Genetics of Multidrug Resistance: Relationship of a Cloned Gene to the Complete Multidrug Resistant Phenotype

James M. Croop, Braydon C. Guild, Philippe Gros, and David E. Housman

INTRODUCTION

The development of drug resistance remains a major cause of failure in cancer chemotherapy. Tumors which are initially sensitive to a chemotherapeutic agent often recur and are resistant to a broad spectrum of cytotoxic drugs. Drug resistant cell lines developed in vitro by stepwise selection with a single chemotherapeutic agent have been useful in understanding the molecular basis of the phenomenon of multidrug resistance. These cell lines display cross-resistance to a broad spectrum of chemotherapeutic agents (1-4). Multidrug resistant cell lines (5-8) designated mdr (8, 9). A 4.5-5.0-kilobase mRNA encoded by a genomic region which contains a small group of related genes of mouse (5), hamster (6, 7), and human origin (8) amplify a chemotherapeutic agents which characterize the multidrug resistant phenotype. The expression of this complementary DNA in transfected clones is dependent upon the number of copies of mdr mRNA, and the selective conditions placed on transfected drug resistant clones. Finally, the drug resistant phenotype is reversed in the transfected clones by verapamil just as drug resistance is reversed in multidrug resistant cell lines.

Previously, we demonstrated that drug sensitive Chinese hamster ovary cells become resistant to Adriamycin (doxorubicin), colchicine, and vinblastine following transfection of the expression vector p91023B containing a mouse cDNA to the genome and the expression of ADR11 can be modulated by releasing an individual clone from selective pressure or by increasing the selective pressure on the clone. The endogenous sequences encoding the multidrug resistance gene are not amplified in transfected drug resistant cell lines (10). We have examined the relationship between gene copy number, expression levels of the mdr mRNA, and the selective conditions placed on transfected clones. The results indicate that the expression of ADR11 is dependent on the number of copies of the cDNA integrated in the genome as well as the selective pressure present during initial selection of the clones. Similar results are observed when an individual clone is removed from selection and then exposed to increased selective pressure in a stepwise fashion. The number of copies of ADR11 in the genome and expression of ADR11 is modulated by the selective conditions placed on the clone. We demonstrate that the cells expressing increased levels of ADR11 are resistant to the same broad spectrum of chemotherapeutic agents which characterize the multidrug resistant phenotype. Finally, we show that the transfected clones expressing ADR11 exhibit collateral sensitivity and reversal of drug resistance when exposed to verapamil in a manner similar to that for multidrug resistant cell lines.

These observations indicate that the drug resistant clones generated by transfection with pBAmdr display many of the characteristics of multidrug resistant cell lines produced by stepwise selection in vitro. It is therefore likely that the mdr mRNA expressed in drug sensitive B-cells encoded by ADR11 encodes a protein which is capable of conveying the character-
istics of multidrug resistant cell lines to previously drug resistant cells.

MATERIALS AND METHODS

Materials. Adriamycin was obtained from Adria Laboratories, Actinomycin D from Merck and Co., and vinblastine sulfate from Eli Lilly and Co. Colchicine, daunomycin, and verapamil were obtained from Sigma Chemical Co. G418 was obtained from GIBCO. Enzymes were obtained from New England Biolabs and Amersham Corp. [α-32P]dCTP and Co. Colchicine, daunomycin, and verapamil were obtained from New England Nuclear.

Transfection of Expression Vectors. The NIH 3T3 y-AM subclone (22) was grown in minimum essential medium (GIBCO Laboratories) supplemented with 10% fetal calf serum, l-glutamine (2 mM), penicillin, and streptomycin. Cells were grown on plastic surfaces at 37°C in an atmosphere containing 5% CO2. This cell line, the drug sensitive parental cell line, was transfected with either 10 µg of pBAmdr, the expression vector containing λDR11, or cotransfected with 10 µg of pBAmdr and 1 µg of an expression vector containing the neomycin resistance gene3 using a standard calcium phosphate technique (23). Similar experiments were performed with λDR11 in the expression vector in the antisense orientation. After 48 h in growth media, the cells transfected with the vector containing λDR11 alone were subcultured in a dilution of 1:20 into selective media containing Adriamycin at 0.05 µg/ml or Adriamycin at 0.1 µg/ml while the cells cotransfected with the neomycin resistance vector were sub cultured into media containing G418 at 1 mg/ml.

