CHARACTERISTICS OF BLEOMYCIN-RESISTANT PHENOTYPES OF HUMAN CELL SUBLINES AND CIRCUMVENTION OF BLEOMYCIN RESISTANCE BY LIBLOMYCIN1


ABSTRACT

Three bleomycin (BLM)-resistant sublines were isolated from a human head and neck squamous cell carcinoma cell line (A-253); these sublines (C-10, D-10, and G-11) were 4-, 9-, and 21-fold resistant to BLM A5, respectively. These sublines were selectively resistant to other members of the BLM class, namely BLM B2, peptomycin, talosimycin S100, and bleomycinic acid; none of the sublines displayed cross-resistance to vincristine, doxorubicin, cis-diaminedichloroplatinum or melphalan; only one subline (G-11) was cross-resistant to X-irradiation. None of the BLM-resistant cell lines demonstrated resistance to the novel BLM analogue liblomycin, which contains a lipophilic terminal amine. The cell cycle distributions of the clonally derived BLM-resistant cell populations were similar to the distribution of the parental cell population. In vitro BLM hydrolysis activity in homogenates of D-10 and G-11 BLM-resistant cell lines was two- to threefold higher than that in homogenates of A-253 or C-10 cells. Nonetheless, no deamido BLM A5 was found associated with any cell type or in the culture medium and more than 80% of the radioactivity in all cell types appeared as unmetabolized BLM A2 by high pressure liquid chromatography. Thus, the appearance of large quantities of the deamido BLM metabolite was not a prominent feature of acquired resistance to BLM in these human tumor cells.

The cellular accumulation of radiolabeled BLM A5 by C-10 and G-11 cells during a 4-h incubation with 14CBLM A5 was 46% that seen with A-253 and D-10 cells. C-10 cells maintained a lower nuclear content of radioactivity than A-253, G-11, or D-10 cells. Initial single strand DNA damage, based upon alkaline elution analysis, was also lower in C-10 cells compared to A-253 cells. D-10 cells, in contrast, exhibited high initial genomic DNA damage but demonstrated a greater repair rate than either A-253 or C-10 cells. Thus, multiple BLM-resistant phenotypes can be obtained from a population of human squamous carcinoma cells, and modification of the terminal amine in the BLM molecule can produce compounds capable of circumventing all of these BLM-resistant phenotypes. Liblomycin, which appears to be a nonclassical BLM, may be a useful therapeutic agent with a spectrum of activity distinct from other members of the BLM class.

INTRODUCTION

The development of resistance to antineoplastic agents represents an important mechanism by which populations of tumor cells evade chemotherapeutic eradication. The mechanisms responsible for acquired resistance in human tumor cells to the natural product BLM1 have not been well investigated. It has been hypothesized (1, 2) that cells become resistant because of reduced BLM uptake, although direct evidence for depressed cellular association in human tumor cells has not been presented. Other investigators (3-5) have proposed that acquired BLM resistance is mediated by increased metabolic drug inactivation by BLM hydrolase. The data supporting this latter hypothesis have been obtained primarily by measuring this enzyme activity in vitro with homogenates from a very limited number of resistant cells. We (6) and others (1, 2) have found a lack of correlation between BLM hydrolyase activity measured in vitro in tumor cell homogenates and BLM-induced cell death. Recent work suggests tumor BLM hydrolyase may differ antigenically from BLM hydrolyase in normal tissue (7). Thus, the factors that regulate the cellular toxicity of BLM remain uncertain and the lack of human tumor cells with acquired BLM resistance has greatly hampered investigations.

In this report we describe the isolation and characterization of the first BLM-resistant cell lines obtained from human tumor cells. Multiple BLM-resistant phenotypes were obtained with cells showing reduced drug association or accelerated DNA repair. In all of the cell lines, cross-resistance is seen to the BLM class of compounds. Interestingly, no cell lines were cross-resistant to liblomycin, which is now defined as a nonclassical BLM.

