



Apoptosis of Cardiomyosites in Heart Disease

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Abstract: Although the notion that apoptosis can act as a pathogenic mediator came somewhat later to cardiovascular medicine than to other fields of medicine, research into the role of apoptosis in heart disease has progressed explosively during the past 10 years. Consequently, we now have much information about apoptosis in cardiac cells, collected in vitro and in vivo under a wide variety of pathological conditions using both experimental animal models and specimens from heart disease patients, and there are a number of excellent reviews dealing with the role of apoptosis in heart disease [1–11].

Among the various cardiovascular ailments, myocardial infarction (MI) is particularly noteworthy for having high rates of both mortality and morbidity. Patients experiencing MI are at risk of sudden death during the acute stage and then ventricular remodeling and heart failure during the chronic stage, with the most critical determinant of the remodeling being the magnitude of the acute infarct (i.e., the number of dead cardiomyocytes resulting from the acute ischemic insult). In addition, other factors, including late death or hypertrophy of cardiomyocytes, fibrosis and the expression of various cytokines, are associated with the continued disease progression during the chronic stage. Notably, apoptosis has been detected in the heart during all stages of MI, suggesting apoptosis may be responsible for a significant amount of cardiomyocyte death during the acute ischemic stage, as well as for a progressive loss of surviving cells during the subacute and chronic stages. Patients who survive large MIs are at especially high risk of developing such failure. Indeed, patients with post-infarct heart failure account for 44% of candidates for cardiac transplantation [12].

Heart failure is the final outcome of a number of heart diseases. Although the mechanisms for cardiac decompensation have not been fully elucidated, loss of cardiac myocytes has been considered important as well as dysfunction of individual cardiac myocytes for one of the mechanisms. Extensive study now indicates that apoptosis is deeply involved in the pathology of almost all types of heart disease, not only ischemia-related ailments (ischemia-reperfusion, the acute and chronic stages of MI, ischemic cardiomyopathy and hibernating myocardium), but also other heart diseases such as dilated cardiomyopathy. Thus, apoptosis appears to be established as one important mode of cardiomyocyte death that is responsible for the development and worsening of heart failure. Other than ischemic hearts and failing hearts, cardiac arrhythmias is an important disease entity where apoptosis is suggested to play a role in the pathogenesis [13–16].

Keywords: apoptosis, myocardial infarction, remodeling, heart failure.

Nevertheless, no therapeutic intervention has yet been established to address the problem of cardiac apoptosis, nor has there ever been a clinical trial aimed at evaluating a therapeutic approach to the

problem. In the present review, we critically assess the relevance of apoptosis to cell death related to MI and heart failure, and the possibility of therapeutic anti-apoptotic interventions.

Basic concept for apoptosis - apoptosis

is a morphological term

Cell death can be classified based on the patho-physiological cause, the molecular mechanism or the morphology of the affected cell (Table 1). Apoptosis is a morphological term coined by Kerr et al. in 1974 [17]. They observed morphological changes in dying cells that were entirely distinct from those of necrosis and speculated that these changes were associated a form of spontaneous cell death. Today, apoptosis is recognized as a fun-damental mechanism, a form of programmed cell death that is regulated physiologically and geneti-cally and plays a central role in development, mor-phogenesis, normal cell turnover, hormone-depen-dent organ atrophy and immune system function (Table 1) [18, 19]. Moreover, it is now apparent that there are many diseases in which abnormal apoptotic processes are etiologically important or in which apoptosis influences the progression or severity of the disease process. For example, abnormally suppressed apoptosis is related to the growth of neoplasms and autoimmune disorders while abnormally induced apoptosis is seen in acquired immunodeficiency disease (AIDS) and various neurodegenerative disorders [20].

In considering the ultrastructural changes that take place during apoptosis, those in the nucleus might be the most impressive. During apoptosis, nuclear chromatin homogenously condenses, attaches to the nuclear membrane and assumes a crescent, half-moon or horseshoe-like appearance (Fig. 1A). The condensed chromatin is glossy and sharply demarcated. Cell shrinkage accompanied by cytoplasmic condensation also occurs until the cell becomes first multi-lobulated and then frag-mented. The nucleus is also fragmented, but other subcellular organelles are preserved in morpholog-ical terms until the final stage. The cell fragments, called apoptotic bodies, are enclosed by the plasma membrane, which appears intact so that the cellular contents are not released, and are rapidly phagocy-tosed by macrophages or neighboring cells. Apoptosis does not lead to inflammation, which is in contrast to necrosis in which an inflammatory response occurs due to rupture of the plasma mem-brane and release of cellular contents.

