

Review

## Myocyte apoptosis in heart failure

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### Abstract

Human heart failure is preceded by a process termed cardiac remodeling in which heart chambers progressively enlarge and contractile function deteriorates. Programmed cell death (apoptosis) of cardiac muscle cells has been identified as an essential process in the progression to heart failure. The execution of the apoptotic program entails complex interactions between and execution of multiple molecular subprograms. Unlike necrosis, apoptosis is an orderly regulated process and, by inference, a logical therapeutic target if intervention occurs at an early stage. To identify potential therapeutic targets, it is imperative to have a full understanding of the apoptotic pathways that are functional in the cardiac muscle. Accordingly, the present review summarizes the apoptotic pathways operative in cardiac muscle and discusses therapeutic options related to apoptosis for the future treatment of human heart failure.

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*Keywords:* Apoptosis; Heart failure; Cell death

### 1. Introduction

Heart failure, the end-stage of various forms of heart disease, is associated with high mortality in modern Western societies [1]. Multiple factors are involved in the etiology of heart failure. A powerful predictor for the development of heart failure is the presence of left ventricular hypertrophy [2]. It is believed that progressive deterioration of the hypertrophied left ventricle (LV), which will eventually precipitate in heart failure, is related to progressive loss of cardiac myocytes [3,4].

During the last few years there has been increasing evidence from human and animal models suggesting that apoptosis or programmed cell death could be a key modulator especially in the transition from “compensato-

ry” hypertrophy to heart failure. Although endothelial cell apoptosis is also frequently found in heart failure biopsies, and could contribute to the ensuing decompensation, this review will focus on programmed cell death of cardiac muscle cells. Cardiomyocyte apoptosis has been documented as a pivotal form of cell death in ischemia and reperfusion damage, with several reports documenting apoptotic rates of 2–12% in the border zone of human myocardial infarcts [5,6]. Taking these values into account, it is not hard to imagine that such dramatic loss of viable tissue can have a disastrous effect on the geometry and function of the left ventricle. These high levels of programmed cell death contrast with the much lower incidence of this form of cell death in post-mortem animal and human biopsies with end-stage failure. Human failing hearts in NYHA classes III–IV typically display apoptotic rates ranging anywhere between 0.12% and 0.70% (see Table 1) [7–14]. Obviously, slight variances in technical approaches to detect PCD account for the

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Table 1  
Incidence of apoptosis in human explant heart failure biopsies

| Reference<br>[#]       | Primary<br>disease |              | Number of apoptotic cells<br>(per 10 <sup>5</sup> cells) |         |
|------------------------|--------------------|--------------|--|---------|
|                        |                    |              | HF   | Control |
| Olivetti<br>et al. [9] | CHF                |              | 232  | 1       |
|                        | ischemic           |              | 243  |         |
|                        | idiopathic         |              | 236  |         |
| Saraste<br>et al. [7]  | CHF                | overall      | 119  | 11      |
|                        | progression        | rapid        | 192  |         |
|                        | to allograft       | intermediate | 93   |         |
|                        |                    | slow         | 26   |         |
| Guerra<br>et al. [10]  | CHF                | female       | 80   | 2       |
|                        | CHF                | male         | 180  |         |
| Narula<br>et al. [11]  | ischemic           |              | 576  | n/a     |
|                        | DCM                |              | 1837   |         |
| Raymart<br>et al. [12] | ischemic           |              | 102  | n/a     |
|                        | DCM                |              | 553  |         |
| Knaapen<br>et al. [13] | ischemic           |              | 1.4  | n/a     |
|                        | DCM                |              | 1.8  |         |
| Latif<br>et al. [14]   | ischemic           |              | 49   | 7.1     |
|                        | DCM                |              | 30   |         |

CHF, chronic heart failure; DCM, dilated cardiomyopathy; HF, heart failure; n/a, not applicable.

differing apoptotic incidences found between the studies. A conceptual question is how loss of 0.1% of contracting muscle could have such a profound influence on overall geometry and function of the complete organ?

To begin to understand this, one should take into account that the documented apoptotic rates were obtained in explanted human tissue, i.e. at one single time point in the disease. If one considers that cellular apoptosis is a process that takes at most 24 h to complete, and that heart failure is a condition that only manifests itself after many years, it is conceivable, that chronic loss of small numbers of cardiomyocytes on a daily basis can have dramatic consequences on myocardial integrity. Alternatively, the low rate at end-stage heart failure does not necessarily reflect the rate during episodes of active disease, especially at phases accompanied by (endocardial) regions with insufficient perfusion and active ischemia.

