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# Neuronal Apoptosis: BH3-Only Proteins the Real Killers?

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At present there is a poor understanding of the events that lead up to neuronal apoptosis that occurs in neurodegenerative diseases and following acute ischemic episodes. Apoptosis is critical for the elimination of unwanted neurons within the developing nervous system. The Bcl-2 family of proteins contains pro- and anti-apoptotic proteins that regulate the mitochondrial pathway of apoptosis. There is increasing interest in a subfamily of the Bcl-2 family, the BH3-only proteins, and their pro-apoptotic effects within neurons. Recently ischemic and seizure-induced neuronal injury has been shown to result in the activation of the BH3-only protein, Bid. This protein is cleaved and the truncated protein (tBid) translocates to the mitochondria. The translocation of tBid to the mitochondria is associated with the activation of outer mitochondrial membrane proteins Bax/Bak and the release of cytochrome C from the mitochondria. ER stress also has been implicated as a factor for the induction of apoptosis in ischemic neuronal injury. The induction of ER stress in hippocampal neurons has been shown to activate expression of *bb3*/*PUMA*, a member of the BH3-only gene family. Activation of PUMA is associated with the activation and clustering of the pro-apoptotic Bcl-2 family member Bax and the loss of cytochrome C from the mitochondria.

**KEY WORDS:** BH3-only proteins; ischemia; ER stress; Bcl-2 family proteins; mitochondria; apoptosis; necrosis.

## INTRODUCTION

Excitotoxic neuron death has been implicated in the pathogenesis of ischemic, traumatic, and epileptic brain injury (Choi, 1994). Following prolonged glutamate receptor overactivation there is extensive necrotic cell death. This is characterized by increased free radical production, a collapse in the mitochondrial membrane potential ($\Delta\psi_m$), disruption of $Ca^{2+}$ homeostasis, ATP depletion, and an increase in cellular volume (Ankarcrona *et al*., 1995; Choi, 1987; Tymianski *et al*., 1993). However, when glutamate receptor activation is only transient, a more delayed cell death may result (Ankarcrona *et al*., 1995; Budd *et al*., 2000; Luetjens *et al*., 2000; Ward *et al*., 2000). This delayed cell death is associated with a release of cytochrome C from the mitochondria and a collapse of the $\Delta\psi_m$ (Budd *et al*., 2000; Lankiewicz *et al*., 2000; Luetjens *et al*., 2000; Ward *et al*., 2000). The molecular mechanism of the mitochondrial cytochrome C release during excitotoxic neuronal cell death remains unresolved.

In the "classical" mitochondrial apoptosis signalling pathway, the release of cytochrome C requires the pro-apoptotic Bcl-2 family members Bax or Bak (Wei *et al*., 2001). These proteins are believed to form pores that make the outer mitochondrial membrane sufficiently permeable for the release of intermembrane proteins, which include cytochrome C (Kuwana *et al*., 2002). For this to occur, Bax and Bak must undergo conformational changes and enter into the outer mitochondrial membrane (Eskes *et al*., 1998; Goping *et al*., 1998). The transcriptional induction or the posttranslational activation of Bcl-2-homolgy domain-3 (BH3)-only proteins is required to trigger the activation of Bax and Bak (Huang and Strasser, 2000), which can be antagonized by the anti-apoptotic Bcl-2 family members Bcl-2 and Bcl-xL.

## BH3-ONLY PROTEINS

The BH-3-only proteins are a subfamily of the Bcl-2 protein family that are essential initiators of programmed

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**Table I.** Mammalian BH3-Only Proteins: Stimuli and Regulation

| BH3-only proteins | Stimulus; upsteam regulators | Mode of activation | Reference |
|---|---|---|---|
| Bad | Growth factor withdrawal; seizure-induced cell death | Phosphorylation | Zha *et al*., 1996; Henshall *et al*., 2001 |
| Bid | Ischemia; seizure-induced cell death | Lipid modification, Proteolytic cleavage | Desagher *et al*., 1999; Wei *et al*., 2001; Plesnila *et al*., 2001 |
| Bik | | Phosphorylation | Biswas and Greene, 2002 |
| Bim | Growth factor withdrawal; FKHRL 1, JNK | Transcriptional induction, Phosphorylation, Translocation | Puthalakath *et al*., 1999; Putcha *et al*., 2001 |
| Blk | | Phosphorylation | Hedge *et al*., 1998 |
| Bmf | Anoikis(= matrix detachment) | Phosphorylation, Translocation | Puthalakath *et al*., 2001 |
| BNIP3 | Hypoxia | Transcriptional induction | Yasuda *et al*., 1998 |
| NIX | Hypoxia | Transcriptional induction | Yasuda *et al*., 1998 |
| Hrk | JNK | Transcriptional induction | Inohara *et al*., 1998 |
| PUMA | p53; ER stress | Transcriptional induction | Yu *et al*., 2001 |
| Noxa | p53; Hypoxia | Transcriptional induction | Oda *et al*., 2000; Kim *et al*., 2004 |
| Spike | | Transcriptional induction | Mund *et al*., 2003 |

