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Multidrug Resistance of DNA-Mediated Transformants Is Linked to Transfer of the Human *mdr*1 Gene

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Mouse NIH 3T3 cells were transformed to multidrug resistance with high-molecular-weight DNA from multidrug-resistant human KB carcinoma cells. The patterns of cross resistance to colchicine, vinblastine, and doxorubicin hydrochloride (Adriamycin; Adria Laboratories Inc.) of the human donor cell line and mouse recipients were similar. The multidrug-resistant human donor cell line contains amplified sequences of the *mdr*1 gene which are expressed at high levels. Both primary and secondary NIH 3T3 transformants contained and expressed these amplified human *mdr*1 sequences. Amplification and expression of the human *mdr*1 sequences and amplification of cotransferred human *Alu* sequences in the mouse cells correlated with the degree of multidrug resistance. These data suggest that the *mdr*1 gene is likely to be responsible for multidrug resistance in cultured cells.

The development of resistance to multiple drugs is a common impediment to successful cancer chemotherapy. This problem has been studied in tissue culture through the development of rodent and human cell lines which express simultaneous resistance to many drugs, such as colchicine, vinblastine, doxorubicin hydrochloride (Adriamycin; Adria Laboratories Inc.), and actinomycin D (3, 4, 16). By use of the technique of in-gel renaturation (21), it has been possible to clone DNA sequences, designated *mdr*, that are commonly amplified in various hamster (13, 14, 22) and human (11, 23, 24) multidrug-resistant cell lines. The cloned human DNA sequence *mdr*1 is expressed at high levels as a 4.5-kilobase (kb) mRNA in several different human multidrug-resistant cell lines (23, 24). A related DNA sequence, *mdr*2, is amplified in some multidrug-resistant cell lines, but no expression of *mdr*2 has been detected (23). Both *mdr*1 and *mdr*2 map to human chromosome 7 (12). Recently, a hamster cDNA clone encoding an antigenic region of a 170-kilodalton membrane glycoprotein (P-glycoprotein) has also been found to recognize amplified and overexpressed sequences in rodent and human multidrug-resistant cells (19). The relationship between the P-glycoprotein and the *mdr* sequences has not yet been established.

To investigate the mechanism of multidrug resistance by an independent approach, we transferred DNA from multidrug-resistant human KB carcinoma cells into sensitive mouse NIH 3T3 cells. Our results demonstrate that expression of multidrug resistance in primary and secondary transformants correlates with the transfer of the *mdr*1 gene but not the closely linked *mdr*2 sequences. These results strongly suggest that the *mdr*1 gene is primarily responsible for multidrug resistance in mammalian cells.

MATERIALS AND METHODS

Tissue culture and drug resistance assays. The human KB carcinoma cell line subclone KB-3-1 (1) and its colchicine-resistant derivative KB-C1.5 (25) (selected and maintained in medium containing 1.5 μg of colchicine per ml) were grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum (M. A. Bioproducts, Inc.). Mouse NIH 3T3 cells were cultured under the same conditions.

Drugs used in this study were purchased from Sigma Chemical Co. For cross resistance tests, cells were plated at 5×10^2 per 35-mm dish, and drugs were added 16 h after cells were plated (1, 25). Colonies were scored after 8 to 10 days of incubation at 37°C. Relative colony-forming ability in the presence of colchicine, vinblastine, or doxorubicin hydrochloride was calculated by dividing the LD_{50} value for each cell line (the concentration of drug reducing cloning efficiency by 50%) by the LD_{50} value for KB-3-1.