MATERIALS AND METHODS

Origin of Drugs and Cells

Peptomycin was provided by the Developmental Therapeutics Program of the National Cancer Institute (Bethesda, MD), bleomycinic acid by Dr. S. M. Hecht (University of Virginia, Charlottesville, VA), and liblomycin (8) by Dr. A. Matsuda (Tokyo, Japan) (Fig. 1). Bleomycin and talosimycin S100 were obtained from the Bristol-Myers Company (Wallington, CT). BLM A2 and BLM B2 were isolated as previously described from Bleomaxine (9, 10). The A-253 cell line, which originated from a human squamous cell carcinoma located in the submaxillary gland (11), was obtained from the American Type Culture Collection (Rockville, MD). To obtain BLM-resistant clones, exponentially growing A-253 cells were detached from monolayers with a solution of 0.25% trypsin (w/v) and 2 mM EDTA in PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4), centrifuged at 200 x g for 5 min, resuspended in McCoy's medium (6 x 105 cells/ml), and exposed at 37°C to 0.04 µM N-methyl-N'-(nitro-N-nitrosoguanidine (Aldrich Chemical, Milwaukee, WI) for 2 h with continuous shaking. Independent studies indicated that, based upon clonal growth, this treatment reduced cell survival by 50%. After the incubation, cells were centrifuged at 200 x g (5 min) and washed once with drug-free McCoy's medium. The cell pellet was resuspended in drug-free McCoy's medium and 10% fetal bovine serum, plated in 150-cm2 cell culture flasks (4 x 106 cells/cm2), and incubated in humidified 5% CO2-95% air at 37°C for 3 days (approximately three population doublings times). The surviving cells were removed from the monolayer by washing with 10 ml of PBS containing 2 mM EDTA (37°C) and subsequently adding 10 ml of PBS containing 0.25% trypsin (w/v) and 2 mM EDTA (37°C). After 3 min the flasks were tapped lightly and 3 ml of McCoy's medium with 10% fetal bovine serum was added. The cell suspension was centrifuged for 3 min at 200 x g and resuspended in McCoy's medium containing 10% fetal bovine serum.

The cells were then plated as previously described (9, 10) in double-
Mycoplasma contamination. Cell growth was visibly disturbed (>0.7 nM). Extensive studies to increase CO2-95% air atmosphere. For X-irradiation studies, cells were irradiated these densities. Various concentrations of drugs were added and cultured dishes were incubated for 4 to 5 days at 37°C under humidified 5% Mg/ml streptomycin, and 0.7 nM of BLM A2 for at least 1 year were determined of BLM A2 that did not affect the rate of cellular proliferation (0.18 cm2 dish). The clonal population was expanded into 25-cm2 flasks and greater were detected in culture dishes containing 3.5 and 7 nM BLM A2 (10s cells/cm2). After 4 weeks in a cell culture incubator at 37°C, 40 and 20 colonies of 80 µm or greater were detected in culture dishes containing 3.5 and 7 nM BLM A2, respectively. Each colony was individually picked using a sterile Pasteur pipet and placed in one well of a 96-well cluster plate with 0.3 ml of drug-free McCoy’s medium and 10% fetal bovine serum. Of the 60 clones isolated, only three achieved confluence (D-10, G-11, and C-10) and these were transferred by trypsin-EDTA treatment to a 9.5-cm² dish. The clonal population was expanded into 25-cm² flasks and after two passages cells were exposed continuously to a concentration of BLM A2 that did not affect the rate of cellular proliferation (0.18 nM). The concentration of BLM A2 in the culture medium was then increased by progressively doubling the drug concentration weekly until cell growth was visibly disturbed (>0.7 nM). Extensive studies to increase further the exposure concentration have not yet been completed. Only resistant cell lines that have been cultured continuously with McCoy’s laboratory indicated no difference in the growth rate of cells plated at initial density of 4 to 8 x 10³ cells/cm²; independent studies in our laboratory indicated no difference in the growth rate of cells plated at these densities. Various concentrations of drugs were added and culture dishes were incubated for 4 to 5 days at 37°C under humidified 5% CO2-95% air atmosphere. For X-irradiation studies, cells were irradiated (250-kV, 220 rads/min, no filtration) immediately after plating in 6-well cluster dishes. After 4 or 5 days the cell density was determined either by Coulter counter or spectrophotometrically by the method of Finlay et al. (12). IC₅₀ (concentration required to inhibit growth by 50%) values were calculated by interpolation from growth inhibition curves.

