Sterile Inflammation and Degradation Systems in Heart Failure

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In most patients with chronic heart failure (HF), levels of circulating cytokines are elevated and the elevated cytokine levels correlate with the severity of HF and prognosis. Various stresses induce subcellular component abnormalities, such as mitochondrial damage. Damaged mitochondria induce accumulation of reactive oxygen species and apoptogenic proteins, and subcellular inflammation. The vicious cycle of subcellular component abnormalities, inflammatory cell infiltration and neurohumoral activation induces cardiomyocyte injury and death, and cardiac fibrosis, resulting in cardiac dysfunction and HF. Quality control mechanisms at both the protein and organelle levels, such as elimination of apoptogenic proteins and damaged mitochondria, maintain cellular homeostasis. An imbalance between protein synthesis and degradation is likely to result in cellular dysfunction and disease. Three major protein degradation systems have been identified, namely the cysteine protease system, autophagy, and the ubiquitin proteasome system. Autophagy was initially believed to be a non-selective process. However, recent studies have described the process of selective mitochondrial autophagy, known as mitophagy. Elimination of damaged mitochondria by autophagy is important for maintenance of cellular homeostasis. DNA and RNA degradation systems also play a critical role in regulating inflammation and maintaining cellular homeostasis mediated by damaged DNA clearance and post-transcriptional regulation, respectively. This review discusses some recent advances in understanding the role of sterile inflammation and degradation systems in HF.

Key Words: Autophagy; Inflammation; Mitochondria; Nucleic acid degradation; Protein degradation

Heart failure (HF) is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or the ejection of blood. Despite progress in cardiovascular research and evidence-based therapies, HF is the leading cause of morbidity and mortality in industrialized countries.1 In most patients with chronic HF, circulating cytokine levels are elevated and positively correlate with the severity of HF and the patient’s prognosis, although infection with microorganisms is not involved.2 Leukocyte infiltration and upregulation of cytokine mRNA levels are observed in the failing heart.3 The vicious cycle of subcellular component abnormalities, inflammatory cell infiltration and neurohumoral activation induces cardiomyocyte injury and death, and cardiac fibrosis, resulting in cardiac dysfunction and HF.4 6

Inflammatory signaling in cardiomyocytes usually occurs as an early response to myocardial injury and entails overproduction of mitochondrial reactive oxygen species (ROS).7 The innate immune system plays a crucial role in acute inflammation caused by microbial infection or tissue damage.2 Pattern recognition receptors recognize not only pathogen-associated molecular patterns but also endogenous molecules released from damaged cells, termed damage-associated molecular patterns (DAMPs). The best-characterized cellular sources of DAMPs are the nucleus (e.g., high-mobility group box 1), mitochondria (e.g., mitochondrial DNA (mtDNA)), and the cytosol (e.g., RNA).8 TLRs are classically divided into 2 groups: cell surface TLRs, such as TLR2 and TLR4, and endosomal TLRs, such as TLR9. TLR2, TLR4, and TLR9 recognize lipoproteins, lipopolysaccharide, and DNA, respectively.2 The inflammasome is a central component of the sterile inflammatory response.9 The NLR family, pyrin domain-containing protein 3 (NLRP3) inflammasome, is the most fully characterized of the inflammasomes and contains the adaptor protein, apoptosis-associated speck-like protein, apoptosis-associated speck-like protein, the proinflammatory caspase, caspase-1, and NLRP3. Once assembled, inactive pro-caspase-1 in the inflammasome complex is auto-processed by proximity to active caspase-1, which subsequently induces the maturation of pro-interleukin (IL)-1β or pro-IL-18 to their active forms.10 11

