Animal Cells and Systems 12: 211-217, 2008

Animal Cells
and
Systems

http://www.AnimalCells.or.kr

Selective Suppression of a Subset of Bax-dependent Neuronal Death by a Cell Permeable Peptide Inhibitor of Bax, BIP

Soo Young Kim, Hyun Kim, and Woong Sun*

Department of Anatomy, College of Medicine, Brain Korea 21, Korea University, Seoul 136-705, Korea

Abstract: Bax, a pro-apoptotic member of Bcl-2 family proteins, plays a central role in the mitochondria-dependent apoptosis. Apoptotic signals induce the translocation of Bax from cytosol into the mitochondria, which triggers the release of apoptogenic molecules such as cytochrome C and apoptosis-inducing factor, AIF. Bax-inhibiting peptide (BIP) is a cell permeable peptide comprised of five amino acids designed from the Bax-interaction domain of Ku70. Because BIP inhibits Bax translocation and Bax-mediated release of cytochrome C, BIP suppresses Bax-dependent apoptosis. In this study, we observed that BIP inhibited staurosporine-induced neuronal death in cultured cerebral cortex and cerebellar granule cells, but BIP failed to rescue granule cells from trophic signal deprivation-induced neuronal death, although both staurosporine-induced and trophic signal deprivation-induced neuronal death are dependent on Bax. These findings suggest that the mechanisms of the Bax activation may differ depending on the type of cell death induction, and thus BIP exhibits selective suppression of a subtype of Bax-dependent neuronal death.

Key words: Bax-inhibiting peptide (BIP), Bax, cerebellar granule neuron, neuronal apoptosis, staurosporine, potassium-serum deprivation

Introduction

A pro-apoptotic member of Bcl-2 family, Bax, is a central player for the neuronal apoptosis during development and following neuronal injury. Essential role of Bax on the apoptotic cell death is well demonstrated by the fact that the genetic deletion of Bax completely rescued neurons from developmental and injury-induced programmed cell death (Sun et al., 2003; Sun and Oppenheim, 2003). In healthy cells, Bax localizes in the cytosols, whereas apoptotic signals induce the translocation of Bax from cytosol into the mitochondria (Wolter et al., 1997), and the mitochondrially translocated Bax irreversibly induces the cytoplasmic release of essential co-factors for apoptosis such as cytochrome C, apopotosis inducing factor (AIF), and Smac/Diablo (Garrido et al., 2006; Kuwana and Newmeyer, 2003; Mignotte and Vayssiere, 1998). For the execution of apoptosis, therefore, it is essential that the modulation of intracellular localization of Bax for the induction/inhibition of apoptosis, and there are several regulating molecules which promote the mitochondrial translocalization of Bax, such as BH3-only members of Bcl-2 family proteins (Bid, Bim, Bok etc) (Fletcher and Huang, 2006; Zhuang and Brady, 2006). In addition to the mitochondrial recruiting factors, some factors suppress the mitochondrial targeting of Bax. For instance, Ku70 is characterized as one of the cytosolic retention factors for Bax (Sawada et al., 2003a; Sawada et al., 2003b). Bax-inhibiting peptide (BIP) is a cell permeable peptide designed based on the Bax-interaction domain of Ku70 (Gomez et al., 2007; Yoshida et al., 2004). BIP is sufficient to inhibit Bax translocation and subsequent Bax-dependent cell death after staurosporine or UV irradiation in HeLa cells (Sawada et al., 2003a; Sawada et al., 2003b). Furthermore, BIP treatment also inhibited neuronal death induced by NGF deprivation (Yu et al., 2003), polyglutamine (Li et al., 2007), and optic nerve transaction (Qin et al., 2004).

We assessed the effect of BIP on the cultured primary cerebral cortex and cerebellar neurons. Surprisingly, whereas BIP inhibited STS-induced neuronal death in cultured cerebral cortex and cerebellar granule cells, BIP failed to rescue granule cells from trophic signal deprivation-induced neuronal death although both type of cell death is dependent on the Bax. These findings suggest that the mechanisms which regulate the localization of Bax, may

*To whom correspondence should be addressed.
Tel: +82-2-920-6153; Fax: +82-2-929-5696
E-mail: woongsun@korea.ac.kr

differ depending on the type of cell death induction, and thus BIP can prevent a subset of Bax-dependent apoptosis.

