Apoptosis in the Heart: About Programmed Cell Death and Survival

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Substantial evidence has accumulated that apoptosis, sometimes called “programmed cell death,” is important in several cardiac diseases. Although most researchers focus on apoptosis in the hope that by understanding its mechanisms one can block this form of cell death, little attention has been given to programmed cell survival.

In recent years, several studies have established that apoptosis, sometimes called “programmed cell death,” occurs in various cardiac pathologies. Apoptosis research has mainly been carried out regarding ischemia-reperfusion injury, myocardial infarction, and heart failure. Currently, we are at a stage at which there is enough evidence to convince everyone that apoptosis is associated with these pathologies. However, important questions about the relative contribution of apoptosis to cell death and its clinical relevance are far from elucidated. In an attempt to identify the areas on which research should focus in the future, we here summarize the most important findings regarding cardiomyocyte apoptosis. Furthermore, we discuss why we should not only study programmed cell death but should also try to identify features of programmed cell survival.

Hallmarks of apoptosis

Apoptosis was originally defined as an energy-dependent form of cell death with distinct phases of ultrastructural morphological features, like nuclear chromatin condensation and cellular shrinking, followed by breakup of the nucleus (karyorrhexis) and cellular budding, with the formation of apoptotic bodies (plasma membrane-delineated cellular particles containing cellular organelles and nuclear fragments), which are rapidly phagocytosed by neighboring cells [Saraste and Pulkki (see Ref. 2) and Ref. 9]. Apoptosis contrasts with nonapoptotic cell death, called oncosis by Majno and Joris (9), which is characterized by depletion of intracellular ATP, cellular swelling, disruption of organelles, and plasma membrane disruption, followed by an inflammatory reaction.
The morphological hallmarks of apoptosis, however, represent only the end stage of various subsequent phases. The initiation phase consists of the activation of the molecular machinery of apoptosis. Very little is known about the exact nature of the triggers. Factors likely to be involved in cardiomyocyte apoptotic cell death are mechanical, such as extensive stretch, and/or elevated concentrations of neurohormonal factors, such as angiotensin II and atrial natriuretic factor (Sabbah; see Ref. 2). Alternatively, it is plausible that multiple intracellular and extracellular alterations that occur during ischemia and/or reperfusion, such as excess of NO, are involved in the induction of apoptosis (Taimor et al.; see Ref. 2).

In the phase preceding the execution or degradation phase of apoptosis (characterized by morphological changes and DNA fragmentation), a specific class of aspartate-specific cysteine proteases becomes activated in a self-amplifying cascade [Saraste and Pulkki (see Ref. 2) and Refs. 6 and 18]. These so-called caspsases can be divided into an upstream and a downstream subgroup. Activation of upstream caspsases, such as caspsases 2, 8, 9, and 10, leads to the proteolytic activation of downstream caspsases, such as caspsase 3 and 7. These downstream caspsases cleave an incompletely characterized set of proteins, such as nuclear proteins, proteins involved in signal transduction, and cytoskeletal proteins. The contribution of the cleavage of the different caspsase targets to the typical apoptotic morphological changes remains to be determined. However, proteolytic cleavage of nuclear proteins, such as lamin B cleavage, is important in inducing the typical nuclear features of apoptosis, like the chromatin condensation into sharply delineated half-moon-shaped chromatin masses. Also, the externalization of phosphatidylserine at the cell membrane is thought to be a consequence of caspsase activity.

Currently, two major pathways leading to caspsase activation are characterized [Saraste and Pulkki (see Ref. 2) and Ref. 6] (Fig. 1). One is a mitochondrial pathway, which involves the mitochondrial release of cytochrome c, the so-called apoptosis-inducing factor (AIF), and probably other factors (like procaspase zymogens of caspsase 2 and 9) into the cytosol (17). Unlike the release of AIF, the release of cytochrome c seems to depend on the opening of the mitochondrial permeability pore, which is associated with a breakdown of the electrochemical gradient ($\Delta\Psi$) over the inner mitochondrial membrane. Cytochrome c can activate procaspase 9 when it is complexed with apoptotic protease activation factor 1 (APAF-1) and dATP, whereas the exact mechanism of the stimulation of DNA fragmentation by AIF remains to be resolved. The second pathway leading to caspsase activation is initiated by ligation of the death receptors. The best-characterized pathways involve binding of Fas ligand (FasL) and tumor necrosis factor (TNF)-$\alpha$ to their respective receptors (Fas and TNFR-1) (12). Caspsase 8 is the most upstream caspsase in these pathways and is activated by a signaling complex at the activated receptors.

