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Heart Muscle and Apoptosis

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1. Introduction

Significant progress has been made in demonstrating the role of apoptosis in various heart diseases, and in elucidating the molecular mechanisms of cardiac apoptosis. Apoptosis has been attributed an essential role in cardiomyopathy. The progressive loss of cardiac myocytes is one of the most important pathogenic components of the heart failure. While initial studies reported unrealistically high levels of cell death, probably due to methodological problems, later work has consistently shown that approximately 80–250 heart muscle cells per 10^5 cardiac nuclei commit suicide at any given time in patients with late-stage dilated cardiomyopathy. In contrast, the base-line rate of apoptosis in healthy human hearts is only one to ten cardiac myocytes per 10^5 nuclei (Anversa & Kajstura, 1998; Yue et al., 1998). Even though the rate of apoptosis in heart failure is relatively low in absolute numbers, it is significantly higher than that in the normal heart, which has essentially negligible baseline apoptosis. Recently, animal models of heart failure incorporating transgenic technology have confirmed that myocyte apoptosis itself is sufficient to induce heart failure. Apoptosis has been implicated in a wide variety of physiological and pathological processes. The importance of apoptosis in cardiovascular system is also becoming increasingly clear, and the inhibition of apoptosis is emerging as a potential therapeutic tool for various forms of cardiovascular disease. In this chapter, we examine the evidence for apoptosis in cardiovascular disease and the molecular mechanisms of cardiac apoptosis.

2. Apoptosis

2.1 Concept and morphologic characteristics of apoptosis

There are two primary pathways by which cells die. The path for accidental cell death is called necrosis. Accidental cell death occurs when cells receive a structural or chemical insult from which they cannot recover. Examples of such insults include ischemia, extremes of temperature, and physical trauma. The hallmark of necrosis is that cells die because they are damaged. In contrast, cells that die by programmed cell death commit suicide actively as the results of activation of a dedicated intracellular program. For programmed cell death, the most commonly described pathway is apoptosis. Apoptosis begins with a signal that can come from within the cell (e.g., detection of radiation-induced DNA breaks) or from without (e.g., a decrease in the level of an essential growth factor or hormone). This pro-apoptotic signal induces the cell to make a decision to

commit suicide. Initially, cells that are committed to undergo programmed cell death are in a latent phase of apoptosis. The latent phase can be subdivided into two stages: a condemned stage, during which the cell is proceeding on a pathway toward death but can still be rescued if it is exposed to anti-apoptotic activities, and a committed stage, beyond which rescue is impossible. Ultimately, the cells enter the execution phase of apoptosis, in which they undergo the dramatic morphologic and physiological changes (Pollard & Earnshaw, 2004). Apoptosis is characterized by a reproducible pattern of structural alterations of both the nucleus and cytoplasm. In order of appearance, these include: (1) Loss of microvilli and intercellular junctions. (2) Shrinkage of the cytoplasm. The cell is smaller in size. The cytoplasm is dense. The organelles, although relatively normal, are more tightly packed. (3) Dramatic changes in cytoplasmic motility with activation of a violent program of blebbing. (4) Loss of plasma membrane asymmetry, with the distribution of phosphatidyl serine being randomized so that it appears in the outer membrane leaflet. (5) Changes in the organization of the cell nucleus, typically involving the hypercondensation of the chromatin. This is the most characteristic feature of apoptosis. The chromatin aggregates peripherally, under the nuclear membrane, into dense masses of various shapes and sizes. The nucleus itself may break up, producing two or more fragments. (6) Formation of cytoplasmic blebs and apoptotic bodies. The apoptotic cell first shows extensive surface blebbing, then undergoes fragmentation into membrane-bound apoptotic bodies composed of cytoplasm and tightly packed organelles, with or without nuclear fragments. (7) Phagocytosis of apoptotic cells or cell bodies, usually by macrophages. The apoptotic bodies are rapidly degraded within lysosomes, and the adjacent healthy cells migrate or proliferate to replace the space occupied by the now deleted apoptotic cell. Because the vesicles remain membrane bound, the cellular contents are never released into the environment. As a result, apoptotic death does not lead to an inflammatory response. (Kumar et al., 2005; Pollard & Earnshaw, 2004).

2.2 Molecular mechanism and pathway description

The process of apoptosis may divided into an initiation phase, during which caspases become catalytically active, and an execution phase, during which these enzymes act to cause cell death. Initiation of apoptosis occurs principally by signals from two distinct but convergent pathways: the extrinsic, or receptor-initiated, pathway and the intrinsic or mitochondrial pathway. Both pathways converge to activate caspases and they may be interconnected at numerous steps.

2.2.1 The extrinsic (death receptors) pathway

This pathway is initiated by engagement of cell surface death receptors on a variety of cells. Death receptors are members of the tumor necrosis factor receptor family (Fas, TNFαR, DR3, DR4, DR5) that contain a cytoplasmic domain involved in protein-protein interactions that is called death domain because it is essential for delivering apoptotic signals.. Death receptor ligands characteristically initiate signaling via receptor oligomerization, which in turn results in the recruitment of specialized adaptor proteins and activation of caspase cascades. Binding of FasL induces Fas trimerization, which recruits initiator caspase-8 via the adaptor protein FADD. Caspase-8 then oligomerizes and is activated via autocatalysis. Activated caspase-8 stimulates apoptosis via two parallel cascades: it can directly cleave and activate caspase-3, or alternatively, it can cleave Bid, a pro-apoptotic Bcl-2 family protein. Truncated