Specific DNA fragmentation at nucleosomal units is one of the most characteristic biochemical features of apoptosis [18]. Apoptotic DNA frag-mentation is detectable using molecular biologi-cal assays, such as DNA gel electrophoresis (Fig. 1B), or in situ labeling of DNA nicks (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP in situ nick end-labeling [TUNEL]) [21] (Fig. 1C) or ligation of DNA strand breaks having one- or two-base 3' overhangs (Taq polymerase-based in situ ligation) [22].

Necrosis is a general term describing another mode of cell death that differs from apoptosis. The common usage of the term “necrosis” is somewhat problematic, however, because 1) dead cells are so severely degraded by the final stage that it cannot be morphologically determined whether they died via apoptosis or necrosis; and 2) necrosis refers only to an irreversible stage of cell death, even though dying cells generally progress from a reversible to an irre-versible stage. To address this issue, Majno and Joris revived an old term, “oncosis,” which refers to cell death accompanied by swelling [23]. They proposed to substitute oncosis for necrosis in cells dying *via* a process involving cellular swelling or dropsy, and contrasted oncosis with apoptosis, which is accom-panied by cellular shrinkage. They then proposed that necrosis be used to refer to the final stage of either apoptosis or oncosis in which advanced degeneration is seen (Fig. 2). We consider this con-cept excellent, so in this review we will use oncosis instead of the more commonly used necrosis The process of apoptosis can be divided into three steps: induction, determination and execution [24]. Induction involves the introduction of apop-totic stimuli to cells. A variety of chemical and mechanical stimuli are capable of inducing apop-to-sis, including the ligands binding to the Fas recep-tor or tumor necrosis factor (TNF) receptor, radia-tion, chemicals, heat and elimination of a growth factor or other hormone. Indeed, activation of path-ways leading to determination may vary greatly depending on cell type and specific cell conditions (*e.g.*, the degree of differentiation and where the cell is in the

cell cycle). During determination, the second step in the apoptotic process, the signal is transferred to the nucleus, where it affects gene expression. Oncogenes such as c-myc, c-fos, bcl-2 and p53, among others, are expressed, yielding products that either promote or suppress expression or activation of proapoptotic and prosurvival mediators. Finally, during execution the DNA fragmentation and the other morphological changes characteristic of apoptosis occur. In contrast to the diverse pathways leading to induction, execution is well conserved in biochemical terms, and involves specific proteolysis by caspases, a family of aspartyl-specific cysteine proteases, and specific DNA fragmentation by deoxyribonucleases (DNases). There are now more than 10 human caspases known, which can be divided into functional groups [25, 26]. Among apoptosis-related DNases, caspase-activated DNase (CAD) and its inhibitor, inhibitor of CAD (ICAD), were discovered simultaneously [27]. Downstream of the caspases is Acinus (apoptotic chromatin condensation inducer in the nucleus), a factor causing condensation of nuclear chromatin [28]. The factors responsible for cellular shrinkage, budding, and fragmentation are still unknown.

Apoptosis of cardiomyocytes

During development, apoptosis contributes to the normal morphogenesis of the heart, as contributes to the morphogenesis of other organs. Apoptotic cardiomyocyte death is known to occur during embryogenesis, while after birth apoptosis is assumed to be involved in the morphogenesis of the conduction system, including the sinus node, AV node and His bundle [29]. In addition, a study in rats found a greater rate of apoptosis of both cardiomyocytes and nonmyocytes in the right ventricle than in the left within days after birth [30]. This is consistent with fact that although there are similar numbers of myocytes in the two ventricles at birth, there is greater number in the mature left ventricle.

Cell death and proliferation are normally in equilibrium in multicellular animals and plants. Consequently, one would expect apoptosis to have only a very limited role in the healthy heart, as the parenchymal cells and terminally differentiated adult cardiomyocytes rarely proliferate, if at all. This prompts one to wonder whether cardiomyocytes might not exhibit the typical apoptosis. Certainly apoptosis accompanied by the typical ultrastructure, caspase-3 activation, and DNA fragmentation can be induced in adult cardiomyocytes by Fas stimulation (Figs. 3 and 4) [31, 32]. Beta-adrenergic pathway stimulation with isoproterenol induces apoptosis that satisfies the morphological criteria [33], and even a simple cell isolation procedure can induce apoptosis in adult cardiomyocytes [34]. Nevertheless, one *in vivo* study showed that cardiac cells (both cardiomyocytes and nonmyocytes) have a significantly lower sensitivity to apoptotic stimulation than liver cells (Fig. 5) [32]. Thus, the heart may be distinct in certain ways, though the rate of clearance of apoptotic cardiomyocytes was similar to that of apoptotic hepatocytes (< 24 hours).

