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

By Peter T. Daniel, Kwok-Tao Pun, Silke Ritschel, Isrid Sturm, J Utta Holler, Bernd Dörken, and Robin Brown

Members of the Bcl-2 gene family have been implicated in the regulation of cell death induced by cytostatic drugs. In some malignancies such as B-cell lymphoma, there is evidence that high expression of Bcl-2 is an independent negative prognostic marker and the overexpression of Bcl-2 has been shown to confer resistance to cytotoxic drugs by preventing drug-induced apoptosis. This function of Bcl-2 can be antagonized by apoptosis-promoting members of the Bcl-2 family. We previously showed that overexpression of Bax restores the chemosensitivity of Bax-deficient breast cancer cell lines. Therefore, we investigated whether the death-promoting Bcl-2 homologue Bik/Nbk can enhance cytostatic drug-induced apoptosis. As a model, we used the T-cell leukemia H9 (CD3+ and CD4+CD8-), which is resistant to corticosteroid-induced cell death and does not express endogenous Bik/Nbk. Sensitivity for drug-induced apoptosis was increased 10- to 39-fold in cells transfected with the full-length coding sequence of Bik/Nbk. In addition, apoptosis induced via CD95/Fas or heat shock was increased to a similar extent. These data show that Bik/Nbk, which, unlike Bax, carries only a BH3 but no BH1 or BH2 domain may be a target to enhance chemosensitivity. The complete suppression of tumor growth in a severe combined immunodeficient mouse xenotransplant model suggests that, in analogy to Bax, Bik/Nbk may function as a tumor suppressor gene.

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nodetection was performed at 4°C by using a polyclonal goat anti-Nbk antibody (Santa-Cruz, Santa Cruz, CA) at 1 µg/mL and visualized by using the ECL detection system (Amersham), according to the manufacturer’s instructions. Glucocorticoid receptor (GR) protein was detected as described above by the use of a polyclonal rabbit antiserum (diluted 1:1,000; Santa-Cruz), which recognized the N-terminus of both GR-alpha (95 kD) and GR-beta (90 kD).

**Northern blot analysis.** Human multiple tissue Northern blots (Clontech) were hybridized with a 32P-α-dCTP (Amersham) random labeled 423bp BamH1-Sma1 fragment of Bik/Nbk in 50% formamide buffer at 43°C for 24 hours, followed by washes in 0.1% SDS 0.1 × standard salt concentration (SSC) buffer at 50°C. The blots were exposed for 10 days with intensifying screens at −70°C.

**RNA preparation, polymerase chain reaction (PCR), and Southern blot analysis.** Total RNA was purified from 5 × 10^6 cells by using the RNAzolB method.16 RNA (4 µg) was used for first strand cDNA synthesis with the GeneAmp RNA PCR kit (Perkin Elmer, Weiterstadt, Germany), according to the manufacturer’s instructions. PCR was performed by using a vector specific T7 forward primer: 5’-TAA-TAC-GAC-TCA-CTA-TAG-GG-3’ and a Bik/Nbk specific reverse primer: 5’-TTC-CAA-AGA-ATC-GAA-GTC-CT-3’ in PCR buffer containing 20 pmol of each primer, 1.5 mmol/L MgCl2, 200 mmol/L dNTPs, and 2.5 units Taq polymerase. Cycling conditions were 30 cycles (94°C, 30 seconds; 60.5°C, 1 minute; 72°C, 1 minute). The PCR fragments were analyzed on a 1.2% gel and identified as Bik/Nbk by Southern blotting onto Hybond N+ membrane (Amersham) and high stringency hybridization using a 32P-γ-ATP labeled oligonucleotide corresponding to the 5’ terminus of Bik/Nbk (4 to 28 bp inclusive).

**Measurement of apoptosis.** After induction of apoptosis by cytotoxic drugs, anti-APO-1 IgG3, anti-CD3, dexamethasone, or heat shock treatment was seen in the heart, brain, skeletal muscle, spleen, kidneys and pancreas (Fig 1A) with lower levels in placenta, epithelial cells (Fig 1). High levels of expression are seen in the tissue distribution with expression being detected mainly in the heart, brain, skeletal muscle, spleen, thymus, ovary, small intestine, colon, and peripheral blood leukocytes. The cell lines Raji (EBV-positive...
type III Burkitt lymphoma) and SW480 (colon adenocarcinoma) had the highest levels of mRNA expression with lower levels in HL-60 and MOLT-4 cells (Fig 1C). Thus, in contrast to the lack of Bik/Nbk expression in nonmalignant lymphoid or colon tissue, Bik/Nbk mRNA appears to be expressed ectopically in some tumor-derived cell lines.