Cell Cycle Distribution and Cloning Assays. Cell cycle distributions were determined on exponentially growing cultures with a Becton Dickinson FACS IV cytometer (13, 14). Clonal growth was evaluated by our previously described double layer agar assay (9) or after plating as monolayer cultures at low density (180 cells/cm²). A-253 and D-10 cells were exposed to various concentrations of BLM A2 continuously or to various doses of X-irradiation. Cells were incubated for 2 weeks and, after staining with 2-(p-iodophenyl)-3-(p-nitrophenoxy)-5-phenyl-tetrazolium chloride (9), the total number of colonies was determined by microscopic examination.

BLM A2 Cellular Association and Metabolism. The cellular accumulation of [³H]BLM A2 was determined as previously described (9) and total cellular radioactivity counted always exceeded by more than 10-fold the background determination. BLM hydrolysis activity was determined in the 105,000 x g supernatant fractions (2 mg protein/ml) from exponentially growing cells using BLM A3 (100 µg/ml) as previously described (9, 10). The radiolabeled material in the medium and associated with cells was examined by HPLC. Approximately 5 x 10⁵ cells were incubated in 1 ml at 37°C with 1 µM of Cu(II) [³H]BLM A2 (10 µCi/ml; DuPont NEN Research Products, Boston, MA) for 60 min. The incubation was terminated by washing the cells twice with 15 ml of ice-cold PBS, resuspending the cells in 0.3 ml of distilled water and lysing by sonication (2 x 5 sec). The medium, which was removed from intact cells before sonication, and the sonicate from cultured cells were centrifuged at 15,600 x g for 5 min, and the supernatant fraction of each was filtered through a 0.45-µm ACRO LC13 membrane filter (Gelman Sciences Inc., Ann Arbor, MI). The filtrate was injected together with unlabeled BLM A2 or BLM dA2 standards onto a Microsorb C₅ column eluted at 1 ml/min as previously described (9, 10). Fractions were collected every 0.3 min and counted by liquid scintillation methods. Recovery for the radioactivity found associated either with cells or in the medium exceeded 90% of the total injected radioactivity and a minimum of 5000 dpm were injected for each sample to ensure accurate counting. Elution of the internal BLM A2 and BLM dA2 standards was monitored by flow fluorescence as described previously (9, 10).

Isolation of Nuclei. Nuclei were isolated by a slight modification of the method of Mimnagh et al. (15) and Roy and Horwitz (16). Exponentially growing cells were detached and resuspended at a concentration of 2-8 x 10⁶ cells/ml in medium containing 10% fetal bovine serum, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4) and 1 µM Cu(II) [³H]BLM A2 (30 µCi/ml). After a 30-min incubation at 37°C, the cells were centrifuged at 1,000 x g for 2 min, washed with 12 ml of drug-free medium and centrifuged as above. The resulting cell pellet was resuspended in 5 ml ice-cold distilled water and 0.5% (v/v) Nonidet P-40 was added. The nuclei were isolated by centrifugation through a 45 µM sucrose at 40,000 x g for 60 min (4°C), resuspended in distilled water (200 µl), dissolved in Protocol (3 ml; DuPont New Research Products, Boston, MA) by heating overnight at 60°C, and the radioactivity in the sample was determined by liquid scintillation counting methods. Recovery of nuclei from cells was greater than 85% as judged by recovery of [³H]thymidine-labeled DNA. The preparations of nuclei were found to be free of cytoplasmic organelles based upon electron microscopy.

Alkaline Elution Techniques and DNA Repair Measurements. Single strand DNA breakage was determined as previously described (9, 10). Exponentially growing cells were incubated with [³H]thymidine, treated with BLM A2 for 1 h, washed, resuspended in ice-cold PBS, and then processed for alkaline elution (9, 10). The DNA of untreated cells was radiolabeled with [³H]thymidine and the cells added to each drug-treated sample as an internal control (10). Rates of DNA repair were assayed as previously described (17). Cells that were preincubated with [³H]thymidine, as mentioned above, were treated with BLM A2 for 1 h, washed free of drug, and resuspended in drug-free buffered saline.
medium and serum for 1 or 2 h. Single strand DNA damage was then measured by alkaline elution techniques (9, 10).

