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Review

Morphologic and biochemical hallmarks of apoptosis

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Abstract

Apoptosis is characterised by a series of typical morphological features, such as shrinkage of the cell, fragmentation into membrane-bound apoptotic bodies and rapid phagocytosis by neighbouring cells. This paper reviews the current knowledge on the molecular mechanisms of apoptosis as they relate to the morphologic hallmarks and their implications for the detection of apoptosis in cardiac tissue. Activation of cysteine proteases called caspases plays a major role in the execution of apoptosis. These proteases selectively cleave vital cellular substrates, which results in apoptotic morphology and internucleosomal fragmentation of DNA by selectively activated DNases. In response to several pro-apoptotic signals, mitochondria release caspase activating factors, that initiate an escalating caspase cascade and commit the cell to die. Members of the Bcl-2 oncoprotein family control mitochondrial events and are able to prevent, or induce, both apoptotic and non-apoptotic types of cell death. This suggests that different types of cell death share common mechanisms in the early phases, whereas activation of caspases determines the phenotype of cell death. Detection of apoptotic cells in tissue samples currently relies on the TUNEL assay. TUNEL-positive cardiomyocytes show morphological features of apoptosis and the typical ladder pattern in DNA electrophoresis. Thus, provided that the staining protocol is carefully standardised, this quantitative methodology provides reproducible results of the occurrence of cardiomyocyte apoptosis in cardiac samples. Recently, potentially more specific assays based on analysis of DNA fragmentation or demonstration of caspase activation have been developed. Applicability of these assays to demonstrate cardiomyocyte apoptosis should be tested.

Keywords: Apoptosis; Myocytes; Mitochondria; Histo(patho)logy

1. Introduction

Apoptosis is characterised by a series of typical morphological events, such as shrinkage of the cell, fragmentation into membrane-bound apoptotic bodies and rapid phagocytosis by neighbouring cells [1]. Internucleosomal fragmentation of genomic DNA has been the biochemical hallmark of apoptosis for many years [2]. The cell death process is executed in an organised fashion reflecting the presence of well preserved molecular pathways. Recently, selective proteolysis of vital cellular substrates has been implicated as the key molecular mechanism of apoptotic changes.

This paper reviews the current knowledge on the molecular mechanisms of apoptosis as they relate to the morphological hallmarks, their implications in the detection of apoptosis and the differentiation between apoptosis and other forms of cell death. We discuss the implications of these issues in the assessment of cardiomyocyte apoptosis.

2. Phases and time course of apoptosis

Under physiological conditions, the occurrence of apoptosis in tissues is typically a rare event. Thus, only a small number of apoptotic cells can be seen at any time point. Studies with cytosolic extracts of cells, which have been induced to undergo apoptosis in a synchronous manner, have shown that apoptosis can be divided into biochemically and morphologically distinct phases [3,4]. In the first,

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pro-apoptotic stimuli trigger activation of the central molecular machinery of apoptosis (initiation phase). In the second, committed or effector phase, the molecular executioner machinery becomes fully activated as shown by the ability of the cytosolic extracts of committed cells to induce apoptotic changes in nuclei [3,4]. Only after this, in the third, or degradation phase, do the hallmarks of apoptosis become evident. These include morphologic changes and DNA fragmentation.

The asynchronous nature of apoptotic death in cell populations is mainly due to the highly variable duration of the initiaton phase. In cell culture videomicroscopy studies the dynamic morphologic changes at the light microscopic level always take place in less than 2 h [5,6]. The point of no return occurs several hours before the appearance of morphologic features [6,7]. In vivo, the duration of an apoptotic cell death has been estimated to be between 6 and 24 h, although it may vary depending on the cell type [1,8]. As a result of the short time scale of apoptosis, only few cells undergoing apoptosis are present at a single time point and the quantitative significance of apoptosis may easily be underestimated.

