A New Cell Death Inhibitor, Bax-inhibiting-peptide (BIP) Derived from Ku70(Animal Biology, Animal Reproduction)

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A New Cell Death Inhibitor, Bax-inhibiting-peptide (BIP) Derived from Ku70

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Summary

Programmed cell death (apoptosis) plays a pivotal role in the homeostasis of multicellular organisms. Bax is a key mediator of apoptosis. Apoptotic stress induces the translocation of Bax from the cytosol to mitochondria, and then Bax induces mitochondria-dependent cell death. Recently, we have found that Ku70 inhibits the translocation of Bax from the cytosol to mitochondria, suggesting that Ku70 inhibits mitochondria-dependent cell death (Sawada et al., 2003b). Moreover, we have designed a new type of cell death inhibitor, Bax-inhibiting-peptide (BIP) derived from Ku70 (Sawada et al., 2003b ; Yoshida et al., 2004). We have demonstrated that BIPs inhibit the cell death induced by anti-cancer drugs, UVC irradiation, and tropic factor deprivation (Sawada et al., 2003b ; Yoshida et al., 2004). BIP directly binds Bax and inhibits the cytotoxic activity of Bax. BIP may become a new tool to control degenerative diseases.

Key words : Apoptosis, Ku70, Bax, Bax-inhibiting-peptide (BIP), cell death inhibitor

Introduction

Apoptosis has become a major research area in the biomedical sciences. This great interest in apoptosis arose due to the recognition that many diseases involve too much apoptosis (e.g., [neuro] degenerative diseases, Parkinson's, Alzheimer's,

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spinal muscular atrophy, AIDS) or too little apoptosis (e.g., cancer [either by virus infection or by DNA mutations such as p53 and Bcl-2] or autoimmune diseases [diabetes type I, encephalomyelitis]). Induction of apoptosis can occur by external or internal stimuli. Two major general pathways of induction of apoptosis exist: the receptor or extrinsic pathway and the mitochondrial or intrinsic pathway (Fig. 1). Both apoptotic signaling pathways converge at the level of the specific proteases-the caspases. Peptide caspase inhibitors can inhibit downstream caspase activation and subsequently apoptosis (reviewed by Lawen, 2003).

There have been numerous reports of anti-apoptotic proteins and cell death inhibitors (Shimizu et al., 2000; Cheng et al., 2002; Lotocki and Keane, 2002; Sawada et al., 2003a, 2003b; Sugioka et al., 2003; de Graaf et al., 2004; Shi, 2004; Yoshida et al., 2004; Ono et al., 2005). One example is the inhibitor of apoptosis protein (IAP) family which includes X-chromosome-linked IAP (XIAP) and TAT-BH4 peptide derived from Bcl-2 Bcl-xL (Shimizu et al., 2000; Cheng et al., 2002; Sugioka et al., 2003; de Graaf et al., 2004; Wrzesien-Kus et al., 2004; Ono et al., 2005). Bcl-2 family proteins control a pivotal step in an evolutionarily conserved pathway of apoptosis, with some members functioning as suppressors and others as promoters of apoptosis (Reed, 1997; Korsmeyer et al., 1999). In mammalian cells, Bcl-2 family proteins control mitochondria-dependent cell death (Reed et al., 1998).

IAPs are molecules controlling the activity of the caspases (Deveraux and Reed, 1999). Several distinct mammalian IAPs, for example XIAP, c-IAP1, c-IAP2 and ML-IAP, have been identified (reviewed by Shi, 2004). Of these, XIAP has the most potent effect in inhibiting caspase-3 and caspase-7 *in vitro*, which is 100-fold stronger than that of c-IAP1 or c-IAP2 (Deveraux and Reed, 1999). In TAT-BH4 peptide, the biochemical role of the conserved N-terminal homology domain (BH4) of Bcl-2 Bcl-xL is pivotal for the inhibition of mitochondria-dependent cell death, which is required for Bcl-2 Bcl-xL to inhibit the release of cytochrome *c* (Shimizu et al., 2000; Sugioka et al., 2003; Ono et al., 2005). TAT-BH4 peptide shows anti-apoptotic activity in cell culture and in rodent models (Shimizu et al., 2000; Sugioka et al., 2003; Ono et al., 2005). However, it is toxic to cells at high concentrations in culture, probably due to the TAT region since HIV-1 TAT protein is known to induce apoptosis (Sugioka et al., 2003).

