Expression and Role of Bcl-xL in Human Hepatocellular Carcinomas

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Transformed hepatocytes survive various apoptotic insults during their growth in vivo. However, molecular mechanisms that inhibit apoptosis and support their survival are not well understood. In this study, we investigated the expression and role of Bcl-xL, an antiapoptotic member of the Bcl-2 family, in human hepatocellular carcinoma (HCC). The Bcl-xL protein was expressed in HepG2, Hep3B, and Huh7 human hepatoma cell lines at high levels, but none of these cells expressed Bcl-2. Down-modulation of Bcl-xL by antisense oligonucleotide activated apoptosis in HepG2 cells in response to cellular stresses induced by staurosporine treatment or by serum starvation. Ectopic expression of transcriptionally active p53 alone was not sufficient for the activation of apoptosis in p53-null Hep3B cells, but apoptosis was induced when endogenous Bcl-xL was simultaneously inhibited by antisense oligonucleotide in these cells. Bcl-xL was expressed in all 20 surgically resected human HCC tissues when examined by Western blot analysis and immunohistochemistry, and levels of its expression were higher in a subset of HCC tissues than those of adjacent nontumor liver tissues or normal livers. We conclude that Bcl-xL expressed in human HCC cells inhibits apoptosis produced by various cellular stresses, such as staurosporine treatment, serum starvation, and p53 activation, and may play an important role in their survival. (HEPATOLOGY 2001;34:55-61.)

Heptocellular carcinoma (HCC), one of the most common cancers in the world, develops from transformed hepatocytes during the course of chronic liver diseases.1,2 When transformed hepatocytes expand in vivo they encounter various microenvironmental stresses, such as hypoxia, decreased growth factor, and lack of nutrient supply, which activate apoptosis.3 In addition, the activation of mitogenic oncogenes, which is important for deregulated proliferation of hepatocytes,4,5 has also been shown to trigger apoptosis.6,7 Therefore, resistance to apoptosis may provide a selective advantage for growth and progression of HCC, but cellular mechanisms to inhibit apoptosis in HCC are not well understood.

It is increasingly recognized that a variety of key events in apoptosis converge on mitochondria, and that mitochondrial release of cytochrome c plays a central role in triggering apoptosis.8 Antiapoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-xL, are mainly located on the outer membrane of mitochondria and inhibit a common pathway of apoptosis, at least in part, by preventing the release of cytochrome c into cytosol.9 The studies of Bcl-2 transgenic mice have shown that the ectopic expression of Bcl-2 in the liver can protect hepatocytes from apoptosis.10,11 However, previous studies using immunohistochemistry have revealed that Bcl-2 is not generally expressed in human hepatocytes.12,13

We investigated the expression and role of Bcl-xL, which possesses an antiapoptotic function similar to that of Bcl-2.14,15 We found that Bcl-xL is highly expressed in human HCC and determined that the endogenously expressed Bcl-xL is important for the inhibition of apoptosis that is initiated by various cellular stresses in HCC-derived cell lines.

MATERIALS AND METHODS

Cell Lines and Tissues. Four human hepatoma cell lines were used: HepG2, which expresses a wild-type p53; Hep3B, which is deficient in p53; Huh7, which expresses p53 with an increased half-life as a result of a point mutation at codon 220; and Focus, which does not express p53.16 Stable Hep3B clones were generated expressing either puromycin resistance alone (BT-2E) or in conjunction with the temperature-sensitive mutant p53val135 (4Bv). The p53 (Val-135) protein exists predominantly in the mutant conformation at 37.5°C and in the wild-type conformation at 32°C. These cell lines have been already described.17 All hepatoma cells were cultured with Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum (Sigma, St. Louis, MO), unless otherwise noted. Twenty pairs of HCCs and adjacent nontumor counterparts were obtained at the time of surgical resection. Ten normal human liver tissues adjacent to hepatic metastatic tumors of colon cancer were obtained at the time of surgery and served as controls.

