth factor induces hypertrophic responses and Stat5 activation in rat ventricular cardiomyocytes. *J Mol Cell Cardiol* 32: 599–610, 2000.
237. Redfern CH, Degtyarev MY, Kwa AT, Salomonis N, Cotte N, Nanevicz N, Feldman N, DEsai K, Vranaizan K, Lee EK, Coward P, Shah N, Warrington JA, Fishman GI, Bernstein D, Baker AJ, and Conklin BR. Conditional expression of a Gi-coupled receptor causes ventricular conduction delay and a lethal cardiomyopathy. *Proc Natl Acad Sci U S A* 97: 4826–4831, 2000.
238. Ren J and Fang CX. Small guanine nucleotide-binding protein Rho and myocardial function. *Acta Pharmacol Sin* 26: 279–285, 2005.
239. Richardson KE, Tannous P, Berenji K, Nolan B, Bayless KJ, Davis GE, Rothermel BA, and Hill JA. Guanosine triphosphatase activation occurs downstream of calcineurin in cardiac hypertrophy. *J Invest Med* 53: 414–424, 2005.
240. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, Martin I, and Nordet P. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. *Circulation* 93: 841–842, 1996.
241. Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P, and Yaffee M. Oxidants in mitochondria: from physiology to diseases. *Biochim Biophys Acta* 1271: 67–74, 1995.
242. Richter C, Park JW, and Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A* 85: 6465–6467, 1988.
243. Ritchie ME. Nuclear factor-kappaB is selectively and markedly activated in humans with unstable angina pectoris. *Circulation* 98: 1707–1713, 1998.
244. Robinson MJ and Cobb MH. Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 9: 180–186, 1997.
245. Rohde LE, Ducharme A, Arroyo LH, Aikawa M, Sukhova GH, Lopez-Anaya A, McClure KF, Mitchell PG, Libby P, and Lee RT. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation* 99: 3063–3070, 1999.
246. Roman BB, Geenen DL, Leitges M, and Buttrick PM. PKC-beta is not necessary for cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* 280: H2264–H2270, 2001.
247. Rosenkranz S. TGF-beta1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res* 63: 423–432, 2004.
248. Rothwarf DM, Zandi E, Natoli G, and Karin M. IKK-g is an essential regulatory subunit of the IκB kinase complex. *Nature* 395: 297–300, 1998.
249. Ruwhof C and van der Laarse A. Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. *Cardiovasc Res* 47: 23–37, 2000.
250. Sabri A, Pak E, Alcott SA, Wilson BA, and Steinberg SF. Coupling function of endogenous alpha(1)- and beta-adrenergic receptors in mouse cardiomyocytes. *Circ Res* 86: 1047–1053, 2000.
251. Sadoshima J, Qiu Z, Morgan JP, and Izumo S. Angiotensin II and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosine kinase, mitogen-activated protein kinase, and 90-kD S6 kinase in cardiac myocytes: the critical role of Ca(2+)-dependent signaling. *Circ Res* 76: 1–15, 1995.
252. Sadoshima J, Xu Y, Slayter HS, and Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 75: 977–984, 1993.
253. Sakata Y, Hoit BD, Liggett SB, Walsh RA, and Dorn GW II. Decompensation of pressure-overload hypertrophy in G alpha q-overexpressing mice. *Circulation* 97: 1433–1435, 1998.
254. Sappino AP, Schurch W, and Gabbiani G. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest* 63: 144–161, 1990.
255. Sarkar S, Leaman DW, Gupta S, Sil P, Young D, Morehead A, Mukherjee D, Ratliff N, Sun Y, Rayborn M, Hollyfield J, and Sen S. Cardiac expression of myotrophin triggers myocardial hypertrophy and heart failure in transgenic mice: changes in gene expression profiles during initiation of hypertrophy and during heart failure measured by DNA microarray analysis. *J Biol Chem* 279: 20422–20434, 2004.
256. Savage DD, Garrison RJ, Kannel WB, Levy D, Anderson SJ, Stokes J III, Feinleib M, and Castelli WP. The spectrum of left ventricular hypertrophy in a general population sample: the Framingham Study. *Circulation* 75: I26–I33, 1987.
257. Scheuermann-Freestone M, Freestone NS, Langenickel T, Hohnel K, Dietz R, and Willenbrock R. A new model of congestive heart failure in the mouse due to chronic volume overload. *Eur J Heart Fail* 3: 535–543, 2001.
