

# Does apoptosis play a role in the progression of heart failure ?

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*The failing heart is characterized by an increase in chamber volume and a reduction in wall thickness, leading to marked reduction in the mass-to-chamber volume ratio. These changes result from extensive myocyte loss, compensatory myocyte hypertrophy, and remodeling of the interstitial compartments. Myocyte loss in heart failure appears to result from the combined effect of myocyte necrosis and apoptosis. DNA strand breaks—the hallmark of apoptosis—have also been recently demonstrated in normal cardiac aging and myocardial infarction. Apoptosis is activated by ischemia and mechanical stress. Reactive oxygen species, atrial natriuretic peptide, angiotensin II, tumor necrosis factor- $\alpha$ , the Fas molecule, and cell-cycle reentry appear to play a role in this process. Intracellular molecular control mechanisms of apoptosis have been evidenced, suggesting therapeutic strategies to prevent apoptosis with a view to increasing survival in patients with cardiac diseases.*

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Despite the high incidence and ominous outcome of heart failure (HF), very little information is available on the mechanisms by which, in many cardiac diseases, pump dysfunction and overt failure progressively develop over time. It is only recently that quantitative results have been collected on the structural characteristics of the hearts of patients in end stage failure examined at the time of cardiac transplantation.<sup>1,2</sup> These data demonstrate that in ischemic and idiopathic dilated cardiomyopathies, despite extreme degrees of hypertrophic growth of the entire heart (by 80% or more in weight), the ventricles have cavity volumes 4 to 5 times larger than controls. Thus, the myocardial mass-to-chamber volume ratio is approximately 50% below the normal range. The myocardium is composed of myocytes almost double in size with a predominant increase in length (of more than 50%) to accommodate the enlarged chamber volume. Ventricular wall thickness, however, remains within control limits, so that the ratio of wall thickness to chamber volume decreases by almost four. Scars, consisting of segmental, replacement, and interstitial fibrosis, occupy between 13% to 28% of the ventricular myocardium, indicating extensive myocyte cell loss. In essence, these findings are consistent with the notion that myocyte cell loss, myocyte hypertrophy, and remodeling of the interstitial

compartment all contribute to the decompensated eccentric ventricular hypertrophy of the failing heart.

Myocyte cell loss in the heart may be attributed to necrosis, apoptosis, or both. Before 1994, only necrosis was described in the myocardium as the outcome of severe and prolonged ischemia. Necrosis is a late morphological event that follows a series of biochemical reactions and functional alterations leading to irreversible myocyte damage. Tiny breaks in the plasmalemmal membrane and amorphous densities in the mitochondria are considered irreversible morphological indexes of this death process. Myocyte necrosis is accompanied by tissue repair involving an inflammatory reaction activated by the release of intracellular materials and the participation of granulocytes, macrophages, and, in later stages, fibroblasts and collagen deposition. The final result is a scar, which may alter the structural and functional properties of the myocardium.

Apoptosis is an earlier event activated by an energy-requiring genetic program in which a  $Ca^{2+}$ -dependent endogenous endonuclease cleaves the DNA in regular fragments of 180 to 200 bp. These characteristic DNA double-strand breaks are associated with intact cytoplasm, indicating that DNA damage is the first event of apoptotic cell death. An additional feature of

apoptosis that differs from necrosis is the disappearance of affected cells with residual apoptotic bodies, but no obligatory reparative fibrosis. This property has been considered a suicidal sacrifice of at-risk cells that may protect the remnant tissue from more deleterious and permanent consequences of the necrotic process.

Although difficulties exist in distinguishing between myocyte cell death by necrosis and by apoptosis, techniques have been developed and used in different experimental conditions to measure the number of myocytes involved in both processes.<sup>3</sup> Apoptosis can be confirmed by the presence of DNA strand breaks in agarose gel electrophoresis. However, in most cases, only qualitative observations have been reported, and the amount of myocyte cell death via this mechanism is still controversial. This is a relevant issue because there is evidence that the entire process of death by apoptosis takes minutes or hours, and, since myocyte proliferation is a limited process, the whole heart may disappear within days or months assuming that apoptotic cell death is an ongoing phenomenon.

The adult human heart contains approximately  $4$  to  $6 \times 10^9$  myocytes, and aging does not affect myocyte cell numbers in the female heart. In contrast, in the male heart, 64 million myocytes/year are lost and this phenomenon is compensated by hypertrophic growth of the remaining viable cells.<sup>4</sup> Despite this reactive growth, the heart weight progressively decreases with time and this imbalance between loss and growth may explain the higher incidence of HF in elderly male subjects compared with females in whom such a loss is not apparent. In Fischer 344 rats, a well-established rat strain for studies on aging,

myocyte cell loss by necrosis and apoptosis has been measured at different ages.<sup>5</sup> In the entire heart, myocyte necrosis precedes apoptosis and involves almost 1500 myocytes at 3 months, increasing to 32 000 myocytes at 24 months.

