Cell Death in Heart Failure

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Heart failure (HF) has become the dominant cardiovascular disorder in the Western world and Japan. Despite significant advances in medical and surgical treatments, HF has become an increasingly frequent reason for hospital admission. HF is preceded by a process called cardiac remodeling, which encompasses the aggregate of compensatory structural and functional changes in response to stress on the chamber wall. Cardiac remodeling involves increased rates of cardiomyocyte cell death. Historically, there are 3 types of cell death: apoptosis, autophagy and necrosis, which have been observed simultaneously in failing human hearts. To prevent the progression of cardiac remodeling and HF, it is imperative to have a full understanding of the cell death mechanism in cardiomyocytes and thus identify potential therapeutic targets.

Much research has focused on the mechanisms of cardiomyocyte cell death in HF and in this review we will summarize the recent findings.

Apoptosis

Apoptosis is the most thoroughly characterized form of programmed cell death and the sequence of molecular events involved in apoptotic cell death is well understood. Apoptosis is defined by characteristic changes: altered nuclear morphology, including chromatin condensation and fragmentation; minor changes in cytoplasmic organelles; cell shrinkage; plasma membrane blebbing; and, apoptotic body formation. There are 2 major apoptotic signaling pathways: the intrinsic and the extrinsic. A wide variety of apoptotic signals, including growth factor deprivation, hypoxia, oxidative stress and DNA damage, activate the intrinsic pathway, which is regulated by members of the Bcl-2 family. The extrinsic pathway is activated when death receptors contain an intracellular death domain, which can recruit caspase 8 subsequently activates downstream effector caspases, such as caspase 3.

In contrast, the extrinsic pathway is activated when death ligands, such as Fas ligand or tumor necrosis factor-α, bind to cognate receptors on the plasma membrane. These receptors contain an intracellular death domain, which can recruit and activate caspase 8 via the adaptor Fas-associated protein with the death domain at the cell surface. Recruitment of caspase 8 subsequently activates downstream effector caspases, such as caspase 3, with no involvement of the Bcl-2 family. Some pathophysiological processes or some stimuli directly or indirectly affect mitochondria and induce cell death.

Apoptosis is extremely rare in the normal myocardium,
with reported rates of 1 apoptotic cardiomyocyte in 10,000–100,000.29 Apoptosis is strikingly increased and plays a significant pathophysiological role in the clinical cardiomyopathies and in experimental models of HF or hypertrophy decomposition.21–28 In human failing hearts in NYHA classes III–IV, apoptotic cells are observed in the range of 0.12–0.70%.29

Recently, multiple stimuli for cardiomyocyte cell death have been identified, including excessive stretch, reactive oxygen species (ROS), β1 adrenergic receptor agonists, angiotensin II, proinflammatory cytokines and cytoskeletal derangements. ROS are involved in a wide variety of cellular functions, including apoptosis. Apoptosis signal-regulating kinase 1 (ASK1) is an ROS-sensitive mitogen-activated protein (MAP) kinase kinase kinase and activates both p38 and c-Jun N-terminal kinase (JNK) pathways by directly phosphorylating and activating MAP kinase (MKK) 4/MKK7 and MKK3/MKK6.30 The ASK1/JNK pathway can inactivate Bcl-2, mediated by its phosphorylation.31 We previously reported that the ROS–ASK1–JNK pathway plays an important role in apoptosis in the heart:32 ASK1 is activated in pressure overloaded and postinfarcted mouse hearts, and ASK1-deficient mice have attenuated cardiac remodeling. The JNK phosphorylation level is significantly increased in pressure overloaded and postinfarcted wild-type mouse hearts. However, the increases in the phosphorylation level of JNK are significantly attenuated in ASK1-deficient mouse hearts. The number of TUNEL (terminal deoxynucleotidyl transferase biotin-dUDP nick end-labeling)-positive myocytes after pressure overload or myocardial infarction is decreased in ASK1-deficient mice compared with wild-type mice. Overexpression of a constitutively active mutant of ASK1 induces apoptosis in isolated rat neonatal cardiomyocytes, whereas neonatal ASK1-deficient cardiomyocytes are resistant to H2O2-induced apoptosis. Thus, the ASK1–JNK pathway plays a pivotal role in regulating left ventricular remodeling by promoting apoptosis, and it has been suggested that inhibition of ASK1 is beneficial for preventing HF.

