

# Molecular and Cellular Mechanisms of Cardiotoxicity

Y. James Kang

Departments of Medicine, Pharmacology, and Toxicology, University of Louisville and Jewish Hospital Heart and Lung Institute, Louisville, Kentucky, USA

Cardiotoxicity resulting from detrimental environmental insults has been recognized for a long time. However, extensive studies of the mechanisms involved had not been undertaken until recent years. Advances in molecular biology provide powerful tools and make such studies possible. We are gathering information about cellular events, signaling pathways, and molecular mechanisms of myocardial toxicologic responses to environmental toxicants and pollutants. Severe acute toxic insults cause cardiac cell death instantly. In the early response to mild environmental stimuli, biochemical changes such as alterations in calcium homeostasis occur. These may lead to cardiac arrhythmia, which most often is reversible. Prolonged stimuli activate transcription factors such as activator protein-1 through elevation of intracellular calcium and the subsequent activation of calcineurin. Upregulation by activated transcription factors of hypertrophic genes results in heart hypertrophy, which is a short-term adaptive response to detrimental factors. However, further development of hypertrophy will lead to severe and irreversible cardiomyopathy, and eventually heart failure. From cardiac hypertrophy to heart failure, myocardial cells undergo extensive biochemical and molecular changes. Cardiac hypertrophy causes tissue hypoperfusion, which activates compensatory mechanisms such as production of angiotensin II and norepinephrine. Both further stimulate cardiac hypertrophy and, importantly, activate counterregulatory mechanisms including overexpression of atrial natriuretic peptide and b-type natriuretic peptide, and production of cytokines such as tumor necrosis factor- $\alpha$ . This counterregulation leads to myocardial remodeling as well as cell death through apoptosis and necrosis. Cell death through activation of mitochondrial factors and other pathways constitutes an important cellular mechanism of heart failure. Our current knowledge of cardiotoxicity is limited. Further extensive studies are warranted for a comprehensive understanding of this field. *Key words:* apoptosis, cardiac hypertrophy, cardiomyopathy, cytokines, gene regulation, heart failure, MAPK, necrosis, PKC, transcription factors. — *Environ Health Perspect* 109(suppl 1):27–34 (2001).

<http://ehpnet1.niehs.nih.gov/docs/2001/suppl-1/27-34kang/abstract.html>

Cardiotoxicity resulting from exposure to environmental toxicants and pollutants has been known for a long time (1). However, a detailed examination of cardiac toxic effects and investigations of the mechanisms of cardiotoxicity had not been undertaken until recently. In fact, cardiotoxicology was not a defined discipline in the past. Two major advances in biomedical research and practice have made an urgent need for cardiotoxicologic studies. First, it is now an exciting time in the history of cardiovascular medicine. New drugs and devices are invented for the treatment of heart disease on a weekly basis (2). Undoubtedly some of these drugs and procedures will have cardiotoxic effects. For every new treatment, it will be essential to thoroughly assess toxic effects on the heart. Second, the application of cutting-edge molecular biology approaches has provided significant and novel insights into cardiac toxicity and its mechanisms (3,4). Mechanistic studies on cardiovascular effects of environmental toxicants and pollutants have emerged at a very fast pace. We have just begun to understand cellular events and molecular mechanisms of cardiotoxicity. A survey of our current knowledge in this exciting field will help to identify gaps that need

to be filled. There are excellent textbooks (5–7) describing basic anatomic, biochemical, and physiologic principles of the heart from humans to experimental animals. Ultrastructural and histologic changes of the heart in toxic injury have also been elaborated in detail (8–10). This review thus only focuses on cellular events, signaling pathways, and molecular mechanisms of cardiotoxicity, with a brief review, if necessary, of bridging knowledge leading to the focused discussion.

## Myocardial Responses to Environmental Toxicants

The foremost changes in the early phase of responses of myocardium to environmental toxicants involve alterations in biochemical reactions. These include the most often described alterations in ionic homeostasis such as changes in intracellular calcium concentrations, which occur in almost all examined exposures to environmental toxicants to date (11,12). Aberrant energy metabolism is another early response to environmental toxicants in the heart, resulting in decreased production and/or enhanced consumption of adenosine triphosphate (ATP) (13,14). Alterations in enzymatic reactions are often described in cardiac toxic responses (15), although we know very little

about these alterations. The early signaling pathways leading to myocardial toxic responses are the recent focus of cardiotoxicologic research (16,17). Detailed descriptions of these pathways and their roles in cardiotoxicity are yet to be explored. It is likely that activation of early genes and signaling pathways is a critical response of myocardial cells to environmental toxic insults (18). The cross-talk between signaling pathways determines the ultimate outcome of myocardial responses to environmental toxicants and pollutants.

Physiologic alterations occur both as early responses to environmental toxicants and as subsequent events in the late development of cardiomyopathy. The most obvious myocardial dysfunction that occurs in the early responses to toxicants is cardiac arrhythmia (19), which often results from the changes in intracellular calcium concentrations and other biochemical alterations, leading to miscommunication between cells and misconduction of electricity (20). These changes, if not accompanied by cardiomyopathy, do not involve myocardial cell death and are reversible. The late phase of cardiac dysfunction, however, often results from cardiomyopathy.

Changes in myocardial morphology take place when extensive toxic insults are imposed on the heart and/or toxic exposures persist on a long-term basis (21–23). Cardiac hypertrophy is often observed as a consequence of long-term toxic insults. The hypertrophy is considered a protective and adaptive response. However, further hypertrophy leads to severe and irreversible cardiomyopathy, eventually resulting in heart failure (24–26).

From cardiac hypertrophy to heart failure, activation of compensatory mechanisms including the sympathetic nerve system and the renin–angiotensin system takes place

---

Address correspondence to Y.J. Kang, Dept. of Medicine, University of Louisville School of Medicine, 511 S. Floyd St., MDR 530, Louisville, KY 40202 USA. Telephone: (502) 852-8677. Fax: (502) 852-6904. E-mail: yjkang01@athena.louisville.edu

The author is a University Scholar of the University of Louisville. The research work cited in this review was supported in part by National Institutes of Health grants CA68125 and HL59225, Established Investigator Award 9640091N from the American Heart Association, and a grant from the Jewish Hospital Foundation, Louisville, Kentucky. The author thanks L. Cai for helping to prepare this review and for constructive discussions.

Received 3 August 2000; accepted 2 November 2000.

(27,28). The compensatory response in turn activates counterregulatory mechanisms such as upregulation of atrial natriuretic peptide (ANP) expression (29,30). These responses collectively lead to extensive biochemical, physiologic, and molecular changes, eventually myocardial remodeling (31–33), and remarkable cell death. These changes ultimately result in heart failure. Figure 1 depicts these mechanisms. Most cardiotoxicologic studies address fundamental and characteristic changes during this stage of myocardial responses to toxic insults. The ultimate targets and the determining units of cardiotoxicity are cardiac cells. In this context, the following sections will be devoted to discussion of the cellular events, molecular mechanisms, and signaling pathways that are involved in the myocardial responses to toxic insults.

## Cellular Events Involved in Cardiotoxicity

Toxic insults trigger a series of reactions in cardiac cells leading to measurable changes in myocardial morphology, biochemistry, and physiology. Mild injuries can be repaired; however, severe injuries lead to cell death in the form of apoptosis and necrosis. If the cell survives the insults, structural and functional adaptations take place.

## Myocardial Apoptosis

Myocardial apoptosis, which is involved in cardiomyopathy, was first recognized in 1994

(34). Recent progress in myocardial research has provided significant insights into the cellular mechanisms of cardiotoxicity. It has been recognized that the loss of cardiac myocytes is a fundamental part of the myocardial injury that initiates or aggravates cardiomyopathy and leads to premature death (35–37). An important mode of myocardial cell loss is apoptosis (38–40), which has been demonstrated in the myocardium of heart failure patients (41,42). In our early studies, we have found that dietary copper restriction causes cardiomyopathy, which is accompanied with apoptosis in rat hearts (43). Myocardial apoptosis has been shown to play an important role in cardiac toxic effects induced by adriamycin (44), an important anticancer agent whose clinical application is limited by its major side effect, cardiotoxicity (45). Exposure of primary cultures of cardiomyocytes to cadmium also induces apoptosis (46).

