Insulin-like Growth Factor 1 (IGF-1) Alters Drug Sensitivity of HBL100 Human Breast Cancer Cells by Inhibition of Apoptosis Induced by Diverse Anticancer Drugs

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ABSTRACT

In this study, we tested the hypothesis that insulin-like growth factor-1 (IGF-1) modulates apoptosis in human breast cancer cells, HBL100, induced by diverse chemotherapeutic drugs. IGF-1 increased cell survival of HBL100 cells treated with 5-fluorouracil (antimetabolite), methotrexate (antimetabolite), tamoxifen (antiestrogen/antiproliferative), or camptothecin (topoisomerase 1 inhibitor) and after serum withdrawal. Elevated cell survival was not due to an increase in cell proliferation by IGF-1, but rather to an inhibition of apoptosis. Evidence for death by apoptosis was supported by cellular morphology and DNA fragmentation. There were no changes observed in Bcl-2 protein or bax mRNA levels. Extracellular matrix (ECM) is known to influence the apoptotic response of cells; therefore, the antiapoptotic effect of IGF-1 on breast cancer cells was examined using different ECMs: laminin, collagen IV, or Matrigel. IGF-1 protected cells from apoptosis induced by methotrexate on all ECMs tested, providing the first evidence that IGF-1 protects against apoptosis in three-dimensional culture systems. These data provide the rationale to search for drugs that lower serum IGF-1 in an effort to improve the efficacy of chemotherapeutic drugs for the treatment of breast cancer.

INTRODUCTION

Most chemotherapeutic drugs kill cancer cells by apoptosis (1); therefore, the successful treatment of human malignancies, such as breast cancer, depends on the response to apoptotic inducers. Physiological modifiers of the response of cancer cells to apoptosis induced by chemotherapy drugs are not well studied. Recently, it has been shown that ECM proteins mitigate apoptosis in normal breast epithelial cells (2). The ECM is rich in growth factors such as IGF-1 that may act as physiological modifiers of apoptosis. The role for the IGF-1 receptor and its ligands IGF-1 and IGF-2 in modulating apoptosis has been demonstrated in a variety of experimental models. Apoptosis is decreased in cell lines that overexpress the IGF-1 receptor (3). Furthermore, apoptosis is induced by the use of antisense oligonucleotides to the IGF-1 receptor (4) and by the expression of a dominant negative mutated IGF-1 receptor (5). Studies with the IGF-1 and IGF-2 ligands have shown that the IGF-l receptor and its ligands result in apoptosis in mouse fibroblasts (7). Use of antisense oligonucleotides to the IGF-2 mRNA reduces pancreatic tumor cell proliferation and enhances apoptosis in vivo (8). Recently, IGF-1 was shown to inhibit apoptosis during mammary gland involution in transgenic mice (9). Hence, manipulation of the IGF-1 receptor and its ligands results in the suppression of apoptosis.

It is possible that IGF-1 inhibits apoptosis in human breast cancer cells, because elevated serum IGF-1 levels are detected in breast cancer patients (10), and the IGF-1 receptor number in breast malignancies is often elevated compared to that of benign tissue, suggesting that expression may correlate with the stage of the disease (11). Stromal cells surrounding breast cancer express IGF-1 and likely supply the tumor cells with IGF-1 through a paracrine pathway (12). Taken together, IGF-1 interacts with breast epithelial cells through endocrine and paracrine pathways; therefore, IGF-1 may modulate apoptosis in breast cancer cells.

Understanding the regulation of apoptosis in breast cancer cells has recently become an area of intense research efforts. Bcl-2 overexpression was shown to protect breast cells from apoptosis induced by Adriamycin (13). In addition, estrogen up-regulates bcl-2 in MCF7 cells (13) and protects against tamoxifen-induced apoptosis (14). On the contrary, there are no changes in bax mRNA in response to estradiol-induced apoptosis (14). Loss of bax mRNA (15) and protein (16) have been correlated with advanced breast cancer and poor response to anticancer drug treatment. Bax restoration experiments have shown that bax sensitizes MCF7 cells to drug- and radiation-induced (18) apoptosis. Additional studies are necessary to unravel the complexities of apoptosis in preneoplastic and neoplastic breast cells.