258. Schluter KD, Goldberg Y, Taimor G, Schafer M, and Piper HM. Role of phosphatidylinositol 3-kinase activation in the hypertrophic growth of adult ventricular cardiomyocytes. *Cardiovasc Res* 40: 174–181, 1998.
259. Schmelzle T and Hall MN. TOR, a central controller of cell growth. *Cell* 103: 253–262, 2000.
260. Schmidt U, Hajjar RJ, Helm PA, Kim CS, Doye AA, and Gwathmey JK. Contribution of abnormal sarcoplasmic reticulum ATPase activity to systolic and diastolic dysfunction in human heart failure. *J Mol Cell Cardiol* 30: 1929–1937, 1998.
261. Schultz JJ, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, and Doetschman T. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest* 109: 787–796, 2002.
262. Sen S, Tarazi RC, and Bumpus FM. Cardiac hypertrophy in spontaneously hypertensive rats. *Circ Res* 35: 775–781, 1974.

263. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, and Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* 14: 2501–2514, 2000.
264. Selvetella G, Hirsch E, Notte A, Tarone G, and Lembo G. Adaptive and maladaptive hypertrophic pathways: points of convergence and divergence. *Cardiovasc Res* 63: 373–380, 2004.
265. Semizarov D, Frost L, Sarthy A, Kroeger P, Halbert DN, and Fesik SW. Specificity of short interfering RNA determined through gene expression signatures. *Proc Natl Acad Sci U S A* 100: 6347–6352, 2003.
266. Sen R and Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46: 705–716, 1986.
267. Sen S, Kundu G, Mekhail N, Castel J, Misono K, and Healy B. Myotrophin: purification of a novel peptide from spontaneously hypertensive rat heart that influences myocardial growth. *J Biol Chem* 265: 16635–16643, 1990.
268. Sen S and Young D. Effect of antihypertensive therapy upon myosin isozyme distribution in spontaneously hypertensive rats. *J Hypertens* 10: 749–756, 1992.
269. Senbonmatsu T, Saito T, Landon EJ, Watanabe O, Price E Jr, Roberts RL, Imboden H, Fitzgerald TG, Gaffney FA, and Inagami T. A novel angiotensin II type 2 receptor signaling pathway: possible role in cardiac hypertrophy. *EMBO J* 22: 6471–6482, 2003.
270. Sentex E, Wang X, Liu X, Lukas A, and Dhalla NS. Expression of protein kinase C isoforms in cardiac hypertrophy and heart failure due to volume overload. *Can J Physiol Pharmacol* 84: 227–238, 2006.
271. Sharov VG, Goussev A, Lesch M, Goldstein S, and Sabbah HN. Abnormal mitochondrial function in myocardium of dogs with chronic heart failure. *J Mol Cell Cardiol* 30: 1757–1762, 1998.
272. Sharp PA. RNAi and double-strand RNA. *Genes Dev* 13: 139–141, 1999.
273. Shima H, Pende M, Chen Y, Fumagalli S, Thomas G, and Kozma SC. Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO J* 17: 6649–6659, 1998.
274. Shubeita HE, McDonough PM, Harris N, Knowlton KU, Glembotski CC, Brown JH, and Chien KR. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes: a paracrine mechanism for myocardial cell hypertrophy. *J Biol Chem* 265: 20555–20562, 1990.
275. Sil P, Gupta S, Young D, and Sen S. Regulation of myotrophin gene by pressure overload and stretch. *Mol Cell Biochem* 262: 79–89, 2004.
276. Sil P, Kandaswamy V, and Sen S. Increased protein kinase C activity in myotrophin-induced myocyte growth. *Circ Res* 82: 1173–1188, 1998.
277. Sil P, Misono K, and Sen S. Myotrophin in human cardiomyopathic heart. *Circ Res* 73: 98–108, 1993.
278. Simonis G, Honold J, Schwarz K, Braun MU, and Strasser RH. Regulation of the isozymes of protein kinase C in the surviving rat myocardium after myocardial infarction: distinct modulation for PKC-alpha and for PKC-delta. *Basic Res Cardiol* 97: 223–231, 2002.
279. Sivasubramanian N, Adhikary G, Sil PC, and Sen S. Cardiac myotrophin exhibits rel/nf-kappa b interacting activity in vitro. *J Biol Chem* 271: 2812–2816, 1996.