DNA-mediated gene transfer. Cotransformation of mouse NIH 3T3 cells was performed by a modification of the calcium phosphate precipitation procedure (28). NIH 3T3 cells (2.5×10^5) seeded 20 to 24 h earlier in Dulbecco modified Eagle medium containing 10% calf serum (Colorado Serum Co.) in a T75 flask were exposed for 16 to 20 h to a calcium phosphate precipitate of 20 μg of genomic DNA and 3 μg of pSV_2neo DNA. The medium was then removed and replaced with fresh Dulbecco modified Eagle medium plus 10% fetal bovine serum. Cells were incubated for 24 h at 37°C to allow phenotypic expression, trypsinized, and replated in medium containing G418 (0.8 mg/ml). Surviving Neo^r colonies were counted after 10 to 12 days, pooled after trypsinization, and selected in medium containing colchicine at 30 ng/ml for 7 days, at 50 ng/ml for 14 days, and at 80 ng/ml for 17 days. Positive colonies were then pooled, and populations were selected for colchicine resistance in several steps up to 1 μg of colchicine per ml. For the secondary transformations, DNA was extracted from the primary NIH 3T3 transformant populations resistant to 1 μg of colchicine per ml, and pSV_2neo DNA was used to cotransform NIH 3T3 cells. Control cells received NIH 3T3 DNA and produced no transformants resistant to 80 ng of colchicine per ml. Neo^r cells were pooled, and colchicine-resistant colonies were isolated after 9 days in medium containing 80 ng of colchicine per ml. Secondary transformants were then grown for 14 days in 80 ng of colchicine per ml, and more

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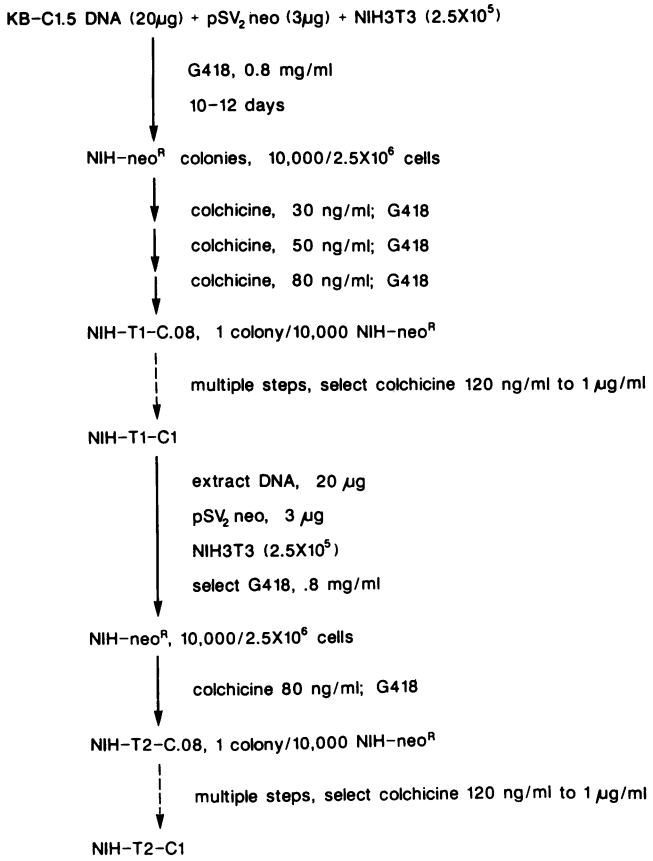

FIG. 1. Schematic representation of the selection of primary and secondary NIH 3T3 transformants by using DNA from multidrug-resistant human cell lines. See the text for details.

resistant populations were selected after growth in increasing concentrations of colchicine up to 1 μg/ml.

Isolation of nucleic acids and filter hybridization. Plasmid pSV$_2$*neo* (27) and plasmid Blur-8 (9) were purified from transformed *Escherichia coli* HB101 cells by the method of Birnboim and Doly (5). Contaminating RNA was removed by equilibrium density centrifugation in CsCl-propidium iodide or CsCl-ethidium bromide gradients. Plasmids were precipitated in 70% ethanol–0.3 M sodium acetate (pH 7). The plasmids used in gene cotransfer experiments were reprecipitated and dissolved in TE buffer (17). Ethanol and salt were removed by dialysis against TE buffer.

