Bcl-G, a Novel Pro-apoptotic Member of the Bcl-2 Family*

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A new member of the Bcl-2 family was identified, Bcl-G. The human BCL-G gene consists of 6 exons, resides on chromosome 12p12, and encodes two proteins through alternative mRNA splicing, Bcl-GL (long) and Bcl-GS (short) consisting of 327 and 252 amino acids in length, respectively. Bcl-GL and Bcl-GS have identical sequences for the first 226 amino acids but diverge thereafter. Among the Bcl-2 homology (BH) domains previously recognized in Bcl-2 family proteins, the BH3 domain is found in both Bcl-GL and Bcl-GS, but only the longer Bcl-Gs protein possesses a BH2 domain. Bcl-Gs mRNA is expressed widely in adult human tissues, whereas Bcl-Gs mRNA was found only in testis. Overexpression of Bcl-Gs or Bcl-Gs in cells induced apoptosis although Bcl-Gs was far more potent than Bcl-GL. Apoptosis induction by Bcl-Gs depended on the BH3 domain and was suppressed by coexpression of anti-apoptotic Bcl-XL protein. Bcl-XL also coimmunoprecipitated with Bcl-Gs but not with mutants of Bcl-Gs in which the BH3 domain was deleted or mutated or with Bcl-GL. Bcl-GS was predominantly localized to cytosolic organelles, whereas Bcl-GL was diffusely distributed throughout the cytosol. A mutant of Bcl-GL in which the BH2 domain was deleted displayed increased apoptotic activity and coimmunoprecipitated with Bcl-XL, suggesting that the BH2 domain autorepresses Bcl-GL.

Bcl-2 family proteins are central regulators of apoptosis (reviewed in Refs. 1–3). Bcl-2 family proteins are conserved throughout the animal kingdom with homologs identified in both vertebrates and invertebrates. These proteins contain up to four conserved Bcl-2 homology (BH) domains, BH1, BH2, BH3, and BH4, which are recognized by their amino acid sequence similarity. Both anti- and pro-apoptotic Bcl-2 family proteins have been identified. These proteins control cell life/death decisions through their effects on events such as mitochondrial release of proteins involved in activation of caspase-family cell death proteases or by binding sequestering caspase-activating proteins (reviewed in Refs. 2–5 and 7–8). Many Bcl-2 family proteins are capable of physically interacting with each other, forming a complex network of homo- and heterodimers, and these physical interactions sometimes play important roles in the opposing effects of pro- and anti-apoptotic members of the family.

The pro-apoptotic members of the Bcl-2 family can be broadly classified into two groups. One group, including Bax, Bak, and Bok in humans, shares structural similarity with the pore-forming domains of certain bacterial toxins and is capable of forming pores in synthetic membranes in vitro (9–12). These proteins exhibit cytotoxic effects independently of their ability to bind other Bcl-2 family proteins including Bcl-2 and other cytoprotective members of the family such as Bcl-XL, Bcl-W, Bfl-1, and Mcl-1. The second group of pro-apoptotic Bcl-2 family proteins varies widely in their amino acid sequences, often containing only a single region of similarity, specifically, the BH3 domain. These “BH3-only” proteins appear to possess no intrinsic or autonomous cytotoxic activity and instead operate as trans-dominant inhibitors of the survival proteins. Their antagonism of proteins such as Bcl-2 and Bcl-XL depends on binding via their BH3 domains to a hydrophobic pocket on target anti-apoptotic proteins (13).

Gene knockout studies in mice have demonstrated nonredundant roles for various Bcl-2 family genes in regulating cell life and death in specific tissues or under particular physiological or pathological circumstances (14–18). Thus, it is important to identify all members of the Bcl-2 family and to delineate the cellular contexts in which they contribute to apoptosis regulation. In this report, we have described the cloning and initial characterization of a new member of the Bcl-2 family, Bcl-G.

