Review

Apoptosis-related genes expressed in cardiovascular development and disease: an EST approach

Mojgan Rezvani, J. David Barrans, Ken-Shwo Dai, Choong-Chin Liew

Institute of Medical Science, The Centre for Cardiovascular Research, Toronto General Hospital, University Health Network, University of Toronto, Ontario, Canada

Department of Laboratory Medicine and Pathobiology, The Centre for Cardiovascular Research, Toronto General Hospital, University Health Network, University of Toronto, Ontario, Canada

Received 6 August 1999; accepted 6 October 1999

Abstract

Apoptosis (programmed cell death) is an important process which, in conjunction with cell proliferation, maintains cell number homeostasis. Although apoptosis has been more extensively investigated in other tissues [1,2], only recently has this process been suspected as a significant contributor to both disease and normal development of the cardiovascular system [3–6]. Grasping a comprehension of the underlying genetic mechanisms of apoptosis is especially crucial considering that cardiac myocytes irreversibly exit the cell cycle and thus fail to proliferate during pathological conditions. Despite great strides in understanding the molecular pathways of apoptosis, there still remain numerous questions to be answered. Identifying key genes that are involved in the regulatory process of apoptosis in the cardiovascular system will serve as a basis for creating more effective therapeutic treatments in cardiovascular disease and provide an understanding of how cardiac development is modulated. This review provides a brief summary of recent data implicating genes that may be involved in apoptosis in the cardiovascular system. It also outlines the continued usefulness of large-scale generation of expressed sequence tags (ESTs) to establish expression profiles from the cardiovascular system and as a means of identifying potentially significant apoptotic regulators previously characterized in other tissues but not as yet in the cardiovascular system. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Apoptosis; Developmental biology; Gene expression; Sequence (DNA/RNA/prot)

1. Introduction

Cell death can occur either by necrosis or apoptosis. Apoptotic and necrotic cells are generally different in morphology and in the sequence of events surrounding their demise. Morphologically, necrosis is characterized by cell lysis and organelle destruction, whereas apoptosis results in cell shrinkage, blebbing, chromatin condensation and DNA fragmentation [7]. The major difference between necrosis and apoptosis is the inflammatory response: cellular debris is accumulated in necrotic cells, whereas in apoptotic cells there exists an efficient degradation and disposal process through phagocytosis by neighboring cells. Apoptosis is essential for normal development through its involvement in controlling cell number homeostasis in conjunction with cell proliferation. However, this balance can be perturbed leading to abnormal, often fatal conditions (such as embryonic lethality) [8,9]; recent studies have sought to identify modulators of apoptosis in an attempt to decipher how the process can be positively or negatively regulated. Early work in the nematode C. elegans [10,11; for reviews see 12,13] has provided...
significant preliminary data in understanding the more complex regulation in higher organisms, such as in mammalian systems.

Apoptosis can be initiated or controlled by developmental and environmental factors such as DNA damage, viral infection, cellular damage and loss of cell–cell or cell–substrate contact; it is also under the regulation of a number of genes. These regulatory genes can be classified into 3 categories:

1. Effectors of apoptosis (e.g., interleukin-1-beta converting enzyme (ICE) family), which are implicated in the onset of apoptosis.
2. Suppressors of apoptosis (e.g., Bcl-2), which are very important for the regulation of apoptosis and are involved in pathogenesis of many diseases such as lymphomas and leukemia [14,15].
3. Intermediate regulators of apoptosis (e.g., Fas/Fas ligand, p53 and c-myc), which can interact with receptor complexes and other apoptotic regulators to induce or suppress apoptosis.

To obtain a profile of genes expressed in the cardiovascular system from a developmental (fetal vs. adult heart) and disease (normal adult vs. hypertrophic heart) perspective, large-scale sequencing of expressed sequence tags (ESTs) from human cardiovascular cDNA libraries has been investigated. To date, our laboratory has sequenced over 51,000 ESTs from these libraries, and a catalogue of over 5000 genes from the cardiovascular system and a profile of their differential expression has been published [16]. From this, genes that are known to be involved in apoptotic pathways – either as effectors, suppressors or intermediate regulators – can be identified in the cardiovascular system and further characterized.