Bid (tBid) translocates to mitochondria, inducing cytochrome c release, which sequentially activates caspase-9 and -3. TNF-α and DR-3L can deliver pro- or anti-apoptotic signals. TNFαR and DR3 promote apoptosis via the adaptor proteins TRADD/FADD and the activation of caspase-8. Interaction of TNF-α with TNFαR may activate the NF-κB pathway via NIK/IKK. The activation of NF-κB induces the expression of pro-survival genes including Bcl-2 and FLIP, the latter can directly inhibit the activation of caspase-8. Some viruses and normal cells produce FLIP, which binds to pro-caspase-8 but cannot cleave and activate the enzyme because it lacks enzymatic activity, and use this inhibitor to protect infected and normal cells from Fas-mediated apoptosis (Kumar et al., 2005). FasL and TNF-α may also activate JNK via ASK1/MKK7. Activation of JNK may lead to the inhibition of Bcl-2 by phosphorylation. In the absence of caspase activation, stimulation of death receptors can lead to the activation of an alternative programmed cell death pathway termed necroptosis by forming complex IIb. (Humphreys & Halpern, 2008; Logue & Martin, 2008; Yuan, 2010)

2.2.2 The intrinsic (mitochondrial) pathway

This pathway of apoptosis is the result of increased mitochondrial permeability and release of pro-apoptotic molecules into the cytoplasm, without a role for death receptors. Growth factors and other survival signals stimulate the production of anti-apoptotic members of the Bcl-2 family of proteins. The Bcl-2 family of proteins regulates apoptosis by controlling mitochondrial permeability. The anti-apoptotic proteins Bcl-2 and Bcl-xL reside in the outer mitochondrial wall and inhibit cytochrome c release. The proapoptotic Bcl-2 proteins Bad, Bid, Bax, and Bim may reside in the cytosol but translocate to mitochondria following death signaling, where they promote the release of cytochrome c. Bad translocates to mitochondria and forms a pro-apoptotic complex with Bcl-xL. This translocation is inhibited by survival factors that induce the phosphorylation of Bad, leading to its cytosolic sequestration. Cytosolic Bid is cleaved by caspase-8 following signaling through Fas; its active fragment (tBid) translocates to mitochondria. Bax and Bim translocate to mitochondria in response to death stimuli, including survival factor withdrawal. Activated following DNA damage, p53 induces the transcription of Bax, Noxa, and PUMA. Upon release from mitochondria, cytochrome c binds to Apaf-1 and forms an activation complex with caspase-9. Although the mechanism(s) regulating mitochondrial permeability and the release of cytochrome c during apoptosis are not fully understood, Bcl-xL, Bcl-2, and Bax may influence the voltage-dependent anion channel (VDAC), which may play a role in regulating cytochrome c release. Mule/ARF-BP1 is a DNA damage activated E3 ubiquitin ligase for p53, and Mcl-1, an anti-apoptotic member of Bcl-2 (Brenner & Mak, 2009; Chalah & Khosravi-Far, 2008; Yuan 2010). The essence of this intrinsic pathway is a balance between pro-apoptotic and protective molecules that regulate mitochondrial permeability and the release of death inducers that are normally sequestered within the mitochondria (Kumar et al., 2005).

2.2.3 The execution phase

The final phase of apoptosis is mediated by a proteolytic cascade, toward which the various initiating mechanisms converge. Caspases, a family of cysteine proteases, are the central regulators of apoptosis. They are mammalian homologues of the ced-3 in the nematode Caenorhabditis elegans. The caspase family, now including more than 10 members, can be divided functionally into two basic groups – initiator and executioner – depending on the

order in which they are activated during apoptosis. Caspases exist as inactive pro-enzymes, or zymogens, and must undergo an activating cleavage for apoptosis to be initiated. Caspases have their own cleavage sites that can be hydrolyzed not only by other caspases but also autocatalytically. After an initiator caspase is cleaved to generate its active form, the enzymatic death program is set in motion by rapid and sequential activation of other caspases. Executioner caspases act on many cellular components. They cleave cytoskeletal and nuclear matrix proteins and thus disrupt the cytoskeleton and lead to breakdown of the nucleus. In the nucleus, the targets of caspase activation include proteins involved in transcription, DNA replication, and DNA repair (Haunstetter & Izumo, 1998; Kumar et al., 2005) (Table 1). Initiator caspases (including caspase-2, -8, -9, -10, -11, and -12) are closely coupled to pro-apoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including caspase-3, -6, and -7), which in turn execute apoptosis by cleaving cellular proteins following specific Asp residues. Activation of Fas and TNFR by FasL and TNF, respectively, leads to the activation of caspase-8 and -10. DNA damage induces the expression of PIDD which binds to RAIDD and caspase-2 and leads to the activation of caspase-2. Cytochrome c released from damaged mitochondria is coupled to the activation of caspase-9. XIAP inhibits caspase-3, -7, and -9. Mitochondria release multiple pro-apoptotic molecules, such as Smac/ Diablo, AIF, HtrA2 and EndoG, in addition to cytochrome c. Smac/Diablo binds to XIAP which prevents it from inhibiting caspases. Caspase-11 is induced and activated by pathological proinflammatory and pro-apoptotic stimuli and leads to the activation of caspase-1 to promote inflammatory response and apoptosis by directly processing caspase-3. Caspase-12 and caspase-7 are activated under ER stress conditions. Anti-apoptotic ligands including growth factors and cytokines activate Akt and p90RSK. Akt inhibits Bad by direct phosphorylation and prevents the expression of Bim by phosphorylating and inhibiting the Forkhead family of transcription factors (Fox0). Fox0 promotes apoptosis by upregulating pro-apoptotic molecules such as FasL and Bim (Degterev & Yuan, 2008; Kurokawa & Kornbluth, 2009; Yuan 2010).