Many *in vivo* studies have shown that only a very small percentage of myocardial cell populations undergo apoptosis under pathologic conditions (47,48). For example, we have observed that less than 0.5% of cells appeared apoptotic in myocardial tissue obtained from copper-deficient mice (49). At first glance, this number seems to be too insignificant to account for myocardial pathogenesis. However, this is a false assessment. In a carefully designed time course study (50), it has been estimated that cardiomyocyte apoptosis may be completed in less than 20 hr in rats. Because the heart is a terminally differentiated organ, myocytes undergoing apoptosis are lost and are not replaced. Thus, the total cell loss can simply be accounted for by the rate of apoptosis plus necrosis. If apoptosis occurs at a constant rate of about 0.5% myocytes a day (49), the potential contribution of apoptosis to the overall loss of myocytes over a long period of time is significant.

## Myocardial Necrosis

The term myocardial necrosis has been widely used to describe myocardial cell death in the past. Myocardial infarction, in particular, was considered a consequence of necrosis (51). It is now recognized that apoptosis contributes significantly to the formation of myocardial infarction (52), although the consequence of apoptosis in this pathogenesis is yet to be defined. However, the significance of necrosis in myocardial pathogenesis cannot be underestimated. The contribution of necrosis to cardiomyopathy induced by environmental toxicants and pollutants is particularly important. A critical issue is how to distinguish between apoptosis and necrosis.

Apoptosis and necrosis were originally described as two distinct forms of cell death that can be clearly distinguished (53).

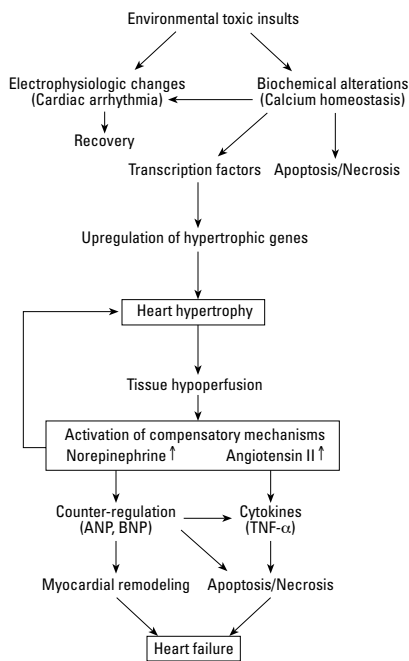
However, these two modes of cell death can occur simultaneously in tissues and cultured cells (54). The intensity and duration of insults may decide the outcome (55). Thus, triggering events can be common for both types of cell death. A downstream controller, however, may direct cells toward a programmed execution of apoptosis. If the apoptotic program is aborted before this control point and the initiating stimulus is severe, cell death may occur by necrosis (56). Alternatively, in acute injury, apoptotic cells can progress along a continuum to eventual necrosis.

It is important to note that apoptosis is an energy-dependent process and that the switch in the decision between apoptosis and necrosis depends on ATP concentrations (57). In particular, conditions causing ischemia to myocardial cells result in significant reduction and eventual depletion of adenine nucleotides. ATP loss of more than 70% of the total pool present in myocardial cells has been shown to cause a switch from apoptosis to necrosis (56,58). This extent of depletion of the ATP pool is often observed in myocardial infarction.

Identification of apoptotic and necrotic cell death processes requires more rigorous methods than have been generally used. Artifacts inherent in techniques that detect double-stranded DNA breaks as well as overlaps between apoptotic and necrotic death programs account for these problems (59,60). Current assays are limited in their capacities to distinguish between apoptosis and necrosis in myocytes. In addition, distinguishing between myocyte and nonmyocyte apoptosis in the myocardium is another problem to overcome. In our recent studies examining adriamycin-induced myocardial apoptosis (61), we used an *in situ* terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay in combination with a dual immunohistochemical detection of  $\alpha$ -sarcomeric actin, a specific protein present in cardiomyocytes. This procedure thus identified whether the TUNEL-positive cells were myocytes. Another procedure we used was immuno-gold TUNEL and electron microscopic examination of the apoptotic cells (61). It is well known that the gold standard for identification of apoptotic cells is morphologic examination by electron microscopy. This immuno-gold TUNEL and electron microscopic procedure thus provides information about cell type and morphologic characteristics of apoptotic cells.

## Myocardial Adaptation

Myocardial adaptation is the general process by which the ventricular myocardium changes in structure and function. This process is often referred to as “remodeling.” During maturation, myocardial remodeling is a normal feature that is a useful adaptation to



**Figure 1.** Myocardial responses to environmental toxic insults leading to heart failure, including important cellular events, signaling pathways, and neurohumoral regulations.

increased demands. However, in response to pathologic stimuli such as exposure to environmental toxicants, myocardial remodeling is adaptive in the short term but maladaptive in the long term and often eventuates in further myocardial dysfunction. The central feature of myocardial remodeling is an increase in myocardial mass associated with a change in the shape of the ventricle (62).

### Molecular Mechanisms Involved in Cardiotoxicity

The most severe and early response of myocardium to overwhelming environmental toxic insults is cell death by apoptosis and necrosis. Myocardial cells, like other cells, are equipped with cell death programs and contain preexisting death machinery. In response to environmental toxic insults, these programs are activated. At the same time, cytoprotective mechanisms are also turned on, such as activation of antiapoptotic factors including the Bcl-2 family. Therefore, whether a cell will die depends on the balance between the activities of cell death programs and the cytoprotective mechanisms. The signaling pathways leading to activation of cell death program are discussed below.

Although cell death is the ultimate end point that is often measured in assessing myocardial responses to environmental toxic insults (63,64), cellular and molecular events leading to myocardial remodeling are critical parameters of cardiotoxicity. The foremost observation of myocardial morphologic change in response to stimuli is heart hypertrophy. Although the hypertrophic response initially is a compensatory mechanism that augments cardiac output, sustained hypertrophy can lead to dilated cardiomyopathy, heart failure, and sudden death (65,66). A series of prototypical molecular responses of cardiomyocytes to environmental stimuli includes an increase in cell size and protein synthesis (67), enhanced sarcomeric organization (68), upregulation of fetal cardiac genes (69,70), and induction of immediate-early genes (71,72). These responses collectively lead to cardiac hypertrophy.

### Myocardial Gene Regulation

Myocardial gene regulation in response to toxic insults is not well understood. However, extensive studies on the activation of transcription factors in cardiac hypertrophy have been undertaken. Because cardiac hypertrophy is the most common response to environmental stimuli, the molecular mechanism involved in gene regulation in cardiac hypertrophy would have implications in myocardial responses to environmental toxicants and pollutants. The transcription factors involved in cardiac hypertrophy include activator protein-1 (AP-1), transcriptional enhancer

factor-1 (TEF-1), serum response factor (SRF), nuclear factors of activated T cells (NFATs), and GATA4. Most of these transcription factors function coordinately in mediating extracellular signaling to regulate hypertrophic gene expression.

AP-1 is a transcription factor composed of *Jun* and *Fos* gene family members (73). The AP-1 binding site is the TRE (12-*O*-tetradecanoylphorbol-13-acetate response element), and the binding of AP-1 to the TRE initiates transcription of the target genes (74). In recent studies, it has been shown that elevated levels of *c-Jun* are associated with the stress induced by ischemia/reperfusion in cardiomyocytes (75). In volume-overload hypertrophy, AP-1 plays an important role in the regulation of Fas and FasL activities (76). Overstretching of myocardium induces Fas expression (77). Fas-dependent signaling pathways are coupled to the activation of AP-1 in isolated cardiomyocytes (76,78). These are pathways that can lead to myocardial cell apoptosis (76–79). However, there are studies showing that activation of AP-1 is independent of the induction of apoptosis (80). AP-1 has been implicated in transcriptional regulation of several genes associated with a hypertrophic response (81,82).

TEF-1 is a transcription factor that has been shown to be activated in  $\alpha_1$ -adrenergic-stimulated hypertrophy of cultured cardiomyocytes (83). It is involved in induction of  $\beta$ -myosin heavy chain (MHC) and  $\alpha$ -skeletal actin fetal isogenes in cardiac hypertrophy (84). SRF has been demonstrated to be both necessary and sufficient to mediate stretch responses in myocardium, leading to induction of *c-fos* expression (85). This stretch-induced activation of SRF was also observed in hypotonic cell swelling, leading to *c-fos* induction (86). Thus, activation of SRF may be the general mechanism of *c-fos* activation in response to increased membrane tension in cardiac myocytes.

