

Role of caspase-independent apoptosis in cardiovascular diseases

Soochan Bae, Bhargavi Yalamarti, Peter M. Kang

Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215

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1. ABSTRACT

Apoptosis plays an important role in various cardiovascular diseases, and inhibition of cardiac apoptosis shows promise as a therapeutic strategy. Caspase, a critical enzyme in the induction and execution of apoptosis, has been targeted to inhibit apoptosis. However a newly recognized caspase-independent apoptosis pathway may also play an important role in cardiac apoptosis. Yet the mechanism and the potential significance of caspase-independent apoptosis in the heart remain poorly understood. In this paper, we reviewed the literatures on the mechanism of caspase-independent apoptosis and its significance in cardiovascular diseases.

2. INTRODUCTION

Apoptosis is a highly regulated mechanism of cell death that plays a fundamental role in various physiological processes in the multi-cellular organism, from nematodes to humans (1). It is implicated in a wide variety of acute pathological processes, such as myocardial infarction (MI), stroke, and sepsis, as well as chronic conditions, such as Alzheimer's, Parkinson's and Huntington's disease. In the last decade, the basic mechanisms of apoptosis have been discovered in greater depth and the role of apoptosis in various disease processes has been increasingly elucidated. In the heart, apoptosis

has been shown to play an important role in numerous pathologic processes, such as MI, reperfusion injury, development of heart failure, and various forms of cardiomyopathies (2, 3). The inhibition of apoptosis as a potential therapeutic strategy is also emerging in cardiovascular disease. Currently, these inhibition strategies have mainly targeted a family of cysteine proteases called caspases, which are highly conserved throughout evolution from invertebrates to humans. However, there is accumulating evidence to suggest that caspase independent pathways play a significant role during cardiac cell death. In this review, we will examine the molecular mechanisms of cardiac apoptosis, focusing particularly on caspase-independent apoptosis and their potential significance in devising effective therapeutic strategies to treat cardiovascular diseases.

3. APOPTOSIS IN CARDIOVASCULAR DISEASES

Heart disease is the leading cause of morbidity and mortality in the developed world. Over 2 million people in the U.S. suffer from congestive heart failure (CHF) with over 400,000 new cases diagnosed every year. The most common cause of CHF in the U.S. is ischemic heart disease, which is the result of an acute or chronic lack of blood supply to the heart. Animal and human studies have demonstrated that apoptosis is associated with acute phases after MI (2) as well as during reperfusion (4). Since cardiomyocytes are terminally differentiated and have little potential for division, preventing cell death has important implications in the treatment of cardiovascular diseases.

In contrast to acute myocardial injury, the pathogenesis of chronic heart failure is characterized by the progressive loss of cardiomyocytes evolving over months to years. Numerous studies involving human and animal models of heart failure suggest that apoptosis may be an important contributor to cardiomyocyte loss in the setting of heart failure (2). However, there still is much controversy surrounding the significance of apoptosis in the development of heart failure. This stems largely from the very low prevalence of apoptosis associated with chronic heart failure (usually less than 1% TUNEL positive cells) (2), and whether apoptosis plays a pathogenic role or is an epiphenomenon associated with end-stage heart failure. However, because of the chronic nature of heart failure, even a very low level of cell death over months to years could be of potential significance to a heart composed of non-dividing cardiomyocytes. It remains to be convincingly proven that apoptosis (or autophagic cell death) contributes significantly to the progression and the development of clinical heart failure.

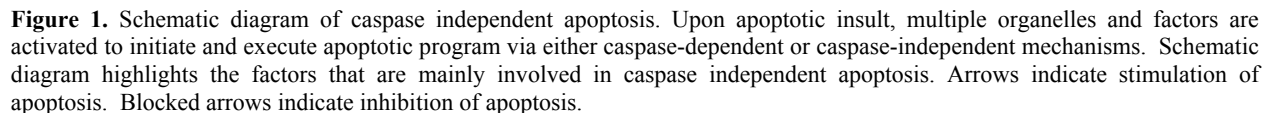
4. OVERVIEW OF CASPASE-DEPENDENT APOPTOSIS

Apoptosis is a tightly regulated cell deletion process that involves complex interactions among various pro- and anti-apoptotic molecules (2, 4, 5). The apoptotic cascade consists of multiple steps that lead to DNA fragmentation. These include the involvement of mitochondrial factors, activation of upstream caspases

followed by activation of downstream caspases; cleavage of various substrates, (i.e. PARP) and finally, DNA fragmentation. Among them, the activation of caspases is believed to be central to the regulation and execution of the apoptotic program. Caspases are group of cysteine proteases that play a critical role in initiating and executing apoptosis. They are produced as zymogens, which are activated via cleavage of their prodomains, and cleave target proteins at specific aspartate residues (6). At least 14 members of the caspase family have been described in mammalian cells thus far, and are grouped into two categories based on structure and function. Initiator caspases, such as caspase-8 and caspase-9, contain a long, functionally important prodomain, and act upstream to initiate and regulate apoptosis. Effector caspases, such as caspase-3, -6 and -7, are characterized by a short prodomain, and act downstream in the common pathway to carry out the final biochemical changes seen in apoptosis. Generally, effector caspases depend on initiator caspases for activation.