Nuclear proteins
Lamin, Rb protein, DNA-dependent protein kinase, 70-kDa subunit of U1 small nuclear ribonucleoprotein, Poly (ADP)-ribosylating protein (PARP), Mdm2
Regulatory proteins
MAPK/ERK kinase kinase 1 (MEKK1), Protein Kinase Cδ, G4-GDI GDP dissociation inhibitor, Sterol regulatory element binding protein, DNA fragmentation factor/inhibitor of caspase-activated DNAse
Cytoskeletal proteins
Fodrin, Gelsolin, Actin, Gas2

Table 1. Downstream targets of Caspases. (Haunstetter & Izumo, 1998)

2.3 Study of apoptosis

Light and electron microscopy are two of the classical techniques for the study of this process. Because of the lack of cellular synchronization in apoptosis and of the fact that the apoptotic cell is rapidly disposed of through phagocytosis, study methods based on

morphologic criteria are adequate for the demonstration of the process, but are not useful for quantifying it. Further to these procedures, the study of DNA fragmentation in agarose gels has been considered to be identificative for apoptosis. A number of techniques take advantage of this DNA fragmentation for labelling the fragments and thus for quantifying the proportion of apoptotic cells. Each DNA fragment has a 3'OH terminal portion. This terminal fragment can be labelled in various ways (for instance, with the help of a modified terminal deoxynucleotidyl transferase), so that the labelling rate is proportional to the degree of DNA fragmentation. In TUNEL assay (terminal deoxyribonucleotidyl transferase [TdT]-mediated dUTP-digoxigeninnick end labeling), TdT transfers a fluorescent nucleotide to exposed breakpoints of DNA. Apoptotic cells that have incorporated the labeled nucleotide are then visualized by fluorescence microscopy or flow cytometry. Apoptotic cells that have extruded some of the DNA have less than their normal diploid content. Automated measurement of the amount of DNA in individual cells by flow cytometry thus produces a population distribution according to DNA content (cytofluorograph) (Rubin et al., 2005). At present, the most widely accepted and standardized technique takes advantage of the changes in the membrane phospholipids that occur early in apoptotic cells (Vermes et al., 1995). The negatively charged membrane phospholipids exposed to the external environment by the apoptotic cell are labeled with fluorochrome-conjugated molecules, and the percentage of fluorescent cells can be easily quantified (Chamond et al., 1999).

2.4 Apoptosis in developmental and physiological processes

Cell death is extremely important in embryonic development, maintenance of tissue homeostasis, establishment of immune self-tolerance, killing by immune effector cells, and regulation of cell viability by hormones and growth factors. Apoptosis is a normal phenomenon that serves to eliminate cells that are not longer needed and to maintain a steady number of various cell populations in tissues. It is important in the following physiologic situations. During molecular maturation of T-cell antigen receptors, immature T cells in the thymus rearrange the genes encoding the receptor α and β chains. Cells with receptors recognizing self-antigens are potentially harmful and are eliminated through programmed cell death. Cells with damaged DNA tend to accumulate mutations, and they are potentially harmful to the organism. DNA damage induces programmed cell death in many cell types. Furthermore one of the mechanisms to eliminate infected cells is through the action of cytotoxic T lymphocytes, which kill cells, by inducing them to undergo programmed cell death. T lymphocytes whose T-cell receptors cannot interact with the spectrum of MHC glycoproteins expressed in a given individual are ineffective in the immune response. Up to 95% of immature T cells die by apoptosis without leaving the thymus. Fetal development involves the sequential appearance and regression of many anatomical structures: some aortic arches do not persist, the mesonephros regresses in favor of the metanephros, interdigital tissues disappear to allow discrete fingers and toes, and excess neurons are pruned from the developing brain. Cells in these conditions serve no purpose in humans and are eliminated by programmed cell death. Excess neurons that do not make appropriate connections have no function and so are eliminated by apoptosis. Up to 80% of neurons in certain developing ganglia die this way (Rubin et al., 2005). Apoptosis also eliminates the constituent cells of mullerian ducts in males. Epithelial cells must die to allow fusion of palate and mammary and prostate cells die when deprived of hormones (Pollard & Earnshaw, 2004).

2.5 Apoptosis and disease

Death by apoptosis is also responsible for loss of cells in a variety of pathologic states. The diseases in which apoptosis has been involved can be divided into two groups: those in which there is an increase in cell survival (or diseases associated to inhibition of apoptosis), and those in which there is an increase in cell death (and hence hyperactive apoptosis) (Chamond et al., 1999). The group of diseases associated to apoptosis inhibition includes those diseases in which an excessive accumulation of cells occurs (neoplastic diseases, autoimmune diseases) (Table 2). It was classically believed that the excessive accumulation of cells in these diseases occurred because of an increased cell proliferation. In recent years, the study of apoptosis in these patients has led to a new and different approach, according to which this accumulation of cells would be due to defective apoptosis. Increased cell apoptosis has also been implicated in the aetiopathogenesis of a number of diseases.