Selection of an appropriate model for studying apoptosis in breast cancer cells is a difficult challenge, because some breast cancer cell lines have a decreased propensity to die by apoptosis (15). For our studies, HBL100 human breast cells were chosen because of their reported propensity to undergo apoptosis after serum withdrawal (15), and because they express the apoptosis proteins bax and bcl-2 (15). In addition, HBL100 cells are immortal, grow in soft agar, stimulate angiogenesis in vivo, and are tumorigenic in nude mice, although only after a high passage number (19). Finally, they express the IGF-1 receptor (11) but do not produce the IGF-1 ligand (12); thus, they are characteristic of other breast cancer cells and breast cancer biopsies. Given these characteristics, HBL100 cells proved to be a useful model for studying growth factor regulation of apoptosis. Our objectives were: (a) to determine whether IGF-1 protected HBL100 cells from apoptosis; (b) to ascertain whether IGF-1 modulated bcl-2 or bax; and (c) to determine whether IGF-1 could mitigate apoptosis on ECMs.

MATERIALS AND METHODS

Cell Viability. HBL100, a human mammary cell line, was obtained from the American Type Culture Collection (Rockville, MD) and grown in DMEM: F12 (phenol red-free; Life Technologies, Inc., Grand Island, NY) supplemented with 5% FCS (HyClone, Logan, UT). The cells were non-tumorigenic in nude mice when 5 × 10⁶ cells/site were delivered by s.c. injection (data not shown). Anticancer drugs and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. All experiments described in this report were done with 10 ng/ml IGF-1 (Upstate Biotechnology, Waltham, MA). For serum withdrawal experiments, 5 × 10⁵ cells were plated in 5% FCS and DMEM:F12 and allowed to attach overnight. The next day, media were removed, cells were rinsed with PBS, and DMEM:F12 with or without IGF-1 was added. Upon drug treatment, cells were prepared as described in the serum withdrawal studies, except that media were changed to 0.5% FCS (a concentration previously determined to maintain viability yet prevent serum withdrawal-induced apoptosis). Cells were treated with camp-
tothecin (0.2 μM), methotrexate (1.0 μM), 5-fluorouracil (100 μM), or tamoxifen (10 μM) in the presence or absence of IGF-1. Camptothecin and tamoxifen killed 50% of the cells after 24 h at those concentrations, whereas it took 48 h for methotrexate and 5-fluorouracil to kill the same number of cells. Hence, cell survival was determined at either 24 or 48 h, depending on the drug. IGF-1 protection against cytotoxicity was confirmed by the MTS assay according to the manufacturer’s instructions (Promega, Madison, WI) with camptothecin (0.06, 0.2, and 0.6 μM). Briefly, 5 × 10^5 cells/well were plated in 96-well plates; the next day, cells were rinsed with PBS and treated with various concentrations of camptothecin; and after 24 h, the cell viability was measured by the addition of MTS tetrazolium, measured at 490 nm with a reference wavelength of 630 nm using a microplate reader (Bio-Rad, Hercules, CA). DMSO, which did not affect cell survival, was used as a vehicle for camptothecin. To determine whether IGF-1 could also protect in the presence of ECM proteins, 5 × 10^5 cells/well were plated in 12-well plates precoated with Matrigel, laminin, or collagen IV (Collaborative Biomedical Products, Bedford, MA) and treated with methotrexate (1 μM) with or without IGF-1. After 96 h, cells were rinsed with PBS and removed from the culture dish with trypsin. Cell number was determined using Coulter counting (Coulter Inc., Hialeah, FL). Statistical significance was determined by Student’s t test.