MATERIALS AND METHODS

Materials

BIP (VPMLK) peptides were synthesized (Peptron, South Korea), and purified by HPLC. The purity of the peptide was >95%. The concentration of the peptides was quantified by UV, lyophilized, dissolved in DW.

Animals

Heterozygous Bax-deficient mice were maintained on a C57BL/6 background. Sibling animals were collected and individually genotyped by PCR as described previously (Knudson et al., 1995). Bax mice were generated from matings between heterozygous (-/-) males and females. For cerebellar granule cultures, postnatal day 7 (P7) mice were used.

Primary neuron cultures

Embryonic cerebral cortex neurons were cultured as previously reported with minor modifications (Sun et al., 2002). Briefly, cerebral hemisphere was isolated from embryonic rats (E17) in cold PBS, and dissociated with 0.25% trypsin, and mechanical trituration. Cells (3×10^5 cells/cm^2) were then plated on the poly-d-lysine coated glass coverslips in Neurobasal media with B-27 supplements (Gibco BRL). Cells were maintained with one-third replacement to fresh media every 3rd days. For staurosporine (STS, Sigma) treatments, 10th days *in vitro* (10 DIV) cells were used, and for kainic acid (KA, Sigma) treatment, 15 DIV neurons were used. For cerebellar granule cell culture, P7 rat cerebellum was dissected, and dissociated with 0.25% trypsin, and pipette trituration. Cells (2×10^5 cells/cm^2) were then plated on the poly-d-lysine coated glass coverslips in DMEM/F12 media with high K+ concentration (25 mM), and 10% heat-inactivated fetal bovine serum (K25+S). In some experiments, cerebellar granule cells were obtained from P7 wild type and Bax-KO mice. On the next day, 10 iM Ara C was added to the media to suppress outgrowth of glial cells. On DIV7, media were replaced by serum-free DMEM/F12 media with 5 mM K^+ (K5-S). Viability of cells was assessed 24 hr after media change.

Cell survival assays

To assess the necrotic and apoptotic neuronal death, propidium iodide (PI, 1 μg/mL) was directly added to the media for 5 min. Because PI is not membrane permeable, only nuclei whose cell membrane was damaged were labeled in red. After washes, cells were fixed with 4% paraformaldehyde for 10 min, and washed with PBS. All nuclei were then stained with Hoechst33342 (blue). Because apoptotic nuclei exhibit either condensed or fragmented morphology, apoptotic neurons can be easily identified. Cells with red and blue nuclei were considered to be necrotic, and blue condensed nuclei to be apoptotic, and blue, large pale nuclei were healthy. Cells exhibiting different nuclear labelings were separately counted and the percentage of apoptotic or necrotic neurons were quantified.

Immunocytochemistry Cells were fixed with 4% paraformaldehyde for 10 min, and washed with PBS three times. Cells were then incubated with blocking solution (10% BSA and 0.2% Triton-X100 in PBS) for 30 min, and rabbit polyclonal anti-Bax (1:500, Pharmingen) and mouse monoclonal anti-cytochrome C (1:1,000, Pharmingen) antibodies were applied for 3 hrs at room temperature. After several washes, Alexa488-conjugated anti-rabbit IgG (1:1,000, Molecular Probes) and Cy3-conjugated anti mouse IgG (1:1,000, Jackson) antibodes were applied for 30 min. Following washes with PBS, cells were then mounted, and the immunofluorescence signal was examined under epi-fluoresence microscope (Zeiss). Images were digitized using CCD camera, and the images were optimized using Adobe Photoshop 5.0 (Adobe).