High-molecular-weight genomic DNA was prepared from NIH 3T3, KB-3-1, KB-C1.5, and primary transformant cell lines by proteinase K-sodium dodecyl sulfate (SDS) digestion, followed by gentle extraction with buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1). RNA was removed with RNase (200 μg/ml) (Sigma) at 37°C for 45 min. Protein was digested with proteinase K (200 μg/ml) (Sigma) for 1 h at 37°C and then purified several times with phenol-chloroform. DNA was precipitated with sodium acetate and ethanol and dialyzed against TE buffer without NaCl. The presence of NaCl was found to reduce transformation efficiencies.

Total cellular RNA was isolated by the methods of Chirgwin et al. (6) and Maniatis et al. (17). Cells were washed twice with phosphate-buffered saline and lysed with guanidine thiocyanate buffer, followed by homogenization in the same buffer. Each homogenate was layered over a CsCl cushion and centrifuged for 20 h at 24,000 rpm in a Beckman SW28 rotor. RNA pellets were purified with phenol-chloroform and then with chloroform-butanol, precipitated twice, and dissolved in TE buffer. Poly(A)$^+$ fractions were selected as described by Aviv and Leder (2).

Restriction endonucleases were purchased from Bethesda Research Laboratories, Inc., and digestions were carried out under the conditions recommended by the supplier.

DNA samples were electrophoresed on 0.8% agarose in 1× TBE buffer and transferred from the gel to either nitrocellulose paper or a Biodyne membrane (Pall) by the method of Southern (26). RNA samples were analyzed by electrophoresis on 1% agarose containing 13.4% formaldehyde and transferred directly to a nitrocellulose filter after two rinses of the gel in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) buffer. For semiquantitative analysis, RNA samples were applied to filters by using a slot blot apparatus (Schleicher & Schnell, Inc.). Filters were baked in a vacuum oven for 2 h at 80°C.

The probes used were labeled to specific activities of 2×10^8 dpm/μg by nick translation (18) or 2×10^9 dpm/μg by oligo labeling (10) with [^{32}P]dCTP (3,000 Ci/mmol) (New England Nuclear Corp.). Baked filters were incubated for 4 to 6 h at 42°C in 50% formamide–5× SSC–10× Denhardt solution–0.1% SDS–100 μg of sonicated salmon sperm DNA per ml and then hybridized overnight in the same solution containing ^{32}P-labeled probe. Filters were washed three times for 10 min each time at room temperature in 2× SSC–0.1% SDS and three times for 20 min each time at 50°C in 0.1× SSC–0.1% SDS, dried, and autoradiographed at −70°C with a Cronex fluorescence enhancer screen (Du Pont Co.). Biodyne filters were hybridized as previously described (23).

RESULTS

DNA-mediated transfer of multidrug resistance. The procedure used to transform NIH 3T3 cells with DNA from multidrug-resistant human KB cells is shown schematically in Fig. 1 (for details, see Materials and Methods). NIH 3T3 cells, like many other rodent cells, give rise to spontaneous multidrug-resistant lines at a relatively high frequency (approximately 10^{-6}), similar to the frequency of DNA-mediated gene transfer with genomic DNA. Therefore, it was necessary to use a two-step protocol to identify the multidrug resistance gene of human origin. In this approach, NIH 3T3 cells are cotransformed with DNA encoding the dominant selectable marker pSV$_2$*neo* and either DNA from multidrug-sensitive cells or DNA from multidrug-resistant cells. Selection for G418 resistance, encoded by pSV$_2$*neo* DNA, results in an enrichment for NIH 3T3 cells carrying cotransformed KB cell DNA.

A library of approximately 10,000 G418-resistant mouse

TABLE 1. Pattern of multidrug resistance in parental KB cells and NIH 3T3 transformants

Cells	Relative resistance[a] to:		
	Colchicine	Vinblastine	Doxorubicin hydrochloride
KB-3-1	1	1	1
KB-C1.5	360	291	59
NIH 3T3	12	10	2.6
NIH-T1-C1	580	134	52
NIH-T2-C1	250	314	57

[a] Determined and expressed as described in Materials and Methods.