In addition, strong scientific evidence now also has been put forward, demonstrating that apoptotic cell death plays a pivotal role in the development of heart failure. Studies using genetically modified mice clearly indicate a direct, causal relation between levels of apoptosis and the progression towards advanced heart failure. Most notably, the group of Kitsis recently generated transgenic mice that express a conditionally active caspase exclusively in the myocardium, and demonstrated that very low levels of myocyte apoptosis (23 myocytes per 10<sup>5</sup> nuclei) caused a lethal, dilated cardiomyopathy in otherwise normal hearts [4]. Importantly, the apoptotic rates were much lower than those observed in failing human hearts (Table 1). Further, even in studies where apoptosis was not the primary focus, apoptosis is often found to

accompany the development of heart disease, [15] while conversely, attenuation of the development of heart failure is often accompanied by inhibition of apoptosis [16]. Therefore, limiting cardiac muscle loss by inhibiting apoptosis may have implications for the treatment of heart failure.

## 2. The cardiac apoptotic pathways

Apoptosis is a highly regulated biological process that regulates the balance between pro-death and pro-survival cell signals, the outcome of which is key for cell fate. Most apoptotic signaling pathways discovered thus far in extra-cardiac cell types have also been found to play a crucial role in induction of apoptosis in the heart, and therefore we will give only a brief overview of the mechanisms here as countless excellent reviews topic these basic mechanisms in detail [17,18].

The key to understanding apoptosis is the activation and function of caspases, a group of cysteinyl-aspartate-directed proteases. In healthy cells, caspases reside in the cytosol as inactive proforms. In most cases, caspases are activated by proteolytic cleavage [19]. Active caspases cleave vital substrates in the cell, leading to cellular demise. In the heart, confirmed caspase substrates include crucial molecules for cellular homeostasis such as  $\alpha$ -actin,  $\alpha$ -actinin,  $\alpha$ / $\beta$ -myosin heavy chain, myosin light chain 1/2, tropomyosin, and cardiac troponins [20]. Cleavage of key molecules for cellular function (execution) is performed by caspase-3, -6 and -7, which, accordingly, are referred to as executioner caspases. Two major apoptotic pathways (i.e. pathways that eventually lead to activity of executioner caspases), the “extrinsic” and “intrinsic” cascades, transduce apoptotic signals in mammalian cells.

The “intrinsic” pathway utilizes mitochondria to propel cell death through opening of the mitochondrial permeability transition pore (MPTP) or rupture of outer mitochondrial membrane, triggering the sudden and complete release of cytochrome *c* and other proteins from the intermembrane space of mitochondria into all other compartments of the cell. The “intrinsic” pathway is primarily activated in myocytes by cellular stimuli such as hypoxia, ischemia–reperfusion, and oxidative stress, which provoke the mitochondrial permeability transition, an increased permeability of the outer and inner mitochondrial membranes [21–23]. The mitochondrial permeability transition pore, a protein complex that spans both membranes, is considered the mediator of this event and consists of at least the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, and cyclophilin-D in the matrix (Fig. 1) [21,23]. Upon permeabilization of the mitochondrion, several intermembrane proteins are released into the cytosol, including cytochrome *c*, Smac/DIABLO, endonuclease G (Endo G), Omim/Htr and