Western Immunoblot Analysis. Cells or tissues were homogenized in a lysis buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 μg/mL aprotinin, 100 μg/mL phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride and phosphate-buffered saline, pH 7.4. Twenty micrograms of protein per lane was electrophoretically separated by sodium dodecyl sulfate-12% polyacrylamide gels, transferred nitrocellulose membrane, and probed with anti-Bcl-x polyclonal antibody (Ab) (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Bcl-2 monoclonal Ab (Transduction Laboratories, Lexington, KY). For the blocking experiment, anti-Bcl-x Ab was incubated with the immunizing peptide (5 μg/mL) before the staining. The blots

Abbreviations: HCC, hepatocellular carcinoma; Ab, antibody; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling; LDH, lactate dehydrogenase; MTT, 3-(4-,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide.

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were developed by an enhanced chemiluminescence system (NEL Life Science Products, Boston, MA). As a control for sample loading, the blot was stripped and reprobed with anti-γ-actin antibody.

Optical densities of bands in each blot were analyzed by using the NIH Image Program, Version 1.61.

**Immunohistochemical Staining.** Ten-micrometer cryostat sections of HCC tissues were fixed with 100% acetone at −20°C for 5 minutes and then incubated with 0.5 μg/ml of the anti–Bcl-x Ab overnight at 4°C. Bound primary Ab was visualized with streptavidin-biotin peroxidase and 0.05% 3,3’-diaminobenzidine-0.02% H₂O₂ solution (Vectastain ABC Kit, Vector, Burlingame, CA) according to the manufacturer’s instructions. The sections were counterstained with 0.5% methyl green. For the blocking control experiment, anti–Bcl-x Ab was incubated with the immunizing peptide (5 μg/ml) before the staining.

**Antisense Oligonucleotide and Transfection.** Oligodeoxynucleotides with a phosphorothioate backbone were synthesized and purified with high performance liquid chromatography (Integrated DNA Technologies, Coralville, IA). The antisense oligonucleotide encodes a sequence complementary to the human bcl-x translation initiation site and extending 3’ downstream for a total of 18 bases. Three different control oligonucleotides were used: (1) a sequence containing a 4-base mismatch as compared with the antisense sequence, (2) a sequence identical to the antisense but in a scrambled order, and (3) a sense sequence. The sequences of these oligonucleotides were as follows (mismatches are underlined): antisense sequence: 5’-CCCGTGTGACACAT-3’; mismatch sequence: 5’-CCCGTGTGACACAT-3’; scramble sequence: 5’-CTGATCGGAGTC-CCTTAG-3’; sense sequence: 5’-ATGTCAGAGCCAACGG3’.

Hepatoma cells grown in 12-well plates were washed 2 times with Opti-MEM 1 medium (Gibco BRL, Gaithersburg, MD). After Opti-MEM 1 medium containing 10 μg/ml lipofectin (Gibco BRL) and each oligonucleotide (400 nmol/L, unless otherwise indicated) was added, they were mixed by swirling the dish. The cells were incubated at 37°C for 4 hours (Hep3B) or 16 hours (HepG2) to complete transfection.

**Cellular Stresses to Hepatoma Cells.** After transfection with each oligonucleotide, hepatoma cells were exposed to 3 different cellular stress-inducing conditions. (1) Two days after transfection, cells were incubated with staurosporine (1 μmol/L) (Calbiochem, San Diego, CA). (2) Immediately after transfection, cells were cultured in the absence of serum. (3) Immediately after transfection, the transcriptionally active p53 protein was induced by incubating the transfected 4Bv cells at 32°C.

**Detection of Oligonucleosomal DNA.** For the quantitative analysis of apoptosis, an enzyme-linked immunosorbent assay (ELISA) kit (Boehringer Mannheim, Mannheim, Germany), which detects oligonucleosomal DNA by anti-histone and anti-DNA Abs was used. Hepatoma cells were cultured in 12-well culture plates. After brief centrifugation, the cells were dissolved in 1 mL of a lysis buffer. The lysate was centrifuged at 200g for 10 minutes, and 20 μL of the supernatant was then subjected to the ELISA test.

**TUNEL Staining.** Hepatoma cells grown in a 4-well chamber slide were fixed with 1% paraformaldehyde. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) was performed using ApopTag peroxidase kit (Intergen, Purchase, NY). The cells were counterstained with 0.5% methyl green.