RESULTS

BIP reduces neuronal apoptosis via inhibiting translocation of Bax and cytochrome C release in cultured cortical neurons

First, we examined whether BIP treatment suppresses the STS-induced cerebral cortex neuronal death (Fig. 1A). In cortical neurons, about 23% neurons remained viable 24 hr after 100 nM STS treatment. On the other hand, co-treatment of BIP (200 μM) significantly suppressed the STS-induced neuronal death. To examine whether anti-apoptotic effect of BIP is mediated by the inhibition of Bax translocation and subsequent cytochrome C release, we performed Bax and cytochrome C immunostaining (Fig. 1B and C). Whereas about 70% neurons exhibited Bax puncta, which is a hallmark for Bax translocation, by 12 hr STS treatment, BIP treatment reduced cells exhibiting Bax puncta, indicating that BIP efficiently blocked Bax translocation. In consistent with this idea, we also found that the cells exhibiting cytochrome C release following STS treatment was reduced by BIP treatment (Fig. 1C). On the other hand, BIP failed to inhibit KA-induced neuronal death (Fig. 2). Following 200 μM KA treatment, there was rapid degeneration of neurons and only 3-5% neurons remained regardless the BIP treatment when examined 24 hr after treatment. Collectively, these results suggest that BIP inhibits STS-induced apoptotic neuronal death, but not KA-induced necrosis of cerebral cortex neurons.

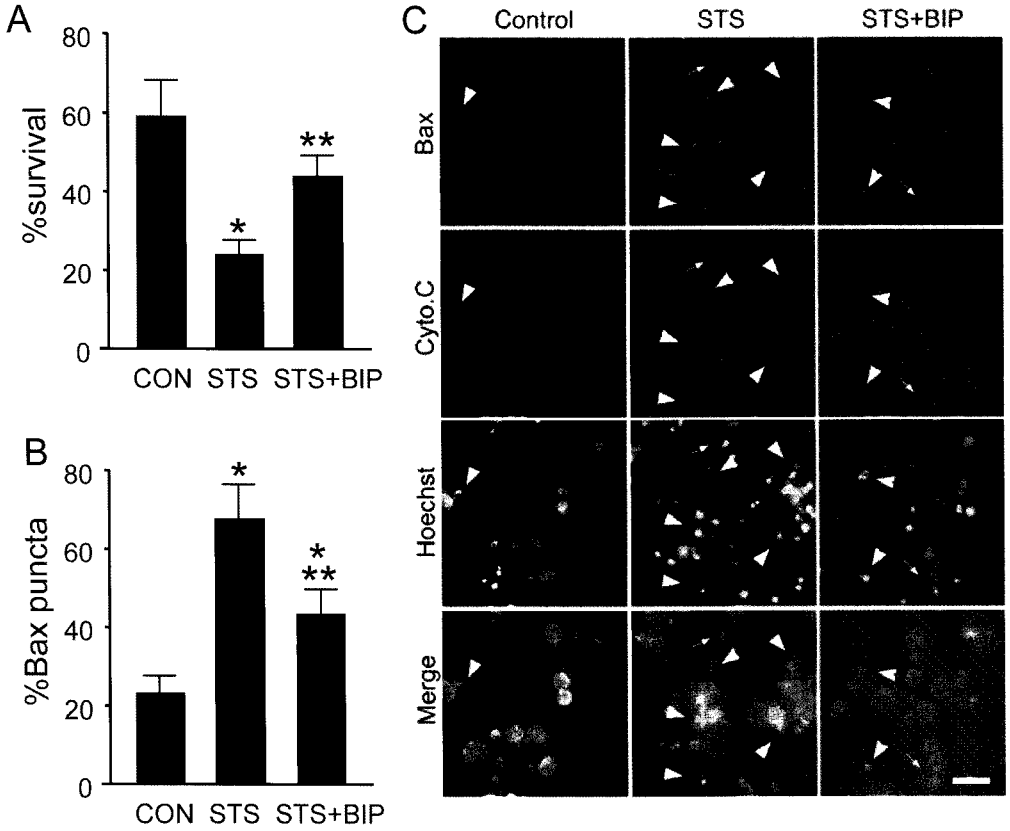

Fig. 1. Effect of BIP on staurosporin (STS) induced cortex neuronal death. (A) Suppression of STS-induced neuronal death by BIP. Cell viability was assessed by the percentage of intact nuclei among total cell nuclei in a field. Data are mean±SE, n=3, *p<0.05 versus control, **p<0.05 versus STS treated neurons in student's t-test comparisons. (B) Quantification of cells exhibiting Bax puncta after 24 h of STS treatments. Data are mean±SE, n=3, *p<0.05 versus control, ***p<0.05 versus STS treated neurons in student's t-test comparisons. (C) Localization of Bax and cytochrome C in cerebral cortex neurons treated with STS in the presence or absence of BIP. STS (100 nM) was treated with BIP in cerebral cortex neurons for 24 h. Co-treatment of BIP (200 μM) suppressed the percentage of condensed nuclei, Bax puncta and cytochrome C release. Cells with cytosolic or puncta forms of Bax are indicated with arrows or arrow heads, respectively. Scale bar=20 μm.