With regard to the above expression pattern where Bik/Nbk was detected preferentially in the epithelial but not the lymphoid compartment (disregarding the ectopic expression in Raji cells), a lymphoid cell system appeared suitable for the functional analysis of Bik/Nbk. The ability of Bik/Nbk to enhance sensitivity for drug-induced apoptosis was, therefore, assessed in the corticosteroid-resistant H9 T-ALL cell line, which does not express detectable levels of endogenous Bik/Nbk as shown by Western blot analysis (Fig 2B).

Therefore, the full-length cDNA of Bik/Nbk was cloned into the pCIN4 vector (pCIN4.Nbk) and stably overexpressed in the H9 cells. Clones selected for resistance to G418 and isolated by limiting dilution were screened for transgene expression by reverse transcription (RT)-PCR followed by Southern blot analysis. In Fig 2A, no exogenous Bik/Nbk could be detected (Fig 2B). In contrast, the clones nos. 2 and 10 show overexpression of the 22.5 kD Bik/Nbk protein. A Gal4-Nbk fusion protein expressed in yeast served as a positive control. However, the colon adenocarcinoma cell line SW480, which shows strong expression of Bik/Nbk RNA (Fig 1C), showed only weak, but detectable Bik/Nbk endogenous protein expression (Fig 2B).

To test for the effect of Bik/Nbk on apoptosis sensitivity, the transfectants were exposed to the cytostatic drugs etoposide, epirubicin, and taxol. After a 72-hour culture, apoptosis was assessed on the single cell level by flow cytometric measurement of the nuclear DNA content. All three clones showed an increased susceptibility for drug-induced apoptosis. Sensitivity for all three drugs was strongly enhanced as compared with the control cells (Fig 3A through C). Comparison of the ED50 concentrations for apoptosis induction shows a 10.1- to 39.3-fold sensitization for drug-induced apoptosis in the Bik/Nbk transfectants as compared with the H9 mock transfectants (Table 1).

In T cells, the CD95 death receptor and its ligand have been implicated in the control of apoptosis.\textsuperscript{14,17,20,21} In addition, drug-induced apoptosis was suggested to depend, in part, on activation of the Fas ligand and subsequent CD95/Fas ligation. Therefore, we assessed the effect of Bik/Nbk on CD95 triggered death of the H9 T cells. CD95/Fas-triggered death was augmented in all three clones (Fig 4B), whereas activation-induced
Bik/Nbk PROMOTES DRUG SENSITIVITY IN ALL

Table 1. ED50 Concentrations for Drug-Induced Apoptosis

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Epirubicin (ng/mL)</th>
<th>Etoposide (ng/mL)</th>
<th>Paclitaxel (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9 3D8 mock</td>
<td>51.3 ± 2.3</td>
<td>710.8 ± 37.4</td>
<td>7.4 ± 1.2</td>
</tr>
<tr>
<td>nbk clone no. 2</td>
<td>4.39 ± 0.92</td>
<td>52.1 ± 5.5</td>
<td>0.73 ± 0.9</td>
</tr>
<tr>
<td>nbk clone no. 10</td>
<td>(11.7)</td>
<td>(13.6)</td>
<td>(10.1)</td>
</tr>
<tr>
<td>nbk clone no. 16</td>
<td>4.1 ± 1.3</td>
<td>18.1 ± 2.3</td>
<td>0.35 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>(12.5)</td>
<td>(39.3)</td>
<td>(21.14)</td>
</tr>
<tr>
<td></td>
<td>4.2 ± 0.3</td>
<td>38.9 ± 7.3</td>
<td>0.41 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>(12.2)</td>
<td>(18.3)</td>
<td>(18)</td>
</tr>
</tbody>
</table>

Cells were exposed to cytostatic drugs for 72 hours. Apoptosis was determined on the single cell level by measuring the DNA content of individual nuclei by flow cytometry. Numbers in brackets indicate the factor of sensitization as compared with the control cells. Data represent the mean of triplicates ± SD.

