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Review Article

Cardiac myocyte apoptosis

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Introduction

Apoptosis, or programmed cell death, is an energy dependent process that effects the controlled removal of a cell. The process is activated during tissue development, such that unneeded structures may be removed and tissues can be shaped and sculpted. It is also activated in response to cellular injury and results in the removal of the injured cell without damaging surrounding tissue. The process of apoptosis differs markedly from necrosis. This is apparent from the characteristic morphological and biochemical features that are peculiar to apoptotic cell death. Increasingly apoptotic cardiac myocyte death is a recognised feature of disease states of the heart including congestive heart failure and acute myocardial infarction, the two most prevalent forms of heart disease in Western society. The inhibition of cardiac myocyte apoptosis in these disease states may salvage viable myocardium and improve ventricular function. This article aims to summarize the morphological changes characteristic of apoptotic cell death and the methods used to identify apoptotic cardiac myocytes. It also addresses the signalling pathways involved in the initiation and control of apoptosis and reviews the evidence for cardiac myocyte apoptosis in disease states of the human heart.

Cell death

For many years cellular injury has been seen in black and white terms. A cell subjected to minor damage recovers, whereas a cell subjected to a more severe injury dies by necrosis. In 1971, a morphologically different form of cell death, termed apoptosis, was described by Kerr *et al*.[1] The term apoptosis is Greek for 'dropping off', as with the leaves of a tree in the autumn. It has since become apparent that apoptosis is an energy requiring form of cell death that is initiated when a cell is either damaged or exposed to death-inducing proteins. Apoptosis is therefore seen as cell suicide. Apoptotic cell death follows well defined time dependent processes which result in changes in the plasma membrane, proteolysis of intracellular proteins, loss of mitochondrial function and characteristic DNA cleavage. These processes are reflected in the morphological changes seen in apoptosis which include cell shrinkage, loss of cell–cell contacts, membrane blebbing, nuclear condensation and finally the breakdown of the cell into apoptotic bodies that are phagocytosed by surrounding cells (Table 1)[2]. The time taken from the initiation of the apoptotic process to the phagocytosis of the dying cell is unknown. Estimates range from hours to days.

Unlike necrotic cell death, apoptosis does not elicit an inflammatory response and plasma membrane integrity is maintained until late in the process. The preservation of membrane integrity limits local tissue damage by preventing the leakage of noxious intracellular contents into the interstitium. This protective effect is an important feature of apoptotic cell death/removal that occurs during development and cellular injury[3].

Detection

The identification of a cell as apoptotic was originally based on morphological criteria[1]. However, artefact and subjective interpretation have necessitated the development of a number of biochemical techniques that identify individual aspects of the apoptotic process (Table 2). Taken together these additional markers add weight to the identification of an apoptotic cell. A combination of three techniques is probably sufficient to identify apoptosis[4]. Some techniques identify changes that occur early in the process such as externalization of phosphatidyl serine at the plasma membrane[5,6] and changes in membrane permeability to vital dyes[7,8]. Other techniques identify changes that occur later in apoptosis. DNA laddering[9,4] and the detection of a reduced cellular DNA content by fluorescence-activated

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Table 1 The morphology of apoptosis and necrosis

Apoptosis	Necrosis
Single/clusters of cells affected	Areas of tissue destroyed
Small, contracted cells	Cellular oedema
Compact, intact organelles	Dilated organelles
Chromatin condensation	Coarse chromatin
Intact plasma membrane	Plasma membrane rupture
Phagocytosis of apoptotic bodies	Cell rupture resulting in inflammation

Table 2 The time dependent appearance of markers of apoptosis and the means of their detection

Marker of apoptosis	Means of detection
Early markers	
Externalisation of phosphatidyl-serine	Annexin V binding and FACS analysis
Caspase activation	Enzymatic assay/Western blot
Cleavage of caspase substrates	Western blot for substrate cleavage e.g. PARP
Late markers	
Inter-histone DNA cleavage	DNA laddering
In situ DNA strand breaks	TUNEL
Decrease in cellular DNA content	Propidium iodide staining and FACS analysis
Morphology	Electron microscopy

cell sorter analysis (FACS)[8] both identify the DNA degradation that occurs late on in the apoptotic process.

The most widely used technique that can be applied to both tissue sections and isolated cells, is terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL)[9,10]. This technique labels the double strand breaks in the DNA (that occur following the activation of an endonuclease) by incorporating a labelled nucleotide at the DNA strand break point. TUNEL is sensitive but recently the specificity has been challenged[11]. As with other techniques TUNEL must be used in combination with additional markers of apoptosis. The advantage of TUNEL is that nuclear and cellular morphology can be assessed at the same time (Fig. 1(a) and (b)).

For the purposes of quantification, whole tissue and isolated cell populations can be given an apoptotic index. To do this both the number of TUNEL labelled myocyte nuclei and the total number of myocyte nuclei need to be identified within a field by microscopic examination. The apoptotic index is expressed as the percentage of TUNEL-positive myocyte nuclei. There are inherent problems with this technique, as TUNEL-labelled nuclei must be identified within the target cell type (e.g. by using antimyosin heavy chain to identify myocytes). Even then apoptotic, peri-myocyte, interstitial cells can result in false positives and there can be labelling of non-specific DNA strand breaks[11]. This problem can be overcome to a large extent by the use of confocal microscopy, which provides the most specific means of identifying TUNEL positive myocytes (Fig. 1, (c) and (d)).