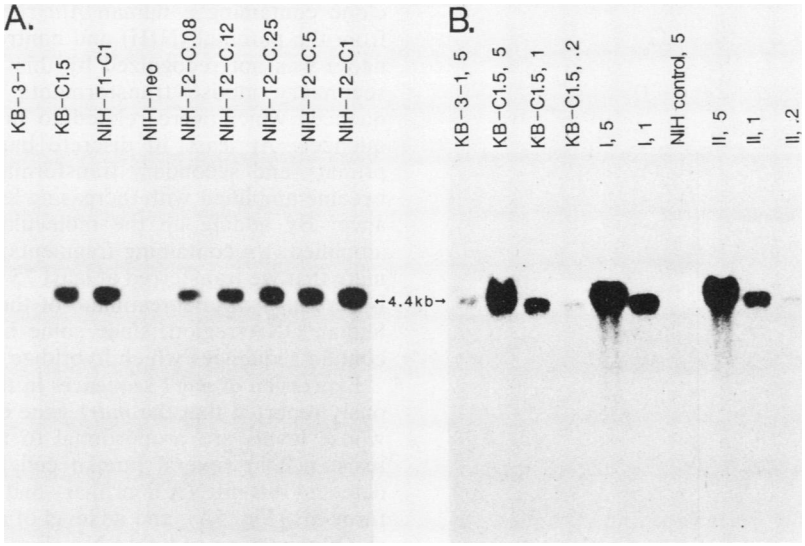

FIG. 2. Southern blot analysis of the *mdr*1 locus in human multidrug-resistant donor cells and transformants. (A) Genomic DNA (2 μg) from each cell line was digested with *Hin*dIII. The conditions for electrophoresis and hybridization were as described in Materials and Methods. Filters were hybridized with the gel-purified 0.8-kb *Pvu*II insert of the pMDR1 clone. KB-3-1 is the wild-type parent; KB-C1.5 is a colchicine-resistant mutant grown in medium containing colchicine (1.5 μg/ml); NIH-T1-C1 represents the primary transformant growing in the presence of colchicine (1 μg/ml); NIH-neo is a mixed population of G418-resistant mouse cells; NIH-T2-C.08 to NIH-T2-C1 represent a series of increasingly colchicine-resistant secondary transformants (the numbers [e.g., .08] represent colchicine selecting concentrations in micrograms per milliliter). (B) Estimation of the level of amplification of *mdr*1 sequences in the primary and secondary transformants relative to the parental KB-3-1 and KB-C1.5 cell lines. The symbols I and II represent primary and secondary transformants, respectively. The intensity of the signal from 5- to 25-fold dilutions of DNA from KB-C1.5 and the transformants was compared with that of the signal from 5 μg of KB-3-1 DNA. The amount of DNA loaded (in micrograms) is indicated at the top of the lanes. DNA was digested with *Hin*dIII. Southern blotting and hybridization were performed as described in Materials and Methods.

cells (each carrying random segments of human DNA) derived from 2.5×10^6 transformed NIH 3T3 cells was prepared by using DNA from control and resistant cells and screened for colchicine resistance. In five independent transformation experiments, only the population of cells which had received DNA from the multidrug-resistant KB cells gave rise to colchicine-resistant colonies (selecting concentration, 30 ng/ml) at a frequency of approximately 1/10,000 G418-resistant colonies (overall frequency, 1/2,500,000 or 4×10^{-7}). In early experiments, we increased the frequency of G418-resistant NIH 3T3 cells by gently shearing the human genomic DNA by a single passage through a 25-gauge needle, but we were unable to obtain any colchicine-resistant colonies by using this protocol, suggesting that the gene(s) we were transferring was very large.