3. Morphologic hallmarks of apoptosis

The definition of apoptosis was first based on a distinct sequence of morphologic features by electron microscopy, described in 1972 by Kerr et al. [1,9]. The onset of apoptosis is characterised by shrinkage of the cell and the nucleus as well as condensation of nuclear chromatin into sharply delineated masses that become marginated against the nuclear membranes. Later on, the nucleus progressively condenses and breaks up (karyorrhexis). The cell detaches from the surrounding tissue and its outlines become convoluted and form extensions. The term budding has been coined for a process whereby the extensions separate and the plasma membrane seals to form a separate membrane around the detached solid cellular material. These apoptotic bodies are crowded with closely packed cellular organelles and fragments of nucleus. The fine structures, including membranes and mitochondria, are well preserved inside the bodies. The apoptotic bodies are rapidly phagocytosed into neighbouring cells, including macrophages and parenchymal cells. Apoptotic bodies can be recognised inside these cells, but eventually they become degraded. If the fragmented cell is not phagocytosed it will undergo degradation which resembles necrosis in a process called secondary necrosis [1,9]. Apoptotic shrinkage, disassembly into apoptotic bodies and engulfment of individual cells characteristically occur without associated inflammation, which would be the consequence of releasing intracellular contents into tissues.

Recent studies indicate that proteolytic cleavages of a set of key proteins by activated caspase proteases play a role in the accomplishment of apoptotic morphology (Table 1) [10]. Although the exact mechanism of how the degradation of these proteins results in apoptotic morphology remains unknown, many target proteins of caspases participate in the formation and regulation of the membrane-associated cortical microfilament cytoskeleton, which is an important determinant of the cell shape (Table 1) [11–20]. Overexpression of the caspase cleaved forms of Gas2 or gelsolin result in dramatic changes in cell shape, resembling apoptosis [18,19]. Also other proteases, such as calpains [11,14], have been implicated in the signalling of the apoptotic changes in the cytoskeleton.

Two protein kinases at the cell-to-cell and cell-to-matrix

Table 1
Structural proteins processed by caspases associated with apoptotic morphology

Protein	Function/localisation	
Actin	Microfilament forming protein with various localisations and functions, i.e. regulation of cell shape in the cortical cytoskeleton	[11–13]
Spectrin/fodrin	Actin cross-linking protein in cortical cytoskeleton	[14–16]
Beta-catenin	Intracellular attachment protein in cell-to-cell junction sites	[17]
Gelsolin	Microfilament fragmenting protein	[18]
Gas2	Microfilament organising protein	[19]
PAK2	Protein kinase involved in regulation of cytoskeleton	[20]
MEKK-1	Regulate cell survival and morphology at cell–matrix and cell–cell contacts sites	[21]
FAK	Regulate cell adhesion at cell–matrix and cell–cell contacts sites	[22]
Keratins 18 and 19	Intermediate filament protein in keratinocytes	[81]
Rabaptin 5	Membrane protein that regulates intracellular vesicle traffic	[104]
Lamin A and B	Intermediate filament that forms the nuclear lamina	[23,24]
NuMa	Mediator of nuclear chromatin–matrix protein interactions	[26]

attachment sites are also targets of caspases [21,22]. Their cleavage by caspases results in further enhancement of the pro-apoptotic signalling [21] and possibly detachment of the cell from the surrounding tissue [22]. Disassembly of the nuclear lamina, the supporting structure of the nuclear envelope, is also an essential feature of nuclear breakdown in apoptosis [3]. This process depends on caspase-mediated degradation of nuclear lamins A and B [23,24]. Proteins involved in the regulation of chromatin structure [25] or interactions between chromatin and nuclear matrix proteins, such as nuclear mitosis associated protein (NuMa) [26], are also cleaved. Caspase proteases have even been implicated in the externalisation of phosphatidyl serine, which mediates the recognition of apoptotic bodies by neighbouring cells [15,27,28].

In cardiomyocytes, several apoptotic features have been reported at the light microscopic level (Fig. 1) (review in [29]). These include condensation of nuclei, shrinkage of the cytoplasm, convolution of outlines, and detachment from the surrounding tissue. Using electron microscopy, morphologic features of apoptosis have been described in cardiomyocytes in the degenerating conduction system [30], in experimental heart failure [31] and in human hibernating myocardium [32]. Apoptotic bodies phagocytosed by macrophages and cardiomyocytes have also been observed, but at this stage the cell type from which the apoptotic body was derived from could not be recognized [30,31].