280. Sledz CA, Holko M, de Veer MJ, Silverman RH, and Williams BR. Activation of the interferon system by short-interfering RNAs. *Nat Cell Biol* 5: 834–839, 2003.
281. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res* 90: 520–530, 2002.
282. Spital A. Diuretic-induced hyponatremia. *Am J Nephrol* 19: 447–452, 1999.
283. Stamm C, Kleine HD, Westphal B, Petzsch M, Kittner C, Nienaber CA, Freund M, and Steinhoff G. CABG and bone marrow stem cell transplantation after myocardial infarction. *Thorac Cardiovasc Surg* 52: 152–158, 2004.
284. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, and Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 361: 45–46, 2003.
285. Steinberg SF, Goldberg M, and Rybin VO. Protein kinase C isoform diversity in the heart. *J Mol Cell Cardiol* 27: 141–153, 1995.
286. Stephens LR, Jackson TR, and Hawkins PT. Agonist-stimulated synthesis of phosphatidylinositol(3,4,5)-trisphosphate: a new intracellular signalling system? *Biochim Biophys Acta* 1179: 27–75, 1993.
287. Stetler-Stevenson WG. Dynamics of matrix turnover during pathologic remodeling of the extracellular matrix. *Am J Pathol* 148: 1345–1350, 1996.
288. Stolovich M, Tang H, Hornstein E, Levy G, Cohen R, Bae SS, Birnbaum MJ, and Meyuhas O. Transduction of growth or mitogenic signals into translational activation of TOP mRNAs is fully reliant on the phosphatidylinositol 3-kinase-mediated pathway but requires neither S6K1 nor rpS6 phosphorylation. *Mol Cell Biol* 22: 8101–8113, 2002.
289. Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell C, Antke C, Sorg RV, Kogler G, Wernet P, Muller HW, and Kostering M. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. *J Am Coll Cardiol* 46: 1651–1658, 2005.
290. Sturgill TW and Wu J. Recent progress in characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. *Biochim Biophys Acta* 1092: 350–357, 1991.
291. Sugden PH and Clerk A. Stress responsive mitogen activated protein kinase in the myocardium. *Circ Res* 83: 345–352, 1998.
292. Sugden PH and Clerk A. Endothelin signalling in the cardiac myocyte and its pathophysiological relevance. *Curr Vasc Pharmacol* 3: 343–351, 2005.
293. Sugden PH and Clerk A. Cellular mechanisms of cardiac hypertrophy. *J Mol Med* 76: 725–746, 1998.
294. Sui G, Soohoo C, Affar eB, Gay F, Shi Y, Forrester WC, and Shi Y. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. *Proc Natl Acad Sci U S A* 99: 5515–5520, 2002.
295. Takahashi H, Takeishi Y, Miyamoto T, Shishido T, Arimoto T, Konta T, Miyashita T, Ito M, and Kubota I. Protein kinase C and extracellular signal regulated kinase are involved in cardiac hypertrophy of rats with progressive renal injury. *Eur J Clin Invest* 34: 85–93, 2004.
296. Takano H, Komuro I, Oka T, Shiojima I, Hiroi Y, Mizuno T, and Yazaki Y. The Rho family G proteins play a critical role in muscle differentiation. *Mol Cell Biol* 18: 1580–1589, 1998.
297. Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, and Walsh RA. Transgenic overexpression of constitutively active protein kinase C epsilon causes concentric cardiac hypertrophy. *Circ Res* 86: 1218–1223, 2000.
298. Tanaka M, Kovalenko SA, Gong JS, Borgeld HJ, Katsumata K, Hayakawa M, Yoneda M, and Ozawa T. Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. *Ann N Y Acad Sci* 786: 102–111, 1996.
299. Tengholm A, Teruel MN, and Meyer T. Single cell imaging of PI3K activity and glucose transporter insertion into the plasma membrane by dual color evanescent wave microscopy. *Sci STKE* 2003: L4, 2003.
300. Thomas CV, Coker ML, Zellner JL, Handy JR, Crumbley AJ III, and Spinale FG. Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy. *Circulation* 97: 1708–1715, 1998.
301. Thurberg BL and Collins T. The nuclear factor-κB/inhibitor of κB autoregulatory system and atherosclerosis. *Curr Opin Lipidol* 9: 387–396, 1998.