The mechanisms of caspase-dependent apoptosis have been reviewed by our group as well as several other investigators (3, 7-13). It is mediated either by mitochondria or by death receptors through the activation of caspase-9 or caspase-8, respectively (2, 4, 5, 14). The extrinsic pathway via the death receptor pathway is initiated by the binding of extracellular death proteins such as Fas L, TNF- α , TRAIL, to their cell surface receptors. Here, the final pathway involves activation of caspase 8/10 pathway, which cleaves procaspase 3, resulting in a proteolytic cascade. The intrinsic, or mitochondrial, pathway is induced by the mitochondria to release cytochrome c into the cytosol upon apoptotic stimulation (15). An activation complex, the apoptosome, is formed with apoptotic protein activating factor-1 (Apaf-1), cytochrome c, dATP, and caspase-9 (16, 17). Apoptosome formation results in the autoprocessing of caspase-9, as well as the activation of downstream caspases, such as caspase-3, to execute the final morphological and biochemical alterations (12, 16). Both pathways ultimately converge on a common downstream pathway, where the final morphological and biochemical alterations characteristic of apoptosis take place (15). Studies also suggest that the endoplasmic reticulum (ER) may play a crucial role in apoptosis. ER-localized pro- and anti-apoptotic Bcl-2 members have been implicated in the regulation of luminal Ca^{2+} levels and the expression of ER resident proteins, thereby strongly affecting apoptotic status of the cells. Caspase-12, found on the cytoplasmic face of the ER, is important for inducing apoptosis in response to prolonged ER stress (18).

In mammalian systems, several caspase inhibitors have been identified. Inhibitor of apoptosis proteins (IAPs) are prototypical inhibitors of caspases (19). However, deletion of x-linked IAP (xIAP) shows no obvious phenotypes in mice suggesting the presence of redundant IAP family members that compensate for the lack of xIAP (20). Inhibition by IAPs are antagonized by the mitochondrial protein Smac (second mitochondria-derived activator of caspase), which promotes cytochrome c-



5. CASPASE-INDEPENDENT APOPTOSIS

contain high levels of endogenous caspase inhibitors, thereby making them relatively resistant to caspase-dependent apoptosis (28, 30). In fact, we, as well as other investigators, have previously reported that various caspase-independent factors, such as apoptosis inducing factor (AIF), endonuclease G (Endo G) and High temperature requirement protein A2 (HtrA2/Omi), could induce apoptosis without mediation of caspases in the heart. These factors normally reside in the mitochondrial intermembrane space. However, in response to apoptotic stimuli, they are released from the mitochondria to the cytosol without concurrent caspase activation, translocate to the nucleus, and cause DNA fragmentation (31-33).

AIF is an evolutionary flavoenzyme and oxidoreductase with homologues in yeast, flies and worms (28, 29, 34). It binds NAD and FAD, but the oxidoreductase activity is not required for apoptotic function. AIF is an NADH-oxidase produced as a 67-kDa protein containing an N-terminal mitochondrial localization signal sequence (28, 29, 34). In mitochondria, AIF is processed into a 57-kDa mature form. It is then released into the cytosol upon apoptotic stimulation and may translocate into the nucleus and induce DNA fragmentation without caspase activation (28). This notion is supported by the fact that microinjection of AIF into cells induced

apoptotic changes, such as chromatin condensation that could not be blocked by zVAD.fmk or the over-expression of Bcl-2 (28, 30, 35). Furthermore, AIF can still trigger DNA fragmentation in Apaf-1 and caspase-9 deficient cells (31), which is probably mediated through direct activation of caspase-3 (36). In the heart, AIF has been implicated in apoptosis induced by oxidative stress, ischemia-reperfusion, and heart failure *in vitro*, as well as *in vivo* (37-39). The mature form of AIF, cleaved by calpain, is released, together with cytochrome c, from the mitochondria to the cytosol in ischemic neonatal cardiomyocytes characterized by caspase-independent DNA degradation (40). We have recently demonstrated significant activation of caspase-independent apoptosis in the Dahl salt sensitive (DSS) rat model of heart failure (39).

The mechanisms that regulate AIF release from the mitochondria are complex and controversial. For example, AIF release may be regulated by either Bcl-2 proteins-dependent or Bcl-2 proteins-independent mechanisms. Several studies have shown that Bcl-2 over-expression prevents both the release of AIF and cell death (41) (28). Furthermore, over-expression of Bax, a pro-apoptotic protein, triggers AIF release (42, 43), and the released AIF is blocked in Bax-deficient neurons upon apoptotic induction (28, 41). In contrast, the Bcl-2-independent mechanism of AIF release involves a poly(ADP-ribose) polymerase-1 (PARP-1), a highly conserved 116-kDa nuclear enzyme, involved in DNA repair (30). The NAD⁺ dependent nuclear DNA repair and ADP-ribosylating enzyme PARP-1 play a key role in the caspase independent form of cell death in myocardial infarction in a number of studies. PARP-1 has been shown to facilitate both the release of AIF from the mitochondria and AIF nuclear translocation without the mediation of Bcl-2 proteins and caspase activation (30, 44). In a murine model of heart failure, PARP inhibition attenuates the development of hypertrophy and the mitochondrial-to-nuclear translocation of AIF (45). Other information suggests PARP-1 might directly or indirectly be involved in the regulation of AIF translocation under conditions of oxidative stress in myocytes (38).

Despite its pro-apoptotic function, AIF has also been shown to possess an essential prosurvival function (46, 47). Homozygous AIF knockout in a mouse is embryonically lethal (48), and AIF knockout embryonic stem (ES) cells exhibit a reduction in mitochondrial respiratory chain complex I activity (49). Moreover, the Harlequin (*Hq*) mouse, which expresses 10-20% of normal AIF levels, exhibits neurodegeneration due to increased neuronal cell death (50). *Hq* mice are prone to damage from ischemia-reperfusion injury and aortic banding-induced heart failure. Also, cardiomyocytes isolated from *Hq* mice are more prone to H₂O₂-induced cell death (51). In addition, Penninger and colleagues demonstrated that a mouse with cardiac and skeletal muscle-specific knockout of AIF develops severe dilated cardiomyopathy and skeletal atrophy accompanied by defective mitochondrial respiratory activity (52).