4. Biochemical hallmarks of apoptosis

4.1. Internucleosomal DNA fragmentation

The biochemical hallmark of apoptosis is degradation of DNA by endogenous DNases, which cut the internucleosomal regions into double-stranded DNA fragments of 180–200 base pairs (bp) [2]. The DNA fragments contain blunt ends [33] as well as single base 3′ overhangs [34]. Internucleosomal fragmentation has been demonstrated with well-characterised apoptotic morphology in a wide variety of situations and cell types (review in [35]).

A variety of caspase substrates are involved in the regulation of DNA structure, repair and replication [25]. The DNase enzymes responsible for the fragmentation during apoptosis include DNA fragmentation factor (DFF40) [36], caspase activated DNase (CAD) [37,38] and in hematopoietic cells NUC70 [39]. DFF40 and CAD are present in normal cells as inactive heterodimers with the inhibitor proteins DFF45 [40] and ICAD (inhibitor of CAD) [38]. These enzymes are selectively activated upon cleavage by caspase 3 [38,40] or by other members of the

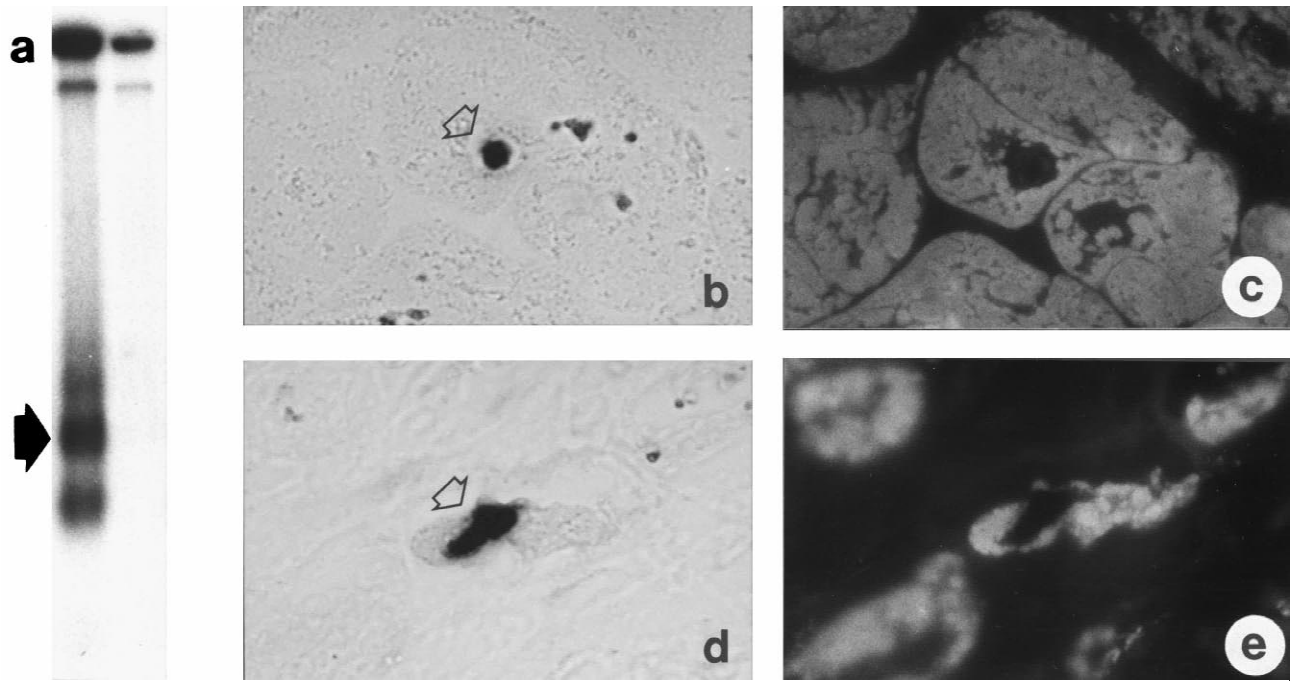

Fig. 1. (a) Ladder pattern of internucleosomal fragmentation in DNA electrophoresis. The DNA is isolated from human myocardium bordering the acutely infarcted tissue and from the remote non-infarcted myocardium of the same heart (left and right lanes, respectively). (b–e) Apoptotic cardiomyocytes detected with TUNEL assay and anti-myosin staining in a cardiomyopathic explanted failing human heart. Positive TUNEL reaction is in the nuclei (b and d, arrows) of the same cells that have been identified as cardiomyocytes by anti-myosin staining (c and e). The nuclei of both cells are condensed, which is typical of apoptosis. Moreover, the cell in panel d is shrunken, its outline is convoluted and detached from the surrounding tissue suggesting that it is in a late stage of apoptosis. Arrow in (a) shows a fragment size of 400 bp, bar in (b–e)=10 μm.