The apoptotic machinery

Apoptosis is a tightly regulated and evolutionary conserved process. Most of our initial understanding of the molecular mechanisms controlling apoptosis came from studies of the development of the nematode *C. Elegans*. A group of cell death (CED) genes were identified that were central to the process of apoptotic cell death[3]. Following the discovery of the CED genes and their corresponding proteins, the mammalian homologues were identified. These, broadly speaking, fall into two groups — the *c*ysteine containing *a*spartate *sp*ecific pro*tease* (caspases), CED-3 homologs, and the Bcl-2 family, CED-9 homologs. The caspases are instrumental in the execution of apoptosis, whereas Bcl-2 proteins can be either pro- or anti-apoptotic and are seen as arbiters of cell survival[12].

Caspases

Prior to the use of the generic term caspase, these enzymes had a bewildering array of unrelated names. Standardized nomenclature has now clarified the situation (Table 3). Caspases are pro-apoptotic and exist as pro-enzymes that are activated by cleavage at specific protein sequences. To date 10 human isoforms have been identified[13]. Following activation these enzymes have proteolytic activity and cleave specific substrates. Caspases activated early in the process (initiators) act on downstream caspases (effectors) in a caspase cascade that affords amplification. Ultimately the caspase cascade results in the cleavage of target intracellular

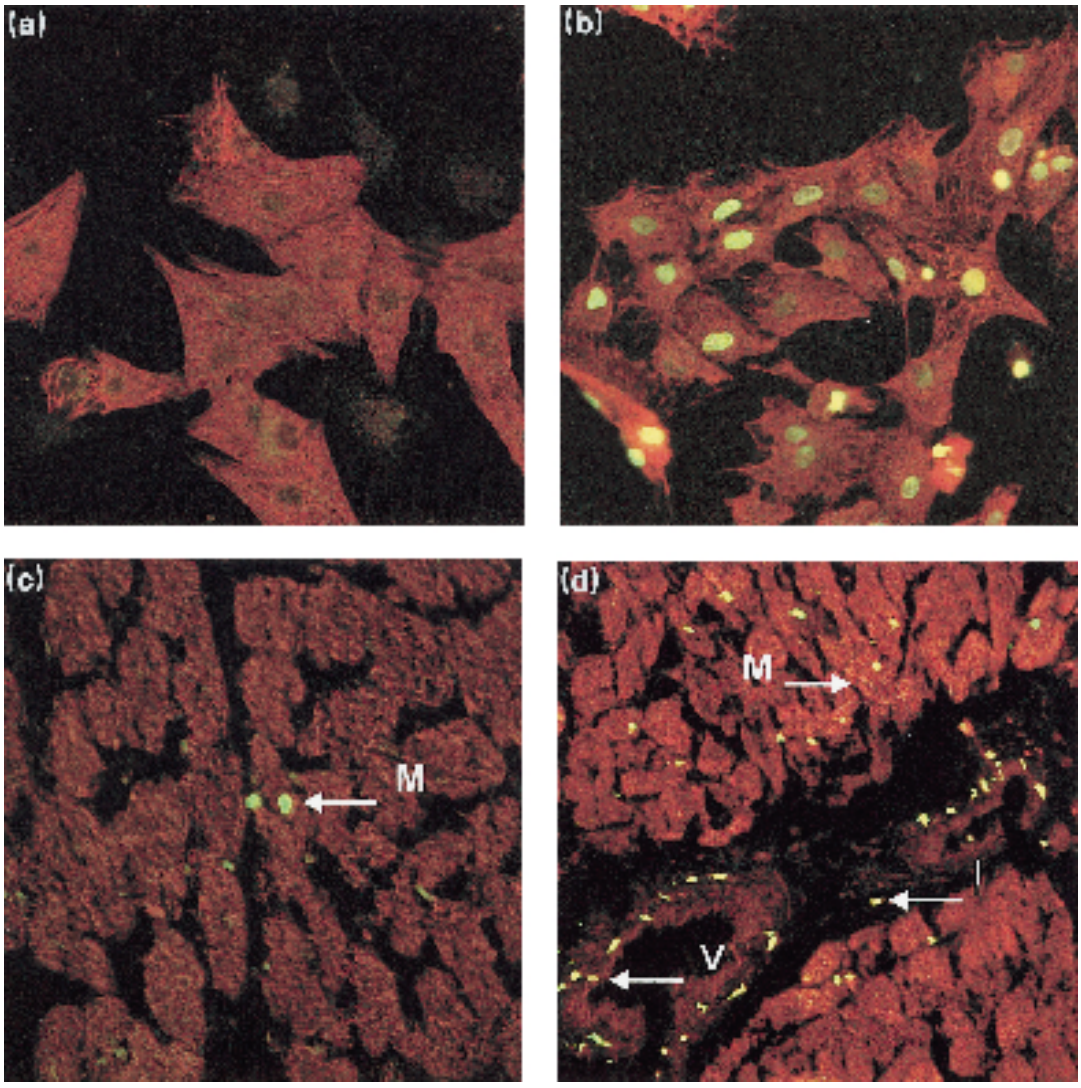

Figure 1 **TUNEL staining. TUNEL staining (green/yellow) of cardiac myocyte nuclei with counter-staining for myosin heavy chain (red). (a) Control neonatal myocytes where the nuclei are not stained. (b) A similar field of cells after exposure to oxidative stress where apoptotic nuclei are clearly labelled. (c) and (d) Tissue sections of an adult rat heart that has been subjected to ischaemia/reperfusion injury. Following cellular injury, apoptotic cell death can occur in single cardiac myocytes (c). This is in contrast to necrotic cell death that kills sheets of connected cells. (d) illustrates the importance of distinguishing TUNEL positive vessel (V) and interstitial (I) cells from positive cardiac myocytes (M) when calculating the apoptotic index.**

