Hypoxia Selection of Death-resistant Cells

A ROLE FOR Bcl-XL

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Received for publication, November 7, 2003, and in revised form, December 12, 2003
Published, JBC Papers in Press, December 15, 2003, DOI 10.1074/jbc.M312225200

Under hypoxia, some cells are irreversibly injured and die, whereas others can adapt to the stress and survive. The molecular and genetic basis underlying cellular sensitivity to hypoxic injury is unclear. Here we have selected death-resistant cells by repeated episodes of hypoxia. The selected cells are cross-resistant to apoptosis induced by staurosporine, azide, and cisplatin. These cells up-regulate Bcl-XL, an anti-apoptotic protein. Bcl-XL interacts with the pro-apoptotic molecule Bax and abrogates its toxicity in mitochondria, resulting in the preservation of mitochondrial integrity, cytochrome c, and cell viability. Down-regulation of Bcl-XL by antisense oligonucleotides or the newly identified Bcl-XL inhibitor chelerythrine restores cellular sensitivity to injury and death. Thus, Bcl-XL is a key molecule for hypoxia selection of death resistance. These findings may have important implications for the development of solid tumors where hypoxia selects for death-resistant cells that are inert to cancer therapy.

Lack of oxygen, i.e. hypoxia, can lead to cell injury and death and determine tissue pathology in ischemic diseases including stroke, myocardial infarction, and acute renal failure (1). On the other hand, cells possess finely tuned systems to sense the drop of oxygen tension and adapt to hypoxic stress (2). One such system is centered around a family of hypoxia-inducible transcription factors (3–7). In addition, survival mechanisms including the expression of death inhibitory genes are activated by hypoxia (8). A good example of hypoxic adaptation is cancer cells within solid tumors (9, 10). During tumor expansion, malformation and malfunction of blood vessels lead to regions of severe hypoxia (11). Although some cells die because of oxygen deficiency, other tumor cells survive the stress and adapt to the hypoxic microenvironment. These cells ultimately become more aggressive and resistant to cell death during cancer therapy (9–12). Hypoxia selection of therapy-irresistant cells may involve the regulation of p53, a tumor suppressor gene, and defects in the cell death machinery (13–17). However, the nature of the selection remains elusive (9, 10). This study was designed to select death-resistant cells through repeated episodes of hypoxia and identify the molecular basis underlying death resistance of the selected cells. We found that hypoxia-selected cells are cross-resistant to cell injury and death induced by different types of insults. These cells up-regulate Bcl-XL, which interacts with the pro-apoptotic molecule Bax and abrogates its toxicity in mitochondria, resulting in the preservation of mitochondrial integrity, cytochrome c and cell viability. Down-regulation of Bcl-XL restores cellular sensitivity to injury and death. Thus, Bcl-XL plays a critical role in hypoxia-selection of death resistance.

MATERIALS AND METHODS

Cells and Antibodies—Immortalized rat kidney epithelial cells were originally from U. Hopfer (Case Western Reserve University, Cleveland, OH). The cells were cultured and plated for experiments as described previously (8, 18, 19). Antibodies were from the following sources. Monoclonal anti-Cyt c (7H8.2C12 and 6H2.B4) and anti-Bcl-XL (clone 4) were from BD Biosciences. Monoclonal anti-Bax (1D1) and anti-Bcl-XL (2H12) were from NeoMarkers (Fremont, CA). Monoclonal (C-2) and polyclonal (ΔC21) anti-Bcl-2 were from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-Bak (NT) was from Upstate Biotechnology (Lake Placid, NY). Polyclonal anti-Bid was from Dr. X. M. Yin at the University of Pittsburgh (Pittsburgh, PA). Polyclonal antibody specific for active caspase-3 was from Dr. A. Srinivasan at Idun Pharmaceuticals Inc. (La Jolla, CA), and all of the secondary antibodies were from Jackson ImmunoResearch (West Grove, PA).