caspase family [41]. Exposure of nuclei to activated CAD or DFF40 is sufficient to induce the nuclear morphologic changes typical of apoptosis [36,37]. Formation of large size (50–300 kbp) DNA fragments precedes internucleosomal fragmentation, but the role of the above mentioned DNases in this process has not been studied [35].

Demonstration of the internucleosomal DNA fragmentation has been a major advance in the detection of apoptosis. The DNA fragments are detectable as a ladder pattern in the electrophoresis of isolated DNA [2]. With the terminal transferase mediated DNA nick end labelling (TUNEL) assay, cells containing DNA strand breaks become visible in light microscopic analysis [8]. The quantification of apoptosis in tissue samples relies on this or related methods.

TUNEL positive cardiomyocytes can be found in cardiac tissue samples in several physiological and pathological conditions (review in [29,42]). However, there has been a substantial variation in the quantitative results obtained in consecutive studies in the same situation. The validity of the TUNEL assay as a method to detect apopotosis has even been questioned [43]. These issues point to methodological complexities associated with the TUNEL assay. DNA damage is not a unique feature of apoptosis, but can occur in necrosis, during repair of reversibly damaged DNA and postmortem autolysis [34,44]. The terminal transferase enzyme adds labelled nucleotides into various kinds of DNA ends [45]. The staining kinetics of the TUNEL assay depends on the accessibility of the DNA strand breaks to the staining reagents. This varies between tissue types and tissue preparation procedures, e.g. fixation and proteolytic pre-treatments [29]. Thus, to avoid false positive or negative results, careful standardisation of the staining conditions is important [29].

Our experience [46,47] is that the optimal positive control of apoptosis is an adjacent myocardial tissue section treated with DNase I enzyme to induce the formation of DNA strand breaks. The tissue sections are heated in sodium citrate (80°C, 30 min) and digested with proteinase K (10 μg/ml, 37°C, 30 min) to expose DNA. To confirm optimal sensitivity and specificity of the assay, the development of the alkaline phosphatase staining reaction should be monitored using the light microscope and terminated upon appearance of intense positivity in the DNase treated control section. These procedures also normalise the staining results for differences in the tissue permeability of the reagents.

Using this methodology, the quantitative results are reproducible ($r=0.88$ for results of repeated procedures) [47] and the TUNEL-positive cardiomyocytes show some features of apoptotic morphology, such as condensed nuclei [46,47] (Fig. 1). In contrast, we have not observed TUNEL staining in cells showing features of coagulative necrosis [46]. In our hands, DNA ladders become demonstrable in myocardial samples when the proportion of TUNEL-positive cardiomyocytes exceeds approximately 0.04% [46] (Fig. 1). Others have shown that postmortem autolysis of 48 h in neural tissue [48] and 24 h in cardiac tissue [49] does not affect the reliability of the TUNEL assay.

4.2. Caspase activation

The fact that interleukin-1β -converting enzyme (ICE) is structurally related to the *Caenorhabditis elegans* cell death protein CED-3, which is necessary for the occurrence of apoptosis in this animal, first suggested that ICE and related enzymes could be implicated in apoptosis also in mammals [50,51]. ICE became the first member of a protease family named as caspases [52], which play a major role in the execution of apoptosis [53–58]. A number of genetic and biochemical studies suggest that caspase activation is essential for the occurrence of the apoptotic phenotype of cell death [59–61]. However, cell death per se is not necessarily dependent on caspases, because cells die through a non-apoptotic morphology even when caspase activity is inhibited [5,7,59,62].