MATERIALS AND METHODS

Cloning of Bcl-G cDNAs—TBLAST searches of the public databases using human Bcl-2 as a query sequence revealed a short EST (GenBank™/EBI no. AW000827) from colonic mucosa of 3 patients with Crohn’s disease, which contains an open reading frame (ORF) encoding sequences similar to the BH2 domain of Bcl-2 family proteins. An oligonucleotide primer (5’-GTAATGTGTCGCCAGCCCAGG-3’) was designed complementary to the EST sequence and used for 5′-RACE, employing the SMART™RACE cDNA Amplification Kit (CLONTECH) and human placental total RNA as template. The 5′-RACE products were subcloned into pCR2.1-TOPO vector using the TOPO™ TA Cloning kit (Invitrogen), and the DNA sequence was determined revealing a complete ORF with start codon within a favorable Kozak sequence context preceded by a 5′-UTR containing stop codons in all three reading frames (GenBank™/EBI accession nos. AF281254 and AF281255). Two additional EST clones, AI478889 and AI240211, were identified by BLAST searches and correspond to overlapping partial Bcl-G cDNAs that contain the 3′-UTR. BLAST searches of GenBank™/EBI also revealed a 190,858 base pair human BAC clone (RPCI11–267J23) in the human BAC clone database (Ref. 19), which contains the BCL-G gene in its entirety.

Plasmids—cDNAs containing the ORFs of Bcl-Gs and Bcl-Gs without additional flanking sequences were generated by PCR using human placental cDNA as a template and the following primers: 5′-GGCTCGAGCCATGGGAGGAGC-3′, sense for both Bcl-GL and Bcl-GS.
A Novel Pro-apoptotic Gene of the Bcl-2 Family

FIG. 1. Sequence analysis of Bcl-G cDNAs. A, the predicted amino acid sequences of the Bcl-Gs and Bcl-G proteins are presented with the BH2 and BH3 domains underlined and residue numbers indicated. The predicted proteins are identical from residues 1–226. The unique C-terminal region of Bcl-GaR is indicated in italics. B, an alignment is presented of the BH3 domains of Bcl-G and several other Bcl-2 family proteins. Identical and similar residues are shown in black and gray blocks, respectively. C, an alignment is presented of the BH2 domains in Bcl-G and several other Bcl-2 family proteins, as above. D, the exon-intron organization of the BCL-G gene is presented. The human BCL-G gene contains 6 exons, spanning a ~30 kilobase region of chromosome 12. Alternative splicing at the 5'-end of exon 5 accounts for the production of the Bcl-Gs and Bcl-GaR proteins, where splice-acceptor sites at nucleotide positions 63,870, 63,797 in BAC clone RPCI 11–267J23 (GenBank®/EBI no. AC00753) are utilized for Bcl-Gs and Bcl-GsR, respectively. The positions of the start and termination codons are indicated, with coding regions in gray blocks and noncoding 5'- and 3'-UTR sequence in open blocks. The BH3 domain is located in exon 4 of both Bcl-Gs and Bcl-GaR, whereas the BH2 domain resides in exon 5 of Bcl-Gs and Bcl-GaR.

The BH3 domain resides in exon 5 of Bcl-G L. AC007537) are utilized for Bcl-G L and Bcl-GS, respectively. The positions of the start and termination codons are indicated, with coding regions in gray blocks and noncoding 5'- and 3'-UTR sequence in open blocks. The BH3 domain is located in exon 4 of both Bcl-Gs and Bcl-GaRs, whereas the BH2 domain resides in exon 5 of Bcl-Gs and Bcl-GaRs.

