Apoptosis is a tightly regulated cell deletion process implicated in a wide variety of physiological and pathological processes! Since the identification of various critical regulators of apoptosis by Robert Horvitz and his colleagues in the roundworm, C. elegans, numerous papers have confirmed that apoptosis is an important aspect of normal organ development and plays a role in a wide variety of physiological and pathological conditions. We review what is currently known about the regulation and the role of apoptosis in various cardiovascular (CV) diseases, and examine some new issues that are emerging in the field of CV apoptosis.

Apoptosis in CV Diseases

Ischemic Heart Disease

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in the developed world. Because cardiac myocytes are terminally differentiated, with very little potential to regenerate, understanding the processes underlying myocyte death and survival is an important task for both cardiologists and cardiovascular researchers. Both animal and human studies have demonstrated that apoptosis is associated with IHD and myocyte apoptosis has been demonstrated in both the acute and chronic phases after myocardial infarction (MI).\(^1,2\) Experimental evidence gathered in vitro and in vivo also suggests a strong link between myocyte apoptosis and oxidative stress.\(^3,4\) Inhibition of apoptosis by a broad-spectrum caspase inhibitor, \(zVAD\).fmk, reportedly reduced the infarct size in the acute ischemic zone in a rat infarction model.\(^5\) Both in vitro and in vivo studies have shown that reoxygenation/reperfusion is a stronger stimulus for apoptosis than hypoxia/ischemia alone.\(^6,7\)

Heart Failure

The pathogenesis of heart failure involves multiple agents, conditions, and events. Among these, the progressive death and loss of cardiac myocytes is one of the most important components. It is natural to suppose that myocyte death is at least in part an apoptotic one. Indeed, numerous human and animal models suggest that apoptosis is a key contributor to myocyte loss in the setting of heart failure.\(^8\) Using transgenic technology, several animal models of heart failure have confirmed that myocyte apoptosis itself is sufficient to induce heart failure. One of the more dramatic and elegant examples involves the cardiac specific overexpression of ligand-activatable caspase-8, an artificial fusion protein consisting of the FK506 binding protein and the catalytic domain of caspase-8.\(^9\) Mice expressing this transgene appear normal at birth, but administration of the dimerization FK1012 results in overwhelming cardiac myocyte apoptosis and rapid death, presumably via caspase-8 and -3 activation. Even without the dimerizer, adult mice with a high expression of the protein develop spontaneous cardiac myocyte apoptosis, leading over time to a lethal dilated cardiomyopathy (DCM). Although the role of caspase-8 in the heart is unclear at this time, that study nevertheless demonstrates that induction of apoptosis can be achieved in the heart.

However, there still is much controversy surrounding the significance of apoptosis in this disease, stemming largely from the limitations of the techniques used to detect apoptosis and the very low levels of apoptosis associated with chronic heart failure (usually less than 1% by TUNEL staining).\(^10\) Consequently, there is some hesitancy in implicating cardiac myocyte apoptosis alone as the critical factor in the development of heart failure. We believe that currently the evidence is overwhelming for the presence of apoptosis in heart failure.\(^11\) Also, because heart failure is chronic disease occurring over years to decades, even a very low level of cell death is of potential significance in a heart composed of nondividing cardiac myocytes. It remains, however, to be convincingly proven that apoptosis contributes significantly to the progression and the development of clinical heart failure.

Cardiac Hypertrophy

In most cases, heart failure is preceded by cardiac hypertrophy. Multiple molecular, hormonal and biochemical investigations have characterized the response of hypertrophied cardiac myocytes to various physiologic and pathophysiologic stimuli. Though numerous mechanisms have been proposed, the signaling pathways that underlie the transition from compensated hypertrophy to decompensated heart failure are incompletely understood. One possible mechanism is that hypertrophy renders cardiac myocytes more sensitive to apoptosis. For example, transgenic mice with overexpression of Gsa signaling develop compensatory hypertrophy at baseline.\(^12\) However, when transgenic females become pregnant, they develop lethal DCM within 1 week after delivery, associated with a markedly increased rate of apoptosis (~26%). Also, Gsa overexpression results in increased sensitivity to apoptotic stimulation, and ultimately leads to cardiomyopathy.\(^13\) Additionally, other hypertrophic signaling molecules, such as angiotensin II, have been shown to promote apoptosis in vitro.\(^14\) Indeed, several