NFAT3 is a member of a multigene family that contains four members, NFATc, NFATp, NFAT3, and NFAT4 (87). These factors bind the consensus DNA sequence GGAAAT as monomers or dimers through a Rel homology domain (88). Unlike the other three members that are restricted in their expression to T cells and skeletal muscle, NFAT3 is expressed in a variety of tissues including the heart (87,89). The role of NFAT3 in cardiac hypertrophy has been demonstrated recently in an elegant study (90). Hypertrophic stimuli such as angiotensin II and phenylephrine cause an increase in intracellular  $Ca^{2+}$  levels in myocardial cells. This elevation in turn results in activation of calcineurin (discussed below). NFAT3 is localized within the cytoplasm and is dephosphorylated by the activated calcineurin. This

dephosphorylation enables NFAT3 to translocate to the nucleus where it can interact with GATA4. NFAT3 can also activate some hypertrophic responsive genes through mechanisms independent of GATA4.

GATA factors are a family of nuclear transcriptional regulatory proteins that are related structurally within a central DNA-binding domain but are restricted in expression to distinct sets of cell types (91). Currently, six different family members have been characterized in vertebrate species. They are GATA1, 2, 3, 4, 5, and 6. Each protein contains two similar repeats of a highly conserved zinc finger of the form CXNCX<sub>6</sub>LWRRX<sub>7</sub>CNAC. The c-terminal repeat constitutes a minimal DNA-binding domain sufficient for sequence-specific recognition of a “GATA” *cis*-element, usually (A/T)GATA(A/G) or a related DNA sequence, present in promoters and/or enhancers of target genes (92). It has been shown that GATA1, 2, and 3 mainly regulate various aspects of hematopoiesis (93), whereas the GATA4, 5, and 6 factors are involved in regulation of cardiogenesis (91). The significance of GATA4 in regulation of hypertrophic response in myocardial cells has been demonstrated recently (94). Cardiac hypertrophy induced by angiotensin II is mediated by an angiotensin II type<sub>1a</sub> receptor (AT<sub>1a</sub>R). A GATA motif was identified in the AT<sub>1a</sub>R promoter. Mutations introduced into the consensus binding site for GATA factor abolished the pressure overload response (94). Moreover, it has been demonstrated that the interactions between AP-1 and GATA4 and between NFAT3 and GATA4 are essential in myocardial hypertrophic responses (90,94).

### Molecular Basis for Alterations in Myocardial Structure and Function

It has been demonstrated that reprogramming of gene expression of  $\beta$ -MHC and  $\alpha$ -skeletal actin is associated with myocardial hypertrophy (69–72,95). Upregulation of these fetal protein isoforms is associated with downregulation of the corresponding adult isoforms,  $\alpha$ -MHC and  $\alpha$ -cardiac actin (96). These molecular processes are correlated with the development of hypertrophy and the thickness of the ventricular wall, which has been shown to result from an increase in cell size due to an increase in the number of sarcomeres and mitochondria within the cell (97). This hypertrophic development leads to tissue hypoperfusion, which in turn results in the activation of compensatory mechanisms such as the sympathetic nerve system and the renin–angiotensin system.

The neurohumoral compensatory response activates counter regulatory hormones such as ANP and b-type natriuretic peptide (BNP) (98). The long-term expression of these hormones, particularly ANP and



BNP, is associated with numerous detrimental biological effects that eventually lead to cardiac failure by way of myocyte dysfunction, apoptosis, and cell loss (99). For instance, the ANP gene is expressed in both atrium and ventricle during embryonic development, but its expression is downregulated in the ventricle shortly after birth, leaving the atrium as the primary site of ANP synthesis within the mature myocardium (100). During myocardial pathologic remodeling, reexpression of ANP in myocytes of the left ventricle takes place (99–101). Recent studies have demonstrated that ANP is a factor responsible for myocardial apoptosis (49,102). Moreover, apoptosis plays a critical role in the development of heart failure (38–42,103,104). Therefore, serum concentrations of ANP have been monitored experimentally and clinically and serve as important indices of heart failure (99–101,105).

## Signaling Pathways Leading to Cardiotoxicity

### Mitochondrial Factors in Myocardial Cell Death

Recent studies (106,107) have focused on mitochondrial factors in myocardial cell death. Current knowledge obtained from cutting-edge experimental approaches suggests that signaling pathways mediating oxidative stress-induced myocardial cell death can be divided into those that trigger the early events of cell loss and those that result in severe, end-stage myocardial cell death. Cytochrome *c* and pro-caspase-3 are preexisting factors that are activated by environmental toxic insults.

Mitochondrial permeability transition (MPT) occurs under toxic insults (108). This MPT behaves like a membrane pore that allows diffusion of solutes < 1,500 Da in size. Although MPT can occur as a temporary event, it can rapidly become irreversible, with the resulting loss of mitochondrial homeostasis and high-amplitude mitochondrial swelling. Because the inner membrane has a larger surface area than the outer membrane, mitochondrial swelling can cause rupture of the outer membrane, releasing intermembrane proteins into the cytosol (109). Among the intermembrane proteins is cytochrome *c*. Another possible mechanism that leads to mitochondrial cytochrome *c* release is the action of Bax, a proapoptotic protein of the Bcl-2 family (110). Overexpression of Bax under oxidative stress conditions has been observed in a number of studies using different organs including the heart (111,112). It has been shown that Bax is translocated from cytosol to mitochondria and forms pores in mitochondrial outer membranes, leaving the inner membranes intact (113).

This mechanism implies that Bax-mediated cytochrome *c* release is independent of MPT (114). The release of cytochrome *c* from mitochondria into the cytosol is a critical initiation step in myocardial apoptosis. Cytochrome *c* aggregates with apoptotic protease-activating factor-1 (another factor released from mitochondria under oxidative stress), procaspase-9, and deoxyadenosine triphosphate (dATP), and subsequently activates caspase-9, which activates caspase-3. In our recent studies, we detected a significant decrease in cytochrome *c* concentrations in mitochondria and a concurrent increase in the cytosol by Western blot analysis in adriamycin-treated cardiomyocytes (115).

To determine the significance of the caspase-3-activated apoptotic pathway in the pathogenesis of cardiomyopathy, we have used a caspase-3-specific inhibitor, Ac-DEVD-cmk, to determine its effect on adriamycin-induced myocardial cell death. Cultured cardiomyocytes isolated from neonatal mice were treated with Ac-DEVD-cmk before adriamycin treatment. This inhibitor efficiently suppressed caspase-3 activity and reduced the number of apoptotic cells in cultures (115).

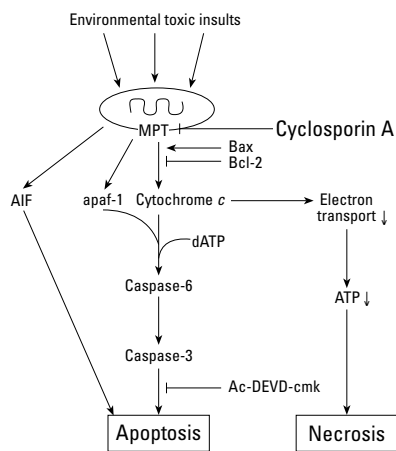
Inhibition of caspase-3-activated apoptotic pathway, however, may not abort ultimate cell death because of necrosis that can also be triggered by MPT and mitochondrial cytochrome *c* release. The loss of cytochrome *c* blocks electron transport, leading to decreased production of ATP and eventually ATP depletion, which results in necrosis. Enhanced necrotic cell death in the presence of caspase-3 inhibitor has been observed in our recent studies examining adriamycin cardiotoxicity. There is another group of proapoptotic factors that are released from mitochondria, but the mechanisms of action of these factors are unclear. These are apoptosis-inducing factors (AIF). It has been shown that AIF may directly translocate to the nucleus after they are released from mitochondria, causing DNA fragmentation (116). These multiple pathways mediated by mitochondrial factors suggest that there is a bewildering diversity of cell death paradigms. In particular, under chronic environmental toxic insults, the relative importance of mitochondrial electron transport defects, MPT, cytochrome *c* leakage, and nonmitochondrial factors needs to be carefully examined. An important clinical consideration is whether caspase inhibitors can offer long-term protection against cell death, as delineated in Figure 2. It is thus important to define the most efficient therapeutic strategy in blocking myocardial cell death rather than in inhibiting a particular cell death program.