How, then, is AIF able to function as both a survival and a death-inducing factor? The clarification of AIF's dual roles was recently elegantly demonstrated using various AIF mutants. Penninger and colleagues first generated a mouse with telencephalon (forebrain)-specific deletion of AIF (*tel AIF*^{-/-}), which demonstrated defective cortical development and reduced neuronal survival due to defects in mitochondrial respiration. This data suggested that AIF is required for neuronal cell survival and normal mitochondrial respiration in neurons (53). They then generated two AIF mutants, a mitochondrially anchored AIF (maAIF) mutant and AIF mutant that harbors a nuclear exclusion signal (neAIF). Reconstitution of wild type AIF in neuronal cells cultured from *tel AIF*^{-/-} mice resulted in robust DNA damage after apoptotic insult. However, reconstitution of maAIF or neAIF mutants in these neurons failed to induce cell death. These findings suggest that during apoptotic stimulation, the proapoptotic function of AIF is recognized when AIF is released from mitochondria and translocates to the nucleus, where it promotes DNA damage. This is highly reminiscent of cytochrome c, which plays a crucial role in oxidative phosphorylation under physiologic conditions, but when released from the mitochondrion in response to apoptotic stimulation, becomes a critical mediator of caspase-dependent apoptosis. It appears, therefore, that the precise function of AIF, like that of cytochrome c, critically depends on mitochondrial release and nuclear translocation.

5.2. Other factors involved in caspase-independent apoptosis

Other evidence of caspase-independent apoptosis is demonstrated in various studies of EndoG. EndoG, a conserved nuclease, is involved in mitochondrial DNA replication with important roles in recombination and repair (54). Similar to AIF, EndoG translocates from the mitochondria to the nucleus during apoptosis and induces DNA fragmentation independent of caspases (55, 56). EndoG and truncated AIF become the essential mediators of apoptosis in a caspase-independent manner in ischemia induced cardiomyocytes (40). Interestingly, EndoG null mice, however, do not have any obvious defects in development or in the regulation of apoptosis (57, 58).

Furthermore, Omi/HtrA2, a mitochondrial serine protease with pro-apoptotic properties, may also contribute to caspase-independent apoptosis (59). One of the crucial substrates of HtrA2/Omi is the antiapoptotic protein HAX-1 (bearing Bcl-2-homology BH1 and BH2 domains), which resides within the mitochondria, presumably in the outer membrane, and may be cleaved by HtrA2/Omi when it is still confined in the mitochondria. Other potential substrates of HtrA2/Omi are Ped/Pea-15 (an inhibitor of the DISC and of stress kinase) and IAPs, which function as endogenous caspase inhibitors (11). There is evidence that HtrA2/Omi also translocates from the mitochondria to the cytosol during ischemia/reperfusion, and a specific HtrA2/Omi inhibitor, ucf-101, has also been shown to attenuate apoptosis and decrease infarct size (60); this mechanism of action, however, is not as well defined.

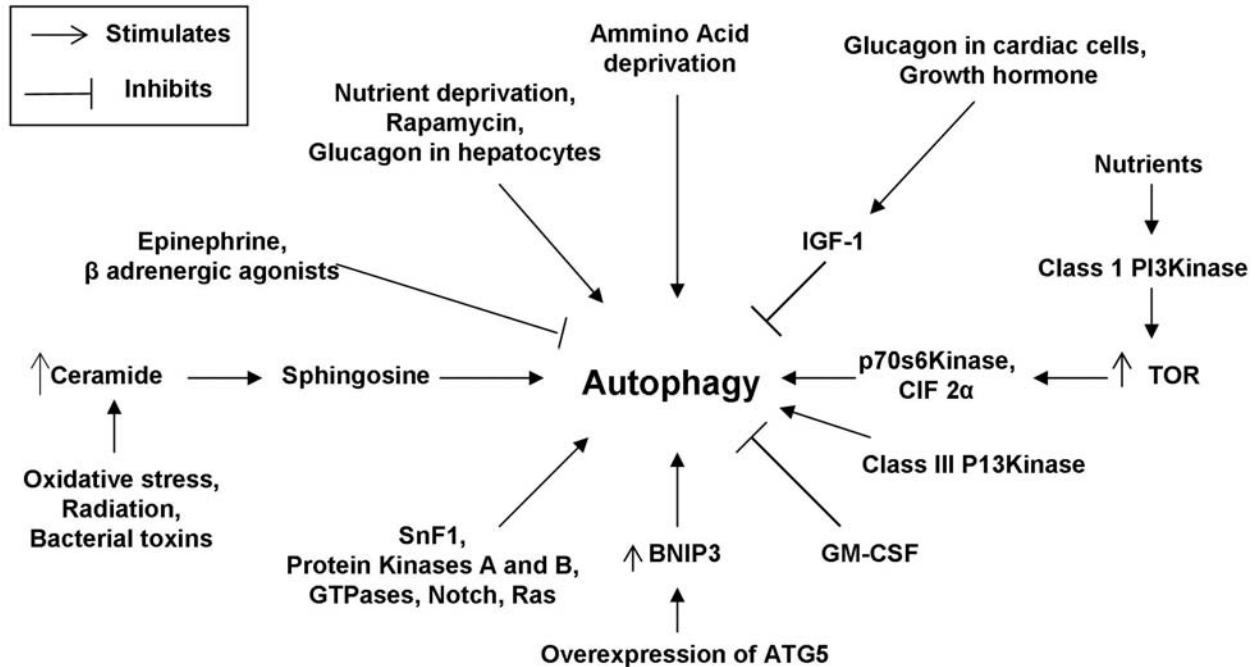


Figure 2. Summary of factors that modulate autophagy in cardiomyocytes. Arrows indicate factors which promote apoptosis. Blocked arrows indicate factors known to inhibit apoptosis.