**Cell Proliferation.** Cells were plated at 2 × 10^4 in a 100-mm^2 culture dish, and the next day, cells were rinsed with PBS and treated with either camptothecin (0.2 μM), tamoxifen (10 μM), methotrexate (1 μM), or 5-fluorouracil (100 μM) in the presence or absence of IGF-1. After exposure for 24 h (camptothecin) or 48 h (methotrexate and 5-fluorouracil), cells were pulse-labeled with BrdUrd (10 μM) for 30 min and then harvested using trypsin and EDTA, washed twice in PBS, fixed in cold 70% ethanol, and stored at 4°C until further processing. Before analysis, cells were incubated with FITC-conjugated anti-BrdUrd (Becton Dickinson, Bedford, MA), treated with RNase A, and stained with propidium iodide (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer’s instructions. DNA analysis was performed using a FACSscan flow cytometer (Becton Dickinson). Gating of the cell population was done to prevent analysis of aggregated cells and to exclude sub-2N cells. At least 10,000 cells/sample were analyzed, and the resultant histograms were plotted using the CellQuest software program (Becton Dickinson). Log growing cells were run as a positive control for normal cell cycle progression.

**Apoptosis.** To distinguish between necrosis and apoptosis, methotrexate was selected as a representative anticancer drug for the induction of cell death. Cells (5 × 10^4) were treated for 48 h with methotrexate (1 μM) in the presence or absence of IGF-1. Approximately 50,000 cells were reserved for analysis of cellular morphology by electron microscopy as described previously (20). For isolation of DNA ladders, cells were rinsed with PBS, homogenized in 100-μl volume of lysis buffer (10 mM EDTA, 50 mM Tris (pH 8.0), 0.5% sodium laurel sarcosine, and 0.5% mg/ml proteinase K), and then incubated at 50°C overnight. RNase A (100 μg/ml) was added to DNA lysates and incubated at 37°C for 1 h. DNA fragments were separated by electrophoresis on a 1% agarose gel in 1× Tris-borate EDTA (50 mM Tris base, 50 mM boric acid, and 1 mM disodium EDTA) at 100 V for 1.5 h and then stained with ethidium bromide (0.5 μg/ml). For the identification of 50-kb DNA fragments, 5 × 10^6 cells were treated as described above and then merged with propidium iodide (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer’s instructions. DNA analysis was performed using a FACSscan flow cytometer (Becton Dickinson). Gating of the cell population was done to prevent analysis of aggregated cells and to exclude sub-2N cells. At least 10,000 cells/sample were analyzed, and the resultant histograms were plotted using the CellQuest software program (Becton Dickinson). Log growing cells were run as a positive control for normal cell cycle progression.

**RESULTS**

Initially, studies were performed to evaluate the ability of IGF-1 to rescue HBL100 cells from apoptosis after serum withdrawal. HBL100 cells readily underwent apoptosis upon serum withdrawal in a time-dependent manner after 24, 48, and 72 h. In contrast, the addition of IGF-1 markedly suppressed the diminution of the cell number by protecting against apoptosis (Fig. 1). After 72 h, IGF-1 protection was attenuated; this is likely due to the diminution of IGF-1. The half-life of IGF-1 in HBL100 cell cultures is not known, but in humans, the half-life of IGF-1 is 12–15 h (23).

To confirm that the cells were dying by apoptosis after serum withdrawal, cells were treated as described above and then fixed and examined for apoptosis based on nuclear condensation and fragmentation. The number of apoptotic cells was determined by counting H&E-stained cells (Fig. 2, insert). In the presence of IGF-1, there were significantly fewer apoptotic cells (Fig. 2). These data provided evidence that IGF-1 was a potent inhibitor of the apoptotic process in HBL100 cells.