RESULTS

Characterization of BLM A2-resistant Cell Lines. Continuous exposure of A-253 and C-10, D-10, and G-11 cells to BLM A2 produced a concentration-dependent inhibition of growth (Fig. 2A). The IC50 for the sensitive A-253 cells was 3.1 nM. The C-10, D-10, and G-11 cells required 11, 27, and 64 nM, respectively, to reduce their final cell number by 50%.

A-253 cells had 38.5% of the total cell population in G1 phase, 36.5% in S phase, and 25.0% in G2/M phase. No significant differences in the cell cycle distribution of the resistant cell lines when grown in the presence of 0.7 nM BLM A2 were noted compared to the parental line grown in the absence of BLM A2 (data not shown). The population doubling time for A-253 cells was 26.6 ± 1.2 h in the absence of BLM A2 and this was similar in D-10 cells grown in the presence of 0.7 nM BLM A2 (28.2 ± 3.9). The G-11 and C-10 cells cultured with 0.7 nM BLM A2 had longer doubling times, i.e., 32.4 ± 3.7 and 37.4 ± 2.3 h, respectively. As seen in Fig. 2 the population doubling time of A-253 cells increases only slightly when these cells were grown in 0.7 nM BLM A2.

The sensitivity and stability of the sublines were examined further. Using clonal growth as an index of responsiveness, the D-10 cells displayed 8- to 10-fold resistance to continuous BLM A2 exposure compared to the parental A-253 cells on plastic (Fig. 3) or soft agar (data not shown). Compared to A-253 cells, the G-11 and C-10 cells also exhibited resistance to continuous exposure to 1.5 nM BLM A2 as measured by clonal growth on plastic (data not shown). The stability of the D-10 subline was determined by clonal growth after 2 months (approximately 60 population doubling times) in drug-free medium and the subline D-10B was found to be approximately twofold less resistant than D-10 cells maintained continuously in 0.7 nM BLM A2 (Fig. 3). C-10 cells appear less stable since removal of BLM A2 for 2 months results in the loss of almost all resistance to BLM A2 (data not shown). The stability of the G-11 cells was not examined.

Sensitivity of BLM A2-resistant Cell Lines to Other Antineoplastic Agents. The parental A-253 cells were equally sensitive to BLM A2, BLM B2, and talisomycin S100 (Table 1). All BLM resistant cell lines displayed cross resistance to compounds in the BLM family; i.e., talisomycin S100, peplomycin, BLM B2, and bleomycytic acid. No subline, however, was cross-resistant to liblomycin (Fig. 2B; Table 1). The resistance was selective for BLM-like compounds, since no cross-resistance to vincristine, melphanal, doxorubicin, and cis-diamminedichloroplatinum was seen with any subline. G-11 cells were found to be slightly cross-resistant to X-irradiation, C-10 cells were collaterally sensitive to X-irradiation, and D-10 cells were equally sensitive. Based upon clonal growth studies, the D-10 cells also displayed no resistance to X-irradiation (data not shown).

BLM Hydrolase Activity and Cellular BLM Metabolism. BLM hydrolase activity was seen with D-10 and G-11 cell homogenates and was two- to threefold higher than that seen with A-253 cells (Table 2). In contrast, the BLM hydrolase activity in C-10 cell homogenates was similar to that of the parental cell line. To determine the biological significance of the altered in vitro BLM hydrolase activity, we next examined the radiolabeled material associated with intact cells. After 1-h incubation of cells with 1 µM [3H]BLM A2, cellular radioac-
tivity was analyzed by reversed-phase HPLC. No significant quantities of BLM dA₂ were seen associated with any cells (Fig. 4). The twin peaks seen in the cellular extracts of G-11 and C-10 cells between fractions 38 and 42 (Fig. 4, C and D) were not noted in other samples from these or other cells and are believed to result from the discontinuous nature of fraction collecting. More than 90% of the total A-253 cell radioactivity coeluted with BLM A₂; almost all of the remaining radioactivity was found in the void volume. The radioactivity from A-253 cells found in the void volume had an apparent molecular mass of 1400 to 1500 using a TSK-20 gel filtration column (data not shown), which is consistent with the size of BLM A₂. The radioactivity in the medium of two resistant sublines also coeluted with BLM A₂ (Fig. 4).