and Bcl-Gs, 5'-CCAACTTTAAGCTACCTCTCAGTTGATATCC-3', antisense for Bcl-Gs; 5'-CCAACTTTTAAATGCGGACATCAAAC-3', antisense for Bcl-Gs. The resulting PCR products were digested with restriction endonucleases and subcloned into the Xhol and HindIII sites of pEGFP-C1 (CLONTECH). A mutant of Bcl-Gs lacking the BH3 domain was created by a two-step PCR method, using the following primers: primer 1, 5'-GGCTTCGAGGACATGGACGCTTGTGACGGCCT-3'; primer 2, 5'-CCGGATCCTCGGCTAGTATTTGTTCTTCTTCAT-3'; primer 3, 5'-CCGGATCCTCGGCTAGTATTTGTTCTTCATCC-3'; and primer 4, 5'-CCAGGCGCTCAATGACCCCATCAACAC-3'. The resulting PCR product was digested with Xhol/BamHI or with BamHI/HindIII respectively and ligated into pEGFP-C1. Site-directed mutagenesis of Bcl-Gs was performed to generate a L216E substitution using the QuikChange™ Site-directed Mutagenesis kit (Stratagene) following the manufacturer’s protocol, with pEGFP-C1/Bcl-Gs plasmid as DNA template and the mutagenic primers: 5'-GCCAAAAATTGTTGAGGAGGAATATTTCTCCAG-3' and 5'-CCACCTGATTCCTCTGAAATTCTCCAGTGTGG-3'. A mutant of Bcl-Gs lacking the BH2 domain was created by PCR using the same forward primer for Bcl-Gs and 5'-GGCTTCGAGGACATCAAAC-3' as the reverse primer.

Measurements of Bcl-G mRNAs—Bcl-G mRNAs were detected by either Northern blotting or RT-PCR. For RT-PCR, we employed multiple tissue cDNA panels (CLONTECH) containing first-strand cDNA generated from 16 different tissues. PCR was performed according to the manufacturer's protocol with the following primers: (a) 5'-primer for both Bcl-G L and Bcl-Gs, corresponding to exon 3, 5'-CTGAGGGTCTC-TTCTTCCAGTCCAAAAGG-3'; (b) 3'-primer for Bcl-G L, corresponding to exon 5, 5'-GGCGGTGAGCTCCTTACAAAGGGCCACC-3'; 3'-primer for Bcl-Gs, corresponding to an alternatively spliced segment of exon 5, 5'-CCAGGGATGGAGGGAAGGCGATGTC-3'. Human glyceraldehyde-3-phosphate dehydrogenase expression was examined by PCR with the following primers: sense, 5'-GGCGGTGAGCTCCTTACAAAGGGCCACC-3'; antisense, 5'-CATGTTGGCCCATGGTCCACCAAC-3'.

Cell Culture, Transfections, and Apoptosis Assay—293T and COS-7 cells were cultured in Dulbecco’s modified Eagle’s high-glucose medium (Irvine Scientific, Santa Ana, CA) containing 10% fetal bovine serum. PC-3 cells were cultured with RPMI 1640 media containing 10% fetal bovine serum. Transfection of cells was performed using SuperFect (Qiagen, Chatsworth, CA). Both floating and adherent cells (after trypsinization) were collected 24 h after transfection, fixed, and stained using 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) for assessing apoptosis based on nuclear fragmentation and chromatin condensation (19, 20).

Commmunoprecipitations and Immunoblotting—Immunoblotting was performed as described previously (19, 20). For communoprecipitations, cells were cultured in 50 mM benzocarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk) to prevent apoptosis. Cells were suspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 mM EDTA, 50 mM NaF, 0.5% Nonidet P-40, 0.1 mM Na3VO4, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride). Lysates (0.2 ml diluted into 1-ml final volume of lysis buffer) were cleared by incubation with 15 ml of protein G-Sepharose 4B (Zymed Laboratories Inc.) and then incubated with 15 μl of polyclonal anti-GFP antibody (Santa Cruz Biotechnology) and 15 μl of protein G at 4 °C overnight. Beads were then washed four times with 1.5 ml of lysis buffer before boiling in Laemmli sample buffer and performing SDS-polyacrylamide gel electrophoresis and immunoblotting.