Hypoxic Incubation (8, 18)—Cells were washed with phosphate-buffered saline, transferred to an anaerobic chamber with 95% N2, 5% CO2, and incubated in glucose-free Krebs-Ringer bicarbonate buffer. This buffer was pre-equilibrated with 95% N2, 5% CO2. EC oxyrase, a biocatalytic oxygen-reducing agent, was added to 1.2 units/ml to the incubation medium to consume residual O2 and maximize the degree of hypoxia (<0.1% O2). After hypoxic incubation, cells were transferred back to full culture medium in 95% air, 5% CO2 for recovery.

Antisense Treatment—The sequences of Bcl-XL antisense and scrambled control were 5'-CCGGTTGCTCTGAGACAT-3' and 5'-CTGATCGAGTCCCTTAG-3', respectively. The antisense sequence was complementary to the translation initiation site of rat Bcl-XL. The oligonucleotides were synthesized to contain a phosphorothioate backbone and purified by high pressure liquid chromatography (Integrated DNA Technologies, Coralville, IA). Transfection was conducted using Oligofectamine (Invitrogen). Cells were examined 24 h after transfection.

Morphological Analysis of Apoptosis (8, 18, 19)—Typical apoptotic morphology including cellular shrinkage and the formation of apoptotic bodies was monitored by light microscopy. Cells were also stained with 5 μg/ml Hoechst 33342 to reveal nuclear condensation and fragmentation by fluorescence microscopy. To quantify apoptosis, five fields with ~200 cells/field in each dish were examined. The experiments were repeated at least four times with duplicate dishes for each condition.

Measurement of Caspase Activity (8, 19)—The enzymatic activity of caspases was measured using the fluorogenic peptide substrate carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD.AFC) (Enzyme Systems Products, Dublin, CA). Cells were extracted with 1% Triton X-100. Lysates of 25-μg protein were added to the

† This work was supported by grants from National Institutes of Health, National Kidney Foundation, and the American Society of Nephrology. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: Cyt c, cytochrome c; DEVD.AFC, carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD, AFC) (Enzyme Systems Products, Dublin, CA); CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; DSP, dithiobis(succinimidyl propionate); STS, staurosporine; WT, wild type; ANOVA, analysis of variance.

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enzymatic reactions containing 50 μM DEVD.AFC. After 60 min of reaction at 37 °C, fluorescence at excitation 360 nm/emission 530 nm was monitored. For each measurement, a standard curve was constructed using free AFC. Based on the standard curve, the fluorescence reading from each enzymatic reaction was translated into the molar amount of liberated AFC to indicate caspase activity.

**In Vitro Reconstitution of Caspase Activation (19)**—Cytosolic capacity for caspase activation was determined by in vitro reconstitution assays. Cytosols were extracted from wild-type or hypoxia-selected cells with 0.05% digitonin and concentrated to 4–5 mg of protein/ml with 3-kDa cutoff microconcentrators. For reconstitution, 1/100 ml of 0.5 mg/ml Cyt c and 1/100 ml of 10 μM dATP were added to 7.5 μl of cytosolic extracts containing 25 μg of protein and incubated for 1 h at 30 °C. The reconstitution mixture was then transferred to 200 μl of caspase activity assays containing 50 μM DEVD.AFC to determine reconstituted caspase activity.

**Cellular Fractionation**—To analyze the subcellular distribution of Cyt c and Bcl-2 family proteins, cells were fractionated into cytosolic and membrane-bound fractions using low concentrations of digitonin, which selectively permeabilizes the plasma membrane to release the cytosol (18–20). Cells were extracted with 0.05% digitonin in isotonic sucrose buffer to collect the cytosol. The digitonin-insoluble fraction was further extracted with 2% SDS to collect the membrane-bound organelar fraction.