Peter M. Kang, MD; Patrick Yue, MD; Seigo Izumo, MD
studies show that angiotensin-converting enzyme (ACE) inhibitors block cardiac apoptosis in vivo.\textsuperscript{18,19}

However, some hypertrophic signaling factors such as cardiotoxin-1, calcitonin, and insulin-like growth factor-1 (IGF-1) seem to be protective against apoptosis.\textsuperscript{20-23} The notion that hypertrophy is a favorable adaptation to stress and that hypertrophied cells are more resistant to apoptotic stimuli is supported by several studies. For example, the cytokine receptor gp130 has been shown, in the setting of hypertrophy, to provide an important survival function in the heart via cardiotoxin-1.\textsuperscript{24} Transgenic mice encoding a hypertrophic phenotype. This notion is supported by a finding that hypertrophy results in a protective effect on cardiac myocytes upon exposure to apoptotic stimuli.\textsuperscript{25} Finally, overexpression of IGF-1 in animals seems to limit infarct size by an antiapoptotic effect.\textsuperscript{22,23}

We speculate that these contradictory findings may be related to the complicated nature of cardiac hypertrophic signaling, and that the apoptotic response (or lack thereof) to a hypertrophic stimulus is most likely dependent on the stimulus. For example, physiologic hypertrophy, such as exercise-induced hypertrophy (ie, the ‘athlete’s heart’), may activate a ‘favorable’ hypertrophic pathway that protects against apoptosis. Conversely, hypertrophy resulting from pathologic stimuli may activate an ‘unfavorable’ hypertrophic phenotype. This notion is supported by a finding that exercise-induced cardiac hypertrophy in rats activated calcitonin, whereas pathological hypertrophy induced by aortic constriction did not.\textsuperscript{25} Although these findings indicate possible strategies to modulate cardiac apoptosis, further investigation is required to clarify these issues. In particular, the transition from hypertrophy to heart failure and its relation to putative favorable and unfavorable hypertrophic signaling events need to be studied to understand the complex and intricate balance between cardiac hypertrophy, apoptosis and heart failure.

**Regulation of Apoptosis**

In general, the molecular mechanism of apoptosis involves the activation of caspases (cysteiny1 aspartate proteases), a family of cysteine proteases that cleave various intracellular target proteins at specific aspartate residues.\textsuperscript{26} The cascades of several specific caspases ultimately converge on a common pathway in which the final morphological and biochemical alterations characteristic of apoptosis take place.

At least 14 members of the caspase family have been described in mammals and are grouped into 2 categories by their structure and function.\textsuperscript{27} ‘Initiator caspases’, such as caspase-8 and -9, contain a long prodomain that is a functionally important interacting domain. They act upstream to initiate and regulate apoptosis, as well as to activate downstream ‘effector caspases’. In contrast, the ‘effector caspases’, such as caspase-3, -6 and -7, are characterized by a short prodomain and they act downstream in the common pathway to carry out the final biochemical changes of apoptosis. Effector caspases generally depend on the initiator caspases for activation, though additional regulatory molecules do exist.

The induction of apoptosis may be mediated either by mitochondria or by death receptors (Fig. 1). In the mitochondrial pathway, an apoptotic insult causes the mitochondria to release cytochrome c.\textsuperscript{28} Released cytosolic cytochrome c, in the presence of dATP, forms an activation complex with apoptotic protein activating factor-1 (apaf-1), and caspase-9.\textsuperscript{29,30} This complex facilitates the autoprocessing of caspase-9, as well as the activation of effector caspases, to execute downstream apoptotic changes.\textsuperscript{29,31} Within the common pathway, there is further downstream regulation by various caspase inhibitors.\textsuperscript{32}

The mitochondrial pathway is tightly regulated by the Bcl-2 superfamily of proteins, which modulate apoptotic responses in various tissues, including the heart. Each member of this family contains one or more B-cell homology (BH) domains; these proteins mediate their effects through these domains. At least 18 members of this family have been identified, and they are classified into antiapoptotic (eg, Bcl-2 and Bcl-xL) and proapoptotic (eg, Bad and Bax).\textsuperscript{33}

The death receptor-mediated pathway involves the binding of a death ligand (eg, FasL, tumor necrosis factor...
(TNF-α) to a membrane-bound death receptor (eg, Fas, TNF-β receptor)4 This binding recruits the death domain (eg, FADD), which in turn activates caspase-8 and subsequent downstream effector caspases. Because of their cellular compartmentalization, the mitochondrial and death receptor pathways have been recently categorized as 'intrinsic' and 'extrinsic', respectively (akin to the intrinsic and extrinsic pathways of the coagulation cascade).

