multiple morphologies of cell death may coexist in the same microscopic field, sometimes even in adjacent cells as illustrated by Kostin et al. Second, the cell may be able to choose its form of death, depending on distinct, highly modifiable, intra- and extracellular conditions. Thus, a low-intensity stress like a shorter period of ischemia or ischemia-reperfusion may lead to apoptotic death while a longer period of insult brings in necrotic death. Similarly, the degree of ATP depletion, amount of PARP cleavage, or rate of mitochondrial permeability transition pore formation could drive a cell toward apoptosis or necrosis or even change one morphology to another. Third, cells sometimes die with a morphology that is intermediate between apoptosis and necrosis and this has been variously termed as necroapoptosis or parapoptosis. Finally, necrosis can be programmed, a feature long thought to be a hallmark of apoptosis. This suggests some commonality between various modes of cell death, which is further supported by the fact that cellular triggers or death signals thought to be predominantly invoked in one form of cell death are sometimes shown to mediate another form of death.

Can Cell Death Morphology Change Its Colors

The authors have previously reported that apoptosis and necrosis occur at comparable rates in the failing heart. They now show that ubiquitylated protein aggregates may mediate cell death at a rate comparable or slightly higher than the other two mechanisms of death. However, it may be artificial to assign pathogenetic significance to quantitative measurements of various forms of cell morphology without a better understanding of the changes in cellular environment. Can one “ice pick” view of cell death explain what happened during evolution to end-stage heart failure? The proportion of cells dying through different mechanisms may be different at various stages in the natural history of the disease. Therapy itself may radically alter the proportions of cells dying of apoptosis, autophagy, and necrosis. ACE inhibitors and beta blockers inhibit apoptotic and to a lesser extent necrotic cell death and thus may make autophagy more prominent. This becomes an important issue since one of the goals of management is to interdict the natural history of heart failure, and identification of a more panoramic view of cell death at earlier time points may be crucial.

This issue of a “snapshot” morphological description becomes even more complex when we assess the effect of interventions specifically directed at death pathways. For instance, caspase-9, a prominent member of the apoptotic pathway, mediates nonapoptotic cell death in the presence of the apoptosis inhibitor zVAD.fm.k. Thus, the same effector machinery mediates different forms of cell death depending on the circumstances. An emphatic example of the interchangeable forms of cell death is seen during embryologic
maturation. Apoptosis is paramount in the developmental process of interdigital resorption. However, digits still mature after inhibiting apoptosis and they do this through necrosis. It thus appears that cells can die in diverse ways and which route it chooses is dependent on signals that are as yet not well known.

**Does Ubiquitylation Play a Role in Myocyte Death in Heart Failure**

One of the most interesting features of the accompanying article is the role of protein ubiquitylation and the ubiquitin-proteasome pathway (UPP) in cell death. The evolutionarily highly conserved ubiquitin system labels substrate proteins with a ubiquitin molecule on lysine residues and targets the marked protein to degradation by the 26S proteosome. Interestingly, while 4 or more ubiquitin molecules condemn a protein to destruction, shorter chains may regulate various survival functions. Immunohistochemical techniques as presented here may not be able to easily discern the reparative versus executioner functions of ubiquitin. Ubiquitylation is a reversible process as deubiquitylating (DUB) enzymes can remove ubiquitin molecules from condemned proteins. Not surprisingly, ubiquitylation intersects with death decisions in a multitude of ways.

The authors observed ubiquitylated protein aggregates in some cardiomyocytes. These aggregates were more prominent in cells showing marked structural changes including autophagic vacuoles. This was accompanied by increased tissue ubiquitin mRNA, polyubiquitylated proteins, increase in E2 Ub conjugation enzyme, no change in E3 Ub ligase, and reduction in two deubiquitylating enzymes. They postulated that defects in this pathway could lead to increased ubiquitylation and protein aggregates to result in cell death by autophagy. Some parts of their hypothesis have evidence in other systems. In neurons after cerebral ischemia, ubiquitylated protein aggregates are commonly found in the dying cells, as opposed to rare deposits in the cells destined to live. Thus, a high density of ubiquitylated protein aggregates in cardiomyocytes may be a marker of doomed cells. What is less proven is their second hypothesis that dysfunction of the ubiquitylation system mediates this cell death. Since over 30 E2 Ub conjugation enzymes, and more than 100 E3 ligases have been identified, it is difficult to postulate direct pathogenetic significance of the changes in only one variety of enzymes. E3 ligase, the main step responsible for ubiquitylation, was not increased in the presence of extensive ubiquitylation of proteins.

**What Renders the “Wounded Cell” a Survivor or a Loser**

It is interesting to note that all forms of cell death in the chronically failing heart, including that mediated by the ubiquitylated protein aggregates, is patchy. This happens at a time when the primary triggers for cell death are expected to be widespread. Most cells exposed to adverse triggers may be protecting themselves to a variable extent and those that cannot do so may be dying. An alternative hypothesis thus could be that the cells, at least those with mild ubiquitylation, were invoking protective responses to avoid cell death and may have died when such responses were overwhelmed. Evidence is accumulating that cells exposed to death signals actively try to protect themselves through a number of mechanisms. When successful, such mechanisms may keep the cell alive, a factor especially important in terminally differentiated cells like the cardiomyocytes. Some of the known mechanisms include release of endogenous antiapoptotic factors to block actions of cytochrome c, or change in PARP and/or ATP levels to modulate the apoptotic pathway. Narula et al have shown that although there is continuous evidence of caspase-8 activation and mitochondrial cytochrome c release in cardiomyopathic hearts, myocytes prevent downstream activation of caspase-3, and lose DNAses to preserve nuclear integrity and defer widespread loss of cytoplasmic proteins (Figure 1). Such a phenomenon represents an interrupted apoptotic cascade in heart failure and possible altered myocardial state of cell survival. There was uninhibited activation of caspase-8 associated with Flip-L's downregulation (competitors of caspase-8), subsequent Bid (caspase-8-mediated inducer of cytochrome c release) truncation, cytochrome c release from mitochondria, and caspase-3 activation. Upregulation of XIAP (inhibitor of active caspase-3) and downregulation of Smac-L (inhibitor of XIAP) restricted active caspase-3. Residual active caspase-3 led to contractile protein cleavage but failed to induce DNA fragmentation due to complete abolition of DNA fragmentation factors (DFF). Such protective mechanisms have now been found within the ubiquitin system where increased E3 ligase activity has been shown to protect against cell death through a selective enhancement of ubiquitin-proteasome-mediated protein degradation.