proteins resulting in either their activation or degradation (Fig. 2). In this way the caspases can (1) recruit and activate proteins involved in the apoptotic process e.g. the caspase-dependent endonuclease[14], (2) inactivate survival proteins e.g. the extracellularly responsive kinases (ERKs)[15] and the DNA repair enzyme poly-(ADP)ribosyl polymerase (PARP)[16] and (3) act directly on cytoskeletal proteins resulting in plasma membrane changes.

Of the ten caspases characterized in humans caspase-3, 8 and 9 are likely to prove more important in cardiac myocyte apoptosis. Caspase-3 represents the final common pathway of the caspase cascade and has been identified in neonatal[17] and adult rat cardiomyocytes[18]. In addition, caspase-3 like activity has been demonstrated in neonatal cardiomyocytes[17] and the caspase-3 inhibitor (ZVAD-fmk) has been shown to attenuate apoptosis in myocardial ischaemia/reperfusion injury[19]. As the initiation of apoptosis is highly conserved, one might expect TNF-α induced apoptosis in cardiac myocytes[13,20] to be mediated by caspase-8 as it is in other cell lines. However, to date, there is no evidence for caspase-8 expression or activity in cardiac myocytes. Caspase-9 is expressed at high levels in the heart, testis and ovary and is implicated in mitochondrial mediated apoptosis which may act to amplify the apoptotic signal[21] or act as an apoptotic signalling pathway in its own right[22]. Caspase-9 is activated by

Table 3 New and old nomenclature of the proteins responsible for the execution of apoptosis

Caspases	Old nomenclature
Caspase-1	Interleukin 1β converting enzyme (ICE)
Caspase-2	ICH-1, Nedd2
Caspase-3	Apopain, CPP32, Yama
Caspase-4	ICH-2, ICErel-II
Caspase-5	ICErel-III
Caspase-6	Mch2
Caspase-7	ICE-Lap3, Mch3
Caspase-8	FLICE, Mch5
Caspase-9	ICE-Lap6, Mch6
Caspase-10	Mch4

Table 4 Examples of pro and anti-apoptotic Bcl-2 proteins

Anti-apoptotic	Pro-apoptotic
Bcl-2	Bax
Bcl-x_l	Bad
A1	Bid
Bcl-w	Bok
CED-9	Bcl-x_s

binding apoptotic protease-activating factor-1 (apaf-1, a CED4 homolog) and cytochrome-*c*[23,24] (both released from the mitochondria) in the presence of ATP. Active caspase-9 cleaves caspase-3 thus causing its activation[22,25]. The roles of the other members of the caspase family in cardiac myocyte apoptosis remain unclear.

The Bcl-2 family

Following the discovery of the human anti-apoptotic protein Bcl-2, an ever-increasing family of related proteins has been identified. So far, 15 human members of the Bcl-2 family have been identified[12]. In contrast to the caspases, the Bcl-2 proteins are not activated by proteolytic cleavage and can be either pro- or anti-apoptotic (Table 4). As well as their role in apoptosis these proteins have a separate role in the control of the cell cycle, with Bcl-2 retarding re-entry into the cell cycle (beyond the scope of this article).

The Bcl-2 proteins influence cell survival primarily by controlling the mitochondrial response to apoptotic signals[26], although direct inhibition of caspase activity may occur[22]. Activation of mitochondrial apoptotic processes is regarded as the 'point of no return' in apoptosis. Involvement of the mitochondria in apoptosis is manifest by the loss of the mitochondrial membrane