A large group of neurodegenerative diseases, among them Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and retinitis pigmentosa, are associated to selective apoptosis of the neurones. This neuronal death appears to be associated to increases susceptibility to apoptosis in these cells. Oxidative stress, mitochondrial defects and neurotoxic agents have been postulated as the inductors of neuronal death. The disease associated to infection by the Human Immunodeficiency Virus (HIV) has been defined as an imbalance between the number of CD4+ lymphocytes and the ability of the bone marrow to generate new mature cells. The CD4+ cells of the HIV(+) patients die through apoptosis when stimulated in vitro. Also, HIV infection of cells from healthy subjects induces apoptosis of CD4+ cells. However, further to this, not only the infected cells but also non-infected cells undergo apoptosis. Replicative exhaustion of the responding cell clones has been demonstrated in a number of diseases (among them AIDS, in which the responding clone is the CD4+ one); in these diseases the responding clone, after an initial phase of intense response to the stimulus, become exhausted and undergo apoptosis (Ameisen, 1995).

Through inhibition of apoptosis	Through excess apoptosis
Cancer	**AIDS**
Colorectal	T lymphocytes
Glioma	**Neurodegenerative diseases**
Hepatic	Alzheimer's disease
Neuroblastoma	Amyotrophic lateral sclerosis
Leukaemias and lymphomas	Parkinson's disease
Prostate	Retinitis pigmentosa
Autoimmune diseases	Epilepsy
Myastenia gravis	**Haematologic diseases**
Systemic lupus erythematosus	Aplastic anaemia
Inflammatory diseases	Myelodysplastic syndrome
Bronchial asthma	T CD4+ lymphocytopenia
Inflammatory intestinal disease	G6PD deficiency
Pulmonary inflammation	**Tissue damage**
Viral infections	Myocardial infarction
Adenovirus	Cerebrovascular accident
Baculovirus	Ischaemic renal damage
	Polycystic kidney

Table 2. Diseases associated to apoptosis

2.6 Apoptosis and cardiovascular disease

The myocardial cells allocate a limited faculty of proliferation and correspondingly, apoptosis is observed infrequently in adult hearts. On the contrary at the duration of organogenesis and in the formation of heart the apoptosis plays an important role, as an example in the formation of septa between the cardiac chambers and the valves. As consequence the defects in apoptosis can constitute basic causative factor of relatives of congenital heart disease. Apoptosis of myocardial cells is also observed afterwards the birth and concretely in the interventricular septum and right ventricular wall, at the duration of passage from the fetal circulation in the adult circulation. Moreover the phenomenon of apoptosis is also distinguished in the conducting system and can lead to congenital heart block, syndrome of long QT, and the existence of accessory pathways (Table 3) (Bennett, 2002). Until recently, the loss of myocytes was attributed to necrosis; however, it is now clear that apoptosis may play an important role in the pathogenesis of a variety of cardiovascular diseases. For instance, apoptosis has been detected in myocardial samples obtained from patients with end-stage congestive heart failure, arrhythmogenic right ventricular dysplasia and myocardial infraction. In addition, apoptosis has been detected in cardiac myocytes under hypoxia/reoxygenetion, mechanical stretch and in animal models of cardiac ischemia/reperfusion injury (Table 4) (Gustafsson & Gottlieb, 2003).

Myocyte
Idiopathic dilated cardiomyopathy
Ischaemic cardiomyopathy
Acute myocardial infarction
Arrhythmogenic right ventricular dysplasia
Myocarditis
Conducting tissues
Pre-excitations syndromes
Congenital complete atrioventricular heart
Long QT syndromes
Vascular
Atherosclerosis
Restenosis after angioplasty / stenting
Vascular graft rejection
Arterial aneurysm formation

Table 3. Apoptosis and cardiovascular disease (Bennett, 2002; Haunstetter & Izumo, 1998)

Stimulus	Signaling pathway	Potential inhibitor
Ischaemia/reperfusion Pressure overload Neurohormonal factors Ischaemia Death receptor ligands	ERK/SARK ERK/SARK G protein coupling Lack of growth factor signaling Adapter molecules / caspases	Activation of ERK, inhibition of SARK signaling Activation of ERK, inhibition of SARK signaling β blockers Activation of Akt/ERK pathways Decoy receptors / receptor antagonists IAPs / caspase inhibitors
ERK, extracellular signal related kinase; IAP, inhibitor of apoptosis protein; SAPK, stress activated protein kinase		

Table 4. Potential inhibitors and signaling pathways of cardiomyocyte apoptosis (Bennett, 2002).