An important target for the activated downstream caspsase 3 is DNA fragmentation factor 45/inhibitor of caspsase-activated DNase (DFF45/ICAD) (11). Its proteolytic inactivation leads to the release and activation of the associated DNA fragmentation...
factor 40/caspase-activated DNase (DFF40/CAD), an endogenous DNase enzyme responsible for the internucleosomal DNA fragmentation during the last phase of apoptosis. The DNA fragmentation results in double-stranded DNA strands that contain single-base 3’ overhangs and blunt ends. The DNA fragments can be detected as a typical ladder pattern after electrophoresis of isolated DNA. Cells with fragmented DNA can be detected with the light microscope with the terminal transferase mediated DNA nick-end labeling (TUNEL) technique. This is the most widely used method for quantification of apoptosis. However, since it can also label nonapoptotic DNA fragmentation, it is also the most criticized method (13, 15).

**Apoptosis in myocardial infarction**

In the context of myocardial infarction, apoptosis is thought to contribute to the total amount of cell death. In myocardial infarction in humans, apoptosis has been observed in three different regions: 1) in the core of the ischemic myocardial area, 2) in the border zone of the infarction, and 3) in the viable myocardium, remote from the ischemic area (Yaoita et al.; see Ref. 2). However, different percentages of TUNEL-positive cells in human infarction have been found, which may be attributable to the time between the analysis and the onset of the infarction and also to the presence or absence of reperfusion. Nevertheless, the highest number of TUNEL-positive cells are consistently present in the infarction border zone. Far fewer positive cells can be noted in the central infarction region (14). This finding contrasts with the observations from experimental models of infarction, with reference to which it has been suggested that even in the central infarct area significant apoptotic cell death occurs (Yaoita et al.; see Ref. 2). However, these TUNEL-positive cells might also represent in part nonapoptotic cell death (13). Discrepant findings have also been reported about the presence of apoptosis in remote noninfarcted segments after recent infarction in humans. Recently, it was shown in an animal model that the increased apoptosis in remote areas after myocardial infarction is associated with an increased expression of the proapoptotic proteins p53 and Bax and of caspase 3. Unfortunately, it is still not clear what the relative contribution of apoptosis to the total amount of cell death is. Hence the pathophysiological significance of apoptosis in myocardial infarction and in the subsequent ventricular remodeling is still not completely understood.

**Clinical relevance of infarction-related myocardial apoptosis**

In attempting to answer the question of whether apoptosis is a clinically important phenomenon in the context of myocardial infarction, one should study the effect of apoptosis inhibition. Several publications have described the decrease in the number of TUNEL-positive cells in response to pharmacological interventions, but, unfortunately, so far few workers have looked at the influence on infarct size (19, 20). Using the non-specific caspase inhibitor ZVAD-fmk, Yaoita et al. (19) showed that both the number of TUNEL-positive cells and the infarct size could be reduced in a rat model of ischemia-reperfusion. In contrast, with specific inhibitors of caspase 1 and 3, only the amount of TUNEL-positive cells was reduced, infarct size having been unaffected (Okamura et al.; see Ref. 2). This raises an important question as to whether inhibiting apoptosis could result in the induction of nonapoptotic cell death. Consequently, future studies will have to focus not only on infarct size reduction but also on the improvement of functional parameters at different time points after infarction.