302. Tian R, Miao W, Spindler M, Javadpour MM, McKinney R, Bowman JC, Buttrick PM, and Ingwall JS. Long-term expression of protein kinase C in adult mouse hearts improves postischemic recovery. *Proc Natl Acad Sci U S A* 96: 13536–13541, 1999.
303. Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, and Smith RD.

Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 45: 205–251, 1993.
304. Tobe S and Epstein M. The use of calcium antagonists in the treatment of hypertensive persons with kidney disease. *Curr Hypertens Rep* 4: 191–194, 2002.
305. Toker A and Cantley LC. Signalling through the lipid products of phosphoinositide-3-OH kinase. *Nature* 387: 673–676, 1997.
306. Tokuda K, Kai H, Kuwahara F, Yasukawa H, Tahara N, Kudo H, Takemiya K, Koga M, Yamamoto T, and Imaizumi T. Pressure-independent effects of angiotensin II on hypertensive myocardial fibrosis. *Hypertension* 43: 499–503, 2004.
307. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, and Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 3: 349–363, 2002.
308. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Tamaki K, Shimokawa H, and Takeshita A. Early induction of transforming growth factor-beta via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 32: 273–279, 1998.
309. Tyagi SC, Campbell SE, Reddy HK, Tjahja E, and Voelker DJ. Matrix metalloproteinase activity expression in infarcted, noninfarcted and dilated cardiomyopathic human hearts. *Mol Cell Biochem* 155: 13–21, 1996.
310. Tyagi SC, Kumar S, and Glover G. Induction of tissue inhibitor and matrix metalloproteinase by serum in human heart-derived fibroblast and endomyocardial endothelial cells. *J Cell Biochem* 58: 360–371, 1995.
311. Tyagi SC, Kumar SG, Haas SJ, Reddy HK, Voelker DJ, Hayden MR, Demmy TL, Schmaltz RA, and Curtis JJ. Post-transcriptional regulation of extracellular matrix metalloproteinase in human heart end-stage failure secondary to ischemic cardiomyopathy. *J Mol Cell Cardiol* 28: 1415–1428, 1996.
312. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De AA, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, and Anversa P. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ Res* 97: 663–673, 2005.
313. Valen G, Yan ZQ, and Hansson GK. Nuclear factor kappa-B and the heart. *J Am Coll Cardiol* 38: 307–314, 2001.
314. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, and Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 274: 787–789, 1996.
315. van Zwieten PA. Comparative mechanisms of action of diuretic drugs in hypertension. *Eur Heart J* 13(suppl G): 2–4, 1992.
316. van Empel VP, Bertrand AT, van der NR, Kostin S, Doevendans PA, Crijns HJ, de WE, Sluiter W, Ackerman SL, and De Windt LJ. Downregulation of apoptosis-inducing factor in harlequin mutant mice sensitizes the myocardium to oxidative stress-related cell death and pressure overload-induced decompensation. *Circ Res* 96: e92–e101, 2005.
317. van RE, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, and Olson EN. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A* 103: 18255–18260, 2006.
318. Vandenburgh D and Kauffman S. In vitro model for stretch-induced hypertrophy of skeletal muscle. *Science* 203: 265–268, 1979.
319. Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, and Waterfield MD. Synthesis and function of 3-phosphorylated inositol lipids. *Annu Rev Biochem* 70: 535–602, 2001.
320. Vecchione C, Fratta L, Rizzoni D, Notte A, Poulet R, Porteri E, Frati G, Guelfi D, Trimarco V, Mulvany MJ, Gabiti-Rosei E, Trimarco B, Cotecchia S, and Lembo G. Cardiovascular influences of alpha1b-adrenergic receptor defect in mice. *Circulation* 105: 1700–1707, 2002.
321. Villarreal FJ, Lee AA, Dillmann WH, and Giordano FJ. Adenovirus-mediated overexpression of human transforming growth factor-beta 1 in rat cardiac fibroblasts, myocytes and smooth muscle cells. *J Mol Cell Cardiol* 28: 735–742, 1996.
322. Waber LJ, Valle D, Neill C, DiMauro S, and Shug A. Carnitine deficiency presenting as familial cardiomyopathy: a treatable defect in carnitine transport. *J Pediatr* 101: 700–705, 1982.
323. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 283: 1482–1488, 1999.
324. Wang J, Wilhelmsson H, Graff C, Li H, Oldfors A, Rustin P, Bruning JC, Kahn CR, Clayton DA, Barsh GS, Thoren P, and Larsson NG. Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. *Nat Genet* 21: 133–137, 1999.
