Role of Apoptosis in Ventricular Remodeling

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Cell loss is a common feature in the failing heart, and this contributes to the relentless progression seen in patients with heart failure. Apoptosis is one of the most common causes of cell loss in animals and humans with heart failure. There is increasing evidence that apoptosis, even while occurring in a low-grade manner, can mediate heart failure. Moreover, inhibiting apoptosis successfully prevents or attenuates heart failure in several animal models. More importantly, apoptosis is one of the few mechanisms that can be easily modulated using pharmacologic or gene therapy approaches. Animal data, obtained in the past few years, have proven the feasibility and success of this approach toward altering the natural history of heart failure. Human studies are pending, but a number of issues such as the type of inhibitor and its optimum timing/dose will need to be resolved before this becomes a reality. Nevertheless, these data will accrue over time, and antiapoptotic therapy is likely to emerge as an important form of heart failure therapy.

Introduction

Congestive heart failure, which affects approximately 1.5% of adults in the United States, is a massive and growing problem [1]. Moreover, despite advances in drug and device therapy, the median survival has remained dismal [2]. Of all the parameters that affect the occurrence and progression of heart failure, changes in ventricular size, shape, and volume that are collectively termed "ventricular remodeling" remain one of the strongest predictors of poor prognosis [3,4]. More importantly, interventions that attenuate ventricular remodeling also improve survival, whereas interventions that worsen remodeling negatively affect prognosis in heart failure despite other beneficial effects [5]. Therefore, the pathophysiologic processes central to ventricular remodeling are being actively investigated and remain an attractive target for intervention.

One of the hallmarks of ventricular remodeling is a progressive worsening of myocardial structure and function even when the stimulus responsible for initial injury is no longer present. This progression was initially attributed to hemodynamic factors such as wall stress or effect of neurohormonal activation, but later evidence confirmed the pivotal role of an absolute reduction in cardiomyocyte number [6]. The main reason for a reduction in cardiomyocyte number appears to be cell death in the presence of nearly nonexistent or inadequate cell regeneration [7•].

Types of Cell Death in Heart Failure

Traditionally, distinct types of cell death based on morphologic characteristics [8] have included necrosis and apoptosis. Necrotic cells show severe disruption of cellular membranes and cell contents, whereas apoptotic cells show preserved cell boundaries with minimal inflammation. More recently, there is increasing evidence for other forms of cell death including those in which there is significant overlap with apoptotic processes [9•]. Additionally, it is now clear that the same heart can show multiple types of cell death, often in the same microscopic field [10]. The type of cell death is often dependent on the time in the natural history when it was studied [11]. Necrosis is a feature of early heart failure, especially of the ischemic variety, but the cause of cell death in chronic heart failure is mainly apoptotic or autophagic [9•,10–12].

Evidence for Apoptosis in Heart Failure

One of the best-studied forms of cell death is apoptosis. There is copious evidence that apoptosis contributes significantly to myocardial cell death during and after a myocardial infarction (MI) [11,12]. Apoptosis has been shown to be prominent in animal [13] and human [14•] MI. The amount of apoptosis is variable and time-dependent. There is also evidence for an increase in many of the apoptosis intermediates, including a colocalization of caspase 3 and apoptosis in the ischemic heart [15]. More

importantly, apoptosis may also contribute to the development and progression of heart failure late after a MI [16]. Apoptosis is found in the chronically failing heart, albeit in a lesser amount than in the immediate post-MI period, and a progressive loss of myocytes through apoptosis has been thought to be important for progression in this syndrome [17]. Some investigators have also found chronic ongoing apoptosis in the hibernating heart, although this is controversial. Some of the data are clouded by methodologic issues, and studies that used very elementary techniques such as terminal transferase uridyl nick end labeling (TUNEL) have overestimated its occurrence [12,18•]. This has been attributed to the fact that TUNEL assays also stain cells undergoing repair and those undergoing apoptosis [19•]. Thus, it is important to use multiple methods of confirming apoptosis to exclude such confounding factors. The second issue in documenting apoptosis is the variability in different models. There is controversy regarding the occurrence of apoptosis in the most studied model—the rat infarct model—with some studies [20], which is in contrast with many other studies, including my own experience, that show very few apoptotic cardiomyocytes. However, the study by Li *et al*. [20] found a significant amount of interstitial apoptosis. Varying time course of apoptosis and methodologic issues could explain this variability. Despite some variability in available data, one can conclude that a low level of apoptosis is continually ongoing in the failing heart and that level of apoptosis correlates with degree of heart failure [21].