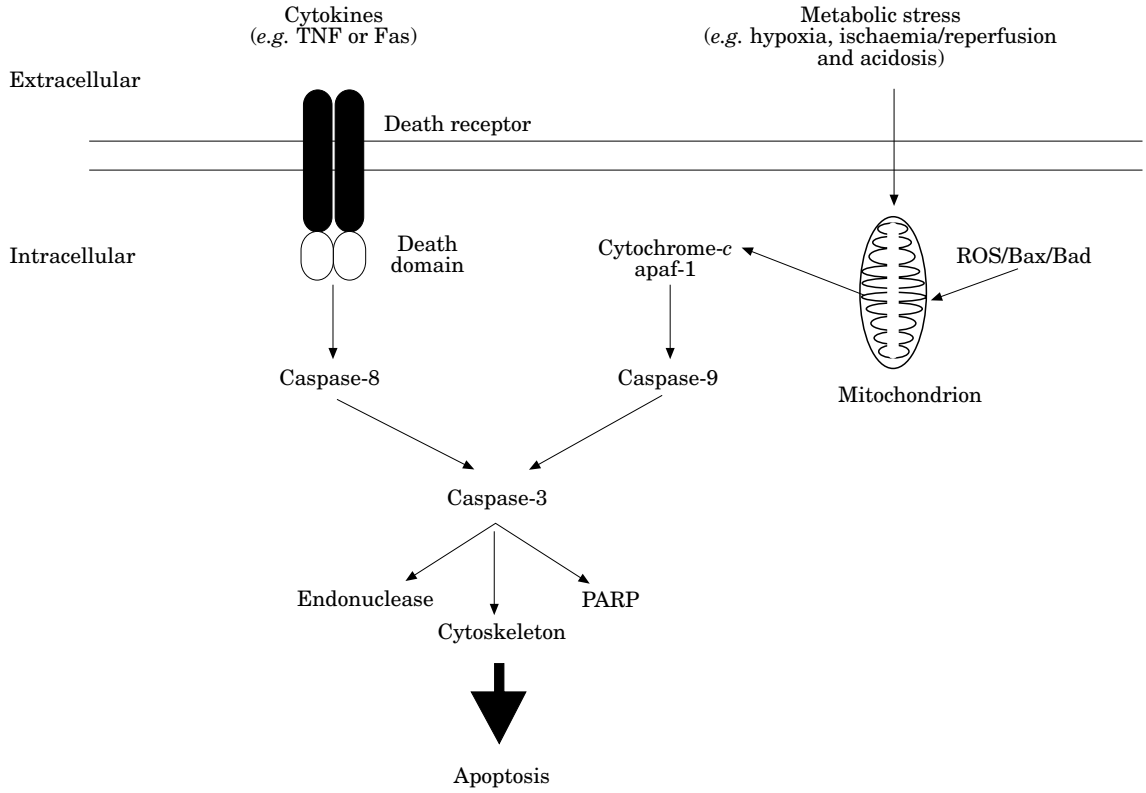

Figure 2 **The caspase cascade. The caspase cascade can be initiated from the cell membrane when death inducing proteins (sFasL or TNF-*α*) bind to their respective death receptors, activating caspases-8. Alternatively, metabolic stressors may activate the caspase cascade via the mitochondria. Release of cytochrome-*c* and apaf-1 from the mitochondria activates caspase-9, which subsequently activates caspase-3. Caspase-3 cleaves multiple intracellular proteins effecting apoptosis.**

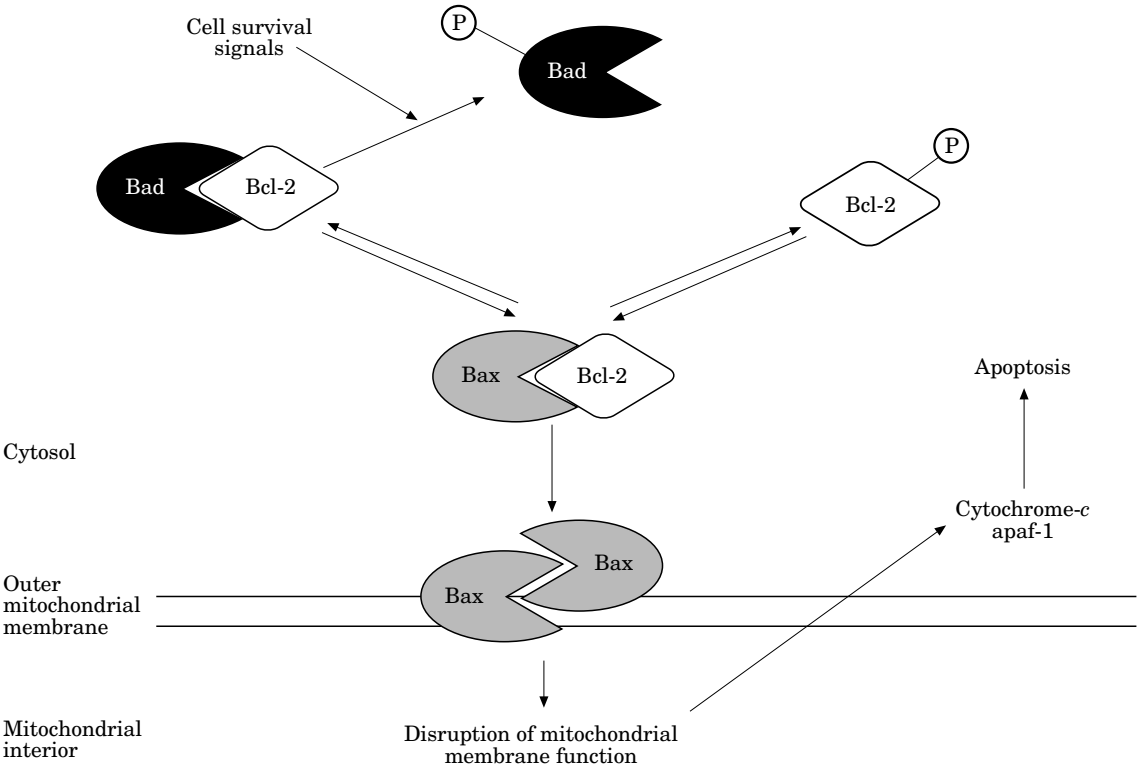

Figure 3 **Bcl-2 family interactions. The Bcl-2 family form homo and heterodimers depending on their relative amounts and phosphorylation status. Bax is released from its Bcl-2 chaperone when Bcl-2 is phosphorylated or when Bad binds to Bcl-2. Free Bax can then form death-inducing homodimers. These translocate to the mitochondria where they disrupt mitochondrial membrane function. Cytochrome-*c* and apaf-1 are subsequently released into the cytosol.**