Other caspase-independent apoptotic mechanisms are also being recognized. Cardiomyocyte death may be induced by hypoxia-acidosis through upregulation of Bnip3, which stimulates the opening of mitochondrial permeability transition pore (MPTP) releasing AIF, cytochrome c, and calcium, without activation of caspases. Bnip3 overexpression also causes extensive fragmentation of the mitochondrial network. Caspase independent pathways involving the PKC epsilon-JNK/p38 MAPK signaling pathway may be actuated by chemical hypoxia induced death in cardiomyocytes (61). In addition, the role of death domain receptor mediated cell death pathways and receptor interacting protein (RIP) kinase is being recognized as caspase independent cell death in cardiomyocytes. Presence of RIP1 prevents the interaction between adenosine nucleotide translocator (ANT) and cyclophilin D. The improper functioning of ANT leads to enhanced ROS production and diminished ATP production (56). Toll like receptor (TLR) stimulated in presence of caspase inhibitors also stimulates caspase independent RIP-mediated cell death.

6. AUTOPHAGY

Autophagy is a dynamic process involving the degradation of long lived proteins and is the only known major regulated mechanism for the disintegration and turnover of unnecessary organelles and cytoplasmic proteins. The term “autophagy” denotes a process of self-cannibalization through a lysosomal degradation pathway. Three basic types of autophagy are recognized in mammalian cells (62). Macroautophagy, the universal form of autophagy, involves the sequestration of target materials into a double membrane enclosed vacuole, which

then fuses with a lysosome. Microautophagy occurs when the components of the cytoplasm directly enter a lysosome through invaginations of the limiting membrane. Lastly, chaperone mediated autophagy is when cytosolic proteins are selectively transported to lysosomes by chaperones such as heat shock protein cognate 73 (HSC73). The hallmark of autophagy is increased lysosomal activity with an increase of Beclin 1, Cathepsin B and D, beta-hexosaminidase, HSC73 and light chain 3II (LC3II) (63).

Autophagy is regulated by several factors that are predominately involved in mammalian target of rapamycin (mTOR) and protein kinase pathways. Genes upstream of TOR kinase encode proteins essential for autophagy (64). (Figure 2) In autophagy, cytoplasmic proteins or dysfunctional organelles are sequestered in a double membrane bound vesicle, termed an autophagosome, and delivered to the liposome by fusion where it is then degraded. Numerous factors stimulate autophagy such as starvation and growth factor deprivation following acute ischemia. The process can be summarized in 4 stages a) induction of autophagy which involves TOR kinase, b) formation of autophagosome, which is controlled by PI3Kinase and phosphatases, c) autophagosome docking and fusion, and d) degradation by lysosomal proteases.

In the heart, autophagy is observed during acute and chronic ischemia, heart failure, as well as aging. Recent findings suggest that autophagy is involved in heart failure caused by dilated cardiomyopathy, valvular heart disease, hypertensive heart disease and chronic ischemia (5, 63, 65, 66). Kostin *et al.* (51), identified the role of ubiquitin proteasomal degradation cascade in a failing human heart. They suggested that a disturbed balance between a high rate

Caspase-independent apoptosis in heart

Table 1. Summary of proteins involved in caspase-independent apoptosis

Factor	Stimulus	Mode of death	Mechanisms/Notes
Beclin 1	I/R	Autophagy	Mediates Autophagy during reperfusion
Urocortin	I/R	Autophagy and Caspase dependent	Is a peptide released from viable cells and mediates through PKC epsilon activation
ATG genes (> 20)	Pressure overload	Protective effects	All steps of autophagy ,i.e. induction (atg 6), Formation of autophagosomes (Atg 8,Atg 12-5)
BNIP3	I/R, Hypoxia	CIA, autophagy	Causes mitochondrial dysfunction and release of cytochrome c, AIF, calcium and MPTP opening
RIP1 Kinase	Death receptor, Toll like receptor	Autophagy	Decreased ANT and Cyclophilin D interaction, Increased ROS
Lysosomal cathepsin B, D	Chronic ischemia	Autophagy	Increases proteolysis i.e. in the last step of autophagy where the contents of autophagolysosome and lysosomal transport proteins are degraded.
HSC 73	Chronic ischemia	Autophagy	Involved in chaperone mediated autophagy.
LC3 –LC3 I LC3 II	Chronic ischemia	Autophagy	Mediate formation of autophagosomes. LC3 gets cleaved by Atg4 to LC3-I, which is activated by Atg 7, transferred to Atg 3, and finally conjugated to phosphatidyl ethanolamine and then recruited to autophagosomal membrane.
AIF Endo G	Cellular stress, Death receptors, BH3 only proteins	CIA, autophagy	Released from mitochondria and translocations to the nucleus
HtrA2/Omi	Ischemia reperfusion	CIA, CDA	Degradates xIAP. Inhibition by ucf-1.
LAMP2	X linked Genetic deficiency	Autophagy	Involved in Rab7-mediated fusion of autophagosomes with lysosomes.
Rab7		Autophagy	Rab 7, a Rab GTPases mediates fusion of autophagosome with the lysosome to allow maturation of late autophagic vacuoles.
GM-CSF	Exogenous administration of GM-CSF	Prevents Autophagy	Mainly via anti-autophagic mechanism rather than anti –apoptosis or regeneration. There was downregulation of TNF-alpha, increased AKT signaling and Matrix mellaoproteases.
Ubiquitin-fusion degradation system 1 (UFD 1)	Downregulation	Autophagy	Downregulation results in massive storage of ubiquitin protein complexes and autophagy