Drug Resistant Phenotype. Colony formation assays were performed with 100 cells plated into 60-mm dishes. Adriamycin was present in the growth media at 0, 12.5, 25, or 50 ng/ml. Colonies were counted at 10 days after staining with 1% methylene blue in phosphate buffered saline:ethanol (1:1, v/v) and washing with water. The percentage of colonies surviving each concentration of Adriamycin relative to the colonies in the plates without Adriamycin is indicated in Table 2. All experiments were done in duplicate.

Drug resistance in mass culture was assayed in 96-well microtiter plates (24). Cells (3000 in 0.2 ml of standard growth media) were plated into each well containing the cytotoxic agent. Experiments were done in duplicate. Final concentrations were: Adriamycin, 480 ng/ml; daunorubicin, 250 ng/ml; colchicine, 50 µg/ml; vinblastine, 25 µg/ml; and actinomycin D, 12.5 ng/ml. Cells were grown for 72 h, fixed in methanol for 15 min, then stained with a 2% crystal violet (J. T. Baker Chemical Co.) solution in ethanol and, finally, rinsed in water. The stained dishes were scanned on a Titertek Multiscan densitometer with a 492 nm filter. The relative absorbance of each well represented the number or remaining cells in the well. Survival is plotted as the percentage of colonies in 5 µM verapamil relative to the colonies plated without verapamil. All experiments were done in duplicate.

RESULTS

Construction of Expression Vectors. The full length mdr cDNA clone λDR11 was inserted into the expression vector pBA which includes Moloney long terminal repeats with an enhancer deletion in the 3’ long terminal repeat and the β-actin promoter (29) (Fig. 1). BclI linkers were ligated to the mdr cDNA which was then cloned into the unique BamHI site of pBA. The β-actin transcriptional elements promote the expression of λDR11. Expression vectors with λDR11 in both sense, pBAmdr, and antisense orientations, pBArdm, were isolated. A similar vector with the neomycin resistance gene (30) inserted at the BamHI site was also constructed.

Selection of Drug Resistant Clones. Transfection of the expression vector with λDR11 in the sense orientation, pBAmdr, yielded colonies which were first visible at approximately 4 weeks during continuous selection with Adriamycin. From 2 to 20 colonies appeared on each plate and these clones were isolated after a total of 5-6 weeks of selection. Colonies were not observed from the cells transfected with λDR11 in the reverse orientation. Colonies 54 and 56 were isolated from cells selected in 0.05 µg/ml Adriamycin and colonies 11 and 13 were isolated from cells selected in 0.1 µg/ml Adriamycin. Colony 13 was maintained in growth media not supplemented with Adriamycin for 4 months (clone 13.C) and was then grown in increasing concentrations of Adriamycin at 0.1, 0.2, and 0.4 µg/ml (clones 13.1, 13.2, 13.4, respectively). Approximately 2-3 weeks were required at each drug concentration before the cells would grow continuously at the new level of selective pressure.

Colonies from the cells cotransfected with the neomycin vector and selected in G418 were isolated after 2 weeks of selection. Colony A7 is a representative of a cotransfected colony.

Analysis of Genomic DNA. DNA was isolated from individual colonies for analysis of the number of copies of the expression vector integrated into the genome. DNA was digested with the restriction endonuclease KpnI. KpnI digestion of the expression vector releases a 3.8- and a 1.8-kilobase fragment containing all of the sequences of λDR11 in the expression vector (Fig. 1). The DNA was electrophoresed, transferred to hybridization

search Laboratories, Inc.) as the RNase inhibitor (28). RNA (10 µg, except for the drug sensitive parental line which was 20 µg) was fractionated on formaldehyde denaturing agarose gels and transferred to a reusable hybridization membrane (Gene Screen Plus; New England Nuclear) by electroblotting in 25 mM sodium phosphate buffer (pH 7).