potential, opening of mitochondrial transition pores, disruption of the electron transport chain, generation of reactive oxygen species and translocation of cytochrome-*c* to the cytoplasm where it activates caspase-9 with the help of apaf-1[23,25,26]. Pro-apoptotic Bcl-2 proteins contribute to this process by disrupting the function of the mitochondrial membranes and their associated proteins. The mechanism remains a subject of conjecture but studies suggest that dimers and higher order structures of some Bcl-2 family proteins may form pores in mitochondrial membranes[26].

The activity of Bcl-2 family proteins is controlled by their phosphorylation status and their ability to form both homo- and heterodimers with other family members[12]. This is illustrated by the interactions between Bcl-2, Bax and Bad (Fig. 3). Bcl-2 binds to and inactivates the pro-apoptotic family member Bax[27] which has the ability to form death-inducing dimers that disrupt mitochondrial membrane function[28]. Bax heterodimers are formed when there is increased expression of Bax[29], a change in Bcl-2 binding affinity for Bax or displacement of Bax by another Bcl-2 binding protein[30].

Phosphorylation of Bcl-2 family proteins affects their binding affinities and intracellular location, thus providing a further means of controlling their activity, Bcl-2 itself is phophorylated[31,32]. The best-characterized example of phosphorylation altering the activity of a Bcl-2 family protein is the phosphorylation (and inactivation) of Bad. Bad activates apoptosis by displacing Bax from Bcl-2 and Bcl-x_l. When Bad is phosphorylated it becomes sequestered in the cytoplasm (binding to the 14-3-3 protein) and is unable to bind to anti-apoptotic Bcl-2 proteins that are associated with the mitochondria[33]. The kinases implicated in Bad phosphorylation include protein kinase B (PKB or Akt)[34,35] and the ERKs[36,37]. Both these kinases can be activated by anabolic/cytoprotective signals such as growth factors.

Evidence supporting the existence of Bcl-2 signalling pathways in cardiac myocytes is sparse, although Bcl-2 has been shown to inhibit ventricular cardiac myocyte death[38]. Increased expression of Bcl-2, with respect to Bax, has been proposed as a potential compensatory mechanism in congestive heart failure[39]. The physiological relevance of the expression of these proteins in the adult human heart remains to be seen.

Initiation, control and inhibition of apoptosis

Apoptosis can be initiated in one of two ways, 'death-receptor' mediated apoptosis and 'non death-receptor'

mediated apoptosis. These pathways have a considerable degree of cross talk and overlap. Death-receptors form a family of proteins that are activated following the binding of extracellular death inducing factors. Non death-receptor mediated apoptotic signalling involves a number of intracellular signalling pathways that usually involve the mitochondria. Whether or not an apoptotic stimulus results in the demise of a cell depends on the cell type, its stage of development and its susceptibility to apoptotic stimuli. A stimulus that effects apoptosis by means of a death-receptor present in one cell type may not be able to do so in another cell type that does not express the receptor.

Individual stimuli involved in cardiac myocyte apoptosis have been identified by in vitro studies. Cardiac myocyte apoptosis has been induced by tumour necrosis factor-α (TNF-α)[40], oxidative stress[41], atrial naturetic peptide[42], hypoxia[43], staurosporine (a protein kinase inhibitor)[17] and stretching[44]. The means by which these stimuli result in caspase activation is far from clear and most of our present information comes from studies in non-myocyte cell lines.

Death-receptor mediated apoptosis

The death-receptors TNF receptor 1 (TNFR1)[20] and the Fas receptor (FasR, also called Fas, CD95 and Apo-1)[45,46] are both expressed in the adult heart. TNFR1 activates the apoptotic process following the binding of TNF-α. The FasR activates the apoptotic process following Fas ligand binding. Fas ligand can be free in the serum (sFasL) or bound to the membrane (FasL) of an apoptosis-inducing cell. Release of FasR from the membrane results in free FasR (also known as sFas or free Fas). sFas can bind to FasR expressed on other cell membranes where it has an anti-apoptotic effect by blocking the binding of pro-apoptotic sFasL/FasL. The ratio of sFas (anti-apoptotic) to sFasL (pro-apoptotic) could be important in disease processes where both these proteins are found in the serum. Other death-receptors (e.g. CAR1, TRAMP and TRAIL) have not been characterized in cardiac myocytes and are not discussed here.

Death-receptors have associated death domain proteins with which they associate following ligand binding and death-receptor dimerization/trimerization. For the FasR and TNFR, these proteins are the TNFR-associated death domain (TRADD) and the Fas-associated death domain (FADD). FADD and TRADD must interact with further proteins, including the receptor-interacting protein[20], to activate caspase-8 which subsequently activates caspase-3[47,48] (Fig. 4). The interaction between death-receptors and death domains can be inhibited by FLICE-inhibitory proteins (FLIPs)[49] which are expressed at very high levels in the heart[50].