CIA=caspase-independent apoptosis, CDA=caspase-dependent apoptosis, ANT = Adenine nucleotide translocator, Atg5=Autophagy related protein 5, AIF=Apoptosis inducing factor, GM-CSF= Granulocyte monocyte - colony stimulating factor, LAMP-2 = Lysosomal associated membrane protein 2, BNIP3 = Bcl-2 interacting protein, RIP1 = Receptor interacting protein, LAMP-2 = Lysosomal associated membrane protein 2, RAB7- Ras associated protein, PKC =Protein Kinase C, xIAP= x-linked inhibitor of apoptosis proteins.

of ubiquitination and inadequate degradation of ubiquitin/protein conjugates may contribute to autophagic cell death (67). In addition, autophagy has been associated with defects in the ubiquitin-proteasomal system in human hibernating myocardium (68). Hein *et al.* observed that autophagy is one of the important factors contributing to the progression of left ventricular systolic dysfunction in the progression of compensated hypertrophy in heart failure (65). In Danon's cardiomyopathy, cardiomyocytes include marked autophagic vacuoles in the cytoplasm, where dysfunction of autophagic process is suggested by deficiency of the lysosomal protein LAMP-2 (69). Interestingly, urocortin inhibits autophagy, which is mediated in part by inhibition of Beclin1 expression and activation of the PI3 kinase/Akt pathway (70). Urocortin is an endogenous cardiac peptide that protects cardiomyocytes from both necrotic and apoptotic cell death following exposure to ischemia/reperfusion injury in both *in vitro* and *in vivo* models. Thus, the overlap between apoptotic and autophagic cell death pathways remains incompletely understood (71).

There are studies, however, that suggest that autophagy is required for survival of cardiomyocyte under pathological conditions. Autophagy may limit the spread of proapoptotic factors such as AIF and cytochrome c from the mitochondria and could be a protective response to sublethal injury. In a study reported by Kuma *et al.*, induction of autophagy is essential for survival of cardiomyocytes during neonatal starvation *in vivo* (72). In

addition, autophagy triggered by ischemia could be a homeostatic mechanism, which could inhibit deleterious effects of chronic ischemia (63). Recently, Matsui and colleagues suggested that autophagy may serve primarily to maintain energy production during acute ischemia, but converts to clearing up damaged organelles during chronic ischemia or reperfusion (73). They demonstrated that ischemia stimulates autophagy through an AMPK-dependent mechanism, whereas ischemia/reperfusion stimulates autophagy through a Beclin 1-dependent, but AMPK-independent, mechanism. Thus, a dual role of autophagy remains to be elucidated in some circumstances of cell death and survival.

7. CONCLUSIONS AND PERSPECTIVE

Apoptosis is implicated in the pathogenesis of numerous cardiovascular diseases, and the inhibition of apoptosis promises to be an extraordinarily important target for therapeutic intervention (10, 74, 75). Although caspase inhibition has shown to reduce myocardial loss and improve cardiac function in various animal models (25, 26), it has not had similar effectiveness in clinical settings. One important issue is that the caspase inhibition strategy is complicated by the activation of caspase-independent apoptosis pathways, which is accompanied by the release of AIF, EndoG, and Omi/HtrA2 from the mitochondria in the absence of caspase activation. Thus, a better understanding of the regulation of the caspases and their relationship with caspase-independent pathways will be

needed to achieve more complete and effective anti-apoptotic therapeutic strategies. Furthermore, addressing specific issues related to the heart will be crucial for a more targeted cardiac anti-apoptotic therapy. Is there a specialized molecular mechanism of apoptosis in the heart that is unique to terminally differentiated cardiomyocytes? Since the baseline level of apoptosis in the heart is extremely low, does the heart have a strong regulatory mechanism that resists apoptosis? What is the significance of apoptosis in the progression of chronic heart failure? Would the supplementation of inhibitors to caspase independent pathways with caspase inhibitors lead to more effective treatment of cardiovascular diseases in clinical setting? Definitive answers to these questions are still lacking, and more research is needed. Further understanding of caspase-dependent and caspase-independent mechanisms will potentially allow us to make a significant impact on how we treat various forms of cardiovascular disease in the future.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