2.6.1 Apoptosis and atherosclerosis

Apoptosis constitutes basic characteristic of vessels remodeling that takes place in organogenesis and in pathological situations like injury and atherosclerosis. The significance of apoptosis in atherosclerosis depends on the stage of the plaque, localization and the cell types involved. Apoptosis of vascular smooth muscle cells (SMC), endothelial cells and macrophages may promote plaque growth and pro-coagulation and may induce rupture, the major consequence of atherosclerosis in humans. Apoptosis of macrophages is mainly present in regions showing signs of DNA synthesis/repair. SMC apoptosis is mainly present in less cellular regions and is not associated with DNA synthesis/repair. Even in the early stages of atherosclerosis SMC become susceptible to apoptosis since they increase different pro-apoptotic factors. Moreover, recent data indicate that SMC may be killed by activated macrophages. The loss of the SMC can be detrimental for plaque stability since most of the interstitial collagen fibres, which are important for the tensile strength of the fibrous cap, are produced by SMC. Rupture of atherosclerotic plaques is associated with a thinning of the SMC-rich fibrous cap overlying the core. Rupture occurs particularly at the plaque shoulders, which exhibit lack of SMCs and the presence of inflammatory cells. Apoptotic SMCs are evident in advanced human plaques including the shoulders regions, prompting the suggestion that SMC apoptosis may hasten plaque rupture. Indeed, increased SMC apoptosis occurs in unstable versus stable angina lesions. Apoptosis of macrophages could be beneficial for plaque stability if apoptotic bodies were removed. Apoptotic cells that are not scavenged in the plaque activate thrombin, which could further induce intraplaque thrombosis (Kockx & Knaapen, 2000). Most apoptotic cells in advanced lesions are macrophages next to the lipid core. Loss of macrophages from atherosclerotic lesions would be predicted to promote plaque stability rather than rupture, since macrophages can promote SMC apoptosis by both direct interactions and by release of cytokines (Bennett, 2002). It can be concluded that apoptosis in primary atherosclerosis is detrimental since it could lead to plaque rupture and thrombosis.

2.6.2 Apoptosis and ischaemia/infarction

Cardiac myocyte death during ischemic injury has been thought to occur exclusively by necrosis, but recently several studies have demonstrated that large numbers of myocytes undergo apoptosis in response to ischemic disorders (Saraste et al., 1997). In humans, apoptosis seems to occur primarily in the border zone of the ischemic region and, according to some studies, in the remote from ischemia regions. However, in vivo animal studies have demonstrated apoptosis both in the ischemic region and the ischemic border zone. Apoptosis of cardiomyocytes occurs in a temporally and spatially specific manner. The central, unperfused region also manifests apoptosis, particularly within the first six hours, although between 6-24 hours necrosis is more common (Bennett, 2002). In contrast, in some studies ischemia caused apoptosis in the ischemic region alone, whereas reperfusion caused a decrease in apoptotic cells in the ischemic region and an increase in apoptotic cells in the ischemic border zone and the remote from ischemia regions. These differences theoretically could be explained by the different methods of measuring apoptosis that were used (Krijnen et al., 2002). Apoptosis in the remote non-infarcted myocardium may be partly responsible for myocardial remodelling and dilatation after myocardial infarction, and may be amenable to treatment. Apoptosis is a highly regulated process in which several regulatory proteins play a significant part. P53 limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses such as DNA damage, hypoxia, and oncogene activation. P53 mediates apoptosis through a linear pathway involving bax activation, cytochrome c release from mitochondria, and caspase activation (Shen & White, 2001). The Bcl-2 family of proteins constitutes a critical checkpoint in cell death. These proteins contain agonists and antagonists of apoptosis and alterations of their ratio determine the life or death of a cell (Anversa & Kajstura, 1998). Proapoptotic proteins include Bax, Bak, Bad, and Bcl-xs whereas Bcl-2 and Bcl-xL are antiapoptotic. Several studies have demonstrated that Bcl-2 protein is induced in salvaged myocytes surrounding infracted areas in the regions at risk in acute stage of infraction. Bcl-2 positive myocytes were not seen in the infracted myocytes in the heart with acute infraction. Bcl-2 positive immunoreactivity was not evident in salvaged myocytes of hearts with old infraction, in chronic ischemic disease or in normal hearts. P53 and Bax protein expression was rare in salvaged myocytes within the risk area at the acute stage of infraction. P53 and Bax positive immunoractivity was evident in the infracted myocytes. P53 protein is induced in salvaged myocytes at the old stage of infraction and in chronic ischemic disease. P53 positive immunoreactivity of normal control heart tissue was slight in most of myocytes. Myocytes exposed to various stress, such as chronic ischemia (salvaged myocytes at the old stage of infraction, myocytes at chronic ischemic disease) induced the overexpression of p53 protein (Tsipis et al., 2007). Consequently, the expression of bcl-2 or P53 protein in myocytes of human hearts with infarction may play an important role in the protection or the acceleration of cellular damage after infarction (Figure 1, Figure 2).