BIP inhibits apoptotic death induced by STS treatment but not serum starvation in cultured cerebellar granule cells

Next, we examined the effect of BIP on the cerebellar granule cell death by potassium-serum deprivation (K5-S). The cultured cerebellar granule cells are normally maintained under the high potassium (25 mM) and 10% serum (K25+S), and Bax-dependent apoptosis takes place when serum is removed from the media with low potassium (5 mM) concentration (D'Mello et al., 1993; Gallo et al., 1987; Nardi et al., 1997). However, surprisingly, we failed to observe significant anti-apoptotic effect of BIP against cerebellar granule cell death induced by K5-S condition (Fig. 3D and G). This effect does not seem to be related to an insufficient concentration of BIP, because higher concentration of BIP (1 mM) treatment or pretreatment of BIP 4 hr before K5-S deprivation failed to exhibit substantial neuroprotecitive effects (data not shown). On the other hand, apoptosis induced by STS treatment in cerebellar granule cells was significantly attenuated by BIP treatment (Fig. 3F and G), similarly to cerebral cortex neurons Whereas anti-apoptotic function of BIP was absent in the K5-S rat granule cells, granule cells from Bax-KO mice did not exhibit any significant cell death in both STS and K5-S induced neuronal cell death condition. These results indicate that BIP can suppress a subset of Bax-dependent neuronal death.

DISCUSSION

It is known that Bax plays critical roles in the execution of neuronal death induced during several types of neurodegenerative diseases. For instance, double mutant of Bax-KO and disease model mice for amyotrophic lateral sclerosis (Gould et al., 2006), Parkinson's disease (Vila et al., 2001) and prion-induced neuronal death (Chiesa et al., 2005) all exhibited marked reduction or absence of neuronal death. Considering these results, it appears that Bax-inhibition may be effective means to protect a subpopulation of neurons from apoptotic cell death during neurodegenerative diseases. In this respect, we addressed the advent of Bax inhibitory peptide (BIP) (Sawada et al.,