Caspases are cysteine proteases that cleave their substrate proteins specifically behind an aspartate residue (review in [63]). They are formed constitutively and are normally present as inactive proenzymes. For the induction of full enzymatic activity, they require cleavage at specific internal aspartate residues, which separate large and small subunits from each other [63]. Studies on substrate specificity, prodomain structure and biological function [10,63–67] have revealed that caspases are activated during apoptosis in a self-amplifying cascade. Activation of the upstream caspases, such as caspases 2, 8, 9 and 10, by pro-apoptotic signals leads to proteolytic activation of the downstream or effector caspases 3, 6 and 7. The effector caspases actually cleave a set of vital proteins and thus, initiate and execute the apoptotic degradation phase including DNA degradation and the typical morphologic features.

Two major pathways of caspase activation have been characterised (Fig. 2). One is initiated by ligation of the death receptors (rev. in [68]), which include e.g. Fas and TNF receptors. Caspase 8 is the most apical caspase in this pathway [65,66], which is activated by a signalling complex at the receptors [68]. The other, mitochondrial pathway (review in [69,70]) integrates apoptotic signals caused by various cytotoxic agents, aberrant oncogene expression [71] and p53 [72]. It is a target of some apoptosis regulating proteins of the Bcl-2 family [73,74]. It also amplifies receptor mediated apoptosis [75]. The key checkpoint of this pathway is the release of cytochrome C and other caspase activating factors, such as the apoptosis inducing factor AIF, into the cytosol from the mitochondrial transmembrane space [69,70,76,77]. Caspase 9 is the most upstream caspase in this mitochondrial apoptotic pathway [67]. Activation of pro-caspase 9 requires a cytosolic complex that includes cytochrome C, the *C.*

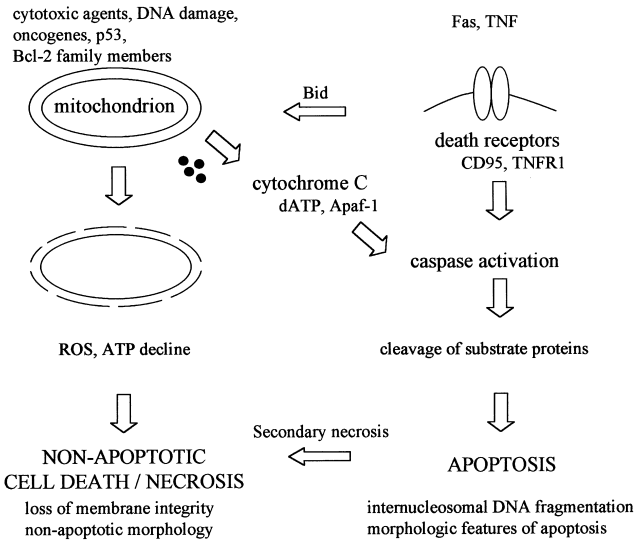

Fig. 2. Major molecular pathways of apoptosis and the relationships between apoptotic and non-apoptotic cell death. Ligation of the death receptors and release of caspase activating factors, such as cytochrome C, from the mitochondria initiate caspase cascades via initiator caspases 8 and 9, respectively. Mitochondria also amplify receptor mediated apoptosis. The activation of caspases determines the phenotype of cell death. However, in some circumstances, apoptosis can progress into secondary necrosis. Apaf-1=apoptosis protease-activating factor, ROS=reactive oxygen species.

elegans death gene CED-4 homologue called apoptosis protease-activating factor (Apaf-1) and dATP [78,79]. Since the mitochondrial morphology remains intact [1], mechanisms of cytochrome C release during apoptosis have been a matter of discussion. Mitochondrial membrane permeability transition and subsequent opening of the permeability transition associated pores [70], and opening of selective release channels before swelling of the mitochondrial matrix, rupture of outer mitochondrial membrane, and permeability transition [69,80] have been suggested.

A novel approach to study the presence of apoptosis is to demonstrate the activation of downstream caspases. This can be detected by western blotting of target proteins that have been cleaved by caspases or by demonstration of caspase activity by enzyme assay. Cleavage site specific antibodies that detect only the cleaved forms of caspase substrates [81–83] or activated forms of caspases have also been generated [84–86]. They can be applied to detect apoptotic cells in tissue sections by light microscopy. In addition, caspase inhibitors have been used to study whether cell death is dependent on caspases.

