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Minireview

Bcl-2 family: Life-or-death switch

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Abstract The Bcl-2 family of proteins that consists of anti-apoptotic and pro-apoptotic members determines life-or-death of a cell by controlling the release of mitochondrial apoptogenic factors, cytochrome *c* and apoptosis-inducing factor (AIF), that activate downstream executional phases, including the activation of death proteases called caspases. Cytochrome *c* release is, thus, central to apoptotic signal transduction in mammals, making study of the mechanism for cytochrome *c* release a major issue. Several models for cytochrome *c* release have been proposed, including rupture of mitochondrial outer membrane and involvement of a specific channel. Here, we provide an overview of recent findings on the role of Bcl-2 family members in the life-or-death decision of a cell.

Key words: Apoptosis; Bcl-2; Mitochondrion; Cytochrome *c*; VDAC

1. Introduction

Apoptosis is the essential physiological mechanism for selective elimination of cells. It plays an integral part in a variety of biological events, including morphogenesis, the process of cell turnover and removal of harmful cells. Apoptotic machinery can also quite easily be activated by a variety of stimuli such as pathological reagents that might cause various diseases. The framework of the apoptotic signal transduction pathway appears to consist of two parts: the private and common. Various pro-apoptotic signals initially activate individual stimulus-specific signaling pathways which eventually converge into a common mechanism that activates death proteases called caspases [1,2]. The common mechanism of apoptosis is negatively regulated by several sets of genes, of which the best characterized is the still growing bcl-2 family [3,4]. This mechanism of apoptosis appears to have been well conserved through evolution: *Caenorhabditis elegans* and mammals share a set of molecules that plays a key role in apoptosis.

Here, we illustrate the recent view of how Bcl-2 family proteins determines life-or-death in a cell.

2. Bcl-2 family proteins as apoptosis regulators

Bcl-2 [5] was initially shown to inhibit cell death induced by IL-3 deprivation [6] and subsequently shown to inhibit cell death induced by various other stimuli including chemotherapeutic agents and heat shock [7]. In recent years, it has been well established that Bcl-2 prevents most forms of apoptotic cell death as well as certain forms of necrotic cell death. A large number of Bcl-2-related proteins have been isolated [3,4] and divided into three categories (Fig. 1):

1. Anti-apoptotic members such as Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, A1 (Bfl-1) and Boo, all of which exert anti-cell death activity and share sequence homology, particularly within four regions, Bcl-2 homology (BH) 1 through BH4, although some members lack an apparent BH4 domain.
2. Pro-apoptotic members such as Bax, Bak, Bad, Mtd (Bok), Diva, which share sequence homology in BH1, BH2 and BH3 but not in BH4, although significant homology at BH4 has been noticed in some members.
3. 'BH3-only proteins', the pro-apoptotic proteins which include Bik, Bid, Bim, Hrk (DP5), Blk and Bnip3, Bnip3L, and share sequence homology only in BH3.

One of the unique features of Bcl-2 family proteins is heterodimerization between anti-apoptotic and pro-apoptotic proteins, which is considered to inhibit the biological activity of their partners [8,9]. This heterodimerization is mediated by the insertion of a BH3 region of a pro-apoptotic protein into a hydrophobic cleft composed of BH1, BH2 and BH3 from an anti-apoptotic protein [10]. In addition to the BH1 and BH2, the BH4 domain is required for anti-apoptotic activity [11]. In contrast, BH3 is essential and, itself, sufficient for pro-apoptotic activity [12].

In addition to the regulation of apoptosis by heterodimerization of anti-apoptotic and pro-apoptotic members of the Bcl-2 family, some protein members have been suggested to regulate apoptosis independently of each other, based on the observations with transgenic and knockout mice [13]. This notion might be consistent with the findings that some Bcl-2 family members such as Bcl-2, Bcl-x_L, and Bax, can form ion channels in synthetic lipid membranes [14–17]. Although one of the BH3-only proteins, Bid, shares very limited sequence homology with Bcl-2 and Bax which have channel-forming ability, it has been shown that the solution structure of Bid is strikingly similar to Bcl-x_L [18–20], and Bid indeed creates an ion channel on synthetic lipid membranes [21]. However, it still remains to be determined whether Bcl-2 family proteins actually form ion channels in vivo and whether these proteins regulate apoptosis via the creation of ion channels.