Apoptosis is important for successful cancer chemotherapy; therefore, experiments were designed to test whether or not IGF-1 protected against apoptosis induced by a variety of anticancer drugs. The anticancer drugs tamoxifen, methotrexate, 5-fluorouracil, and camptothecin were selected to chemically induce apoptosis in 0.5% FCS and DMEM:F12 (minimal amount of serum necessary to prevent the activation of apoptosis by serum withdrawal). In the presence of IGF-1, there were approximately 25% more surviving cells with all of

**Western Blot Analysis.** Cells (5 × 10^4) were plated in T150 culture flasks; the next day, the cells were rinsed with PBS and placed in 0.5% DMEM:F12 containing 0.2 μM camptothecin; and cells were collected at 2, 4, 8, 12, and 24 h in the presence or absence of IGF-1. Floating and attached cells were harvested and pooled, and proteins were isolated by homogenization in ice-cold lysis buffer [1% Triton X-100, 2 mM EDTA, 2 mM EDTA, 1 mM NaF, 50 mM Na2MoO4, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 15 μM (4-aminophenyl)-methanesulfonyl fluoride in 20 mM HEPES (pH 7.4)]. Western blot analysis was performed as described previously (21). Bcl-2 antibody (Dako Corp., Carpenteria, CA) was used for analysis. A positive control for BCL-2 was provided by analyzing lysates from a stably transfected cell line, S49/BCL-2 (a generous gift from Dr. John Cidlowski, National Institute of Environmental Health Sciences). Prestained molecular weight markers were run to estimate the size of the proteins detected (Bio-Rad).

**Northern Blot Analysis.** Total RNA was isolated according to the manufacturer’s instructions (Qiagen, Chatsworth, CA). Separation of 10 μg of total RNA was performed on a 1% agarose gel and transferred according to Sambrook et al. (22). QuickHyb hybridization solution (Stratagene, La Jolla, CA) was used for the prehybridization (30 min at 68°C) and hybridization (68°C for 1 h). Nitrocellulose was washed with high stringency (3 × SSC and 0.1% SDS) followed by 30 min at 60°C with 0.1× SSC and 0.1% SDS. Bar and β-actin probes were generated by reverse transcription-PCR amplification and purification of MCF7 mRNA. Breast cell lines that have been reported to have relatively high (MCF7) and low (MDA-MB-435) bar mRNA (15) were analyzed for method validation (data not shown). The levels of mRNA in those cell lines were consistent with the reported results.
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Fig. 2. Inset, H&E-stained cells undergoing apoptosis (arrow). The quantification of apoptotic cells is represented in the graph below. Statistical significance was determined using Student’s t test.

![Graph showing quantification of apoptotic cells](image)

To address whether the increase in cell number with IGF-1 was a result of cell proliferation or an inhibition of apoptosis, cells were treated as described in the cell viability experiments, pulse-labeled with BrdUrd, and analyzed by flow cytometry to determine whether IGF-1 was acting as a mitogen. IGF-1 did not stimulate cell proliferation as measured by BrdUrd incorporation for cell cycle analysis in low serum or in the presence of camptothecin, 5-fluorouracil, methotrexate, or tamoxifen (Table 1). These data were interpreted based on the observation that there was no increase in S-phase or G2 phase of the cell cycle. An arrest (accumulation of cells) in G1 was observed in low serum and after drug treatment. Less than 2N nuclei were gated during the analysis, and this fraction contained dead and dying cells. The dual-labeled nuclei (BrdUrd and propidium iodide) prevented the analysis of a clear sub-2N peak characteristic of apoptosis. As expected, log growing cells were undergoing rapid cell proliferation reflected by changes in the cell cycle parameters.

To determine whether IGF-1 modulated apoptosis, methotrexate-treated cells were evaluated for apoptosis by cellular morphology and DNA fragmentation. Morphological changes indicative of apoptosis were observed in cells by electron microscopy. There was condensed chromatin that aligned with the nuclear membrane (Fig. 5a), whereas the perinuclear and cytoplasmic membranes remained intact. Nucleolar remnants were evident (arrow), but mitochondria and endoplasmic reticulum were maintained (Fig. 5b). Envelopment of apoptotic bodies (arrow) by adjacent normal cells was also noticed (Fig. 5c). Similar results were observed for camptothecin, tamoxifen, and 5-fluorouracil (data not shown). Signatures of apoptosis could also be found in patterns of DNA fragmentation. Cells floating in the media after treatment with methotrexate for 48 h produced both small DNA fragments (Fig. 6a, Lane 1). No DNA ladders were detected in the viable cells that remained attached to the tissue culture dish (Fig. 6a, Lane 2). There were more cells dying by apoptosis than there were cells remaining attached and viable, therefore slightly more DNA was loaded in Lane 1 as compared to Lane 2. We concluded from these experiments that the drugs tested (Fig. 3). IGF-1 protection was confirmed in a dose-dependent manner using camptothecin at 0.06, 0.2, and 0.60 μM by the MTS assay (Fig. 4).

