Abnormally Banded Chromosomal Regions in Doxorubicin-resistant B16-BL6 Murine Melanoma Cells

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ABSTRACT

B16-BL6 murine melanoma cells were selected for cytogenetic evaluation during the stepwise development of increasing resistance in vivo to the antitumor antibiotic, doxorubicin (DOX). Karyotypic studies demonstrated extensive heteroploidy with both numerical and structural abnormalities which were not present in the parental DOX-sensitive B16-BL6 cells. Trypsin-Giemsia banding revealed the presence of several marker chromosomes containing abnormally banding regions (ABRs) in the 44-fold B16-BL6 DOX-resistant subline. These ABRs appeared to be more homogeneously staining at the higher DOX concentrations. Length measurements (ABR index) in seven banded metaphases indicated a direct correlation with increasing DOX concentration. When the DOX-resistant cells were grown in drug-free medium for 1 yr, the drug-resistant phenotype generally declined in parallel with the level of resistance and the ABR index. DOX-induced cytogenetic damage examined by sister chromatid exchange methodology in parental B16-BL6 cells indicated a linear sister chromatid exchange:DOX dose-response relationship. However, after continuous treatment of parental B16-BL6 cells with DOX (0.01 μg/ml) for 30 days, sister chromatid exchange scores were found to return to base-line values. The B16-BL6 resistant cells demonstrated a cross-resistant phenotype with A/-trifluoroacetyladriamycin-14-valerate, actinomycin D, and the Vinca alkaloids, but not with l-/β-D-arabinofuranose. The results suggest that ABR-containing chromosomes in DOX-resistant sublines may represent cytogenetic alterations of specific amplified genes involved in the expression of DOX resistance. Further studies are required to identify and define the possible gene products and to correlate their relationship to the cytotoxic action of doxorubicin.

INTRODUCTION

Adriamycin, DOX, is used clinically in the treatment of many human tumors including soft tissue and bone sarcomas, and breast, ovarian, and lung carcinomas (1). The usefulness of DOX, however, is often limited by the development of resistance to the drug and by cardiotoxicity. The antitumor activity of DOX is clearly multifactorial including (for reviews, see Refs. 2 and 3): (a) DNA intercalation; (b) inhibition of DNA and RNA polymerases; (c) alteration of tumor growth and cell cycle kinetics; (d) reduction to a semiquinone radical and the production of a hydroxyl radical which may damage the DNA; (e) modulation of the cell membrane activities with or without cellular entry; (f) inhibition of the mitochondrial electron transport system; and (g) covalent binding with DNA topoisomerase II to mediate DNA damage (4). The molecular mechanism(s) of DOX resistance is presently unknown (3); however, in vivo studies with DAU-resistant Ehrlich ascites tumor (5) and DAU- and DOX-resistant P388 murine leukemia (6, 7) suggest reduced cellular accumulation and retention of the drug as possible mechanisms of resistance to the cytotoxic effects of DAU and DOX.

Mechanisms of drug resistance fall into three general categories (8). The first group includes mutations which alter the target enzyme affinity. To date this group has not been associated with gene amplification. However, gene amplification has been observed in response to cytotoxic agents in several eukaryotic cell systems by either the overproduction of the drug target enzyme or by aberrant drug transport (for reviews, see Refs. 8 and 9). For example, drug-resistant cells that overexpress target-specific proteins by selective gene amplification include: (a) amplified DHFR genes in MTX resistance; (b) the trifunctional catalyst for the carbamyl P-synthetase, aspartate transcarbamylase, and dihydroorotase reactions of pyrimidine synthesis genes in N-(phosphonomethyl)-L-aspartate-resistant cells; and (c) hydroxymethyl CoA reductase in compactin-resistant cells. More recently, evidence has been presented wherein enhanced efflux of antibiotics resulted from duplication of a chromosomal sequence in tetracycline-resistant Escherichia coli (10). In addition, cytogenetic alterations associated with gene amplification have been described in resistance due to altered drug transport with Vinca alkaloids (11, 12), maytansine (13), COL (14–16), and DOX (16, 17). In situ hybridization studies have demonstrated that the amplified gene sequences may be localized to either expanded intrachromosomal regions that stain uniformly, known as HSRs, or to extrachromosomal, acentric chromatin paired elements known as DMs (reviewed in Refs. 8, 9, 11, and 18). ABRs have been recognized in tumor cell populations expressing low levels of antifolate resistance (18) as well as in Vinca alkaid-resistant cells (19). Thus, a relationship is apparent among several drug-resistant phenotypes and karyotypic manifestations associated with gene amplification.