Cellular and Nuclear Association of [3H]BLM A₂. The cellular accumulation of [3H]BLM A₂ was examined next (Fig. 5). Steady state levels of radioactivity were reached with all cells within 30 min. C-10 and G-11 cell lines had approximately 1/5 of the steady state levels seen with A-253 and D-10 cells.

Table 2 Determination of BLM hydrolase activity in cellular homogenates
The in vitro formation of BLM dA₂ was determined by HPLC with the protein in the 105,000 x g supernatant fraction as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Cell line</th>
<th>µg BLM dA₂/h x mg protein</th>
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<tbody>
<tr>
<td>A-253</td>
<td>1.09 ± 0.10³</td>
</tr>
<tr>
<td>D-10</td>
<td>2.88 ± 0.19³</td>
</tr>
<tr>
<td>G-11</td>
<td>2.92 ± 0.41³</td>
</tr>
<tr>
<td>C-10</td>
<td>1.03 ± 0.15</td>
</tr>
</tbody>
</table>

³ Mean ± SEM, N = 5 to six determinations.
³ P < 0.05 compared to A-253 cells using ANOVA and the Least Significant Difference Test (23).

Consistent with their lower steady state values, the C-10 cells also had a lower nuclear content of radioactivity compared to A-253 or D-10 cells (Table 3). Interestingly, G-11 cells had a nuclear content of radioactivity that was statistically indistinguishable from that seen in A-253 and D-10 cells, suggesting that lower total cell content does not always lead to reduced nuclear content. In all of these cell lines, more than 85% of the total cellular radioactivity was found associated with the nonnuclear fraction. Due to the small quantity of radioactive material present in the nuclear fraction, we were unable to analyze the radioactivity by HPLC.

DNA Damage and Repair. Incubation of A-253 cells for 1 h with 5 µM BLM produced 321 rad-equivalents of single strand DNA damage (Table 4). Consistent with the lower nuclear content, C-10 cells displayed less DNA damage (230 rad-equivalents) than the wild-type cells. D-10 cells exhibited considerably more initial DNA lesions than the A-253 cells. Within 1 h after removal of BLM, between 40 to 65% of the initial DNA damage in A-253 and C-10 cells was repaired while more than 80% of the initial injury in D-10 cells was repaired. Similarly, more than 90% of the initial DNA damage seen in D-10 cells resolved by 2 h, while only 50 to 70% of the initial DNA damage was repaired in C-10 and A-253 cells. Thus, at 2 h DNA damage in A-253 and C-10 cells was threefold greater than that in D-10 cells. DNA damage was not determined in G-11 cells.

DISCUSSION

Resistance is believed to be a major factor limiting the usefulness of our current chemotherapeutic agents. We have
cells (2, 5, 19). were incubated in suspension at 37°C with 1 \( \mu M \) \([3H]BLM A2\) (10 Ci/mmol) for various times. Cells were washed once with ice-cold medium containing serum and then centrifuged through oil to remove radioactivity in the medium. The accumulation of radioactivity was measured by liquid scintillation techniques as described in "Materials and Methods." Each value is the mean of six to 12 determinations. Bars, SEM.

Table 3 Nuclear content of radioactivity after incubation with \([3H]BLM A2\).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Nuclear content (dpm/10^6 cell)</th>
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<tbody>
<tr>
<td>A-253</td>
<td>8030 ± 160</td>
</tr>
<tr>
<td>D-10</td>
<td>8230 ± 130</td>
</tr>
<tr>
<td>G-11</td>
<td>6650 ± 150</td>
</tr>
<tr>
<td>C-10</td>
<td>4940 ± 660*</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) compared to A-253 cells using ANOVA and the Least Significant Difference Test (23).

Table 4 Effect of BLM on DNA integrity and DNA repair.

<table>
<thead>
<tr>
<th>Hours after removal of BLM</th>
<th>Rad-equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-253</td>
</tr>
<tr>
<td>0</td>
<td>321</td>
</tr>
<tr>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
</tr>
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devolved sublines of human tumor cells that have several features making them particularly suitable for mechanistic studies of BLM and BLM resistance. The human A-253 cells were derived from a cell type that normally responds to BLM in vivo (18). The sublines have levels of resistance that are clinically relevant and have been maintained for more than 2 years in culture. The cell cycle distributions in the sublines are similar to the parental line. Thus, different cytokinetic properties cannot explain the resistant phenotype. Furthermore, at least one subline (D-10) retained resistance to BLM A2 even after 60 population doubling times in drug-free medium.