### Cytokines and Other Factors in Myocardial Death Signaling

Cytokines have been shown to be associated with the pathogenesis of acute coronary

syndromes (117) and chronic heart failure (118). In both cases, myocardial cell loss is the essential feature of the disease. Increased serum concentrations of proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) have been found in chronic heart failure (119). Another biomarker of chronic heart failure is the increased serum concentrations of Fas ligand (120). Both TNF- $\alpha$  and Fas ligand induce apoptosis of cardiomyocytes in various experimental studies (120,121). Other cytokines such as interleukin-6 are also found in the serum of patients with failing hearts (122–124).

TNF- $\alpha$  is the most studied cytokine in myocardial cell death signaling pathways. Cardiomyocytes are both the source and the target of this cytokine (125). The proinflammatory cytokines interleukin-1, interleukin-2 and interferon- $\gamma$  can induce TNF production from target cells, including cardiomyocytes (116). The most important role of TNF- $\alpha$  in chronic heart failure is the induction of myocardial cell apoptosis (125–127). The pathway leading to TNF- $\alpha$ -induced myocardial apoptosis is mediated by TNF receptors, TNFR1 and TNFR2 (128). Activation of these receptors leads to activation of caspase-8 (129). A BH3 domain-containing proapoptotic Bcl2 family member, BID, is then cleaved by caspase-8 (130). The truncated BID is translocated from cytosol to mitochondria, inducing first the clustering of mitochondria around the nuclei and release of cytochrome *c*, and then the loss of mitochondrial membrane potential, cell shrinkage, and nuclear condensation, i.e., apoptosis (130). Caspase-8 also directly activates caspase-3, leading to apoptosis (131).



**Figure 2.** Mitochondrial factor-mediated apoptosis and necrosis. Note that mitochondrial cytochrome *c* release can lead to activation of caspase-3 as well as decreased production of ATP. Inhibition of caspase-3 by Ac-DEVD-cmk would thus inhibit the apoptotic pathway but enhance the ATP-depletion-induced necrosis if the stimulus for cytochrome *c* release persists. Cyclosporin A can inhibit MPT formation and thereby suppress cytochrome *c* release.

Interleukin-6 is a multifunctional pro-inflammatory cytokine that mediates both immune and inflammatory responses. Elevated serum concentrations of interleukin-6 have been observed in patients with heart failure (126,132). A new member of the interleukin-6 receptor family of cytokines, cardiotrophin-1, has been cloned recently (133). This is added to the list of other members of this family, including interleukin-6, interleukin-11, leukemia inhibitory factor, oncostatin, and ciliary neurotrophic factor. Cardiotrophin-1 acts as a nerve growth factor and also induces hypertrophy of cardiac myocytes (134,135). Interestingly, cardiotrophin-1 has been shown to inhibit cytokine-induced apoptosis *in vitro* (136). Further studies are needed to investigate its role in myocardial disease conditions.

Endothelin-1 is importantly involved in cardiomyopathy. It is produced within both failing and nonfailing hearts from several types of cells, including endothelial cells (137), endocardium, and myocytes (138). It has been considered that limited expression of endothelin-1 within the myocardium is an adaptive response to stress, providing increased inotropic support for the cardiac myocyte (139,140) as well as increasing the rate of myocyte protein synthesis (141). Overexpression of endothelin-1, however, eventually becomes maladaptive by producing focal vasospasm, myocytosis, and increased myocardial fibrosis (142). In a rat model, it has been shown that myocardial infarction is accompanied with overexpression of endothelin-1 and that selective inhibition of the endothelin-A receptor with a specific antagonist (BQ-123) improved long-term survival of these animals (143).

### Sarcoplasmic Reticulum Pathway Leading to Apoptosis

This pathway was identified recently (144). It was shown that caspase-12 is localized in the endoplasmic reticulum (ER) and activated by ER stress. This stress includes disruption of ER calcium homeostasis and accumulation of excessive proteins in the ER. Mice deficient in caspase-12 are resistant to ER stress-induced apoptosis. Therefore, caspase-12 mediates an ER-specific apoptotic pathway. This pathway would have a large impact on myocardial cells and should be investigated in the future.

### Role of Calcium in Cardiotoxicity

This topic has been the most discussed and investigated in cardiotoxicologic research. However, our understanding of the role of calcium in cardiotoxicity is rather superficial. When carefully examining the current literature, one finds that there are few mechanistic studies that specifically probe the role of

calcium in cardiotoxicity. Yet, numerous studies have implicated intracellular  $Ca^{2+}$  as a signal for cardiac responses to environmental toxic insults (145–147). In response to myocardial stress by environmental stimuli, calcium concentrations are increased in the myocardial cells (148). This is consistent with the speculation that  $Ca^{2+}$  coordinates physiologic responses to stresses. Many other speculations are derived from the studies examining the role of calcium in toxicologic responses in other systems. The unique action of calcium in cardiotoxicity, however, has to be studied specifically.

Recently there has been a breakthrough in understanding the role of calcium in mediating myocardial hypertrophic signals (90). A sustained increase in intracellular  $Ca^{2+}$  concentrations activates calcineurin. Calcineurin is a ubiquitously expressed serine/threonine phosphatase that exists as a heterodimer, comprised of a 59-kDa calcium-binding catalytic A subunit and a 19-kDa  $Ca^{2+}$ -binding regulatory B subunit (149). Activation of calcineurin is mediated by binding of  $Ca^{2+}$  and calmodulin to the regulatory and catalytic subunits, respectively. A toxicologic significance of calcineurin is that it is activated by a sustained  $Ca^{2+}$  elevation and is insensitive to transient  $Ca^{2+}$  fluxes such as those that occur in response to cardiomyocyte contraction (90).

Numerous studies have demonstrated important roles for Ras, mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) signaling pathways in myocardial responses to hypertrophic stimuli (150,151). All these signal transduction pathways are associated with an inotropic increase in intracellular  $Ca^{2+}$  concentrations (152). The coordinating role of calcium in cardiac hypertrophic response was speculated in a recent study (90) as follows. Hypertrophic stimuli such as angiotensin II and phenylephrine cause an elevation of intracellular  $Ca^{2+}$  that results in activation of calcineurin. A series of reactions occurs through the activated calcineurin, including dephosphorylation of NFAT3 and its translocation to nucleus where it can interact with GATA4 (90). Calcineurin could also act through an NFAT-independent mechanism to regulate myocardial hypertrophy.

### Protein Kinase C and the Myocardial Signaling Pathway

PKC and the myocardial signaling pathway are among the most extensively studied topics in cardiac research. Most cardiotoxicologic studies have adapted the concept of cardiac physiologic studies in examining the role of PKC in mediating toxic signals. Several excellent reviews are available on PKC in myocardial signaling pathways leading to cardiac hypertrophy and heart failure (153–157).

Therefore, this topic will not be included in this review.

### Oxidative Stress and Mitogen-Activated Protein Kinases

Oxidative stress and MAPKs are involved in myocardial remodeling and play a major role in the development of cardiotoxicity. Among the MAPKs, p38 MAPK has been extensively studied in myocardial apoptosis. The p38 MAPK is a subfamily of the MAPK superfamily and is stress responsive. This subfamily consists of p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$ , and p38 $\delta$  (158,159). Recent studies have identified p38 MAPK as an important group of signaling molecules that mediate environmental stress responses in various cell types (160,161). In noncardiac cells, p38 MAPK has been implicated in gene expression, morphologic changes, and cell death in response to endotoxin, cytokines, physical stress, and chemical insults (162–164). In cardiac cells, it has been reported that p38 MAPK is associated with the onset of apoptosis in ischemia-reperfusion-treated hearts (165,166). In particular, transfection experiments using primary cultures of neonatal rat cardiomyocytes have shown that p38 $\alpha$  is critically involved in myocyte apoptosis (167). In any event, the common observation is that p38 MAPK activation is associated with accumulation of reactive oxygen species generated under stress conditions. In this context, we have examined the possible role of p38 MAPK in mediating adriamycin-induced myocardial apoptosis.