**Immunoblot Analysis**—To analyze the subcellular distribution of Cyt c and Bcl-2 family proteins, cytosolic and membrane fractions extracted from the same amounts (~1 × 10^6) of cells were subjected to electrophoresis. For other immunoblots, 50 μg of protein was loaded for each lane. Electrophoresis and blotting were conducted using NuPAGE gel systems (Invitrogen) (8, 18, 19). After incubation with primary and horseradish peroxidase-conjugated secondary antibodies, antigens on the blots were revealed by exposure to chemiluminescent substrates (Pierce).

**Immunoprecipitation (18, 21)**—Cells were extracted directly with 2% CHAPS to collect whole cell lysates or subjected to sequential extractions with 0.05% digitonin and 2% CHAPS to collect cytosolic and membrane-bound fractions, respectively. The zwitterionic detergent CHAPS was used for extraction to avoid nonionic detergent-induced association between Bcl-2 family proteins (22). Proteins extracted from ~5 × 10^6 cells were precleared by incubation with normal serum and agrose protein A/G (Santa Cruz Biotechnology). The precleared lysates were subsequently incubated with 1 μg of immunoprecipitation antibody and 30 μl of agrose protein A/G. Immunoprecipitates were collected by centrifugation and dissolved in 2% SDS sample buffer for further analysis.

**Fig. 1. Selection of death-resistant cells by repeated episodes of hypoxia.** a, cell death during seven sequential cycles of hypoxia. Cells were subjected to 6.5 h of severe hypoxia in glucose-free buffer and recovered in culture medium. Cell death was estimated morphologically following hypoxic incubation. b, cross-resistance of hypoxia-selected cells to cell death induced by azide, STS, and cisplatin. WT (□) and hypoxia-selected cells (HSC, ■) were exposed to 10 mM azide for 3 h, 1 μM STS for 5 h, or 5 μM cisplatin for 24 h. Cell death was determined morphologically. Caspase activity was measured enzymatically using DEVD.AFC as substrates. Data are expressed as means ± S.E. (n = 4). *, significant difference between wild-type and hypoxia-selected cells.

**c**, cell morphology. After 5 h of STS incubation, cells were stained with Hoechst 33342. Phase-contrast and nuclear staining images were captured by light and fluorescence microscopy.
immunoblot analysis. To detect the interaction between Bcl-X\textsubscript{L} and Bax, co-immunoprecipitation was conducted. Cell lysates were immunoprecipitated with a rabbit polyclonal anti-Bcl-X\textsubscript{L} antibody, and the immunoprecipitates were analyzed for Bax by immunoblots using a mouse anti-Bax antibody or vice versa.

**Immunofluorescence (18, 19, 21)**—Cells grown on collagen-coated glass coverslips were fixed in a modified Zamboni’s fixative and permeabilized with 0.1% SDS. The cells were then incubated with 5% normal goat serum for blocking and exposed to primary antibodies (monoclonal mouse anti-Cyt\textsubscript{c} and polyclonal rabbit anti-active caspase-3) and secondary antibodies (cyanine 3-labeled goat anti-mouse IgG and fluorescein isothiocyanate-labeled goat anti-rabbit IgG). Images were captured by fluorescence microscopy.

**Protein Cross-linking (21, 23)**—Cells were incubated for 30 min under constant mixing with 1 mM dithiobis(succinimidyl propionate) (DSP), a cell-permeable bifunctional cross-linker (Pierce). The cells were subsequently lysed with 0.05% digitonin to release cytosol. Digitonin-insoluble membrane fractions were collected and subjected to electrophoresis under non-reducing conditions for immunoblot analysis.

**Statistics**—Data were expressed as means ± S.E. (n ≥ 4). Statistical differences between means were determined using analysis of variance (ANOVA) by two-tailed tests. p < 0.05 was considered to reflect significant differences.

