Basic mechanisms in apoptosis and heart failure

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Conflicts of interest: None.

Abstract

The syndrome of heart failure may arise from different causes but there are features that are common to all: myocardial fibrosis, desensitization of β-adrenergic receptor signalling, excitation-contraction uncoupling, myosin isoform switch, altered energy utilization and ''cell loss''. These unifying features imply that the molecular pathways that underpin them are activated in most, if not all, instances of heart failure. Good response to therapy such as angiotensin receptor or β-adrenergic receptor blockade, is also not selective to only specific causes of heart failure. Thus many molecular mechanisms now form important targets in the heart failure drug discovery pipeline.

The best-understood form of "cell loss" in the myocardium is APOPTOSIS. Both evolutionarily conserved pathways of apoptosis: extrinsic and intrinsic, are activated in heart failure. Apoptotic cell death involves the recruitment of death activating complexes whose formation is dependent on specific protein motifs called death domain motifs. Apoptotic cell death also characteristically requires the activation of caspases* that selectively cleave proteins, eventually leading to cell disassembly. This review covers the basic mechanisms involved in apoptosis and heart failure.

■ *Heart Metab.* 2010;47:5–8.

Keywords: Apoptosis, caspases, death domain motifs, heart failure

Introduction

Molecular mechanisms in heart failure are increasingly well understood [1], and many aspects of these mechanisms currently form important targets in the heart-failure drugs-discovery pipeline [2]. Although heart failure may result from a variety of heterogeneous causes, it is striking that these are nonetheless linked by distinct unifying features such as myocardial fibrosis, desensitization of β-adrenergic receptor signaling, excitation–contraction uncoupling, myosin isoform switch, and altered energy utilization [2]. ''Cell loss'' is another unifying feature found in nearly all forms of cardiomyopathy [3–5], and loss of myocytes is predicted to decrease contractility and promote cell slippage, wall thinning, and chamber dilatation. Currently, the best understood form of cell loss or cell death in heart failure is apoptosis. This review aims to cover the mechanisms of the basic science involved in apoptosis.

Apoptosis is a regulated mode of cell death in multicellular organisms [6]. It is critical for sculpting tissue during development, and is also activated when tissues are exposed to injury, when irreparable damage is done and affected cells have to be eliminated. Apoptosis is often contrasted to necrosis, in which cell death involves cell lysis and produces a surrounding inflammatory response. Recently, however, tightly regulated processes in necrosis have also been identified [7]. Even so, there are biochemical and morphological changes that classically characterize apoptosis: cellular and nuclear shrinkage, chromatin condensation, cell membrane blebbing, formation of apoptotic bodies, and DNA fragmentation [8]. Most of these

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features, best seen on electron microscopy, remain the hallmarks of apoptotic cell death.

There are two evolutionarily conserved pathways of apoptosis. The *extrinsic* pathway utilizes cell-surface death receptors and links external stimuli to intracellular apoptotic cell death machinery. The *intrinsic* pathway involves the mitochondria and endoplasmic reticulum, which, again, sense stimuli and transduce signals to execute apoptosis via another distinct set of molecules.

The extrinsic apoptotic pathway

In the extrinsic pathway, death ligands (such as FasL) interact and bind with their respective cell-surface death receptors (such as Fas ligand receptor), reorganizing the inactive receptor and stimulating the recruit-