Mammalian cell culture lines resistant to VCR (11, 12, 19, 20), ACT D (12), maytansine (13), COL (14, 15, 21), cytochalasin D (22), teniposide (23), and DOX (17, 24) have been reported to contain HSRs or DMs when exposed to high drug concentrations. In vitro and in vivo studies have demonstrated DOX-resistant cells to be characteristically cross-resistant to this heterogeneous group of pharmacological agents with unrelated structures and mechanisms of action. Although the molecular basis for the multidrug-resistant phenotype or PDR is not understood, the PDR phenotype suggests that the primary defect(s) may be localized in the cell membrane. Several reports, some with karyotypic changes, have provided evidence that increased drug resistance correlates with the increased expression of M, 150,000–180,000 plasma membrane glycoprotein family (11, 15, 25–27). However, direct proof that the overexpressed glycoprotein(s) is the sole mediator of PDR has not yet been established.
This paper describes the cytogenetic studies of B16-BL6 murine melanoma cells during the stepwise development of DOX resistance. Several large marker chromosomes containing ABRs were observed in the 44-fold B16-BL6 DOX (0.25 µg/ml)-resistant subline. Furthermore, the length of the ABRs in the resistant sublines is correlated with the degree of DOX resistance, and the karyotype of the 44-fold resistant cells was found to gradually decline in the absence of selection pressure over a 1-yr period. A preliminary report has been presented (28).

MATERIALS AND METHODS

Cell Line and Culture Conditions. The B16-BL6 murine melanoma cell line was a gift from Dr. Isaiah J. Fidler, M. D. Anderson Hospital and Tumor Institute, Houston, TX. The B16-BL6 parental and resistant sublines were maintained in MEM supplemented with 5% fetal bovine serum (HyClone Laboratories, Logan, UT), nonessential amino acids, vitamin solution, 2 mM l-glutamine, streptomycin (100 µg/ml), penicillin (100 units/ml), and 0.1 mM sodium pyruvate. All tissue culture media and supplements were obtained from M. A. Bioproducts, Walkersville, MD. Cells were subcultured weekly following treatment with trypsin-EDTA and incubated at 37°C in a humidified atmosphere consisting of 5% CO₂ plus 95% air. The B16-BL6 cells were routinely tested for the presence of Mycoplasma contamination as described by Chen (29).

Selection Scheme. Log phase cultures of B16-BL6 cells were treated continuously with DOX, and the drug concentration was increased 2- to 4-fold when the cells demonstrated adaptive growth patterns to a fixed extracellular DOX concentration (approximately 12 to 20 wk). No mutagenic pretreatment was used. At selected DOX levels, the resistant sublines were partially characterized, and stock cultures were stored in liquid nitrogen. Resistant sublines were grown without drug for at least 2 wk prior to experimental analysis.

Cytogenetic Studies. Log phase cultures were treated by the synchronized cytogenetic method described by Yunis (30) or by the standard method with a 2-h Colcemid (GIBCO, Grand Island, NY) pretreatment (0.10 µg/ml) prior to cell harvest. Briefly, trypsinized cells were centrifuged, resuspended in 0.075 M KCl for 30 min, and fixed with a 3:1 (v/v) methanol:glacial acetic acid solution. Cells were resuspended in 0.5 to 1.0 ml of fixative and dropped onto cold, wet slides from a distance of 3 ft. The slides were either GTG banded (31) or CNG banded (32). At least 30 metaphases were examined for each cytogenetic analysis. Murine chromosome nomenclature followed the convention described by Nessebitt and Franke (33). The terminology used to describe the aberrant chromosomes was that defined by Biedler et al. (34). Chromosomal lengths were measured in seven GTG-banded karyotypes with an opisometer in each of the following DOX-resistant sublines: 0.10 µg/ml; 0.25 µg/ml; 0.50 µg/ml; and 1.00 µg/ml. For Markers II to VII, the results were expressed as a ratio of the ABR length to the total length of the cell's chromosome complement. ABR index changes were analyzed by regression analyses.