Fig. 3. Cell survival in the presence of IGF-1 (10 ng/ml) and select anticancer drugs. HBL100 cells were treated with camptothecin, methotrexate, 5-fluorouracil, or tamoxifen at concentrations of 0.2, 1.0, 100, and 10 μM, respectively. Cells were tested at a concentration and time course that would kill 50% of the cells. Camptothecin and tamoxifen killed 50% of the cells after 24 h, whereas methotrexate and 5-fluorouracil killed 50% of the cells after 48 h (n = 5; P < 0.05).

![Graph showing cell survival](image)
trexate (0.01 μM), 5-fluorouracil (100 nM), or tamoxifen (10 nM) in the presence or absence of IGF-I (10 ng/ml). Cells grown in 5% serum were a positive control for cell proliferation and mitogenicity. Sub-2n cells were gated during the analysis to exclude apoptotic cells and cellular aggregates.

Table I Cell proliferation indices using BrdUrd labeling

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% &lt;2N</th>
<th>% G1 phase</th>
<th>% S-phase</th>
<th>% G2 phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% serum</td>
<td>3.5</td>
<td>44.5</td>
<td>37.8</td>
<td>14.2</td>
</tr>
<tr>
<td>0.5% serum</td>
<td>27.0</td>
<td>67.8</td>
<td>4.6</td>
<td>0.6</td>
</tr>
<tr>
<td>0.5% serum/IGF-1</td>
<td>44.2</td>
<td>49.9</td>
<td>5.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>37.2</td>
<td>51.2</td>
<td>11.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Camptothecin/IGF-1</td>
<td>32.2</td>
<td>56.3</td>
<td>10.9</td>
<td>0.6</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>39.9</td>
<td>47.5</td>
<td>11.6</td>
<td>1.0</td>
</tr>
<tr>
<td>5-Fluorouracil/IGF-1</td>
<td>40.8</td>
<td>45.9</td>
<td>11.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>26.9</td>
<td>61.5</td>
<td>11.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Methotrexate/IGF-1</td>
<td>34.8</td>
<td>52.5</td>
<td>12.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>38.3</td>
<td>50.2</td>
<td>10.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Tamoxifen/IGF-1</td>
<td>38.5</td>
<td>49.7</td>
<td>10.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

data that the appearance of distinct 180-bp fragments of DNA in Lane 1 is evidence that methotrexate treatment induced cells to die by apoptosis. IGF-I suppression of apoptosis could not be correlated with changes in the production of small DNA fragments due to the limitations in the sensitivity of the assay, i.e., the assay was not sensitive enough to detect a 25% change. Large 50-kb DNA fragments were also isolated (Fig. 6b). Very few 50-kb fragments were observed in the sample taken from log growing cells (Fig. 6b, Lane 2) compared to those in cells treated with methotrexate (Fig. 6b, Lane 3). When the cells were treated with IGF-1 and methotrexate, the formation of 50-kb DNA fragments was suppressed (Fig. 6b, Lane 4). We and others\(^3\) have found that typically 50-kb DNA fragments run slightly ahead of the molecular weight standard. It is possible that the standard is not exactly 50 kb and that cellular endonucleases may produce slightly smaller pieces of DNA or that the electrophoresis conditions are causing the fragments to run a little bit faster.