ment of adaptor proteins [such as Fas-associated via death domain (FADD)], which in turn recruits procaspase-8 into a multiprotein complex called the death-inducing signaling complex (DISC) [9]. Clustering of these interacting proteins within the DISC promotes autoproteolytic processing and activation of the caspase-8 by induced proximity [10]. In some cells, processed caspase-8 is sufficient to activate the other downstream effector caspases directly, leading to the execution phase of apoptosis (see below). In other cells, activation of downstream effector caspases further requires the amplification loop, where caspase-8 mediates cleavage of the proapoptotic Bcl-2 family member, Bid, which subsequently releases mitochondrial proapoptotic factors [11], linking the extrinsic pathway to the intrinsic pathway of apoptosis (see below). *Figure 1* summarizes the interlinked pathways of the extrinsic and intrinsic apoptotic cascades.
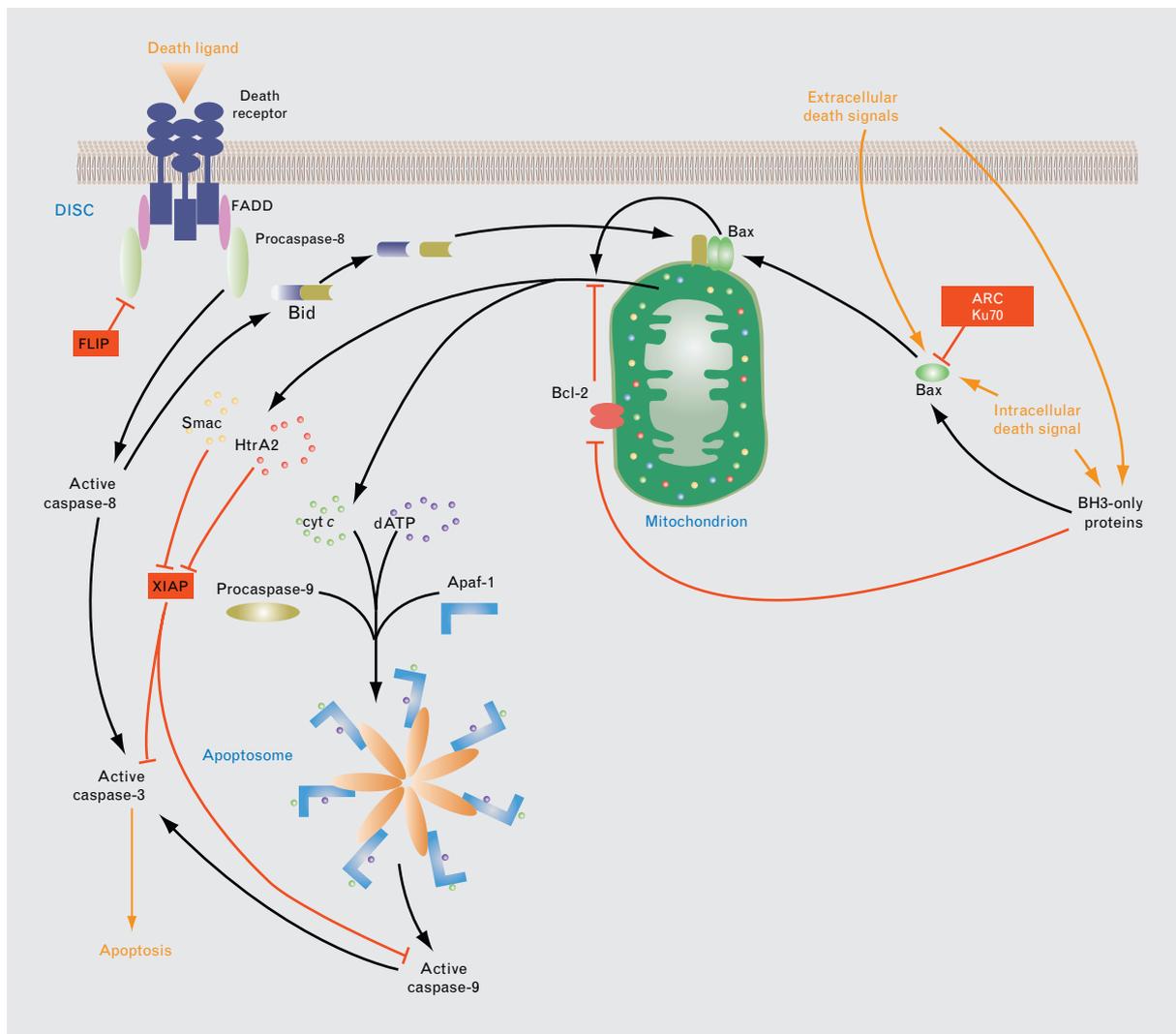

Figure 1. The apoptotic cascade. ARC, apoptosis repressor with a caspase recruitment domain; cyt c, cytochrome c*; DISC, death-inducing signaling complex; FADD, Fas-associated via death domain; FLIP, FADD-like interleukin-1b-converting enzyme-like inhibitory protein; XIAP, X-linked inhibitor of apoptosis. (Adapted from Foo et al [27], with permission.).

Formation of the multiprotein DISC complex is critically mediated by interactions between "death domain motifs" that are present on each of the components in the protein complex (eg, Fas, FADD, caspase-8). Death domain interactions are characteristic in the formation of "death" complexes in both pathways of apoptosis. FasL–Fas interaction and DISC complex formation are regulated by the soluble endogenous decoy receptor, DcR3 [12], by various Fas isoforms lacking the death domain [13], or by soluble FasL generated by proteolytic processing or alternative splicing [14]. The *FasL* gene is often transcriptionally inactive, and upregulated by transcription factors such as nuclear factor kappa B (NFκB), and nuclear factor of activated T cells (NFAT) [15]. Expression of *Fas* may, similarly, be regulated by the transcription factor, p53 [16]. The extrinsic pathway of apoptosis is also held in check by the endogenous anti-apoptotic FADD-like interleukin-1b-converting enzyme-like inhibitory protein (FLIP) [17]: FLIP binds to and inhibits procaspase-8. The protein, Itch, is a ubiquitin E3 ligase for FLIP, mediating FLIP ubiquitination and degradation. In death receptor tumor necrosis factor-α signaling, Jun kinase-mediated activation of Itch is proapoptotic because it leads, downstream, to FLIP protein degradation [18].