To obtain secondary transformants, we isolated DNA from NIH 3T3 primary transformants which had been selected in increasing concentrations of colchicine for the purpose of amplifying the transferred human gene. We repeated the two-step transformation protocol, including the addition of new pSV_2neo DNA and the generation of a new population of 10,000 NIH 3T3 colonies which were resistant to G418. Although the overall frequency of transformation to multidrug resistance was not higher in these secondary transformants, it was possible to select colonies directly with 80 ng of colchicine per ml, indicating transfer of a higher level of resistance than we had observed with primary transfer of multidrug resistance into NIH 3T3 cells. The control DNA for this transfer came from NIH 3T3 cells and produced no multidrug-resistant colonies when selection was done with 80 ng of colchicine per ml.

Both primary and secondary colchicine-resistant transformants were tested for multidrug resistance. All transformed cell lines were multidrug resistant, and the patterns of cross resistance to colchicine, vinblastine, and doxorubicin hydrochloride for parental and transformant lines were similar (Table 1).

Amplification of *mdr*1 sequences in transformants. Because we have previously shown that multidrug-resistant human cells have an amplified *mdr*1 gene (23, 24), we isolated DNA from primary and secondary transformant lines to see if the human *mdr*1 sequences were present in these transformants. Figure 2 shows autoradiograms of a Southern blot analysis of DNA digested with *Hin*dIII and probed with an 800-base-pair insert from the pMDR1 plasmid containing a segment of the human *mdr*1 gene. Specific hybridization to a 4.4-kb *Hin*dIII fragment was seen in the multidrug-resistant parent (KB-C1.5) and in primary (NIH-T1-C1) and secondary (NIH-T2-C.08 to NIH-T2-C1) NIH 3T3 transformants (Fig. 2A). Increased amplification of this fragment correlated with increased levels of resistance. In the exposure of the autoradiogram shown in Fig. 2A, single-copy levels of the *mdr*1 fragments in the parental, multidrug-sensitive KB-3-1 cell line were not observed, but single-copy levels were easily observed in longer exposures (e.g., see Fig. 2B). In contrast, no hybridization to DNA from the NIH 3T3 parental cell line or control G418-resistant populations (NIH-neo) was seen in these experiments. Figure 2B shows a Southern blot of DNA at various dilutions, indicating that the extent of amplification of the 4.4-kb *Hin*dIII fragment of the *mdr*1 gene in primary and secondary transformants selected for resistance to 1 μg of colchicine per ml was comparable to that seen in the multidrug-resistant KB-C1.5 parent (approximately 20- to 25-fold).

To prove that the bands hybridizing to the pMDR1 probe corresponded to the transferred human *mdr*1 gene and not to

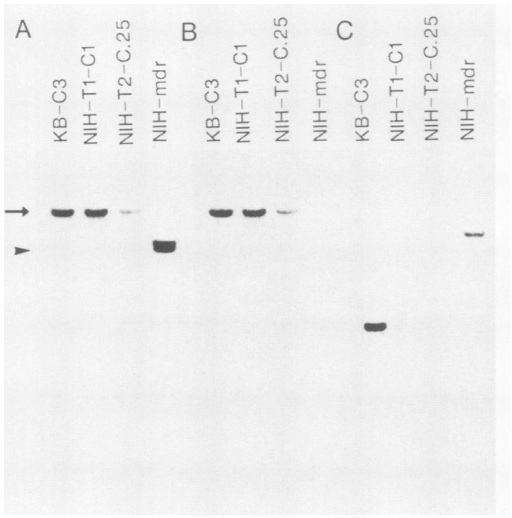

FIG. 3. Multidrug-resistant transformants containing the human *mdr*1 gene. Each lane contains 5 μg of genomic DNA digested with *Eco*RI and transferred onto a Biodyne membrane (Pall) after electrophoresis on 1% agarose. Hybridization with gel-purified inserts of pMDR1 (A and B) and pMDR2 (C) was done under the conditions of low (A and C) and high (B) hybridization stringencies as previously described (23). The arrow indicates the position of the human *Eco*RI genomic fragment which hybridized to pMDR1, and the arrowhead indicates the mouse genomic fragments which hybridized to this probe at low stringency. NIH-mdr is DNA from an NIH cell line selected for multidrug resistance.