2. Genes involved in apoptosis – a cardiovascular perspective

The control of apoptosis has been linked to a variety of genes and gene families. Differential expression of these genes between tissues, developmental and disease states and, indeed, between organisms, has been studied to establish pathway(s) of apoptotic regulation. From our data, we have extracted and modified two specific functional subsets of genes identified in the cardiovascular system [16], namely ‘apoptosis’ (Table 1a) and ‘DNA synthesis/replication’ (Table 1b). In Table 1a, the genes were further characterized based on their mode of apoptotic regulation as described previously in the literature, namely ‘effectors’, ‘suppressors’ or ‘intermediate regulators’ of apoptosis. The two subsets were selected since both apoptosis and DNA synthesis/replication are involved in controlling cell number homeostasis.

The genes and gene families identified in our database are described here, including recent data from those characterized further in our laboratory. Individual genes are classified according to their function as described in the literature.

2.1. Interleukin-converting enzyme (ICE) family: caspases

The discovery of death effector genes in C. elegans, namely ced-3 and ced-4 [17,18], provided an important foundation for understanding how apoptosis could be carried out in higher organisms. The ced-3 protein was found to be homologous to a mammalian cysteine protease known as interleukin-1beta-converting enzyme (ICE), now considered a prototype of the caspase (ICE/Ced-3) family of proteases. To date there are at least 14 known human members of this family [19], and studies have shown that caspases are primary death effectors that can be inhibited to block apoptosis [20]. Consequent to cellular apoptotic induction, caspase proforms are proteolytically cleaved to generate activated forms of the enzyme. Typically, these enzymes are activated by other members of the ICE family such as the positive regulator caspase-2 (ICH-1/NEDD-2), whose proform is cleaved by a caspase-3 (CPP32)-like protease [21].

Recently, caspases have been found to play an important role in regulating apoptosis in the cardiovascular system (for a review, see Ref. [22]), particularly in vascular smooth muscle cells [23] and cardiac cells. In vitro, apoptosis induced in cultured rat myocytes was attenuated with ZVAD-fmk, a caspase-specific inhibitor [24]; caspase-3 was found to be present in staurosporine-induced apoptotic cells, implicating this family member as an effector of apoptosis.

Two members of the ICE family may provide further insight into a link between apoptosis regulation and cardiac development. Ich-1L and Ich-1S, also known as caspase-2, has been uncovered through random sequencing of a heart cDNA library [16]. These genes represent two Ich-1 mRNA species that have been reported through alternative splicing: Ich-1L, a gene encoding a 435 amino acid protein that induces programmed cell death; and Ich-1S, a truncated version of Ich-1L whose overexpression suppresses apoptosis [25].

2.2. Bcl-2 family

Spawning from previous work in the nematode C. elegans, a homologue to the death suppressor gene ced-9 was identified in mammals which was found to be significant in maintaining cell survival in human B-cell lymphoma [14,26,27]. Named Bcl-2, this gene served as a prototype for a large family of related apoptotic regulators, including various isoforms and homologues (e.g., Bcl-xL/ Bcl-XS, Bax, Bad, Bak, Bik, etc.) which function to promote death or, like Bcl-2, possess anti-apoptotic activi-
A striking feature of the Bcl-2 family (and one that has helped pave the way for the discovery of novel family members) is the ability of the molecules to form homodimers and heterodimers, a trait that appears to play a significant role in controlling apoptosis [28,29]. Identifying the differential expression of these genes is crucial in understanding how cell number balance is upset in cardiovascular development and/or disease.