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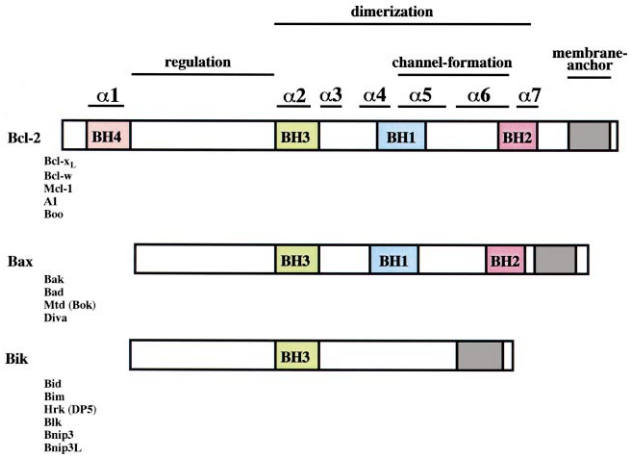

Fig. 1. Schematic drawing of Bcl-2 and related proteins. BH1 to BH4 are conserved sequence motifs. Several functional domains of Bcl-2 are shown (see text). α1–α7 Indicate helices identified in Bcl-x_L, in which a core of two hydrophobic helices α5 and α6 is surrounded by five amphipathic helices. The region between α1 and α2 is called a loop which appears to have a regulatory role. A membrane-anchoring domain is not carried by all members of the family.

3. Prevention of the release of mitochondrial apoptogenic factors by Bcl-2/Bcl-x_L

There is accumulating evidence that the mitochondria play an essential role in many forms of apoptosis [22] by releasing apoptogenic factors, such as cytochrome *c* [23–25] and apoptosis-inducing factor (AIF) [26] from the intermembrane space into the cytoplasm, which activates the downstream executional phase of apoptosis. AIF, which is a flavoprotein with a significant homology to bacterial oxidoreductases, has an ability to induce apoptotic morphological changes of the nucleus in a caspase-independent manner [26], probably representing a minor pathway for apoptotic nuclear changes. On the other hand, cytochrome *c* directly activates caspases [23] by binding to a cytoplasmic protein Apaf-1 (Ced-4 homologue) via the C-terminal WD-40 repeat domain in the presence of ATP or dATP, resulting in an oligomer complex [27] which recruits pro-caspase-9, in turn inducing the self-cleavage/activation of caspase-9 [27,28].

The release of AIF is dependent upon the occurrence of membrane permeability transition (PT)[29] characterized by membrane potential ($\Delta\psi$) loss [30], while cytochrome *c* release can be either dependent [31,32] or independent [24,25,33] on PT. Both Bcl-2 and Bcl-x_L prevent all mitochondrial changes including cytochrome *c* release and $\Delta\psi$-loss [31,32,34]. However, Bcl-x_L but probably not Bcl-2 has an additional ability to prevent caspase activation by sequestering Apaf-1 [35,36]. Although this design is similar to the mechanism by which CED-4 (Apaf-1 homologue) is regulated by CED-9 (Bcl-2 homologue) in *C. elegans* [37], the physiological role of Bcl-2 family proteins to sequester Apaf-1 in preventing caspase activation was recently questioned, based upon the failure to detect a physiologically relevant level of stable interaction between Apaf-1 and Bcl-2/Bcl-x_L [38].

As for the process of cytochrome *c* release, three models have been proposed. One is that cytochrome *c* release is mediated by physical rupture of the mitochondrial outer membrane, resulting from mitochondrial swelling (Fig. 2a) [39,40], although previous studies suggest that mitochondrial swelling rarely accompanies apoptosis. The other models involve cytochrome *c* release through specific channels (Fig. 2b,c).

4. Mechanism for cytochrome *c* release through a channel

A system with isolated mitochondria and recombinant Bcl-2 family proteins has proven very useful for understanding mechanisms of how Bcl-2 family proteins regulate apoptotic mitochondrial changes. It has been shown that addition of recombinant Bax or Bak to isolated mitochondria induces cytochrome *c* release and $\Delta\psi$-loss [31,32,41]. Because these effects can be inhibited by cyclosporin A [31,32], which targets mitochondrial cyclophilin D, the cyclosporin A-sensitive PT pore has been implicated in Bax/Bak-mediated cytochrome *c* release and $\Delta\psi$-loss. Consistently, Bax/Bak-mediated cytochrome *c* release and $\Delta\psi$-loss are reportedly inhibited by other PT inhibitors such as bonkrekic acid that targets ANT and calcium depletion [31,32,34]. There were controversial reports that Bax/Bak-mediated cytochrome *c* release is independent of $\Delta\psi$-loss, but this discrepancy arises from the difference in experimental conditions used as discussed below. Thus, it is fair to say that Bax/Bak-mediated cytochrome *c* release and $\Delta\psi$-loss can at least be regulated by the PT pore. The PT pore is an oligo-protein channel, consisting of porin (or the voltage-dependent anion channel, VDAC) on the outer membrane, adenine nucleotide translocator (ANT) on the inner membrane, and matrix protein cyclophilin D [42]. Bax has been shown to interact with VDAC [32,43] and ANT [43,44]. VDAC has recently been determined to be one of the functional targets for Bcl-2 family proteins [43]: Bcl-x_L closes the VDAC channel on liposomes, whereas Bax/Bak appears to open the channel so that cytochrome *c* passes though the channel (Fig. 2c). An essential role of VDAC in Bax/Bak-mediated cytochrome *c* was confirmed by the observation that Bax/Bak induces cytochrome *c* release from wild type but not VDAC-1-deficient yeast mitochondria [43]. How does Bax/Bak open the VDAC pore size? Bax/Bak might induce a conformational change of VDAC to form a larger

