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REVIEW

BIK, the founding member of the BH3-only family proteins: mechanisms of cell death and role in cancer and pathogenic processes

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BIK is the founding member of the BH3-only family pro-apoptotic proteins. BIK is predominantly localized in the ER and induces apoptosis through the mitochondrial pathway by mobilizing calcium from the ER to the mitochondria and remodeling the mitochondrial cristae. BIK-mediated apoptosis is mediated by selective activation of BAX. BIK also induces non-apoptotic cell death in certain cell types by unknown mechanisms. BIK is non-essential for animal development, but appears to be functionally redundant for certain developmental functions with BIM. BIK is implicated in the selection of mature B cells in humans. BIK is a pro-apoptotic tumor suppressor in several human tissues and its expression in cancers is prevented by chromosomal deletions encompassing the *Bik* locus or by epigenetic silencing. BIK appears to be a critical effector in apoptosis induced by toxins, cytokines and virus infection. Several anti-cancer drugs transcriptionally activate *Bik* gene expression through transcriptional pathways dependent on factors such as E2F and p53 or by removal of epigenetic marks on the chromatin. BIK appears to be a prominent target for anti-cancer drugs that inhibit proteasomal functions. BIK has also been used as a therapeutic molecule in gene therapy-based approaches to treat difficult cancers.
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Introduction

BIK (BCL-2 interacting killer), was the first member of the BH3-only pro-apoptotic proteins (Boyd *et al*., 1995). In addition to BCL-2, it was also shown to strongly interact with other anti-apoptotic proteins, adenovirus E1B-19K, BCL-xL and EBV-BHRF1 and to exhibit a potent cell death activity when expressed autonomously. The cell death activity was suppressed by coexpression of BCL-2, BCL-xL, E1B-19K and EBV-BHRF1, suggesting that BIK might be a common target for cellular and viral anti-apoptosis proteins. Although other BH3-only proteins such as BNIP1, BNIP3 (Boyd *et al*., 1994) and BAD (Yang *et al*., 1995) were identified earlier as proteins that interacted with viral and cellular anti-apoptotic proteins in two hybrid screenings, BIK was the first protein in which the cell death activity was linked to the conserved BCL-2 homology domain 3 (BH3) (Boyd *et al*., 1995; Chittenden *et al*., 1995) and thus considered as the founding member of BH3-only family proteins. BIK was shown to share two domains, the BH3 domain and the trans-membrane domain with other BCL-2 family proteins and specific mutations within the BH3 domain strongly reduced the pro-apoptotic activity (Boyd *et al*., 1995; Chittenden *et al*., 1995). BIK was also subsequently identified as an E1B-19K-interacting protein, where it was designated as NBK (Han *et al*., 1996). An ortholog of BIK referred as BLK (BIK-Like Killer) was identified from the mouse EST data bank (Hegde *et al*., 1998). Although BIK is highly conserved among primates, in other mammalian species the amino acid sequences outside the BH3 domain exhibit some sequence variations. However, based on secondary structure predictions all mammalian BIK proteins appear to be structurally similar. The gene for human BIK is encoded in chromosome 22q13.3 (Verma *et al*., 2000). The human BIK protein is a phosphoprotein (Verma *et al*., 2001). BIK is predominantly localized in the ER (Germain *et al*., 2002) and appears to mediate apoptosis signaling to the mitochondria (Germain *et al*., 2005). The mechanisms by which BIK induces cell death and its role in normal cells and in cells undergoing pathological stress including cancer are reviewed here.

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BIK protein

BIK is a 160 aa protein and contains a trans-membrane domain (TM), in addition to the BH3 domain (Figure 1). Predictions based on primate genome sequence analyses have suggested a larger isoform of 202 residues (XB_525617). However, the existence of such an isoform remains to be demonstrated. Secondary structure prediction suggested that BIK might form six α helices with exposed BH3 domain (McDonnell *et al*., 1999). Predictions based on the analysis of mean hydrophobicity and

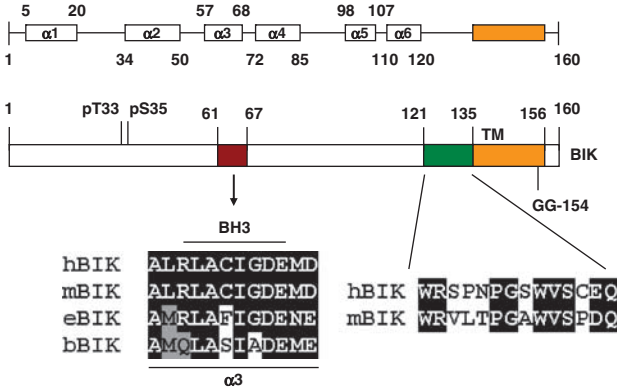

Figure 1 Domain structure of BIK protein. Three domains of human BIK and their homologies to BIK proteins from different animal species are shown. The predicted secondary structure of hBIK (shown on the top) is based on McDonnell *et al*. (1999). The α3 region encompassing the BH3 domain is highly conserved and the sequences of the α3 region of human, murine, equine and bovine BIK are indicated. The C-terminal domain (aa 121–135) that is required for maximal pro-apoptotic activity of hBIK is conserved only in mBIK. The phosphorylation sites at Thr33 and Ser35 and the RHBDD1 cleavage site ($G_{153}G_{154}$) in the transmembrane (TM) domain are indicated.

mean net charge suggested that BIK may have a well-defined structure although most other BH3-only members (except BID) appear to be unstructured (Hinds *et al*., 2007). Phylogenetic analysis of BCL-2 family proteins suggested that BIK may be more closely related to BAK (Aouacheria *et al*., 2005). Mutational analyses indicated that the core BH3 domain was crucial for the apoptotic activity (Boyd *et al*., 1995; Chittenden *et al*., 1995; Elangovan and Chinnadurai, 1997; Mathai *et al*., 2002). The TM domain was dispensable for the apoptotic activity at least under conditions of transient overexpression (Elangovan and Chinnadurai, 1997). In addition to the BH3 domain, a C-terminal domain adjacent to the TM was also important for maximal cell death activity in transient assays. The C-terminal motif resembles the substrate recognition motif present in several caspases (Elangovan and Chinnadurai, 1997) and a sequence element in bacterial hemolysins. This domain is conserved only in human and murine BIK proteins and its functional significance is not clear. hBIK was shown to be phosphorylated on Thr(33) and Ser(35) by a casein kinase II-like kinase and mutations that prevented phosphorylation reduced the cell death activity and interaction with anti-apoptosis proteins (Verma *et al*., 2001). Mutations that mimicked phosphorylation (Thr, Ser→Asp) enhanced the cell death activity of BIK and interaction with BCL-xL and BCL-2 (Li *et al*., 2003). hBIK was reported to be localized predominantly in the ER (Germain *et al*., 2002). However, mitochondrial localization of hBIK (Han *et al*., 1996) and mBIK (Hegde *et al*., 1998) has also been reported. Only modest steady state levels of BIK protein were generally observed in most cultured cell lines. Several reports have indicated that BIK was down-regulated by proteasomal degradation (Marshansky *et al*., 2001; Nikrad *et al*., 2005; Zhu *et al*., 2005b). A recent report identified a serine protease of the Rhomboid family, RHBDD1 that released BIK by cleaving the C-terminal TM domain from the membrane at a GG (aa 153–154) motif (Wang *et al*., 2008). Although RHBDD1 was shown to localize predominantly in the mitochondria, it was suggested that it might be translocated to the ER to cleave ER-associated BIK. In cells treated with the proteasome inhibitor Bortizomib (Velcade) which was reported to increase the level of BIK (Nikrad *et al*., 2005; Zhu *et al*., 2005b; Yeung *et al*., 2006; Li *et al*., 2008). BIK appeared as a doublet (Hur *et al*., 2006; Wang *et al*., 2008), suggesting that the lower MW band was destined for proteasomal degradation. Depletion of RHBDD1 by the use of siRNA increased the level of full length BIK and apoptosis. These results suggested that RHBDD1-targeted BIK for proteasomal degradation by liberating BIK through regulated transmembrane proteolysis (Brown *et al*., 2000). Therapeutic intervention with proteasome-mediated downregulation of BIK was suggested as one of the modes of action of proteasome inhibitors that are currently in clinical trials to treat a variety of human cancers.