- Kerr J. F., A. H. Wyllie & A. R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)
- Kang P. M., A. Haunstetter, H. Aoki, A. Usheva & S. Izumo: Morphological and molecular characterization of adult cardiomyocyte apoptosis during hypoxia and reoxygenation. *Circ Res* 87, 118-125 (2000)
- Kang P. M. & S. Izumo: Apoptosis in heart: basic mechanisms and implications in cardiovascular diseases. *Trends Mol Med* 9, 177-182 (2003)
- Ohno M., G. Takemura, A. Ohno, J. Misao, Y. Hayakawa, S. Minatoguchi, T. Fujiwara & H. Fujiwara: "Apoptotic" myocytes in infarct area in rabbit hearts may be oncotic myocytes with DNA fragmentation: analysis by immunogold electron microscopy combined with In situ nick end-labeling. *Circulation* 98, 1422-1430 (1998)
- Knaapen M. W., M. J. Davies, M. De Bie, A. J. Haven, W. Martinet & M. M. Kockx: Apoptotic versus autophagic cell death in heart failure. *Cardiovasc Res* 51, 304-312 (2001)
- Nicholson D. W. & N. A. Thornberry: Caspases: killer proteases. *Trends Biochem Sci* 22, 299-306 (1997)
- Abraham M. C. & S. Shaham: Death without caspases, caspases without death. *Trends Cell Biol* 14, 184-193 (2004)
- Green D. R. & J. C. Reed: Mitochondria and apoptosis. *Science* 281, 1309-1312 (1998)
- Haunstetter A. & S. Izumo: Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res* 82, 1111-1129 (1998)
- Kang P. M. & S. Izumo: Apoptosis and heart failure: A critical review of the literature. *Circ Res* 86, 1107-1113 (2000)
- Kroemer G. & S. J. Martin: Caspase-independent cell death. *Nat Med* 11, 725-730 (2005)
- Slee E. A., M. T. Harte, R. M. Kluck, B. B. Wolf, C. A. Casiano, D. D. Newmeyer, H. G. Wang, J. C. Reed, D. W. Nicholson, E. S. Alnemri, D. R. Green & S. J. Martin: Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9- dependent manner. *J Cell Biol* 144, 281-292 (1999)
- Thornberry N. A. & Y. Lazebnik: Caspases: enemies within. *Science* 281, 1312-1316 (1998)
- Aikawa R., I. Komuro, T. Yamazaki, Y. Zou, S. Kudoh, M. Tanaka, I. Shiojima, Y. Hiroi & Y. Yazaki: Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. *J Clin Invest* 100, 1813-1821 (1997)
- Liu X., C. N. Kim, J. Yang, R. Jemmerson & X. Wang: Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86, 147-157 (1996)
- Li P., D. Nijhawan, I. Budihardjo, S. M. Srinivasula, M. Ahmad, E. S. Alnemri & X. Wang: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, 479-489 (1997)
- Zou H., W. J. Henzel, X. Liu, A. Lutschg & X. Wang: Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90, 405-413 (1997)
- Maag R. S., S. W. Hicks & C. E. Machamer: Death from within: apoptosis and the secretory pathway. *Curr Opin Cell Biol* 15, 456-461 (2003)
- Salvesen G. S. & C. S. Duckett: IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 3, 401-410 (2002)
- Harlin H., S. B. Reffey, C. S. Duckett, T. Lindsten & C. B. Thompson: Characterization of XIAP-deficient mice. *Mol Cell Biol* 21, 3604-3608 (2001)
- Brocheriou V., A. A. Hagege, A. Oubenaissa, M. Lambert, V. O. Mallet, M. Duriez, M. Wassef, A. Kahn, P. Menasche & H. Gilgenkrantz: Cardiac functional improvement by a human Bcl-2 transgene in a mouse model of ischemia/reperfusion injury. *J Gene Med* 2, 326-333 (2000)
- Ekhterae D., Z. Lin, M. S. Lundberg, M. T. Crow, F. C. Brosius, 3rd & G. Nunez: ARC inhibits cytochrome c release from mitochondria and protects against hypoxia-induced apoptosis in heart-derived H9c2 cells. *Circ Res* 85, e70-77 (1999)
- Koseki T., N. Inohara, S. Chen & G. Nunez: ARC, an inhibitor of apoptosis expressed in skeletal muscle and heart that interacts selectively with caspases. *Proc Natl Acad Sci U S A* 95, 5156-5160 (1998)
- Han Y., Y. S. Chen, Z. Liu, N. Bodyak, D. Rigor, E. Bisping, W. T. Pu & P. M. Kang: Overexpression of HAX-1 protects cardiac myocytes from apoptosis through caspase-9 inhibition. *Circ Res* 99, 415-423 (2006)
- Yaoita H., K. Ogawa, K. Maehara & Y. Maruyama: Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor [In Process Citation]. *Circulation* 97, 276-281 (1998)
- Laugwitz K. L., A. Moretti, H. J. Weig, A. Gillitzer, K. Pinkernell, T. Ott, I. Pragst, C. Stadele, M. Seyfarth, A. Schomig & M. Ungerer: Blocking caspase-activated apoptosis improves contractility in failing myocardium. *Hum Gene Ther* 12, 2051-2063 (2001)