Confocal Microscopy—GFP-expressing cells were incubated with 50 nM Mitotracker Red CMXROS (Molecular Probes) for 30 min at 37 °C in culture medium. The cells were then washed with phosphate-buffered saline, fixed in 4% formaldehyde, and imaged by confocal microscopy using a Bio-Rad MRC 1024 instrument (19, 21).

RESULTS

Identification and Sequence Analysis of the BCL-G gene and cDNAs—A short EST was identified during searches of the public databases, which when conceptually translated revealed a polypeptide sequence with similarity to the BH2 domain of Bcl-2 family proteins. Full-length cDNAs were obtained revealing two potential transcripts containing ORFs for proteins of 327 and 252 amino acids, respectively, which we have termed Bcl-G L and Bcl-Gs, respectively. The predicted Bcl-G L and Bcl-Gs proteins are identical for the first 226 amino acids and then diverge thereafter. Comparison of the predicted amino acid sequences of Bcl-G L and Bcl-Gs with Bcl-2 family proteins revealed the presence of a candidate BH3 domain in both Bcl-G L and Bcl-Gs (Fig. 1, A and B) and the presence of a BH2...
domain in Bcl-G, but not in Bcl-Gs (Fig. 1, A and C). Using the Bcl-G cDNA sequences, the human genomic database was searched, revealing a BAC clone from chromosome 12p12 containing the BCL-G gene. Comparison with the cDNA sequences suggests a 6-exon structure for the BCL-G gene. The Bcl-GL and Bcl-Gs cDNAs can be accounted for by an alternative mRNA splicing mechanism in which different splice acceptor sites associated with exon 5 are employed, resulting in a change in the distal reading frame (Fig. 1D).

**Expression of Bcl-G L and Bcl-G S mRNAs—** Northern blotting demonstrated the presence of Bcl-G transcripts of ~1.5–2.5 kilobase pairs in length in several adult human tissues (Fig. 2A), but the highest levels of Bcl-G mRNA by far were found in male gonad (testis), thus prompting the moniker “Bcl-Gonad” (Bcl-G). Because Northern blotting failed to resolve the mRNAs encoding Bcl-GL and Bcl-Gs, we designed RT-PCR assays using primers specific for Bcl-GL and Bcl-Gs sequences associated with exon 5. The amplified bands corresponding to Bcl-GL and Bcl-Gs were excised and sequenced, confirming the validity of the RT-PCR strategy (not shown). Bcl-Gs mRNA was clearly detected in lung, pancreas, prostate, and testis, with lower levels present in some other tissues (Fig. 2B). In contrast, Bcl-Gs mRNA was uniquely expressed in testis. RT-PCR amplification of a control mRNA, glyceraldehyde-3-phosphate dehydrogenase, demonstrated loading of nearly equivalent amounts of mRNA from each tissue.

**Induction of Apoptosis by Bcl-G Proteins—** To assess the effects of Bcl-G proteins on apoptosis, various cell lines, including COS-7, HEK293T, and PC3, were transiently transfected with plasmids encoding Bcl-GL or Bcl-Gs. For most experiments, Bcl-GL and Bcl-Gs were expressed as GFP fusion proteins so that successfully transfected cells could be conveniently identified (Fig. 3A), but similar results were obtained when FLAG-epitope tags were employed instead (not shown). Overexpression of the shorter Bcl-Gs protein reproducibly induced apoptosis in all cell lines tested (Fig. 3A) and other methods (not shown). In contrast, Bcl-Gs was more variable and less efficient at inducing apoptosis in these transient transfection assays. Immunoblot analysis of lysates from transfected cells demonstrated that the less potent effects of Bcl-GL could not be accounted for by lower levels of protein production (Fig. 3A). Indeed, Bcl-GL protein accumulated to levels ~10-fold higher in cells compared with Bcl-Gs, suggesting that Bcl-Gs is a far more potent apoptosis inducer. Analysis of the same blots with an anti-tubulin antibody confirmed loading of essentially equivalent amounts of total protein for each sample, thus validating the results. In additional transfection experiments, Bcl-GL failed to demonstrate cytoprotective activity in side by side comparisons with Bcl-2 and Bcl-XL (data not presented). Also, when Bcl-GL was coexpressed with Bcl-Gs in cells, no synergy with or nullification of Bcl-Gs-induced apoptosis was observed.