Apoptosis seemed to be the predominant cell death pathway in this system. It was therefore possible that IGF-1 protected against apoptosis by up-regulation of the antiapoptotic protein bcl-2 and/or down-regulation of the proapoptotic gene bax. Cells were treated with camptothecin (0.2 μM) for 2, 4, 8, 12, and 24 h in the presence or absence of IGF-1 and then analyzed by Western blot analysis for bcl-2 protein expression (Fig. 7). There were no changes observed in bcl-2 protein expression after treatment with camptothecin and IGF-1 or camptothecin alone for various times. Similar results were observed when cells were treated with methotrexate and after serum withdrawal (data not shown). Next, total RNA was isolated from cells treated with camptothecin, tamoxifen, methotrexate, and 5-fluorouracil, with and without IGF-1, to evaluate changes in bax mRNA levels. IGF-1 did not down-regulate the expression of bax mRNA in the presence of the selected anticancer drugs. Furthermore, the anticancer drugs themselves did not change the expression of bax compared to that of untreated cells. Finally, in the absence of any drug treatment, IGF-1 did not affect bax expression (Fig. 8). Taken together, IGF-1 does not seem to protect against apoptosis by changing the relative amounts of bcl-2 or bax. Bax protein was not evaluated due to a lack of specificity of commercially available antibodies.

The ECM proteins laminin and collagen IV are found in the stromal tissue of the breast; therefore, plates coated with these proteins were used to ask whether or not IGF-1 modulates apoptosis in a stroma-like environment. After a 96-h exposure to 1 μM methotrexate in the presence of IGF-1, there were 36 and 43% more surviving cells on collagen IV-coated (Fig. 9A) and laminin-coated (Fig. 9B) plates, respectively, compared to cultures treated with 1 μM methotrexate alone. The same experiment was repeated on an ECM containing growth factors, which are known components of stromal tissue. Matrigel-coated plates were used for this purpose, and our results showed that IGF-1 treatment resulted in a 29% increase in cell survival (Fig. 9C). In summary, IGF-1 consistently suppressed drug-induced cell death in stroma-like tissue culture conditions.

**DISCUSSION**

IGF-1 may be a double-edged sword for breast cancer because not only is IGF-1 mitogenic to breast tissue (24), but, as our data shows, IGF-1 also inhibits apoptosis in HBL100 cells induced by serum withdrawal and by the clinically relevant and functionally diverse anticancer drugs camptothecin (topoisomerase I inhibitor), tamoxifen (antiestrogen/antiproliferative), methotrexate (antimetabolite), and 5-fluorouracil (antimetabolite). In a similar study, IGF-1 protected MCF7 breast cancer cells from puromycin, actinomycin D, 5-fluorouracil, cisplatin, and Adriamycin, although the mechanism of cell death in those experiments was reported as necrosis (25). Geier et al. (25) reported that DNA ladders were not detectable; we found that the DNA ladders were particularly difficult to detect and required modification of our standard protocol. They also reported that transmission electron microscopy of MCF7 cells showed morphology of necrosis, although late-stage apoptosis can look like necrosis.\(^4\) In our experience, the detection of apoptosis in human breast cancer cells has proven to be more difficult than that in other apoptosis-sensitive cells such as the preneoplastic Syrian hamster embryo cells (20). We illustrated that the cells were dying by apoptosis using a number of methods: DNA fragmentation; pulse field gel electrophoresis; light microscopy; and, finally, electron microscopy. The most definitive method, in our opinion, is analysis by electron microscopy; therefore, we tested methotrexate as well as tamoxifen and camptothecin by this method. In each case, signatures of apoptosis represented by nuclear condensation and emargination were evident. Taken together, IGF-1 protects both preneoplastic (HBL100) and neoplastic (MCF7) breast cell lines from cell death induced by a number of structurally and mechanistically distinct anticancer drugs.

Our data show that IGF-1 is not mitogenic for the HBL100 cells in

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\(^3\) J. Cidlowski, personal communication.

\(^4\) G. A. Preston, personal communication.
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Fig. 6. a, DNA ladders were observed in the media harvested from cells treated with methotrexate (1 μM) for 48 h (Lane 1), whereas no DNA ladders were detected in the viable cells that remained attached to the dish (Lane 2). b, Pulse field gel electrophoresis of DNA isolated from cells growing in log phase (Lane 2), methotrexate (1.0 μM) for 48 h (Lane 3), or IGF-1 (Lane 4). λ-DNA was run in Lane 1 as a molecular weight marker.