27. Zheng T. S., S. Hunot, K. Kuida & R. A. Flavell: Caspase knockouts: matters of life and death. *Cell Death Differ* 6, 1043-1053 (1999)
28. Lorenzo H. K., S. A. Susin, J. Penninger & G. Kroemer: Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. *Cell Death Differ* 6, 516-524 (1999)
29. Penninger J. M. & G. Kroemer: Mitochondria, AIF and caspases--rivaling for cell death execution. *Nat Cell Biol* 5, 97-99 (2003)
30. Sharp T. V., H. W. Wang, A. Koumi, D. Hollyman, Y. Endo, H. Ye, M. Q. Du & C. Boshoff: K15 protein of Kaposi's sarcoma-associated herpesvirus is latently expressed and binds to HAX-1, a protein with antiapoptotic function. *J Virol* 76, 802-816 (2002)
31. Cande C., F. Cecconi, P. Dessen & G. Kroemer: Apoptosis-inducing factor (AIF): key to the conserved caspase-independent pathways of cell death? *J Cell Sci* 115, 4727-4734 (2002)
32. Cregan S. P., V. L. Dawson & R. S. Slack: Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene* 23, 2785-2796 (2004)
33. Lockshin R. A. & Z. Zakeri: Caspase-independent cell death? *Oncogene* 23, 2766-2773 (2004)
34. Susin S. A., H. K. Lorenzo, N. Zamzami, I. Marzo, B. E. Snow, G. M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D. R. Goodlett, R. Aebersold, D. P. Siderovski, J. M. Penninger & G. Kroemer: Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397, 441-446 (1999)
35. Yang J., X. Liu, K. Bhalla, C. N. Kim, A. M. Ibrado, J. Cai, T. I. Peng, D. P. Jones & X. Wang: Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 275, 1129-1132 (1997)
36. Lakhani S. A., A. Masud, K. Kuida, G. A. Porter, Jr., C. J. Booth, W. Z. Mehal, I. Inayat & R. A. Flavell: Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science* 311, 847-851 (2006)
37. Kasahara H., T. Ueyama, H. Wakimoto, M. K. Liu, C. T. Maguire, K. L. Converso, P. M. Kang, W. J. Manning, J. Lawitts, D. L. Paul, C. I. Berul & S. Izumo: Nkx2.5 homeoprotein regulates expression of gap junction protein connexin 43 and sarcomere organization in postnatal cardiomyocytes. *J Mol Cell Cardiol* 35, 243-256 (2003)
38. Chen M., Z. Zsengeller, C. Y. Xiao & C. Szabo: Mitochondrial-to-nuclear translocation of apoptosis-inducing factor in cardiac myocytes during oxidant stress: potential role of poly(ADP-ribose) polymerase-1. *Cardiovasc Res* 63, 682-688 (2004)
39. Siu P. M., B. S., N. Bodyak, D. Rigor & P. M. Kang: Response of caspase-independent apoptotic factors to high salt diet-induced heart failure. *Journal of Molecular and Cellular Cardiology* (2007)
40. Bahi N., J. Zhang, M. Llovera, M. Ballester, J. X. Comella & D. Sanchis: Switch from Caspase-dependent to Caspase-independent Death during Heart Development: essential role of endonuclease G in ischemia-induced DNA processing of differentiated cardiomyocytes. *J Biol Chem* 281, 22943-22952 (2006)
41. Cregan S. P., A. Fortin, J. G. MacLaurin, S. M. Callaghan, F. Cecconi, S. W. Yu, T. M. Dawson, V. L. Dawson, D. S. Park, G. Kroemer & R. S. Slack: Apoptosis-inducing factor is involved in the regulation of caspase-independent neuronal cell death. *J Cell Biol* 158, 507-517 (2002)
42. Arnoult D., P. Parone, J. C. Martinou, B. Antonsson, J. Estaquier & J. C. Ameisen: Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome c release in response to several proapoptotic stimuli. *J Cell Biol* 159, 923-929 (2002)
43. Arnoult D., B. Gaume, M. Karbowski, J. C. Sharpe, F. Cecconi & R. J. Youle: Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. *Embo J* 22, 4385-4399 (2003)
44. Joseph B., P. Marchetti, P. Formstecher, G. Kroemer, R. Lewensohn & B. Zhivotovsky: Mitochondrial dysfunction is an essential step for killing of non-small cell lung carcinomas resistant to conventional treatment. *Oncogene* 21, 65-77 (2002)
45. Xiao C. Y., M. Chen, Z. Zsengeller, H. Li, L. Kiss, M. Kollai & C. Szabo: Poly(ADP-Ribose) polymerase promotes cardiac remodeling, contractile failure, and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure. *J Pharmacol Exp Ther* 312, 891-898 (2005)
46. Modjtahedi N., F. Giordanetto, F. Madeo & G. Kroemer: Apoptosis-inducing factor: vital and lethal. *Trends Cell Biol* 16, 264-272 (2006)
47. Porter A. G. & A. G. Urbano: Does apoptosis-inducing factor (AIF) have both life and death functions in cells? *Bioessays* 28, 834-843 (2006)
48. Joza N., S. A. Susin, E. Daugas, W. L. Stanford, S. K. Cho, C. Y. Li, T. Sasaki, A. J. Elia, H. Y. Cheng, L. Ravagnan, K. F. Ferri, N. Zamzami, A. Wakeham, R. Hakem, H. Yoshida, Y. Y. Kong, T. W. Mak, J. C. Zuniga-Pflucker, G. Kroemer & J. M. Penninger: Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* 410, 549-554 (2001)
49. Vahsen N., C. Cande, J. J. Briere, P. Benit, N. Joza, N. Larochette, P. G. Mastroberardino, M. O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J. M. Penninger, H. Schagger, P. Rustin & G. Kroemer: AIF deficiency compromises oxidative phosphorylation. *Embo J* 23, 4679-4689 (2004)
50. Klein J. A., C. M. Longo-Guess, M. P. Rossmann, K. L. Seburn, R. E. Hurd, W. N. Frankel, R. T. Bronson & S. L. Ackerman: The harlequin mouse mutation downregulates apoptosis-inducing factor. *Nature* 419, 367-374 (2002)
51. van Empel V. P., A. T. Bertrand, R. van der Nagel, S. Kostin, P. A. Doevendans, H. J. Crijns, E. de Wit, W. Sluiter, S. L. Ackerman & L. J. De Windt: Downregulation of apoptosis-inducing factor in harlequin mutant mice sensitizes the myocardium to oxidative stress-related cell death and pressure overload-induced decompensation. *Circ Res* 96, e92-e101 (2005)
52. Joza N., G. Y. Oudit, D. Brown, P. Benit, Z. Kassiri, N. Vahsen, L. Benoit, M. M. Patel, K. Nowikovsky, A. Vassault, P. H. Backx, T. Wada, G. Kroemer, P. Rustin & J. M. Penninger: Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. *Mol Cell Biol* 25, 10261-10272 (2005)