**Apoptosis in heart failure**

Although a causal relationship is far from proven, it became evident from studies with animal models of experimentally induced heart failure that the process of ongoing left ventricular dysfunction with reference to heart failure is accompanied by an ongoing loss of cardiomyocytes. Also, studies of end-stage failing human hearts have suggested the existence of cardiomyocyte apoptosis, regardless of the predisposing factor (Sabbah; see Ref. 2). Indeed, TUNEL-positive cardiomyocytes have been detected in hearts of patients with ischemic cardiomyopathy (ICM) and idiopathic dilated cardiomyopathy (IDC) (Table 1). Cardiomyocyte apoptosis has also been detected in dog models of heart failure (Table 1).

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**TABLE 1.** Examples of published percentages of apoptotic cardiomyocytes in human failing hearts and animal models of heart failure or associated risk factors.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cause/Risk Factor</th>
<th>Detection</th>
<th>%Apoptotic Cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Aging</td>
<td>TUNEL</td>
<td>0.001 at 3 mo; 0.008 at 24 mo</td>
<td>Kajstura et al. <em>Am J Physiol Heart Circ Physiol</em> 271: H1212–H1228, 1996</td>
</tr>
<tr>
<td>Dog</td>
<td>Microembolization</td>
<td>TUNEL</td>
<td>0.46 bordering infarcts; 0.017 in remote areas</td>
<td>Sharov et al. <em>Am J Pathol</em> 148: 141–149, 1996</td>
</tr>
<tr>
<td></td>
<td>ICM</td>
<td>TUNEL</td>
<td>0.243</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>IDC</td>
<td>TUNEL</td>
<td>0.01</td>
<td>Sabbah H. <em>Cardiovasc Res</em> 45: 704–712, 2000</td>
</tr>
<tr>
<td></td>
<td>ICM</td>
<td>TUNEL</td>
<td>0.031; 0.05 bordering infarcts</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>IDC + ICM</td>
<td>TUNEL/Taq</td>
<td>0.16 males, 0.076 females</td>
<td>Guerra et al. <em>Circ Res</em> 85: 856-866, 1999</td>
</tr>
<tr>
<td></td>
<td>EM</td>
<td>0.25 males, 0.08 females</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IDC, idiopathy dilated cardiomyopathy; ICM, ischemic cardiomyopathy; TUNEL, terminal transferase mediated DNA nick-end labeling; EM, electron microscopy.
When heart failure was induced by either multiple intracoronary microembolization or rapid ventricular pacing, the majority of TUNEL-positive cells were found in areas bordering old microinfarcted regions with replacement fibrosis. Also, in a study of human heart failure, the higher rates of apoptosis in ICM than in IDC were attributed to the high incidence of TUNEL-positive cells in regions bordering old infarcts. These findings suggest that infarcted regions predispose bordering areas to apoptotic cell death (Sabbah; see Ref. 2). The triggers for the increased apoptosis in infarct border zones are not known, but it is possible that hypoxia and increased stretch, two factors known from in vitro studies to elicit apoptosis, are involved (6). Myocardial stretch is also present under ventricular overload leading to left ventricular dilatation. Both left ventricular hypertrophy and dilatation have been associated with cardiomyocyte apoptosis; however, as with the progression of heart failure, a causal relationship has not yet been established. Other factors that are believed to play a key role in triggering apoptosis in heart failure are increased levels of angiotensin II, norepinephrine, and cytokines such as TNF-α (Sabbah (see Ref. 2) and Ref. 6).