Treatment with adriamycin significantly induced apoptosis in neonatal cardiomyocyte cultures and activated p38 MAPK (61). That p38 MAPK was involved at least in part in the adriamycin-induced myocyte apoptosis was demonstrated by two important observations (61). First, a time-course analysis revealed that p38 MAPK activation preceded the onset of apoptosis. A sensitive and early apoptosis detection method of Annexin V-FITC has been used to detect the onset of myocyte apoptosis. It was demonstrated that as early as 30 min after adriamycin treatment, myocyte apoptosis occurred. The early detection of p38 MAPK activation by a sensitive FITC-conjugated anti-phospho-p38 antibody and confocal microscopy was observed 20 min after adriamycin treatment. Second, application of SB203580, a specific inhibitor of p38 MAPK, significantly inhibited adriamycin-induced myocyte apoptosis. Because SB203580 acts as a specific inhibitor of p38 $\alpha$  and p38 $\beta$  but not p38 $\gamma$  and p38 $\delta$ , the involvement of the former specific isoforms of p38 MAPK in the adriamycin-induced myocyte apoptosis are implicated. Recent studies have identified that p38 $\alpha$  is specifically involved in apoptosis of neonatal rat cardiomyocytes in primary cultures and

p38 $\beta$  mediates hypertrophy of these cells (167). Further studies are required to determine which specific isoform(s) of p38 MAPK are essential in the signaling pathway of adriamycin-induced apoptosis.

An important novel observation in our recent studies is that metallothionein (MT), an important antioxidant, inhibited both apoptosis and p38 MAPK activation by adriamycin in cardiomyocytes (61). Although adriamycin-induced apoptosis was partially inhibited (by 50%), the activation of p38 MAPK was almost completely blocked by MT. Taken together, these observations suggest that oxidative stress-activated p38 MAPK plays a crucial role in myocardial apoptosis.

## Conclusion

Myocardial responses to detrimental environmental insults lead to myocardial apoptosis or necrosis, remodeling, and heart failure. The current understanding of the cardiac toxic effects is, however, superficial. Most of the cardiotoxicologic studies have been descriptive. Any agent, either physical or chemical, can trigger myocardial responses. However, these responses may not necessarily be toxic. On the other hand, there is no single agent that only triggers toxic responses. Of note, cellular responses to toxic insults always start with initiation of protective mechanisms. The toxic end points are the ultimate outcome of the balance between cytoprotection and the insult. Therefore, it is critical to identify the true toxic end point of cardiotoxicity. Many intermediate responses, however, can lead to either protective or injurious responses. For example, p38 MAPK activation can lead to cytoprotection against oxidative stress by activating transcription factors that upregulate protective mechanisms or cause cell death by activating apoptotic programs. It is well known that multiple signaling pathways exist and are activated in response to stresses. One cannot define which pathway mediates the toxic response or the protective action. In this context, inhibition of one pathway may activate the other. For instance, inhibition of caspase-3 can abort apoptosis if caspase-3 is a key element in a particular apoptotic signaling pathway. This inhibition may shift the apoptotic program to necrotic if mitochondrial cytochrome c release is a critical upstream event that leads to caspase-3 activation. Thus, an important consideration in preventing myocardial cell death induced by environmental toxicants and pollutants is not an inhibition of a particular cell death program but rather the ultimate survival of a cell. Further extensive studies of the molecular mechanisms and signaling transduction pathways of cardiotoxicity are warranted. Advances in molecular biology and the available cutting-edge experimental approaches

will provide powerful tools for such studies. It is probable that significant insights into cardiotoxicity will be obtained in the near future. A comprehensive understanding of cardiotoxicity will greatly contribute to the advance of cardiac medicine.