Roles of Bax in pathogenesis

In the intrinsic pathway, pro-apoptotic Bcl-2 family proteins such as Bax play a key role in mitochondria-dependent cell death (Jurgensmeier et al., 1997; Green, 2000). Bax normally resides in the cytosol in a quiescent state. In

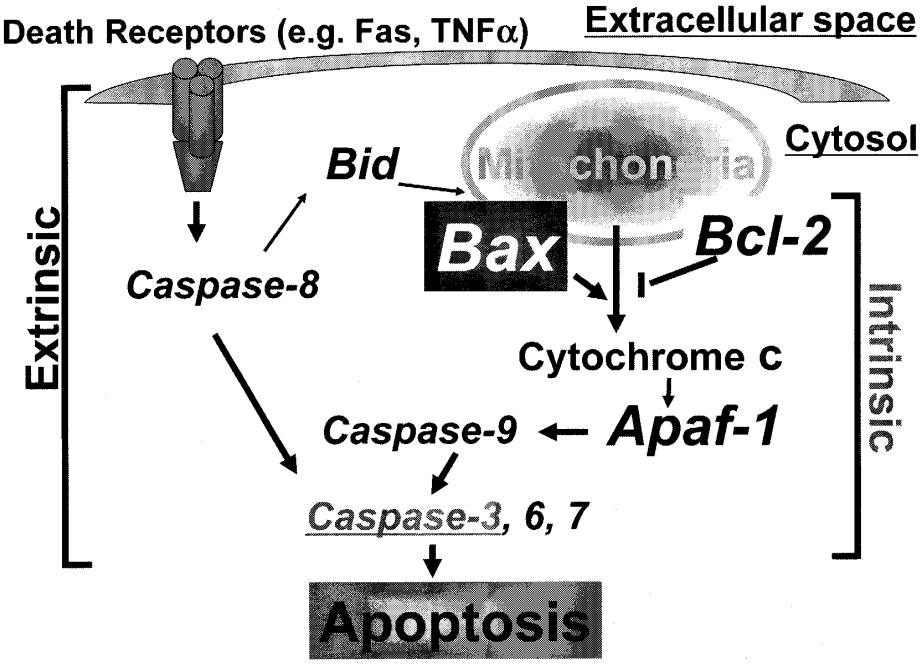

FIG 1. Extrinsic and intrinsic apoptosis pathways.

response to apoptotic stimuli, however, Bax translocates into mitochondria and promotes the release of cytochrome *c*, possibly by forming a pore in the mitochondrial outer membrane (Spierings et al., 2005). Cytochrome *c* and ATP are cofactors in the activation of apoptotic protease-activating factor-1 (Apaf-1). Activated Apaf-1, cytochrome *c* and procaspase-9 form an apoptosome, leading to the cascade of caspases that execute apoptosis (Zou et al., 1997 ; Green, 2000) (Fig. 1).

Bax-mediated cell death is implicated in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and ischemia/reperfusion-induced organ damage (Kim et al., 2001 ; Shults et al., 2005 ; Takuma et al., 2005). Moreover, experiments with transgenic Bax mice suggest that Bax also plays an important role in cell death during normal retinal development (Pequignot et al., 2003). During embryogenesis, increased expression of Bax is positively correlated with apoptosis in granulosa cells of rat and human ovaries and in luteal cells of bovine and rabbit ovaries (Tilly et al., 1995 ; Rueda et al., 1997 ; Goodman et al., 1998 ; Kugu et al., 1998 ; Dharmarajan et al., 1999). Interestingly, the oocytes of Bax-deficient mice are resistant to apoptosis induced by exposure to chemotherapy (Perez et al., 1997). Consequently, to protect oocytes and neurons during embryogenesis or degenerative diseases, the activation of Bax should be prevented.

Discovery of the interaction of Bax and Ku70

A yeast-based functional screening system has been employed to search for Bax inhibitors (Xu and Reed, 1998; Xu et al., 2000), resulting that Ku70 was cloned as a potential Bax suppressor protein (Sawada et al., 2003b). Ku70 is a 70 kDa subunit of Ku, which also comprises a 80 kDa subunit (Ku80) (reviewed by Downs and Jackson, 2004). Ku has DNA-end joining activity required for double-strand break (DSB) repair; Ku also plays a role as a DNA-binding unit of the DNA-dependent protein kinase holoenzyme (DNA-PK), a sensor of damaged DNA (Downs and Jackson, 2004). Although the DNA repair function of Ku70 explains its role in the nucleus, Ku70 has been also found in the cytosol (Fewell and Kuff, 1996). Our recent study has identified a cytosolic function for Ku; Ku70 binds Bax and inhibits Bax-mediated apoptosis by preventing its translocation to mitochondria (Sawada et al., 2003b). Furthermore, the previous study demonstrated that a portion of the carboxy-terminal domain of Ku70 (amino acids 578-609) is sufficient to mediate this activity and that Ku70 does not need to be in a complex with Ku80 to function in this manner (Sawada et al., 2003b). Recently, Cohen et al. (2004) have discovered that acetylation of Ku70 in the cytosol is one of the mechanisms by which Ku70 dissociates from Bax, leading to Bax-mediated cell death.