2.6.3 Apoptosis and heart failure

Congestive heart failure occurs as a late manifestation in diverse cardiovascular diseases characterized by volume or pressure overload and significant loss of contractile muscle mass. Cardiac output is initially maintained in these disorders by the development of compensatory myocardial hypertrophy and dilatation. However, the early mechanical adaptations to growth stimulus soon fall short of adequate compensation. The mechanism by which compensatory
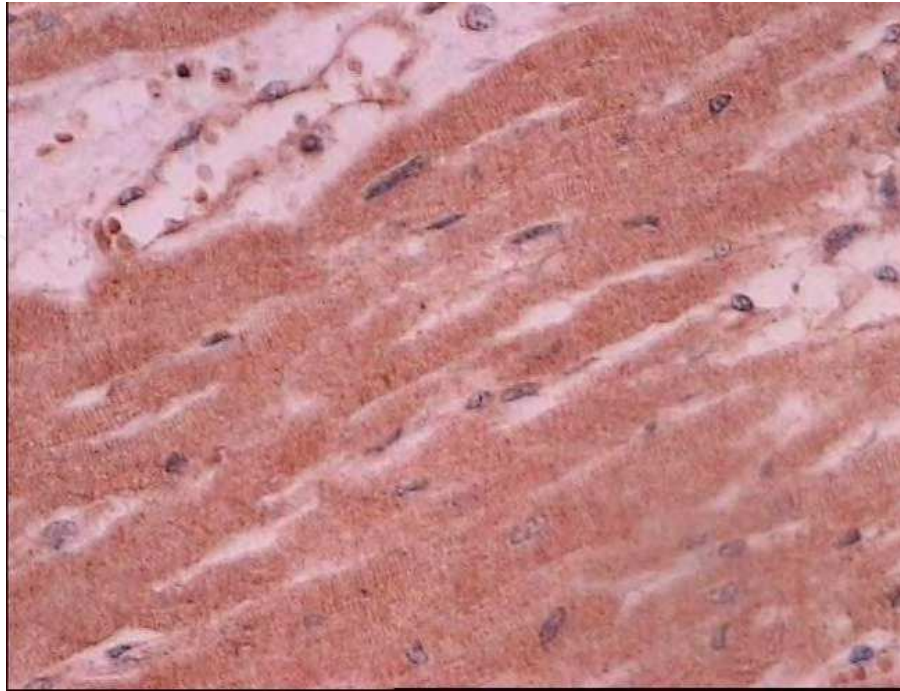
Fig. 1. Immunohistochemical staining for Bcl-2 in myocardial infarction (X400).
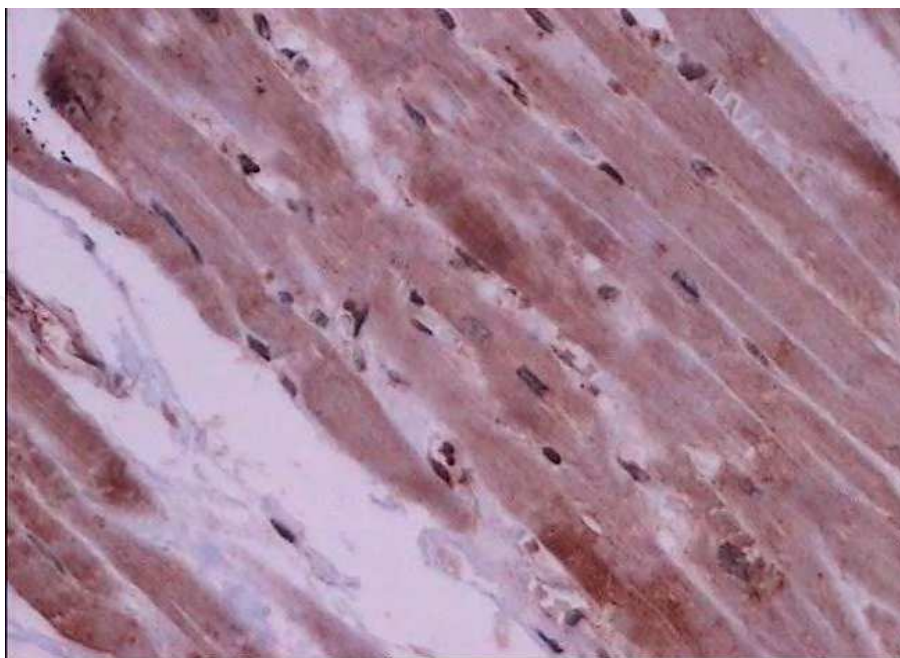

Fig. 2. Immunohistochemical staining for P53 in myocardial infarction (X400).

response triggered by myocardial failure culminates in myocardial dysfunction is not clear. In the past few years, several scientists have proposed apoptosis as the basis of the inexorable decline in ventricular systolic function (Narula et al., 2000). Although the initial studies reported unrealistically high levels of apoptosis in failed heart (as much as 35%), recent studies show an apoptosis rates of <1% (TUNEL-positive cells) during heart failure (Kang & Izumo, 2000). Because of the limitations with TUNEL staining and the difficulties in interpreting these findings, the use of TUNEL alone to detect the presence of apoptosis is not sufficient to define the role of apoptosis in heart failure. Later studies have consistently shown that approximately 80–250 heart muscle cells per 10^5 cardiac nuclei commit suicide at any given time in patients with late-stage dilated cardiomyopathy. In contrast, the base-line rate of apoptosis in healthy human hearts is only one to ten cardiac myocytes per 10^5 nuclei (Anversa & Kajstura, 1998; Yue et al., 1998). Even though the rate of apoptosis in heart failure is relatively low in absolute numbers, it is significantly higher than that in the normal heart, which has essentially negligible baseline apoptosis. Recently, animal models of heart failure incorporating transgenic technology have confirmed that very low levels of myocyte apoptosis, levels that are four- to tenfold lower than those seen in human heart failure, are sufficient to cause a lethal, dilated cardiomyopathy (Wencker et al., 2003).