We also reported that chronic inhibition of ASK1 activation by transcoronary gene transfer using recombinant adeno-associated virus can attenuate the progression of cardiac remodeling in TO-2 cardiomyopathic hamsters.33 Inhibition of ASK1 reduces the number of apoptotic cells and selectively attenuates JNK activation. Thus, suppression of ASK1 may constitute a novel therapeutic strategy for the treatment of patients with HF. On the other hand, we have reported that the phosphorylation of p38, which is also downstream of ASK1, is increased to a similar degree in both wild-type mice and ASK1-deficient mice after pressure overload or myocardial infarction.22 We have also reported that p38 plays a critical role in the cardiomyocyte survival pathway.34 In response to pressure overload to the left ventricle, cardiac-specific p38α-deficient mice developed cardiac dysfunction and heart dilatation, accompanied by massive cardiac fibrosis and the appearance of apoptotic cardiomyocytes. These findings suggest that other, not yet identified, MAP kinase kinase kinases can activate p38 and promote cell survival mechanisms.

**Necrosis**

Necrosis is classically characterized by early plasma membrane rupture and dilatation of cytoplasmic organelles, in particular the mitochondria.35,36 Necrosis is often defined in a negative manner, as death lacking the characteristics of programmed cell death and thus accidental and uncontrolled.37 However, it has recently become clear that necrotic cell death is well controlled and programmed. Mitochondria play an important role in energy production, Ca2+ homeostasis and cell death.

Mitochondrial membrane permeability transition (MPT), also known as mitochondrial depolarization, is defined as the loss of transmembrane potential of the mitochondrial inner membrane and is regulated by Ca2+-dependent increase in the permeability of the mitochondrial membrane.38,39 MPT leads to the loss of the proton gradient and the shutdown of ATP generation through oxidative phosphorylation, resulting in mitochondrial swelling and the rupture of the outer membrane. Thus, MPT is considered to be a key event and is thought to occur after the opening of a putative channel complex, which has been termed the permeability transition pore and which consists of the voltage-dependent anion channel, the adenine nucleotide translocator (ANT), cyclophilin D (CypD) and other molecules.40,41 Permeability transition pores open in the mitochondrial inner membrane in response to stimuli such as increased intracellular Ca2+, inorganic phosphate, alkaline pH, and ROS. Because MPT still occurs in the ANT-deficient mitochondria in response to several MPT inducers, such as calcium ionophores, ANT may not be essential for MPT.42 CypD is a cyclophilin family of peptidyl prolyl-cis, trans-isomerases and a structural component of the MPT pore.43 CypD resides in the mitochondrial matrix, but associates with the inner mitochondrial membrane during MPT.

Oxidative stress has been implicated in necrotic cell death in cardiac pathologies such as ischemia–reperfusion (IR) injury,44 and HF.45 Olfactory stress-induced necrosis is considered to be caused by MPT pore opening and ATP depletion.46,47 Recently, CypD-deficient mice showed a high level of resistance to IR-induced cardiac injury.48 In control hearts, IR injury caused significant necrotic damage, but in CypD deficient hearts, the infarct area was dramatically reduced. Consistently, lactate dehydrogenase release is almost completely inhibited in CypD-deficient hearts. The CypD-dependent MPT regulates some forms of necrotic death, but not apoptotic death, by using CypD-deficient mitochondria and cells. These findings suggest that Cyp-D-mediated MPT is a key effector of cellular necrosis. Several pharmacological agents, in particular cyclosporine A (CsA), can inhibit the loss of mitochondrial membrane potential.49 The mitochondrial target of CsA is CypD. However, CsA also inhibits calcineurin; for example, loss of calcineurin activity enhances apoptotic cell loss in the heart.50 Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis mediated through CypD attenuates muscular dystrophy.50 Thus, the CsA analogs that do not inhibit calcineurin might be advantageous for attenuating MPT and the loss of cells by necrosis in select forms of HF.45 Using ASK1-deficient mice, we found that ASK1 is involved in necrosis, as well as apoptosis, and that ASK1-dependent necrosis is likely to contribute to myocardial cell death in the IR heart.51 In control hearts, IR injury causes significant necrotic damage; however, the infarct area is dramatically reduced in ASK1-deficient hearts. The mechanism of ASK1-dependent necrosis remains to be elucidated.