Apoptosis in myocardial infarction

Apoptosis among *in vivo* cardiomyocytes was first reported by Gottlieb *et al.* [35]. In that case, apoptosis was induced by ischemia/reperfusion in rabbit hearts. The apoptosis was identified based on the presence of fragmented DNA in electrophoretic gels, on *in situ* nick end-labeling assays and on electron microscopic analysis showing the presence of clumped chromatin scattered within the nucleus. In the same year, an *in vitro* experiment showed that hypoxia induced DNA fragmentation accompanied by overexpression of Fas mRNA in cultured neonatal cardiomyocytes [36], and somewhat later persistent ischemia without reperfusion was shown to induce a substantial number of apoptotic cardiomyocytes in areas of acute infarction in rat hearts [37]. In humans, DNA fragmentation was detected in cardiomyocytes from hearts autopsied following fatal MI [38]. Since publication of these studies, numerous other studies on the relation between apoptosis and myocardial ischemia and infarction have been published, for the most part confirming the aforementioned observations [5]. Regardless of the cause of cardiomyocyte death (*i.e.*, ischemia, reperfusion or both) studies conducted with several animal models and on tissues from humans suggest that both apoptosis and necrosis are responsible for acute ischemia-induced cardiomyocyte death [39–44].

It is important to note, however, that all of the evidence for apoptosis in cardiomyocytes cited above

is indirect, based on detection of DNA fragmentation and/or so-called apoptosis-related factors, and that there is little evidence of apoptotic ultrastructure, which is the gold standard for diagnosing apoptosis. Indeed, the electron microphotograph appearing in the pioneering study by Gottlieb *et al.* [35] shows not an apoptotic cell but a typical oncotic cardiomyocyte frequently found when a heart is reperfused after ischemia; it is a well-known example of contraction band necrosis [45, 46]. Thus, the simple use of TUNEL-positivity and DNA ladder detection for determination of apoptosis can result in misunderstandings as to the mode of cell death, as has repeatedly been cautioned [47–53]. There are several reasons for this. First, TUNEL detects both single-strand and double-strand DNA breaks with free 3'-OH terminals, and may therefore detect not only apoptosis-induced DNA breaks but also ones induced by oncosis. Second, DNA ladders may not be specific for cardiomyocyte apoptosis *in vivo* since nonmyocytes cells, whose numbers greatly exceed those of myocytes in myocardial tissue, may contaminate tissue samples. In addition, DNA ladders are also detected in typical oncosis models created by direct plasma membrane damage [50]. Third, DNA fragmentation is not necessarily related to apoptotic morphology in the nucleus [54, 55]. Notably, the same warning was recently raised in the field of neurology, as it was found that neuronal cell death due to ischemic or degenerative disorders is unrelated to apoptosis, despite the fact that the neurons showed positive TUNEL reactions [56, 57].

We emphasize the importance of using electron microscopy to assess apoptotic morphology, particularly preservation of membrane integrity. When we employed electron microscopy combined with TUNEL (EM-TUNEL), we found TUNEL-positivity in cardiomyocytes that had already exhibited irreversible oncosis with ruptured plasma membranes several hours earlier (Fig. 6) [58]. We found no cardiomyocytes exhibiting apoptotic ultrastructure in infarcted areas. Thus, the so called “apoptotic cardiomyocytes” in the infarcted areas were actually irreversibly oncotic cells with fragmented DNA. This implies that although some final steps in the apoptotic process may be activated in infarcted tissue, this activation likely has no relevance to the extent of infarction already determined by irreversibly oncotic cardiomyocytes. Furthermore, although loss of mitochondrial permeability transition has been observed in the heart rendered ischemia/reperfusion and linked to cardiomyocyte apoptosis [59, 60], a recent study denied that linkage [61], supporting the doubt of cardiomyocyte apoptosis during ischemia/reperfusion.