an amplified endogenous mouse *mdr* gene, we used a multidrug-resistant subline of NIH 3T3 cells (NIH-mdr) isolated in our laboratory (unpublished data). This subline has amplified the mouse homologs of the human *mdr*1 and *mdr*2 DNA sequences. Figure 3A shows that the *Eco*RI fragments hybridizing to pMDR1 under conditions of low hybridization stringency (4 × SSC–0.5% SDS at 65°C in the last wash) were the same size (13.5 kb) in multidrug-resistant human KB-C3 cells and in both primary and secondary NIH 3T3 transformants. In the multidrug-resistant mouse cell line, however, the human pMDR1 probe hybridized to two different amplified bands of 7.8 and 7.5 kb. In other experiments (data not shown), the same bands could be detected in mouse cells at a single copy. When the filter was washed under conditions of high hybridization stringency (0.1× SSC–0.5% SDS at 65°C), the 7.8- and 7.5-kb mouse bands disappeared, but the 13.5-kb band in the KB-C3 cells and the transformants was still detectable (Fig. 3B), indicating that the human *mdr*1 gene and not the mouse *mdr* gene was amplified in the transformants.

The same blot was hybridized with the pMDR2 clone (23) containing a segment of the human *mdr*2 region under conditions of low hybridization stringency (Fig. 3C). This probe hybridized to a 4.5-kb *Eco*RI fragment in human DNA and to a 10.5-kb *Eco*RI fragment in mouse DNA, corresponding to the amplified mouse homolog of *mdr*2. However, no bands hybridizing to pMDR2 were detected in the DNA of primary and secondary transformants, indicating that *mdr*1 but not the linked *mdr*2 sequences was transferred to these cells.

Additional evidence that the exogenous human genes are amplified in these transformants came from an analysis of the cotransferred human *Alu* highly repetitive sequences. Figure 4 shows Southern blots hybridized with the Blur-8 clone containing a human *Alu* repetitive sequence. DNA from the parental (NIH) and control NIH 3T3 cells (NIH-neo^R) was not recognized by this probe, but primary and secondary mouse transformants contained discrete sequences which were related to human *Alu* repetitive sequences. At least 10 discrete bands were seen in both primary and secondary transformants, and most of these became amplified with increasing levels of multidrug resistance. By adding up the molecular masses of all of the amplified *Alu*-containing fragments, it was possible to estimate that we transferred at least 75 kb of human DNA. This is certainly an underestimate of the size of the transferred human DNA region, since some human fragments do not contain sequences which hybridize with the Blur-8 probe.

Expression of *mdr*1 sequences in transformants. We previously reported that the *mdr*1 gene encodes a 4.5-kb mRNA whose levels are proportional to the degree of multidrug resistance in several human cell lines (23, 24). We also detected this mRNA in primary and secondary mouse transformants (Fig. 5A), and its level of expression, as shown by slot blot analysis of total RNA (Fig. 5B), seemed to correlate well with the degree of multidrug resistance. One interesting feature of this analysis was the finding that shortly after selection with 80 ng of colchicine per ml, the secondary transformants expressed relatively low levels of *mdr*1 mRNA [Fig. 5B, row NIH-T2-C.08(1)], but after continued selection for several weeks with 80 ng of colchicine per ml [Fig. 5B, row NIH-T2-C.08(2)], the levels of expression increased dramatically.

To prove that the 4.5-kb mRNA expressed in the mouse transformants was of human origin, we compared the hybridization properties of human and mouse *mdr*1 mRNAs on the same Northern blot. The major species of mouse *mdr*