The intrinsic apoptotic pathway

The intrinsic pathway transduces extracellular and intracellular stimuli, including nutrient depletion, radiation, hypoxia, oxidative stress, ischemia-reperfusion, and DNA damage. Direct signaling from each of these is unclear, but they converge on the pivotal event of mitochondrial outer membrane permeabilization (MOMP) [19].

At the mitochondria, MOMP often follows dissipation of the mitochondrial inner transmembrane potential ($\Delta\varphi m$), which may involve opening of the mitochondrial permeability transition pore [19]. A separate mechanism for MOMP involves members of the Bcl-2 family of proteins acting at the outer mitochondrial membrane. *Bcl-2* (the mammalian homologue of *ced-9*) is the prototype of the important family of genes in this intrinsic pathway of apoptosis [20]. *Bcl-2* family members share Bcl-2 homology (BH) domains. The BH123 (multidomain) members, Bax and Bak, are proapoptotic proteins that, upon apoptotic stimuli, undergo conformational change, oligomerize, and translocate to the mitochondrial outer membrane, promoting MOMP. Cells lacking the *Bax* and *Bak* genes fail to undergo MOMP, reflecting the critical role for these multidomain proteins in the intrinsic pathway of apoptosis. The other subfamily, BH3-only proteins, are proapoptotic and can activate Bax/Bak either directly (effectors; eg, Bid and Bim) or by interfering with anti-apoptotic Bcl-2 family members (sensitizers; eg, Puma, Noxa, Bad). Anti-apoptotic Bcl-2 family proteins such as Bcl-2 itself, Bcl-xL and Mcl-1 prevent MOMP by sequestering BH3-only proteins, and probably also Bax and Bak themselves. Recently, p53 was shown to have a non transcription-related role in apoptosis by translocating to the mitochondria and directly promoting the oligomerization of Bax and Bak (acting like an effector), or by binding and neutralizing anti-apoptotic members Bcl-xL and Mcl-1 (acting like a sensitizer) [21]. An emerging theme is of other nuclear proteins that function in the cytosol through interaction with Bcl-2 family proteins; these include Ku70 which, apart from being involved in DNA repair, also inhibits the translocation of Bax to the mitochondria [22]. Nuclear protein TR3 also binds Bcl-2 and promotes MOMP [23]. Histone 1.2, released from the nucleus after DNA damage, triggers MOMP – again via an interaction with Bcl-2 family members [24].

After MOMP, critical apoptogens (eg, cytochrome *c*) are released from the mitochondrial intermembrane space into the cytosol [25]. When in the cytosol, cytochrome *c* binds to the adaptor protein, Apaf-1. Apaf-1 oligomerizes into an apoptosome, which recruits and activates caspase-9. Like DISC in the extrinsic pathway, recruitment of caspase-9 in the apoptosome is dependent on "death domain motifs" on both caspase-9 and Apaf-1 [19]. As with caspase-8, activated caspase-9 activates downstream effector caspases and leads to the execution phase of apoptosis. This sequence of events is depicted at bottom left in *Figure 1*.

Caspases

This family of cysteine proteases recognize specific peptide sequences and cleave proteins only after an aspartic acid residue. The specificity of caspases is consistent with the characteristic that apoptosis does not involve indiscriminate protein digestion, but rather a selection of proteins is cleaved in a coordinate manner, resulting in cell disassembly. Specific substrates of downstream effector caspases (traditionally believed to be 3 and 7) include inhibitor of caspase-activated DNase (the inhibitor of the nuclease responsible for DNA fragmentation), the nuclear lamina, and cytoskeleton regulators such as focal adhesion kinase. In unstressed conditions, effector caspases are inhibited by endogenous X-linked inhibitor of apoptosis (XIAP). Upon apoptotic stimuli, another set of mitochondrial apoptogens (Smac/DIABLO and Omni/HtrA2) are released from the mitochondria [26]. These bind and inactivate XIAP and thereby activate effector caspases.

Significance of apoptosis in heart failure

Heart failure is characterized by a very low, but significantly increased prevalence of myocyte apoptosis (0.08–0.25% in individuals with dilated cardiomyopathy, compared with 0.001–0.002% in controls) [27]. Several mouse models demonstrate that myocyte apoptosis itself directly causes dilated cardiomyopathy [28–30], and increased apoptosis may mark the transition from compensated hypertrophy to decompensated cardiomyopathy [31]. Moreover, both extrinsic and intrinsic pathways of apoptosis operate in the stressed myocardium [27]. Loss of survival signals demonstrates that accelerated apoptosis aggravates cardiomyopathy [29], and overexpression of anti-apoptotic proteins such as Bcl-2 [32] provides proof of concept that apoptosis is a valid target for the design of novel heart failure therapy.

* see glossary for definition of these terms. ■

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