**Lactate Dehydrogenase Release Assay.** The release of lactate dehydrogenase (LDH) into the culture medium was assessed by using a commercial kit (Promega, Madison, WI). The results were expressed as the percentage of cell death calculated using the following formula: % Cell death = 100 × (Sample LDH release − culture medium background)/(maximum LDH release − culture medium background).

The maximum LDH release was determined using LDH values released from 0.9% Triton-X100–treated cells in the same experimental condition.

**Cell Viability Assay.** Cell viability was assessed by a cell proliferation assay kit (Promega) based on the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) assay. Preparation of S-100 Fraction and Western Analysis of Cytochrome C. HepG2 cells grown in 6-well plates were washed once in phosphate-buffered saline, resuspended in 1 mL of an isotonic buffer (250 mmol/L sucrose, 10 mmol/L KCl, 1.5 mmol/L MgCl₂, 1 mmol/L EDTA, 1 mmol/L EGTA, 50 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 20 mmol/L HEPES, pH 7.5), and homogenized for 40 strokes with a Dounce homogenizer type B. Samples were centrifuged at 600g for 10 minutes at 4°C to eliminate nuclei and unbroken cells. The resulting supernatant was further centrifuged at 100,000g for 1 hour at 4°C. The supernatant was collected and incubated on ice with an equal volume of 20% trichloroacetic acid. After 15 minutes, the supernatant was spun at 13,000g for 15 minutes at 4°C; the pellet was then rinsed in 1 mL ethanol and diethyl ether (1:6) on ice. The pellet was then resuspended in Laemmli buffer and one fourth volume of samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by Western blot using mouse anti-cytochrome c monoclonal Ab (1 μg/ml) (7H8.2C12) (PharMingen, San Diego, CA).

**Statistical Analysis.** Data were expressed as mean ± SD and compared using Bonferroni’s t test among groups if one-way ANOVA showed P < .01. The difference was considered statistically significant if P < .01.

**RESULTS**

**Bcl-xL, but Not Bcl-2, Is Expressed in Human Hepatoma Cell Lines.** Expression of Bcl-xL and Bcl-2 was investigated by Western analysis in HepG2, Hep3B, Huh7, and Focus human hepatoma cell lines. Bcl-xL protein was expressed in all these human hepatoma cells (Fig. 1A). The levels of expression were high in HepG2, Hep3B, and Huh7, but it was relatively low in Focus hepatoma cells. Of the Bcl-xL protein in hepatoma cells migrated as doublet bands with molecular masses of approximately 30 and 32 kd. Both 30 and 32 kd bands were specifically reactive to anti–Bcl-x Ab, because they became undetectable in the presence of the immunizing peptide (Fig. 1B) and because they were inhibited by transfection of bcl-x antisense oligonucleotide (Fig. 1C). Bcl-xS, a proapoptotic molecule derived from the bcl-x gene by alternative splicing, was not detected in any of the hepatoma cells examined, although the anti–Bcl-x Ab used in this Western analysis recognized both Bcl-xL and Bcl-xS (data not shown). Bcl-2 protein was not detected in any of the hepatoma cells examined (Fig. 1A). This observation on Bcl-2 expression was consistent with a previous immunohistochemical study by Charlotte et al.

**Inhibition of Endogenous Bcl-xL by Antisense Oligonucleotide Activates Apoptosis in Hepatoma Cells.** To examine the role of Bcl-xL in hepatoma cells, an antisense oligonucleotide targeted to the bcl-x initiation codon was transfected into HepG2 cells using a lipofectin transfection reagent. Mismatch, scramble, and sense oligonucleotides were used as controls. Western analysis revealed that antisense treatment (200-800 nmol/L) reduced the expression of Bcl-xL bands in a dose-dependent manner (Fig. 1C). We used 400 nmol/L of antisense oligonucleotide for subsequent studies, which resulted in an approximately 9-fold reduction in expression of Bcl-xL protein.