Fig 4. Sensitization of H9 cells for cell death induced by Fas/CD95, CD3 crosslinking, or dexamethasone. (A) Activation-induced cell death on CD3 crosslinking by immobilized anti-CD3 monoclonal antibody (MoAb) (clone OKT3). Plates were coated with OKT3 (coating concentration 0.1 to 100 ng/mL) as described.14,17 (B) Induction of cell death by CD95/Fas triggering by (soluble) anti-CD95 MoAb (clone anti-APO-1 IgG3). (C) Cell death induction by dexamethasone, which was added to the cultures at concentrations from 10⁻⁸ to 10⁻⁵ mol/L. Cell death was determined on the single-cell level by measuring the DNA content of individual nuclei by flow cytometry. Data represent the mean of triplicates ± SD. (A through C) H9 control cells (○), Bik/Nbk clone no. 2 (●), Bik/Nbk clone no. 10 (■), Bik/Nbk clone no. 16 (▲). Medium control in (A) or cultures incubated with FII23c isotype-matched control antibody (0.12 μg/mL) in (B): H9 control cells (○), Bik/Nbk clone no. 2 (●), Bik/Nbk clone no. 10 (■), Bik/Nbk clone no. 16 (▲).

Apoptotic cells are known to shrink and this leads to a decrease of the forward light scatter (FSC) as measured by flow cytometry. In addition, late stage apoptotic cells show an increased membrane permeability, which was determined by addition of propidium iodide (PI). Thus, cells with a decrease in FSC and an increased uptake of PI can be considered as apoptotic.18 In comparison and in clear contrast to anti-CD3–induced H9 cells, neither H9 3D8 cells nor Bik/Nbk H9 transfectants showed such signs of apoptosis after induction with dexamethasone, thereby showing that dexamethasone does not induce apoptosis in these cells (not shown). We were also unable to observe induction of a DNA ladder after induction with dexamethasone, unlike CD3 or CD95/Fas triggering which induce activation of genomic DNA fragmentation.22

To further exclude that dexamethasone does not induce apoptosis in the H9 T cells because of defects in GR expression and function we performed a Western blot analysis for GR expression in H9 3D8 and the Bik/Nbk transfectants (Fig 4A). There was no difference in GR-alpha (95 kD) or GR-beta (90 kD) expression levels. In addition, the GR expression is known to be under control of steroid responsive elements in T cells.23 Thus, exposure of H9 T cells to dexamethasone led to an induction of GR expression in both the H9 3D8 control cells and the Bik/Nbk transfectants (Fig 5B and C). Additional evidence for the presence of a functional GR receptor and signaling pathway come from the observation that glucocorticoids induce promoter activation in H9 T cells transfected with retroviral long terminal repeat promoter constructs containing GR-response elements.24 Therefore, the GR receptor and signaling pathway appear to be intact in the H9 T cells and the Bik/Nbk clones.

Thus, Bik/Nbk could promote sensitivity toward drug-induced apoptosis and CD95/Fas-mediated death, but could not render the steroid refractory H9 cells sensitive for steroid-induced apoptosis.

To assess the effect of Bik/Nbk on another physiologic
apoptotic stimulus, and in addition to the death induced by cytostatic drugs, we investigated the response to heat shock (Table 2). The cells were incubated for 4 hours at temperatures ranging from 37°C to 45°C. After heat shock, the cells were cultured for a further 24 to 48 hours at 37°C. Cell death by apoptosis was induced at temperatures of 39°C and above. Apoptosis was detectable in the H9 3D8 mock transfectants (Table 2).

Table 2. Heat-Shock-Induced Apoptosis

<table>
<thead>
<tr>
<th>Hours</th>
<th>Temperature</th>
<th>H9 3D8</th>
<th>Clone No. 2</th>
<th>Clone No. 10</th>
<th>Clone No. 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>37</td>
<td>2.4 ± 0.5</td>
<td>2.1 ± 0.6</td>
<td>2.3 ± 0.7</td>
<td>2.8 ± 3.2</td>
</tr>
<tr>
<td>24</td>
<td>41</td>
<td>10.9 ± 1.1</td>
<td>16.9 ± 1.2</td>
<td>23.1 ± 3.1</td>
<td>18.2 ± 2.1</td>
</tr>
<tr>
<td>48</td>
<td>37</td>
<td>2.4 ± 0.9</td>
<td>2.1 ± 3.4</td>
<td>3.3 ± 3.3</td>
<td>2.8 ± 3.7</td>
</tr>
<tr>
<td>48</td>
<td>41</td>
<td>15 ± 0.6</td>
<td>28 ± 4.2</td>
<td>38.7 ± 3.5</td>
<td>32.5 ± 3.9</td>
</tr>
</tbody>
</table>

Cells were heated for 4 hours from 37°C to 45°C. Subsequently, the cells were cultured for 24 to 48 hours at 37°C until apoptosis was determined on the single cell level by measuring the DNA content of individual nuclei by flow cytometry. Data represent the mean of triplicates ± SD.