Quality control mechanisms at both the protein and organelle levels, such as elimination of apoptogenic proteins and damaged mitochondria, maintain cellular homeostasis. An imbalance between protein synthesis and degradation is likely to result in cellular dysfunction and disease. Three major protein degradation systems have been identified, namely the cysteine protease system, including calpain, autophagy, and the ubiquitin proteasome system (UPS).12 14 Increased wall stress results in the accumulation of damaged mitochondria and apoptogenic proteins and then promotes cardiomyocyte death in the failing heart. Elimination of damaged mitochondria by autophagy is also important for maintenance of cellular homeostasis (Figure 1).15 17 Autoph-
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Involved in the development and progression of chronic HF. Furthermore, inflammation mediated by the innate immune system is involved in the pathogenesis of HF. The circulating levels of tumor necrosis factor (TNF) have been shown to be elevated in severe chronic HF. In most patients with chronic HF, circulating cytokines are elevated and the elevated cytokine levels correlate with the severity of HF and prognosis. We observed massive infiltration of CD45-positive leukocytes, including CD68-positive macrophages and Ly6G-positive neutrophils, in wild-type mouse failing hearts in response to pressure overload by thoracic transverse aortic constriction (TAC), and attenuation of inflammatory cell infiltration and HF in TAC-operated TLR9-deficient mice compared with TAC-operated wild-type mice (Figure 1). In contrast, we recently reported that TLR9 prevented cardiac rupture after myocardial infarction in mice independent of inflammation, possibly by promoting proliferation and differentiation of cardiac fibroblasts. Both TLR2 and TLR4 are strongly upregulated in chronic dilated cardiomyopathy and HF. Activation of TLR2 and TLR4 eventually leads to reduction of ejection fraction through nuclear factor-κB (NF-κB)-dependent mechanisms. The NLRP3 inflammasome effector, caspase-1, is upregulated in murine and human failing hearts. Genetic ablation of Nlrp3 in cardiac-specific calcineurin transgenic mice, which exhibit dilated cardiomyopathy, reduces pro-inflammatory cytokine maturation and cardiac inflammation, and improves systolic performance.

Sterile Inflammation and the Innate Immune System in HF

Activation of the neurohumoral and sympathetic systems, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress and impaired calcium handling are involved in the development and progression of chronic HF. Furthermore, inflammation mediated by the innate immune system is involved in the pathogenesis of HF. The circulating levels of tumor necrosis factor (TNF) have been shown to be elevated in severe chronic HF. In most patients with chronic HF, circulating cytokines are elevated and the elevated cytokine levels correlate with the severity of HF and prognosis. We observed massive infiltration of CD45-positive leukocytes, including CD68-positive macrophages and Ly6G-positive neutrophils, in wild-type mouse failing hearts in response to pressure overload by thoracic transverse aortic constriction (TAC), and attenuation of inflammatory cell infiltration and HF in TAC-operated TLR9-deficient mice compared with TAC-operated wild-type mice (Figure 1). In contrast, we recently reported that TLR9 prevented cardiac rupture after myocardial infarction in mice independent of inflammation, possibly by promoting proliferation and differentiation of cardiac fibroblasts. Both TLR2 and TLR4 are strongly upregulated in chronic dilated cardiomyopathy and HF. Activation of TLR2 and TLR4 eventually leads to reduction of ejection fraction through nuclear factor-κB (NF-κB)-dependent mechanisms. The NLRP3 inflammasome effector, caspase-1, is upregulated in murine and human failing hearts. Genetic ablation of Nlrp3 in cardiac-specific calcineurin transgenic mice, which exhibit dilated cardiomyopathy, reduces pro-inflammatory cytokine maturation and cardiac inflammation, and improves systolic performance.
Protein Degradation in HF

Cysteine Proteases

Calpain are Ca2+-sensitive cysteine proteases that degrade intracellular substrates, including cytoskeletal proteins, and are implicated in a wide variety of biological functions, including cell migration, cell cycle regulation, mitogenic and differentiation signaling, and apoptosis. Calpains are expressed ubiquitously and are heterodimers consisting of a large catalytic subunit encoded by \textit{Capml} for \( \mu \)-calpain and \textit{Capn}2 for m-calpain, respectively, along with a common small regulatory subunit encoded by \textit{Capn4}/\textit{Capn1}. Many studies have demonstrated that calpain inhibition contributes to cardioprotective effects. Cardiac-specific calpain 4/\textit{Capn}l-deficient mice show inhibition of NF-xB protein signaling/inflammation, and reduced cardiac remodeling after myocardial infarction. In contrast, we reported that calpains protected the heart from hemodynamic stress induced by TAC and were required for plasma membrane maintenance using our cardiac-specific calpain 4/\textit{Capn}1-deficient mice. Caspases consist of a cysteine protease cascade that is well defined as an essential proteolytic pathway to induce apoptosis. In addition to apoptosis, caspases are involved in tissue differentiation, regeneration, neural development, genome stability maintenance, autophagy, and inflammation. Caspase-1, an inflammatory caspase, plays a crucial role in NLRP3 inflammasome activation by cleaving pro-IL-1\( \beta \) or pro-IL-18 for maturation. Caspase-1 is upregulated in murine and human failing hearts.