## REFERENCES AND NOTES

- Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer* 32:302–314 (1973).
- Hieble JP. Adrenoceptor subclassification: an approach to improved cardiovascular therapeutics. *Pharm Acta Helv* 74:163–171 (2000).
- Robbins J. Remodeling the cardiac sarcomere using transgenesis. *Annu Rev Physiol* 62:261–287 (2000).
- James J, Robbins J. Molecular remodeling of cardiac contractile function. *Am J Physiol* 273:H2105–H2118 (1997).
- Schlant RC, Alexander RW. *The Heart: Arteries and Veins*. New York:McGraw-Hill, 1994.
- Navaratnam V. *Heart Muscle: Ultrastructural Studies*. Cambridge, UK:Cambridge University Press, 1987.
- Challice CE, Viragh S. *Ultrastructure of the Mammalian Heart*. New York:Academic Press, 1973.
- Mergner WJ, Jones RT. Ultrastructure and histology of the heart and cardiovascular system in toxic injury. In: *Principles of Cardiac Toxicology* (Baskin SI, ed). Boca Raton, FL:CRC Press, 1991:167–207.
- Herman EH, Ferrans VJ. Overview of morphological changes induced by the toxic effects of drugs on the cardiovascular system. In: *Principles of Cardiac Toxicology* (Baskin SI, ed). Boca Raton, FL:CRC Press, 1991:607–681.
- Ferrans VJ, Sanchez JA, Herman EH. Pathologic anatomy of animal models of the cardiotoxicity of anthracycline. In: *Cancer Treatment and the Heart* (Muggia FM, Greer JL, eds). Baltimore: The Johns Hopkins University Press, 1992: 89–113.
- Symanski JD, Gettes LS. Drug effects on the electrocardiogram. A review of their clinical importance. *Drugs* 46:219–248 (1993).
- Earm YE, Ho WK, So I. Effects of adriamycin on ionic currents in single cardiac myocytes of the rabbit. *J Mol Cell Cardiol* 26: 163–172 (1994).
- Lemasters JJ, Nieminen AL, Qian T, Trost LC, Herman B. The mitochondrial permeability transition in toxic, hypoxic and reperfusion injury. *Mol Cell Biochem* 174:159–165 (1997).
- Abas L, Bogoyevitch MA, Guppy M. Mitochondrial ATP production is necessary for activation of the extracellular-signal-regulated kinases during ischaemia/reperfusion in rat myocyte-derived H9c2 cells. *Biochem J* 349:119–126 (2000).
- Depre C, Taegtmeyer H. Metabolic aspects of programmed cell survival and cell death in the heart. *Cardiovasc Res* 45: 538–548 (2000).
- Sadoshima J, Qiu Z, Morgan JP, Izumo S. Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and c-fos gene expression in cardiac myocytes. *EMBO J* 15:5535–5546 (1996).
- Piano MR. Cellular and signaling mechanisms of cardiac hypertrophy. *J Cardiovasc Nurs* 8:1–26 (1994).
- Cheng TH, Shih NL, Chen SY, Wang DL, Chen JJ. Reactive oxygen species modulate endothelin-1-induced c-fos gene expression in cardiomyocytes. *Cardiovasc Res* 41:654–662 (1999).
- Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, et al. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11:11–17 (2000).
- Rosen MR. Cardiac arrhythmias and antiarrhythmic drugs: recent advances in our understanding of mechanism. *J Cardiovasc Electrophysiol* 6:868–879 (1995).
- He SY, Matoba R, Sodesaki K, Fujitani N, Ito Y. Morphological and morphometric investigation of cardiac lesions after chronic administration of methamphetamine in rats. *Nippon Hoigaku Zasshi* 50:63–71 (1996).
- Ho AK, Duffield R. 6-Hydroxydopamine-induced developmental cardiac alterations in morphology, calmodulin content, and K(2+)-mediated [Ca(2+)](i)Transient of chicken cardiomyocytes. *J Mol Cell Cardiol* 32:1315–1326 (2000).
- de Oliveira CF, Cintra KA, Teixeira SA, De Luca IM, Antunes E, De Nucci G. Development of cardiomyocyte hypertrophy in rats under prolonged treatment with a low dose of a nitric oxide synthesis inhibitor. *Eur J Pharmacol* 391:121–126 (2000).
- Maass A, Leinwand LA. Animal models of hypertrophic cardiomyopathy. *Curr Opin Cardiol* 15:189–196 (2000).
- Kohno I, Komori S, Yamamoto K, Sano S, Ishihara T, Umetani K, Sawanobori T, Ijiri H, Tamura K. Hypertrophic cardiomyopathy complicated with cardiac amyloidosis. *Intern Med* 39:637–640 (2000).
- Perennec J, Willemain M, Pocholle P, Hatt PY, Crozatier B. Cardiac ultrastructural abnormalities in Syrian hamsters with spontaneous cardiomyopathy or subjected to cardiac overloads. *Basic Res Cardiol* 87:54–64 (1992).
- Piano MR, Bondmass M, Schwertz DW. The molecular and cellular pathophysiology of heart failure. *Heart Lung* 27:3–9 (1998).
- Holtz J. Pathophysiology of heart failure and the renin-angiotensin system. *Basic Res Cardiol* 88(suppl 1):183–201 (1993).
- Francis GS, Chu C. Compensatory and maladaptive responses to cardiac dysfunction. *Curr Opin Cardiol* 10:260–267 (1995).
- Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *New Engl J Med* 339:321–328 (1998).
- Swynghedauw B. Molecular mechanisms of myocardial remodeling. *Physiol Rev* 79:215–262 (1999).
- Sonnenblick EH, Anversa P. Models and remodeling: mechanisms and clinical implications. *Cardiologia* 44:609–619 (1999).
- St John Sutton M, Plappert T. Myocardial texture in hypertrophic remodeling: new insight into ventricular load and function? *J Hum Hypertens* 14:7–8 (2000).
- Gottlieb RA, Bureson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 94:1621–1628 (1994).
- Lorell BH. Transition from hypertrophy to failure. *Circulation* 96: 3824–3827 (1997).
- Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: experimental observation and clinical implications. *Circulation* 81:1161–1172 (1990).
- Francis GS, Carlyle WC. Hypothetical pathways of cardiac myocyte hypertrophy: response to myocardial injury. *Eur Heart J* 14:49–56 (1993).
- Sabbah HN, Sharov VG. Apoptosis in heart failure. *Prog Cardiovasc Dis* 40:549–562 (1998).
- Sharov VG, Sabbah HN, Shimoyama H, Goussev AV, Lesch M, Goldstein S. Evidence of cardiocyte apoptosis in myocardium of dogs with chronic heart failure. *Am J Pathol* 148:141–149 (1995).
- Li Z, Bing OHL, Long X, Robinson KG, Lakatta EG. Increased cardiomyocyte apoptosis during the transition to heart failure in the spontaneously hypertensive rat. *Am J Physiol* 272: H2313–H2319 (1997).
- James TN. Normal and abnormal consequences of apoptosis in the human heart. *Circulation* 90:556–573 (1994).
- Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Loreto CD, Beltrami CA, Krajewski S, et al. Apoptosis in the failing human heart. *N Engl J Med* 336:1131–1141 (1997).
- Yin X, Saari JT, Kang YJ. Copper deficiency induces apoptosis in the heart of rats. *Proc N D Acad Sci* 92:23 (1995).
- Kang YJ. Suppression of doxorubicin-induced apoptosis in the metallothionein overexpressing heart of transgenic mice. *Proc Am Assoc Cancer Res* 38:192 (1997).
- Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol Ther* 47:219–231 (1990).
- El-Sherif L, Wang G-W, Kang YJ. Suppression of cadmium-induced apoptosis in metallothionein-overexpressing transgenic mouse cardiac myocytes. *FASEB J* 14:A1520 (2000).
- Gorza L, Menabo R, Di Lisa F, Vitadello M. Troponin T cross-linking in human apoptotic cardiomyocytes. *Am J Pathol* 150: 2087–2097 (1997).
- Schaper J, Lorenz-Meyer S, Suzuki K. The role of apoptosis in dilated cardiomyopathy. *Herz* 24:219–224 (1999).
- Kang YJ, Zhou Z-X, Wu H, Wang G-W, Saari JT, Klein JB. Metallothionein inhibits myocardial apoptosis in copper-deficient mice: role of atrial natriuretic peptide. *Lab Invest* 80: 745–757 (2000).
- Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest* 74:86–107 (1996).
- Eliot RS, Clayton FC, Pieper GM, Todd GL. Influence of environmental stress on pathogenesis of sudden cardiac death. *Fed Proc* 36:1719–1724 (1977).
- Yaoita H, Ogawa K, Maehara K, Maruyama Y. Apoptosis in relevant clinical situations: contribution of apoptosis in myocardial infarction. *Cardiovasc Res* 45:630–641 (2000).
- Wyllie AH. Death from inside out: an overview. *Philos Trans R Soc Lond B Biol Sci* 345:237–241 (1994).
- Kajstura J, Liu Y, Baldini A, Li B, Olivetti G, Leri A, Anversa P.