Several studies have provided evidence of caspase activation in cardiomyocyte death. Active forms of caspases 2, 3 and 7 are generated in the ischemic–reperfused myocardium [87]. Caspase 3 substrate cleavage has been observed under oxidative stress, in ischemic–reperfused myocardium, during acute cardiac allograft rejection and in explanted failing human hearts [87–90]. Moreover, activated form of caspase 3 has been shown to colocalise with the TUNEL-positive cardiomyocytes in ischemic–reperfused myocardium [84]. Recently, translocation of cytochrome C, the major intermediate activator of caspases, from mitochondria into cytoplasm has been observed in explanted failing human hearts [90] and in response to oxygen radical treatment [88]. Caspase inhibitors have been shown to prevent cardiomyocyte death in response to simulated ischemia in vitro [91] and in ischemic–reperfused myocardium together with reduction of myocardial infarct size [87,92].

5. Apoptosis and necrosis: the spectrum of cell death

The differences between apoptosis and other types of cell death have recently been a matter of discussion [93]. Apoptosis is usually contrasted to necrosis, which is considered as the general appearance of cell death following rapid loss of cellular homeostasis (accidental cell death) [1,9,93]. The term oncosis has been coined for cell death caused specifically by ischemia [93]. These types of cell death are characterised by swelling due to accumulation of water and electrolytes, early plasma membrane rupture and disruption of cellular organelles, including mitochondria. Budding and formation of apoptotic bodies are absent and the nuclear chromatin is irregularly clumped. Leakage of intracellular contents induces an inflammatory response. Phagocytosis of the remnants of dead cells is delayed until accumulation of inflammatory cells. The resulting lesions contain groups of contiguous necrotic cells and at later time points inflammatory leukocytes.

The exact morphology of necrotic cells is highly variable, depending on the features of the causative agent. Detection of non-apoptotic cell death relies mainly on the morphologic appearance of the lesions and on the use of assays that demonstrate cell membrane damage, such as uptake of a tracer dye or leakage of intracellular enzymes. However, the term necrosis is actually imprecise [93], as it not only refers to changes occurring during cell death but also to degradation after cells have died. Even apoptotic cells undergo secondary necrosis after the distinct apoptotic morphology has already been accomplished [1].

Recent biochemical studies have weakened the strong contrast between apoptosis and necrosis (Fig. 2) [69,70]. An emerging view is that the mitochondrial changes would be the critical step in commitment to both apoptotic and non-apoptotic types of cell death (Fig. 2). First, the mitochondrial permeability transition is involved in apoptosis and in many examples of well characterised necrosis [70]. Second, the anti-apoptotic proteins of the Bcl-2 family, which act on the mitochondrial level by blocking the release of cytochrome C, prevent both apoptotic and non-apoptotic cell death [5,7,62]. In contrast, the pro-apoptotic family members such as Bax, induce both types

of cell death [94]. Thus, it appears that apoptosis and necrosis may share common mechanisms in the early stages of cell death. The activation of caspases would finally determine whether the cell death is phenotypically apoptotic or necrotic [59,69]. Still, the lack of ATP as well as increasing intensity of the stimulus may result in abortion of apoptosis and consequently, in a mixture of apoptotic and necrotic features [69,95,96]. Similarly, caspase mediated cell death can serve as a precursor of secondary necrosis [69].

The lack of uniform criteria to differentiate between apoptosis and other types of cell death has caused confusion in determining the relative contributions of each form of cell death, particularly in situations when apoptosis and necrosis are thought to co-exist. A typical example is acute myocardial ischemia–reperfusion injury [46,49,97–100]. The kinetics of cell death is very rapid as an area of myocardial necrosis evolves in the central ischemic area during the first hours–days after the onset of ischemia [97,98]. Apoptotic DNA fragmentation has been found in the very early phases of the process in the central ischemic areas and at later times in the border zones [46,98]. Moreover, there is a population of cells showing both internucleosomal DNA fragmentation and membrane damage typical of apoptosis and necrosis, respectively [98]. The proportion of apoptotic cells in the ischemic areas has been highly variable. Moreover, the classical apoptotic morphology has been absent in two consecutive studies [99,100]. Several possible explanations to this have been proposed. First, if the same stimulus induced both apoptosis and necrosis in a dose-dependent fashion [96], higher intensity of ischemia in the central areas could favour necrosis. Second, since the completion of the apoptotic program requires energy, progression of the process could be aborted by the loss of high energy ATP in severely ischemic tissue [95]. Even a specific association of apoptosis with reperfusion has been suggested [99,101]. Third, it has been suggested that biochemical pathways mediating both types of cell death could be activated in the same cell [100].