The involvement of Bcl-2 family members in ischemia and oxidative stress is significant [30,31], although the exact mechanism by which apoptosis is signalled remains a mystery. Early work reported that Bcl-2 may play an important role in preventing cell death through the scavenging of free radicals, but a direct involvement in myocardial ischemia may not be as significant as suggested previously, considering that it also functions to attenuate apoptosis under anaerobic conditions [29]. Consequently, alternative mechanisms of apoptosis in this condition have been investigated. Both $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ were found to induce apoptosis in isolated cardiac cells but without a concomitant increase in Bcl-2 or Bax protein levels; furthermore, $\text{H}_2\text{O}_2$ induces an upregulation of Bad protein, following which, Bad and Bax form heterodimers with Bcl-2 [32]. It appears, however, that this pathway is independent from those triggered by free radicals (e.g., $\text{O}_2^-$) and suggests a more complex underlying mechanism of apoptotic response. Support for this lies in the findings that Bcl-2 and NFkappaB are differentially regulated in

Table 1
In Silico Northern analysis of known genes expressed in the human cardiovascular system: a) genes involved in apoptosis; and b) genes involved in cell division (DNA synthesis/replication). Modified from Hwang and Dempsey et al., Circulation 1997;96:4146–4203. URL: http://americanheart.org/Scientific/pubs/scipub/genome.html

<table>
<thead>
<tr>
<th>a) Genes involved in apoptosis</th>
<th>Accession</th>
<th>F</th>
<th>A</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effectors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo-2 ligand</td>
<td>U57059</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apoptotic death agonist (BID) isolog*</td>
<td>U75509</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bcl-x isolog</td>
<td>X82537</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>death receptor 3</td>
<td>U72763</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAF1 isolog</td>
<td>U39643</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interleukin-1-beta converting enzyme isoform beta (IL1BCE)</td>
<td>U13697</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA-3 (apoptosis-related) isolog</td>
<td>D50465</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nip1</td>
<td>U15172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nip2</td>
<td>U15173</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nip3</td>
<td>U15174</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nip3 homolog*</td>
<td>U15174</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive regulator of programmed cell death ich-1L</td>
<td>U13021</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>programmed cell death-2/Rp8 homolog (PDCD2)</td>
<td>S78085</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stanniin isolog</td>
<td>M81639</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zinc-finger protein requiem (req) isolog</td>
<td>U10435</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zinc-finger protein requiem homolog ubi-d4</td>
<td>U43920</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Suppressors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apoptosis inhibitory protein</td>
<td>U19251</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apoptotic cell death regulator DAD-1</td>
<td>D15057</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2 binding component 6 (bbcb6)</td>
<td>U66879</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2 related</td>
<td>U27467</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative regulator of programmed cell death ich-1S</td>
<td>U13022</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intermediate Regulators</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fas isolog</td>
<td>U10289</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myeloid differentiation primary response protein MyD88</td>
<td>U70451</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myeloid differentiation primary response protein MyD88 homolog</td>
<td>U70451</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myeloid differentiation protein (MCL1)</td>
<td>L08246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDAG51 isolog**</td>
<td>U44088</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF receptor-1 associated protein (TRADD)</td>
<td>L41690</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-R2-TRAF signalling complex protein**</td>
<td>L49431</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p38-2G4 isolog</td>
<td>X84789</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53 activated fragment-1</td>
<td>U03106</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued on next page)
response to ischemia and reperfusion. During repeated cyclic episodes of short-term ischemia followed by a short period of reperfusion, cardiomyocyte apoptosis and DNA fragmentation were reduced; associated with increased expression of Bcl-2 mRNA and activation of NFkappaB [33]. Thus, Bcl-2 and its family members appear to have crucial roles in the progression of apoptosis during ischemia, although the involvement of each component remains to be fully elucidated.