**Autophagy**

Autophagy has evolved as a conserving process for bulk...
degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles. In a nutrient-deprived cell, autophagy is a cell-survival mechanism. There are 3 main autophagic pathways: macroautophagy, microautophagy, and chaperon-mediated autophagy. The term “autophagy” refers to macro-autophagy, unless otherwise specified. Autophagy involves sequestration of cytosolic constituents, including proteins and organelles, in autophagosomes and degradation in lysosomes. Autophagy is controlled by autophagy-related genes, many of which are involved in autophagosome formation. In general, autophagy is thought to be a non-selective degradation system. This feature is in marked contrast to the ubiquitin–proteasome system, which specifically recognizes only ubiquitinated proteins for proteasomal degradation. Recent studies have demonstrated a variety of physiological and pathophysiological roles in autophagy, such as adaptation to nutrient deprivation, intracellular clearance of protein and organelles, development, anti-aging, elimination of microorganisms, cell death, tumor suppression, and antigen presentation. Autophagy appears to modulate both cell viability and death.

Autophagy has been observed in both hypertrophied myocardium and failing myocardium caused by dilated cardiomyopathy, valvular disease, and ischemic heart disease. In patients with terminal HF secondary to ischemic cardiomyopathy or dilated cardiomyopathy, cellular degeneration with granular cytoplasmic ubiquitin inclusion is detected in 0.3% of the cardiomyocytes. Human failing hearts with idiopathic dilated cardiomyopathy exhibited morphological features of autophagic, apoptotic and necrotic cells. In human hibernating myocardium, degenerated cardiomyocytes with autophagic vacuoles and nuclear disassembly are also observed. Cardiomyocytes obtained from a UM-X7.1 hamster model of human dilated cardiomyopathy contain typical autophagic vacuoles, including degraded mitochondria, glycogen granules, and myelin-like figures. Degenerated cardiomyocytes showing characteristics of autophagy have also been observed in diphtheria toxin receptor transgenic mice. However, it remains unclear whether autophagy is a sign of failed cardiomyocyte repair or is a suicide pathway for failing cardiomyocytes.

In genetically engineered cell lines and mice, autophagy appears to play a protective role in cardiomyocytes. Enhancing autophagy by beclin-1 overexpression reduces Bax activation and protects against IR injury in cardiac HL-1 cells. LAMP2-deficient mice show excessive accumulation of autophagic vacuoles and impaired autophagic degradation of long-lived proteins, resulting in cardiomyopathy. We have also reported that temporally controlled, cardiac-specific, Atg5-deficiency in tamoxifen-treated Atg5 flox/flox; MLC2v-Cre+ mice causes cardiac dysfunction and left ventricular dilation 1 week after TAC. Polysubiquitinated proteins accumulate, ER stress is increased and apoptosis is promoted in Atg5-deficient hearts. Four weeks after TAC, autophagy is upregulated in failing wild-type hearts. These results indicate that constitutive autophagy in the heart under baseline conditions is a homeostatic mechanism for maintaining cardiomyocyte size and global cardiac structure and function, and that upregulation of autophagy in the failing heart is an adaptive response that protects cells from hemodynamic stress.

Autophagy may be protective during ischemia, but may be detrimental during reperfusion. Autophagy is induced by ischemia and is further enhanced by reperfusion. Autophagy resulting from ischemia is accompanied by AMP-activated protein kinase (AMPK) activation and is inhibited by dominant-negative AMPK. In contrast, autophagy during reperfusion is accompanied by upregulation of beclin-1 but not by activation of AMPK. Induction of both autophagy and cardiac injury during IR are attenuated in heterozygous beclin-1-deficient mice, accompanied by a decrease in apoptosis.