**The BH3 Domain of Bcl-Gs Is Required for Its Pro-apoptotic Activity—** The Bcl-Gs protein contains a BH3 domain but lacks other regions of homology with Bcl-2 family proteins. Structural studies indicate that BH3 domains represent amphipathic α-helices in which the hydrophobic surface of the α-helices of apoptosis-inducing BH3 peptides bind to a pocket on survival proteins such as Bcl-XL (22). We, therefore, compared the apoptosis-inducing activity of the wild-type Bcl-Gs protein with mutants lacking the BH3 domain (ΔBH3) or in which leucine 216 within the BH3 domain of Bcl-Gs was chosen for mutation to the charged glutamic acid, based on comparisons with previously described BH3 mutagenesis experiments demonstrating a critical requirement for the analogous leucine in other pro-apoptotic Bcl-2 family proteins (23, 24).

Wild-type Bcl-Gs potently induced apoptosis when overexpressed in COS-7, PC3, HEK293T, and other cell lines, whereas Bcl-Gs-ΔBH3 and Bcl-Gs-L216E did not (Fig. 3B and data not shown). Immunoblot analysis confirmed production of the Bcl-Gs-ΔBH3 and Bcl-Gs-L216E proteins at levels exceeding the amounts of wild-type Bcl-Gs protein. We conclude, therefore, that the BH3 domain of Bcl-Gs is critical for its pro-apoptotic activity.

**The BH2 Domain of Bcl-Gs Negatively Regulates Its Pro-apoptotic Activity—** Compared with Bcl-Gs, Bcl-GsL only weakly induces apoptosis. Bcl-GsL contains a BH2 domain not found in Bcl-Gs. A mutant of Bcl-GsL lacking the BH2 domain was created. Bcl-GsL-ΔBH2) induced apoptosis in COS-7 cells as potently as Bcl-Gs (Fig. 3C). Thus the BH2 domain negatively regulates the pro-apoptotic activity of Bcl-Gs.

**Bcl-Gs Associates with Bcl-XL in a BH3-dependent Manner—** The pro-apoptotic activity of BH3-only members of the Bcl-2 family depends on their ability to dimerize with and suppress the activity of survival proteins such as Bcl-XL (reviewed in Ref. 13). We, therefore, explored whether Bcl-GsL and Bcl-Gs are capable of associating with other Bcl-2 family proteins by immunoprecipitation assays. Bcl-Gs association with the survival proteins Bcl-XL and Bcl-2 was readily detected by coin-
munoprecipitation using lysates from transiently transfected cells, whereas no association with pro-apoptotic proteins Bax, Bak, Bid, or Bad was observed (Fig. 4A and not shown). Interaction of Bcl-Gs with Bcl-2 and Bcl-Xl, but not with Bax or Bak, was also confirmed by yeast two-hybrid assays (not shown). Yeast two-hybrid assays also suggested that no homo- or heterodimerization occurred among the Bcl-Gs and Bcl-Gl proteins. In contrast, association of the longer Bcl-Gs protein with Bcl-2 or Bcl-Xl was not easily detected by immunoprecipitation assays (Fig. 4A). With much longer x-ray film exposure times, however, small amounts of Bcl-Xl were observed in association with Bcl-Gs immunocomplexes, suggesting either low affinity binding of Bcl-Gs to Bcl-Xl or implying that only a small portion of total Bcl-Gs proteins are competent to bind Bcl-Xl (not shown). The interaction of Bcl-Gs with Bcl-Xl was BH3-dependent, as determined by comparisons of wild-type Bcl-Gs with the Bcl-Gs-ΔBH3 and Bcl-Gs-ΔL16E proteins (Fig. 4, B and C). Thus, the pro-apoptotic activity of Bcl-Gs correlates with its ability to bind Bcl-Xl. When the BH2 domain of Bcl-Gs was deleted, this mutant Bcl-Gs associated with Bcl-Xl (Fig. 4D), thus providing further evidence that the BH2 domain autorepresses this protein.