Regulation of BIK expression

Northern blot analysis showed a restricted pattern of expression of *Bik* in human tissues (Daniel *et al*., 1999). Higher levels of expression were seen in the kidneys and pancreas compared with other organs. Although *Bik* expression was not detectable in normal colon and lymphoid tissues, certain cell lines derived from these tumor tissues contained higher levels of *Bik* mRNA. In mouse, higher levels of *Bik* expression was observed in the hematopoietic compartment and in organs such as the kidney, liver, lung and heart. In general, *Bik* expression was increased in epithelial cells in both human and mouse. The sequence variations between the human and mouse *Bik* promoter regions may contribute to some of the differential patterns of expression.

In addition to post-translational control of BIK protein expression at the level of protein stability, the expression of the *Bik* gene was shown to be regulated at the level of transcription under different contexts. Such a regulated expression appears to be consistent with the prediction that BIK might be constitutively active as a result of the exposed BH3 domain (McDonnell *et al*., 1999). The human *Bik* gene was reported to be a transcriptional target for p53 (Mathai *et al*., 2002). The expression of one of the adenovirus E1A proteins that promotes the accumulation of p53 (in addition to activation of E2F) induced accumulation of *Bik* mRNA and protein. Further, infection of p53-null human epithelial cancer cells with an adenovirus vector that expressed *wt* p53 increased the levels of *Bik* mRNA and protein whereas the mutant p53 did not. Although stimuli such as γ radiation and treatment with the topoisomerase inhibitor doxorubicin (that upregulate endogenous p53) were shown to activate BIK expression

in human KB cells, the p53-dependency was not examined (Mathai *et al*., 2005). However, both agents were reported to enhance the levels of *Bik* mRNA in a p53-dependent fashion in MCF7 breast cancer cells by employing siRNA-mediated depletion of p53 by another group (Hur *et al*., 2006). Although the *hBik* promoter (Verma *et al*., 2000) contains degenerate p53 binding sites, in promoter assays no p53 response was detected (Mathai *et al*., 2002; Hur *et al*., 2006). It is possible that the degenerate p53-binding sites might be functional under conditions of p53 overexpression. Other studies have reported the activation of *Bik* expression by genotoxic stress in cells that lacked functional p53. A rapid, albeit modest activation of *Bik* was observed in a p53-deficicent human lymphoma cell line treated with the DNA topoisomerase inhibitor, camptothecin (Paquet *et al*., 2004). An increase in *Bik* expression also occurred in a p53-null human colon cell line (HCT116 $p53^{-/-}$) treated with chemotherapeutic drugs adriamycin and cisplatin (Real *et al*., 2006). Thus, it appears that human *Bik* is activated by genotoxic stimuli through p53-dependent and -independent mechanisms. In murine cells no *Bik* activation was observed as a result of genotoxic stress or by p53 overexpression (Coultas *et al*., 2004).

A gene profiling study of an estrogen-dependent breast cancer cell line revealed specific upregulation of *Bik* expression during apoptosis caused by estrogen starvation or exposure to antiestrogens, such as fulvestrant (Hur *et al*., 2004). Suppression of BIK expression by siRNA-mediated depletion diminished fulvestrant-induced apoptosis linking BIK to antiestrogen-induced apoptosis. The antiestrogen-induced upregulation of *Bik* mRNA was linked to p53 as siRNA-mediated p53 knockdown as well as p53-dominant negative mutant abolished *Bik* mRNA accumulation (Hur *et al*., 2006). Surprisingly, this activity of p53 appeared to be unrelated to its DNA-binding activity as fulvestrant treatment did not enhance the DNA-binding activity of p53 in a gelshift assay with oligonucleotide probes containing p53-binding sites. A 2 kb *Bik* promoter region was also not responsive in the promoter assay. The mode of p53-mediated upregulation of *Bik* mRNA accumulation in breast cancer cells exposed to antiestrogen remains to be elucidated.

In cells treated with adriamycin (Real *et al*., 2006) and in cells infected with adenovirus (Subramanian *et al*., 2007) *Bik* was shown to be transcriptionally activated by the E2F pathway. The *hBik* promoter contains an E2F-binding site at position −104. The direct involvement of E2F1 in transcriptional activation of *Bik* was demonstrated by promoter assays, by ChIP and electrophoretic mobility-shift assays. These results indicated that *Bik* was a pro-apoptotic target for E2F transcription factors that are activated during genotoxic stress and viral infection.

BIK protein expression was activated in human and murine epithelial cells treated with INFγ (Mebratu *et al*., 2008), a cytokine that mediates its effect through the transcription factor STAT1. In mouse $STAT1^{-/-}$ airway epithelial cells that were treated with INFγ, BIK expression was reduced compared with $STAT1^{+/+}$ cells, suggesting that BIK expression was increased as a result of STAT1 activation by INFγ. However, the mechanism of STAT1-dependent activation of BIK expression is unclear. Although the *Bik* promoter contains several STAT1-binding sites, reporter-based promoter assays failed to detect a response to INFγ treatment. The possibility that cooperation with other transcription factors and/or post-transcriptional mechanisms may contribute to increased BIK expression in response to INFγ remains to be elucidated. Treatment of B-lymphoma cell lines with another cytokine, TGF-β resulted in increase in mRNA levels of *Bik* that was also accompanied by a reduction in the level of *Bcl-xL* mRNA (Saltzman *et al*., 1998; Spender *et al*., 2009). *Bik* was shown to be a direct transcriptional target for TGF-β through recruitment of the transcription factor, Smad to the consensus Smad-binding sites in the *Bik* promoter (Spender *et al*., 2009). ChIP assays and electrophoretic mobility-shift assays revealed binding of the Smad 3/4 complex to a Smad-binding site at −1055. These results indicated that BIK was a mediator of TGF-β-induced apoptosis. An increased expression of BIK was observed in human B cell lymphoma that underwent apoptosis as a result of ligation of surface IgM (Jiang and Clark, 2001). The increase in the BIK expression was shown to be regulated both at transcriptional as well as at a post-transcriptional level. The increase in *Bik* mRNA was abolished by treatment with cyclosporin A suggesting a calcium/calcineurin-dependent pathway whereas a sustained BIK expression was dependent on both the calcium/calcineurin and the PI3K-dependent pathways. The regulated expression of BIK appears to be critical for the selection of mature B lymphocytes.