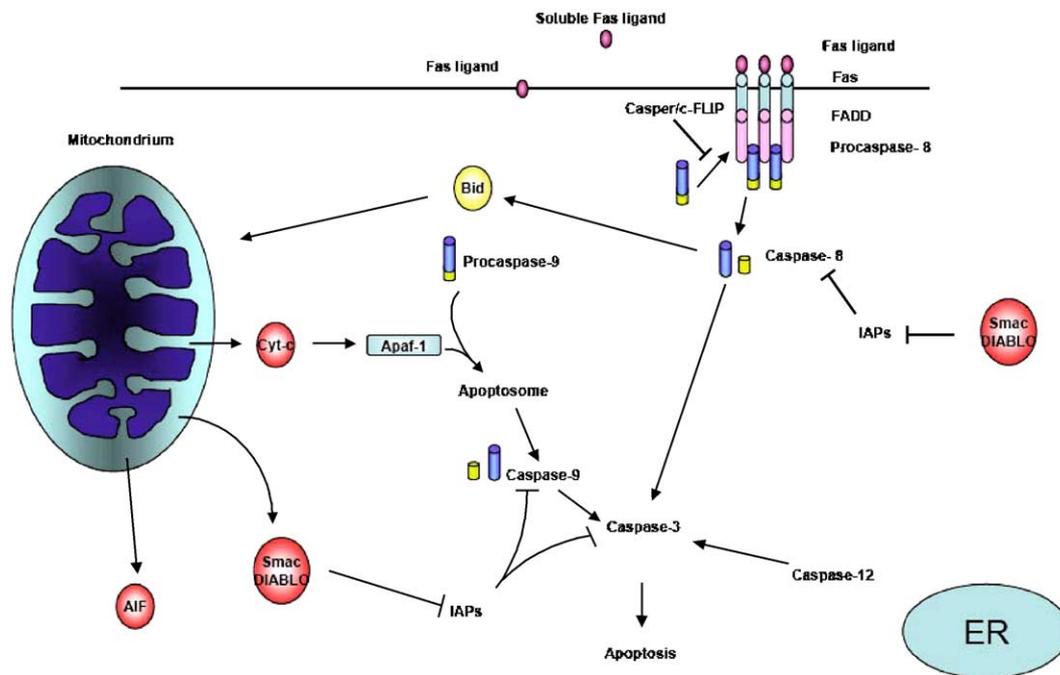


Fig. 1. Apoptotic pathways. Two major apoptotic pathways are active in mammalian cells, including the cardiac muscle cell. Mitochondria play a key role in the “intrinsic” pathway. The mitochondrial death pathway is mediated by intracellular and extracellular death-signals that impinge upon mitochondria leading to the disruption of normal mitochondrial physiology, leading to opening of the mitochondrial permeability transition pore (MPTP). The MPTP is a non-specific pore comprised of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase (ANT) and cyclophilin D as well as other proteins. The MPTP permits the movement of small molecules (<1.5 kDa) between the cytosol and the mitochondrial matrix. Upon permeabilization of the mitochondrion, several intermembrane proteins are released into the cytosol, including cytochrome *c*, Smac/DIABLO, endonuclease G (Endo G), Omim/Htr and apoptosis-inducing factor (AIF). Specifically, cytochrome *c* binds to the cytosolic protein apaf1 facilitating formation of the “apoptosome” complex, which results in caspase-9 activation that in turn provokes caspase-3 activation. Secondly, the “extrinsic” death-receptor pathway is triggered by binding of members of the death-receptor superfamily, such as Fas/CD95 and TNF- $\alpha$  to their cognate receptors, which induces receptor clustering. Activation of the cognate receptors triggers recruitment of the adaptor molecule, FADD. This results in the generation of a death-inducing signaling complex (DISC), which is capable to activate caspase-8 and caspase-3. The “extrinsic” and “intrinsic” pathways converge upon the effector caspases, resulting in the cleavage of substrates and cell death. Connections between the “intrinsic” and “extrinsic” pathways exist through caspase-8 mediated cleavage of Bid, resulting in its translocation to mitochondria, where it activates Bak and promotes cytochrome *c* release. Caspases can be inhibited by inhibitory of apoptosis (IAP)-proteins, which in turn can be inhibited by Smac/DIABLO.

apoptosis-inducing factor (AIF) (Fig. 1). Once released, cytochrome *c* binds to the cytosolic protein apaf1 facilitating formation of the “apoptosome” complex, which results in caspase-9 activation that in turn provokes caspase-3 activation [24]. Smac/DIABLO indirectly activates caspases by sequestering caspase-inhibitory proteins, [25] while release of Endo G and AIF from mitochondria results in their translocation to the nucleus where they either directly or indirectly facilitate DNA fragmentation [26].

The “extrinsic” apoptotic pathway entails the death-receptor pathway, triggered by members of the death-receptor superfamily, such as the Fas receptor or the tumor necrosis factor  $\alpha$  receptor (TNFR) (Fig. 1). Binding of the transmembrane protein Fas ligand to its cognate receptor induces receptor clustering and formation of a death-inducing signaling complex (DISC) [27]. This complex recruits multiple procaspase-8 molecules via the adaptor molecule FADD (Fas-associated death domain protein), resulting in proximity-induced caspase-8 activation [28]. Fas ligand is abundantly present in

adult cardiomyocytes and its expression increases in response to pathologic stimuli [29]. Cardiac overexpression of Fas ligand results in accentuated apoptosis in vitro, while Lpr mice, which lack Fas, display less apoptosis and a reduced infarct size in I/R studies [30]. Circulating Fas ligand levels are elevated in human heart failure, which may reflect activation of the Fas–Fas ligand system [31].