- Coronary artery constriction in rats: necrotic and apoptotic myocyte death. *Am J Cardiol* 82:30K–41K (1998).
55. Leist M, Nicotera P. The shape of cell death. *Biochem Biophys Res Commun* 236:1–9 (1997).
  56. Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 185:1481–1486 (1997).
  57. Eguchi Y, Shimizu S, Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* 57:1835–1840 (1997).
  58. Leist M, Single B, Naumann H, Fava E, Simon B, Kuhnle S, Nicotera P. Inhibition of mitochondrial ATP generation by nitric oxide switches apoptosis to necrosis. *Exp Cell Res* 249:396–403 (1999).
  59. McCarthy NJ, Evan GI. Methods for detecting and quantifying apoptosis. *Curr Top Dev Biol* 36:259–278 (1998).
  60. Sgong R, Gruber J. Apoptosis detection: an overview. *Exp Gerontol* 33:525–533 (1998).
  61. Kang YJ, Zhou Z-X, Wang G-W, Burid A, Klein JB. Suppression by metallothionein of doxorubicin-induced cardiomyocyte apoptosis through inhibition of p38 mitogen-activated protein kinases. *J Biol Chem* 275:13690–13698 (2000).
  62. Anversa P, Ricci R, Olivetti G. Myocyte cell loss and myocyte hypertrophy in the aging rat heart. *J Am Coll Cardiol* 7:1140–1149 (1986).
  63. Pentel PR, Jentzen J, Sievert J. Myocardial necrosis due to intraperitoneal administration of phenylpropanolamine in rats. *Fundam Appl Toxicol* 9:167–172 (1987).
  64. Limaye DA, Shaikh ZA. Cytotoxicity of cadmium and characteristics of its transport in cardiomyocytes. *Toxicol Appl Pharmacol* 154:59–66 (1999).
  65. Katz A. The cardiomyopathy of overload: an unnatural growth response in the hypertrophied heart. *Ann Intern Med* 121:363–371 (1994).
  66. Anversa P, Capasso JM. Cardiac hypertrophy and ventricular remodeling. *Lab Invest* 64:441–445 (1991).
  67. Mann DL, Kent RL, Cooper GT. Load regulation of the properties of adult feline cardiocytes: growth induction by cellular deformation. *Circ Res* 64:1079–1090 (1989).
  68. Morgan HE, Baker KM. Cardiac hypertrophy. *Circulation* 83:13–25 (1991).
  69. Schwartz K, Boheler KR, De la Bastie D, Lompre AM, Mercadier J-J. Switches in cardiac muscle gene expression as a result of pressure and volume overload. *Am J Physiol* 262:R364–R369 (1992).
  70. Rozych JD, Barnes MA, Schmid PG, Zile MR, McDermott PJ, Cooper G. Load effects on gene expression during cardiac hypertrophy. *J Mol Cell Cardiol* 27:485–499 (1995).
  71. Chien KR, Knowlton KU, Zhu H, Chien S. Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. *FASEB J* 5:3037–3046 (1991).
  72. Kolbeck-Ruhmkorff C, Zimmer H-G. Proto-oncogene expression in the isolated working rat heart: combination of pressure and volume overload with norepinephrine. *J Mol Cell Cardiol* 27:501–511 (1995).
  73. McMahon SB, Monroe JG. Role of primary response genes in generating cellular responses to growth factors. *FASEB J* 6:2707–2715 (1992).
  74. Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* 249:1266–1272 (1990).
  75. Brand T, Sharma HS, Fleishmann KE, Duncker DJ, McFalls EO, Verdouw PD, Schaper W. Proto-oncogene expression in porcine myocardium subjected to ischemia and reperfusion. *Circ Res* 71:1351–1360 (1992).
  76. Wollert KC, Heineke J, Westernmann J, Ludde M, Fiedler B, Zierhut W, Laurent D, Bauer MKA, Schulze-Osthoff K, Drexler H. The cardiac Fas (APO-1/CD95) receptor/Fas ligand system: relation to diastolic wall stress in volume-overload hypertrophy in vivo and activation of the transcription factor AP-1 in cardiac myocytes. *Circulation* 101:1172–1178 (2000).
  77. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed cell death. *J Clin Invest* 96:2247–2259 (1995).
  78. Tanaka M, Ito H, Adachi S, Akimoto H, Hishikawa T, Kasajima T, Marumo F, Hiroe M. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. *Circ Res* 75:426–433 (1994).
  79. Maulik N, Goswami S, Galang N, Das DK. Differential regulation of Bcl-2, AP-1 and NF- $\kappa$ B on cardiomyocyte apoptosis during myocardial ischemic stress adaptation. *FEBS Lett* 443:331–336 (1999).
  80. Lenczowski JM, Dominguez L, Eder AM, King LB, Zacharchuk CM, Ashwell JD. Lack of a role for jun kinase and AP-1 in Fas-induced apoptosis. *Mol Cell Biol* 17:170–181 (1997).
  81. Paradis P, MacLellan WR, Belaguli NS, Schwartz RJ, Schneider MD. Serum response factor mediates AP-1-dependent induction of the skeletal  $\alpha$ -actin promoter in ventricular myocytes. *J Biol Chem* 271:10827–10833 (1996).
  82. Kovacic-Milivojevic B, Wong VSH, Gardner DG. Selective regulation of the atrial natriuretic peptide gene by individual components of the activator protein-1 complex. *Endocrinology* 137:1108–1117 (1996).
  83. Kariya K, Karns LR, Simpson PC. An enhancer core element mediates stimulation of the rat beta-myosin heavy chain promoter by an alpha 1-adrenergic agonist and activated beta-protein kinase C in hypertrophy of cardiac myocytes. *J Biol Chem* 269:3775–3782 (1994).
  84. Karns LR, Kariya K, Simpson PC. M-CAT, CarG, and Sp1 elements are required for alpha 1-adrenergic induction of the skeletal alpha-actin promoter during cardiac myocyte hypertrophy. Transcriptional enhancer factor-1 and protein kinase C as conserved transducers of the fetal protein in cardiac growth. *J Biol Chem* 270:410–417 (1995).
  85. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 75:977–984 (1993).
  86. Sadoshima J, Izumo S. Signal transduction pathways of angiotensin II-induced c-fos gene expression in cardiac myocytes in vitro. *Circ Res* 73:424–438 (1993).
  87. Rao A, Luo C, Hogan PG. Transcription factors of the NF-AT family: regulation and function. *Annu Rev Immunol* 15:707–747 (1997).
  88. Rooney JW, Hodge MR, McCaffrey PG, Rao A, Glimcher LG. A common factor regulates both Th1- and Th2-specific cytokine gene expression. *EMBO J* 13:625–633 (1994).
  89. Hoey T, Sun YL, Williamson K, Xu X. Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins. *Immunity* 2:461–472 (1995).
  90. Molkenin JD, Lu J-R, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93:215–228 (1998).
  91. Yamamoto M, Ko LJ, Leonard MW, Beug H, Orkin SH, Engel JD. Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. *Genes Dev* 4:1650–1662 (1990).
  92. Evens T, Reitman M, Felsenfeld G. An erythrocyte-specific DNA-binding factor recognizes a regulatory sequence common to all chicken globin genes. *Proc Natl Acad Sci U S A* 85:5976–5980 (1988).
  93. Orkin SH. GATA-binding transcription factors in hematopoietic cells. *Blood* 80:575–581 (1992).
  94. Evens T. Regulation of cardiac gene expression by GATA-4/5/6. *Trends Cardiovasc Med* 7:75–83 (1997).
  95. Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci U S A* 85:339–343 (1988).
  96. Mercadier JJ, Bouveret P, Gorza L, Schiaffina S, Clark WA, Zak R, Swynghedauw B, Schwartz K. Myosin isoenzymes in normal and hypertrophied human ventricular myocardium. *Circ Res* 53:52–62 (1983).
  97. Ferrans VJ. Cardiac hypertrophy: morphological aspects. In: *Growth of the Heart in Health and Disease* (Zak R, ed). New York:Raven Press, 1984:187–239.
  98. Komuro I, Yazaki Y. Control of cardiac gene expression by mechanical stress. *Annu Rev Physiol* 55:55–75 (1993).
  99. Ferrari R, Ceconi C, Curello S, Ferrari F, Confortini R, Pepi P, Visioli O. Activation of the neuroendocrine response in heart failure: adaptive or maladaptive process? *Cardiovasc Drugs Ther* 10:623–629 (1996).
  100. McKenzie JC, Kelley KB, Merisko-Liversidge EM, Kennedy J, Klein RM. Developmental pattern of ventricular atrial natriuretic peptide (ANP) expression in chronically hypoxic rats as an indicator of the hypertrophic process. *J Mol Cell Cardiol* 26:753–767 (1994).
  101. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 90:195–203 (1994).
  102. Wu C-F, Bishopric NH, Pratt RE. Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. *J Biol Chem* 272:14860–14866 (1997).
  103. Kang PM, Izumo S. Apoptosis and heart failure: a critical review of the literature. *Circ Res* 86:1107–1113 (2000).
  104. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw B-A. Apoptosis in myocytes in end-stage heart failure. *New Engl J Med* 335:1182–1189 (1996).
  105. Yoshibayashi M, Saito Y, Nakao K. Brain natriuretic peptide versus atrial natriuretic peptide—physiological and pathophysiological significance in children and adults: a review. *Eur J Endocrinol* 135:265–268 (1996).
  106. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 281:1309–1312 (1998).
  107. Orrenius S, Burgess, DH, Hampton, MB, Zhivotovskiy B. Mitochondria as the focus of apoptosis research. *Cell Death Differ* 4:427–428 (1997).
  108. Kroemer G, Zamzami N, Susin SA. Mitochondrial control of apoptosis. *Immunol Today* 18:44–51 (1997).
  109. Reed JC, Jurgensmeier JM, Matsuyama S. Bcl-2 family proteins and mitochondria. *Biochim Biophys Acta* 1366:127–137 (1998).
  110. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 281:1322–1324 (1998).
  111. Cook SA, Sugden PH, Clerk A. Regulation of bcl-2 family proteins during development and in response to oxidative stress in cardiac myocytes: association with changes in mitochondrial membrane potential. *Circ Res* 85:940–949 (1999).
  112. Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H. Expression of bcl-2 protein, an inhibitor of apoptosis, and bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. *Circulation* 94:1506–1512 (1996).
  113. Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci U S A* 95:4997–5002 (1998).
  114. Saikumar P, Dong Z, Patel Y, Hall K, Hopfer U, Weinberg JM, Venkatachalam MA. Role of hypoxia-induced Bax translocation and cytochrome c release in reoxygenation injury. *Oncogene* 17:3401–3415 (1998).
  115. Wang GW, Zhou ZX, Kang YJ. Effect of metallothionein on doxorubicin-induced apoptosis in cardiomyocytes: role of cytochrome C-activated caspase-3. *Toxicol Sci* 54(Abstract Issue):114 (2000).
  116. Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N, Prevost M-C, Leber B, Andrews D, Penninger J, et al. Mitochondrial-nuclear translocation of AIF in apoptosis and necrosis. *FASEB J* 14:729–739 (2000).
  117. Pulkki KJ. Cytokines and cardiomyocyte death. *Ann Med* 29:339–343 (1997).
  118. Shan K, Kurrelmeier K, Seta Y, Wang F, Dibbs Z, Deswal A, Lee-Jackson D, Mann DL. The role of cytokines in disease progression in heart failure. *Curr Opin Cardiol* 12:218–223 (1997).
  119. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, Mann DL. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation* 93:704–711 (1996).
  120. Nishigaki K, Minatoguchi S, Seishima M, Asano K, Noda T, Yasuda N, Sana H, Kumada H, Takemura M, Noma A, et al. Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure. *J Am Coll Cardiol* 29:1214–1220 (1997).
  121. Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Voipio-Pulkki LM. Apoptosis in human acute myocardial infarction. *Circulation* 95:320–323 (1997).
  122. Lange GL, Schreiner GF. Immune mechanisms of cardiac disease. *N Engl J Med* 330:1129–1135 (1994).
  123. Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: the cytokine hypothesis. *J Card Fail* 2:243–249 (1996).
  124. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the studies of left ventricular dysfunction (SOLVD). *J Am Coll Cardiol* 27:1201–1206 (1996).
  125. Meldrum DR. Tumor necrosis factor in the heart. *Am J Physiol* 274:R577–R595 (1998).
  126. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, Gielesboski CC, Quintana PJE, Sabbadini RA. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes: involvement of the sphingolipid signaling cascade in cardiac cell death. *J Clin Invest* 98:2854–2865 (1996).
  127. Ferrari R. The role of TNF in cardiovascular disease. *Pharmacol Res* 40:97–105 (1999).
  128. Vanderabeele P, Deleereq W, Beyaert R, Fiers W. Two tumor necrosis factor receptors: structure and function. *Trends Cell Biol* 5:392–399 (1995).