Apoptosis in heart failure

Large MIs lead to severe chronic heart failure with unfavorable remodeling of the left ventricle that is characterized by ventricular dilation and diminished cardiac performance [62]. The ultimate size of acute infarct, which can be determined within several hours after the onset [63], is the most critical determinant of subsequent heart failure. However, many other factors, including late cardiomyocyte death or hypertrophy, fibrosis and expression of various cytokines are also associated with disease progression [64–67]. When subjected to a chronic load, the heart maintains an appropriate functional level through cardiomyocyte hyperfunctionality and hypertrophy. Within the loaded cardiomyocytes, a cascade of protein kinases is activated, which in turn leads to augmented gene transcription and protein synthesis and then to induction of cardiomyocyte hypertrophy. If the load is excessive or long lasting, however, cardiac hypertrophy decompensates into cardiac dilation and failure. The mechanisms responsible for cardiac decompensation are not well understood but likely involve progressive contractile dysfunction and/or progressive cardiomyocyte degeneration and death. Loss of cardiomyocytes during either the acute or chronic stage of MI directly contributes to contractile dysfunction. In addition, apoptosis is now suggested to be involved in the acute ischemic death of cardiomyocytes, as well as in the dropout of surviving cardiomyocytes during the chronic stage of MI.

Narula *et al.* and Olivetti *et al.* found apoptotic cardiomyocytes (TUNEL-positive cardiomyocytes) in the explanted hearts obtained during cardiac transplantation from patients with heart failure resulting from ischemic or dilated cardiomyopathy; they suggested that the loss of cardiomyocytes via apoptosis may contribute to the progression of heart failure [68, 69]. Subsequent studies showed 1) that the incidence of apoptotic cardiomyocytes exhibiting fragmented DNA in TUNEL assays is increased significantly at the borders of subacute and old infarcts, though there is wide variation in

the frequency (0.05%–35%) [70, 71]; 2) that persistent and progressive loss of cardiomyocytes in areas neighboring infarcts due to apoptosis occur during the subacute stage (up to 60 days after the onset) of MI [72]; and 3) that the incidence of cardiomyocyte apoptosis was significantly reduced when the responsible coronary artery was recanalized [73]. In that regard, the term “hibernation” refers to a chronic condition of severe energy deprivation in the myocardium due to chronic or repetitive under-perfusion associated with reversible contractile dysfunction [74, 75]. Elsässer *et al.* [76] showed the presence of apoptosis in human hibernating myocardium and postulated its possible role in tissue deterioration, though Dispersyn *et al.* [77] were unable to confirm this finding; they observed cardiomyocyte dedifferentiation, but not extensive degeneration through apoptosis, in human chronic hibernating myocardium under the electron microscope.

Left coronary artery ligation in experimental animals leads to MI and subsequent development of progressive heart failure. In these animals, cardiomyocytes with fragmented DNA were found among in the apparently surviving myocytes at the border of old infarcted tissue in rats and mice [66, 78–82]. Moreover, some studies reported a relation between the rate of apoptosis among cardiomyocytes (incidence of TUNEL-positive cardiomyocytes) and left ventricular remodeling (dilation). Likewise, using dogs, Sharov *et al.* showed that 3 to 4 months after surgery, there were significantly larger numbers of apoptotic cardiomyocytes in hearts with MIs induced by transcatheter coronary embolization, mainly distributed at the borders of the infarction scars, than in sham-operated hearts [83]. Such coronary embolization was also associated with development of heart failure, caspase-3 activation, and increased numbers TUNEL-positive cardiomyocytes in sheep [84, 85]. Moreover, Chen *et al.* reported apoptosis in subendocardial regions of hibernating areas of the pig heart and emphasized the importance of ongoing apoptotic cell death in this situation [86].