More recently, the discovery of another proapoptotic member of the Bcl-2 family, termed Bcl-2 interacting protein (BID), has connected the 2 pathways.35,36 The full length BID protein is usually located in the cytosol, but activation of caspase-8 cleaves BID into truncated BID (tBID), which has been shown to translocate to the mitochondria and trigger cytochrome c release via interactions, most likely, with Bak and Bax.35

In addition, various caspase inhibitors have been identified in mammalian systems. One group of molecules are the inhibitors of apoptosis proteins (IAPs)32 and are characterized by an homologous domain called the baculoviral IAP repeat (BIR); each IAP contains 1–2 BIR domains. So far, at least 7 IAP-like molecules have been described in humans. Smac/DIABLO is an another recently identified novel mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition;38,39 Upon apoptotic stimulation, Smac/DIABLO escapes into the cytosol where it promotes caspase-9 activation by binding to IAP, thus removing its inhibition. Interestingly, Smac/DIABLO has been shown to have high levels of expression in the heart.38,39

Apoptotic Signaling in the Heart

Mitochondrial Pathway

Numerous reports suggest that in the setting of oxidative stress in the heart, the primary apoptotic pathway is mediated by the mitochondrial.12,40–43 In support of this, the level of Bcl-2 has been shown to be upregulated after acute ischemic insult44,45 and overexpression of Bcl-2 in cardiac myocytes, both in vitro and in vivo, has been shown to protect against apoptosis.12,42,43 The ratio between Bcl-2 and Bax may be an important contributor to the increased rate of apoptosis in cardiac myocytes,38,47 and this is supported by the reversal of Bcl-2/Bax ratio in heart after left ventricular (LV) assist device placement.48

Death Receptor Pathway

Components of the death receptor-mediated apoptotic pathway have also been shown to be upregulated in cardiac myocytes during myocardial ischemia and heart failure.49–51 In immune-mediated cardiomyopathy, cardiac apoptosis is associated with increased expression of the Fas/FasL system52 but the death receptor-mediated pathway in the heart may not be a dominant player in many settings of myocyte apoptosis. Indeed, cardiac specific overexpression of both TNF-β and FasL does not result in increased myocyte apoptosis.53,54 The importance of this pathway is further challenged by the fact that there are high levels of death receptor pathway inhibitors in the heart.55,56 FLICE-inhibitory protein (FLIP), which shows a high sequence homology to caspase-8, has been shown to inhibit FADD-mediated recruitment of caspase-8 for subsequent activation of the death receptor complex.55 Apoptosis repressor with caspase recruitment domain (ARC), an inhibitor that contains a caspase recruitment domain (CARD), interacts with upstream caspases, specifically to block caspase-2 and -8 (ie, components of the death receptor pathway), but does not block caspase-1, -3 or -9.56 Interestingly, ARC also has been shown to inhibit cytochrome c release by hypoxia-induced apoptosis in heart-derived H9c2 cells.57 These findings suggest that although the death receptor-mediated pathway may be important in certain situations (notably in autoimmune-mediated heart failure), it may typically be more difficult to activate in cardiac myocytes. We speculate that this may not be the predominant pathway in more common forms of heart failure, such as ischemic cardiomyopathy.

MAP Kinase/JNK

C-Jun N-terminal kinase (JNK) has been shown to be activated after apoptotic stimulation, and is a potential candidate for apoptotic signaling in the heart.58 Reoxygenation and reperfusion are known to activate JNK in the heart.59,60 and inhibition of the JNK pathway has been shown to abrogate apoptosis induced by these stressors.60,61 Several mechanisms have been proposed for JNK-mediated apoptosis, one of which is the activation of c-Jun transcriptional activity by phosphorylation. In that case, the Fas ligand is upregulated in a MEKK1- and c-Jun-dependent manner.62,63 Also, JNK phosphorylation of Bcl-2 and Bcl-xl has been implicated.64 However, this has been challenged by the demonstration of an antiapoptotic effect of Bcl-2 phosphorylation by JNK.65 More recently, the critical role of JNK in ultraviolet (UV)-induced apoptosis was shown using Jnk1-Jnk2-deficient double null mouse embryonic fibroblasts.66 In that study, the JNK pathway was found to be dispensable for Fas-induced receptor-mediated apoptosis, but absolutely necessary for UV-induced Bid translocation, cytochrome c release, mitochondrial dysfunction and apoptosis. Of note, no phosphorylation of Bcl-2 family proteins was detected.66 Therefore, the biological consequences of Bcl-2 phosphorylation by JNK at this time remain unclear.

