Does Autophagy Mediate Cardiac Myocyte Death During Stress?

Jihoon Nah, Álvaro F. Fernández, Richard N. Kitsis, Beth Levine, Junichi Sadoshima

Although autophagy generally promotes survival of cardiac myocytes, it can also promote cardiac myocyte death under some conditions. Here, we describe how activation of autophagy leads to death of cardiac myocytes, introduce autosis as a novel and unique form of cell death by autophagy, and discuss the functional significance of autophagic cell death in cardiac myocytes.

Autophagy is an evolutionarily conserved mechanism for the degradation of cellular components and organelles by lysosomes. Because autophagy is capable of eliminating large protein aggregates and even damaged organelles, it is a unique component of cellular quality control mechanisms. Autophagy also plays an important role in the maintenance of cellular energetics by recycling amino acids and fatty acids for ATP production. One can speculate that these properties of autophagy are particularly advantageous in terminally differentiated cardiac myocytes because protein aggregates and damaged intracellular organelles are not diluted through cell division in these cells and cardiac myocytes have especially high energetic demands. Consistent with these functions, a large number of studies have supported the notion that autophagy is protective in the heart at baseline and in response to stress. However, strong evidence also exists that the activation of autophagy in some situations induces cell death. For example, cardiac myocyte death is attenuated by interventions that inhibit activation of autophagy in some models of ischemia/reperfusion, pressure overload, doxorubicin-induced cardiomyopathy, and excessive mitophagy in response to activation of Parkin. Nevertheless, the cell-death–promoting effects of autophagy in the heart remains controversial, in part, because of technical issues (see below) and because of the general belief that autophagy is solely an adaptive mechanism. Here, we discuss the induction of cardiac myocyte death by autophagy in the heart in particular pathological conditions.

Autophagic cell death has been described as massive cytoplasmic vacuolization without nuclear condensation. However, a limitation of this purely morphological definition is that the presence of autophagic vacuoles in dying cells (cell death with autophagy) does not necessarily mean that autophagy is the cause of cell death (cell death by autophagy). Autophagy in this situation may simply represent an unsuccessful attempt of the cell to save itself. Loss-of-function experiments in which autophagy is blocked are needed to distinguish whether autophagy is functioning in a given situation as a pathogenic or survival mechanism. A caveat to this approach, however, is that none of the interventions currently available to inhibit autophagy are completely specific. Although it is not possible to completely circumvent this problem, the employment of a combination of several approaches to suppress autophagy may strengthen the conclusion that autophagy is mediating cell death. Investigating this issue is important because death of cardiac myocytes is a critical component in the development of heart failure. Below, we discuss other considerations on the concept of autophagic cell death.

One of the concepts supporting the existence of autophagic cell death is that levels of autophagy in a cell must be appropriate to maintain cellular homeostasis. This suggests that supraphysiological levels of autophagy may induce excessive destruction of cellular components, thereby causing cell death. In fact, one can induce this condition in cultured cells with artificial interventions, such as high doses of the cell-permeable peptide TAT-Beclin 1, which induces autophagy by mobilizing endogenous Beclin 1. Excessive activation of mitophagy can also kill cardiac myocytes. The notion that massive destruction induces cellular suicide is appealing, but several issues must be considered. First, thought needs to be given as to how an initially adaptive mechanism becomes excessive. For example, autophagy during myocardial ischemia/reperfusion is activated, in part, through increases in oxidative stress. Activation of autophagy is presumably at least initially adaptive in this condition because oxidative stress induces protein misfolding, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction, all of which must be eliminated urgently. However, oxidative stress can be strongly amplified through reactive oxygen species–induced release of reactive oxygen species, which may in turn induce autophagy beyond physiological levels. A similar dose dependence is found in ER stress. Although the unfolded protein response activated by ER stress is initially protective, it can activate cell death when the level of ER stress becomes excessively high or is sustained.