Apart from the mitochondrial and death receptor pathways, additional genetic programs must exist, as apoptosis is still present in mice defective in both caspase-8 (crucial for the death receptor pathway) and caspase-9 (crucial for the mitochondrial pathway) [32]. Another pathway is initiated by caspase-12. Caspase-12 is localized to the endoplasmic reticulum (ER) and is specifically activated by ER stress [33]. The ER is an organelle that ensures correct protein folding, however, under various conditions, such as glucose deprivation or disturbance of intracellular calcium homeostasis, unfolded proteins accumulate in the ER lumen and provoke ER stress [34].

### 3. Regulation of apoptosis

The Bcl-2 family of proteins has emerged as a key regulatory component of the cell death process [35,36]. The growing Bcl-2 family consist of death antagonists (Bcl-2, Bcl-xL) and death agonists (Bax, Bak), which function primarily to protect or disrupt the integrity of the mitochondrial membrane and control the release of (pro)apoptotic intermembrane proteins (Fig. 2) [18]. Another class of death effectors, called BH3-only proteins, serves as ligands to activate pro-apoptotic Bcl-2 family members or inactivate anti-apoptotic Bcl-2 members. Gene targeting of the Bcl-2 family has demonstrated that some of these molecules have unique functions in cell death. Overexpression of Bcl-2 in cardiomyocytes prevents the loss of the electro-potential of the mitochondrial membrane, and prevents the release of mitochondrial intermembrane proteins. Overexpression of Bcl-2 is cytoprotective, attenuating apoptosis induced by p53, a transcription factor activated in response to DNA damage [37]. Bcl-2 overexpression also protects against hypoxia/reoxygenation induced apoptosis in cardiomyocytes in vitro and cardiac ischemia/reperfusion injury in vivo [38,39].

The functions of the executioner caspases are modulated by another set of proteins, the IAPs (inhibitor of apoptosis proteins). The inhibitors of apoptosis proteins (IAPs)

comprise a family of proteins that oppose caspases, and include XIAP, survivin, HIAP1, HIAP2, NAIP, Livin, Ts-IAP and Apollon (Fig. 3). Among the IAPs, XIAP (X chromosome-linked IAP) is the best-characterized and most potent caspase inhibitor, as it has been demonstrated to target initiator caspase-9 and effector caspase-3 and caspase-7 [40]. It has been demonstrated that in the failing human heart, IAPs, such as XIAP, are downregulated, theoretically rendering the cardiomyocytes more sensitive to apoptosis [41].

A factor involved in the regulation of IAPs entails Smac/DIABLO [42,43]. Smac/DIABLO is released from the mitochondria along with cytochrome *c* during apoptosis. Binding of Smac/DIABLO to IAPs disrupts the binding of IAPs to caspases and relieves the inhibition of apoptosis [44]. Recent studies demonstrate that during cardiac ischemia/reperfusion Smac/DIABLO translocates from the mitochondria to the cytosol, [45] suggesting that Smac/DIABLO and its pro-death function, is operative in the cardiac muscle.

Another novel protein identified to have similar characteristics in antagonizing IAP function is named Omi/HtrA2 [46,47]. Until very recently, this pro-apoptotic candidate molecule was not studied in the heart, but its function was demonstrated in a study where mice were treated with ucf-101, a compound that shows high selectivity against the serine protease activity of Omi/HtrA2