129. Schneider P, Tschopp J. Apoptosis induced by death receptors. *Pharm Acta Helv* 74:281–286 (2000).
130. Li H, Zhu H, Xu C-J, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94:491–501 (1998).
131. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Kramer PH, Peter ME. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J* 17:1675–1687 (1998).
132. Tsutamoto T, Hisanaga T, Wada A, Maeda Y, Fukui D. Plasmin concentration of interleukin-6 as a marker of prognosis in patients with chronic congestive heart failure. *Circulation* 90:381 (1994).
133. Pennica D, King KL, Shaw KJ, Luis E, Rullamas J, Luoh S, Darbonne WC, Knutzon DS, Yen R, Chien KR, et al. Expression cloning of cardiotrophin-1, a cytokine that induces cardiac myocyte hypertrophy. *Proc Natl Acad Sci U S A* 92:1142–1146 (1995).
134. Cheng Z, Pennica D, Wood WI, Chien KR. Cardiotrophin-1 displays early expression in the murine heart tube and promotes cardiac myocyte survival. *Development* 122:419–428 (1996).
135. Wollert KC, Taga T, Saito M, Narazaki M, Kishimoto T, Glembocki CC, Vernalis AB, Heath JK, Pennica D, Wood WI, et al. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series via gp130/leukemia inhibitory factor receptor-dependent pathways. *J Biol Chem* 271:9535–9545 (1996).
136. Cheng Z, Knowlton K, Chen J, Hoshijima M, Brown JH, Chien KR. Cardiotrophin-1 (CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for myocardial cell hypertrophy. *J Biol Chem* 272:5783–5791 (1997).
137. Nishida M, Springhorn JP, Kelly RA, Smith TW. Cell-cell signaling between adult rat ventricular myocytes and cardiac microvascular endothelial cells in heterotypic primary culture. *J Clin Invest* 91:1934–1941 (1993).
138. Ito H, Hirata Y, Adachi S, Tanaka M, Tsujino M, Koike A, Nogami A, Marumo F, Hiroe M. Endothelin-1 is an autocrine/paracrine factor in the mechanism of angiotensin-II-induced hypertrophy in cultured rat cardiomyocytes. *J Clin Invest* 92:398–403 (1993).
139. Kelly RA, Eid H, Kramer BK, O'Neill M, Liang BT, Reers M, Smith TW. Endothelin enhances the contractile responsiveness of adult rat ventricular myocytes to calcium by a pertussis toxin-sensitive pathway. *J Clin Invest* 86:1164–1171 (1990).
140. Sakai S, Miyauchi T, Sakurai T, Kasuya Y, Ihara M, Yamaguchi I, Goto K, Sugishita Y. Endogenous endothelin-1 participate in the maintenance of cardiac function in rats with congestive heart failure: marked increase in endothelin-1 production in the failing heart. *Circulation* 93:1214–1222 (1996).
141. Jones LG, Rozich JD, Tsutsui H, Cooper G. Endothelin stimulates multiple responses in isolated adult ventricular cardiac myocytes. *Am J Physiol* 263:H1447–H1454 (1992).
142. Weber KT, Sun Y, Guarda E. Structural remodeling in hypertensive heart disease and the role of hormones. *Hypertension* 23:869–877 (1994).
143. Sakai S, Miyauchi T, Kobayashi M, Yamaguchi I, Goto K, Sugishita Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. *Nature* 384:353–355 (1996).
144. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yanker BA, Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid- $\beta$ . *Nature* 403:98–103 (2000).
145. Shier WT, DuBourdieu DJ. Sodium- and calcium-dependent steps in the mechanism of neonatal rat cardiac myocyte killing by ionophores. I: The sodium-carrying ionophore, monensin. *Toxicol Appl Pharmacol* 116:38–46 (1992).
146. Torason M, Wey HE, Richards DE, Mathias PI, Krieg E. Altered  $Ca^{2+}$  mobilization during excitation-contraction in cultured cardiac myocytes exposed to antimony. *Toxicol Appl Pharmacol* 146:104–115 (1997).
147. Buck ED, Lachnit WG, Pessah IN. Mechanisms of delta-hexachlorocyclohexane toxicity. I: Relationship between altered ventricular myocyte contractility and ryanodine receptor function. *J Pharmacol Exp Ther* 289:477–485 (1999).
148. Sleight P. Calcium antagonists during and after myocardial infarction. *Drugs* 51:216–225 (1996).
149. Stemmer PM, Klee CB. Dual calcium ion regulation of calcineurin by calmodulin and calcineurin B. *Biochemistry* 33:6858–6866 (1994).
150. Jalili T, Takeishi Y, Walsh RA. Signal transduction during cardiac hypertrophy: the role of G  $\alpha_q$ , PLC  $\beta$  I, and PKC. *Cardiovasc Res* 44:5–9 (1999).
151. Sugden PH, Clark A. "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res* 83:345–352 (1998).
152. Ho PD, Zechner DK, He H, Dillmann WH, Glembocki CC, McDonough PM. The Raf-MEK-ERK cascade represents a common pathway for alteration of intracellular calcium by Ras and protein kinase C in cardiac myocytes. *J Biol Chem* 273:21730–21735 (1998).
153. Bogoyevitch MA, Sugden PH. The role of protein kinases in adaptational growth of the heart. *Int J Biochem Cell Biol* 28:1–12 (1996).
154. Puceat M, Vassort G. Signalling by protein kinase C isoforms in the heart. *Mol Cell Biochem* 157:65–72 (1996).
155. Dorn GW II, Brown JH. Gq signaling in cardiac adaptation and maladaptation. *Trends Cardiovasc Med* 9:26–34 (1999).
156. Wang J, Liu X, Arneja AS, Dhalla NS. Alterations in protein kinase A and protein kinase C levels in heart failure due to genetic cardiomyopathy. *Can J Cardiol* 15:683–690 (1999).
157. Balasubramanyam M, Mohan V. Signal transduction during cardiac hypertrophy: new insights. *Indian Heart J* 52:226–232 (2000).
158. Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J. Characterization of the structure and function of a new mitogen-activated protein kinase (p38 $\beta$ ). *J Biol Chem* 271:17920–17926 (1996).
159. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372:739–746 (1994).
160. Lechner C, Zahalka MA, Giot JF, Moller NP, Ullrich A. ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation. *Proc Natl Acad Sci U S A* 93:4355–4359 (1996).
161. Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, Young PR, Lee JC. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett* 364:229–233 (1995).
162. Wang XZ, Ron D. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* 272:1347–1349 (1996).
163. Tan Y, Rouse J, Zhang A, Cariati S, Cohen P, Comb MJ. FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. *EMBO J* 15:4629–4642 (1996).
164. Lin Q, Schwartz J, Bicana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science* 276:1404–1407 (1997).
165. Yin T, Sandhu G, Wolfgang CD, Burrier A, Webb RL, Rigel DF, Hai T, Whelan J. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. *J Biol Chem* 272:19943–19950 (1997).
166. Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, BenLevy R, Ashworth A, Marshall CJ, Sugden PH. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ Res* 79:162–173 (1996).
167. Wang Y, Huang S, Sah VP, Ross J, Brown JH, Han J, 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).