Mechanisms of BIK-induced cell death

The mechanism of cell death induced by BIK has been investigated in epithelial cancer cell lines by different groups. Overexpression of hBIK by adenovirus vectors revealed that BIK-induced apoptosis occurred through the activation of caspase-9 and the mitochondrial release of cytochrome *c* (Tong *et al*., 2001; Germain *et al*., 2002; Gillissen *et al*., 2003). In transient transfection studies, the cell death activity of mBIK (BLK) was also shown to be mediated by the mitochondrial pathway as mBIK-induced cell death was inhibited by a dominant-negative mutant of caspase-9. Thus, BIK-induced cell death in epithelial cancer cells appears to follow the prototypical mitochondrial caspase-9-dependent pathway.

The mechanism of BIK-induced cell death in epithelial cancer cells has been further refined through studies by Shore and colleagues. They have shown that ER-localized BIK-induced apoptosis through the mitochondrial pathway that resulted in cytochrome *c* release (Germain *et al*., 2002, 2005; Mathai *et al*., 2005). The release of ER Ca^{2+} mediated by BIK was dependent on the localization of conformationally activated

BAX/BAK in the ER and was deficient in BAX/BAK double knockout mouse kidney cells (Mathai *et al*., 2005). They demonstrated that BIK-induced Ca^{2+} release from the ER resulted in the recruitment of the mitochondrial fission protein DRP1 (Dynamin-related protein 1) from the cytosol to the mitochondria and remodeling of the inner mitochondrial membrane cristae (Germain *et al*., 2005). BIK (*wt*) expression caused the opening of the mitochondrial cristae tubules whereas the expression of a BH3 mutant (L61G) of BIK was deficient in cristae opening linking the activity of the BH3 domain to cristae opening. BIK-induced cristae remodeling was inhibited by dominant-negative DRP1 as well as by an inhibitor of mitochondrial Ca^{2+} uptake (Ru360). These results illuminated the essential role of the Ca^{2+}-dependent GTPase activity of DRP1 in BIK-mediated opening of the cristae thereby mobilizing cytochrome *c* for cytosolic release through organelle fragmentation (Figure 2). These results were consistent with previous studies that established the role of DRP1-mediated mitochondrial transformation in cytochrome *c* release during apoptosis (Frank *et al*., 2001; Scorrano *et al*., 2002). The mechanism of BIK-mediated cristae opening was different from that of tBID-mediated cristae remodeling that did not require the BH3 domain of tBID (Scorrano *et al*., 2002). The Ca^{2+}-dependent release of cytochrome *c* was consistent with an *in vitro* study using isolated mitochondria where the release of cytochrome *c* mediated by recombinant BIK was suppressed by Ca^{2+} chelation (Shimizu and Tsujimoto, 2000). The Shore group also observed the cooperation between BIK and the mitochondrial BH3-only member NOXA. The expression of hNOXA did not induce cytochrome *c* egress whereas the coexpression of BIK and NOXA induced conformational activation of BAX and accelerated cytochrome *c* release. Although the mechanism of such cooperation between the two BH3-only proteins which localized at different organelles is not known, it is possible that it may be related to cooperative activation of BAX by BIK and liberation of BAK from the complex with MCL-1 by NOXA (Willis *et al*., 2005).

In contrast to the apoptotic cell death induced by BIK in epithelial cancer cells, BIK-induced non-apoptotic cell death in other cell types. The cell death induced by BIK was independent of the activation of common

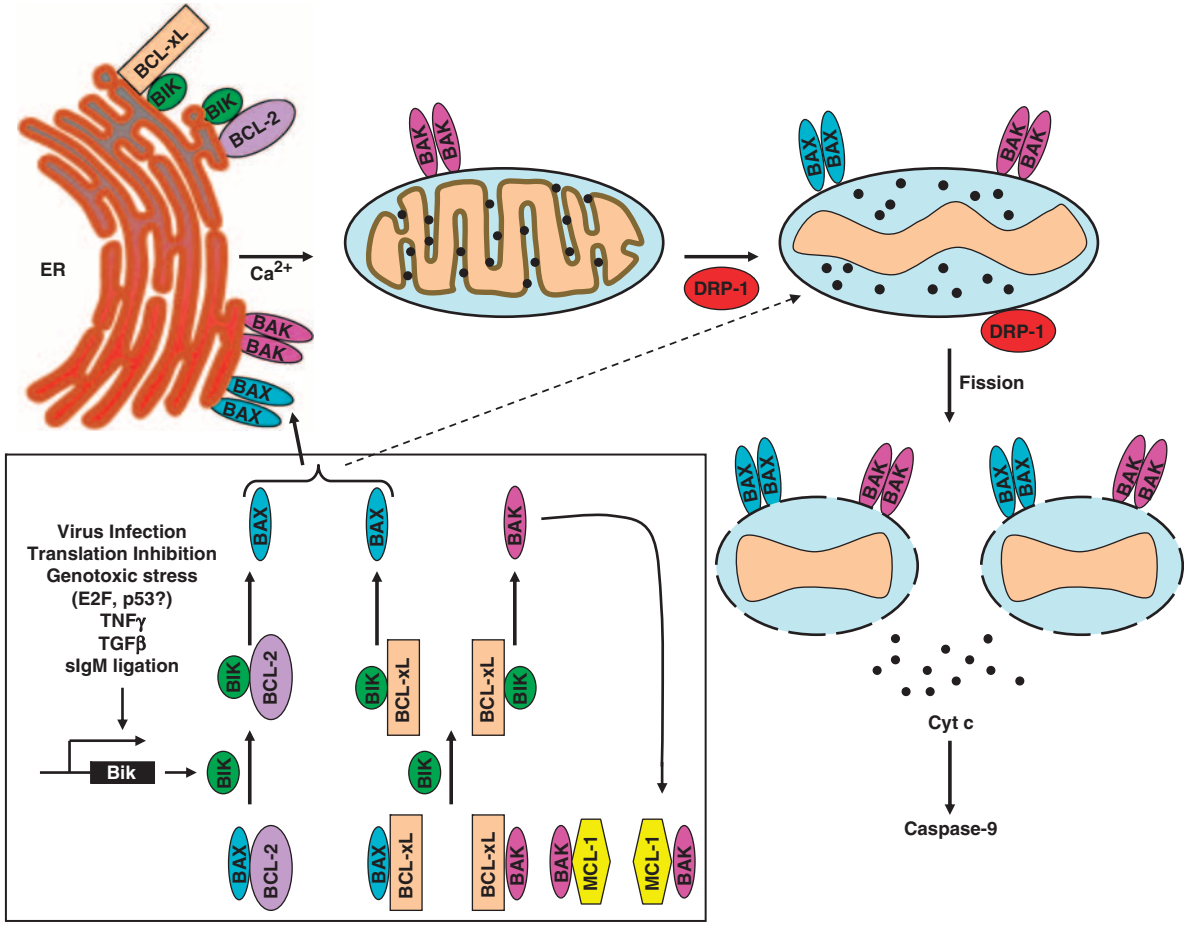

Figure 2 Model for BIK-induced apoptosis in epithelial cancer cells. In the boxed area, various stimuli that transcriptionally activate the expression of endogenous *Bik* gene are indicated. The mode of BAX activation through interaction of BIK with BCL-xL and BCL-2 is also shown in the boxed panel. The mode of mitochondrial cristae remodeling, mitochondrial fission and the release of cytochrome *c* mediated by ER-associated BIK is illustrated and is based on Germain *et al*. (2005). The mitochondrial uptake of Ca^{2+} released from the ER is depicted to recruit the cytosolic GTPase, DRP-1 to the mitochondria to mediate mitochondrial fission.