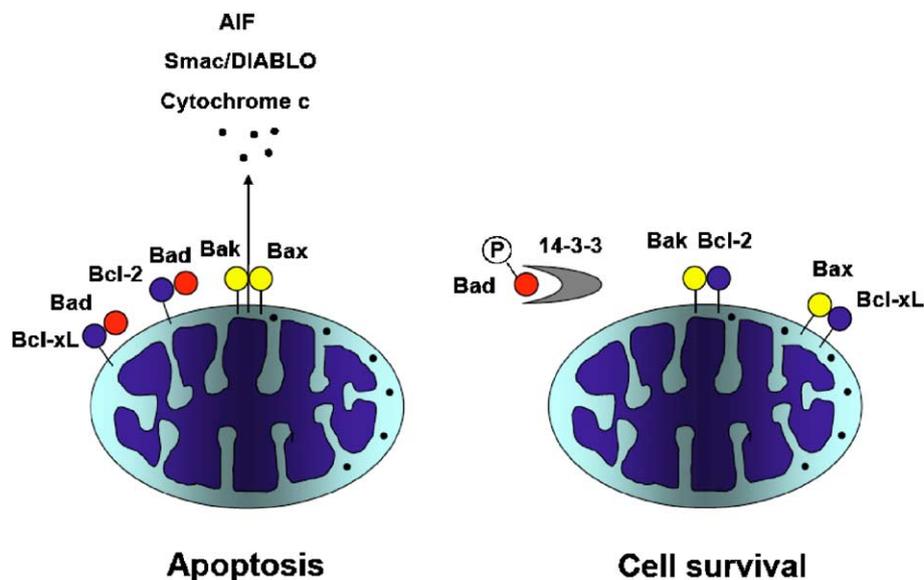


Fig. 2. Function of Bcl-2 family proteins. Named after the founding member of the family, which was isolated as a gene involved in B-cell lymphoma (hence the name *bcl*), the Bcl-2 family is comprised of well over a dozen proteins, which have been classified into three functional groups. Members of the first group, such as Bcl-2 and Bcl-xL, are characterized by four short, conserved Bcl-2 homology (BH) domains (BH1–BH4). They also possess a C-terminal hydrophobic tail, which localizes the proteins to the outer surface of mitochondria with the bulk of the protein facing the cytosol. The key feature of group I members is that they all possess anti-apoptotic activity, and protect cells from death. In contrast, group II consists of Bcl-2 family members with pro-apoptotic activity. Members of this group, which includes Bax and Bak, have a similar overall structure to group I proteins, containing the hydrophobic tail and all but the most N-terminal, BH4 domain. Group III consists of a large and diverse collection of proteins whose only common feature is the presence of the ~12–16-amino-acid BH3 domain. The Bcl-2 family of proteins function primarily to protect or disrupt the integrity of the mitochondrial membrane and control the mitochondrial release of pro-apoptotic proteins like cytochrome *c*, AIF and Smac/DIABLO. Anti-apoptotic Bcl-2 members (Bcl-2, Bcl-xL) protect the mitochondrial membrane. In response to environmental cues, these anti-apoptotic proteins engage another set of pro-apoptotic proteins of the Bax subfamily (which includes Bax, Bak), normally loosely residing on the mitochondrial outer membranes or the cytosol. The interaction between Bak and Bax proteins results in oligomerization and insertion into the mitochondrial membrane of the complete complex.

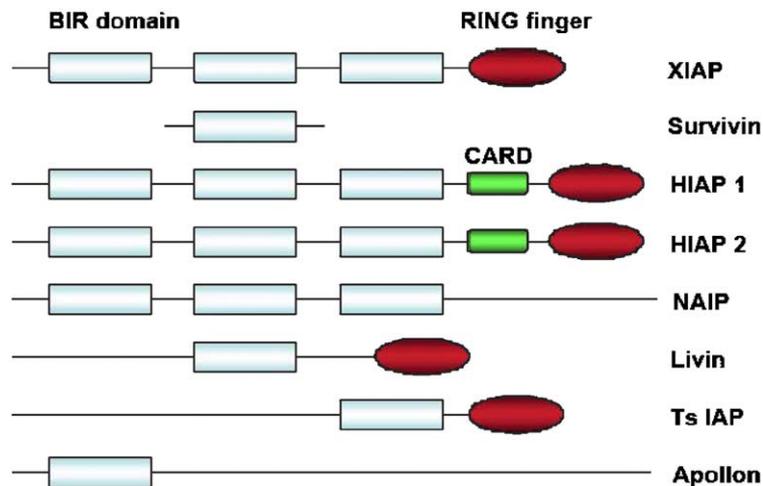


Fig. 3. Inhibitor of Apoptosis (IAP) gene family. IAP proteins are characterized by the presence of one or more baculoviral IAP repeats (BIR) motifs, the number of BIR domains is variable, but all found near the N-terminus. Some IAPs also contain a C-terminal RING zinc-finger domain, which has an ubiquitin E3 ligase activity. HIAP1 and HIAP2 have conserved the caspase recruitment domain (CARD), the function of the domain in these molecules is currently unknown. BIR, baculovirus IAP repeat; CARD, caspase-recruitment domain; NAIP, neuronal apoptosis inhibitory protein.