Bax-inhibiting-peptides (BIPs) derived from Ku70

Based on the finding that Ku70 binds Bax and inhibits Bax-mediated apoptosis (Sawada et al., 2003b) and that the Bax-binding domain of Ku70 is mapped to amino acids 578–583 (VPMLKE), some Bax-inhibiting-peptides

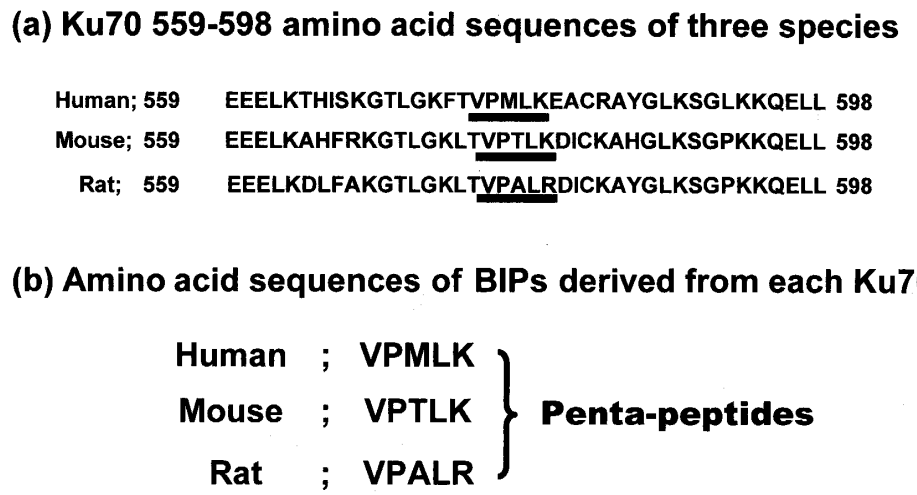

FIG 2. Sequence alignment of the C-terminus of Ku70 (a), and Bax inhibiting peptides (BIPs) derived from each Ku70 (b) in humans, mice and rats.

(BIPs) have been developed as VPMLK, VPTLK and VPALR in humans, mice and rats, respectively (Sawada et al., 2003a ; Yoshida et al., 2004) (Fig. 2). These pentapeptides derived from domains of each Ku70 are able to permeate cells and to suppress Bax-mediated apoptosis induced by staurosporine (STS), UVC irradiation and anti-cancer drugs in several types of cells (Sawada et al., 2003a; Yoshida et al., 2004). Bax binds to the BIPs derived from the human, mouse and rat Ku70 (Sawada et al., 2003a ; Yoshida et al., 2004). Human version of BIP (VPMLK) as well as Ku70 can rescue NGF-derived primary culture neuron from cell death (Yu et al., 2003). In addition, BIPs inhibit tropic factor (e.g., hormones and growth factors) deprivation-induced apoptosis in cumulus cells of three species (mice, rats and pigs) and myeloid cells (32D (EpoR wt)) (Yoshida et al., 2004). It is well established that Bax plays a key role in tropic factor deprivation-induced cell death (Deckwerth et al., 1996 ; Vaux and Korsmeyer, 1999 ; Rathmell et al., 2003). Moreover, in an *in vivo* system, BIP prevents apoptosis of retinal ganglion cells (RGCs) after optic nerve transection (ONT) in adult Wistar rats (Qin et al., 2004).

Conclusions and Future direction

Bax is an important factor in cell death since Bax-mediated cell death is known to be involved in several types of degenerative conditions including ovarian follicle atresia, Alzheimer's disease and Parkinson's disease (Perez et al., 1999 ; Shults, 2005 ; Takuma et al., 2005). The cell-permeable Bax-inhibiting-peptides (BIPs) derived from Ku70 may provide valuable information required for development of new therapeutics to control apoptosis-related diseases. For a clinical application in protecting against cell damage during degenerative diseases and infertility, further improvement in the peptide sequence or chemical modification of BIPs will be required to avoid immune reactions and to control the half-life of BIPs *in vivo*.

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