Cytogenetic studies of parental B16-BL6 and B16-BL6 DOX (0.25 µg/ml)-resistant cells implanted in C57BL/6 mice were performed as described by Gibas et al. (35). Ten metaphases were examined per tumor.

SCE Analysis. Exponentially growing cells were exposed to a final concentration of 10 µg of 5-bromo-2′-deoxyuridine (Sigma Chemical Co., St. Louis, MO) per ml for two cell cycles of DNA replication (~30 h). Duplicate cultures were grown in the dark and harvested by the standard cytogenetic method as described above. B16-BL6 parental cells were exposed to either 0.01, 0.02, 0.05, or 0.10 µg of DOX per ml for 24 h prior to cell harvest. B16-BL6 parental cells treated continuously with DOX (0.01 µg/ml) for 4 wk during the selection of DOX-resistant cells were also studied. Untreated control cultures were analyzed concomitantly for spontaneous or base-line SCEs to prevent erroneous interpretation due to temperature fluctuations, batch differences in media or reagents, or the photolysis of 5-bromo-2′-deoxyuridine or DOX. A single lot of fetal bovine serum was used throughout the study.

Differential staining of the sister chromatids was performed by a modification of the fluorescence-plus-Giemsa technique (36). Briefly, the slides are stained with Hoechst 33258 (5 µg/ml) (Calbiochem, La Jolla, CA) in a 2x SSC solution, rinsed in distilled water, coverslipped with 2x SSC buffer, placed slanted in a 60-mm Petri dish containing 2x SSC, and illuminated under a UV lamp for 2 h. The slides were rinsed in distilled water and stained for 20 min with a 4% Giemsa stain.

For each DOX concentration, 30 metaphases from duplicate cultures were analyzed. Each point of exchange was counted as one SCE. Because of the heterogeneous cell population and variability in the chromosome number per metaphase, the data were expressed as SCEs per chromosome (37). To normalize the data, the mean SCE:chromosome ratio of the untreated control (base line) was subtracted from each individual test SCE:chromosome ratio for the appropriate day of study.

Drug Sensitivity Assays. Cytotoxic effects of DOX were determined by treating cells for 3 h with varying concentrations of DOX (0.01 to 10.00 µg/ml), washing twice with a balanced salt solution, and reincubating for an additional 72 h at 37°C in supplemented MEM. Cell counts from triplicate flasks were determined by trypan blue dye exclusion using a hemacytometer. The average cell number at each drug concentration expressed as a percentage (mean ± SE) of the untreated control was plotted against the drug concentration on a logarithmic scale. Tests for the statistical significance of differences between the slopes of each subline followed regression analysis.

Cross-resistance studies were performed by the method described above with AD-32, ACT D, ARA C, VCR, and VBL. AD-32 was a gift from Dr. M. Israel, University of Tennessee, Memphis, TN. DOX, ACT D, ARA C, and VBL were dissolved in sterile distilled water, and working solutions were prepared in MEM. Dimethyl sulfoxide was used to dissolve and make dilutions of AD-32. The final concentration of dimethyl sulfoxide in control and treated cultures was 1%. Control flasks contained equal volumes of the drug-free diluent.

RESULTS AND DISCUSSION

The multistep selection scheme of B16-BL6 murine melanoma cells to increasing concentrations of DOX is summarized in Fig. 1. Doubling times for both the B16-BL6 parental cells and the DOX (0.25 µg/ml)-resistant subline were approxi-
mately 15 h. The 44-fold B16-BL6 DOX (0.25 μg/ml)-resistant subline exhibited the typical pattern of cross-resistance to ACT D, AD-32, VCR, and VBL (Fig. 2) which is characteristic of PDR. No significant cross-resistance to aRA C was detected (P = 0.08).