**RESULTS AND DISCUSSION**

Our overall strategy was to subject cells to cycles of hypoxic challenge to kill vulnerable cells and select resistant colonies. To this end, kidney epithelial cells were exposed to severe hypoxia for 6.5 h in glucose-free medium. The cells were then returned to full culture medium for recovery. After recovery, surviving cells were subjected to further hypoxic cycles. Hypoxia led to cell death mainly in the form of apoptosis (18). Cell death during the first hypoxic cycle was ~75%, and the death rate decreased to <5% after seven sequential selections (Fig. 1a). The selected cells were also resistant to cell injury induced by other stimuli including the respiration inhibitor azide, the

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**Fig. 2. Death resistance of hypoxia-selected cells occurs at the mitochondrial level.**

(a) Cyt\textsubscript{c} release from mitochondria is ameliorated in hypoxia-selected cells. WT and hypoxia-selected cells (HSC) were incubated with 1 mM STS for 0–6 h. Cells were then fractionated into cytosolic and membrane-bound organellar fractions for immunoblot analysis of Cyt\textsubscript{c}. b, immunofluorescence confirming that Cyt\textsubscript{c} release was suppressed in hypoxia-selected cells. WT and HSC were incubated with STS for 5 h before fixation for Cyt\textsubscript{c} immunofluorescence. Control cells without STS exposure had punctated mitochondrial staining of Cyt\textsubscript{c} (data not shown). c, accumulation of Bax in mitochondria is suppressed in hypoxia-selected cells. WT and HSC were incubated with 1 mM STS for 0–6 h. Cells were then fractionated into cytosolic and membrane-bound organellar fractions for immunoblot analysis of Bax. d, Bax oligomerization is blocked in hypoxia-selected cells. WT and HSC were incubated with 1 mM STS for 5 and 6 h, respectively. The treatments were chosen to accumulate comparable amounts of Bax to mitochondrial fractions in these two types of cells. The cells were then exposed to DSP for cross-linking. Mitochondrial fractions were extracted for immunoblot analysis of Bax under non-reducing conditions. For lanes 2 and 4, samples were incubated with 5 mM dithiobis (DTT) for 30 min before loading. e, cytosol of hypoxia-selected cells is competent for caspase activation. Cytosolic fractions isolated from WT and HSC were incubated for 1 h in the absence (+Cyt\textsubscript{c}) or presence (+Cyt\textsubscript{c}) of exogenous Cyt\textsubscript{c}. The incubation mixtures were then added to enzymatic reactions containing DEVD-AFC to measure caspase activity.
cancer therapy drug cisplatin, and the broad-spectrum protein kinase inhibitor staurosporine (STS) (Fig. 1b). For example, 5 h of STS induced 62% apoptosis in wild-type cells (WT) but only 13% in hypoxia-selected cells. The activation of caspases, a family of cysteine proteases involved in apoptosis (24), was also significantly suppressed in the selected cells (Fig. 1b). Representative images of the cells are shown in Fig. 1c. Phase-contrast microscopy showed cellular shrinkage and fragmentation, typical morphological features of apoptosis. Nuclear staining with Hoechst 33342 demonstrated chromosomal condensation and nuclear fragmentation. Again, compared with wild type, apoptosis was significantly less in hypoxia-selected cells. Thus, after seven cycles of hypoxia, we have selected cells that are resistant to cell injury and death induced by different types of insults.