[47]. Omi/Htr antagonism resulted in marked protection against ischemia/reperfusion injury of the murine heart [48]. Whether Omi/HtrA2 antagonism is similarly efficacious in the setting of heart failure remains to be determined, but this novel pharmacological agent may hold promise as a therapeutic device.

XIAP-interacting protein-1 (XAF1) seems able to interact with and inhibit specifically one IAP; XIAP [49]. One hypothesis is that the inhibition actually occurs in the nucleus. Since activated caspase-9 can disrupt the nuclear membrane, XIAP, bound to the caspase-3 complex, could translocate into the nucleus where XAF1 would relieve the XIAP inhibition, allowing the caspases to proceed with the apoptotic process [50]. Interestingly, XAF1 is abundantly expressed in the heart, suggesting a possible important role for XAF1 in regulating cardiac apoptosis, and by its mere abundance in the heart, would provide a rationale why this organ is relatively susceptible to cell death [49].

ARC (apoptosis regulator with caspase recruitment domain), a gene uncovered by Nunez and associates, is predominantly expressed in skeletal and cardiac muscle [51]. ARC is an apoptosis repressor with a caspase recruitment domain (CARD) and interacts with caspase-2, caspase-8, but not with caspase-1, -3, or -9. Overexpression of ARC inhibits apoptosis induced by Fas, TNF $\alpha$ , and caspase-8, but not by caspase-9 [51]. Furthermore, hypoxia in cell culture induces loss of endogenous ARC in the cytosol and was associated with translocation of ARC from the cytosol to intracellular membranes, release of cytochrome *c* from the mitochondria, activation of caspase-3 and cleavage of PARP [52]. Overexpression of ARC maintains cytosolic levels of ARC and inhibits all apoptotic features, including cytochrome *c* release [52]. Because caspase inhibitors fail to inhibit cytochrome *c* release, these studies predict that ARC acts upstream of caspase activation

in this model of apoptosis, perhaps at the mitochondrial level.

#### 4. Therapeutic implications

Many of the illnesses in Western societies can be attributed directly or indirectly to dysregulation of programmed cell death. Disorders in which defective regulation of apoptosis contributes to disease pathogenesis or progression involves either cell accumulation, in which cell eradication or cell turnover is impaired (e.g. cancer), or cell loss, in which the cell suicide program is inappropriately triggered (e.g. heart failure, neurodegenerative diseases, inflammation, stroke, type I diabetes, CNS injury). Obviously, heart failure would be classified as one of the clinical disorders where apoptosis should be actively antagonized to limit cell loss. Recent approaches also consider stem cell or progenitor cell therapies, which are aimed at altogether replacing functional myocytes rather than inhibiting cell death of pre-existing muscle cells (see for a series of recent reviews on this topic a spotlight issue of Cardiovascular Research, issue 58(2), 2003). The therapeutic options discussed in this session exclusively deal with the salvage of existing cardiac muscle. Table 2 presents a number of apoptosis-based therapeutic approaches, which are being tested in clinical trials for a variety of disorders. Below we will discuss a number of additional apoptosis targets, which may be considered for the development of anti-apoptosis clinical strategies for heart failure.

The neurohormonal axis (renin-angiotensin system or RAS and sympathetic system) is (over) active in the failing heart and neurohormonal antagonism is an important therapeutic tool in protecting the failing myocardium. Interestingly, neurohumoral stimulation is closely inter-