There is a fascinating link between endoplasmic reticulum (ER) stress, ubiquitylation, and cell survival (or death) that might explain some of the findings in the accompanying study. In this scenario, various stimuli (including ischemia, altered redox state, or abnormal calcium homeostasis, which are prevalent in the failing myocardium) cause ER stress and accumulation of unfolded protein in the ER. This invokes a series of protective responses, such as the unfolded protein response (UPR), which help the cell to survive by reducing the entry of new proteins into the ER as well as increasing protein translocation out of it. Retrotranslocated proteins have sticky hydrophobic ends and need to be rapidly marked for degradation failing, which they accumulate in the cell as protein aggregates. There is increased degradation of ER-translocated proteins in the proteosome through the ubiquitin pathway, which seeks to restore ER function and reduce ER stress. Indeed, there is evidence for ER stress in the ischemic myocardium.

Prolonged or overwhelming ER stress can lead to protein aggregates (as in the Kostin study) and apoptotic or necrotic cell death suggesting an overlap between protein aggregates and usual forms of cell death. Protein aggregates can turn off synthesis of many important proteins, including those in the UPP, and mediate necrotic cell death or increase apoptotic signaling and thus mediate apoptotic death; this once again suggests a common link among various forms of cell death including that through ubiquitylated protein aggregates. It is interesting that preconditioning, a significantly
protective phenomenon, prevents protein accumulation and reduces cell death. Myocytes accumulated ubiquitylated proteins in presence of normal tissue proteosome activity in the present study. There could have been too much misfolded protein translocation to ER-associated degradation (ERAD) pathway from the sick and stressed ER. Thus, the cell death seen in this study might reflect a prolonged UPR and ultimate overwhelming of the ERAD rather than a primary defect in the ubiquitin/deubiquitylation system (Figure 2).

It is intriguing that the authors found no costaining with activated caspase-3 or TUNEL positivity in ubiquitin-laden cells. Ubiquitylation is a major mechanism to modulate apoptosis. Ubiquitylation or deubiquitylation of apoptotic proteins (like caspases) or its regulators (Bcl2, p53, NF-κB) has been shown to both increase or reduce apoptosis depending on which factor gets ubiquitylated. For example, p53, which is a highly proapoptotic factor in most tissues, is marked for degradation through ubiquitylation. Similarly, ubiquitin mediated modulation of Bcl2 and its pro- and antiapoptotic family members can regulate apoptosis depending on the circumstances. Finally, the endogenous antiapoptotic proteins (IAPs) have ubiquitylation activity and can inhibit apoptosis by ubiquitylating caspases, the effector machinery in apoptotic cell death. Also, the definition of...
TUNEL-based apoptosis may not be entirely appropriate in failing cardiomyocytes.22

Speculations on Life and Death
Based on the discussions above, the following conclusions can be drawn. First, multiple death signals are active at any given time within the same organ or tissue. Second, cells likely to die invoke multiple protective mechanisms. Finally, doomed cells, once compensatory mechanisms are overwhelmed, may succumb to intended death pathways, crossover to other morphological variety, or die of a hybrid form of death.33,34 One could speculate that crosstalk in death pathways might be dependent on a number of associated factors such as the energy state of cell and intensity of damage. Preserved energy states and lower intensity stimuli may drive a cell toward “energy hungry” death pathways like apoptosis and possibly UPP. Overwhelming UPR, which too is an energy-dependent process, may lead to ubiquitylated protein aggregation and thus cell death if UPR is not turned off. On the other hand, exhaustion of energy during these (energy-requiring processes) and exaggeration of severity of inducing stimuli may upset calcium homeostasis and mediate necrosis. Repletion of the cytosolic ATP pool before irreversible damage may redirect the death program toward energy-dependent death pathways.35

Death by any or many mechanisms is a loss of contractile muscle mass, and largely irreparable. Natural adaptation of myocytes (and other terminally differentiated cells), particularly in chronic/degenerative disorders (such as heart failure, Alzheimer’s disease) associated with upregulation of protective factors (or downregulation of death factors), renders the cells into a state of suspended animation.21,36 The provocative hypothesis provided above for excessive ubiquitylation and one proposed by us earlier of interrupted apoptotic cascade support the survival instincts of cardiomyocytes. Such adaptive processes may significantly retard the rate of loss of muscle mass and allow reversibility of contractile function to an extent. Therefore, the list of the skillful protective mechanisms in cardiomyocytes that include preconditioning, hibernation, repetitive stunning, stunning, and interrupted apoptosis continues to grow. Better understanding of death-defying mechanisms should allow better avenues of intervention and hence functional reversibility. Whoever said that the expectations of Egyptian pharaohs were unrealistic?

References


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Death Hath a Thousand Doors To Let Out Life…
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