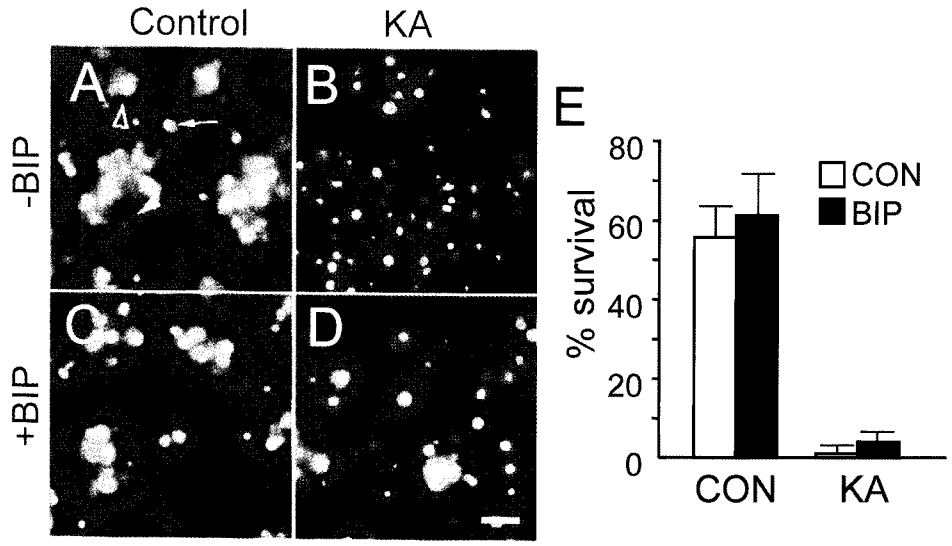

Fig. 2. Effect of BIP on cell viability after Kainic acid (KA) treatment. (A-D) DIV15 cerebral cortex neurons were treated with KA (200 μM) (B and D) in the presence (C and D) or absence (A and B) of BIP (200 μM). Cell death was examined by Hoechst (blue) and PI (red) staining. Filled arrowhead indicates intact neuron, arrow indicates apoptotic neurons, and empty arrowhead indicates membrane disrupted necrotic neuron. (E) Quantification of cell viability after KA treatment with BIP. Cell viability was assessed by the percentage of intact nuclei among total cell nuclei. Data are mean±SE, n=3. Scale bar=20 μm.

2003a; Sawada et al., 2003b), and examined whether BIP treatment could block the apoptosis of two different types of CNS neurons. Our major finding in this study is that BIP selectively inhibits STS-induced apoptotic death of neurons but not apoptosis induced by neurotrophic signal-deprivation (K5-S) in cerebellar granule cell. It is somewhat surprising, because both conditions are Bax-dependent, and apoptosis in both conditions was completely blocked in Bax-KO neurons. However, it is unlikely that neurotrophic signal-deprivation universally promotes cell death which cannot be protected by BIP. It has been reported that BIP can protect retinal ganglion neurons from apoptosis induced by optic nerve transection (Qin et al., 2004). Nerve transection typically deprives the source of trophic signals for retinal neurons, and it is assumed that ganglion neurons undergo apoptosis via trophic signal deprivation cascade. In this respect, at least some neurons may be protected by BIP from deprivation-induced apoptosis.

Although it is required to be explored with further experimental examinations, differential efficacy of BIP on STS- vs. deprivation-induced neuronal death suggests that these two conditions promote different signal cascade for apoptosis execution and activation of Bax. Localization of Bax is tightly regulated by, at least, two independent mechanisms: the cytosolic retention and mitochondrial targeting. Mitochondrial targeting of Bax protein is regulated by the induction of BH3-only proteins, such as Bim, tBid, Bad etc (Leber et al., 2007; Levine et al., 2008). Upon stimulation of cell death signaling, the activation of these BH3-only proteins was mediated by multiple mechanisms, such as transcriptional activation (Bim), proteolytic cleavage (tBid) or phosphorylation (Bad). Depending on the stimuli, different sets of BH3-only proteins are activated by different stimuli. For instance, p53-dependent apoptosis induced by glucocorticoids or staurosporine in immune cells appears to be mainly mediated by PUMA and Noxa (Villunger et al., 2003), whereas trophic signal deprivation promotes the transcriptional activation of Bim in neurons (Putcha et al., 2001; Whitfield et al., 2001). On the other hand, BIP targets the binding site for the Ku70 in the N-terminus of Bax, which interferes with the structural change of Bax that are required for the translocation of Bax into mitochondria (Gomez et al., 2007; Sawada et al., 2003b). Therefore, it is plausible that the BH3-only proteins or other Bax-recruiting factors which are activated by deprivation condition may be able to over-ride the interference of BIP with Bax activation. Whereas Bax-translocation via reduction of Ku70 interaction may be efficiently repressed by BIP, Bax-translocation via induction of mitochondrial recruiting factors may be less affected by BIP treatment. In consistent with our observation, it was reported that BIP failed to protect sympathetic neurons from GDNF-deprived neuronal death, although it efficiently blocked NGF-deprived neuronal apoptosis (Yu et al., 2003). Although GDNF-deprivation may induce Bax-independent neuronal death, our result also raises the possibility that GDNF-deprivation induced neuronal death is Bax-dependent, but BIP-independent apoptosis. This point is required to be addressed in the future.