Table 2  
Examples of (pre)clinical trials with apoptosis-based therapies

| Drug             | Company                     | Target                                  | Type                 | Effect on apoptosis | Disease             | Status |
|------------------|-----------------------------|---|----------------------|---------------------|---------------------|--------|
| IDN6556          | Idun Pharmaceuticals        | Caspases                                | Small molecule       | anti                | Acute liver injury  | Ph II  |
| VX-799           | Vertex Pharmaceuticals      | caspase-1                               | Small molecule       | anti                | Arthritis           | Pre    |
| CGP3466B         | Novartis                    | GAPDH                                   | Small molecule       | anti                | Parkinson's disease | Ph II  |
| Genasense        | Genta                       | BCL-2                                   | Antisense technology | pro                 | Cancer              | Ph II  |
| Vitaxin          | Applied Molecular Evolution | $\alpha\text{v}\beta\text{3}$ -integrin | Humanized antibody   | pro                 | Angiogenesis        | Ph III |
| INGN201          | Introgen Pharmaceuticals    | p53                                     | Adenovirus           | pro                 | Cancer              | Ph III |
| E1A gene therapy | Targeted genetics           | E1A                                     | Adenovirus           | pro                 | Cancer              | Ph III |
| MS-275           | National Cancer Institute   | HDAC                                    | Small molecule       | anti                | Cancer              | Ph I   |

GAPDH, glyceraldehyde-3-phosphatase dehydrogenase; E1A, adenovirus E1A protein; HDAC, histone deacetylase.

twined with cardiac muscle apoptosis, and a number of its antagonists can modify apoptosis. For example, angiotensin II has potent apoptotic effects in cardiomyocytes which can be blocked with ATII blockers [53]. Similarly, norepinephrine causes apoptosis, while apoptosis can be attenuated with beta blockers [54]. Indeed, chronic use of both ACE inhibitors and beta blockers can prevent apoptosis in animal models [55]. Conclusively, these arguments suggest that clinical strategies aimed at protecting the failing heart from the noxious effects of RAS and catecholamine stimulation already indirectly prevent cardiac muscle apoptosis. In fact, it is likely that the potent anti-apoptotic effects of carvedilol may have something to do with its unparalleled efficacy in treating heart failure.

Another logical target to modulate cardiomyocyte apoptosis more directly in the failing heart would be the executioner caspases. First, caspase inhibition is possible through classical organic pharmacological approaches using a spectrum of small molecule caspase inhibitors. At this moment, broad-spectrum caspase inhibitors are already being evaluated in clinical trials to determine their usefulness as broad hepatoprotectant drugs in delaying or preventing the progression of hepatitis to cirrhosis and other conditions that may destroy the liver. Indeed, much evidence points to the beneficial aspects of caspase inhibitors in acute ischemia–reperfusion-induced cardiac injury. For example, ZVADfmk, a broad-spectrum and irreversible caspase inhibitor has been shown to reduce apoptosis and infarct size in an ischemia–reperfusion rat model, [56] coupled to a 72% reduction in the number of apoptotic cells in the treatment group.

However, caspase-inhibition may also be of benefit in the slower LV remodeling processes that provoke heart failure. Using a pig model of transient coronary occlusion to study cardiac remodelling and LV function, Yarbrough et al. demonstrated that delivery of a pan-caspase inhibitor significantly altered ventricular remodeling in the inhibitor group [57]. It should be noted that in this study the caspase inhibitors were delivered relatively early (upon reperfusion) and that apoptosis, nor caspase inhibition, were directly measured. However, similar benefits were reported from anti-caspase therapy in rats with artery occlusion, demonstrating reduced cardiomyocyte apoptosis in the remote myocardium and attenuated ventricular remodeling [58].

Finally, Kitsis's group recently demonstrated that anti-apoptotic therapy is also beneficial in transgenic mice with non-ischemic cardiomyopathy [59]. Collectively, from these recent pre-clinical studies it is conceivable that caspase inhibitors can have a remarkably positive effect on infarct size, cardiac muscle death in the remote myocardium and LV remodeling in heart failure animal models.

Besides caspases, other cellular targets in the apoptotic pathway also hold promise as future therapeutic devices in heart failure. Aurintricarboxylic acid (ATA) is an inhibitor that targets endonucleases, which are situated relatively downstream in the apoptotic pathways, and provoke DNA strand breaks. ATA was recently shown to significantly reduce the number of apoptotic cells in the peri-necrotic myocardium of an ischemia–reperfusion dog model. At the same time, Bcl-2 was found to be significantly increased, while Bax and activated caspase-3 were significantly reduced [60]. ATA treatment translated into functional benefit in the form of substantial enhancement in segmental shortening and segmental work in the area-at-risk myocardium, and improved endothelial function and myocardial perfusion.