caspases such as caspase 6/7, caspase-9 and the release of cytochrome *c* in malignant glioma (Naumann *et al*., 2003) and melanoma (Oppermann *et al*., 2005) cell lines. In the case of glioma cell lines, BIK-induced cell death was suppressed by high concentrations of the broad spectrum caspase inhibitor zVAD-fmk and the anti-apoptosis protein XIAP suggesting an involvement of some caspase-related activities. The death activity in these cells was only partially suppressed by BCL-2/BCL-xL overexpression, raising the possibility that BIK-induced cell death might be independent of engagement of BCL-2/BCL-xL. The precise mechanism of cell death in these neuronal and ectodermal cancer cells remains unknown.

A form of non-apoptotic death was also observed in *Bcl-2*$^{-/-}$ mouse embryo fibroblasts (Rashmi *et al*., 2008). Overexpression of BIK in *Bcl-2*$^{-/-}$ cells resulted in enhanced cell death compared with *Bcl-2*$^{+/+}$ cells in the absence of activation of caspase-9 and 3 and detectable release of cytochrome *c* from the mitochondria. The extent of BIK-induced cell death was augmented by treatment with the pancaspase inhibitor, zVAD-fmk in these cells. The cell death induced by BIK exhibited certain autophagic features, such as cytosolic vacuoles, punctate distribution of LC3 and enhanced expression of Beclin-1 and was inhibited by pharmacological inhibitors of autophagy. Although the mechanism of this specific mode of cell death induced by BIK is not known, the possibility that BIK may selectively liberate Beclin-1 from BCL-xL remains to be investigated.

BAX-dependent apoptosis signaling by BIK

There is an obligate requirement of BAX and BAK for the manifestation of the pro-apoptotic activity of various BH3-only proteins. Mouse embryo fibroblasts doubly deficient for both BAX and BAK were shown to be resistant to apoptosis induced by ectopic expression of various BH3-only proteins such as BID, BIM and NOXA whereas mouse embryo fibroblasts singly deficient for either BAX or BAK were not (Cheng *et al*., 2001; Wei *et al*., 2001; Zong *et al*., 2001). Although the functional redundancy model for BAX and BAK is widely accepted, the pro-apoptotic activity of BIK was shown to be dependent only on BAX in several human epithelial cancer cell lines (Theodorakis *et al*., 2002; Gillissen *et al*., 2003). The exclusive BAX-dependency for BIK-induced cell death was not due to any functional deficiency in BAK expression as ectopic overexpression of BAK did not confer sensitivity to BIK in BAX-null cells (Theodorakis *et al*., 2002). Further, these cells exhibited sensitivity to a BAK-dependent pro-apoptotic protein BCL-xS (Gillissen *et al*., 2007). A potential mechanism underlying the BAX selectivity for BIK-induced apoptosis was recently reported (Figure 2, boxed panel). In healthy cells, BAK was shown to be sequestered by BCL-xL and MCL-1 (Willis *et al*., 2005). Gillissen *et al*. (2007) showed that in coimmunoprecipitation studies BIK expressed from an adenovirus vector interacted with endogenous BCL-xL and not with MCL-1 and thus failed to disrupt the BAK-MCL-1 complex. Further, the stability of MCL-1 was also enhanced in BIK-expressing cells. Displacement of the BH3 domain containing E3 ligase, MULE (Zhong *et al*., 2005) from MCL-1 by other BH3 ligands appeared to be a contributing factor in MCL-1 stabilization (Warr *et al*., 2005). In light of the inability of BIK to interact with MCL-1 in the above experiments, it appeared that BAK liberated from the BCL-xL complex by BIK might contribute to increased accumulation of MCL-1. The role of MCL-1 in dictating the BAX-dependent mode of action was further substantiated by siRNA-mediated depletion of MCL-1 in BAX-null cells resulting in sensitivity to BIK. As BIK was also shown to efficiently interact with BCL-2 (Boyd *et al*., 1995; Elangovan and Chinnadurai, 1997), it is likely that BIK might displace BAX from the BCL-2/BAX complex as well. The result of Gillissen *et al*. (2007) in which overexpressed BIK in BAX-null human cancer cells (DU145) did not interact with endogenous MCL-1 is different from the results of Shimazu *et al*. (2007) who reported the interaction between transfected BIK and endogenous MCL-1 in 293 cells. As 293 cells express E1B-19K that has been demonstrated to be in constitutive complex with BAK (Sundararajan *et al*., 2001; Lomonosova *et al*., 2002), it is possible that a fraction of MCL-1 is not complexed with BAK in these cells. Thus, the available evidence suggests that BIK may activate BAX by the 'indirect mode' that is, interaction with pro-survival proteins (see accompanying paper by Giam *et al*., in this issue on page S128) by selective displacement of BAX from BCL-xL and BCL-2. A caveat to the above model of BAX activation by BIK is that most BAX molecules in normal cells were reported to be in a free monomeric form (Willis *et al*., 2005). As BAX was originally identified as a BCL-2-binding protein (Oltvai *et al*., 1993), the possibility that at least a fraction of BAX might be present in complex with BCL-2/BCL-xL cannot be ruled out. Although the data of Gillissen *et al*. (2007) is consistent with the 'displacement' model, other potential mechanism(s) of BAX activation by BIK cannot be ruled out.

BIK in physiological and pathological cell death

Mice with homozygous deletion in the *Bik* locus were shown to be viable without overt developmental defects (Coultas *et al*., 2004), possibly reflecting functional redundancy with other BH3-only members. For example, concomitant deletion of *Bik* and *Bim* was shown to arrest spermatogenesis in mice suggesting both BH3-only members play a redundant role in normal testicular development (Coultas *et al*., 2005). In humans, BIK appears to play a critical role in B-cell homeostasis. In a gene expression profiling study of B-cell germinal center reaction, activation of *Bik*, in addition to other pro-apoptotic genes such as *FAS* was detected during naive B cell to centroblast transition and remained upregulated in the memory B cells (Klein *et al*., 2003). As

discussed earlier, in studies using B-lymphoma cells, increased expression of BIK was observed in cells that underwent apoptosis as a result of ligation of surface IgM (Jiang and Clark, 2001). These studies suggested that BIK may be important in apoptosis selection of mature human B lymphocytes.

Critical roles for BIK during certain pathological insults have been reported. The cytokine IFNγ which has been shown to induce apoptosis to resolve inflammation-induced airway hyperplasia was shown to mediate its pro-apoptotic activity through the action of BIK (Mebratu *et al*., 2008). Treatment with IFNγ-induced increase in *Bik* mRNA and protein in human and murine airway epithelial cells. The apoptotic activity of IFNγ was diminished in cells transduced with *Bik* shRNA and in murine cells derived from $Bik^{-/-}$ animals. The cell death activity of BIK was attributed to its ability to interact with activated ERK1/2 in a BH3-dependent fashion and to inhibit nuclear translocation of ERK1/2. These results suggested that BIK may be important in the elimination of supernumerary of inflammation-damaged cells in the airway.