It has been long believed that apoptosis does not occur in terminally differentiated cells such as adult cardiomyocytes. However, all mechanisms responsible for induction of apoptosis are operative in myocytes and are particularly activated during heart failure. Actually, the onset of myocardial failure leads to systemic and myocardial neurohumoral alterations and cytokine expression to maintain cardiac output. Upregulation of these adaptive responses also induces growth response and leads to compensatory myocardial hypertrophy and dilatation. Cardiac myocytes differentiate and withdraw from the cell cycle during the neonatal period, and persistent growth stimulus in the adult myocardium (such as that in heart failure) is perceived as a contradictory genetic demand, and programmed cell death occurs (Narula et al., 2000). P53 (tumor suppressor protein) is involved in the regulation of cell cycle progression in response to DNA damage. This p53 typically causes the cell to delay its entry into S phase until the damage has been repaired. P53 also is involved in triggering an apoptotic response in instances in which the damage is too severe to repair. P53 is a transcriptional regulator of the bcl-2 and bax genes. P53 mediates apoptosis through a linear pathway involving bax transactivation, Bax translocation from the cytosol to membranes, cytochrome c release from mitochondria, and caspase-9 activation, followed by the activation of caspase-3, -6, and -7 (Kim et al., 1994; Shen & White, 2001). P53 down-regulates the antiapoptotic gene product Bcl-2 and up-regulates the proapoptotic gene product Bax. Immunohistochemistry of p53 and antiapoptotic Bcl-2 protein demonstrated higher levels of both of these proteins in heart failure as compared with normal hearts (Figure 3, Figure 4). Tsipis et al. have observed that the percentage of p53- and bcl-2 positive samples in the end-stage dilated cardiomyopathy was 100% (20/20 diseased group samples). A 2- and 2.5-fold increase in p53 and bcl-2 positive samples was observed in the diseased group as compared with the control group. The diseased group had a larger number of samples with strong p53 staining as compared with the control group, which demonstrated weak p53 staining. Bcl-2 staining in the positive samples of the diseased group was generally weak as in the control group (Tsipis et al., 2010). Latif and colleagues, in a quantitation of the bcl-2 family of proteins after Western Immunoprobing, demonstrated a 2.9- and 5.35-fold increase in the levels of Bax and of Bcl-2, respectively,
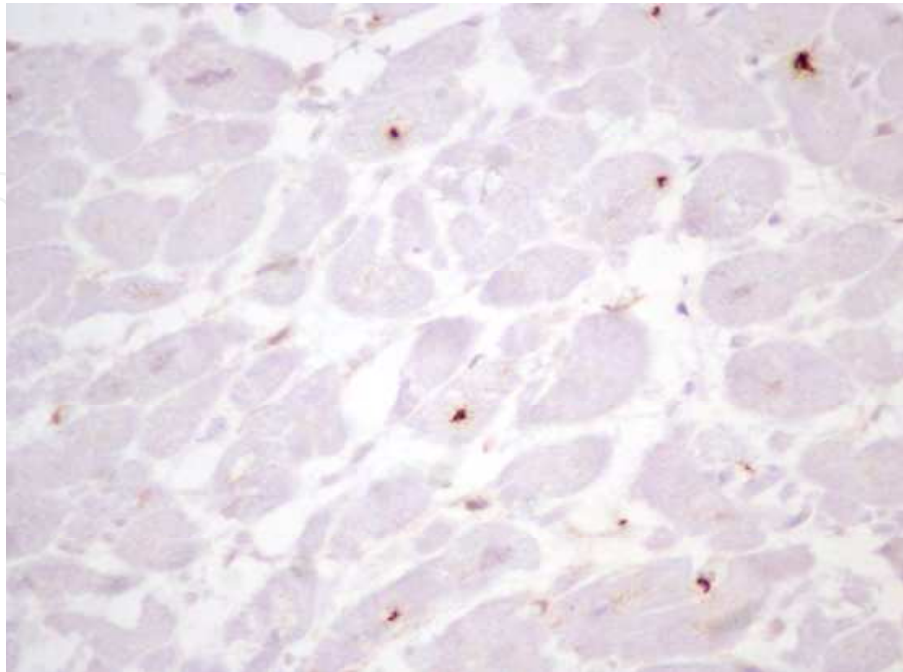
Fig. 3. Immunohistochemical staining for p53 protein in dilated cardiomyopathy (X400).
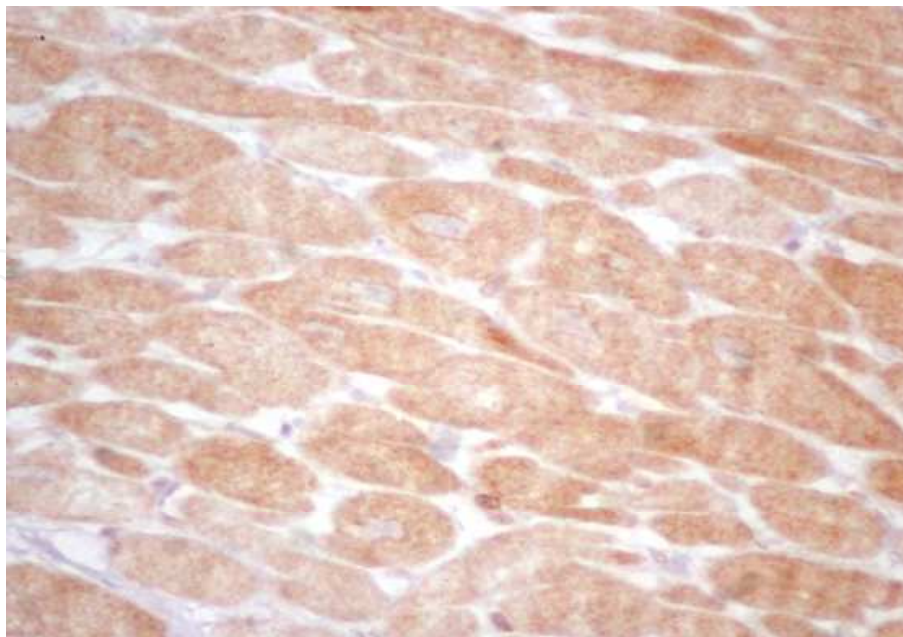