325. Wang XS, Diener K, Jannuzzi D, Trollinger D, Tan TH, Lichenstein H, Zukowski M, and Yao Z. Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. *J Biol Chem* 271: 31607–31611, 1996.
326. Wang Y, Huang S, Sah VP, Ross JJ, Brown JH, Han J, and Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 273: 2161–2168, 1998.
327. Watanabe A, Arai M, Yamazaki M, Koitabashi N, Wuytack F, and Kurabayashi M. Phospholamban ablation by RNA interference increases Ca2+ uptake into rat cardiac myocyte sarcoplasmic reticulum. *J Mol Cell Cardiol* 37: 691–698, 2004.
328. Watanabe K, Fujii H, Takahashi T, Kodama M, Aizawa Y, Ohta Y, Ono T, Hasegawa G, Naito M, Nakajima T, Kamijo Y, Gonzalez FJ, and Aoyama T. Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor alpha associated with age-dependent cardiac toxicity. *J Biol Chem* 275: 22293–22299, 2000.
329. Wenzel S, Taimor G, Piper HM, and Schluter KD. Redox-sensitive intermediates mediate angiotensin II-induced p38 MAP kinase activation, AP-1 binding activity, and TGF-beta expression in adult ventricular cardiomyocytes. *FASEB J* 15: 2291–2293, 2001.
330. Whitmarsh AJ, Yang SH, Su MS, Sharrocks AD, and Davis RJ. Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. *Mol Cell Biol* 17: 2360–2371, 1997.
331. Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, and Molkentin JD. Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. *Circ Res* 94: 110–118, 2004.
332. Wolf SC, Amend T, Risler T, Amann K, and Brehm BR. Influence of endothelin receptor antagonists on myocardial protein kinase C isoforms in uraemic cardiomyopathy. *Clin Sci (Lond)* 103(suppl 48): 276S–279S, 2002.
333. Wollert KC and Drexler H. Cell-based therapy for heart failure. *Curr Opin Cardiol* 21: 234–239, 2006.
334. Wong SC, Fukuchi M, Melnyk P, Rodger I, and Giaid A. Induction of cyclooxygenase-2 and activation of nuclear factor-kappaB in myocardium of patients with congestive heart failure. *Circulation* 98: 100–103, 1998.
335. Wu N and Ataai MM. Production of viral vectors for gene therapy applications. *Curr Opin Biotechnol* 11: 205–208, 2000.
336. Wymann MP, Zvelebil M, and Laffargue M. Phosphoinositide 3-kinase signaling: which way to target? *Trends Pharmacol Sci* 24: 366–376, 2003.
337. Yamaoka S, Courtois G, Bessia C, Whiteside ST, Weil R, Agou F, Kirk HE, Kay RJ, and Israel A. Complementation cloning of NEMO, a component of the IκB kinase complex essential for NF-κB activation. *Cell* 93: 1231–1240, 1998.
338. Yan M, Dai T, Deak JC, Kyriakis JM, Zon LI, Woodgett JR, and Templeton DJ. Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. *Nature* 372: 798–800, 1994.
339. Yao S, Chen S, Clark J, Hao E, Beattie GM, Hayek A, and Ding S. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *Proc Natl Acad Sci U S A* 103: 6907–6912, 2006.
340. Yazaki Y and Komuro I. Role of protein kinase system in the signal transduction of stretch-mediated myocyte growth. *Basic Res Cardiol* 87(suppl 2): 11–18, 1992.

341. Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol* 19: 623–629, 2000.
342. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, and Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 108: 2070–2073, 2003.
343. Zandi E, Chen Y, and Karin M. Direct phosphorylation of IκB by IKKa and IKKb: discrimination between free and NF-κB-bound substrate. *Science* 281: 1360–1363, 1998.
344. Zandi E, Rothwarf DM, Delhase M, Hayakawa M, and Karin M. The IκB kinase complex (IKK) contains two kinase subunits, IKKa and IKKb, necessary for IκB phosphorylation and NF-κB activation. *Cell* 91: 243–252, 1997.
345. Zaragoza D, Ghavidel A, Heitman J, and Schultz MC. Rapamycin induces the G0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. *Mol Cell Biol* 18: 4463–4470, 1998.
346. Zechner D, Thuerauf DJ, Hanford DS, McDonough PM, and Glembotski CC. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. *J Cell Biol* 139: 115–127, 1997.
347. Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, Tiranti V, and DiDonato S. Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNA(Leu)(UUR). *Lancet* 338: 143–147, 1991.
348. Zhang B, Dhillon S, Geary I, Howell WM, Iannotti F, Day IN, and Ye S. Polymorphisms in matrix metalloproteinase-1, -3, -9, and -12 genes in relation to subarachnoid hemorrhage. *Stroke* 32: 2198–2202, 2001.
349. Zhang H, Stallock JP, Ng JC, Reinhard C, and Neufeld TP. Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes Dev* 14: 2712–2724, 2000.
350. Zhang W, Anger T, Su J, Hao J, Xu X, Zhu M, Gach A, Cui L, Liao R, and Mende U. Selective loss of fine tuning of Gq/11 signaling by RGS2 protein exacerbates cardiomyocyte hypertrophy. *J Biol Chem* 281: 5811–5820, 2006.
351. Zhang Y, Bloem LJ, Yu L, Estridge TB, Iversen PW, McDonald CE, Schrementi JP, Wang X, Vlahos CJ, and Wang J. Protein kinase C betaII activation induces angiotensin converting enzyme expression in neonatal rat cardiomyocytes. *Cardiovasc Res* 57: 139–146, 2003.
352. Zhao Y, Samal E, and Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 436: 214–220, 2005.
353. Zheng CF and Guan KL. Activation of MEK family kinases requires phosphorylation of two conserved Ser/Thr residues. *EMBO J* 13: 1123–1131, 1994.
354. Zhou P, Sun LJ, Dotsch V, Wagner G, and Verdine GL. Solution structure of the core NFATC1/DNA complex. *Cell* 92: 687–696, 1998.
355. Zhu Z, Zhu S, Liu D, Cao T, Wang L, and Tepel M. Thiazide-like diuretics attenuate agonist-induced vasoconstriction by calcium desensitization linked to Rho kinase. *Hypertension* 45: 233–239, 2005.
356. Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, Makita N, Iwanaga K, Zhu W, Kudoh S, Toko H, Tamura K, Kihara M, Nagai T, Fukamizu A, Umemura S, Iiri T, Fujita T, and Komuro I. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* 6: 499–506, 2004.
357. Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, and Yazaki Y. Both Gs and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J Biol Chem* 274: 9760–9770, 1999.

Address reprint requests to:
Dr. Subha Sen
Molecular Cardiology
Lerner Research Institute
Cleveland Clinic Foundation
9500 Euclid Avenue
NB50
Cleveland, OH 44195

E-mail: sens@ccf.org

Date of first submission to ARS Central, September 28, 2006; date of acceptance, January 24, 2007.

This article has been cited by:

1. Xavier Palomer, Eva Capdevila-Busquets, David Álvarez-Guardia, Emma Barroso, Mercè Pallàs, Antoni Camins, Mercy M. Davidson, Ana Planavila, Francesc Villarroya, Manuel Vázquez-Carrera. 2012. Resveratrol induces nuclear factor-#B activity in human cardiac cells. *International Journal of Cardiology* . [CrossRef]
2. Subir Kumar Maulik, Santosh Kumar. 2012. Oxidative stress and cardiac hypertrophy: a review. *Toxicology Mechanisms and Methods* 1-8. [CrossRef]
3. Yamuna Devi Paila, Ekta Jindal, Shyamal K. Goswami, Amitabha Chattopadhyay. 2011. Cholesterol depletion enhances adrenergic signaling in cardiac myocytes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1808**:1, 461-465. [CrossRef]
4. X.-L. Meng, J.-S. Shen, S. Kawagoe, T. Ohashi, R. O. Brady, Y. Eto. 2010. Induced pluripotent stem cells derived from mouse models of lysosomal storage disorders. *Proceedings of the National Academy of Sciences* **107**:17, 7886-7891. [CrossRef]
5. Dan-Li Yang, Hai-Gang Zhang, Ya-Li Xu, Yun-Hua Gao, Xiao-Jiao Yang, Xue-Qin Hao, Xiao-Hui Li. 2010. Resveratrol inhibits right ventricular hypertrophy induced by monocrotaline in rats. *Clinical and Experimental Pharmacology and Physiology* **37**:2, 150-155. [CrossRef]