Clinical relevance of heart failure-associated apoptosis

Although not fully understood, the pathophysiology of heart failure is thought to be multifactorial. Several mechanisms, such as impaired excitation-contraction coupling, neurohormonal deregulation, altered calcium homeostasis, and altered composition of the extracellular matrix (connective tissue), are likely to be involved in the pathogenesis and progression of the disease (Sabbah; see Ref. 2). It is not known what the contribution of cardiomyocyte apoptosis is in this respect. The fact that, in a recent study (15), both oncosis and apoptosis were found at comparable rates in explanted hearts from both end-stage ICM and end-stage IDC patients makes the interpretation of apoptosis even more obscure in terms of mechanistic relevance. To interpret the currently available data, we need knowledge about the presence and degree of apoptosis in mild-to-moderately failing human hearts and in compensated hypertrophic human hearts. Alternatively, the effect of currently common therapeutic interventions like angiotensin-converting enzyme (ACE) inhibition and β-adrenergic antagonistic approaches should be evaluated with respect to their effect on apoptosis in human heart failure. ACE inhibition through long-term treatment with enalapril has been shown to attenuate apoptosis in dogs with microembolization-induced heart failure and in spontaneously hypertensive rats. Norepinephrine-induced apoptosis in isolated rat cardiomyocytes was blocked with propanolol (Yaoita et al.; see Ref. 2). It is not clear, however, whether the putative antiapoptotic effects of some β-antagonists are related to their interference with β-adrenoceptors. Apart from their β-adrenergic action, some β-blockers, like carvedilol and nebivolol, have been shown to possess antioxidant properties. Since oxidative stress is thought to be a trigger of apoptosis, it can be hypothesized that antioxidant properties lie at the basis of the reported antiapoptotic action of carvedilol (20). Nevertheless, further extensive research is needed for a better understanding of the role of apoptosis in heart failure and the influence of conventional pharmacological approaches on cell death and functional outcome.

Apoptosis in chronic hibernating myocardium

In the thinking about apoptosis and nonapoptotic cell death, it is often suggested that the two types of cell death can be triggered by the same factors but that the degree of the insult determines whether the jeopardized cells will die by oncosis or apoptosis. If this idea is correct, it can be assumed that even a lesser insult than is necessary to cause apoptosis would trigger changes in the cell. This sublethal injury is likely
to trigger several protective mechanisms, which can be subsumed under the term “programmed cell survival” (Fig. 2). As explained by Dépré and Taegtmeyer (2), an example of programmed cell survival is the initial response of the heart to moderate ischemia. Through modification of its gene expression and an enhanced anaerobic glucose metabolism, the resistance of the heart to the ischemic insult is increased. Preconditioning and hibernation are typical examples of this adaptive response (8). In the context of chronic hibernation, the described dedifferentiation with the initiation of a fetal gene program should also be considered as adaptive (4). There is a change in the expression not only of genes that are involved in energy metabolism but also of genes that code for contractile and structural proteins (1). Hence the cardiomyocytes change from an efficient contractility state to an energy-sparing state. The associated structural changes could therefore be interpreted as purely adaptive and not degenerative (4). This situation contrasts sharply with that observed in moderate ischemia. Through modification of its gene expression, the described dedifferentiation with the initiation of a fetal gene program should also be considered as adaptive (4). There is a change in the expression not only of genes that are involved in energy metabolism but also of genes that code for contractile and structural proteins (1). Hence the cardiomyocytes change from an efficient contractility state to an energy-sparing state. The associated structural changes could therefore be interpreted as purely adaptive and not degenerative (4). This situation contrasts sharply with that observed in terminal stages of heart failure, in which not only the adult isoforms but also the fetal isoforms of some proteins are downregulated. Examples are the downregulation of both adult α-myosin heavy chain (MHC) and fetal β-MHC and of both the adult GLUT4 glucose transporter and the fetal GLUT1 isoform. It is thought that these changes constitute a final maladaptive response predisposing to cell death, possibly through apoptosis (Dépré and Taegtmeyer; see Ref. 2). Although cardiac hibernation is believed to be a protective response of the chronically or repetitively underperfused myocardium (8), the limited research into apoptosis in chronic hibernating myocardium so far revealed discrepant findings (Dispersyn et al.; see Ref. 2). Some publications suggest that apoptosis is an important feature of chronic hibernating myocardium, and others claim that in this context apoptosis does not occur at all or only to a very limited extent (4, 16). Probably most, if not all, of the discrepancies can be explained by different patient and tissue selection criteria used in these investigations (4). Nevertheless, so far important questions as to whether cardiomyocyte dedifferentiation represents a stable adaptive state enabling the cardiomyocytes to survive under unfavorable circumstances for a prolonged period of time, and whether dedifferentiation precedes degeneration (possibly through apoptosis) remain unsettled.