Autophagy

Macroautophagy (commonly referred to as autophagy) is a conserved process from yeast to mammals for the bulk degradation and recycling of long-lived proteins and organelles. Intracellular components are surrounded by double membrane-bound autophagic vesicles that fuse with lysosomes to form autolysosomes for degradation. Controlled by autophagy-related proteins (Atgs), such as Atg5, Beclin 1, Atg7, and microtubule-associated protein 1 light chain 3 (LC3), autophagy plays an essential role in maintaining cellular homeostasis. When cardiac-specific Atg5-deficient mice are subjected to TAC, the mice develop cardiac dysfunction and left ventricular dilatation within 1 week. The mice show an accumulation of poly-ubiquitinated protein, increased endoplasmic reticulum stress, and the promotion of apoptosis.

We reported that autophagy was upregulated in the failing wild-type mouse heart 4 weeks after TAC, although Sadoshima’s group showed that cardiac autophagy was suppressed in the failing heart 30 days after TAC. Damaged mitochondria generate ROS and the myocardial ATP level drops in the failing heart, leading to AMP-activated protein kinase (AMPK) signaling activation. Both ROS accumulation and AMPK signaling activation upregulate autophagy. Autophagy has been reported in failing myocardium caused by dilated cardiomyopathy, valvular disease, and ischemic heart disease. Dead and dying cardiomyocytes displaying characteristics of autophagy have also been observed in animal models such as UM-7.1 cardiomyopathic hamsters. From this observation, the question remains as to whether autophagy is a sign of failed cardiomyocyte repair or is a suicide pathway for failing cardiomyocytes. MitDNA contains inflammatory unmethylated CpG motifs. We reported that mtDNA that escapes from autophagy caused inflammation and HF, and that the TLR9 signaling pathway played a crucial role in recognizing undergraded mtDNA and modulating sterile inflammation in stressed cardiomyocytes. When mtDNA was insufficiently degraded in autolysosomes by DNase II ablation in stressed cardiomyocytes, cytokines such as IL-1\( \beta \) and IL-6 were generated and CD45-positive cells, including CD68-positive and Ly6G-positive cells, showed massive infiltration (Figure 1). We recently reported that autophagy via the tuberous sclerosis complex 2 (TSC2)-mechanistic or mammalian target of rapamycin complex 1 signaling pathway plays an important role in maintenance of cardiac function and mitochondrial quantity and size in the heart using cardiac-specific TSC2-deficient mice. Furthermore, mechanical unloading of the failing human heart by left ventricular assist device support (with a mean duration of 214 days) decreased markers of autophagy. These findings suggest that autophagy is an adaptive mechanism in the failing heart.

Chaperone-mediated autophagy is also an important pathway involved in protein degradation and can be activated under stressed conditions. When chaperone-mediated autophagy is inhibited, autophagy is upregulated. Chaperone-mediated autophagy compensates when autophagy is inhibited. Danon’s cardiomyopathy is the result of a deficiency in lysosome-associated membrane protein 2, which plays a role in chaperone-mediated autophagy.

Ubiquitin Proteasome System (UPS)

The UPS is the main ATP-dependent protein degradation machinery in the cytosol and nucleus of eukaryotic cells and is responsible for the degradation of 80–90% of intracellular proteins. The UPS degrades proteins through the covalent attachment of the 8.5-kDa protein ubiquitin to target substrates and then through the 26S proteasome. The covalent modification of target proteins by ubiquitin occurs via an enzymatic cascade that includes an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligase, which both recruits protein substrates and mediates the attachment of ubiquitin. Poly-ubiquitinated proteins are increased in human cardiomyopathies and chronic HF. However, proteasome activity in both hypertrophic cardiomyopathic and failing human hearts is impaired, suggesting that proteasome functional insufficiency plays a major role in cardiac pathogenesis. In addition, enhancement of proteasomal function protects against cardiac proteinopathy and oxidative stress. Mechanical unloading with a left ventricular assist device support increases proteasome activity. An alternative form of the 20S proteasome, known as the immunoproteasome, is highly efficient proteolytic machinery derived from the constitutive proteasome and is abundantly expressed in immune cells. The immunoproteasome is also expressed in nonimmune cells during inflammation. The immunoproteasome is induced by interferon-\( \gamma \) and TNF-\( \alpha \) and plays an important role in the degradation of oxidized proteins. All 3 immunoproteasome-specific subunits are upregulated under acute exposure to oxidative stress in cultured cells.