Myocyte apoptosis was restricted to the left ventricle and involved 140 myocytes at 3 months and less than 1000 cells at 24 months. Myocyte cell loss was associated at 16 and 24 months of age with ventricular dysfunction and failure. Thus, in men and rats, a progressive drop-off of cells occurs with aging, explaining at least in part the functional deterioration.

In the terminal stages of failing human hearts, myocyte apoptosis, documented by DNA strand breaks, was found to account for an average of 2318 myocyte nuclei per million, a figure 232 times higher compared to controls. In 77% of apoptotic myocyte nuclei, characteristic morphologic features of apoptosis were found by confocal microscopy.<sup>6</sup> Additionally, DNA laddering was used to document DNA fragmentation biochemically. In several cases, myocardial fibrosis indicative of previous necrotic cell death was present and ranged from 1% to 44%. No difference was found in the amount of apoptosis among ischemic and idiopathic dilated cardiomyopathies and valvular disease.

The circumstantial evidence for the occurrence of apoptosis in cardiac diseases resulting in HF does not explain the stimulus that may trigger myocyte apoptotic cell death. It has been demonstrated that oxygen deprivation, a very well-known cause of necrosis in the myocardium, is able to elicit myocyte apoptosis. Experimentally, apoptosis precedes necrosis after coronary artery ligation in the rat.<sup>3</sup> In the hearts of patients who died shortly after acute myocar-

dial infarction and intractable congestive heart failure, DNA fragmentation was found in 12% of myocyte nuclei in the region bordering the infarcted area, while in only 1% of cells was apoptosis in progress in the remote myocardium.<sup>7</sup> However, following extensive myocardial infarction, in addition to the ischemic event, the remaining viable tissue is exposed to an abrupt elevation in diastolic transmural stress that persists during the healing phases and cannot return to normal in the absence of external intervention. This is because chamber volume increases and ventricular wall thickness decreases. Wall thinning is characterized by a reduced number of cells across the wall, a phenomenon termed side-to-side lateral cell slippage, which is seen in both human and rodent failing hearts. In an attempt to interpret the mechanism of myocyte translation from the inner to the outer layers of the ventricular wall, it has been suggested that stress may be concentrated on a single cell of a hypothetical ring of myocytes, producing irreversible damage and eventually death of the target cell.<sup>8</sup>

More recently, data have accumulated to show that in conditions in which abnormal stress is applied to the myocardium, reactive myocyte growth is associated with apoptotic death signals. Overstretching papillary muscles *in vitro*, as well as simulating the diastolic overload *in vivo*, produces myocardial remodeling and apoptotic myocyte cell death.<sup>9</sup> Increased systolic and diastolic wall stress is equally effective in inducing myocyte apoptosis in animals and humans.<sup>10</sup> Terminal stages of HF that are accompanied by severe and prolonged stress are associated with myocyte loss by apoptosis.<sup>6,7</sup> Interestingly, after a period of well-compensated cardiac hypertrophy



in the spontaneously hypertensive rat model, cardiac failure develops only in some animals in which a greater degree of cardiac myocyte apoptotic cell loss can be demonstrated.<sup>11</sup> Reducing the pressure overload on these hearts by angiotensin-converting enzyme inhibition decreases the amount of apoptosis.

Thus, there is no doubt that stress may play a critical role, but very limited information is available on the signal transduction pathway followed by the stimulus from the surface of the cell to the nucleus and on how mechanical, hormonal, or chemical signals can be transformed into intracellular messages. Myocyte apoptosis appears to be modulated by multiple factors promoting or opposing myocyte death. Among others, the members of the Bcl-2 family have been quite extensively evaluated, although their role is still unclear.<sup>3,6,9</sup> Bcl-2, which promotes cell survival, is decreased in HF, whereas Bax, a gene of the same family promoting apoptosis, is unchanged. The precise mechanisms by which the two members of the same family have opposite effects is still unclear. The suggestion has been made that apoptosis is the result of the ratio between proapoptotic and antiapoptotic proteins that probably act as homodimers and heterodimers. Fas antigen, a cell surface molecule that belongs to the tumor necrosis factor and nerve growth factor receptor family, can also stimulate apoptotic myocyte cell death, and Bcl-2 may interfere with this process. Acute coronary occlusion or hypoxia are able to upregulate Fas expression in the ischemic myocytes.<sup>3</sup> However, passive overstretching of isolated papillary muscles produces Fas overexpression, but myocyte apoptosis seems to be dependent upon superoxide anion