In ultrastructural terms, however, there is no evidence of apoptosis in cardiac myocytes in hearts failing as a result of ischemic heart disease or any other origin. The detection of apoptosis in heart failure has generally entailed the use of indirect methods such as DNA fragmentation and apoptosis-related factors such as caspases. Activation of caspases is strongly indicative of apoptosis because there is no conclusive evidence that caspase-independent apoptosis exists [87]. Especially, evidence of activated caspase-3, one of the effector caspases, is considered useful for detection of apoptosis. Activated caspase-3 was detected by several reports in cardiomyocytes of failing hearts due to dilated cardiomyopathy [88] or ischemic heart disease [72, 73]. However, such positive data were challenged [89, 90] and the specificity remains undetermined because of too high rate of the positive cells [91]: more than 25% of the myocytes were active caspase-3-positive at the site of infarction [72, 73]. Thus, it is still unclear whether the detected active caspases in the previous reports really indicated apoptosis. In fact, the incidences of apoptotic cardiomyocytes detected by TUNEL or activated caspase-3 in diseased hearts are too high for these cells to be considered dead; considering that cardiomyocyte replication in adults is rare and that apoptosis progresses rapidly, such a continuous and massive loss of cardiomyocytes without corresponding replication would rapidly lead to clinical crisis. Nevertheless, no such clinical crisis is observed in most patients. Using electron microscopic TUNEL, we showed that in biopsy specimens from the hearts of patients with dilated cardiomyopathy, TUNEL-positive cells were neither apoptotic nor oncotic, but were living cells (Fig. 7) [92]. We suggested that such cardiomyocytes were undergoing DNA repair because they always expressed proliferating cell nuclear antigen (PCNA), an indicator of DNA replication and repair [93], but never Ki-67, a replication-associated antigen [94]. Furthermore, a subsequent study reported expression of the splicing factor SC-35 in the TUNEL-positive cardiomyocytes in hearts of patients with dilated cardiomyopathy, indicating them to be alive [95], which we confirmed not only in failing hearts such as dilated cardiomyopathy and postmyocarditis but also in other heart diseases (*i.e.*, hypertrophic cardiomyopathy and hypertensive heart disease) [90].

Knowledge about apoptotic signaling has been largely obtained from studies using proliferating or undifferentiated cells; apoptotic signaling in cardiomyocytes is far less well understood. It is generally assumed, however, that apoptotic signaling is essentially similar among cells. Fig. 8 schematically summarizes the apoptotic signal transduction pathways in cardiomyocytes.

In the heart rendered acute ischemia, a substantial number of genes and their products (more than

100) are alternately regulated or activated/inactivated; among these are the so-called apoptosis-related factors. In humans, for example, Bcl-2 is overexpressed in the surviving cardiomyocytes around acute infarcts, which is substituted by Bax during the chronic stage of the infarction [97]. Fas is also reportedly overexpressed in cardiomyocytes neighboring acute infarcts in a rat infarction model [40, 98]. Plasma concentrations of soluble Fas are increased in parallel to the severity of heart failure in humans [99], as is soluble Fas ligand [100], but there may not be a direct temporal or spatial relationship between Fas expression and cardiomyocyte apoptosis in infarcted hearts [101].

The present review does not intend to describe molecular biological aspects of cardiomyocyte apoptosis in detail. For further understanding that field, please refer to other excellent reviews [1, 4–7, 11, 102]. strategies to prevent cardiomyocyte cell death. In Fig. 5, possible therapeutic interventions are indicated by underlines. Of those, the most important and best studied approach may be caspase inhibition. For example, when Yaoita et al. applied a caspase inhibitor, YVAD.fmk, to a rat acute MI model, they observed not only a reduction in the numbers of TUNEL-positive cardiomyocytes but also a reduction in acute infarct size [103]. This finding was subsequently confirmed using other types of caspase inhibitor [104–107], though two studies using a rabbit model (including ours) could not confirm that caspase inhibitors reduced infarct size [108, 109]. There is also a study showing caspase inhibition to have a beneficial effect on postinfarction functional recovery, though this effect appears to be independent of the anti-apoptotic effect, suggesting caspase inhibitors have actions other than inhibition of apoptosis [110]. Conversely, still another study reported an infarct-reducing effect of a caspase inhibitor without functional recovery [111]. Clearly, the therapeutic effect of caspase inhibitors on acute MI remains controversial.

In the actual clinical settings, a number of large-scale trials have shown effectiveness of angiotensin converting enzyme (ACE) inhibitors and β -blockers for preventing development and progress of heart failure after infarction [112–115], although it is unclear whether the beneficial effects mechanically depend on the anti-apoptosis. In contrast, a clinical benefit of TNF- α antibodies on the prognosis of moderate-to-severe chronic heart failure was ruled out by results of recent clinical trials [116, 117].