RNA-DNA hybridization was carried out for 18 h at 65°C in the presence of 1 M NaCl, 1% sodium dodecyl sulfate, 10% dextran sulfate, 400 ng of denatured salmon sperm DNA per ml, and 107 cpm of 32P-labeled hybridization probe. Blots were washed to a final stringency of 60 mM NaCl-60 mM sodium citrate-0.1% sodium dodecyl sulfate at 65°C and exposed to preflashed film for 1 to 72 h with and without an intensifying screen prior to densitometric analysis.

Studies with Verapamil. The effect of verapamil was analyzed in both the presence and the absence of the cytotoxic agent Adriamycin. To assay the cloning efficiency of the parental and the transfected drug resistant cell lines, 100 cells were plated in 60-mm dishes in growth media containing either 0 or 5 µM verapamil. In experiments which included Adriamycin, the cells were grown at the concentration of Adriamycin at which the drug resistant clone was normally maintained. Colonies were counted at 10 days after staining with 1% methylene blue in phosphate buffered saline:ethanol (1:1, v/v) and washing in water. Survival is plotted as the percentage of colonies in 5 µM verapamil relative to the colonies in the plates without verapamil. All experiments were done in duplicate.

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membranes, and probed with λDR11 to identify copies of the cDNA transfected into the cells (Fig. 2). The same membrane was rehybridized with Fis-1 (31), a single copy gene, to normalize the amount of DNA in each lane (data not shown).

The endogenous mouse sequences of the mdr gene(s) which hybridize to λDR11 are clearly seen as a series of high molecular weight bands in the nontransfected parental cell line (Fig. 2, Lanes a and j) and the transfected colonies which have been in drug selection (Fig. 2, Lanes b-i, k). There appears to have been no amplification of the endogenous sequences in the transfected colonies when the lanes are normalized for the amount of DNA loaded. DNA from each of the transfected colonies contained the predicted 3.8- and 1.8-kilobase restriction fragments which hybridize to λDR11, neither of which are present in the parental cell line. The intensity of hybridization of these restriction fragments indicates that the number of copies of λDR11 present in the genome at each level of selective pressure was thus dependent upon the amount of continued selective pressure. The endogenous mdr sequences did not appear to increase in copy number when normalized for the amount of DNA loaded in each lane.

Just as the number of copies of the transfected mdr cDNA correlated with the presence or absence of selective pressure, the amount of 4.5–5-kilobase mRNA also was dependent on the concentration of Adriamycin. Increasing concentrations of Adriamycin from 0.1 to 0.2 and finally 0.4 μg/ml resulted in the loss of XDR11 copies from 8 to 3 (Fig. 2, Lane K).

Comparison of the clones selected at 0.05 μg/ml Adriamycin to those selected at 0.1 μg/ml Adriamycin indicates that the amount of mdr mRNA expressed was also dependent on the selective pressure placed on the cells. Cell lines with a similar number of copies of the introduced mdr cDNA expressed more of the specific mdr mRNA if the clone had been selected at higher concentrations of Adriamycin (Table 1).

Analysis of Selective Pressure. Clone 13 was chosen to analyze the effect of selective pressure on a transfected multidrug resistant cell line. Clone 13 was grown in growth media not supplemented with Adriamycin for 4 months, clone 13.C, which resulted in the loss of λDR11 copies from 8 to 3 (Fig. 2, Lane K; Table 1). Clone 13.C was then grown in increasing concentrations of Adriamycin from 0.1 to 0.2 and finally 0.4 μg/ml resulting in amplification of the transfected mdr cDNA from 3 to 9 to 11 copies of λDR11 in the genome of clones 13.1, 13.2, and 13.4, respectively (Fig. 2, Lanes g-i; Table 1). The number of copies of the expression vector in the genome was thus dependent upon the amount of continued selective pressure. The endogenous mdr sequences did not appear to increase in copy number when normalized for the amount of DNA loaded in each lane.