6. Implications for studying apoptosis in cardiomyocytes

Increasing evidence shows that cardiomyocyte apoptosis occurs in various disease conditions. However, the exact significance of apoptosis and other types of cell death remains to be studied. Table 2 summarises some features of cardiomyocytes that must be taken into account when the results of in vitro apoptosis experiments are to be interpreted. Moreover, to address these questions a consensus on the definition of apoptosis and methodological issues associated with quantification of apoptosis would be needed.

To quantify apoptotic cardiomyocytes in tissue samples, a method with high signal to noise ration is required. Quantification requires analysis of a large number of high power microscopic fields because the number of apoptotic cells may be very small [47] and highly variable in different parts of the sample [45]. The cardiomyocyte origin of the cells undergoing apoptosis should be confirmed by the presence of surrounding myofibers (see Fig. 1). This can be done using either non-specific counterstaining or specific antibodies, which are likely to be more sensitive in case of very small apoptotic bodies. The current method of choice for quantification of apoptotic

Table 2
Special features of cardiomyocytes with possible influence on apoptosis

Feature	Biological factors	Technical factors
Few if any mitoses	High level expression of inhibitors of apoptosis, such as ARC [105] No physiological need of apoptosis to counterbalance mitosis	Low levels of apoptotic cells Difficulty in obtaining sufficient tissue samples
Many nuclei per cell	Correlation of nuclear and cellular apoptosis?	Reliability of nuclear apoptosis assays?
Organised contractile machinery for highly specialised function	May influence morphology, critical proteins may be different than in other cell types Overstretching mediates apoptosis [106]	Morphological criteria may differ from other cell types Assays based on detection of caspase substrate degradation must be validated
High, continuous utilization of ATP for contractile work	Energy dependent execution of apoptosis may be very susceptible to disturbed metabolic state	Difficult standardisation of in vivo energetical conditions
Multicellular tissue architecture	Intercellular signals as mediators of apoptosis?	Cell type identification necessary

cardiomyocytes is the TUNEL assay, provided that is carefully standardised (see above).

Confirmation of apoptosis is then based on other criteria, such as demonstration of the internucleosomal DNA fragmentation in electrophoresis or analysis of the morphological features. Electrophoresis does not specify the cell types undergoing apoptosis and it is not sensitive enough to detect small quantitative differences. Morphologic criteria are considered as the most reliable evidence of apoptosis. However, demonstration of complete apoptotic morphology by a single method is difficult. Nuclear condensation, shrinkage of the cell and fragmentation into apoptotic bodies can be visualised using light microscopy, which is suitable for screening of large numbers of cells. Electron microscopy is required to demonstrate the loss of intact intracellular structures. The major limitation of electron microscopy is that studying large numbers of cells is not feasible. Since the proportion of cells in the degradation phase of apoptosis at a single time point is small, finding even a single apoptotic cell by electron microscopy may be difficult. On the other hand, the use of morphologic criteria has some inherent limitations. The exact sequence of morphological changes may vary in different cell types. For example, there is less formation of apoptotic bodies in cells with a stiff intracellular structure [9].

Novel detection methods of apoptosis include the in situ ligation assay and assays based on the demonstration of caspase activation. By the in situ ligation assay using Taq polymerase it is possible to detect the single base DNA overhangs in apoptotic cells [34]. These DNA fragments are not found in necrotic tumour tissue and after non-apoptotic events, such as treatment of cells with hydrogen peroxide or exposing them to prolonged post-mortem autolysis [34]. Thus, assays using Taq polymerase are suggested to be very specific for the detection of apoptosis in tissue samples. In the myocardium, equal results have been obtained using both TUNEL- and Taq polymerase assays [102,103]. Activation of caspases is a potentially specific feature of apoptosis, as it reflects the underlying molecular changes. Recent studies have shown that methods based on demonstration of caspase activation can be applied to detect apoptotic cells in tissue sections by light microscopy. However, sufficient quantitative accuracy and reproducibility of these assays remains to be studied. In addition to detection of apoptosis, caspase inhibitors could be used to probe caspase-dependence of cardiomyocyte death in response to various apoptotic stimuli.

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