**Bcl-Gs Is Associated with Cytosolic Organelles—** Many Bcl-2 family proteins, such as Bcl-2, Bcl-Xl, and Bak, contain a hydrophobic stretch of amino acids near their carboxyl terminus that anchors them in intracellular membranes of mitochondria, endoplasmic reticulum, or nuclear envelope (reviewed in Refs. 1–3). However, some pro-apoptotic Bcl-2 family proteins such as Bax, Bid, and Bim are found in the cytosol and must be induced to translocate to mitochondria and other organelles where the Bcl-2-family proteins to which they dimerize reside (25–28). We explored the intracellular locations of the Bcl-Gs and Bcl-Gl protein by two-color confocal microscopy analysis of cells expressing GFP-tagged proteins. GFP-Bcl-Gs protein was located diffusely throughout cells, similar to GFP control protein (Fig. 5). In contrast, Bcl-Gs was found in a punctate cytosolic pattern (Fig. 5), and partially colocalized with a mitochondria-specific dye (Mitotracker). Surprisingly, deletion of the BH3 domain from Bcl-Gs did not disrupt the punctate distribution (Fig. 5), indicating that other regions of the Bcl-Gs protein are sufficient for subcellular targeting. Subcellular fractionation experiments confirmed these observations, demonstrating association of Bcl-Gs and Bcl-Gs-ΔBH3 predominantly with organelle-containing heavy membrane fractions, with scant amounts in the soluble cytosolic compartment (not shown).

**DISCUSSION**

We describe here a new member of the BCL-2 gene family in humans, BCL-G. The BCL-G gene potentially encodes two protein products, Bcl-Gs and Bcl-Gl. Bcl-2 family proteins contain up to four conserved BH domains. The shorter Bcl-Gs protein contains only the BH3 domains, similar to several other pro-apoptotic Bcl-2 family proteins, including Bad, Hrk, Bik, Bim, Blk, Noxa(APR), and Egl1 (reviewed in Refs. 13, 29, 30). In contrast, the longer Bcl-Gl protein contains BH2 and BH3 domains. No other examples of Bcl-2 family proteins are known that combine BH2 and BH3 domains in the absence of BH1. Though the Bad protein was originally suggested to contain a BH2 domain (31) and has been shown to possess the BH3 domain, inspection of the BH2 region reveals very little similarity of amino acid sequence with other BH2 domains (32). In contrast, the BH2 of Bcl-Gs contains a stretch of 8 of 8 residues showing identity or conservative amino acid substitutions with the BH2 domains of other family members. By comparison, the Bad sequence reveals only 3 of 8 identical or similar amino acids in the same region. Thus, Bcl-Gs defines a novel structural variant within the BCL-2 family of apoptosis-regulating proteins.

The production of different protein isoforms by alternative mRNA splicing is a common feature of BCL-2 family genes, including BCL-2, Bcl-X, MCL-1, BAX, and BIM (33–37). Unlike BCL-X, which encodes a long and short protein, Bcl-Xl and Bcl-Xs possessing anti-apoptotic and pro-apoptotic functions, respectively, the longer isoform of Bcl-G did not display anti-apoptotic activity. When overexpressed, Bcl-Gs induced modest and variable increases in apoptosis, whereas the shorter Bcl-Gs protein consistently exhibited potent cytotoxic activity. This behavior is reminiscent of the proteins encoded by the BIM gene, which include Bim-short (BimSs), Bim-long (BimLS), and Bim-Extra-Long (BimEL) (34). The longer proteins, BimSs and BimEL, are sequestered in complexes with dynein light-chain...
(DLC) in association with microtubules, thus preventing them from interacting with target proteins such as Bcl-XL on the surface of mitochondria and other organelles (26). In contrast, because the shortest isoform, BimS, does not associate with DLC, it is free to interact with Bcl-XL, Bcl-2, and other survival proteins and hence displays far more potent apoptotic activity when overexpressed in cells. By analogy, the longer Bcl-GL protein could be sequestered in an inactive complex with an unidentified protein.