Just as the number of copies of the transfected mdr cDNA correlated with the presence or absence of selective pressure, the amount of 4.5–5-kilobase mRNA also was dependent on the concentration of Adriamycin. Increasing concentrations of Adriamycin from 0.1 to 0.2 to 0.4 μg/ml resulted in increased expression of the mdr mRNA species which hybridized to the mdr sequences. This correlates with the predicted size of the mRNA transcript from the expression vector. The 4.5–5-kilobase mRNA species in the transfected clones was approximately 3–800-fold more abundant than the endogenous mdr mRNA species in the parental cell line (Table 1). The endogenous mdr mRNA species could be just detected at base line levels when twice the amount of RNA from the drug sensitive parental cell line was loaded (Fig. 3, Lane a). There appears to be a general correlation between the overexpression of the mdr mRNA species and the copy number of λDR11 present in the genome at each level of selective pressure (Table 1). Higher copy numbers of λDR11 correlated with increased levels of expression of the mRNA at each level of selective pressure.

Analysis of Drug Resistance. One clone from each of the selection methods, G418 for the clones cotransfected with the neomycin vector or Adriamycin at either 0.05 or 0.1 μg/ml, was analyzed in a colony forming assay in the presence of increasing concentrations of Adriamycin (Table 2). All of the clones displayed resistance to Adriamycin when compared to the parental cell line. Resistance was proportional to the concentration of Adriamycin used in selecting the clone. Clone A7 which had been selected in G418 was least resistant, clone 56 which had been selected in 0.05 μg/ml of Adriamycin was intermediate in resistance, and clone 13 which had been selected in 0.1 μg/ml of Adriamycin was most resistant. This correlated with the amount of specific mdr mRNA detected in each of the clones.

Five of the transfected colonies were analyzed in mass culture for resistance to the chemotherapeutic agents which characterize the multidrug resistant phenotype. Cytotoxicity was analyzed by exposing 2 × 10^5 cells in microtiter wells to Adriamycin, daunorubicin, colchicine, vinblastine, and actinomycin D for 72 h and determining the number of cells surviving. All five clones were resistant to this broad spectrum of cytotoxic agents (Fig. 4).

Analysis of Selective Pressure. Clone 13 was chosen to analyze the effect of selective pressure on a transfected multidrug resistant cell line. Clone 13 was grown in growth media not supplemented with Adriamycin for 4 months, clone 13.C, which resulted in the loss of λDR11 copies from 8 to 3 (Fig. 2, Lane K; Table 1). Clone 13.C was then grown in increasing concentrations of Adriamycin from 0.1 to 0.2 and finally 0.4 μg/ml resulting in amplification of the transfected mdr cDNA from 3 to 9 to 11 copies of λDR11 in the genome of clones 13.1, 13.2, and 13.4, respectively (Fig. 2, Lanes g-i; Table 1). The number of copies of the expression vector in the genome was thus dependent upon the amount of continued selective pressure. The endogenous mdr sequences did not appear to increase in copy number when normalized for the amount of DNA loaded in each lane.
Fig. 2. Southern analysis of genomic DNA of transfected clones. DNA was digested with KpnI and probed with λDR11. KpnI digestion releases a 3.8- and a 1.8-kilobase (kb) size fragment from pBAmdr containing the sequences of λDR11 (arrows). High molecular weight bands represent the endogenous mdr gene. Lane a, parental cell line; Lane b, clone A7; Lane c, clone 54; Lane d, clone 56; Lane e, clone 11; Lane f, clone 13; Lane g, clone 13.1; Lane h, clone 13.2; Lane i, clone 13.4; Lane j, parental cell line; Lane k, clone 13.C.