The UPS acts as a protein degradation system but also regulates the NF-xB signaling pathway. The inhibitor of NF-xB (IxB) is ubiquitinated and degraded by the UPS, which in turn allows nuclear translocation of NF-xB. NF-xB is an inducible transcription factor that coordinates specific gene expression programs to affect the regulation of multiple physiological functions such as mediators of
Mitochondrial Degradation in HF

Damaged Mitochondria in HF
Mitochondrial fusion and fission are fundamental processes necessary for the health of mitochondria. Mitochondria undergo constant fusion and fission to change their shape and size to meet the metabolic demand in a cell. Mitochondrial fusion serves to mix and unify the mitochondrial compartment for the maintenance of mitochondrial functions. Mitochondria in the failing myocardium exhibit misalignment, disorganized cristae, membrane disruption, and aggregation. Small, damaged mitochondria are increased in the failing heart. Damaged mitochondria induce accumulation of ROS and apoptogenic proteins, and subcellular inflammation, resulting in cardiomyocyte death and eventually HF. Mitochondria in the failing myocardium exhibit decreased activity of electron transport complexes, decreased capacity for oxidative phosphorylation and increased oxidative stress. As mentioned before, when mtDNA is not completely degraded by autophagy, the remaining mtDNA induces inflammation in the heart. Mitochondria in the failing myocardium also exhibit increased levels of oxidized mtDNA, reduced mtDNA replication and depletion of mtDNA. Mitochondria released into the cytosol may also be involved in inflammation in the heart.

Mitophagy
Autophagy was initially believed to be a non-selective process. However, it has been revealed that there are selective types of autophagy, including mitophagy. Mitochondrial fission (mitochondrial fragmentation) is part of the mitochondrial degradation mechanism (mitophagy) and creates smaller mitochondria from dysfunctional mitochondria because the size of autophagosomes containing mitochondria is limited. Dnm1, a yeast homolog of dynamin-related protein 1, is a dynamin-related GTPase required for mitochondrial fission, and is recruited to the degrading mitochondria through its interaction with Atg11. Dnm1 mutants impair Atg11 binding and partially suppress mitophagy in yeast, suggesting a close relationship between mitochondrial fission and mitophagy.

Mitophagy is important for clearing mitochondria under basal conditions and in response to stress. There are two major pathways involved in regulating mitophagy: phosphatase

RNA Degradation
The RNA degradation system also plays an important role in regulating inflammation mediated by post-transcriptional regulation (Figure 1). The best-characterized cis element is ARE, which is localized in the 3' untranslated regions. ARE-binding proteins have been shown to regulate mRNA stability via the ARE-mediated pathway. These proteins include HuR, tristetraprolin (TTP), butyrate response factor 1, butyrate response factor 2, ARE RNA-binding protein 1 and KH-type splicing regulatory protein. Whereas HuR stabilizes ARE-containing mRNAs, other decay factors promote ARE-mediated decay. TTP destabilizes cytokine-encoding mRNA, such as TNF-α, by the binding to AREs of a set of mRNAs. We previously reported that the signal transducer and activator of transcription 6 (STAT6)-deficient mice showed left ventricular dilatation and cardiac dysfunction with increased apoptotic response 1 week after TAC. Prolonged induction of TNF- α mRNA was observed 1 and 2 days after TAC in STAT6-deficient hearts, whereas TNF-α mRNA was only transiently induced in wild-type hearts. TTP was induced 1 day after TAC in wild-type mice, but not in STAT6-deficient mice. These findings suggest that the sustained induction of TNF- α regulated by TTP via STAT6 may play a significant role in the development and progression of chronic HF.