**Crosstalk Between Apoptosis, Necrosis and Autophagy**

Autophagy seems to be required to (1) increase protein turnover, (2) remove damaged organelles such as mitochondria undergoing MPT, which is defined as the loss of transmembrane potential of the mitochondrial inner membrane, and (3) maintain ER as a cytoprotective mechanism. With increasing stress, pro-apoptotic factors are released from mitochondria undergoing MPT. In particular, in the absence of autophagy, the accumulation of polysubiquitinated proteins may be responsible for increased ER stress, resulting in apoptosis. Excessive autophagic activity induced by severe stimuli can destroy a large fraction of the cytosol and organelles and release lysosomal enzymes and possibly other factors that promote cell death, leading to apoptotic cell death. Under extreme stress, MPT occurs in all mitochondria and the intracellular supply of ATP is exhausted, leading to necrotic cell death. Thus, the dead and dying cells can simultaneously show characteristics of autophagy, apoptosis and necrosis.

Recent reports have demonstrated crosstalk between the apoptotic, necrotic and autophagic pathways. Several signaling pathways that are induced by common cellular stressors regulate both autophagy and apoptosis. ROS not only trigger apoptosis but are also essential for autophagy and specifically regulate Atg4 activity. Increases in the cytosolic-free Ca2+ concentration not only activate pro-apoptotic signals but also potently induce autophagy by activating calmodulin-dependent kinase kinase.

Members of the beclin-1 and Bcl-2 family serve as a point of crosstalk between the autophagic and apoptotic pathways. Beclin 1, which is a mammalian autophagy gene, was originally identified as a Bcl-2 interacting protein. Beclin 1 directly interacts not only with Bcl-2 but also with other anti-apoptotic Bcl-2 family proteins such as Bcl-xL. Bcl-2 inhibits beclin-1-dependent autophagy in yeast and mammalian cells. Cardiac Bcl-2 transgenic expression also inhibits autophagy in murine heart cells. Bcl-2 inhibits the formation of the beclin-1/Wsp34 PI3 kinase complex and activates mTOR, which inhibits beclin-1-dependent autophagy. Beclin 1, which is a mammalian autophagy gene, was originally identified as a Bcl-2 interacting protein. Beclin 1 directly interacts not only with Bcl-2 but also with other anti-apoptotic Bcl-2 family proteins such as Bcl-xL. Bcl-2 inhibits beclin-1-dependent autophagy in yeast and mammalian cells. Cardiac Bcl-2 transgenic expression also inhibits autophagy in murine heart cells. Bcl-2 inhibits the formation of the beclin-1/Wsp34 PI3 kinase complex and activates mTOR, which inhibits beclin-1-dependent autophagy. Beclin 1, which is a mammalian autophagy gene, was originally identified as a Bcl-2 interacting protein. Beclin 1 directly interacts not only with Bcl-2 but also with other anti-apoptotic Bcl-2 family proteins such as Bcl-xL. Bcl-2 inhibits beclin-1-dependent autophagy in yeast and mammalian cells. Cardiac Bcl-2 transgenic expression also inhibits autophagy in murine heart cells. Bcl-2 inhibits the formation of the beclin-1/Wsp34 PI3 kinase complex and activates mTOR, which inhibits beclin-1-dependent autophagy.
beclin-1-associated class III PI3 kinase activity. UVRAG is a positive, and Bcl-2 a negative, regulator of the beclin-1–class III PI3 kinase complex. Under stressed conditions, Bcl-2 weakly associates with the beclin-1–class III PI3 kinase complex, whereas UVRAG remains in the complex, resulting in amplified autophagy. Although HSpin1, the kinase complex, whereas UVRAG remains in the complex, Bcl-2 weakly associates with the beclin-1–class III PI3 kinase activity. UVRAG under lethal stressed conditions.

47. Halestrap A. A pore way to die.