Table 1 Integration and expression of λDR11 in transfected multidrug resistant clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Vector copies</th>
<th>RNA level</th>
</tr>
</thead>
<tbody>
<tr>
<td>A7</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>54</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>56</td>
<td>6</td>
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</tr>
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</tr>
<tr>
<td>13.1</td>
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<td>186</td>
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<tr>
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<td>9</td>
<td>300</td>
</tr>
<tr>
<td>13.4</td>
<td>11</td>
<td>801</td>
</tr>
</tbody>
</table>

expression of the specific mdr message quite dramatically in clones 13.1, 13.2, and 13.4 (Fig. 3, Lanes g-i) while continued culture in Adriamycin-free media resulted in a significant reduction in the amount of the transcript in clone 13.C (Table 1).

Increasing the selective pressure of Adriamycin by continuous growth of clone 13.C in 0.1, then 0.2, and finally 0.4 μg/ml increased resistance to the anthracyclines and the Vinca alkaloids (Fig. 4).

Reversal of Drug Resistance with Verapamil. Verapamil has previously been shown to reverse the multidrug resistant phenotype in a wide variety of multidrug resistant cell lines (21). To analyze the effects of verapamil on the multidrug resistant phenotype conveyed by λDR11, the derivatives of clone 13 growing in 0.1, 0.2, and 0.4 μg/ml of Adriamycin and the drug sensitive parental cell line were grown in 0 or 5 μM verapamil in the presence or absence of Adriamycin. Fig. 5 indicates that when grown in the absence of Adriamycin the multidrug resistant clones displayed a modest amount of toxicity to verapamil relative to the drug sensitive parental cell line. Drug resistance was completely abolished by verapamil when the multidrug resistant clones were grown in the presence of Adriamycin at the concentration at which each clone was normally maintained, as indicated in Fig. 5.

DISCUSSION

The isolation of molecular probes from multidrug resistant cell lines has started to lead to an understanding of the mechanism of multidrug resistance. These cell lines contain double...
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Table 2 Colony forming assay of transfected clones
Parental cell line (Am) and clones A7, 56, and 13 were compared for the ability to form colonies when grown in increasing concentrations of Adriamycin. Values are the percentage of colonies surviving at each concentration of Adriamycin relative to the colonies in the plates without Adriamycin.

<table>
<thead>
<tr>
<th>Concentration of Adriamycin</th>
<th>Clone</th>
<th>12.5 ng/ml</th>
<th>25 ng/ml</th>
<th>50 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>27</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>40</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>69</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 4](image)

Fig. 4. Analysis of multidrug resistant phenotype of transfected clones. Mass cultures of $2 \times 10^5$ cells in microtiter wells exposed to cytotoxic agents were stained and scored for survival after 72 h. Survival of the parental cell line (Am) and clones A7, 54, 56, 11, 13, 13.1, 13.2, and 13.4 in Adriamycin (480 ng/ml), daunorubicin (250 ng/ml), colchicine (50 ng/ml), vinblastine (25 ng/ml), and actinomycin D (12.5 ng/ml). Ordinate, percentage of survival in drug compared to the respective control plated without drug.

![Figure 5](image)

Fig. 5. Effects of verapamil on colony formation. ■, parental cell line (Am) and clones 13.1, 13.2, and 13.4 grown without Adriamycin; □, same lines grown with Adriamycin at the concentration in which each clone is normally maintained. Ordinate, percentage of colonies surviving in 5 μM verapamil relative to the number of colonies surviving without verapamil.

Minute chromosomes or homogeneously staining regions (33–35) which suggest gene amplification. Using molecular techniques, independently derived multidrug resistant cell lines have been shown to amplify a common region of the genome (36). We have isolated a series of unique cDNA clones from a drug sensitive mouse cell line complementary to the mRNA encoded in the amplified region (6) and overexpressed in multidrug resistant cell lines (5–8). The cDNA clones, as well as previous Southern hybridization data (6), indicate that a family of closely related genes are amplified in multidrug resistant cell lines. One of these cDNA clones, XDR11, is capable of conveying the complete multidrug resistant phenotype. The human cDNA analogue to λDR11 has also recently been cloned and sequenced (16). The human cDNA, mdr1, and λDR11 share very high homology at both the nucleic acid and predicted amino acid level (data not shown). It is not clear if λDR11 and mdr1 represent transcripts from the same gene or different members of the family of mdr genes. Cross-hybridization studies with molecular probes to gp170 (7) and the human cDNA clone indicate that the mdr1 cDNA is also closely related to the cDNA encoding gp170 (37).