**Clinical relevance of apoptosis in chronic hibernation**

The main limitation in answering the question of whether apoptosis occurs in chronic hibernating myocardium and whether this has clinical consequences is the limited tissue availability. Needle biopsies from properly selected patients are not only scant but also very small. It is therefore difficult to get accurate estimates of the rate of apoptosis, especially when a very low rate is expected. Indeed, one of the hallmarks of hibernating myocardium is the functional recovery after revascularization of the myocardium. This suggests that, if present, apoptosis would only occur in a very low percentage of the cardiomyocytes, far less than the amount of dedifferentiated cells found in the majority of patients with hibernating myocardium (Dispersyn et al.; see Ref. 2).

**Other aspects of programmed cell survival**

As already mentioned, preconditioning represents a well-known form of programmed cell survival. Several mechanisms are implicated in the biphasic short-term protection that brief ischemic periods confer to the cells, and activation of ATP-sensitive potassium channels (K<sub>ATP</sub>) is thought to play an important role (8). K<sub>ATP</sub> activation might be protective by reducing the action potential duration, thereby lowering cellular calcium overload. It was recently shown that even ischemic periods of several hours may trigger protective mechanisms. Prolonged simulated ischemia resulted in a transient inhibition of nitric oxide-induced apoptosis in isolated cardiomyocytes (Taimor et al.; see Ref. 2). The upregulation of stress proteins has also been reported to occur after ischemic preconditioning and was associated with an attenuation ischemia-reperfusion-induced apoptotic cell death. Indeed, heat-shock proteins such as Hsp70, Hsp27, and αB-crystallin are known to protect against ischemic damage; however, there is no evidence that these stress proteins play a role in chronic situations like chronic hibernation. In an animal model, a severe reduction in coronary flow reserve was required to upregulate Hsp70 mRNA (5). In human end-stage heart failure, only Hsp60 was found to be upregulated, whereas the levels of Hsp27, Hsp70, and Hsp90 were unchanged with respect to nonfailing hearts. It would be interesting to determine the expression levels of heat-shock proteins in mild-to-moderate heart failure.

Recently, various trophic factors have captured the attention of researchers owing to their possible role in cardiomyocyte survival. Factors like insulin-like growth factor-I (IGF-I), cardiотrophin-1 (CT-1), and neuregulins were shown to be associated with decreased apoptosis in various experimental setups that induce cell death, like those based on ischemia, serum deprivation, induction of reactive oxygen species, and stretch (7). Moreover, these factors elicit hypertrophic growth of isolated cardiomyocytes and influence protein expression patterns, with upregulation of some fetal proteins. The exact molecular pathways that mediate the cellular protection remain to be elucidated, but the activation of phosphatidylinositol 3-kinase and subsequent activation of the serine-threonine kinase Akt seems to be a common denominator. Final effector mechanisms might involve an upregulation of ant apoptotic proteins, such as Bcl-2 and members of the inhibitors of apoptosis (IAP) family, the attenuation of an upregulation of pro apoptotic proteins, such as Bax and p53, or the prevention of caspase activation, for example by Akt phosphorylation of Bad (7). Recently, inflammation-related cytokines such as TNF-α and interleukin-6 were also implicated in cytoprotection. A review by Sack et al. (2) states that several observations suggest that low levels of TNF-α have a beneficial role with reference to acute or subacute hemodynamically induced and/or ischemia-reperfusion-induced biomechanical stress. The molecular mechanisms

**“Recently, inflammation-related cytokines . . . were also implicated in cytoprotection.”**
behind the protective properties are unknown but may involve the activation of nuclear factor-kB, which promotes the expression of cytoprotective genes. The expression levels of the cytokines seem to be important in determining whether they exert a beneficial or detrimental effect. The modulation of cytokine levels might therefore give rise to potential therapeutic approaches for the treatment of myocarditis, cardiac ischemia, and heart failure (Sack et al.; see Ref. 2).