Nonmyocytes as the target

What was infarcted myocardial tissue containing dead cardiomyocytes and infiltrating inflammatory cells during the acute stage is instead comprised of scar tissue containing few cellular components by the chronic stage. This transformation is mediated during the subacute stage by granulation tissue comprised of numerous myofibroblasts and a rich vasculature [118, 119]. During the subacute phase of MI, the non-myocyte population increases due to both proliferation and infiltration, affecting postinfarction remodeling through the synthesis of extra-cellular matrix and/or the secretion of cytokines and local hormones that interact with cardiomyocytes and nonmyocytes alike [120]. As evidenced by ultrastructure and DNA fragmentation, apoptosis is the mechanism by which non-myocytes present in infarcted tissues, including leukocytes, myofibroblasts, macrophages and endothelial cells, are eliminated from the subacute infarction (Figs. 9 and 10) [121, 122].

This raises the question, what would happen if this apoptosis, which occurs during the natural course of healing, was artificially inhibited? We recently reported that blockade of apoptosis in granulation tissue using the pancaspase inhibitor Boc-Asp.fmk significantly improves survival rate and mitigates both ventricular remodeling and dysfunction during the chronic stage in infarct-bearing rats (Fig. 11) [123]. Possible mechanisms to explain such beneficial effects might be preservation of infarcted wall thickness, which could reduce wall stress, and preservation of myofibroblasts, which could promote infarct contraction. It was also found that in mice, granulation tissue cell apoptosis is at least in part Fas/Fas ligand-dependent [124]. More recently, we found that transforming growth factor- β (TGF- β) could accelerate the granulation tissue cell apoptosis in infarcted area, and that the inhibition of TGF- β signal during the subacute stage of MI mitigated the post-MI ventricular remodeling and heart failure [125]. Taken together, these findings imply the utility of a new therapeutic strategy against postinfarction heart failure - i.e., postinfarction blockade of granulation tissue cell apoptosis - which would be applicable in patients who missed the chance for coronary reperfusion therapy at

the acute stage of their MI [126].

Autophagy in heart disease

Autophagic cell death is another form of pro-grammed cell death that has recently attracted attention [127]. Although autophagy was orig-inally thought to be a physiological process for eliminating unnecessary subcellular organelles, as apoptosis is for eliminating unnecessary cells, cells do die via autophagic mechanisms, and terminally differentiated cells, like neurons and cardiomyocytes, are thought to be more sensitive to autophagy than other cell types [128, 129]. In contrast to apoptosis, autophagic cell death is caspase-independent. Vacuolar degeneration is its most characteristic feature, with cells exhibiting large vacuoles (autophagosomes) containing degraded mitochondria and myelin-like struc-tures. Cathepsin family proteins, the proteases within lysosomes, play important roles here. Recently, cardiomyocyte death showing char-acteristics of autophagy was reported in human heart failure due to dilated cardiomy-opathy, valvular heart disease, hypertensive heart disease, and chronic ischemia [95, 130–133]. Notably, the incidence of such autophagic cardiomyocytes in failing hearts was reportedly greater than the incidence of apoptotic cells. We have also shown abundant and severe autophagic degeneration in car-diomyocytes of the UM-X7.1 strain hamster, an animal model of human dilated cardiomyopathy (Fig. 12) [134]. In this study, we sug-gested a linkage between autophagic degener-ation and cell death. Strictly speaking, howev-er, it is still undetermined whether abnormal autophagy is the primary cause of cardiomy-ocyte death, the result of a failed compensato-ry mechanism, or a phenomenon unrelated with pathogenesis in heart failure. Additional investigation will be necessary to determine the precise role of autophagic cardiomyocyte death in heart disease

Concluding remarks

Although more than 10 years have passed since the first description of apoptosis in MI, this topic continues to attract much attention. Perhaps because apoptosis is considered to be more con-trollable than necrosis, not only its scientific sig-nificance but also the expectation of a therapeutic application drives research on the topic. In that context, the present review may seem somewhat cautionary, but the fact remains that there are many hurdles to surmount before regulation of apoptosis can be clinically applied in the treat-ment of MI and any other heart disease. The most critical issue may be definitive detection of car-diomyocyte apoptosis in pathological hearts, which has not yet been achieved during any stage of MI. Electron microscopy is the most definitive approach, but it is technically difficult and time-consuming, and it is not a good technique for quantification. Development of a more convenient but highly selective assay would be desir-able. In addition, it is still unclear to what extent apoptosis contributes to the pathogenesis or aggravation of heart disease - i.e., it remains unknown whether a reduction of apoptosis would actually improve outcomes in heart disease? Finally, manipulation of apoptosis almost certain-ly entails some risk of adverse effects. All of these issues will have to be resolved in the future.

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