We have previously shown that λDR11 will convey the multidrug resistant phenotype to drug sensitive cells in the expression vector p91023b which includes sequences capable of amplification (9). We report here that λDR11 can convey the complete multidrug resistant phenotype in an expression vector where the β-actin promoter drives the transcription of the λDR11 cDNA. The specific features of p91023b, other than λDR11, are not required for drug resistance.

Analysis of the multidrug resistant phenotype conveyed by the expression vector indicated that each of the transfected clones was resistant to a broad range of chemotherapeutic agents. Five clones were analyzed using a rapid cytotoxicity assay which evaluates mass population of cells. All five of the clones were resistant to Adriamycin, daunoymycin, colchicine, vinblastine, and actinomycin D. Although there were differences in the numbers of copies in the genome of the expression vector and differences in the expression of λDR11 in each clone, cell survival was relatively similar. This assay indicates that a broad range of drug resistance will permit similar survival of cells in mass culture during a short exposure to a cytotoxic agent. This is in contrast to cytotoxicity measured in the colony formation assay where colony formation was directly related to selective pressure and the level of specific mdr mRNA. Survival in mass cultures for relatively short exposures to cytotoxic agent presumably is dependent on a number of factors in addition to drug resistance. Growth rates of individual clones, secretion of growth factors, and uptake of drug per cell may only be a few of the potential factors which determine cell survival. The complex interactions determining cell survival which occur in mass populations of cells rather than the ability of single isolated cells to develop colonies in the colony formation assays may actually be more representative of cell kill kinetics which occur in complex tissues. Previous observations have indicated that a small amount of resistance in tissue culture may produce large amounts of resistance in vivo (38).

Clone A7 deserves special attention. It is the result of cotransfection of the expression vector containing λDR11 and the expression vector containing the neomycin resistance gene. This clone was selected with G418 which is not included in the multidrug resistant phenotype. Without prior selection in Adriamycin, clone A7 is resistant to multiple chemotherapeutic agents indicating that selective pressure is not an absolute requirement for the development of the multidrug resistant phenotype. Multidrug resistance has developed in this clone from the increased amount of the mdr specific mRNA transcribed from λDR11 in pBAmr. It is worth noting again that λDR11 was cloned from a cDNA library generated from a cell line which was not drug resistant (10) and thus represents the

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mRNA from the normal gene. This would indicate that small increments in expression of either the normal mdr gene or the cDNA λDR11 alone is capable of conveying resistance to multiple chemotherapeutic agents.

The endogenous mdr gene copies did not appear to amplify during the initial selection of the clones or during modulation of the selective pressure. Increasing the selective pressure on clone 13 resulted in preferential amplification of the construct containing λDR11 rather than amplification of the endogenous mdr sequences. The ability to amplify the λDR11 construct indicates that the signal responsible for amplification of mdr genes can be recognized and processed by the sequences contained in λDR11. The preferential amplification of λDR11 over the endogenous sequences would also suggest that a more complex series of events is required to initiate amplification of the mdr gene than the cDNA construct. This would indicate that when the exogenous mdr cDNA is introduced into the genome it integrates into sites which are favorable for the amplification process. Preferential amplification of the introduced cDNA as opposed to amplification of the endogenous mdr gene also occurred when λDR11 was introduced into drug sensitive cells in the expression vector p91023B (9). Similarly the endogenous mdr gene was not amplified during selection in cytotoxic drug when the mdr gene was transferred from drug resistant cells to drug sensitive cells via either chromosome transfer (5) or DNA mediated gene transfer (39, 40).