Members of the Bcl-2 family are key regulators of apoptosis. Overexpression of Bcl-2 in the B-cell compartment of transgenic mice leads to B-cell hyperplasia. Subsequent dysregulation of genes such as c-myc can lead to the development of B-cell lymphoma. The ability of Bcl-2 to prevent apoptosis is antagonized by the proapoptotic members of the Bcl-2 family. Tissue hyperplasia and tumor promotion can be achieved by the inactivation of such proapoptotic genes as demonstrated by the phenotype of Bax knockout mice and transgenic Bax k.o. mice carrying a truncated SV40 large T antigen. The mechanism of action of the proapoptotic genes is nevertheless unclear, although recent evidence has implicated the APAF-1 gene, a homologue of the Caenorhabditis elegans ced-4 gene. 

In this model, Bcl-xL, caspase-9, and APAF-1 form a ternary complex. The role of Bax in this complex could be to compete for binding to the caspase activating protein APAF-1 and effect cell death. Additional data show that Bcl-2 prevents apoptosis by the inactivation of such proapoptotic genes as demonstrated by the phenotype of Bax knockout mice and transgenic Bax k.o. mice carrying a truncated SV40 large T antigen. 

The mechanism of action of the proapoptotic genes is nevertheless unclear, although recent evidence has implicated the APAF-1 gene, a homologue of the Caenorhabditis elegans ced-4 gene.30 In this model, Bcl-xL, caspase-9, and APAF-1 form a ternary complex. The role of Bax in this complex could be to compete for binding to the caspase activating protein APAF-1 and effect cell death. Additional data show that Bcl-2 prevents mitochondrial permeability shift transition and that Bax may directly activate mitochondria resulting in the induction of the mitochondrial permeability transition and the release of cytochrome C.34

Table 3. Suppression of Tumor Growth by Bik/Nbk

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tumor Volume ± SD (cm³)</th>
<th>Tumor Take Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9 3D8</td>
<td>2.49 ± 0.27</td>
<td>7/7</td>
</tr>
<tr>
<td>Clone no. 2</td>
<td>0</td>
<td>0/6</td>
</tr>
<tr>
<td>Clone no. 10</td>
<td>0</td>
<td>0/6</td>
</tr>
<tr>
<td>Clone no. 16</td>
<td>0</td>
<td>0/6</td>
</tr>
</tbody>
</table>

SCID mice were injected s.c. with 10⁷ cells at t = 0. H9 3D8: n = 7 animals, transfectants: n = 6. Tumor growth was measured at 9 weeks after xenotransplantation in two dimensions with calipers and tumor volumes were calculated as described.29

Fig 5. Expression and induction of the GR in H9 cells. (A) Western blot analysis for GRα (95 kD, upper band) and GRβ (90 kD, lower band) expression. Lane 1, H9 3D8 cells; lane 2, H9 Bik/Nbk clone no. 16, lane 3, clone no. 10; lane 4, clone no. 2. (B) Western blot analysis for induction of GR expression by dexamethasone in H9 3D8 cells. Lane 1, medium control; lane 2, 24-hour culture in the presence of dexamethasone (10⁻⁶ mol/L); lane 3, 48 hours; lane 4, 72-hour induction. (C) Induction of GR expression by dexamethasone in H9 Bik/Nbk clone no. 16. Lane 1, medium control; lane 2, 24-hour culture in the presence of dexamethasone (10⁻⁶ mol/L); lane 3, 48 hours; lane 4, 72 hours.