As described above and earlier in detail [61,62], several reactive pathways implicated in hypertrophic growth modulation of the cardiac muscle, also impinge upon specific targets in the intrinsic and extrinsic apoptotic pathways. Insulin-like growth factor-1 (IGF-1) is an important survival growth factor in the myocardium. Several animal studies report potent anti-apoptotic actions of IGF-1 in the heart and improvement of cardiac function in animal models of cardiomyopathy [61]. IGF-1 inhibits cardiomyocyte apoptosis by attenuating Bax induction and caspase-3 activation [63,64].

Finally, oxidative stress is common in heart disease and it can trigger the “intrinsic” apoptotic pathways via multiple mechanisms including an increase in p53, Bax and Bad translocation to the mitochondria, release of cytochrome *c*, and caspase activation [65]. Reactive oxygen species (ROS) are formed at an accelerated rate in the failing myocardium. In fact, intrinsic oxidative stress was demonstrated to increase with age in the heart, due to both age-related impairment of transcriptional responses to oxidant stress [66], diminished expression of antioxidant defense enzymes such as glutathione peroxidase and MnSOD, and as a result

of age-dependent cardiac mitochondrial dysfunction [67]. For example, mice deficient in SOD2, a mitochondrial anti-oxidant enzyme, spontaneously develop dilated cardiomyopathy, which can be largely overcome by the administration of catalase-SOD-mimetics [68]. Similarly, mice with cardiac-specific overexpression of a dominant negative form of thioredoxin, a cytosolic anti-oxidant, display reduced defense against oxidative stress and accelerated progression to heart failure in response to pressure-overload [69]. In a rat infarct model, probucol, an anti-oxidant, prevented internucleosomal DNA fragmentation, and upregulation of p53, Bax and caspase-3 protein expression [70]. Interestingly, this inhibition also occurred in the remote non-ischemic myocardium—a fact that could be important in view of LV ventricular remodeling during heart failure development. Since a large number of commonly used, safe compounds have potent anti-oxidant as well as anti-apoptotic effects, this form of therapy may likely find early introduction in the repertoire of heart failure drugs.

The above examples demonstrate promising effects of inhibiting apoptosis on the development of cardiovascular disease. Promising additional therapeutic targets could consist of the muscle-enriched protein ARC, in the inhibitor of apoptosis protein-family (IAPs) such as XIAP and survivin, or the proteins that regulate IAPs such as Smac/DIABLO, Omi/HtrA2 and XAF1. However, understanding the structure and mode of function of this relatively new group of proteins in more detail will become a first, critical step in developing therapeutic agents.

Recent research has disentangled the complex processes of the apoptotic signaling pathways in the myocardium, and appointed potential new therapeutic targets, although the development of novel approaches, aimed at counteracting cardiomyocyte apoptosis in heart failure, encounters a number of practical limitations. Unfortunately, the cell death pathway contains very few conventional drug targets, such as enzymes and small-ligand receptors. Therefore, other strategies such as gene and anti-sense therapy should be considered besides the classical pharmacological approaches.

Apart from these practical limitations, theoretical restrictions also exist; e.g. can apoptosis be selectively modulated in one organ or cell type without adverse effects on other key systems? Counteracting apoptosis will be beneficial in the treatment of diseases such as heart failure and neurodegenerative disorders; activating apoptosis is, however, essential in treating disorders where there is insufficient cell death, such as cancer. Systemic inhibition of apoptosis may therefore result in increased tumorigenic potential in extra-cardiac and neuronal tissues in patients already affected by serious afflictions such as heart failure. Another practical obstacle before these agents can be safely introduced in clinical testing is the need for further information about the most appropriate timing of anti-apoptotic therapy. As evidenced above, the vast majority of pre-clinical studies where anti-apoptotic strategies were

evaluated applied the drugs early in the course of injury. It will become of interest to design studies where the efficacy of anti-apoptosis regimens are tested in later stages, when animal models of heart failure are in more advanced stages of cardiomyopathy. Another important issue, before embarking on clinical trials, is the availability of techniques to evaluate actual myocyte loss (and protection thereof), preferably in a non-invasive manner. To this end, non-invasive imaging approaches [71,72] with high enough resolution to detect apoptotic alterations at the cellular level, should be further developed for clinical use. To accomplish this, a more thorough understanding of the molecular pathways that initiate and execute apoptosis is imperative in designing successful anti-apoptotic therapies in humans. Promising targets have already been identified and the ability to develop apoptosis-based therapeutics that modulate the survival of the cardiac muscle cell in human heart failure are definitely within reach.

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