53. Cheung E. C., N. Joza, N. A. Steenaart, K. A. McClellan, M. Neuspiel, S. McNamara, J. G. MacLaurin, P. Rippstein, D. S. Park, G. C. Shore, H. M. McBride, J. M. Penninger & R. S. Slack: Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis. *Embo J* 25, 4061-4073 (2006)
54. Li L. Y., X. Luo & X. Wang: Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* 412, 95-99 (2001)
55. Chen Z., C. C. Chua, Y. S. Ho, R. C. Hamdy & B. H. Chua: Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice. *Am J Physiol Heart Circ Physiol* 280, H2313-2320 (2001)
56. van Loo G., P. Schotte, M. van Gurp, H. Demol, B. Hoorelbeke, K. Gevaert, I. Rodriguez, A. Ruiz-Carrillo, J. Vandekerckhove, W. Declercq, R. Beyaert & P. Vandenabeele: Endonuclease G: a mitochondrial protein released in apoptosis and involved in caspase-independent DNA degradation. *Cell Death Differ* 8, 1136-1142 (2001)
57. David K. K., M. Sasaki, S. W. Yu, T. M. Dawson & V. L. Dawson: EndoG is dispensable in embryogenesis and apoptosis. *Cell Death Differ* 13, 1147-1155 (2006)
58. Irvine R. A., N. Adachi, D. K. Shibata, G. D. Cassell, K. Yu, Z. E. Karanjawala, C. L. Hsieh & M. R. Lieber: Generation and characterization of endonuclease G null mice. *Mol Cell Biol* 25, 294-302 (2005)
59. Suzuki Y., K. Takahashi-Niki, T. Akagi, T. Hashikawa & R. Takahashi: Mitochondrial protease Omi/HtrA2 enhances caspase activation through multiple pathways. *Cell Death Differ* 11, 208-216 (2004)
60. Kong S. W., N. Bodyak, P. Yue, Z. Liu, J. Brown, S. Izumo & P. M. Kang: Genetic expression profiles during physiological and pathological cardiac hypertrophy and heart failure in rats. *Physiol Genomics* 21, 34-42 (2005)
61. Wang S., M. Miura, Y. K. Jung, H. Zhu, E. Li & J. Yuan: Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* 92, 501-509 (1998)
62. Terman A., B. Gustafsson & U. T. Brunk: Autophagy, organelles and ageing. *J Pathol* 211, 134-143 (2007)
63. Yan L., D. E. Vatner, S. J. Kim, H. Ge, M. Masarekar, W. H. Massover, G. Yang, Y. Matsui, J. Sadoshima & S. F. Vatner: Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci U S A* 102, 13807-13812 (2005)
64. Levine B. & J. Yuan: Autophagy in cell death: an innocent convict? *J Clin Invest* 115, 2679-2688 (2005)
65. Hein S., E. Arnon, S. Kostin, M. Schonburg, A. Elsasser, V. Polyakova, E. P. Bauer, W. P. Klovekorn & J. Schaper: Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 107, 984-991 (2003)
66. Shimomura H., F. Terasaki, T. Hayashi, Y. Kitaura, T. Isomura & H. Suma: Autophagic degeneration as a possible mechanism of myocardial cell death in dilated cardiomyopathy. *Jpn Circ J* 65, 965-968 (2001)
67. Kostin S., L. Pool, A. Elsasser, S. Hein, H. C. Drexler, E. Arnon, Y. Hayakawa, R. Zimmermann, E. Bauer, W. P. Klovekorn & J. Schaper: Myocytes die by multiple mechanisms in failing human hearts. *Circ Res* 92, 715-724 (2003)
68. Elsasser A., A. M. Vogt, H. Nef, S. Kostin, H. Mollmann, W. Skwara, C. Bode, C. Hamm & J. Schaper: Human hibernating myocardium is jeopardized by apoptotic and autophagic cell death. *J Am Coll Cardiol* 43, 2191-2199 (2004)
69. Tanaka M., N. Yamasaki & S. Izumo: Phenotypic characterization of the murine Nkx2.6 homeobox gene by gene targeting. *Mol Cell Biol* 20, 2874-2879 (2000)
70. Valentim L., K. M. Laurence, P. A. Townsend, C. J. Carroll, S. Soond, T. M. Scarabelli, R. A. Knight, D. S. Latchman & A. Stephanou: Urocortin inhibits Beclin1-mediated autophagic cell death in cardiac myocytes exposed to ischaemia/reperfusion injury. *J Mol Cell Cardiol* 40, 846-852 (2006)
71. Hamacher-Brady A., N. R. Brady & R. A. Gottlieb: The interplay between pro-death and pro-survival signaling pathways in myocardial ischemia/reperfusion injury: apoptosis meets autophagy. *Cardiovasc Drugs Ther* 20, 445-462 (2006)
72. Kuma A., M. Hatano, M. Matsui, A. Yamamoto, H. Nakaya, T. Yoshimori, Y. Ohsumi, T. Tokuhisa & N. Mizushima: The role of autophagy during the early neonatal starvation period. *Nature* 432, 1032-1036 (2004)
73. Matsui Y., H. Takagi, X. Qu, M. Abdellatif, H. Sakoda, T. Asano, B. Levine & J. Sadoshima: Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circ Res* 100, 914-922 (2007)
74. Garg S., J. Narula & Y. Chandrasekhar: Apoptosis and heart failure: clinical relevance and therapeutic target. *J Mol Cell Cardiol* 38, 73-79 (2005)
75. Logue S. E., A. B. Gustafsson, A. Samali & R. A. Gottlieb: Ischemia/reperfusion injury at the intersection with cell death. *J Mol Cell Cardiol* 38, 21-33 (2005)

Abbreviations: MI: myocardial infarction, CHF: congestive heart failure, Apaf-1: apoptotic protein activating factor-1, ER: endoplasmic reticulum, IAPs: inhibitor of apoptosis proteins, xIAP: x-linked IAP, ARC: apoptosis repressor with caspase recruitment domain, HAX-1: HS-1 associated protein-1, AIF: apoptosis inducing factor, EndoG: endonuclease G, HtrA2/Omi: high temperature requirement protein A2, DSS: Dahl salt sensitive, PARP-1: poly(ADP-ribose) polymerase-1, ES: embryonic stem, *Hq*: Harlequin, maAIF: mitochondrially anchored AIF, neAIF: nuclear exclusion signal AIF, MPTP: mitochondrial permeability transition pore, RIP: receptor interacting protein, ANT: adenosine nucleotide translocator, HSC73: heat shock protein cognate 73, LC3II: light chain 3II, mTOR: mammalian target of rapamycin.

Key Words: Apoptosis, Caspase, Apoptosis inducing factor, Heart, Autophagy, Review

Send correspondence to: Peter M. Kang, MD, Cardiovascular Division, Beth Israel Deaconess Medical Center, 330 Brookline Ave, SL-423C, Boston, MA 02215, Tel: 617-667-4865, Fax: 617-975-5201, E-mail: pkang@bidmc.harvard.edu

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