Infection of mammalian cells with bacterial and viral pathogens generally inhibit cellular protein synthesis resulting in apoptosis of infected cells. One of the toxins produced by *Escherichia coli*, MazF that inhibited protein synthesis by cleavage of cellular mRNA-induced apoptosis through BIK (Shimazu *et al*., 2007). The use of cells derived from mutant mice deficient in various BH3-only members such as *Bik*, *Bim*, *Puma* and *Noxa* revealed that MazF-induced cell death was dependent only on *Bik*. Similar effects were observed when cells were treated with a general translation inhibitor, cycloheximide. These results highlight the general importance of BIK in apoptosis induced by pathogens and toxins.

The results of Shimazu *et al*. (2007) also suggested that BIK may be important in viral pathogenesis because shutoff of host cell protein synthesis is a prominent feature in cells infected with several different viruses. The authors showed that the infection of mouse kidney cells from $Bik^{-/-}$ mice with an apoptogenic mutant of adenovirus type 5 did not manifest the apoptotic cytopathic effect. A different independent study identified prominent transcriptional activation of *Bik* during adenovirus-induced apoptosis in human cells (Subramanian *et al*., 2007). This study demonstrated that the transcriptional activation of *Bik* was mediated by the E2F pathway. Specific depletion of BIK in adenovirus-infected cells reduced the level of apoptosis induced by an apoptogenic viral mutant that lacked the anti-apoptosis protein E1B-19K. Although it is not known whether *Bik* was activated in adenovirus-infected mouse cells by an E2F-dependent mechanism, the conservation of E2F-binding sites in both human and mouse promoters might be an indication of a common mode of activation. In light of the results from the two groups, it would be interesting to directly determine the role of BIK in apoptosis induced by other viruses. It should be noted that BIK was shown to interact with the viral anti-apoptosis protein EBV-BHRF1 (Boyd *et al*., 1995), in addition to E1B-19K (Boyd *et al*., 1995; Han *et al*., 1996).

Although viruses appear to target certain BH3-only members, such as BIK to inhibit apoptosis through sequestration by their anti-apoptosis proteins, the pathogen *Chlamydia trachomatis* was shown to target BH3-only proteins for degradation in infected cells (Dong *et al*., 2005). It is known that infection with *C. trachomatis*, a leading cause for blindness and sexually transmitted diseases in certain parts of the world, results in an inflammatory response without significant cellular apoptotic response. Chlamydial infection was also shown to confer resistance to heterologous apoptotic stimuli (Fan *et al*., 1998). In human cells infected with *C. trachomatis*, the BH3-only proteins BIK, BIM and PUMA were degraded without any effect on some other BH3-only members (BID and BAD that are normally activated by post-transcriptional modifications). There was also no change in the multi-domain pro-apoptotic (BAX and BAK) and anti-apoptotic proteins (BCL-2 and BCL-xL). Although the specific consequence of degradation of BH3-only proteins in Chlamydial pathogenesis remains to be established, the above study suggested a novel mode of suppression of apoptotic response by pathogenic bacteria.

BIK and human cancer

Bik as a tumor suppressor

There are several reports suggesting that the *Bik* gene may serve as a pro-apoptotic tumor suppressor in specific tissues. *Bik* was shown to be expressed at high levels in normal kidney epithelia (Daniel *et al*., 1999) whereas in renal cell carcinomas the expression of the *Bik* gene was inactivated by loss of heterozygosity at the *Bik* locus in chromosome 22q13.2 and by epigenetic promoter silencing (Sturm *et al*., 2006). A genome-wide analysis of genetic changes in human gliomas identified a deleted segment in chromosome 22q encompassing the *Bik* gene (Bredel *et al*., 2005). Short chromosomal deletions of the region containing the *Bik* locus were also reported in colorectal cancers (Castells *et al*., 1999) and in head and neck cancers (Reis *et al*., 2002). The *Bik* gene was shown to be frequently mutated in peripheral B-cell lymphomas (Arena *et al*., 2003). Mutations in both intronic and exonic regions of the *Bik* gene were identified. Although no mutation was detected in exon 3 coding for the BH3 domain, other exonic mutations were concentrated in the region coding for BIK between amino acids 43 and 134 that was previously shown to be important for maximal pro-apoptotic activity of BIK (Elangovan and Chinnadurai, 1997).

A microarray analysis showed prominent activation of *Bik* mRNA expression among over 350 apoptosis-related genes in four different human lung, prostate and renal carcinoma cell lines that were treated with a DNA methyltransferase 1 (DNMT1) inhibitor (5′-Aza-2′-deoxycytidine) and a histone deacetylase inhibitor (Depsipeptide) (Dai *et al*., 2006). These drugs had a

synergistic effect in augmenting the level of BIK protein expression. A different study also reported increase in *Bik* mRNA and protein in human cancer cells that were treated with DNMT1 antisense oligonucleotides (Milutinovic *et al*., 2004). A microarray analysis of a multiple myeloma cell line treated with the demethylating drug zebularine also provided evidence that the *Bik* gene was epigenetically silenced (Pompeia *et al*., 2004). Although it is unclear whether the effect of the inhibitors of epigenetic modifications is directly related to changes at the *Bik* promoter or through indirect effects, the above studies suggested that the transcription of the *Bik* gene was silenced in different cancer cell lines. As discussed earlier, the expression of *Bik* was reported to be silenced in estrogen-dependent breast cancer cells and was activated by treatment with antiestrogens, resulting in BIK-mediated apoptosis and that cells resistant to antiestrogen-induced apoptosis did not express *Bik* (Hur *et al*., 2004, 2006). Several reports have shown that in cancer cells that constitutively express *Bik* mRNA, the BIK protein was actively targeted by the proteasomal machinery (Marshansky *et al*., 2001; Nikrad *et al*., 2005; Zhu *et al*., 2005b; Yeung *et al*., 2006; Li *et al*., 2008). Thus, genomic deletions at the *Bik* locus, selective silencing of the *Bik* gene expression and/or post-transcriptional downregulation of BIK protein by proteasomal degradation and efficient apoptosis upon BIK re-expression reinforce the notion that *Bik* is a tumor suppressor.

Bik as a prognostic marker

Paradoxically, *Bik* expression was found to be high in certain sporadic breast tumors (Garcia *et al*., 2005). Also poor prognosis of non-small cell lung cancers (NSCLC) was shown to correlate with high expression of *Bik* (Lu *et al*., 2006). In the case of NSCLC, the increase in *Bik* expression also coincided with increase in *Bcl-2* expression. It is possible that the pro-apoptotic activity of BIK might be kept in check by BCL-2 during tumorigenesis and chronic low-level cell death mediated by runaway BIK may lead to tumor cell adaptation and evolution of aggressive tumor cells. As overexpression of *Bik* and *Bcl-2* in NSCLC was also accompanied by overexpression of caspases (caspase 8 and caspase 10) associated with inflammation and immune response, it is possible that the battle between the pro-apoptotic (BIK) and anti-apoptotic (BCL-2) molecules may lead to inflammatory responses and tumor progression. Interestingly, overexpression of another BH3-only member, *Bnip3* was also reported to be associated with poor prognosis in breast cancers (Sowter *et al*., 2003) and in NSCLC (Giatromanolaki *et al*., 2004) (see paper by Chinnadurai, Vijayalingam and Gibson, in this issue on page S114).