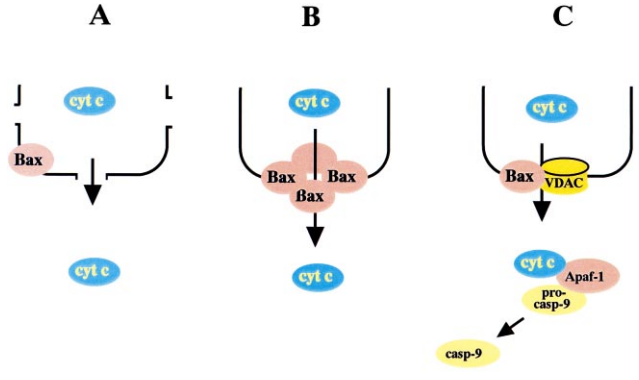

Fig. 2. Mechanisms for cytochrome *c* release during apoptosis. Three mechanisms are proposed by which cytochrome *c* is released during apoptosis: (A) physical rupture of the outer membrane, (B) a channel formed by pro-apoptotic Bcl-2 family members such as Bax, (C) a novel channel formed by VDAC and pro-apoptotic Bcl-2 family members such as Bax. Molecular nature of the novel VDAC-Bax channel is not determined but might be a composite channel. Once cytochrome *c* is in the cytoplasm, it activates caspase(s).

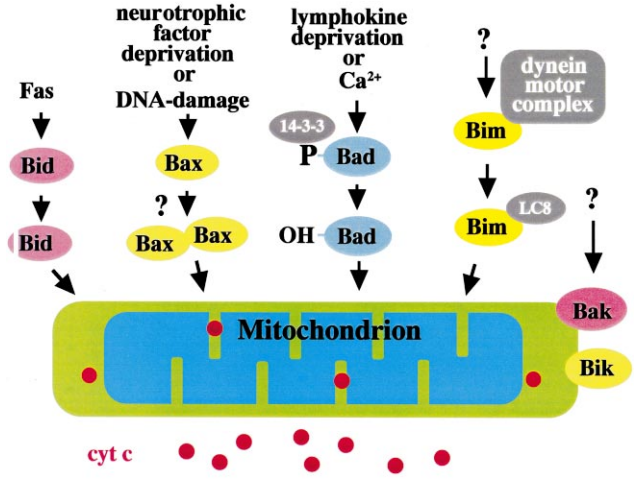

Fig. 3. Convergence of different apoptotic signals into the mitochondria via divergent pro-apoptotic members of the Bcl-2 family. Different pro-apoptotic Bcl-2 family members might receive different death-signals via various regions and transduce the signals to the mitochondria via the conserved BH3 domains.

pore or be involved in forming a larger pore together with VDAC.

Kroemer's group has described that Bax modulates ANT activity [44], although how Bax actually induces cytochrome *c* release by interacting with ANT was not sufficiently explained. It may be presumed that enhanced opening of ANT induced by Bax might result in mitochondrial swelling, leading to outer membrane rupture. Studies with yeast mutants revealed that none of the three ANTs is essential for Bax/Bak-mediated cytochrome *c* release from isolated mitochondria [45], which would exclude ANTs altogether in Bax/Bak-mediated cytochrome *c* release.

It was shown that unlike Bax and Bak, BH3-only proteins such as Bid and Bik do not directly affect VDAC activity on liposomes [46], suggesting that the BH3-only proteins function through pro-apoptotic Bcl-2 family members such as Bax and Bak or through a totally different mechanism. The former possibility might be supported by the findings that Bid induces a conformational change of Bax and Bak, as assessed by an increased accessibility of some antibodies to Bax and Bak which would otherwise be non-reactive [47]. However, the following might support the latter possibility: Bax/Bak-induced cytochrome *c* release is significantly different from Bid (or Bik)-mediated cytochrome *c* release. Notable dissimilarities are the concomitant occurrence of $\Delta\psi$-loss with Bax/Bak-mediated but not Bid/Bik-mediated cytochrome *c* release and the inhibition of Bax/Bak-mediated but not Bid/Bik-mediated cytochrome *c* release by PT inhibitors and respiratory chain inhibitors [46].