Karyotypic studies were performed in the parental B16-BL6 cells and four DOX-resistant B16-BL6 sublines (0.10, 0.25, 0.50, and 1.00 μg of DOX per ml). The B16-BL6 parental cells were characterized by a modal number of 74 chromosomes with a modal range of 59 to 62 chromosomes was observed in the cells of these heterogeneous subpopulations. These findings indicate either the loss of chromosomes or the presence of multiple complex cytogenetic rearrangements. Fig. 4 is a representative karyotype from the 44-fold B16-BL6 DOX-resistant, 0.25-μg/ml subline which is characterized by several marker chromosomes (Mar II–IV) containing ABRs. With increasing DOX resistance, several additional marker chromosomes evolved, and the ABRs appeared to become more homogeneously staining. The marker chromosomes (Fig. 5; Markers II–VII) were categorized according to their length, GTG, and CNG banding patterns. Measurements of seven GTG-banded karyotypes at the four DOX drug levels (Fig. 5; Markers II-VII) were categorized according to their length, GTG, and CNG banding patterns. Measurements of seven GTG-banded karyotypes at the four DOX drug levels (Fig. 5) were analyzed for their length and degree of DOX resistance (Table 1). DMs were observed in only 10% of the metaphases screened in the DOX-resistant subline cultured in the presence of DOX (1.00 μg/ml).

Since the abnormal banding pattern precluded the identification of the derivative chromosome(s), a modification of the stepwise selection protocol using lower DOX concentrations (B16-BL6 parental → 0.01 μg → 0.025 μg → 0.05 μg of DOX per ml) was undertaken. At DOX resistance of 0.05 μg/ml, different yet distinct cytological ABR markers which demonstrated a repetitive pattern were present in all metaphases (n = 50) in one of three forms: a large ring marker chromosome; 2 identical marker chromosomes with 5 CNG-positive banding regions interspersed by ABRs; or a fusion of the markers to form one long derivative chromosome with twice as many heterochromatic banding regions (Fig. 6). These findings are in agreement with the variable appearing ABRs found in several different MTX-resistant sublines expressing less than 87-fold increases of DHFR activity (18).

Several independently derived cell lines resistant to various selecting agents at high drug concentrations have limited tumorigenic potential (11, 38) thus preventing their use as model systems for new in vivo therapy-resistant studies. The B16-BL6 DOX (0.25 μg/ml)-resistant subline exhibited a tumorigenic incidence in syngeneic C57BL/6 mice comparable to the parental sensitive line. Karyotypic examination confirmed the presence of either the B16-BL6 parental or resistant subline tumor origin in 5 of 5 tumor-implanted footpads for each of the cell lines.

To assess the stability of the DOX-resistant phenotype, the DOX (0.25 μg/ml)-resistant subline was cultured in the absence of drug for 12 mo (~500 doublings). After 6 mo in drug-free medium, there was no significant change in the drug resistance phenotype or the ABR index. However, when grown in the absence of selection pressure for 12 mo, the ABR index decreased from 0.137 ± 0.020 to 0.105 ± 0.014 (P = 0.07) (Table 1). Drug sensitivity studies indicated a 68% decrease in the relative drug resistance by a comparison of the ID50 of the B16-BL6 DOX-resistant (0.25 μg/ml) subline continuously exposed to drug to that of its counterpart cultured for 1 yr in the absence of DOX (Fig. 2). Although the DOX-resistant phenotype is unstable, phenotypic reversion and a decrease in the ABR length are more gradual than the rapid reversion associated withacentromeric DMs (8, 9, 12, 14, 17).

![Fig. 2. DOX sensitivity and cross-resistant studies to various antineoplastic agents in the murine parental B16-BL6 cells (O); the DOX (0.25 μg/ml-resistant subline continuously exposed (CE) to drug (•); and the DOX (0.25 μg/ml)-resistant variant grown in drug-free (DF) medium for 1 yr (©). Points, mean of at least two separate experiments; three replicates were analyzed per concentration. Drug sensitivity assays were carried out as described in "Materials and Methods" with the exception of a 12-h drug exposure for VCR and VBL. The cross-resistance as estimated from the ID50 of the lines was as follows: ~25-fold for VCR (P = 0.0001) and VBL (P = 0.005); 10-fold cross-resistance to ACT D (P = 0.0001); 5-fold cross-resistance to AD-32 (P = 0.02). No cross-resistance to ARA C (P = 0.08) was observed. Bars, SE.](image-url)
ABRs IN B16-BL6 DOX-RESISTANT CELLS

Fig. 3. Karyotype of parental B16-BL6 cells. The modal number is 74 chromosomes. The marker chromosomes (labeled mar 1-14) have the following karyotypic designations: mar 1, T(3G;13A5); mar 2, R(10.16); mar 3, T(3F3; 16B2); mar 4, T(XDr:15A2); mar 5, I(12.12); mar 6, T(9A5.12); mar 7, T(9C.12); mar 8, Dp(14C?); mar 9, Df(5C1-D); mar 10, I(19.19); mar 11, Df(14B-D3); mar 12, T(3G;16A?); mar 13, Df(13B-D3)?; mar 14, centric fragment.