DISCUSSION

Overexpression of Bik/Nbk decreases tumorigenicity of H9 T lymphoma cells in mice. Therefore, we tested whether the ectopic overexpression of Bik/Nbk decreases tumorigenicity of H9 T lymphoma cells in such a xenotransplantation model in SCID mice. Cells (10⁷) were injected subcutaneously (s.c.) into the inguinal region. Tumor growth was observed starting at week 6 after transplantation (Table 3). The H9 3D8 mock transfected cells grew to large local s.c. tumors. Some mice developed macroscopic lymph node dissemination. Hind limb paralysis was observed as previously encountered in the case of Nalm-6 pre-B-ALL xenotransplantation.25 Mice were, therefore, sacrificed 9 weeks after transplantation. In contrast, no tumors developed in the mice transplanted with Bik/Nbk transfectants (Table 3). These mice remained tumor free for a further 3 months after the mice transplanted with H9 control cells had to be sacrificed.
In previous experiments, we observed that breast cancer cells have a defect in Bax expression. The reconstitution of Bax in the breast cancer cells restored apoptosis sensitivity for serum starvation or Fas-triggered death. Additional experiments showed that Bax, as well, increased the sensitivity of the breast cancer cells to apoptosis induced by exposure to DNA damaging agents such as anthracyclin drugs. In metastatic colorectal cancer, we recently described that the lack of Bax is a negative prognostic factor, especially in those patients carrying a wild-type p53 gene. In contrast to the broad expression of Bax and Bak, the expression of Bik/Nbk seems to be restricted to epithelial tissues. Given the proapoptotic properties, Bik/Nbk could be involved in tissue specific maintenance of homeostasis in these adult tissues as suggested for Bcl-2 or Bax. In this context, it was surprising to find that some of the cell lines tested showed constitutive overexpression of Bik/Nbk. In the case of Raji, this overexpression shows a good correlation with a high sensitivity for Fas-triggered apoptosis. Mutations in the Bik/Nbk gene like in the case of Bax, p53, or deregulated activation of the Bik/Nbk gene by EBV-encoded factors (in the case of Raji), may also be the cause for the ectopic expression. Nevertheless, we cannot exclude that this overexpression is paralleled by defects in the downstream death signaling cascade and concomitant deregulation of the upstream death effector Bik/Nbk.

In the Bik/Nbk transfectants, we observed an increase in Bik/Nbk mRNA, express only low levels of endogenous Bik/Nbk protein. H9 T cells do not express detectable levels of endogenous Bik/Nbk and are resistant to glucocorticoid-induced apoptosis. Therefore, we investigated in this model whether the overexpression of Bik/Nbk in stably transfected cells increased the sensitivity to cytostatic drug-induced apoptosis and rendered them sensitive to steroid-induced cell death. This question is of interest in light of data that suggest that primary resistance to steroid-induced cell death is an important negative prognostic factor that may predict treatment failure in acute lymphocytic leukaemia.

In H9 cells stably transfected with Bik/Nbk, we observed sensitization of the cells to cytostatic drug-induced cell death as compared with the parental line and the mock transfecants. This increase of drug sensitivity amounted to a 10- to 39-fold reduction in the ED50 for the topoisomerase inhibitors epirubicin and etoposide as well as for the microtubule disrupting drug paclitaxel (taxol). These results show and confirm that the proapoptotic properties, Bik/Nbk could be involved in tissue specific maintenance of homeostasis in these adult tissues as suggested for Bcl-2 or Bax. In this context, it was surprising to find that some of the cell lines tested showed constitutive overexpression of Bik/Nbk. In the case of Raji, this overexpression shows a good correlation with a high sensitivity for Fas-triggered apoptosis. Mutations in the Bik/Nbk gene like in the case of Bax, p53, or deregulated activation of the Bik/Nbk gene by EBV-encoded factors (in the case of Raji), may also be the cause for the ectopic expression. Nevertheless, we cannot exclude that this overexpression is paralleled by defects in the downstream death signaling cascade and concomitant deregulation of the upstream death effector Bik/Nbk.

In a xenotransplantation SCID mouse model, Bik/Nbk overexpressing cells were unable to form tumors. The data obtained from these experiments are in line with our previous observations that reconstitution of Bax expression in xenotransplanted breast cancer cells decreases tumor formation in SCID mice. The clonogenic potential of H9 T cells can, thus, be abrogated by the overexpression of Bik/Nbk. This is in line with the decreased clonogenicity (data not shown) and elevated spontaneous (background) apoptosis of the Bik/Nbk transfectants in vitro. In this context, it would be interesting to examine whether Bik/Nbk expression is lost or downregulated during tumorigenesis as we observed for Bax in breast cancer and high-grade metastatic colorectal cancer.

In the Bik/Nbk transfectants, we observed an increase in sensitivity to CD95 triggered cell death. Thus, Bik/Nbk not only sensitizes cells to drug-induced apoptosis, but also to CD95/Fas-triggered apoptosis, ie, one of the major physiologic programmed cell death pathways in lymphoid cells. In malignant disease, recent observations have suggested that drug-induced cell death by a variety of compounds, including antimetabolites, leads to induced expression of CD95/Fas and FasL. The Bik/Nbk data are consistent with this model and suggest that Bik/Nbk acts on a pathway that sensitizes the cells to apoptosis that is common to both stimuli. The Bik/Nbk enhancement of cell death under these circumstances is also in line with results that suggest CD95/Fas triggered death can be inhibited by overexpression of Bcl-2 and can be enhanced by Bax expression.