Besides its effect on VDAC in cytochrome *c* release, Bax itself has been shown to form a large channel with a conductance at nS level on the planar lipid bilayer [17], which might be large enough for cytochrome *c* to pass through (Fig. 2b), but can not be determined until experimentally demonstrated.

5. Potential loss and VDAC

In addition to being an essential factor in cytochrome *c* release, VDAC has been shown to also be required for apoptotic $\Delta\psi$-loss [43] whereas ANTs are again dispensable [45]. Since VDAC is a component of the PT pore, VDAC probably communicates with other channels on the inner membrane: opening VDAC sufficiently for cytochrome *c* release might trigger the opening of inner membrane channels, resulting in $\Delta\psi$-loss. Given that Bax/Bak directly interacts with ANT and that there seem to be several ANT-like proteins in the mitochondria, such as the Pi/OH^- antiporter and uncoupling proteins, these channels might be directly involved in $\Delta\psi$-loss. Since $\Delta\psi$-loss is Ca^{2+}-dependent, the Ca^{2+} depletion prevents $\Delta\psi$-loss, which might explain occurrence of Bax/Bak-induced cytochrome *c* release without $\Delta\psi$-loss [31,33]. Whether or not Ca^{2+} is required for cytochrome *c* release seems to depend on different mitochondrial preparations and experimental conditions that might affect the activity or structure of the PT pore. Ca^{2+} eventually induces PT, probably resulting in the enhancement of cytochrome *c* release at least with isolated mitochondria. However, it should be noted that, in cells, the interaction of Bax with VDAC might not trigger all of what is seen in the isolated mitochondria during PT. For example, swelling might be just an extreme reaction of the in vitro PT. Since VDAC and ANT are in the PT pore complex, an ANT inhibitor bonkrekic acid (assumed to close ANT channel) would conceivably prevent VDAC opening, thereby inhibiting cytochrome *c* release. Alternatively, Bax might induce $\Delta\psi$-loss by a totally different mechanism, which might be indirectly regulated by the PT pore.

6. Bcl-2 family proteins: a possible convergence point for various life-death signals

In living cells, apoptotic mitochondrial changes are predominantly prevented by anti-apoptotic Bcl-2 members. During apoptosis, the pro-apoptotic Bcl-2 family members are activated, apparently undergoing a conformational change [47], possibly leading to the exposure of the pro-apoptotic BH3 domain through several mechanisms, including dephosphorylation (e.g. Bad) [48] and proteolytic cleavage by caspases (e.g. Bid) [49,50]. Dephosphorylation by phosphase(s) such as calcineurin also frees Bad from cytoplasmic 14-3-3 to translocate to the mitochondria. In addition to Bid and Bad, Bax and Bim, which mainly localize in the cytoplasm in living cells, translocate to the mitochondria to exert pro-apoptotic activity during apoptosis [51–53]. Cytoplasmic Bim (inactive) is bound to LC8 dynein light chain and thereby sequestered to the microtubules-associated dynein motor complex [54]. Bax translocation to the mitochondria involves homodimerization. Different pro-apoptotic members play a major role in initiating cytochrome *c* release in certain settings of apoptosis: Bid is implicated in Fas-mediated apoptosis, Bax in DNA damage-induced apoptosis and neurotrophin deprivation-induced death and Bad in lymphokine deprivation-induced cell death in certain cells.

Given that the activities of anti-apoptotic and pro-apoptotic members of the Bcl-2 family are regulated by different mechanisms, such as various binding proteins with apparently distinct functions and post-translational modifications, such as phosphorylation [55] and proteolytic cleavage [56], the Bcl-2 family might constitute a convergence point for various apoptosis-regulating signals [4]. The presence of a number of BH3-only proteins with a high degree of heterogeneity might

suggest that they receive different death signals through heterogeneous regions of the proteins and that all information converges to the mitochondria via a common BH3 domain (Fig. 3). Thus, the presence of heterogeneity and a common element such as the BH3 domain seem to make it possible for a variety of death signals to be integrated into a common pathway on the mitochondria.

7. In summary

Apoptotic mitochondrial changes, including release of cytochrome *c* and potential loss leading to AIF release, are central to apoptotic signal transduction. Anti-apoptotic members and pro-apoptotic members interact with each other to suppress the activity of their partners and also function independently to directly regulate these apoptogenic mitochondrial changes by interacting with VDAC and possibly other mitochondrial proteins as well. Thus, Bcl-2 family proteins constitute a major life-or-death decision point on the mitochondria.

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