Besides interactions with sequestering proteins, the activity of pro-apoptotic Bcl-2 family proteins can be suppressed by other mechanisms, including post-translational modifications. For example, the Bad protein is inactivated by phosphorylation. This protein can be directly or indirectly phosphorylated by several protein kinases, including PKA, PKB (Akt), Raf1, and Pak1, thus preventing it from dimerizing with target proteins such as Bcl-2 and Bcl-XL (reviewed in Refs. 30, 38). The intracellular location of Bad varies, depending on its phosphorylation state, with phosphorylated Bad residing in the cytosol and unphosphorylated Bad associated with mitochondria and other intracellular organelles where Bcl-2 and Bcl-XL are located. In this regard, the Bcl-GL protein contains candidate phosphorylation sites for PKA and PKC, including some not found in Bcl-GS. However, we have been unable to demonstrate in vivo phosphorylation of Bcl-GL in pilot experiments (unpublished observations).

Another post-translational modification shown previously to active latent pro-apoptotic Bcl-2 family proteins is proteolysis. Specifically, the Bid protein contains a N-terminal domain of 58 amino acids that masks its BH3 domain, reducing its ability to dimerize with other Bcl-2 family proteins. Upon cleavage by caspases, however, removal of the N-terminal domain exposes the BH3 domain and is associated with translocation of Bid from the cytosol to mitochondria, where it induces cytochrome c release and apoptosis (27, 28). Whereas Bcl-GL contains candidate caspase recognition sites, we have been unable to demonstrate significant cleavage of Bcl-GL in vitro using purified active caspases or in cells during apoptosis (unpublished observations). We cannot exclude the possibility, however, that a specific caspase not yet tested is capable of cleaving and activating Bcl-GL.

Though possessing no hydrophobic region that might anchor it in membranes, the Bcl-GS protein was constitutively associated with intracellular organelles. Interestingly, removal of the BH3 domain did not interfere with organellar-targeting of Bcl-GS but did abolish dimerization with Bcl-XL. Thus, the BH3
domain apparently is not responsible for association of Bcl-Gs with intracellular organelles. This BH3-independent targeting of Bcl-Gs differs from some other BH3-only Bcl-2 family proteins such as Bad, where it has been observed that removal of the BH3 domain abrogates binding to anti-apoptotic Bcl-2 family proteins as well as association with mitochondria (39).

The BCL-G gene resides on chromosome 12p12, a region deleted in ~50% of prostate cancers, ~30% of ovarian cancers, and ~30% of childhood acute lymphocytic leukemias (ALLs) (40–42). Given that at least one of the protein products of the BCL-G gene exhibits pro-apoptotic function, it is possible that BCL-G represents a tumor suppressor gene. However, thus far, we have detected neither somatic mutations in the exons of BCL-G nor evidence of deletion of both BCL-G alleles in tumor cell lines or primary tumor specimens (data not shown). Further studies are required therefore to determine whether loss of BCL-G expression occurs in tumors by means other than somatic alterations in gene structure and DNA sequence such as changes in gene methylation or aberrant transcriptional or post-transcriptional regulation.

Investigation of the tissue distribution of Bcl-Gs and Bcl-Gs mRNAs by RT-PCR revealed that Bcl-Gs mRNA is found in several normal adult tissues, whereas Bcl-Gs was detected only with intracellular organelles. This BH3-independent targeting domain apparently is not responsible for association of Bcl-G S protein with mitochondria (39).

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