Interestingly, numerous studies indicate that JNK can be either proapoptotic or antiapoptotic, depending on the cell type. In fact, some reports show that JNK is antiapoptotic in neonatal cardiac myocytes67 and in differentiated mouse embryonic stem cells.68 This antiapoptotic effect of JNK in those studies may be explained by differences in the cell culture systems. In particular, the antiapoptotic JNK function in embryonic stem cell-derived cardiac myocytes (which resemble fetal or neonatal cardiac myocytes) depends on transcriptional control69 but the proapoptotic effect of JNK does not.66 One could speculate, then, that there are different molecular mechanisms underlying the opposite effects of JNK.

PI-3K/Akt

In neuronal and hematopoietic cells, the rescue of these cells from apoptosis by IGF-1, nerve growth factor, and interleukin-3 seems to be critically dependent on signaling mechanisms that involve PI-3K/Akt.69,70 Phosphoinositide-3 kinase (PI-3K) is a well-characterized kinase that is implicated in a variety of cell processes, including apoptosis, and the downstream signaling cascade of PI-3K involves the activation of the serine/threonine kinase Akt.71 Akt is expressed in almost all tissues and has been shown to suppress apoptotic death induced by growth factor withdrawal and loss of cell adhesion in a number of cell
The antiapoptotic mechanism of Akt possibly involves a proapoptotic regulator, Bad, which translocates from the outer mitochondrial membrane to the cytosolic compartment upon phosphorylation by Akt. There, it is rendered inactive by being sequestered in a complex with a chaperone-like protein 14-3-3. Akt also has been shown to inactivate caspase-9 by phosphorylating it.

Both PI-3K and Akt are abundantly expressed in the heart. In an experimental model of MI using transgenic mice overexpressing IGF-1, IGF-1 overexpression reduced LV dilation, functional impairment, and myocyte apoptosis after MI. The cardioprotective effect of IGF-1 may be mediated via the PI-3K/Akt pathway and there are numerous in vitro and in vivo studies demonstrating that activation of the PI-3K/Akt pathway is associated with increased cardiac myocyte survival and improved cardiac function during ischemia/reperfusion.

### Knock-out Mice Studies

Knock-out mice have become an important tool in the study of the functional significance of the target genes. Many apoptosis-related genes have been deleted, yielding a variety of phenotypes, and the studies have suggested that the factors controlling apoptosis are tissue specific and vary