Fig. 7. BCL-2 protein expression as a function of time. a, camptothecin-treated cells in the absence (Lanes 1, 3, 5, 7, and 9) or presence of IGF-1 (Lanes 2, 4, 6, 8, and 10) after 2, 4, 8, 12, and 24 h. DMSO used as a vehicle for camptothecin was run as a negative control (Lane 11), and S49/BCL-2 was run as a positive control (Lane 13).

low serum. Furthermore, IGF-1 is not mitogenic when the cells are then treated with camptothecin, 5-fluorouracil, methotrexate, or tamoxifen. Similarly, IGF-1 is not mitogenic to BALB/c 3T3 fibroblasts when treated with the topoisomerase 2 inhibitor etoposide (7). IGF-1 is therefore able to protect both epithelial and mesenchymal cells from apoptosis in the absence of mitosis.

The mechanism(s) whereby IGF-1 suppresses apoptosis remains unknown. We report here that neither bcl-2 protein nor bax mRNA is affected by IGF-1. It is possible that protein-protein interactions are attenuated by IGF-1, because bcl-2 and bax form heterodimers and homodimers (26). Such changes would not be detected by the methods used in these studies. Lack of responsiveness of bcl-2 and bax may be due to the absence of functional p53 in HBL100 cells, because p53 has
been shown to regulate both these genes (27). HBL100 cells have been reported to express the SV40 large T antigen due to viral insertion (28), and it has been shown that SV40 large T antigen inactivates p53 (29).

IGF-1 and IGF-II encounter the breast epithelial cells from both endocrine and paracrine pathways. In this study, we demonstrate that IGF-1 protects against drug-induced cell death when cells are plated on ECM comprised of Matrigel, laminin, or collagen IV. The ECM provided a stroma-like environment to allow us to ask whether paracrine exposure, i.e., the media addition of IGF-1, could modulate apoptosis. It is noteworthy that Matrigel contains growth factors such as IGF-1, basic fibroblast growth factor, epidermal growth factor, platelet-derived growth factor, and transforming growth factor β (product specification sheet, Collaborative Biomedical Products) and that these growth factors are also present in human breast stroma (30), although the bioavailability of IGF-1 or any of the other growth factors in this matrix has not been tested. Based on our studies using ECM, it is possible that in vivo, serum IGF-1 suppresses drug-induced apoptosis. If reduction in serum IGF-1 resulted in a 20—40% increase in cell killing/day, this might significantly contribute to the effectiveness of cancer chemotherapies. In addition, IGF-2 is often overexpressed in the stromal tissue from breast cancer patients (30), and it also uses the IGF-1 receptor for signal transduction. Hence, it is also possible that a reduction in IGF-2 would also sensitize breast cancer cells to apoptosis.

These data suggest that IGF-1-lowering drugs or receptor blockers may enhance chemotherapy for breast cancer patients and lead to improved therapy. Drugs such as Suramin, (31, 32), Octreotide, (33), fenretinide (34), and tamoxifen (34) disrupt IGF-1 synthesis or binding and therefore may be useful for sensitizing breast cancer cells to apoptosis. It is noteworthy that systemic modulation of IGF-1 is fortunately associated with relatively few side effects in human patients (35). Receptor blockers are also an option. Disruption of the IGF-1 receptor using antisense oligonucleotides slows the growth of breast cancer cell line MCF7 (36). Although principally more difficult, interrupting the IGF-1 receptor is more desirable than modifying systemic IGF-1 because it would affect endocrine and paracrine sources of IGF-1 and IGF-2.

Our studies have focused on the HBL100 cells as a model; therefore, the interpretation of these data should remain within this context. Additional studies are necessary to address the role of IGF-1 in apoptosis in preneoplastic and neoplastic breast tissues. We conclude that IGF-1 is important in regulating anticancer drug-induced apoptosis in HBL100 cells and propose that disrupting the IGF-1 signaling pathway may improve breast cancer chemotherapy.

REFERENCES

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