BIK in anticancer strategies

Since activation of BIK expression induced efficient cell death in estrogen-responsive breast cancer cell lines (Hur *et al*., 2004, 2006), modulating BIK expression could prove to be the basis of an interventional approach to treat estrogen receptor-positive human breast cancers. As mentioned earlier, treatment with various chemotherapeutic agents such as adriamycin (Panaretakis *et al*., 2002; Real *et al*., 2006) and camptothecin (Paquet *et al*., 2004) was reported to activate BIK expression in p53-positive as well as in p53-negative cells. Treatment with DNMT1 inhibitors and histone deacetylase inhibitors which activate *Bik* expression from the endogenous (silenced) *Bik* locus and the use of proteasomal inhibitors that stabilize BIK protein (in addition to other BH3-only members such as BIM and NOXA) appear to be highly promising anticancer approaches. The efficacy of proteasome inhibitors was also reported to be enhanced in combination with other chemotherapeutic agents such as TRAIL (Nikrad *et al*., 2005; Zhu *et al*., 2005a), cisplatin (Li *et al*., 2008) and inositol hexakisphosphate (Diallo *et al*., 2008).

Several reports linked the lack of BIK expression to drug resistance during chemotherapeutic treatments. Although many of the conventional drug therapy fails to overcome the impasse of chemoresistance, using novel interventional strategies such as overexpression of BIK through exogenously introduced transgene expression could constitute an attractive approach to treat drug-resistant cancers. BIK overexpression in drug-resistant breast cancer cells that were deficient in the mitochondrial apoptosis pathway resulted in reversion of chemoresistance (Radetzki *et al*., 2002). Vector-mediated BIK expression in a T-ALL (acute lymphoblastic/lymphocytic leukemia) cell line conferred sensitivity to cortico-steroid-induced cell death (Daniel *et al*., 1999). Adenovirus-mediated BIK expression was shown to inhibit tumor formation by chemoresistant human prostate and colon cancer cell lines in a mouse xenotransplant model (Tong *et al*., 2001). As pointed out earlier, *in vitro* studies have suggested potential BIK-based cancer gene therapy approaches to kill difficult cancers such as melanoma (Oppermann *et al*., 2005) and glioblastoma (Naumann *et al*., 2003). Busulfan is a major conditioning chemotherapeutic agent used during bone marrow transplantation in patients with chronic or acute myelogenous leukemia. In busulfan-resistant leukemic cell lines and in clinical samples expression of *Bik* among other apoptotic genes such as *Bnip3* was found to be downregulated whereas anti-apoptotic genes such as *Bcl-2* and *Bcl-xL* were upregulated (Valdez *et al*., 2008). Therapeutic approaches to activate the pro-apoptotic BH3-only molecules including *Bik* and/or gene therapy-based approaches may improve the clinical outcome of chemotherapy treatments.

Systemic administration of *wt Bik* gene or a phosphomimetic mutant version ($T_{33}S_{35} \rightarrow D$, BikDD) (that exhibited enhanced apoptotic activity) through cationic liposome particles was shown to inhibit growth and metastasis of human breast cancer cells in the mouse orthotopic xenograft model (Zou *et al*., 2002; Li *et al*., 2003). A similar approach using a tumor-specific BikDD expression construct was shown to exhibit significant anti-tumor effects on pancreatic cancer and prolong survival in xenograft and orthotopic mouse models of pancreatic tumors without toxicity (Xie *et al*., 2007).

Conclusions

Since the discovery of BIK, a number of BH3-only members have been identified and their physiological role as effectors of the canonical animal cell apoptosis pathway has been established. Although BIK deficiency does not appear to affect the normal developmental processes in the small animal model (possibly as a result of functional redundancy with other BH3-only cousins such as BIM) BIK is implicated in certain normal and pathological functions in human cells. Particularly, the potential role of BIK in viral pathogenesis is intriguing and could be a novel target in antiviral strategies. Since BIK appears to function as a tumor suppressor in human and appears to be activated by small molecule drugs that reactivate epigenetically silenced *Bik* gene or by other chemotherapeutic drugs that activate p53-independent transcriptional pathways, anti-cancer strategies focused on augmenting BIK expression appear to be attractive. Because proteasomal inhibitors have been shown to dramatically increase BIK protein accumulation, the combination of transcriptional modulators and proteasomal inhibitors appear to be very promising. The BIK-based gene therapy approaches also hold promise for treatment of difficult cancers such as brain and pancreatic cancers.

Conflict of interest

G Chinnadurai is currently receiving grant support from the National Cancer Institute (NCI, Rockville, MD, USA). G Chinnadurai holds a US patent (#5858678) on apoptosis regulating proteins BIK, BNIP3, BNIP1 and BNIP2 (Bip1A, Nip1-3). S Vijayalingam and R Rashmi have declared no conflicts of interest.

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References

Aouacheria A, Brunet F, Gouy M. (2005). Phylogenomics of life-or-death switches in multicellular animals: Bcl-2, BH3-Only, and BNip families of apoptotic regulators. *Mol Biol Evol* **22**: 2395–2416.

Arena V, Martini M, Luongo M, Capelli A, Larocca LM. (2003). Mutations of the BIK gene in human peripheral B-cell lymphomas. *Genes Chromosomes Cancer* **38**: 91–96.

Boyd JM, Gallo GJ, Elangovan B, Houghton AB, Malstrom S, Avery BJ *et al*. (1995). Bik, a novel death-inducing protein shares a distinct sequence motif with Bcl-2 family proteins and interacts with viral and cellular survival-promoting proteins. *Oncogene* **11**: 1921–1928.

Boyd JM, Malstrom S, Subramanian T, Venkatesh LK, Schaeper U, Elangovan B *et al*. (1994). Adenovirus E1B 19 kDa and Bcl-2 proteins interact with a common set of cellular proteins. *Cell* **79**: 341–351.

Bredel M, Bredel C, Juric D, Harsh GR, Vogel H, Recht LD *et al*. (2005). High-resolution genome-wide mapping of genetic alterations in human glial brain tumors. *Cancer Res* **65**: 4088–4096.

Brown MS, Ye J, Rawson RB, Goldstein JL. (2000). Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell* **100**: 391–398.

Castells A, Ino Y, Louis DN, Ramesh V, Gusella JF, Rustgi AK. (1999). Mapping of a target region of allelic loss to a 0.5-cM interval on chromosome 22q13 in human colorectal cancer. *Gastroenterology* **117**: 831–837.

Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T *et al*. (2001). BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol Cell* **8**: 705–711.

Chittenden T, Flemington C, Houghton AB, Ebb RG, Gallo GJ, Elangovan B *et al*. (1995). A conserved domain in Bak, distinct from BH1 and BH2, mediates cell death and protein binding functions. *EMBO J* **14**: 5589–5596.