Several proteins have been identified that are directly involved in the inhibition of apoptosis. Among them, the Bcl-2 family members Bcl-2 and Bcl-xL are the best characterized. They are capable of inhibiting the release of the proapoptotic factors ALF and cytochrome c from the mitochondria (17). Other antiapoptotic mechanisms of Bcl-2 are thought to consist of 1) an direct antioxidant effect, 2) inhibition of proapoptotic Bcl-2 family members, like Bak and Bak, and 3) the inhibition of caspase activation through an APAF-1-dependent mechanism (6). The results of Maulik et al. (10) are encouraging in terms of potential therapeutic roles, since they show that ischemic preconditioning causes an upregulation of Bcl-2 and that this upregulation is associated with a reduction in apoptosis. IAP-1, IAP-2, and XIAP are members of the IAP family. These proteins are capable of inhibiting low levels of downstream caspase activation and thus of inhibiting both death receptor- and cytochrome c-mediated apoptosis. The IAPs presumably play a part in inhibiting inadvertent apoptosis, but it is not yet known how the expression levels of these proteins can be influenced and what effect this modulation has on cell death and cardiac function (6, 7). Finally, some proteins have been identified that can interfere with receptor-mediated caspase activation. Proteins referred to as FLIPs [fas-associated death domain (FADD)-like interleukin-1-converting enzyme (FLICE) inhibitory proteins] are homologs of caspase 8 and 10, which do not possess the activity of caspases. Another muscle-specific homolog of caspases 2 and 8 is the apoptosis repressor with caspase-recruitment domain (ARC). It is probable that these proteins competitively inhibit upstream caspase activation in the death receptor pathway. Besides these non-functional caspase homologs, so-called decoy receptors have been identified. An example is a TNF-related apoptosis-inducing ligand (TRAIL) receptor without cytoplasmic domain, necessary for the apoptotic signal transduction. Also, soluble forms of Fas and TNFR-1 exist, although it is not clear whether these are capable of scavenging receptor ligands and thereby inhibiting apoptosis induction (6, 7). Whether this growing class of proteins with direct antiapoptotic effects can be used therapeutically in the future needs to be explored. Again, it is not known yet how these proteins can be modulated (the only available method seems to be modulation of gene expression), and even more uncertain is their effect on cardiomyocyte survival and cardiac function. Nevertheless, further investigation in this area may yield interesting and potentially clinically important results.

Conclusion

Hoping that inhibition of cardiomyocyte apoptosis would provide a new means of preventing cardiac disease, researchers have intensified their efforts in this area in the past few years. However, a critical question needs to be asked: what are we really looking at? Recent reports suggest that the TUNEL method is not specific for the detection of apoptotic cells and that, through its use, oncocytic cardiomyocytes and even living cardiomyocytes undergoing DNA repair may be labeled. (13, 15). This finding, together with the fact that very few studies so far have yielded convincing ultrastructural evidence of apoptosis, should incite us to consider the possibility that apoptosis is not as important as we currently believe it to be. Moreover, the apparently low rates of apoptosis in recent reports of human heart failure (Table 1) also raise serious questions. As Schaper et al. (15) have pointed out, in a chronic process like heart failure, a percentage that is apparently so low would lead to unlikely high amounts of cardiomyocyte loss over a period of one year. If we are overestimating the true incidence of apoptosis, the hypothetical therapeutic benefit of apoptosis inhibition is limited. Apart from this consideration, it is still not clear whether preventing apoptosis would lead to functional improvement, because it might be that nonapoptotic cell death occurs when apoptosis is inhibited. It follows that interfering with the apoptotic machinery to prevent apoptotic cell death might not be the only or even the best strategy to follow. Although a largely unexplored terrain, all of the endogenous survival mechanisms addressed in this paper constitute an attractive field of research alongside the elucidation of the mechanism of apoptosis. Understanding endogenous protective mechanisms, stimulating them so as to prevent activation of the apoptotic cascade, might be an attractive alternative.

References