We then investigated why the selected cells were more resistant to cell injury. The observation that these cells became cross-resistant to different types of insults suggests that molecular alterations in the common death pathways might be responsible. Two major pathways for apoptosis have been delineated: 1) the death receptor-mediated intrinsic pathway and 2) the mitochondrion-mediated extrinsic pathway (25–27). Earlier studies including ours indicate that hypoxia, azide, and STS induce apoptosis through the extrinsic pathway (18, 19). It has been shown that these stimuli activate pro-apoptotic Bcl-2 family proteins to permeabilize the outer membrane of mitochondria, leading to the release of apoptogenic proteins such as Cyt c. Cyt c, after being released, binds Apaf-1 in the cytosol and activates caspase-9, which proteolytically activates downstream executioner caspases (18, 19, 28). Thus, we first determined whether hypoxia-selected cells were resistant to Cyt c release, a hallmark of outer membrane damage in mitochondria (29–32). Without treatment, Cyt c was detected exclusively in the mitochondrial fraction in both wild-type and selected cells (Fig. 2a, lanes 1 and 5). STS incubation for 4 h led to the release of Cyt c into the cytosol in wild-type cells (lane 2) but not in hypoxia-selected cells (lane 6). Longer STS treatment led to further Cyt c release in wild-type cells (lanes 3 and 4), whereas the release in hypoxia-selected cells, although becoming detectable, was much lower (lanes 7 and 8). The results were confirmed by immunofluorescence, demonstrating that significantly fewer cells leaked Cyt c into the cytosol in hypoxia-selected group during STS incubation (Fig. 2b).

Targeting of mitochondria by pro-apoptotic proteins (e.g., Bax, Bak, and Bid) can be a primary cause of outer membrane leakage (31–34). Our earlier work has suggested a critical role for Bax in mitochondrial damage during hypoxia and STS-induced apoptosis (18, 19). Thus, to determine why hypoxia-
selected cells were resistant to Cyt c release, we examined the targeting of mitochondria by Bax. STS treatment led to the accumulation or targeting of Bax to mitochondria (Fig. 2c, lanes 1–4), and importantly, Bax movement was suppressed in hypoxia-selected cells (lanes 5–8). For example, by the end of 4 h of STS, significant amounts of Bax moved to the mitochondrial fraction in wild-type cells (lane 2) but not in hypoxia-selected cells (lane 6). We further analyzed the oligomerization status of Bax, because permeabilization of the mitochondrial outer membrane appears to depend on the formation of Bax oligomers (31, 32). To reveal the oligomers, cells were subjected to chemical cross-linking with DSP to preserve protein-protein interactions and the mitochondrial fractions were extracted for immunoblot analysis of Bax. As shown in Fig. 2d, significant amounts of Bax oligomerized in wild-type cells after 5 h of STS treatment (lane 1). The oligomers were resolved into monomers when the samples were treated with dithiothreitol, a reducing reagent that breaks up the disulfide bond in DSP (lane 2). In sharp contrast, Bax in hypoxia-selected cells did not form oligomers (lanes 3 and 4). Thus, hypoxia-selected cells were resistant to injury and death at the mitochondrial level, showing less Bax accumulation, oligomerization, outer membrane damage, and

FIG. 4. Down-regulation of Bcl-XL in hypoxia-selected cells restores their sensitivity to injury. a, down-regulation of Bcl-XL by a specific antisense oligonucleotide. Hypoxia-selected cells were transfected with Bcl-XL antisense or scrambled sequence at 0, 0.1, and 0.4 μM. Whole cell lysates were analyzed for Bcl-XL by immunoblotting. As a control for sample loading and transferring, the blot was reprobed for β-actin. b, Bcl-XL antisense restores the sensitivity of hypoxia-selected cells to injury and death. WT, HSC, hypoxia-selected cells transfected with Bcl-XL antisense (HSC+antisense), and hypoxia-selected cells transfected with scrambled sequence (HSC+scrambled) were incubated with 1 μM STS for 5 h. Cell death was estimated by cellular and nuclear morphology. Data are expressed as means ± S.E. (n = 4). * significantly different from wild-type groups. c, Bcl-XL antisense promotes Bax translocation to mitochondria in hypoxia-selected cells during STS incubation. Hypoxia-selected cells transfected with Bcl-XL antisense or scrambled sequence were incubated with STS for 5 h. The cells were then fractionated into cytosolic and membrane-bound fractions for immunoblot analysis of Bax. d, Bcl-XL antisense promotes Cyt c release in hypoxia-selected cells during STS treatment. Hypoxia-selected cells transfected with Bcl-XL antisense or scrambled sequence were incubated with STS for 5 h. The cells were then fractionated into cytosolic and membrane-bound fractions for immunoblot analysis of Cyt c. e–l, hypoxia-selected cells with or without Bcl-XL antisense transfection were incubated with STS for 5 h. The cells were fixed and processed for immunofluorescence of Cyt c (e and i) and active caspase-3 (f and j). The same cells were also stained with Hoechst 33342 to reveal nuclear morphology (g and k). h and l are superimposed images of elfg and iljh, respectively.
Bcl-X<sub>L</sub> in Hypoxia Selection of Death Resistance