<table>
<thead>
<tr>
<th>Gene</th>
<th>Viability</th>
<th>Major phenotypes</th>
<th>Cardiac phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase-1</td>
<td>Yes</td>
<td>No IL-1β and IL-18 processing.</td>
<td>None</td>
<td>Kuida et al, 1995; Li et al, 1995; Fantuzzi et al, 1998</td>
</tr>
<tr>
<td>Caspase-2</td>
<td>Yes</td>
<td>Oocytes resistant to drug-induced apoptosis.</td>
<td>None</td>
<td>Bergeron et al, 1998</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>Perinatal death</td>
<td>Defective apoptosis in neuronal progenitor cells.</td>
<td>None</td>
<td>Kuida et al, 1996</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>Embryonic death</td>
<td>Fibroblasts resistant to death-receptor mediated apoptosis.</td>
<td>Myocardial thinning, reduced trabeculation</td>
<td>Varfolomeev et al, 1998</td>
</tr>
<tr>
<td>Caspase-11</td>
<td>Yes</td>
<td>No IL-1β and IL-18 processing.</td>
<td>None</td>
<td>Wang et al, 1998</td>
</tr>
<tr>
<td>Caspase-12</td>
<td>Yes</td>
<td>Defective endoplasmic reticulum mediated apoptosis.</td>
<td>None</td>
<td>Nakagawa et al, 2000</td>
</tr>
<tr>
<td>Bcl-2 proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bak</td>
<td>Yes</td>
<td>No gross phenotypic abnormality.</td>
<td>None</td>
<td>Lindsten et al, 2000</td>
</tr>
<tr>
<td>Bax</td>
<td>Yes</td>
<td>Germ cell, lymphoid, and neuronal hyperplasia, male infertility.</td>
<td>None</td>
<td>Knudson et al, 1995</td>
</tr>
<tr>
<td>Bax+Bak</td>
<td>Perinatal death (10% survival to adulthood)</td>
<td>Interdigital webs, imperforate hymen, excess growth of neuronal and hematopoietic cell lines.</td>
<td>None</td>
<td>Lindsten et al, 2000</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Death at 2–5 weeks</td>
<td>Polycystic kidney disease, neuronal loss, lymphoplasia</td>
<td>None</td>
<td>Nakayama et al, 1993</td>
</tr>
<tr>
<td>Bcl-w</td>
<td>Yes</td>
<td>Ablation of germ cells, increased intestinal apoptosis in response to 5-FU.</td>
<td>None</td>
<td>Pritchard et al, 2000</td>
</tr>
<tr>
<td>Bcl-x</td>
<td>Embryonic death (E13)</td>
<td>Neuro/erythroid apoptosis.</td>
<td>None</td>
<td>Motoyama et al, 1995</td>
</tr>
<tr>
<td>Bid</td>
<td>Yes</td>
<td>Resistance to Fas-induced hepatic failure.</td>
<td>None</td>
<td>Yin et al, 1999</td>
</tr>
<tr>
<td>Death receptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fas</td>
<td>Yes</td>
<td>Autoimmune disease, lymphadenopathy, liver hyperplasia.</td>
<td>None</td>
<td>Adachi et al, 1995</td>
</tr>
<tr>
<td>FasL</td>
<td>Yes</td>
<td>Autoimmune disease, lymphadenopathy, lymphoid hyperplasia.</td>
<td>None</td>
<td>Takahashi et al, 1994</td>
</tr>
<tr>
<td>TNF-R1</td>
<td>Yes</td>
<td>Resistance to Listeria monocytogenes.</td>
<td>None</td>
<td>Rothe et al, 1993</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apaf-1</td>
<td>Perinatal death</td>
<td>Brain hyperplasia, craniofacial abnormalities, derangements of optic and inner ear development.</td>
<td>None</td>
<td>Cecconi et al, 1998</td>
</tr>
<tr>
<td>p53</td>
<td>Yes</td>
<td>Multiple malignancies, onset at 6–9 months.</td>
<td>None</td>
<td>Jacks et al, 1994</td>
</tr>
<tr>
<td>xIAP</td>
<td>Yes</td>
<td>No gross phenotypic abnormality.</td>
<td>None</td>
<td>Harlin et al, 2001</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; apaf-1, apoptosis protein activating factor 1; FADD, fas-associated death domain; IAP, inhibitor of apoptosis protein; IL, interleukin; TNF-R1, tumor necrosis factor receptor 1.
proteins (eg, Bcl-2, Bcl-w, Bcl-xL) have phenotypes characterized by decrements in growth and development of various tissues.84–86 Evidence of functional redundancy in the Bcl-2 superfamily comes from combined Bax/Bak knock-out mice, which, in comparison to the relatively mild phenotype of the individual knock-outs, produce interdigitations, cytokine maturation and cell growth and differentiation. These mice exhibit variable phenotypes ranging from normal to embryonic lethality (Table 1). Caspase-1, -2, -11 and -12 knock-out mice do not have a significant decrease in viability, but they do have minor defects in the processing of apoptosis.87–89 In contrast, caspase-3- and caspase-9-deficient mice show profound defects in apoptosis; both die in the perinatal period.90–92 In addition, caspase-9-deficient mice show defective apoptosis through the mitochondrial-mediated pathway,93,94 which is consistent with the finding that caspase-9 is activated by the Apaf-1/cytochrome c complex. Caspase-8-deficient mice also die as embryos, and are characterized by severe developmental defects, including a thinned myocardium and a dramatic reduction in the number of hematopoietic precursors.95