Consistent with these data, we have identified several Bcl-2 family members in our heart databases. In addition to Bcl-2 itself and related proteins, other genes that have been found to be expressed in the cardiovascular system (Table 1a) including Bcl-2-binding component 6, Bak,
Bcl-x, and BID (identified in a human endothelial cell library; data not shown). Bak and Bcl-x have recently been implicated in cytokine-induced cardiac myocyte apoptosis [34]. BID, a BH3 domain-containing death agonist protein [35], binds to other family member proteins (especially Bax and Bcl-2) to induce apoptosis [36].

2.3. Apoptosis-related genes identified in the cardiovascular system

Through the course of cDNA library sequencing, we have identified apoptosis-related genes (Table 1) previously characterized in other tissues or organisms, but whose role in the cardiovascular system is either newly-emerging or poorly recognized at this time. With further study these genes may provide significant insight into the mechanisms and pathways of apoptosis in cardiac tissue.

2.3.1. Effectors

2.3.1.1. MA-3 MA-3 is a novel mouse gene whose level was found to be induced in apoptosis-induced mouse cell lines (including thymocytes, T cells, B cells and pheochromocytoma) [37]. The MA-3 mRNA was expressed throughout mouse adult tissues, especially in the thymus. The MA-3 gene appears to be highly conserved during evolution in vertebrates and in Drosophila.

2.3.1.2. Nip family The Nip family of proteins were identified using a yeast-two hybrid screen which sought to identify factors interacting with adenovirus E1B 19 kDa protein and its functional substitute, Bcl-2 [38]. Thus, interaction of Nip with these two proteins appears to contribute to cell survival [38]. However, Nip 3 (nineteen kDa interacting protein-3) is a homodimerizing Bcl-2 binding protein [39] and a potent mitochondrial membrane-bound pro-apoptotic regulator found to overcome suppressor effects of Bcl-2 [40].

2.3.1.3. Stannin Stannin is a protein involved in the neurotoxicity of trimethyltin, a potent chemical that damages neurons in the nervous system [41]. Stannin is highly expressed in apoptotic neuronal cells, implying a role for this protein in neurovascular pathology. Although Northern blot failed to detect any appreciable levels of stannin mRNA in rat heart [42], the finding of a stannin isolog in a human fetal heart cDNA library reveals the benefits of large-scale sequencing in discovering low-expressed genes, as stannin may be expressed at a level below the detection limit of Northern blot.

2.3.2. Suppressors

2.3.2.1. DAD-1 In hamster cell lines, a mutant form of DAD-1 was found to induce apoptosis, suggesting a role for this protein as an apoptotic suppressor [43]. In silico analysis has shown that DAD-1 is more highly expressed in cardiac hypertrophy compared to normal adult heart, and thus may play a role in controlling cell numbers during disease. In fact, of the apoptotic regulators identified in our database, DAD-1 was the only gene found to be expressed in a hypertrophic heart library (Table 1).

2.3.2.2. Apoptosis inhibitory protein Neuronal apoptosis inhibitory protein (NAIP) appears to play a key role in modulating apoptosis directly, receptor-associated proteins and signal transducers work in concert to regulate cell death initiated by extracellular agonists. Two receptor systems involved in this network are the tumor necrosis factor receptor (TNFR1) and Fas/APO-1 which, after stimulation from receptor ligands, signal activation of apoptosis (for review see Ref. [45]). Recent receptor-binding studies have identified a family of ligands, which interact with an 80 amino acid region on the receptor known as the ‘death domain,’ that trigger the pro-apoptotic response: TNF-alpha, FasL, Apo-2 [46], FADD/MORT1 (which is recruited to Fas upon binding and interacts with FLICE/MACH/Mch5; see refs. [47–51]), RIP and TRADD.