Cyt<sub>c</sub> release. We further determined whether these cells were also resistant at levels downstream of Cyt<sub>c</sub> release, i.e. in the cytosol. To this end, we isolated cytosol from wild-type and hypoxia-selected cells and compared their capacity of caspase activation after adding exogenous Cyt<sub>c</sub> (Fig. 2c). Clearly, in the presence of the same amounts of Cyt<sub>c</sub>, greater caspase activity was reconstituted in the cytosol of hypoxia-selected cells than the wild-type. Thus, death resistance of hypoxia-selected cells did not occur in the cytosol, rather it was at the level of mitochondria.

To identify the mitochondrial changes responsible for death resistance of hypoxia-selected cells, we focused on the Bcl-2 family proteins. Bcl-2 family proteins are important regulators of apoptosis (30–35). A major acting site for these proteins is the mitochondria. We first compared the expression and intra-
cellular distribution of the proteins in wild-type and hypoxia-selected cells by immunoblotting. As shown in Fig. 3a, both types of cells had similar levels of expression for Bax, Bak, and Bid, the three pro-apoptotic Bcl-2 family proteins. Subcellular distribution of these proteins in wild-type and hypoxia-selected cells also appeared similar, with the majority of Bax and Bid in the cytosol and Bak in the membrane-bound organellar fraction (Fig. 3a). Bcl-2 expression was low and barely detectable by immunoblotting in both cell types (Fig. 3a). Nevertheless, immunoprecipitation revealed similar levels of Bcl-2 expression (Fig. 3b). On the other hand, the expression of Bcl-X<sub>L</sub>, another well documented anti-apoptotic protein (30–35), was noticeably higher in hypoxia-selected cells (Fig. 3a). Bcl-X<sub>L</sub> was detected in the cytosol as well as in the membrane fraction enriched with mitochondria (Fig. 3a). Densitometric analysis of the immunoblots indicated that Bcl-X<sub>L</sub> in the selected cells was 5.7-fold higher than that of wild type (Fig. 3c). Up-regulation of Bcl-X<sub>L</sub> in hypoxia-selected cells was further confirmed by immunoprecipitation (Fig. 3b). Pro- and anti-apoptotic Bcl-2 family proteins may antagonize each other through heterodimerization (30–36). Our co-immunoprecipitation experiments suggest that, in hypoxia-selected cells, Bcl-X<sub>L</sub> might directly interact with Bax. As shown in Fig. 3d, precipitation with an anti-Bcl-X<sub>L</sub> antibody pulled down not only Bcl-X<sub>L</sub> but also Bax from whole cell lysates, cytosolic extracts, and to a less extent, membrane extracts (lanes 1–3). Consistently, anti-Bax antibodies precipitated Bax as well as Bcl-X<sub>L</sub> (Fig. 3d, lanes 4–6). The results suggest that Bcl-X<sub>L</sub> may interact with Bax and antagonize its toxicity, leading to the preservation of mitochondrial integrity and cell viability.