Coultas L, Bouillet P, Loveland KL, Meachem S, Perlman H, Adams JM *et al*. (2005). Concomitant loss of proapoptotic BH3-only Bcl-2 antagonists Bik and Bim arrests spermatogenesis. *EMBO J* **24**: 3963–3973.

Coultas L, Bouillet P, Stanley EG, Brodnicki TC, Adams JM, Strasser A. (2004). Proapoptotic BH3-only Bcl-2 family member Bik/Blk/Nbk is expressed in hemopoietic and endothelial cells but is redundant for their programmed death. *Mol Cell Biol* **24**: 1570–1581.

Dai Z, Liu S, Marcucci G, Sadee W. (2006). 5-Aza-2′-deoxycytidine and depsipeptide synergistically induce expression of BIK (BCL2-interacting killer). *Biochem Biophys Res Commun* **351**: 455–461.

Daniel PT, Pun KT, Ritschel S, Sturm I, Holler J, Dorken B *et al*. (1999). Expression of the death gene Bik/Nbk promotes sensitivity to drug-induced apoptosis in corticosteroid-resistant T-cell lymphoma and prevents tumor growth in severe combined immunodeficient mice.PG—1100-7. *Blood* **94**: 1100–1107.

Diallo JS, Betton B, Parent N, Peant B, Lessard L, Le Page C *et al*. (2008). Enhanced killing of androgen-independent prostate cancer cells using inositol hexakisphosphate in combination with proteasome inhibitors. *Br J Cancer* **99**: 1613–1622.

Dong F, Pirbhai M, Xiao Y, Zhong Y, Wu Y, Zhong G. (2005). Degradation of the proapoptotic proteins Bik, Puma, and Bim with Bcl-2 domain 3 homology in Chlamydia trachomatis-infected cells. *Infect Immun* **73**: 1861–1864.

Elangovan B, Chinnadurai G. (1997). Functional dissection of the pro-apoptotic protein Bik. Heterodimerization with anti-apoptosis proteins is insufficient for induction of cell death. *J Biol Chem* **272**: 24494–24498.

Fan T, Lu H, Hu H, Shi L, McClarty GA, Nance DM *et al*. (1998). Inhibition of apoptosis in chlamydia-infected cells: blockade of mitochondrial cytochrome *c* release and caspase activation. *J Exp Med* **187**: 487–496.

Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F *et al*. (2001). The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* **1**: 515–525.

Garcia N, Salamanca F, Astudillo-de la Vega H, Curiel-Quesada E, Alvarado I, Penaloza R *et al*. (2005). A molecular analysis by gene expression profiling reveals Bik/NBK overexpression in sporadic breast tumor samples of Mexican females. *BMC Cancer* **5**: 93.

Germain M, Mathai JP, McBride HM, Shore GC. (2005). Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. *EMBO J* **24**: 1546–1556.

Germain M, Mathai JP, Shore GC. (2002). BH-3-only BIK functions at the endoplasmic reticulum to stimulate cytochrome *c* release from mitochondria. *J Biol Chem* **277**: 18053–18060.

Giatromanolaki A, Koukourakis MI, Sowter HM, Sivridis E, Gibson S, Gatter KC *et al*. (2004). BNIP3 expression is linked with

hypoxia-regulated protein expression and with poor prognosis in non-small cell lung cancer. *Clin Cancer Res* **10**: 5566–5571.

Gillissen B, Essmann F, Graupner V, Starck L, Radetzki S, Dorken B *et al*. (2003). Induction of cell death by the BH3-only Bcl-2 homolog Nbk/Bik is mediated by an entirely Bax-dependent mitochondrial pathway. *EMBO J* **22**: 3580–3590.

Gillissen B, Essmann F, Hemmati PG, Richter A, Oztop I, Chinnadurai G *et al*. (2007). Mcl-1 determines the Bax dependency of Nbk/Bik-induced apoptosis. *J Cell Biol* **179**: 701–715.

Han J, Sabbatini P, White E. (1996). Induction of apoptosis by human Nbk/Bik, a BH3-containing protein that interacts with E1B 19K. *Mol Cell Biol* **16**: 5857–5864.

Hegde R, Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. (1998). Blk, a BH3-containing mouse protein that interacts with Bcl-2 and Bcl-xL, is a potent death agonist. *J Biol Chem* **273**: 7783–7786.

Hinds MG, Smits C, Fredericks-Short R, Risk JM, Bailey M, Huang DC *et al*. (2007). Bim, Bad and Bmf: intrinsically unstructured BH3-only proteins that undergo a localized conformational change upon binding to prosurvival Bcl-2 targets. *Cell Death Differ* **14**: 128–136.

Hur J, Bell DW, Dean KL, Coser KR, Hilario PC, Okimoto RA *et al*. (2006). Regulation of expression of BIK proapoptotic protein in human breast cancer cells: p53-dependent induction of BIK mRNA by fulvestrant and proteasomal degradation of BIK protein. *Cancer Res* **66**: 10153–10161.

Hur J, Chesnes J, Coser KR, Lee RS, Geck P, Isselbacher KJ *et al*. (2004). The Bik BH3-only protein is induced in estrogen-starved and antiestrogen-exposed breast cancer cells and provokes apoptosis. *Proc Natl Acad Sci USA* **101**: 2351–2356.

Jiang A, Clark EA. (2001). Involvement of Bik, a proapoptotic member of the Bcl-2 family, in surface IgM-mediated B cell apoptosis. *J Immunol* **166**: 6025–6033.

Klein U, Tu Y, Stolovitzky GA, Keller JL, Haddad Jr J, Miljkovic V *et al*. (2003). Gene expression dynamics during germinal center transit in B cells. *Ann N Y Acad Sci* **987**: 166–172.

Li C, Li R, Grandis JR, Johnson DE. (2008). Bortezomib induces apoptosis via Bim and Bik up-regulation and synergizes with cisplatin in the killing of head and neck squamous cell carcinoma cells. *Mol Cancer Ther* **7**: 1647–1655.

Li YM, Wen Y, Zhou BP, Kuo HP, Ding Q, Hung MC. (2003). Enhancement of Bik antitumor effect by Bik mutants. *Cancer Res* **63**: 7630–7633.

Lomonosova E, Subramanian T, Chinnadurai G. (2002). Requirement of BAX for efficient adenovirus-induced apoptosis. *J Virol* **76**: 11283–11290.

Lu Y, Lemon W, Liu PY, Yi Y, Morrison C, Yang P *et al*. (2006). A gene expression signature predicts survival of patients with stage I non-small cell lung cancer. *PLoS Med* **3**: e467.

Marshansky V, Wang X, Bertrand R, Luo H, Duguid W, Chinnadurai G *et al*. (2001). Proteasomes modulate balance among proapoptotic and antiapoptotic Bcl-2 family members and compromise functioning of the electron transport chain in leukemic cells. *J Immunol* **166**: 3130–3142.

Mathai JP, Germain M, Marcellus RC, Shore GC. (2002). Induction and endoplasmic reticulum location of BIK/NBK in response to apoptotic signaling by E1A and p53. *Oncogene* **21**: 2534–2544.

Mathai JP, Germain M, Shore GC. (2005). BH3-only BIK regulates BAX,BAK-dependent release of Ca2+ from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death. *J Biol Chem* **280**: 23829–23836.