The Fas/TNF receptor system is rapidly becoming well understood and recent studies have implicated members of this protein family in a variety of cardiovascular diseases: a) dilated cardiomypathy was found to have a significant positive correlation with Fas expression and apoptosis from the myocytes of patients with this condition [52]; b) increased FasL expression in inflammatory disease, reflecting the role of apoptosis in autoimmune myocarditis [53–55]; c) Fas was found to be expressed in a fetal heart cDNA library [16]. Within the complex of the Fas receptor system, FAF1 associates with the cytoplasmic domain of FAS and has been found to potentiate apoptosis in mice [56]. TDAG51 (see Table 1a; identified in a human aorta cDNA library, results not shown) also interacts with Fas to induce apoptosis in T-lymphocytes [57]. Further studies will confirm their involvement in cardiovascular apoptosis.

The TNF-alpha receptor system is especially interesting for modulating apoptosis as it appears to serve a dual function: not only does it induce apoptosis through mediators such as FADD, it also can act as an apoptotic suppressor via the activation of NFkappaB. Originally identified in baculoviruses, homologous mammalian inhibitory apoptotic proteins (IAPs) have been identified and characterized. IAP1 and IAP2 interact with a heterocom-
2.4.1. Apoptotic genes studied in our laboratory

2.4.2. Zinc finger proteins

Recent studies have implicated members of the zinc finger protein (ZFP) family of transcription factors in the positive or negative regulation of apoptosis. For example, extensive apoptosis has been reported in cells a) lacking GATA-1 function [64], and b) overexpressing PAG608 [65], Sp1 [66], ZK1 [67], Requiem [68] and WT1 [69]. Although several ZFPs have been functionally linked to apoptosis, the precise regulatory networks of apoptotic pathways are still not well understood. To understand the pathological mechanisms of heart failure and the involvement of apoptosis, extensive studies of cardiovascular ZFP regulatory networks are required. As an initial step toward this goal, a recently established profile of ZFPs from heart cDNA libraries could be used as a significant resource of ZFP expression data [70]. Elucidating the function of these and other genes identified in the profile may pave the way in understanding the role of ZFPs in apoptotic regulatory pathways of the heart and will help clarify the pathogenesis of cardiovascular disease.

2.4.3. APC

The tumor suppressor protein adenomatosis polyposis coli (APC) was first identified in the cardiovascular system in April, 1996, (accession number N85172; Ref. [16]) and its expression confirmed by RT–PCR [77,78]. In an attempt to characterize the role of APC in the cardiovascular system, we have generated preliminary data from a recent study that indicates a possible involvement of APC in the apoptotic process in vitro (manuscript submitted for publication). Inhibition of APC expression by antisense oligonucleotides drastically altered the cellular proliferation rate, reducing the number of cells during the course of the experiment. In addition, there appeared to be higher cell death in antisense treatment by virtue of a greater number of detached cells. This suggests that APC may also be involved in programmed cell death (i.e., apoptosis) similar to what has been previously observed with many other tumor suppressors. For example, transcription factors such as c-myc are intimately associated with cellular proliferation as its constitutive expression increases the susceptibility of cells to apoptosis [79]. Interestingly, a recent study has shown that APC is involved in the c-myc pathway [80], providing further evidence for a possible link between APC and apoptotic regulation.

2.4.4. p53

The tumor suppressor DNA-binding protein p53 is widely known as an intermediate effector of apoptosis. It is involved in mechanisms of growth arrest and apoptosis, and may stimulate cell death in response to DNA damage [71,72]; conversely, p53-induced apoptosis can be inhibited by members of the Bcl-2 family [73]. Exposure of myocytes to H2O2 and O2− – and thus stimulating apoptosis – has resulted in increased levels of p53 protein [32]. p53 activates Bax and represses Bcl-2, and may work to induce apoptosis through upregulation of the renin–angiotensin system, as observed in rat cardiomyocytes which showed increased levels of angiotensinogen and angiotensin II AT1 receptor, and a consequent 14-fold increase in angiotensin-II expression [74]. Our preliminary RT–PCR results have indicated a differential expression of p53 mRNA during human heart development as well as in cardiac hypertrophy (unpublished data; results not shown). Finding therapeutic agents that may control the level of p53-induced apoptosis may be very important in reducing the consequences of cardiac injury (for review see Ref. [75]).