6. Gabriela Placoná Diniz, Marcela Sorelli Carneiro-Ramos, Maria Luiza Morais Barreto-Chaves. 2010. Thyroid Hormone Increases TGF-#1 in Cardiomyocytes Cultures Independently of Angiotensin II Type 1 and Type 2 Receptors. *International Journal of Endocrinology* **2010**, 1-7. [CrossRef]
7. Mark W. M. Schellings, Geert C. Almen, E. Helene Sage, Stephane Heymans. 2009. Thrombospondins in the heart: potential functions in cardiac remodeling. *Journal of Cell Communication and Signaling* **3**:3-4, 201-213. [CrossRef]
8. Rebecca H. Ritchie, Jennifer C. Irvine, Anke C. Rosenkranz, Ruchi Patel, Igor R. Wendt, John D. Horowitz, Barbara K. Kemp-Harper. 2009. Exploiting cGMP-based therapies for the prevention of left ventricular hypertrophy: NO• and beyond. *Pharmacology & Therapeutics* **124**:3, 279-300. [CrossRef]
9. Jing-Chao Lu, Wei Cui, Hai-Lin Zhang, Fan Liu, Mei Han, De-Min Liu, Hong-Ning Yin, Kai Zhang, Jun Du. 2009. Additive beneficial effects of amlodipine and atorvastatin in reversing advanced cardiac hypertrophy in elderly spontaneously hypertensive rats. *Clinical and Experimental Pharmacology and Physiology* **36**:11, 1110-1119. [CrossRef]
10. Gabriela Placoná Diniz, Marcela Sorelli Carneiro-Ramos, Maria Luiza Morais Barreto-Chaves. 2009. Angiotensin type 1 receptor mediates thyroid hormone-induced cardiomyocyte hypertrophy through the Akt/GSK-3#/mTOR signaling pathway. *Basic Research in Cardiology* **104**:6, 653-667. [CrossRef]
11. Barbara K#apci#ska, Ewa Sadowska-Kr#pa, S#awomir Jagsz, Andrzej Sobczak, Ma#gorzata #endzian-Piotrowska, Jan Górski, Józef Langfort. 2009. Short-term effects of electrically induced tachycardia on antioxidant defenses in the normal and hypertrophied rat left ventricle. *The Journal of Physiological Sciences* **59**:3, 199-206. [CrossRef]
12. Narasimman Gurusamy , Subhendu Mukherjee , Istvan Lekli , Claudia Bearzi , Silvana Bardelli , Dipak K. Das . 2009. Inhibition of Ref-1 Stimulates the Production of Reactive Oxygen Species and Induces Differentiation in Adult Cardiac Stem Cells. *Antioxidants & Redox Signaling* **11**:3, 589-599. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
13. Vladimír Krys#tof, Ivo Chamrád, Radek Jorda,, Jir#í Kohoutek. 2009. Pharmacological targeting of CDK9 in cardiac hypertrophy. *Medicinal Research Reviews* n/a-n/a. [CrossRef]
14. Dan-Li Yang, Hai-Gang Zhang, Ya-Li Xu, Yun-Hua Gao, Xiao-Jiao Yang, Xue-Qin Hao, Min Su, Xiu-Qin Wang, Xiao-Hui Li. 2009. G#q-Protein Carboxyl Terminus Imitation Polypeptide (GCIP)-27 Inhibits Right Ventricular Hypertrophy Induced by Monocrotaline in Rats. *Biological & Pharmaceutical Bulletin* **32**:3, 376-381. [CrossRef]
15. X. Palomer, D. Alvarez-Guardia, R. Rodriguez-Calvo, T. Coll, J. C. Laguna, M. M. Davidson, T. O. Chan, A. M. Feldman, M. Vazquez-Carrera. 2008. TNF- reduces PGC-1 expression through NF- B and p38 MAPK leading to increased glucose oxidation in a human cardiac cell model. *Cardiovascular Research* **81**:4, 703-712. [CrossRef]
16. Yoshiaki Kawase, Roger J Hajjar. 2008. The cardiac sarcoplasmic/endoplasmic reticulum calcium ATPase: a potent target for cardiovascular diseases. *Nature Clinical Practice Cardiovascular Medicine* **5**:9, 554-565. [CrossRef]
17. M SUSSMAN. 2007. “AKT”ing Lessons for Stem Cells: Regulation of Cardiac Myocyte and Progenitor Cell Proliferation. *Trends in Cardiovascular Medicine* **17**:7, 235-240. [CrossRef]