Alternatively, anti-apoptotic mechanisms by transient blockade of Bax function by BIP and absence of Bax protein in Bax-KO neurons may be different. Although

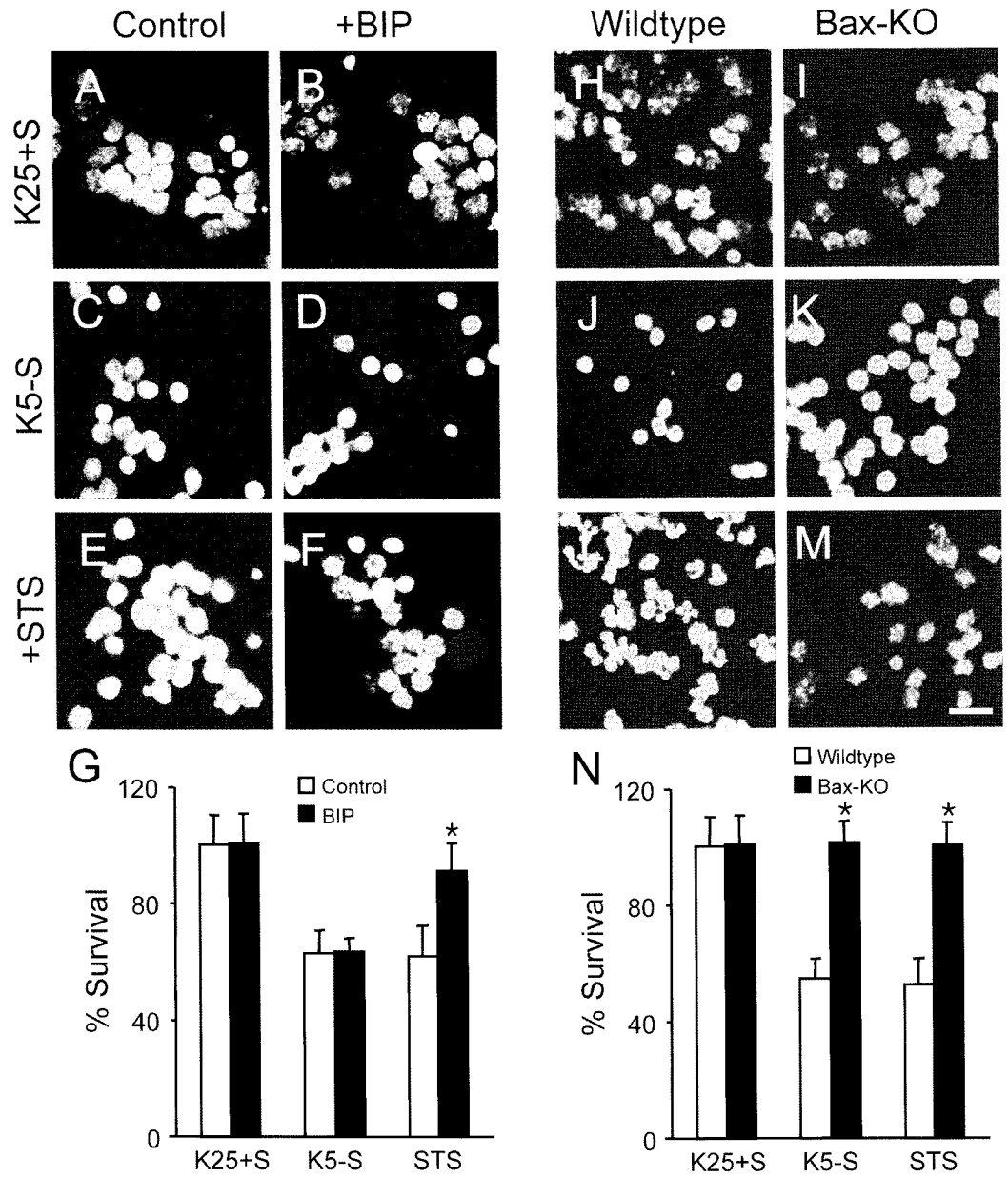

Fig. 3. Effect of BIP on potassium-serum deprived (K5-S) vs. STS treated cerebellar granule cells. (A-F) DIV7 cerebellar granule cells were treated by serum-free media with 5 mM K+ (K5-S) (C and D) or STS (400 nM) (E and F) in the presence (B, D and F) or absence (A, C and E) of BIP (200 μM). Representative pictures were taken of granule cell nuclei stained with Hoechst. (G) Cell viability was assessed by the percentage of intact nuclei among total cell nuclei. Data are mean±SE, n=3 *p<0.05 versus control. (H-N) Bax-KO (I, K and M) or WT (H, J and L) cerebellar granule cells were treated by K5-S (J and K) or STS (L and M). (N) Cell viability was assessed by the percentage of intact nuclei among total cell nuclei. Data are mean±SE, n=3 *p<0.05 versus control. Scale bar=20 μm.