There are knock-out animals specific for molecules in the death receptor pathway of apoptosis. Gene targeting experiments with Fas and Fas ligand have demonstrated increased nonneoplastic lymphoproliferation, and an increased propensity for autoimmune diseases.96,97 Morphologically, these mice develop liver hyperplasia and lymphadenopathy. Clues to the importance of FADD come from its ability to Listeria monocytogenes infection.99

Finally, the putative roles of other molecules in the apoptotic machinery have been confirmed by knock-out studies. p53-deficient mice, though initially viable, develop multiple types of neoplasms (often in the same animal) and die between 6 and 9 months of age.100 Deletion of Apaf-1 results in perinatal death accompanied by craniofacial abnormalities, exencephaly, and deranged optic and inner ear development, exencephaly, and deranged optic and inner ear abnormalities, including a thinned myocardium and a dramatic reduction in the number of hematopoietic precursors.95

Table 2  Reported Cardiovascular Studies Using Microarrays

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of genes</th>
<th>Model</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA microarray</td>
<td>588</td>
<td>Mouse experimental MI</td>
<td>Changes in genes involved in apoptosis, muscle development,</td>
<td>Lyn et al, 2000</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>Rat CHF after experimental MI</td>
<td>Upregulation of genes involved in stress response and wound</td>
<td>Sehl et al, 2000</td>
</tr>
<tr>
<td></td>
<td>-4,000</td>
<td>Rat experimental MI</td>
<td>Changes in gene involved in cell cycle, ECM,</td>
<td>Stanton et al, 2000</td>
</tr>
<tr>
<td></td>
<td>1,860</td>
<td>Mouse hypertrophy</td>
<td>Upregulation of genes involved in cytosome, intracellular</td>
<td>Friddle et al, 2000</td>
</tr>
<tr>
<td></td>
<td>-4,200</td>
<td>Mouse myocarditis</td>
<td>Upregulation of genes involved in ECM, host defense,</td>
<td>Taylor et al, 2000</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>Rat CHF after experimental MI; NRVC exposed to IGFBP-3</td>
<td>IGFBP-3 upregulated in first model; RT-PCR used to analyze expression in NRVC; upregulation of secreted factors.</td>
<td>Henson et al, 2000</td>
</tr>
<tr>
<td>Oligonucleotide</td>
<td>~7,000</td>
<td>Explanted failed human hearts</td>
<td>Changes in genes involved in metabolism, protein degradation, protein synthesis, stress response, and structure.</td>
<td>Yang et al, 2000</td>
</tr>
<tr>
<td>microarray</td>
<td>7,000</td>
<td>ACE inhibitor administration in rat experimental MI</td>
<td>Upregulation of genes involved in complement, ECM, inflammation, and wound healing.</td>
<td>Jin et al, 2001</td>
</tr>
<tr>
<td>cDNA microarray</td>
<td>1,193</td>
<td>Exposure of NRVC to IGF-1</td>
<td>Changes in genes involved in cell cycle, intracellular</td>
<td>Liu et al, 2001</td>
</tr>
<tr>
<td></td>
<td>~8,800</td>
<td>4 transgenic mouse models of hypertrophy</td>
<td>No single gene consistently upregulated. Upregulation of genes involved in apoptosis in the GluG transgenic mouse heart.</td>
<td>Arnow et al, 2001</td>
</tr>
<tr>
<td>Incyte DNA microarray</td>
<td>~11,000</td>
<td>Mouse transplant rejection</td>
<td>Upregulation of genes involved in rejection.</td>
<td>Saiura et al, 2001</td>
</tr>
<tr>
<td>cDNA subtractive</td>
<td>194</td>
<td>Transient ischemia in pigs</td>
<td>Upregulation of genes involved in survival.</td>
<td>Deppe et al, 2001</td>
</tr>
<tr>
<td>hybridization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; cDNA, complementary DNA; CHF, congestive heart failure; ECM, extracellular matrix; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; MI, myocardial infarction; NRVC, neonatal rat ventricular cardiomyocyte; RT-PCR, reverse transcriptase-polymerase chain reaction.
ear development, similar to caspase-9 knock-out mice.\textsuperscript{101} In contrast to these 2 proapoptotic molecules, the antiapoptotic molecule XIAP has a benign knock-out phenotype, with no identifiable abnormalities, suggesting the presence of redundant IAP family members that compensate for the lack of XIAP.\textsuperscript{102}

Interestingly, knock-out mice do not exhibit global suppression of cell death, but rather tissue- and cell type-specific or stimulus-dependent effects. These functions are generally related to maintaining homeostasis by regulating apoptosis and cellular proliferation. Unfortunately, some knock-out mice exhibit perinatal or early post-natal lethality and consequently, it is impossible to assess the functional significance of these apoptotic modulators in a tissue specific manner in adult animals. Therefore, tissue specific deletion of these molecules in the postnatal period (eg, via the Cre-loxP targeted gene deletion technology) will be necessary to elucidate their functional significance in adults.