Analysis of the levels of mdr specific mRNA in the transfected clones indicates that expression of λDR11 is dependent on both the number of copies integrated into the genome and the amount of selective pressure placed on the individual clone. Increasing the copy number of λDR11 correlates with increasing the amount of mRNA expression at any constant level of selective pressure. This is seen when comparing clone 54 with clone 56 or clone 11 with clone 13 (Table 1). However, increasing the amount of selective pressure can dramatically increase the amount of mRNA expressed when a similar number of copies of the expression vector are incorporated into the genome. A comparison of clones 54, 13, and 13.4 highlights the effect of selective pressure on the amount of mRNA present in the cell. Each clone has a similar number of copies of λDR11 but the expression of the mdr specific mRNA is dependent upon the amount of selective pressure. Previous analysis of λDR11 in p91023B indicated that all of the increased levels of mdr specific mRNA originated from the expression vector (9). The possibility exists, however, that some of the increase in mdr specific mRNA in the pBAmdr transfecants is due to increased transcription of the endogenous sequences since the sizes of the endogenous and pBAmdr transcript are similar. These data would suggest that expression of λDR11 is related to both copy number and amount of selective pressure. Furthermore this indicates that there may be different signals for amplification of the expression vector and increasing expression of mRNA from the expression vector. Both of these signals can apparently be recognized and processed by the sequences in λDR11.

Reversal of the multidrug resistant phenotype has been observed with a broad range of agents. Calcium antagonists, including verapamil (41), calmodulin inhibitors (41), quinidine (42), synthetic isoprenoids (43), tamoxifen and other triparanol analogues (44), and Tween (45), are all capable of potentiating the toxicity of chemotherapeutic agents in multidrug resistant cell lines. Cornwell et al. (46) have recently presented data suggesting that verapamil may bind to gp170 resulting in loss of drug resistance. Although the exact mechanism by which drug resistance is reversed is not clear, at least two components are involved. The agents which reverse the multidrug resistant phenotype will increase drug toxicity in both drug sensitive and drug resistant cell lines in the presence of cytotoxic agents (21). In addition, verapamil has also been shown to be more toxic to multidrug resistant cell lines than to drug sensitive parental cell lines even in the absence of cytotoxic agents (47), a phenomenon termed collateral sensitivity (48). It has been difficult to determine whether the increased cytotoxicity to chemotherapeutic agents is due to increased collateral sensitivity in the presence of cytotoxic agents or whether potentiating the actions of cytotoxic agents by reversing the drug resistance mechanism is responsible for cell death. The multidrug resistant colonies generated by transfection with λDR11 display both characteristics of verapamil. The transfected colonies exhibited modest toxicity to low doses of verapamil when cultured in the absence of cytotoxic agent while the drug sensitive parental cell line did not exhibit toxicity at low concentrations. When the multidrug resistant colonies generated by transfection were cultured in the presence of the cytotoxic agent Adriamycin, drug resistance was completely abolished when verapamil was present in the media. These observations would suggest that the gene product encoded by λDR11 alone enables the transfected multidrug resistant colonies to respond to verapamil in a manner similar to other multidrug resistant cell lines.

The mouse cDNA λDR11 appears to convey many of the characteristics of multidrug resistant cell lines to previously drug sensitive cells. The hallmark of the cell lines is the drug resistant phenotype which includes resistance to the anthracyclines, Vinca alkaloids, and actinomycin D. The clones transfected with the λDR11 cDNA are resistant to each of these drugs. Clones which have been transfected with λDR11 respond at the molecular level by modulating the number of copies of the cDNA in the genome based on the stringency of selection in a manner analogous to the number of copies of the endogenous mdr gene present in multidrug resistant cell lines during stepwise selection or reversion out of selection (5-8). Finally, the transfected clones respond to verapamil just as multidrug resistant cell lines do by displaying both collateral sensitivity and reversal of drug resistance. Although multiple mechanisms of drug resistance have been suggested in some multidrug resistant cell lines (49), these data would suggest that over-expression of the protein encoded by λDR11 alone is responsible for the characteristics of the multidrug resistant cell lines and most importantly increased expression by itself is sufficient for drug resistance.

REFERENCES

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