Degradation of Nucleic Acids in HF
DNA Degradation
Circulating mitochondrial DAMPs cause inflammatory responses to injury. Circulating mtDNA is increased after myocardial infarction and ischemia-reperfusion injury. The release of mtDNA into the cytosol during mitochondrial dysfunction or apoptosis leads to NLRP3 inflammasome activation in macrophages. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that interacts with DNA directly and utilizes GTP and ATP to generate cyclic GMP-AMP capable of STING activation, resulting in activation of type I interferon and other immunomodulatory molecules. Dicer, a mitochondrial enzyme, is the key enzyme in mtDNA degradation. Dicer plays an important role in mtDNA degradation in autolysosomes. We recently reported that mtDNA degraded insufficiently in autolysosomes by cardiac-specific ablation of Dnase II led to severe myocardial inflammation mediated through TLR9 activation and cardiac dysfunction during pressure overload. These findings suggest that mtDNA may induce inflammation and cell death in cardiomyocytes (Figure 1). Furthermore, Dnase II activity was upregulated in hypertrophied hearts, but not in failing hearts, suggesting that upregulation of Dnase II activity may be a therapeutic target for HF.

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Inflammation and Degradation Systems

and tension homolog-induced putative kinase 1 (PINK1)/PARK2 (known as Parkin)-mediated mitophagy, and receptor-mediated mitophagy. PINK1 and PARK2 degrade damaged mitochondria in response to a reduction in the mitochondrial membrane potential and they do not interact with components of the autophagy machinery. The PINK1 protein level is markedly reduced in end-stage human failing hearts. PINK1-deficient mice develop left ventricular dysfunction and evidence of pathological cardiac hypertrophy as early as 2 months of age, and they have greater levels of oxidative stress and impaired mitochondrial function.

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PARK2-deficient mice show only mild phenotypes regarding behaviour and normal cardiac function, but disorganized and smaller mitochondria in the heart under basal conditions.

Regarding receptor-mediated mitophagy, Atg32 in yeast, and Bcl-2-like protein 13 (Bcl2-L-13), BCL2 adenovirus E1B interacting protein 3 (BNIP3), BNIP3-like (NIX/BINP3L), and FUN14 domain containing 1 (FUNDC1) in mammals localize on the outer membrane and promote mitophagy by directly binding to components of the autophagy machinery, such as Atg8 in yeast or LC3 in mammals. NIX/BINP3L is involved in specific types of mitophagy for mitochondrial elimination from reticulocytes. FUNDC1 is involved in hypoxia-induced mitophagy. BNIP3 and NIX/BINP3L play dual roles in the regulation of mitophagy as well as cell death in the heart. Their regulatory mechanism is not fully understood, but both BNIP3 and NIX/BINP3L mediate the progression of cardiac diseases through cardiomyocyte death.

Atg32 is essential for mitophagy through its interaction with Atg8 and a scaffold protein, Atg11 in yeast, but no Ag32 homolog was identified in mammalian cells until recently. We recently reported that Bcl2-L-13, also known as Bcl-rambo, is a functional mammalian Atg32 homolog that mediates mitophagy and mitochondrial fragmentation.

The second WXXLI motif at residues 273–276 was a functional LC3-interacting region. Bcl2-L-13 was essential for a mitochondrial uncoupler, carbonyl cyanide m-chlorophenyl hydrazone-induced mitochondrial fragmentation and mitophagy. Bcl2-L-13 induced mitophagy in PARK2-deficient cells, indicating that PARK2 is not essential for Bcl2-L-13-induced mitophagy. All Bcl2 homology domains (BH) 1–4 were involved in Bcl2-L-13-induced mitochondrial fragmentation. The mutation study of Bcl2-L-13 at serine 272 to alanine showed that phosphorylation at serine 272 was involved in LC3 binding and mitophagic activity, but not mitochondrial fragmentation. Because no mammalian homolog of Atg11 has been identified, Bcl2-L-13 may have dual functions of Atg32 and Atg11 or bind to an unidentified mammalian homolog of Atg11 as a scaffold protein. However, it is unclear whether Bcl2-L-13 plays a physiological role in the heart (Figure 2).

Closing Remarks

We have described some recent advances in our understanding of the role of sterile inflammation and degradation systems in HF. However, there are still unanswered questions, such as (1) how sterile inflammation is regulated in the failing heart, and (2) how the degradation systems are activated and regulated in the failing heart. Because ROS and apoptogenic proteins leak from damaged mitochondria, it is important to elucidate how to regulate mitophagy, including the physiological role of Bcl2-L-13 in the heart. A better understanding of the unanswered questions will potentially generate a novel therapy for preventing or slowing the development and progression of HF.

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Conflict of Interest

The authors declare no competing financial interests.

References

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Figure Bcl2-L-13 plays a physiological role in the heart (Figure 2).