formation.<sup>9</sup> p53 and Waf-1 may promote myocyte apoptosis directly or through the renin-angiotensin system.<sup>11</sup> The interleukin-1 $\beta$ -converting enzyme family, which, in humans, includes caspase 1 to caspase 10, seems to have a direct role in myocyte cell death.<sup>12</sup> Tumor necrosis factor- $\alpha$  is elevated in patients with HF, is known to signal through functionally active receptors on myocyte sarcolemma, and may induce myocyte apoptosis *in vitro* by increasing intracellular sphingolipids.<sup>12</sup> Atrial natriuretic peptide provokes apoptosis in neonatal myocytes, suggesting an important role of this molecule alone or in combination with other neuroendocrine effectors.<sup>13</sup> Angiotensin II has been able to increase apoptotic cell death in isolated myocytes through enhanced Ca<sup>2+</sup> entry into the cytoplasm. This effect was inhibited by specific angiotensin-1 (AT<sub>1</sub>) receptor blockade.<sup>14</sup> Angiotensin-converting enzyme inhibition, by reducing the pressure overload on the hypertrophic hypertensive rat heart, seems to be able to protect against apoptosis.<sup>11</sup> Pioneering studies to promote myocyte survival from apoptotic cell death with growth factors are promising.<sup>15</sup>

Finally, the existence of similarities between apoptosis and the different phases of the cell cycle has been noted for some time in several cell systems and, as an extreme view, apoptosis has been considered an unsuccessful mitotic process. Although adult cardiac myocytes have been considered as being post-mitotic cells, several studies in humans and animals have demonstrated that HF is associated with enhanced DNA synthesis in myocyte nuclei.<sup>16</sup> This phenomenon is also accompanied by upregulation of molecular markers for cell-cycle progression and mitotic figures. The simultaneous presence of apop-

osis and DNA synthesis in HF supports the concept that in order to maintain pump function, extremely stressed, still viable myocytes could be induced to reenter the cell cycle. However, this maneuver may result in myocyte proliferation and/or death depending upon the ability to produce intact or damaged DNA. In this regard, it is well known that cell-cycle progression in the face of DNA damage is, in general, a potent stimulus to apoptotic suicidal induction in order to maintain intact the genomic DNA. In the heart, the suicidal apoptotic program of some myocytes in stressful conditions may be viewed as a positive attempt to avoid more dangerous consequences produced by necrotic cell death.

The hypothesis that apoptosis is involved in the progression of various cardiac diseases leading to irreversible failure is based on several facts: (i) heart failure develops more frequently in patients with hypertension and cardiac hypertrophy and survivors from acute myocardial infarction, conditions in which apoptotic myocyte cell death has been demonstrated; (ii) the presence of programmed myocyte cell death has been found in the myocardium of patients in end-stage cardiac failure; (iii) myocyte apoptosis can be elicited in isolated myocytes exposed to substances present at high levels during the developmental phases of HF; (iv) elevations in mural stress characteristic of HF of various origins may induce myocyte apoptosis; (v) agents that ameliorate survival in humans with HF reduce apoptotic myocyte cell death; and (vi) aging of the heart resulting in HF is accompanied by apoptotic myocyte cell loss.

In the majority of these conditions, however, necrotic myocyte cell death is present, and the final destiny of the failing heart should be attributed

to the cumulative effects of both death processes on the number of dying or surviving cells. The advantage of recognizing that apoptosis is consistently involved in abnormalities underlying heart dysfunction depends on the possible prevention of myocyte loss by pharmacological intervention or gene therapy. Once general and specific mechanisms, gene inducers and protectors, modulators, and other variables involved in apoptosis have been discovered, strategies to promote myocyte rescue will be generated, thereby preventing cell loss by this mechanism. Since the amount of myocyte apoptosis is not trivial in the pathologies where it has been found, patient survival or death may depend on the number of myocytes preserved from dying.

In conclusion, cardiac myocyte cell death by apoptosis is found in different diseases that progress acutely or chronically to HF, decreasing the number of viable myocytes. Thus, the answer to the original question—does apoptosis play a role in the progression to HF?—is yes. On the other hand, apoptosis cannot be considered the sole factor responsible for the progression of cardiac diseases to dysfunction and failure. Necrosis is also present, and the total amount of myocyte cell loss, in addition to the anatomical and structural remodeling of the myocardium, is a prominent factor in pump dysfunction. However, the complexity of events involved in the genesis of HF cannot be attributed to any one process alone, and gene expression, regulation of contractility, changes in the cytoskeleton and extracellular matrix, ion homeostasis, and other unknown factors may all contribute to cardiac failure. In any event, there is still the hope that therapeutic efforts aimed at myocyte preservation

from apoptotic cell death may provide a further tool to improve prognosis in HF.

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