To further determine the role of Bcl-X<sub>L</sub> in death resistance of hypoxia-selected cells, we down-regulated its expression using a specific antisense oligonucleotide (37). In hypoxia-selected cells, the antisense inhibited Bcl-X<sub>L</sub> expression without affecting the expression of β-actin (Fig. 4a, lanes 1–3). A control oligonucleotide with scrambled sequence was not effective in down-regulating Bcl-X<sub>L</sub> (Fig. 4a, lanes 4–6). We then examined the effect of Bcl-X<sub>L</sub> antisense on apoptosis induced by STS. The results are summarized in Fig. 4b. Without STS treatment, apoptosis was always below 10%. In line with previous observations, STS incubation for 5 h led to 57% apoptosis in wild-type cells but only 17% in hypoxia-selected cells. Of significance, down-regulation of Bcl-X<sub>L</sub> by antisense restored the sensitivity of hypoxia-selected cells to injury. As a result, 60% apoptosis was now induced by STS. The scrambled sequence showed no effect (Fig. 4b). These results suggest a critical role for Bcl-X<sub>L</sub> in death resistance of hypoxia-selected cells. We further showed that Bcl-X<sub>L</sub> antisense drastically promoted Bax targeting of mitochondria in hypoxia-selected cells (Fig. 4c, lane 4), which was accompanied by outer membrane damage, shown as increased release of Cyt<sub>c</sub> (Fig. 4d, lane 4). By immunofluorescence, we confirmed that significantly more cells leaked Cyt<sub>c</sub> into the cytosol in the antisense-treated group (Fig. 4i) compared with the scrambled sequence group (Fig. 4e).

Importantly, the cells with leaked Cyt<sub>c</sub> activated caspases (Fig. 4, f and j) and developed apoptotic morphology including nuclear condensation and fragmentation (Fig. 4, g and k). This was clearly shown by superimposition of the images (Fig. 4, h and i). The results indicate that antisense down-regulation of Bcl-X<sub>L</sub> in hypoxia-selected cells restored their sensitivity to cell injury and death. We further tested the effects of chelerythrine, a newly identified pharmacological inhibitor of Bcl-X<sub>L</sub> (38). Chelerythrine increased apoptosis in hypoxia-selected cells at concentrations of 1–5 μM (data not shown). Together, these results suggest a critical role for Bcl-X<sub>L</sub> in death resistance of hypoxia-selected cells.

In conclusion, we have selected death-resistant cells through repeated episodes of hypoxia. These cells are cross-resistant to cell death and injury induced by different types of insults. Death resistance of the cells occurs at the level of mitochondria, and a critical molecular determinant appears to be Bcl-X<sub>L</sub>. Up-regulation of Bcl-X<sub>L</sub> in hypoxia-selected cells preserves the integrity of mitochondria and cellular viability, probably by antagonizing pro-apoptotic molecules such as Bax. The findings may have implications for the pathogenesis of chronic or fluctuating hypoxia, conditions found in solid tumors. In solid tumors, malformation and malfunction of blood vessels lead to the development of hypoxic microenvironments (9–12). Under these conditions, some cells die because of oxygen deficiency, whereas others survive the stress and become resistant to injury and death during cancer therapy (11, 13, 14). Notably, elevated expression of Bcl-X<sub>L</sub> is detected in solid tumors such as colorectal adenocarcinomas, Kaposi’s sarcoma, and multiple myeloma (39–41). Thus, hypoxia-mediated selection of Bcl-X<sub>L</sub> may provide a mechanism for tumor expansion and resistance to cancer therapy.

Acknowledgments—We thank Dr. M. A. Venkatachalam at the University of Texas Health Science Center at San Antonio for discussion. We thank Drs. P. McNeil and R. Markowitz at Medical College of Georgia for critical reading of the paper. We also thank Dr. X. M. Yin at the University of Pittsburgh School of Medicine and Dr. A. Srivastavan at Idun Pharmaceuticals Inc. for antibodies to Bid and active caspase-3.

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Bcl-X<sub>L</sub> in Hypoxia Selection of Death Resistance

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