It should be noted, however, that massive cellular destruction may not be the only means by which autophagy can kill cells. Several other scenarios exist. First, death may be induced by autophagic degradation of molecules involved in cell survival. Autophagy can target specific proteins for degradation through
interaction between the protein targets and certain adapter molecules for LC3, such as p62/SQSTM1. For example, autophagy degrades dBruce, an inhibitor of apoptosis in flies and, in fibroblasts, the antioxidant catalase. Second, the components of the autophagic or mitophagic machinery can directly affect cell survival and death through nonautophagic functions. Direct crosstalk between autophagy-related (Atg) proteins and the death machinery has been reviewed previously. Although the second scenario involves induction of cell death with autophagy, they may not involve induction of cell death by autophagy. Third, different forms of autophagy may affect the activities of one another. For example, suppression of generalized autophagy is cardioprotective in mouse models of type 1 diabetes mellitus by stimulating mitophagy. If the reciprocal relationship also operates, it is possible that excessive activation of autophagy may inhibit mitophagy, thereby depriving the cell of the protection it entails.

Thus, autophagy may be involved in death of cardiac myocytes through multiple mechanisms. In general, death of cardiac myocytes occurs when autophagy is activated in excess. The question then arises as to what are the morphological or biochemical features common in cell death induced by autophagy. Liu et al have introduced the concept of a novel form of cell death by autophagy, termed autosis, which is characterized by unique morphological features without features of apoptosis or necrosis. Autosis can be induced by TAT-Beclin 1 and starvation in HeLa cells in vitro and in response to cerebral hypoxia–ischemia in hippocampal neurons in neonatal rats in vivo, whereas it is inhibited by knockdown of Atg13 or Atg14, as well as treatment with 3-methyladenine, an inhibitor of autophagy. Cells dying by autosis exhibit 2 phases of morphological changes: phase 1 with gradual changes and phase 2 with abrupt changes, collapse, and death. In phase 1a, convoluted nuclei, dilated and fragmented ER, and increased numbers of autophagosomes, autolysosomes, and empty vacuoles are observed. In phase 1b, a swollen perinuclear space, which contains cytoplasmic materials and electron-dense mitochondria, is observed. In phase 2, the number of ER, autophagosomes, and autolysosomes is drastically decreased and focal nuclear concavity and focal ballooning of the PNS are observed. Currently, the molecular mechanism by which autosis induces death is not well understood. Importantly, however, Na⁺,K⁺-ATPase participates in regulating autosis, as cardiac glycosides, chemical inhibitors of the Na⁺,K⁺-ATPase, inhibit autosis and dramatically reduce brain damage in neonatal rats subjected to cerebral hypoxia–ischemia. Moreover, knockdown of the Na⁺,K⁺-ATPase inhibits starvation-induced autosis in cultured cells. To date, the presence of autosis has not been shown in cardiac myocytes. It should be noted that the α-subunit of the Na⁺,K⁺-ATPase expressed in the rodent heart is not inhibited by the classic cardiac glycoside drugs (eg, digoxin) used in humans. Thus, other approaches will need to be used to study autosis using mouse models. One important feature of autosis is that the death cannot be prevented in the presence of bafilomycin A1, a vacuolar proton ATPase inhibitor. Thus, stimulation of early stages of autophagosome formation, such as consumption of ER membranes as a source of autophagosomes, rather than massive destruction at lysosomes, may be the cause of cell death with the characteristic nuclear morphology. This raises the possibility that even strong accumulation of autophagosomes alone without increases in autophagic flux can induce cell death. Identification and characterization of autosis in the heart would help to elucidate when and how autophagy participates in death of cardiac myocytes and myocardial injury in response to stress. In addition, because cardiac glycosides are available to block the Na⁺,K⁺-ATPase in humans, it is important to determine whether this protein is rate limiting in cardiac myocyte death mediated by autosis. Furthermore, if this is the case, it may be possible to develop novel, small-molecule inhibitors of this protein with reduced arrhythmogenic properties.

In summary, increasing lines of evidence suggest that cardiac myocytes can be killed by autophagy (Figure). Further
investigation is required to determine whether autophagic cell death occurs in pathophysiologically relevant conditions in the heart and, if so, what the underlying mechanisms are and how they can be prevented. Establishing a mouse model of cardiac autosis would be helpful to address these questions. The list of cellular functions mediated by autophagy is expanding rapidly from the aforementioned quality control and energetic balance to cellular defense, immunity, reprogramming, differentiation, cell–cell communication, and more. With this in mind, it may be that autophagy affects survival and death of cardiac myocytes both directly and indirectly through these many functions.

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References


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