McDonnell JM, Fushman D, Milliman CL, Korsmeyer SJ, Cowburn D. (1999). Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. *Cell* **96**: 625–634.

Mebratu YA, Dickey BF, Evans C, Tesfaigzi Y. (2008). The BH3-only protein Bik/Blk/Nbk inhibits nuclear translocation of activated ERK1/2 to mediate IFN gamma-induced cell death. *J Cell Biol* **183**: 429–439.

Milutinovic S, Brown SE, Zhuang Q, Szyf M. (2004). DNA methyltransferase 1 knock down induces gene expression by a mechanism independent of DNA methylation and histone deacetylation. *J Biol Chem* **279**: 27915–27927.

Naumann U, Schmidt F, Wick W, Frank B, Weit S, Gillissen B *et al*. (2003). Adenoviral natural born killer gene therapy for malignant glioma. *Hum Gene Ther* **14**: 1235–1246.

Nikrad M, Johnson T, Puthalalath H, Coultas L, Adams J, Kraft AS. (2005). The proteasome inhibitor bortezomib sensitizes cells to killing by death receptor ligand TRAIL via BH3-only proteins Bik and Bim. *Mol Cancer Ther* **4**: 443–449.

Oltvai ZN, Milliman CL, Korsmeyer SJ. (1993). Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74**: 609–619.

Oppermann M, Geilen CC, Fecker LF, Gillissen B, Daniel PT, Eberle J. (2005). Caspase-independent induction of apoptosis in human melanoma cells by the proapoptotic Bcl-2-related protein Nbk/Bik. *Oncogene* **24**: 7369–7380.

Panaretakis T, Pokrovskaja K, Shoshan MC, Grander D. (2002). Activation of Bak, Bax and BH3-only proteins in the apoptotic response to doxorubicin. *J Biol Chem* **21**: 21.

Paquet C, Schmitt E, Beauchemin M, Bertrand R. (2004). Activation of multidomain and BH3-only pro-apoptotic Bcl-2 family members in p53-defective cells. *Apoptosis* **9**: 815–831.

Pompeia C, Hodge DR, Plass C, Wu YZ, Marquez VE, Kelley JA *et al*. (2004). Microarray analysis of epigenetic silencing of gene expression in the KAS-6/1 multiple myeloma cell line. *Cancer Res* **64**: 3465–3473.

Shimizu S, Tsujimoto Y. (2000). Proapoptotic BH3-only Bcl-2 family members induce cytochrome *c* release, but not mitochondrial membrane potential loss, and do not directly modulate voltage-dependent anion channel activity. *Proc Natl Acad Sci USA* **97**: 577–582.

Sowter HM, Ferguson M, Pym C, Watson P, Fox SB, Han C *et al*. (2003). Expression of the cell death genes BNip3 and NIX in ductal carcinoma *in situ* of the breast; correlation of BNip3 levels with necrosis and grade. *J Pathol* **201**: 573–580.

Spender LC, O'Brien DI, Simpson D, Dutt D, Gregory CD, Allday MJ *et al*. (2009). TGF-beta induces apoptosis in human B cells by transcriptional regulation of BIK and BCL-X(L). *Cell Death Differ* (in press).

Sturm I, Stephan C, Gillissen B, Siebert R, Janz M, Radetzki S *et al*. (2006). Loss of the tissue-specific proapoptotic BH3-only protein Nbk/Bik is a unifying feature of renal cell carcinoma. *Cell Death Differ* **13**: 619–627.

Subramanian T, Vijayalingam S, Lomonosova E, Zhao LJ, Chinnadurai G. (2007). Evidence for involvement of BH3-only proapoptotic members in adenovirus-induced apoptosis. *J Virol* **81**: 10486–10495.

Sundararajan R, Cuconati A, Nelson D, White E. (2001). Tumor necrosis factor-alpha induces Bax-Bak interaction and apoptosis, which is inhibited by adenovirus E1B 19K. *J Biol Chem* **276**: 45120–45127.

Theodorakis P, Lomonosova E, Chinnadurai G. (2002). Critical requirement of BAX for manifestation of apoptosis induced by multiple stimuli in human epithelial cancer cells. *Cancer Res* **62**: 3373–3376.

Tong Y, Yang Q, Vater C, Venkatesh LK, Custeau D, Chittenden T *et al*. (2001). The pro-apoptotic protein, Bik, exhibits potent antitumor activity that is dependent on its BH3 domain. *Mol Cancer Ther* **1**: 95–102.

Valdez BC, Murray D, Ramdas L, de Lima M, Jones R, Kornblau S *et al*. (2008). Altered gene expression in busulfan-resistant human myeloid leukemia. *Leuk Res* **32**: 1684–1697.

Verma S, Budarf ML, Emanuel BS, Chinnadurai G. (2000). Structural analysis of the human pro-apoptotic gene Bik: chromosomal localization, genomic organization and localization of promoter sequences. *Gene* **254**: 157–162.

Verma S, Zhao LJ, Chinnadurai G. (2001). Phosphorylation of the pro-apoptotic protein BIK: mapping of phosphorylation sites and effect on apoptosis. *J Biol Chem* **276**: 4671–4676.

Wang Y, Guan X, Fok KL, Li S, Zhang X, Miao S *et al*. (2008). A novel member of the Rhomboid family, RHBDD1, regulates BIK-mediated apoptosis. *Cell Mol Life Sci* **65**: 3822–3829.

Warr MR, Acoca S, Liu Z, Germain M, Watson M, Blanchette M *et al*. (2005). BH3-ligand regulates access of MCL-1 to its E3 ligase. *FEBS Lett* **579**: 5603–5608.

Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ *et al*. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* **292**: 727–730.

Willis SN, Chen L, Dewson G, Wei A, Naik E, Fletcher JI *et al*. (2005). Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. *Genes Dev* **19**: 1294–1305.

Xie X, Xia W, Li Z, Kuo HP, Liu Y, Ding Q *et al*. (2007). Targeted expression of BikDD eradicates pancreatic tumors in noninvasive imaging models. *Cancer Cell* **12**: 52–65.

Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. (1995). Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell* **80**: 285–291.

Yeung BH, Huang DC, Sinicrope FA. (2006). PS-341 (bortezomib) induces lysosomal cathepsin B release and a caspase-2-dependent mitochondrial permeabilization and apoptosis in human pancreatic cancer cells. *J Biol Chem* **281**: 11923–11932.

Zhong Q, Gao W, Du F, Wang X. (2005). Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell* **121**: 1085–1095.

Zhu H, Guo W, Zhang L, Wu S, Teraishi F, Davis JJ *et al*. (2005a). Proteasome inhibitors-mediated TRAIL resensitization and Bik accumulation. *Cancer Biol Ther* **4**: 781–786.

Zhu H, Zhang L, Dong F, Guo W, Wu S, Teraishi F *et al*. (2005b). Bik/NBK accumulation correlates with apoptosis-induction by bortezomib (PS-341, Velcade) and other proteasome inhibitors. *Oncogene* **24**: 4993–4999.

Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. (2001). BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. *Genes Dev* **15**: 1481–1486.

Zou Y, Peng H, Zhou B, Wen Y, Wang SC, Tsai EM *et al*. (2002). Systemic tumor suppression by the proapoptotic gene bik. *Cancer Res* **62**: 8–12.