Apoptosis and Contractile Dysfunction in Heart Failure

The classical view is that the apoptotic cell dies and this contributes to heart failure through a reduced contractile cell mass. There might also be other mechanisms through which the apoptotic cascade could mediate heart failure. Because much of the apoptotic machinery can cleave contractile proteins, including actin, myosin, and troponins [22], it is possible that activation of apoptotic pathways could mediate contractile dysfunction that is to some extent independent of cell death. Caspase 3 can degrade actinin, a component of the Z band that holds thin filaments together, and other scaffold proteins; loss of such proteins can disadvantage synchronized filament function. Indeed, caspase 3 overexpression has been shown to directly reduce the contractile performance of the left ventricle [23], and the degree of myosin cleavage correlates with the contractile performance of the heart [24]. Moreover, caspase inhibition improves contractile function in part through reduced contractile protein loss [25]. Not all cells showing apoptotic changes die immediately; Narula *et al*. [26] recently postulated that such cells in which systolic dysfunction may precede any breakdown of DNA or irreversible cell death might show contractile dysfunction—a condition they termed "zombie myocytes." This suggests that apoptosis may play a much broader role in heart failure—it might also cause contractile failure in surviving cells in addition to loss of contractile cell mass itself.

Evidence That Interruption of Apoptosis Is Beneficial

The past 10 years provided evidence that apoptosis occurred in the failing heart and contributed significantly to myocardial cell death and the development/progression of heart failure [26]. However, much of this was an extrapolated association, and some critical issues remained unresolved; there was little evidence that one could induce a controlled degree of apoptosis to generate a predictable degree of ventricular remodeling. Conversely, there was little to suggest that inhibiting apoptosis could impede progression of heart failure. The past few years have provided new and exciting evidence that apoptosis is pivotal in the progression of heart failure. More importantly, these studies provide evidence that inhibiting apoptosis is a promising therapeutic strategy.

Induction of apoptosis mediates heart failure

One of the most convincing pieces of evidence that apoptosis plays an independent and important role in the development of heart failure comes from two recent, classic papers by Wencker *et al*. [27••] and Hayakawa *et al*. [28]. Wencker *et al*. [27••] created a transgenic model of cardiac-specific caspase 8 overexpression in which caspase 8 activation could be triggered using a specific ligand. They found that a low level of caspase 8 overexpression was associated with a low level of cardiomyocyte apoptosis (average of 23 cardiomyocytes per 10^5 nuclei in these hearts, compared with 80+ cardiomyocytes per 10^5 nuclei in failing human hearts and 1–2 cardiomyocytes per 10^5 nuclei in healthy hearts), and this was sufficient for inducing severe heart failure over a period of time. Greater expression was associated with greater apoptosis and failure. Caspase inhibition or a point mutation that abolished caspase activity prevented heart failure. This was one of the most conclusive pieces of evidence that apoptosis contributes to heart failure irrespective of the reason for apoptosis. Hayakawa *et al*. [28] (the same group) has now presented similar evidence in a completely different model of heart failure. Cardiac-restricted overexpression of Gα (q) in mice results in a fatal form of peripartum cardiomyopathy that is thought to be related to apoptosis. Administering a broad caspase inhibitor to these animals early in pregnancy reduces caspase 3 activity and apoptosis, and this was accompanied by improved function and remodeling. Although attenuation of remodeling was partial in these rats, caspase inhibition completely abolished premature mortality related to peripartum cardiomyopathy. This again conclusively suggests

that apoptosis can mediate various forms of heart failure and that modulating apoptosis ameliorates heart failure. The other interesting feature of this model is that Gα (q) is part of a pathway used by multiple neurohormones that are increased in heart failure syndromes. This suggests a link between apoptosis and many of the common triggers in heart failure.

Other types of studies also provide strong supporting evidence for the role of apoptosis in ventricular remodeling. Activating caspases, whether through overexpression of caspase activators, such as mammalian sterile 2–like kinase 1, which is a substrate and activator of caspase 3 [29], or cardiac-specific caspase 3 itself [30], results in severe cardiomyopathy through apoptotic mechanisms. In the same way, ablating apoptosis signal-regulating kinase, which plays an important role in regulating left ventricular (LV) remodeling by promoting apoptosis [31] or caspase 1 [32] (which is not apoptotic by itself but is related to activation of other intermediates), attenuates apoptosis and ventricular remodeling.