Signalling through death-receptors and their associated proteins may be the means by which TNF-α induces cardiac myocyte apoptosis[40], although TNF-α induced oxidative stress may represent an alternative pathway[20,51]. In the clinical setting of congestive heart failure death-receptor apoptotic signalling pathways may prove to be important as both TNF-α[20,52] and sFasL[53]/sFas[54] are chronically elevated.

Non death-receptor mediated apoptosis

Apoptosis is a ubiquitous process and can be initiated by death-receptor and non-death-receptor signalling pathways. Non death-receptor signalling pathways are particularly important in cell lines not expressing death-receptors at the plasma membrane. Whether, or not, a cell dies by apoptosis following the activation of non death-receptor pathways depends on the cell's ability to integrate death and survival signals. Some of the more important death and survival signals are derived from proto-oncogenes[55], protein kinase B (PKB or akt)[34] and the mitogen-activated protein kinase (MAPK) superfamily[56–58].

Until recently, proto-oncogenes (e.g. ras and myc) were regarded as proteins that activated anabolic/proliferative signalling pathways. Paradoxically, it now appears that these proteins can also increase a cell's susceptibility to apoptosis, such that aberrant cell growth can be curtailed by the initiation of the primed apoptotic process[55,59]. Ras is one such proto-oncogene that has a dual role, acting in both a pro and anti-apoptotic manner. Ras can inactivate the pro-apoptotic protein Bad[36] protecting the cell but can also activate the pro-apoptotic protein p53[59] priming the cell for apoptosis. Cross talk between growth and death pathways poses an attractive model for the involvement of apoptosis in the transition from cardiac hypertrophy to cardiac failure although the role of p53 in cardiac myocyte apoptosis remains a topic of debate[60,61].

The role of the MAPKs in the control of apoptosis is rapidly becoming a field in its own right. The three families that make up the MAPK superfamily, the extracellularly-responsive kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38-MAPKs, have different activities with respect to apoptosis[15]. The ERKs are ostensibly anti-apoptotic, which would compliment their previously described cytoprotective role[62]. Experimental evidence supports this hypothesis. Inhibition of ERK activity increases cardiac myocyte death secondary to oxidative damage[41] and the ERKs are implicated in the phosphorylation/inactivation of Bad[37]. The JNKs appear to be pro-apoptotic and experiments suggest JNK signalling is involved in apoptosis secondary to oxidative stress[63], TNF-α[64] and in animal models of AMI[65]. Recently, a novel kinase that acts upstream in the JNK/p38-MAPK signalling pathways has been identified. It has been named the apoptosis signal regulating kinase-1 (ASK-1) and is involved in TNF-α induced apoptosis[51,66]. The role of the p38-MAPKs remains controversial with studies reporting it to have no effect[63], to be pro-apoptotic[67,68] and to be anti-apoptotic[69,70].