We then investigated the effect of bcl-x antisense oligonucleotide on apoptosis that was induced by staurosporine treatment in HepG2 cells. Figure 2A depicts the percent viability of antisense-transfected HepG2 cells that were cultured for 12 hours with or without staurosporine. Compared with control
oligonucleotides, antisense oligonucleotide significantly decreased the viability of HepG2 cells that were incubated with staurosporine, but did not affect their viability without staurosporine. Since degradation of chromosomal DNA into oligonucleosomal fragments is a characteristic biochemical marker of apoptosis, histone-associated oligonucleosomal DNA fragment within the cells was determined quantitatively by ELISA to assess apoptosis. Figure 2B shows the oligonucleosomal DNA detected in the cytoplasm of HepG2 cells that were treated for 4 to 8 hours with staurosporine. Compared with control oligonucleotides, transfection of antisense oligonucleotide significantly increased the amount of oligonucleosomal DNA in staurosporine-treated HepG2 cells, indicating that the induction of apoptosis by staurosporine is inhibited by Bcl-xL endogenously expressed in HepG2 cells. The effect of staurosporine on the cytosolic release of cytochrome c was also examined, because staurosporine has been shown to induce apoptosis by translocation of mitochondrial cytochrome c into cytosol. As shown in Figure 2C, cytosolic cytochrome c was increased in the S-100 fraction of

![Figure 2](https://example.com/fig2.png)

**FIG. 2.** Increased susceptibility of HepG2 cells to staurosporine by down-modulation of Bcl-xL. HepG2 cells were transfected with either bcl-xL antisense (AS), mismatch (MS), scramble (SCR), or sense (S) oligonucleotide, and cultured with 10% fetal bovine serum containing media for 2 days. (A) Viable cells were determined by MTT assay at 12 hours after treatment with (left) or without (right) staurosporine. Results are the mean ± SD of values obtained by determination of 5 experiments. The vertical axis indicates the percent viability of cells compared with that of scramble oligonucleotide-treated cells without staurosporine (*P < .01, AS- vs. control oligonucleotide-treated cells that were cultured with staurosporine, respectively, by the Bonferroni t test after significant ANOVA, n = 5). (B) Histone-associated DNA fragment within the cells was determined at 0, 4, and 8 hours after the staurosporine treatment. The levels of oligonucleosomal DNA were expressed as an arbitrary unit that was determined by relative ratio to that found in scramble oligonucleotide-treated cells before staurosporine treatment (*P < .01, AS- vs. control oligonucleotide-treated cells, respectively, by Bonferroni t test after significant ANOVA, n = 5). (C) Detection of cytochrome c in the S-100 fraction of HepG2 cells. The S-100 fraction was prepared from HepG2 cells 4 hours after staurosporine treatment. Accumulation of cytochrome c was examined by Western analysis. G3PDH served as a control for sample loading.

![Figure 1](https://example.com/fig1.png)

**FIG. 1.** Western analysis of Bcl-xL in hepatoma cell lines. (A) Western analysis of Bcl-xL and Bcl-2 in human hepatoma cell lines (HepG2, Hep3B, Huh7, and Focus). Jurkat human T leukemia cell line, which expresses both Bcl-xL and Bcl-2, served as a positive control. G3PDH served as a control for sample loading. (B) Competitive inhibition of Bcl-xL bands by immunizing peptide. The blots were stained with anti-Bcl-x Ab that had been incubated with either immunizing peptide or nonspecific peptide. Immunizing peptide specifically blocks Bcl-xL bands that migrate at 30 and 32 kd. (Lane 1, #16 nontumor liver; lane 2, #16 HCC; lane 3, #19 nontumor liver; lane 4, #19 HCC; lane 5, #23 normal liver; lane 6, HepG2 cells) (see Fig. 5). (C) Western analysis of Bcl-xL in HepG2 cells 2 days after the transfection with bcl-xL antisense (AS), mismatch (MS), scramble (SCR), or sense (S) oligonucleotide. G3PDH served as a control for sample loading.
HepG2 cells that were treated with bcl-x antisense and staurosporine, whereas control oligonucleotides did not induce the cytosolic accumulation of cytochrome c in staurosporine-treated HepG2 cells.

The effect of bcl-x antisense oligonucleotide was also examined in serum-starved HepG2 cells. Figure 3A shows the result of the MTT assay. Antisense treatment significantly reduced the viability of HepG2 cells under serum-free condition. Figure 3B depicts TUNEL staining of HepG2 cells 1 day after serum starvation. Transfection of bcl-x antisense oligonucleotide substantially increased the number of TUNEL-positive cells, compared with those of control oligonucleotides in serum-starved HepG2 cells.