The effect of Bik/Nbk overexpression was examined in another physiologic model of cell death. We observed an enhancement of cell killing after heat shock, in contrast to treatment with anti-CD3 and dexamethasone in which killing was unaffected. This was surprising in the case of the anti-CD3-mediated death, because this is considered to be mediated by the CD95/Fas ligand. Nevertheless, there is evidence that additional, Fas-independent pathways participate in the activation-induced cell death on CD3-crosslinking, which could be Fas-independent, such as other members of the death ligand receptor superfamilies, eg, tumor necrosis factor receptor-mediated signals. In addition we were not able, as previously shown, to completely abrogate CD3-triggered apoptosis by addition of high-affinity blocking anti-CD95/Fas antibodies to the culture. These and many other findings from other groups show that the activation-induced cell death of T cells is not mediated exclusively by the CD95/Fas receptor/ligand interaction. This is also in line with the fact that antigen receptor-triggered apoptosis in B cells is clearly independent from CD95/Fas, which further corroborates the fact that additional, CD95/Fas-independent signals participate in antigen receptor-mediated cell death in lymphoid cells.

Thus, Bik/Nbk differentially sensitized the T-lymphoma cells to a variety of apoptotic stimuli. This might be related to structural properties of Bik/Nbk.

The Bcl-2 family protein Bik/Nbk contains only one of the signature domains of the Bcl-2-family, the BH3 domain. The BH3 domain is conserved both in the proapoptotic and the antiapoptotic Bcl-2 family proteins. The BH3 domain of the proapoptotic proteins may serve a dual function. It appears to be essential for their cell death activity and for mediating homo-
dimerization with antiapoptosis proteins.\(^3,5,43,44\) Because BH3-alone containing proapoptotic proteins (Bik/Nbk, Bid, Hrk, Bad, Mtd) share only the BH3 domain in common, it has been discussed whether the BH3 domain is a death effector module and is postulated to elicit its cell death activity by inactivating the antiapoptotic proteins through heterodimerization. This is supported by the observation that the BH3 domain is an apoptosis effector in a cell-free system.\(^4\) However, mutational analysis of the BH3 domain in Bik\(^40\) and a novel proapoptotic Bcl-2 homologue, Mtd,\(^45\) suggests that heterodimerization via the BH3 domain with survival proteins alone is insufficient to explain their cell death inducing activity. Nevertheless, Bik/Nbk may also function as a naturally dominant negative antagonist whose role in the cell is to bind to and inactivate antiapoptotic genes, eg, Bcl-2 and Bcl-xL, like in the case of Bad. Given the above described capability of members of the Bcl-2 family to induce apoptosis even in the absence of a functional BH3 domain we would favor the direct induction of the mitochondrial apoptotic signaling cascade and subsequent activation of caspases. Such a view of a direct action of Bik/Nbk, independent from dimerization to Bcl-2 or Bcl-xL, is supported by data where the BH3 domain in Bad or Bak was deleted.\(^27,46\) This prevents homodimerization of Bad but the BH3-deleted mutants retain their capability to enhance apoptosis.

The Bik/Nbk data presented here establish that Bik/Nbk differentially controls the cellular apoptotic response, depending on the type of induction stimulus. In our previous work, we showed that overexpression of Bad may enhance chemosensitivity.\(^2\) Recent data show that such an effect of Bad may be caused by the direct activation of the mitochondrial death cascade.\(^34\) Such an effect appears to be independent from the interaction with Bcl-2/Bcl-x via the BH1/BH2 domains, which would be in line with our findings that the proapoptotic Bcl-2 homologue Bik/Nbk, which lacks the BH1 and BH2 domain, enhances drug and Fas-mediated apoptosis. Taken together, these data support the role of Bik/Nbk as a direct death effector not only in propagation of cell death on DNA damage, but also for CD95/Fas crosslinking. Such a differential control of different apoptotic pathways could be mediated by functional domains apart from the BH3 signature domain.

Finally, the fact that Bik/Nbk can sensitize cells to drug-induced apoptosis suggests that upregulation of such tissue-specific dominant negative Bcl-2–like protein might yield a therapeutic strategy to overcome drug resistance and tumors refractory to cytotoxic therapies.

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