cell death through the activation of Bax and Bak. All the members of this protein family contain a short amino acid (9-16 amino acids) BH3- domain, however they do not posses a very strong structural homology (Huang and Strasser, 2000). The BH3 domain is essential for the binding of these BH3-only proteins to both pro- and antiapoptotic members of the Bcl-2 family. In *C. elegans* a single BH3-only protein, EGL-1, is required for the initiation of programmed cell death (Conradt and Horvitz, 1998), however, in mammals there are at least 12 BH3-only proteins (see Table I). The diversity of these BH3-only proteins is reflected in the different modes of activation. They operate by either blocking the actions of the anti-apoptotic proteins (Bcl-2 and Bcl-xL), there-by facilitating apoptosis, or by promoting apoptosis through the activation of pro-apoptotic proteins (Bax and Bak).

## Excitotoxic Injury

In recent years it is becoming more evident that BH3-only proteins are integrally involved with the apoptotic neuronal death cascade. Neurons from mice deficient of the BH3-only protein Bid have been shown to be resistant to ischemic injury in vivo, as well as hypoxic and excitotoxic injury in vitro (Plesnila *et al*., 2001). The activation of Bid and the subsequent cleavage of Bid to the truncated form (tBid) is an essential component of most forms of receptor-mediated apoptosis (Li *et al*., 1998; Luo *et al*., 1998). During an ischemic-episode- or seizure-induced neuronal death, Bid is truncated to its 15-kDa form that targets the mitochondria (Henshall *et al*., 2001; Plsenila *et al*., 2001). This cleavage of Bid has been shown to occur through caspase-8 (Li *et al*., 1998; Luo *et al*., 1998). tBid is then translocated to the mitochondria, where it activates Bax or Bak and induces the release of cytochrome C from the mitochondria (Eskes *et al*., 2000; Wei *et al*., 2000). As caspase activation may only be very marginal in excitotoxic neuronal apoptosis (Armstrong *et al*., 1997; Budd *et al*., 2000; Lankiewicz *et al*., 2000) this suggests that the activation of Bid may trigger excitotoxic neuronal injury through another pathway (Chen *et al*., 2001; Stoka *et al*., 2001). There is also increasing evidence that Bim may play a significant role in the regulation of neuronal vulnerability in seizure-induced neuronal injury (Shinoda *et al*., in press)

## ER Stress

It has been suggested that ER stress may also contribute to the induction of neuronal apoptosis injury following ischemia (Paschen and Frandsen, 2001). ER stress is the term given to any condition that results in the accumulation of unfolded or malfolded proteins within the ER lumen (Kauffman, 1999). In rodent cells, ER-stress-induced cell death has been shown to involve the activation of ER-resident caspase-12, which subsequently activates executioner caspases such as caspase-3 (Nakagawa and Yuan, 2000; Nakagawa *et al*., 2000). However, there is

increasing evidence that ER stress may activate the mitochondrial apoptotic pathway that results in the release of cytochrome C from the mitochondria (Annis *et al*., 2001; Häcki *et al*., 2000; Wei *et al*., 2001). This requires an increase in the permeability of the outer mitochondrial membrane that is triggered by Bax and Bak (Desagher and Martinou, 2000; Wei *et al*., 2001). In a recent study, Reimertz *et al*. (2003) used tunicamycin to induce ER stress in hippocampal neurons. Tunicamycin prevents protein glycosylation and results in the buildup of malformed proteins within the lumen of the ER. This in turn results in the characteristic unfolded protein response (UPR). Following tunicamycin there is an increase in the expression of a number of different genes that are typically involved in the UPR response, such as *BIP* and *GRP 94* (for a review see Kaufman *et al*., 1999). These molecular chaperones help relieve ER stress by promoting protein folding and keeping proteins in a folding competent state. Following ER stress induced by tunicamycin, there is induction of the bbc3/PUMA protein and subsequent activation of the mitochondrial apoptosis pathway indicated by the release of cytochrome C from the mitochondria (Reimertz *et al*., 2003). In addition to ER stress, Bbc3/PUMA is also activated after transient forebrain ischemia. Furthermore, overexpression of Bbc3/PUMA is sufficient to trigger apoptosis in neuronal cells, and cells deficient in *bbc3*/*PUMA* showed dramatically reduced apoptosis in response to ER stress (Reimertz *et al*., 2003).

It is apparent that the BH3-only proteins play an integral part in the apoptotic pathways that are associated with ischemic and seizure-induced neuronal injury. Through a more detailed understanding of these pathways we will gain new insight into the regulatory pathways that control neuronal injury and through this we may find the new targets for future drug development.

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