Our results clearly indicate that BLM resistance need not produce cross-resistance to other natural products. The cross-resistance profile of our human sublines, in fact, indicates phenotypes that are highly selective for compounds in the BLM family. This is consistent with observations on nonhuman cells (2, 5, 19).

Because previous studies (4, 5, 18) suggested that inactivation of BLM maybe a mechanism of resistance, we examined the ability of all cells to inactivate BLM. The only known site on BLM that is metabolized is the carboxamide moiety of the \( \beta \)-alanine region of the molecule, which is deamidated (6, 7, 18) and at least 100-fold less active than BLM A2 (2, 7). Our results, like those of other investigators (4, 5), indicate that selection for a BLM-resistant phenotype can generate at least some cells with elevated in vitro levels of BLM hydrolase. C-10 cells did not, however, display elevated BLM hydrolase activity in vitro; this indicates other resistance mechanisms must be operative. In our other sublines, the importance of the elevated BLM hydrolase activity in producing the acquired BLM-resistance phenotype is unclear, because we were unable to detect significant quantities of BLM dA2 in intact cells in culture or in the medium. Roy and Horwitz (16) also reported no evidence of metabolism of \([3H]BLM A2\) with cultured HeLa cells. We cannot exclude, however, the possibilities that extensive metabolism occurred in subcellular drug compartments, such as the nucleus, or that the deamidated product might be metabolized further. Thus, further work is required to understand what role BLM hydrolase may have in determining resistance of tumor cells.

In all of the human cell types examined, cellular BLM A2 association reached equilibrium within 30 min. Similar kinetics of BLM association have been noted in other cell types by other investigators (16, 20). In two of our three sublines, i.e., C-10 and G-11, impaired cellular association of BLM A2 was seen. Such a reduction would be predicted if membrane alterations and/or decreased cellular uptake were mechanisms of BLM resistance (1, 2). C-10 cells had lower levels of nuclear radioactivity and DNA damage after BLM exposure compared to A-253 or D-10 cells. A similar decrease in nuclear radioactivity has been reported for rat cells with natural resistance to BLM (21). The D-10 subline was clearly distinguishable from C-10 and G-11 cells since it displayed identical amounts of cellular radioactive BLM A2 as compared to the A-253 cells. The resistance to BLM in D-10 cells may reflect an enhanced ability to repair DNA damage. Zuckerman et al. (19) identified a BLM-resistant subline of B16 melanoma cells that withstood or repaired single strand DNA breaks effectively. Based upon their cross-resistance to \( \alpha \)-irradiation (19), these B16 cells were similar to our G-11 cells but different from our D-10 subline. The D-10 cells did exhibit elevated initial DNA scissions and recent work by Smith (22) suggests that such enhanced single strand damage to genomic DNA may paradoxically stimulate cellular recovery and increase resistance to BLM lethality.

Thus, acquired BLM resistance in human tumor cells is associated with several resistant phenotypes. We have identified a new BLM analogue, liblomycin, which is equally active against all of these BLM-resistant phenotypes. This makes liblomycin unique among the compounds tested in the BLM family. Prior to liblomycin, alterations in the terminal amine moiety had not yielded substantially novel compounds. Based on our results with BLM-resistant cells, liblomycin may represent a useful agent with a therapeutic profile distinct from other BLM-like agents previously described.

ACKNOWLEDGMENTS

We are grateful to Drs. Said M. Sebti and James H. Harrison for their helpful comments.

Note Added In Proof

After submission of this manuscript, Urade et al. (Cancer, 61: 1501-1507, 1988) reported the establishment of three BLM-resistant human carcinoma cell lines.
REFERENCES

Characteristics of Bleomycin-resistant Phenotypes of Human Cell Sublines and Circumvention of Bleomycin Resistance by Liblomycin

John S. Lazo, I. Deborah Braun, David C. Labaree, et al.