The mechanisms by which gene amplification may result are unknown. One proposal suggests that agents that affect DNA synthesis, repair, or recombination may facilitate gene amplification events by unequal sister chromatid exchanges (8, 9). This phenomenon may occur by a repetitive, staggered, mispairing recombination process. Such a model would be supported by complex chromosomal rearrangements as those seen in the B16-BL6 DOX-resistant cells. To assess the cytogenetic damage of DOX, parental B16-BL6 murine melanoma cells were treated for 24 h with DOX concentrations of 0.01, 0.02, 0.05, and 0.10 µg/ml as well as continuously for 4 wk with DOX (0.01 µg/ml). No mitotic figures were observed at 0.10 µg/ml, presumably because the B16-BL6 sensitive cells were blocked in the G2 phase of the cell cycle (39). The SCE results are presented in Fig. 7. The variability in SCE scores is consistent with a heterogeneous tumor cell population. The results indicate the SCE frequency of the parental cells exposed to the drug for 24 h varied directly and linearly with DOX concentration (P = 0.0001). However, after 4 wk of continuous drug treatment at a DOX concentration of 0.01 µg/ml, the SCE effects (0.23 ± 0.06 SCE/chromosome) were indistinguishable from base-line scores (0.22 SCE/chromosome). It was also of interest that chromosomal fragmentation and asymmetrical radial formations increased with drug dose in these murine melanoma cells. These results are in agreement with DOX having been established as a potent SCE inducer in Chinese hamster cells (40) and human lymphocytes (40, 41). Furthermore, the adaptive behavior pattern of the B16-BL6 parental cells as shown by SCE studies after a continuous exposure to DOX (0.01 µg/ml) for 1 mo may be an indication of the capacity of these melanoma cells to tolerate small dose increases of DOX. However, whether such mechanisms are responsible for the putative gene amplification events associated with DOX resistance remains to be answered.

Few in vitro solid tumor model systems developed specifically for DOX resistance have reported cytological evidence of gene amplification (17, 24). The presence of multiple minutes and an HSR on chromosome 4 was first reported by Massino and Kopnin in 270-fold DOX-resistant Djungarian hamster cells continuously exposed to DOX (0.80 µg/ml) (24). In addition,
Fig. 4. Karyotype of the B16-BL6 DOX (0.25 μg/ml)-resistant subline. This representative karyotype is characterized by 61 chromosomes. Marks 9–14 were also present in the parental karyotype. Markers A and B are random chromosomal changes; mar A appears to be a derivative of No. 9, whereas mar B is a translocation involving chromosomes 12 and 14. Mar I, Mar II, Mar III, and Mar IV are markers unique to the DOX-resistant cells.

Howell et al. (17) have reported the isolation of an unstable, 3000-fold DOX-resistant Chinese hamster V79 mutant cell line characterized by the presence of approximately 4 DMs/cell. In both studies, amplified DNA sequences which appear to be unique to DOX resistance were identified (16, 42).

Molecular genetic studies are needed to confirm and identify any putative amplified gene sequences in the B16-BL6 DOX-resistant cells. Possible candidates for amplified genes in DOX-resistant cells are related to the PDR phenotype as well as DOX resistance. The overproduction of a detoxification enzyme, glutathione S-transferase, has been reported in a human breast DOX-resistant cell line (43). A second candidate is a M, 19,000–26,000 cytosol protein (pI = 5.7) that is overproduced in VCR-resistant cells (12, 19), COL- and DOX-resistant cells (44), and ACT D-resistant cells (12). Another cytosol protein (M, 30,000; pI = 5.2) has been described in VBL-resistant human leukemia cells (27). Possibly these proteins are related, and the variability in molecular weight and isoelectric point may be accounted for by species, cell type, or technical variations.