In silico Northern analysis has identified a gene, WAF1, which is directly induced by p53. It contains a p53-binding site in its promoter region and was shown to reduce human tumors in culture [76].

3. Summary and future directions

Large-scale EST sequencing of heart cDNA libraries has proven to be a successful means of identifying key regulatory genes involved in cardiovascular development and disease [16,81,82]. This method has allowed us to compare expression patterns of cardiovascular genes from
different categories within the database, such as in this review where we compare genes involved in apoptosis and DNA synthesis/cell division.

As with other well-documented apoptotic regulatory proteins, understanding the involvement of novel cardiac cell modulators is a critical undertaking, considering that in humans, myocytes irreversibly exit the cell cycle just before birth. Cardiomycytes are especially prone to abnormal imbalances in cell numbers, such as the case in myocardial infarction, in which prolonged deprivation of oxygen leads to local necrosis of cardiomycytes. This is particularly damaging to the health of the organism because of the inability of cardiomycytes to re-enter a proliferating mitotic cell cycle thus preventing replacement of lost tissue. Instead, the damage is patched up with non-contracile fibroblasts that form fibrous scar tissue. In North America, where cardiovascular disease represents the prime cause of death, cardiac research at the molecular level has been focused on elucidating mechanisms underlying cardiomycyte re-entry into the cell cycle. Since proliferation and apoptosis work in concert to balance cell number, the focus of investigations should also be directed toward understanding mechanisms regulating apoptosis and the manner in which these mechanisms intertwine with those modulating cell cycle re-entry. This is supported by the observation that the general expression pattern of genes regulating DNA synthesis and replication mirror those that are involved in apoptotic pathways (Table 1a and b). How these cells reach a state of apoptotic inducement, and thus upsetting the inherent balance, may provide significant evidence of regulatory effects in cell number homeostasis.

By establishing a profile of cardiovascular gene expression, and identifying those genes involved in modulating this balance, gaining insight into the mechanisms of apoptosis becomes a much simpler task. The use of bioinformatics has provided important preliminary data for studying at the bench level the effects of these candidate genes in a more convenient fashion. One limitation to this approach is the amount of data needed to accurately arrive at conclusions of differential gene expression. Indeed, large-scale sequencing offers the ability to establish a trend of expression patterns between libraries if the number of ESTs generated is significant enough; careful statistical analysis of individual gene expression (e.g., determining Poisson probabilities; see Ref. [16]) and supplementary confirmatory work at the bench level (such as with RT–PCR on more interesting clones; Ref. [78]) can contribute to a more robust result. Gene expression profiles will no doubt lead to a better understanding and further hypotheses into the regulatory pathways of apoptosis and disease. Further investigation into novel genes may reveal previously unknown apoptotic regulators. In the future, this groundwork will be increasingly beneficial for designing more effective therapeutic interventions in counteracting apoptosis with the hopes of successfully treating cardiovascular disease.

Acknowledgements

The Cardiac Gene Unit (URL: http://www.tcgu.med.utoronto.ca) was established in memory of Nigel M.S. Martin. This work was supported by the Heart and Stroke Foundation of Ontario, the Medical Research Council of Canada and Spectral Diagnostics, Inc. MR and K-SD were recipients of Heart and Stroke Foundation Traineeships. MR and JDB were recipients of University of Toronto Open Fellowships. We would also like to thank Mr. Adam Dempsey and Dr. Noel Pabalan for their critical comments and suggestions in the preparation of this manuscript.

References

[18] Yuan J, Horvitz HR. The Caenorhabditis elegans cell death gene


[51] Chinnaiyan AM, Tepper CG, Seldin MF et al. FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced apoptosis. J Biol Chem 1996;271(9):4961–4965.