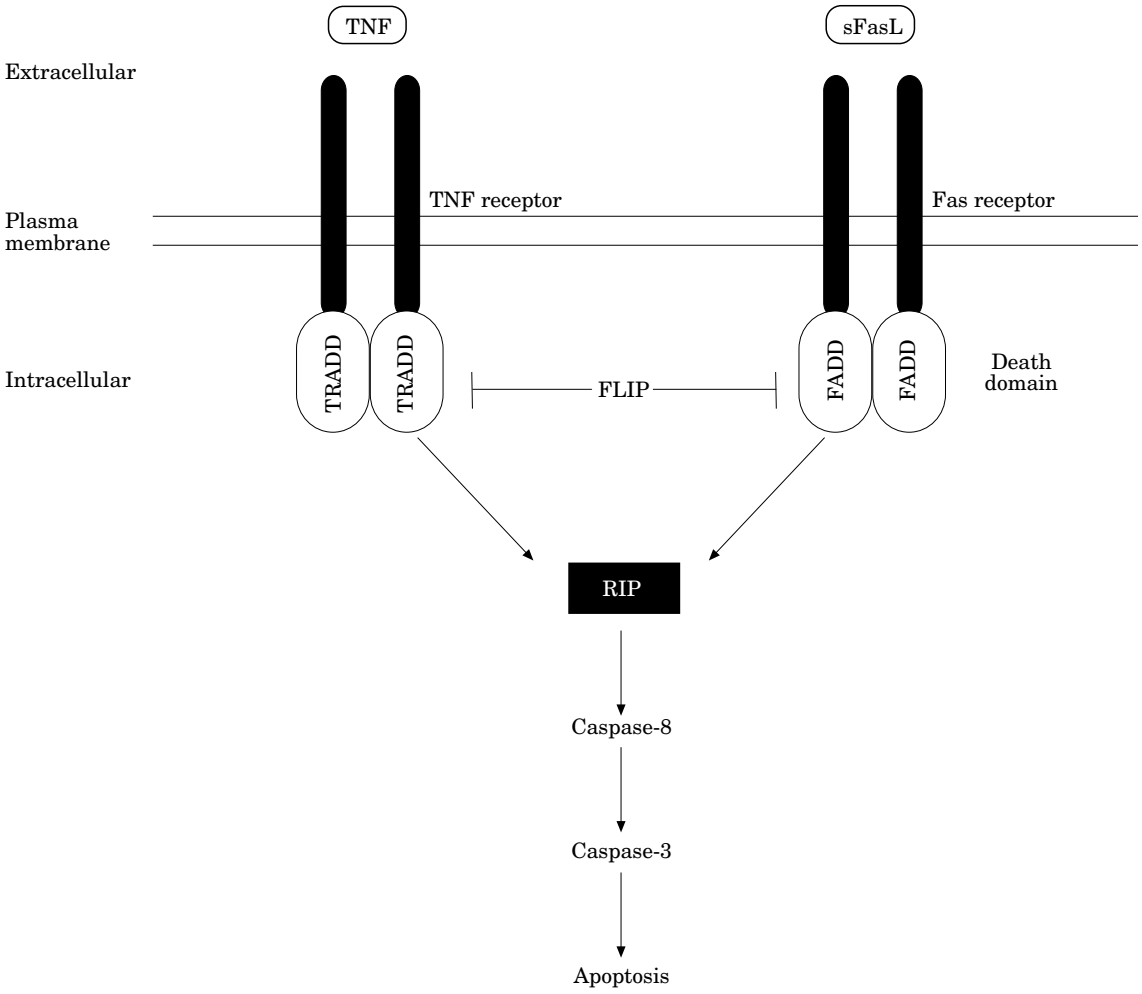

Figure 4 **Death-receptor signalling. Death-receptors, TNFR and FasR (also called Fas or CD95), activate caspase-8 following the binding of their respective ligands and the formation of death-receptor homodimers/trimers. The interaction of the death-receptors with their associated death domain proteins (FADD and TRADD) can be prevented by the anti-apoptotic protein FLIP which is expressed at high levels in the heart.**

The characterization of the cross talk between the proto-oncogenes, PKB, the MAPKs and the apoptotic machinery remains in its infancy. However, a picture of inter-related and inter-dependent growth and death signalling pathways is increasingly apparent (Fig. 5).

Inhibition of apoptosis

The pathways described above act to modify the pro- and anti-apoptotic activities of the caspases and the Bcl-2 family. There is a group of proteins that directly inhibit pro-apoptotic caspase activity. One such protein, the apoptosis repressor with caspase recruitment domain (ARC), is expressed only in heart and skeletal muscle[71]. ARC directly inhibits the activity of the 'initiator' caspase, caspase-8. Another group of proteins that act in a similar way, on 'effector' caspases, are the human inhibitor of apoptosis gene products (IAPs). These proteins act to directly inhibit caspase-3 and caspase-7 activity[72]. The factors involved in the control of the IAPs are poorly characterized, however, transcription of IAPs may be regulated by nuclear factor κB (NF-κ(B))[73,74].

Clinical implications

Apoptotic cell death, or lack of it, has been the subject of intense investigation for many years within the field of oncology. There is now a growing body of evidence, morphological and biochemical, demonstrating the existence of apoptotic cardiac myocyte death in disease states of the adult human heart. Apoptotic cardiac myocyte death has now been identified in tissue from patients with congestive heart failure[39,75], acute myocardial infarction[76,77] arrhythmogenic right ventricular dysplasia[78] and myocarditis[53]. Conversely, lack of cardiac myocyte apoptosis has been implicated in