Other favorable gene products include the apparent members of the overexpressed membrane-bound glycoprotein gene family demonstrated in multidrug-resistant cell lines (11, 15, 25–27, 45). The most extensively studied of these is the amplified high-molecular-weight plasma membrane glycoprotein(s) (M, 150,000–180,000) which correlates well with the degree of drug resistance. Recently, amplified P-glycoprotein (M, 170,000) gene sequences have been demonstrated to be actively expressed and localized to a HSR-bearing chromosome in a 650-fold COL-resistant Chinese hamster ovary cell line (15). The availability of the appropriate complementary DNA probes should provide an approach to identify, localize, and define the pres-
Fig. 5. DOX-resistant marker chromosomes. The markers are shown first by their GTG-banding pattern followed by their CNG-banding pattern. MAR I is a fusion product of the two copies of Mar I found in the parental karyotype and is not considered an ABR-containing chromosome. MAR II–MAR VII are categorized as the ABR-containing chromosomes. MAR VII may be a shortened derivative of MAR VI (this chromosome was only found at the highest level of DOX resistance).

Table 1 Mean ABR length measurements in parental sensitive and DOX-resistant sublines of B16-BL6 mouse melanoma

<table>
<thead>
<tr>
<th>Sublines</th>
<th>Relative resistance</th>
<th>Chromosomes/ cell</th>
<th>ABR index</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16/parent</td>
<td>1</td>
<td>71 ± 3.4*</td>
<td>0</td>
</tr>
<tr>
<td>B16/DOX (0.10 µg/ml)</td>
<td>10</td>
<td>62 ± 1.7</td>
<td>0.110 ± 0.019</td>
</tr>
<tr>
<td>B16/DOX (0.25 µg/ml), DF*</td>
<td>14</td>
<td>57 ± 3.6</td>
<td>0.105 ± 0.014</td>
</tr>
<tr>
<td>B16/DOX (0.25 µg/ml)</td>
<td>44</td>
<td>57 ± 6.6</td>
<td>0.137 ± 0.020</td>
</tr>
<tr>
<td>B16/DOX (0.50 µg/ml)</td>
<td>60</td>
<td>60 ± 2.2</td>
<td>0.156 ± 0.013</td>
</tr>
<tr>
<td>B16/DOX (1.00 µg/ml)</td>
<td>176</td>
<td>60 ± 1.8</td>
<td>0.191 ± 0.020</td>
</tr>
</tbody>
</table>

* The relative resistance is defined as the ID₅₀ of the resistant sublines divided by the ID₅₀ of the parental line after a 3-h pulse exposure to DOX.

* The chromosome counts of the seven GTG-banded karyotypes used for the length measurements.

* The length of the ABR-containing chromosomes compared to the total length of the cell's chromosome complement.

* Mean ± SD.

* DF, the DOX-resistant (0.25 µg/ml) variant subline maintained in DOX-free medium for 12 mo.

Fig. 6. Marker chromosomes in B16-BL6 DOX (0.05 µg/ml)-resistant cells. Three different DOX-resistant markers were identified by GTG banding in this resistant subline. A repetitive pattern is demonstrated by the CNG-banded marker. These studies indicate reproducible gene amplification-associated cytogenetic rearrangements are possible in DOX-resistant B16-BL6 cells.

Fig. 7. SCE frequencies in the B16-BL6 DOX-sensitive cells. Duplicate cultures containing a final concentration of 10 µg of 5-bromo-2'-deoxyuridine per ml after a 24-h DOX drug exposure were scored. Sixty metaphases were analyzed at each drug concentration. The mean SCE:chromosome ratio of the untreated control (base line) was subtracted from each individual test SCE:chromosome ratio for the appropriate day of study to normalize the data. The SCE frequency of the sensitive cells varied directly and linearly with DOX concentrations (P = 0.0001). Points, the adjusted SCE:chromosome means; bars, SD.

ence of these and other possible gene products. Further investigations are warranted to ensure that the amplified gene sequences are transcriptionally active, to explain the variability in the cross-resistant patterns, and to describe any possible relationship the putative gene product candidates may have to one another.

Based on these preliminary findings, the B16-BL6 DOX-resistant subline appears to be a promising in vitro and in vivo model system for studying the mechanisms of doxorubicin resistance and its associated genetic regulatory mechanisms in solid tumors. An opportunity to study the apparent complex genetic alterations underlying the various biochemical changes associated with the development of DOX resistance could provide a basis for the understanding of the cellular and molecular mechanisms involved in multidrug resistance.
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