Bax-KO neurons develop grossly normal, there are several reports that Bax-KO cells exhibit long-term adaptive compensatory alterations. For instance, Bax-KO cells maintain significantly less amount of calcium in the ER, due to the leakage of ER calcium via IP3 receptor (Oakes et al., 2005; Shi et al., 2005). In fact, cellular calcium dynamics is critically associated with the survival and apoptosis of cerebellar granule cells (Moran et al., 1999; Toescu, 1999; Yao et al., 1999; Zhong et al., 2004). Furthermore, it is reported that Bax plays additional roles other than the modulation of cell death, which include the modulation of mitochondrial dynamics (Karbowski et al., 2006) and fate decision of neural stem cells (Jang et al., 2006). Therefore, either long-term compensatory modification or apoptosis-independent function of Bax may influence the extent of neuronal death, which cannot be protected by acute blockade of Bax activation by BIP.

ACKNOWLEDGMENT

This work was supported by Korean Ministry of Education, Science and Technology grant ROI-2008-000-10357-0.

REFERENCES

Chiesa R, Piccardo P, Dossena S, Nowoslawski L, Roth KA. Ghetti B, and Harris DA (2005) Bax deletion prevents neuronal loss but not neurological symptoms in a transgenic model of inherited prion disease. *Proc Natl Acad Sci USA* 102: 238-243.

D'Mello SR, Galli C, Ciotti T, and Calissano P (1993) Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. *Proc Natl Acad Sci USA* 90: 10989-10993.

Fletcher JI and Huang DC (2006) BH3-only proteins: orchestrating cell death. *Cell Death Differ* 13: 1268-1271.

Gallo V, Kingsbury A, Balazs R, and Jorgensen OS (1987) The role of depolarization in the survival and differentiation of cerebellar granule cells in culture. *J Neurosci* 7: 2203-2213.

Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, and Kroemer G (2006) Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 13: 1423-1433.

Gomez JA, Gama V, Yoshida T, Sun W, Hayes P, Leskov K, Boothman D, and Matsuyama S (2007) Bax-inhibiting peptides derived from Ku70 and cell-penetrating pentapeptides. *Biochem Soc Trans* 35: 797-801.

Gould TW, Buss RR, Vinsant S, Prevette D, Sun W, Knudson CM, Milligan CE, and Oppenheim RW (2006) Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J Neurosci* 26: 8774-8786.

Jang JW, Boxer RB, and Chodosh LA (2006) Isoform-specific ras activation and oncogene dependence during MYC- and Wnt-induced mammary tumorigenesis. *Mol Cell Biol* 26: 8109-8121.

Karbowski M, Norris KL, Cleland MM, Jeong SY, and Youle RJ (2006) Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443: 658-662.

Knudson CM, Tung KS, Tourtellotte WG, Brown GA, and Korsmeyer SJ (1995) Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 270: 96-99.

Kuwana T and Newmeyer DD (2003) Bcl-2-family proteins and the role of mitochondria in apoptosis. *Curr Opin Cell Biol* 15: 691-699.

Leber B, Lin J, and Andrews DW (2007) Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes. *Apoptosis* 12: 897-911.

Levine B, Sinha S, and Kroemer G (2008) Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy* 4: 600-606.

Li Y, Yokota T, Gama V, Yoshida T, Gomez JA, Ishikawa K, Sasaguri H, Cohen HY, Sinclair DA, Mizusawa H, and Matsuyama S (2007) Bax-inhibiting peptide protects cells from polyglutamine toxicity caused by Ku70 acetylation. *Cell Death Differ* 14: 2058-2067.

Mignotte B and Vayssiere JL (1998) Mitochondria and apoptosis. *Eur J Biochem* 252: 1-15.

Moran J, Itoh T, Reddy UR, Chen M, Alnemri ES, and Pleasure D (1999) Caspase-3 expression by cerebellar granule neurons is regulated by calcium and cyclic AMP. *J Neurochem* 73: 568-577.