**Down-modulation of Bcl-xL Sensitizes Hep3B to p53-Induced Apoptosis.** Enforced expression of Bcl-xL has been shown to inhibit the apoptosis in transcriptionally active p53 gene-transfected cells, but it is not clear if p53-induced apoptosis is inhibited by Bcl-xL that is endogenously expressed in hepatoma cells. To investigate a physiologic role of Bcl-xL in p53-induced apoptosis, bcl-x antisense oligonucleotide or control oligonucleotide was transfected into Hep3B/4Bv and BT-2E clones, and then cultured at either 37.5°C or 32°C. Figure 4A shows the result of LDH release at 3 days after transfection. Antisense treatment significantly increased cell death in 4Bv clones at 32°C (*P < .01, AS- vs. control oligonucleotide-treated cells, respectively, by the Bonferroni t test after significant ANOVA, n = 5). (B) Histone-associated DNA fragment within 4Bv cells determined at 2 days after the transfection using ELISA. The amount of oligonucleosomal DNA was expressed as the ratio to that in scramble oligonucleotide-treated cells (*P < .01, AS- vs. control oligonucleotide-treated cells, respectively, by the Bonferroni t test after significant ANOVA, n = 5).
oligonucleotides were transfected into Hep3B/4Bv clones that express inactive p53 at 37.5°C and its active form at 32°C, and into Hep3B/BT-2E clone that expresses puromycin resistance gene alone. The oligonucleotide-transfected Hep3B/4Bv and BT-2E clones were cultured for 3 days either at 37.5°C or 32°C and cell death was examined by LDH release. As shown in Fig. 4A, antisense treatment significantly increased the death of 4Bv cells at 32°C, but not at 37.5°C. Expression of transcriptionally active p53 (32°C) did not produce cell death in control oligonucleotide-transfected 4Bv cells. A previous study also showed that expression of wild-type p53 alone did not activate apoptosis in p53-null Hep3B cells. There was no difference in cell death of BT-2E clones when they were cultured at 37.5°C or at 32°C, indicating that the difference of culture conditions did not affect the cell death rate under our experimental conditions. Cell death was also examined by DNA fragmentation using oligonucleosomal DNA detection ELISA (Fig. 4B). Antisense treatment significantly increased the amount of oligonucleosomal DNA in 4Bv clones at 32°C, but not at 37.5°C. These findings indicate that resistance of Hep3B to p53-mediated apoptosis is due, at least in part, to the endogenous expression of Bcl-xL.

**Expression of Bcl-xL in Human HCC Tissues.** Expression of Bcl-xL was examined by Western analysis in 20 pairs of surgically resected human HCC tissues and adjacent nontumor tissues as well as 10 normal livers (Fig. 5). All human HCC, nontumor liver, and normal liver tissues expressed Bcl-xL as a doublet band with molecular masses of approximately 30 and 32 kd. Both 30 and 32 kd bands were specifically reactive to anti-Bcl-x Ab, because they became undetectable in the presence of the immunizing peptide (Fig. 1B). Whereas levels of Bcl-xL expression corresponding to both bands were similar between normal livers and nontumor liver tissues in each blot, subpopulation of HCC tissues expressed Bcl-xL at higher levels. In 6 of 20 cases, the tumor to nontumor ratio of optical density of Bcl-xL blot adjusted by G3PDH was more than 2-fold. Figure 6A depicts typical immunoperoxidase staining of Bcl-xL in moderately differentiated HCC tissue and an adjacent nontumor counterpart. Most if not all HCC cells were stained with anti–Bcl-x Ab and showed a diffuse cytosolic cellular staining pattern. The staining in tumor cells was substantially stronger than that in hepatocytes present in the adjacent nontumor tissue (Fig. 6A), and it was competitively inhibited by the immunizing peptide (Fig. 6B). Because the Bcl-xL was not detected in HCC tissues by Western analysis using the same anti–Bcl-x Ab (data not shown), the diffuse cellular staining pattern in tumor cells represents the in vivo expression of Bcl-xL protein in HCC.