**DNA Array Analysis**

Traditionally, the study of the molecular mechanisms of disease has been complicated by the intricate nature of the pathophysiologic processes in question, which often involve multiple pathways and molecules. Notably, the molecular mechanisms of various CV diseases, such as cardiac hypertrophy and failure, involve the activation of numerous interdependent pathways. Previously, investigators found it difficult to effectively study these pathways because the available technology was unable to analyze more than a few genes at once. However, with the recent discovery of the complete sequence of the human genome, as well as the genomes of other organisms, new high-throughput approaches to studying these complex pathways have been made possible. By using multiple cDNA or oligonucleotide samples placed on a glass slide (called a GeneChip), investigators can analyze several thousand full-length genes or expressed oligonucleotide sequences at once.

Already, initial studies using microarray technology have yielded interesting results regarding the pathogenesis of various CV diseases (Table 2). In vitro exposure of cardiac myocytes to IGF-1 has resulted in increased expression of genes involved in the cell cycle, secreted factors, intracellular signaling, protein synthesis, and metabolism.\textsuperscript{103} With the notable exception of cell cycle factors, in vivo exposure of mice to pharmacologic hypertrophic stimuli has demonstrated similar findings.\textsuperscript{104} cDNA microarray analysis of experimental MI models have shown that, in the setting of ischemia, increased expression of genes involved in inflammation, wound healing, structural proteins, metabolism and survival predominates.\textsuperscript{105–108} Evidence from heart failure studies reveal increases in the expression of genes involved in myocyte degradation, metabolism, and stress response, as well as a decrease in genes encoding structural proteins.\textsuperscript{109–111} Also, analysis of 4 different transgenic mouse models of cardiac hypertrophy did not reveal consistent upregulation of any single gene, although a correlation was seen between the number of differentially expressed genes and the degree of hypertrophy.\textsuperscript{112} Interestingly, within that same study, the Gaq transgenic mouse heart revealed an increased expression of apoptosis genes, and cDNA microarray analysis of a murine model of myocarditis revealed an increased expression of host defense and viral replicative genes.\textsuperscript{113} Finally, an oligonucleotide microarray analysis in a murine model of cardiac transplant rejection showed an increased expression of genes related to the rejection response.\textsuperscript{114}

Despite the promise of GeneChips, there are several limitations inherent in this technology. The first is cost. Because GeneChips are very new, the price of each chip tends to be impractical for most studies, but as the use of this technology becomes more widespread, the cost will likely decrease. The second limitation is that many sequences placed on the microarray represent unknown ESTs. Although this may be useful to identify novel genes or pathways, it also complicates the analysis. The third and perhaps most troublesome limitation is the difficulty in data analysis. Simple interpretation, such as using an arbitrarily determined differential gene expression level as a cutoff value, has been the most widely used method. However, the expression of a gene does not always correlate with its biological or functional significance. The baseline expression level, the persistence of expression and the time and location of expression are critical in accurately interpreting the significance. Therefore, sampling at various spatial components and time points are required for the accurate interpretation of DNA array analysis. That said, however, DNA microarray analysis does contribute insight into groups of genes that are activated with certain manipulations, and provides important clues and potentially novel directions for future analysis.

**Conclusions**

It is apparent that apoptosis plays a role in a variety of CV diseases, but more work is required to understand the molecular mechanisms that govern the process in the heart. Genetic and organizational studies should be explored further, using the knockout mouse system and microarray GeneChip analysis, and pharmacological studies using large animal models should also be encouraged. The initial analytical work must be carried out in well-defined experimental frameworks that are tissue-targeted and time-specific with clear quantitative endpoints. Only then will we be able to conduct meaningful human studies to answer whether the inhibition of apoptosis in heart disease translates into clinical benefit.

**Acknowledgment**

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**References**

Apoptosis in Cardiovascular Disease