Nardi N, Avidan G, Daily D, Zilkha-Falb R, and Barzilai A (1997) Biochemical and temporal analysis of events associated with apoptosis induced by lowering the extracellular potassium concentration in mouse cerebellar granule neurons. *J Neurochem* 68: 750-759.

Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M. Pozzan T, and Korsmeyer SJ (2005) Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci USA* 102: 105-110.

Putcha GV, Moulder KL, Golden JP, Bouillet P, Adams JA. Strasser A, and Johnson EM (2001) Induction of BIM, a proapoptotic BH3-only BCL-2 family member, is critical for neuronal apoptosis. *Neuron* 29: 615-628.

Qin Q, Patil K, and Sharma SC (2004) The role of Bax-inhibiting peptide in retinal ganglion cell apoptosis after optic nerve transection. *Neurosci Lett* 372: 17-21.

Sawada M, Hayes P, and Matsuyama S (2003a) Cytoprotective membrane-permeable peptides designed from the Bax-binding domain of Ku70. *Nat Cell Biol* 5: 352-357.

Sawada M, Sun W, Hayes P, Leskov K, Boothman DA, and Matsuyama S (2003b) Ku70 suppresses the apoptotic translocation of Bax to mitochondria. *Nat Cell Biol* 5: 320-329.

Shi J, Parada LF, and Kernie SG (2005) Bax limits adult neural stem cell persistence through caspase and IP3 receptor activation. *Cell Death Differ* 12: 1601-1612.

Sun W, Funakoshi H, and Nakamura T (2002). Localization and functional role of hepatocyte growth factor (HGF) and its receptor c-met in the rat developing cerebral cortex. *Brain Res Mol Brain Res* 103: 36-48.

Sun W, Gould TW, Vinsant S, Prevette D, and Oppenheim RW (2003) Neuromuscular development after the prevention of naturally occurring neuronal death by Bax deletion. *J Neurosci* 23: 7298-7310.

Sun,W and Oppenheim RW (2003) Response of motoneurons to neonatal sciatic nerve axotomy in Bax-knockout mice. *Mol Cell Neurosci* 24: 875-886.

Toescu EC (1999) Activity of voltage-operated calcium channels in rat cerebellar granule neurons and neuronal survival. *Neuroscience* 94: 561-570.

Vila M, Jackson-Lewis V, Vukosavic S, Djaldetti R, Liberatore G, Offen D, Korsmeyer SJ, and Przedborski S (2001) Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proc Natl Acad Sci USA* 98: 2837-2842.

Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, Adams JM, and Strasser A (2003) p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 302: 1036-1038.

Whitfield J, Neame SJ, Paquet L, Bernard O, and Han J (2001) Dominant-negative c-Jun promotes neuronal survival by reducing BIM expression and inhibiting mitochondrial cytochrome c release. *Neuron* 29: 629-643.

Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, and Youle RJ (1997) Movement of Bax from the cytosol to mitochondria

during apoptosis. *J Cell Biol* 139: 1281-1292.

Yao CJ, Lin CW, and Lin-Shiau SY (1999) Roles of thapsigargin-sensitive Ca2+ stores in the survival of developing cultured neurons. *J Neurochem* 73: 457-465.

Yoshida T, Tomioka I, Nagahara T, Holyst T, Sawada M, Hayes P, Gama V, Okuno M, Chen Y, Abe Y *et al.* (2004) Bax-inhibiting peptide derived from mouse and rat Ku70. *Biochem Biophys Res Commun* 321: 961-966.

Yu LY, Jokitalo E, Sun YF, Mehlen P, Lindholm D, Saarma M, and Arumae U (2003) GDNF-deprived sympathetic neurons die via a novel nonmitochondrial pathway. *J Cell Biol* 163: 987-997.

Zhong J, Deng J, Huang S, Yang X, and Lee WH (2004) High K+ and IGF-1 protect cerebellar granule neurons via distinct signaling pathways. *J Neurosci Res* 75: 794-806.

Zhuang J and Brady HJ (2006) Emerging role of Mcl-1 in actively counteracting BH3-only proteins in apoptosis. *Cell Death Differ* 13: 1263-1267.

[Received November 3, 2008; accepted December 9, 2008]