**DISCUSSION**

Enforced expression of anti-apoptotic Bcl-2 family protein has been shown to suppress apoptosis in response to a wide variety of stimuli such as activation of mitogenic oncogenes, hypoxia, withdrawal of trophic factors, and loss of adherence. In addition, the bcl-xL transgene was shown to promote SV40 large T antigen–mediated transformation of pancreatic β cells and to increase the incidence of carcinogen-induced skin tumor. Therefore, the enforced expression of Bcl-xL appears to facilitate tumorigenesis in cooperation with other tumor-promoting events. However, the physiologic role of Bcl-xL, which is endogenously expressed in tumor cells, is not clear. We found that Bcl-xL is highly expressed in various hepatoma cells (Fig. 1A) as others have previously reported. In the present study, we sought to investigate the role of Bcl-xL that is expressed in hepatoma cells using an antisense oligonucleotide approach. As shown in Figs. 2 and 3, down-modulation of Bcl-xL activated apoptosis of HepG2 cells that were exposed to staurosporine or serum starvation. These results are in agreement with the previous observation by Luo et al. that the reduced Bcl-xL expression by antisense bcl-xL gene transfection rendered HepG2 cells susceptible to apoptosis during Taxol or doxorubicin treatment. Down-modulation of Bcl-xL also produced accumulation of cytochrome c in the cytosolic fraction of HepG2 upon staurosporine treatment, suggesting that the release of mitochondrial cytochrome c is blocked by endogenously expressed Bcl-xL. Hepatoma cell lines expressed not only Bcl-xL but also bcl-w and mcl-1 (Takehara et al. unpublished data), both of which have been shown to possess anti-apoptotic functions. Importantly, down-modulation of

**Fig. 5.** Bcl-xL expression in human HCC. Expression of Bcl-xL was assessed by Western analysis in 20 pairs of HCC (T), adjacent nontumor liver counterparts (NT) (#1-#20), and 10 normal livers (normal) (#21-#30). Bcl-xL migrates as doublet bands of 30 kd and 32 kd. G3PDH served as a control for sample loading. Optical density of each band was analyzed by NIH Image. T/NT represents the tumor to nontumor ratio of optical density of Bcl-xL adjusted by G3PDH in HCC and adjacent nontumor liver pairs.
Bcl-xL was sufficient for the induction of apoptosis in response to cellular stresses, indicating that Bcl-xL expressed in hepatoma cells plays an integral role in suppressing apoptosis when they are exposed to proapoptotic conditions.

Induction of apoptosis in response to a variety of cellular stresses is mediated at least in part by the tumor suppressor gene p53. Although HCC may acquire resistance to apoptosis by inactivating the p53 gene function, mutations or deletion of p53 gene are not generally found until the late stages of HCC. In this study, we found that Bcl-xL is highly expressed in both wild-type p53-positive (HepG2) and p53-negative hepatoma cell lines (Hep3B, Huh7) (Fig. 3B). Most significantly, Hep3B, which was resistant to p53-activated apoptosis, experienced apoptosis by p53 activation only when endogenous Bcl-xL was inhibited by bcl-x antisense. These results suggest that Bcl-xL can function as a suppressor of p53-activated apoptosis in HCC.

Elevated expression of Bcl-xL has been reported previously in some human malignancies including colorectal adenocarcinoma, stomach cancer, Kaposi's sarcoma, and multiple myeloma. In this report, we also established that Bcl-xL is highly expressed in a subset of human HCC tissues as well as in human hepatoma cell lines (Figs. 1A and 5). Although the balance between proapoptotic and antiapoptotic Bcl-2 family proteins is important for the regulation of apoptosis, proapoptotic proteins including Bax, Bad, and Bid were expressed in HCC tissues at the levels similar to those in adjacent non-tumor or normal liver tissues (Takehara et al. unpublished data). Therefore, dysregulated expression of Bcl-xL, but not Bax, Bad, nor Bid, may play an important role in the regulation of apoptosis in HCC.

In conclusion, we showed that endogenous Bcl-xL inhibits apoptosis produced by various stress-inducing conditions, such as staurosporine treatment, serum starvation, and p53 activation. We propose that Bcl-xL expressed in HCCs may function as a pivotal apoptosis antagonist during their growth in vivo.

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