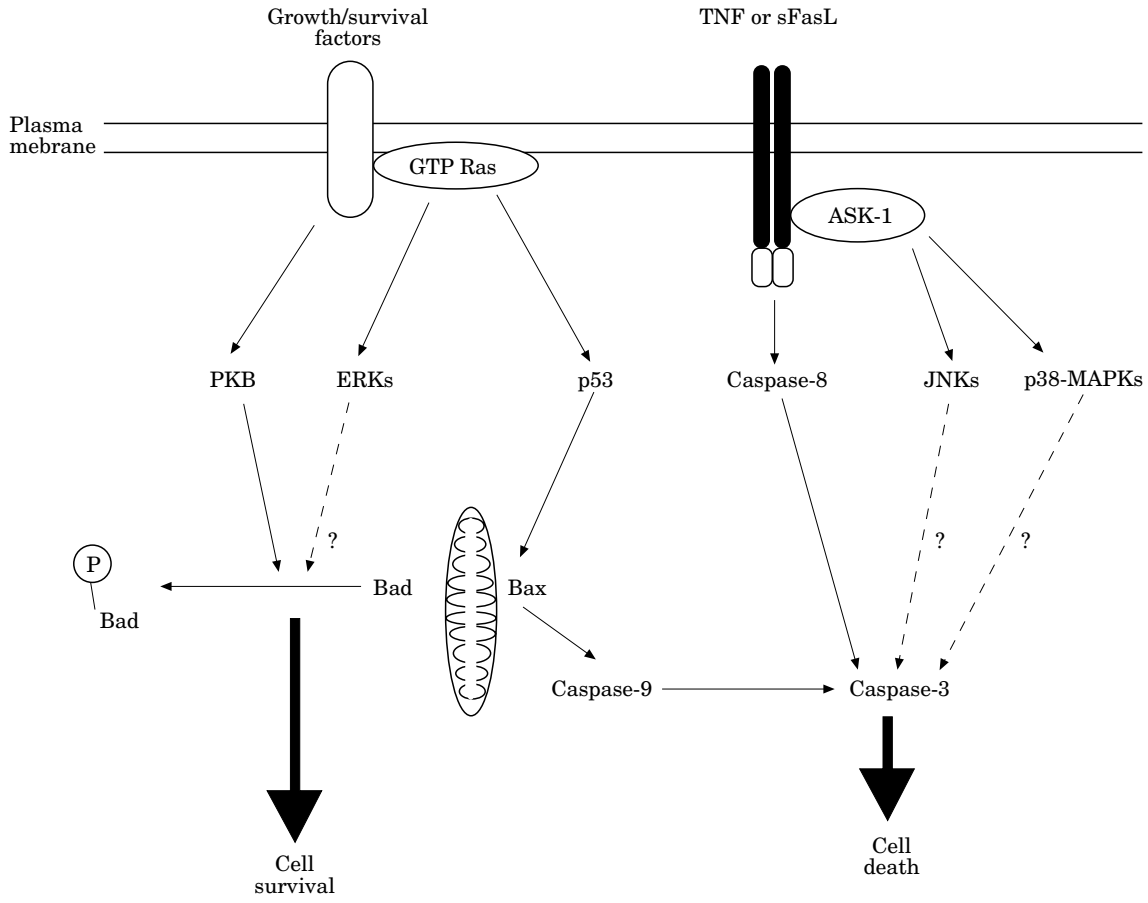

Figure 5 **The inter-relation of stress/survival pathways with the Bcl-2 family and the caspases. TNF-*α* binding to the TNFR activates ASK-1, which subsequently activates the SR-MAPKs. These in turn (predominantly the JNKs) activate caspase-3 resulting in apoptosis. The proto-oncogene ras appears to have a dual role. Ras can promote apoptosis through p53, which increases the amount of death inducing Bax. Conversely, ras can also inhibit apoptosis through the activity of PKB/ERKs that phosphorylate and inactivate the pro-apoptotic protein BAD. The fate of the cell thus depends on the complex integration of multiple death and survival signals.**

congenital defects of the conducting system and in the cardiac abnormalities associated with Noonan's syndrome[79].

Initial values for the percentage of apoptotic cardiac myocytes in congestive heart failure were, in retrospect, too high at 35%[75]. Subsequently values of 0·23%[39] or lower have been proposed using confocal microscopy and electron microscopy evaluation. The observation that cardiac myocyte apoptosis occurs in congestive heart failure is supported by data from animal models. Cardiac myocyte apoptosis occurs in heart failure in dogs following micro-embolization[80] and in pacing induced cardiomyopathy[81]. This form of cell death also occurs in transition from hypertrophy to heart failure in the spontaneously hypertensive rat[82].

The amount of apoptosis that occurs in acute myocardial infarction is less clear. The variation in the quantification of apoptosis in acute myocardial infarction may reflect the different times of collection of autopsy samples as well as differences in methodologies between studies. Apoptosis does occur in the infarcted area, the border zone and in the distal myocardium in acute myocardial infarction and this is supported by animal data[83]. In the clinical setting of acute myocardial infarction, thrombolysis provides another means of inducing apoptotic cell death in cardiac myocytes. Oxidative stress that occurs during reperfusion injury has been shown to induce apoptosis, over and above that induced by ischaemia[18,84]. The degree of apoptotic cell death that occurs following ischaemia/reperfusion may be reduced by the use of caspase inhibitors[19], anti-oxidants[85] and ischaemic preconditioning[86]. This may have therapeutic implications.

There are a likely to be a number of factors involved in cardiac myocyte apoptosis in the clinical setting of congestive heart failure and acute myocardial infarction. In congestive heart failure it is possible that myocytes are sensitized, through BCL-2 family activity/inactivity, to apoptotic stimuli. In acute myocardial infarction cardiac myocyte apoptosis can be seen within hours of

the event. This suggests a more direct activation of the caspase cascade rather than subtle changes in the cells' death/survival signalling pathways. The individual factors triggering the apoptotic process are unknown but oxidative stress, that occurs in both these conditions, is likely to play a major role[87,88]. Other stressors that may be involved include TNFα[52], sFasL[53], ANP[42], angiotensin[60] and wall stress[44].

Therapeutic intervention to reduce the loss of cardiac myocytes through programmed cell death could salvage viable myocardium in both myocardial infarction and congestive heart failure. To an extent, this may already occur in clinical practice in the form of angiotensin converting enzyme inhibitors and anti-oxidants, of which carvedilol may have particular relevance[89,90]. More specific and efficacious inhibition of cardiac myocyte apoptosis may translate into clinical benefit. In the long term, gene therapy may provide a means of preventing apoptosis in cardiac myocytes. Research in this area is also important with regard to cardiac myocyte proliferation as the apoptotic proteins are increasingly recognised as inseparable from the cell cycling machinery.

Conclusions

Cardiac myocyte apoptosis occurs in human heart disease. Provisional evidence from animal models suggests this form of cell death plays an important pathophysiological role in heart disease. Prevention of this form of cell death (preserving functional myocardium) may have major implications in the treatment of acute myocardial infarction and congestive heart failure. However, the development of specific inhibitors of cardiac myocyte apoptosis is currently restricted by our limited understanding of the signalling processes involved. Inhibition of caspases and manipulation of Bcl-2 family protein activities are potential therapeutic targets for the prevention of cardiac myocyte apoptosis.

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