Apoptosis is also triggered by extrinsic pathways in the failing heart. Studies manipulating these ligand-activated pathways of apoptosis also show a similar benefit of inhibiting apoptosis. Engel *et al.* [33] studied whether overexpression of tumor necrosis factor (TNF), a known mediator of the extrinsic pathway to apoptosis in the failing heart, would mediate ventricular remodeling through an apoptotic mechanism. Mice with cardiac-restricted overexpression of TNF were studied serially to correlate apoptosis with LV remodeling (measured using magnetic resonance imaging) during the transition from LV hypertrophy to LV dilatation. TNF-mediated cardiomyocyte apoptosis adversely remodeled the ventricle. Induction of apoptosis signaled the transition to heart failure in absence of myocyte necrosis. Myocyte apoptosis correlated well with wall thining (but surprisingly not with LV dilatation). More conclusively, broad-based caspase inhibition decreased myocyte apoptosis, with consequent attenuation of wall thinning and remodeling. This suggests that apoptosis conclusively contributes to ventricular remodeling and that inhibiting apoptosis attenuates heart failure. In this regard, a recent study further showed that inhibiting TNF using a soluble p75 TNF receptor fusion protein (0.5 mg/kg subcutaneously twice weekly) also attenuated apoptosis and ventricular remodeling [34].

Inhibiting apoptosis attenuates heart failure

There had been accumulating evidence that antiapoptotic pharmacologic therapy had beneficial effects in ischemic reperfusion [35,36] and sepsis [37]. However, until recently, there was little evidence that such pharmacologic therapy altered the natural history of acute or chronic heart failure in nontransgenic models. There is some question of purity of transgenic models, and showing efficacy of caspase inhibition in garden-variety, everyday models is most important. Two recent studies seem to prove that reducing cell loss through apoptosis significantly attenuates ventricular remodeling [25,38••]. My colleagues and I have recently shown that long-term in vivo caspase inhibition protects against myocardial contractile protein cleavage and better preserves LV systolic function after a MI. The caspase inhibitor was started at the time of left anterior descending artery ligation (which created moderate-sized infarcts) and continued for 28 days. Compared with controls administered the vehicle, caspase inhibitor therapy significantly reduced caspase 3 activation (55%), troponin I cleavage (51%), and apoptosis (60%). LV end-diastolic pressure (8 ± 0.9 vs 13 ± 1.6 mm Hg; $P < 0.05$) was lower, and the ejection fraction was higher (39% and 46% greater at 1 and 4 weeks after MI; $P < 0.05$) in the rats subjected to caspase inhibition. Heart weight/body weight ratios and LV dimensions were better, whereas fibrosis was lower (by 46%) in the caspase inhibitor–treated animals. These results suggest that modulating caspase activity and apoptosis provides significant benefit in the post-MI period [25]. Another study has recently shown the protective effect of reducing infarct segment apoptosis even when therapy was started at a later time point after MI [38••]. In that study, when treatment was started 3 days after MI and continued for 12 weeks, inhibiting apoptosis in the infarct segment granulation tissue had profound effects on ventricular remodeling over the long term; the authors attributed this to a better formed scar. An important finding of this study was a reduction in overall mortality after caspase inhibition. In a subsequent study, the same group demonstrated that much of the apoptosis in the granulation tissue was mediated by the extrinsic pathway through Fas/Fas ligand interaction. Inhibition of this pathway using adenovirus carrying soluble Fas inhibited apoptosis, improved scar thickness, and decreased remodeling [20]. This opens up gene therapy avenues to modulate apoptosis in heart failure. Given the paucity of studies in the literature, the details regarding the optimum inhibitor and timing of its use remain to be worked out. Nevertheless, it is clear that apoptosis can be manipulated using pharmacologic therapy with significant benefits, and this might be a promising therapy in the future.

Conclusions

The past year has provided significant evidence that apoptosis is pivotal in the development and progression of heart failure. It has also provided strong evidence that attenuating apoptosis alters the natural history of heart failure in various ischemic and nonischemic models. Pharmacologic methods to inhibit apoptosis are still in their infancy and need more studies. More specifically, we need to obtain crucial data regarding the best type of therapy, and we must optimize the dose and timing of such intervention. Because physiologic processes also

use apoptosis and some immune functions also invoke apoptosis, it is possible that antiapoptotic therapy may have unexpected consequences. Thus, we also need to know the downsides of short- and long-term antiapoptotic therapy. Nevertheless, antiapoptotic therapy is here to stay, and the next 5 to 10 years may see the beginning of early human trials.

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