Fig. 4. Immunohistochemical staining for bcl-2 in dilated cardiomyopathy (X400).

in patients with dilated cardiomyopathy (Latif et al., 2000). Narula and colleagues demonstrated a release of cytochrome c from mitochondria in patients with heart failure (Narula et al., 2000). In the study of Tsipis et .al, increased expression of P53 protein was seen, but p53 up-regulates the proapoptotic gene product Bax (Tsipis et al., 2010). Elevated levels of Bax and Bak may mediate the release of cytochrome c, as it has been demonstrated that Bax and Bak accelerate the opening of the voltage-dependent anion channel (Latif et al., 2000; Shimizu et al., 1999). These results suggest that increased expression of p53 may be associated with apoptosis in heart failure of end-stage dilated cardiomyopathy. Moreover, various factors present in the failing myocardium have been shown to stimulate apoptosis in cardiac myocytes. Such factors include inflammatory cytokines, reactive oxygen species, nitric oxide, hypoxia, reperfusion, growth factors, and mechanical stretch (Foo et al., 2005). Ventricular decompensation and failure impose an elevated diastolic load on myocytes, resulting in stretching of sarcomeres and the stimulation of multiple second messenger systems which have been linked to the initiation of myocyte reactive hypertrophy in the pathologic heart. Abnormal levels of resting tension may lead to the local release of angiotensin II (Ang II) and the induction of programmed cell death in the myocardium. Sarcomere elongation in vitro results in Ang II release and activation of p53 and p53-dependent genes (Leri et al., 1998). Moreover, overstretching appears to be coupled with oxidant stress, expression of Fas, programmed cell death, architectural rearrangement of myocytes, and impairment in force development of the myocardium (Cheng et al., 1995). Using Western blotting, Olivetti et al. demonstrated a 2.4-fold increase in bcl-2 in patients with heart failure (Olivetti et al., 1997). However, the expression of Bax protein was not altered in the diseased group. This low expression of Bax protein may represent the prevalence of bcl-2 compensatory mechanism. The elevated presence of p53-positive cells, as demonstrated by immunohistochemistry, suggest that apoptosis may be significantly higher in dilated cardiomyopathy than that in the normal heart. On the other hand, increased expression of the antiapoptotic protein bcl-2 in human myocardium with dilated cardiomyopathy may be a compensation for the loss of myocytes and a possible compensatory antiapoptotic mechanism in the diseased group (Tsipis et al., 2010). In conclusion the etiology of heart failure in dilated cardiomyopathy involves multiple agents. The heart failure involves not only the contractile dysfunction, but also the progressive loss of myocytes by apoptosis. The elevated expression of proapoptotic is associated with progressive loss of myocytes in heart failure, and the increased expression of antiapoptotic proteins represent a possible compensatory mechanism. The prevalence of the apoptotic mechanism or this of compensatory antiapoptotic may influence the evolution of heart failure in cardiomyopathy.

3. Conclusions

Cells are poised between survival and apoptosis, and their fate rests on a balance of powerful intracellular and extracellular forces, whose signals constantly act upon and counteract each other. In many circumstances, apoptosis is a self-protective programmed mechanism that leads to the suicide of a cell when its survival is deemed detrimental to the organism. In other instances, apoptosis is a pathological process that contributes to many disorders. Thus, the pharmacological manipulation of apoptosis represents an active frontier of drug development. Recognition of the inducing mechanisms of

apoptosis could open up ways to inhibit cell death in cardiovascular tissues and possibly help to define targets for future drug design. Furthermore, end-stage events of apoptosis, such as the activation of downstream caspases are essentially uniform in all cell types; although some regulatory mechanisms may be unique to cells in cardiovascular tissues. Elucidation of proapoptotic and antiapoptotic mechanisms in cardiomyocytes and vascular smooth muscle cells could delineate potential targets for intervention. In conclusion, various factors present in the diseased myocardium have been shown to stimulate apoptosis in cardiac myocytes. Changes in the induction of genes promoting or opposing apoptosis may modulate the total amount of myocyte damage. There is still a need to clarify the role played by different genetic and environmental factors implicated in cell death or survival.

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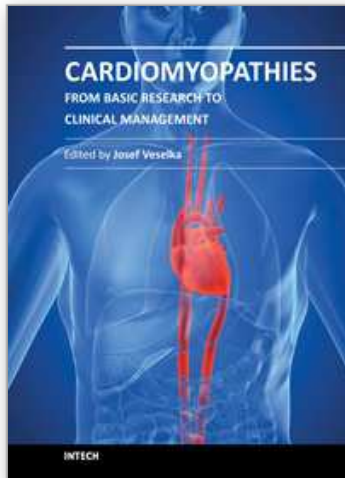
Cardiomyopathies - From Basic Research to Clinical Management
Edited by Prof. Josef Veselka

Cardiomyopathy means "heart (cardio) muscle (myo) disease (pathy)". Currently, cardiomyopathies are defined as myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of a coronary artery disease, hypertension, valvular heart disease or congenital heart disease sufficient to cause the observed myocardial abnormalities. This book provides a comprehensive, state-of-the-art review of the current knowledge of cardiomyopathies. Instead of following the classic interdisciplinary division, the entire cardiovascular system is presented as a functional unity, and the contributors explore pathophysiological mechanisms from different perspectives, including genetics, molecular biology, electrophysiology, invasive and non-invasive cardiology, imaging methods and surgery. In order to provide a balanced medical view, this book was edited by a clinical cardiologist.

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