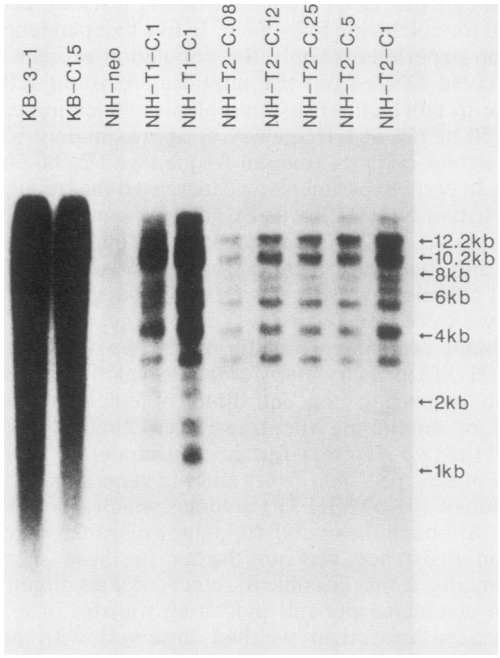

FIG. 4. Analysis by Southern hybridization of primary and secondary transformants containing human *Alu*-related repetitive sequences. DNA was digested with *Hin*dIII and probed with the human repetitive sequence probe Blur-8. Mouse (2 μg) and human (0.2 μg) DNAs were used. See the legend to Fig. 2 for an explanation of the lane headings.

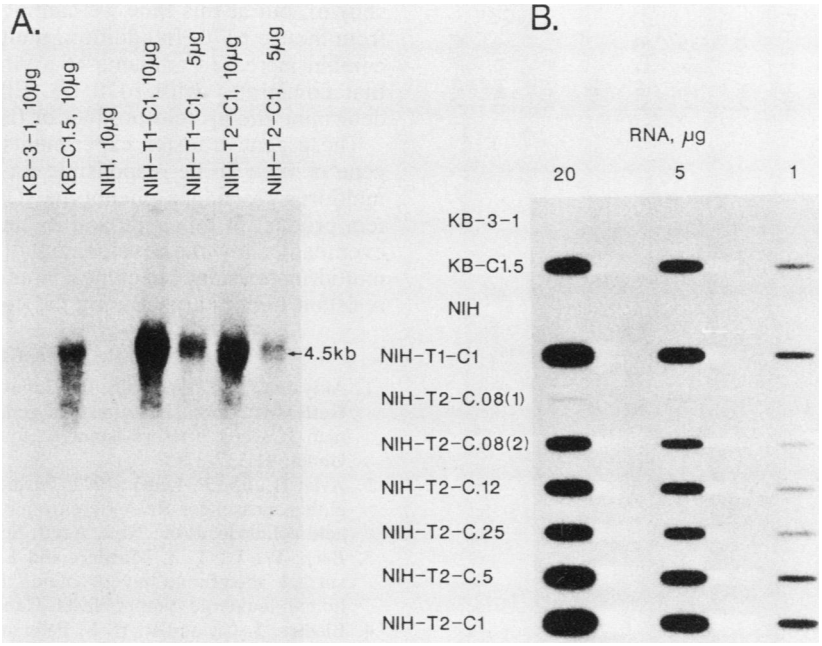

FIG. 5. Expression of human *mdr*1 sequences in mouse NIH 3T3 transformants. (A) Northern hybridization of total RNA from the parental donor cells and transformants. (B) Slot blot hybridization. Filters were probed with the pMDR1 0.8-kb *Pvu*II insert. The amount of RNA loaded is indicated at the top of the lanes and was confirmed by agarose gel electrophoresis of RNA samples with visualization of 28S and 18S RNA. NIH is RNA from untransformed NIH 3T3 cells. NIH-T2-C.08(1) is RNA from a population of transformants selected 2 weeks earlier in colchicine (80 ng/ml), and NIH-T2-C.08(2) is RNA from the same cell population grown in the selective medium for an additional 3 weeks.

mRNA moved slightly faster in this gel system (Fig. 6; data not shown), and the major mRNA species in the transformants showed the same migration as human *mdr*1 mRNA. The additional slower-migrating mRNA species seen in the transformants in this experiment was observed in several other multidrug-resistant KB cell lines (data not shown) and was therefore not unique to the transformants. The faster-migrating mRNA species were usually not seen and may represent degraded mRNA. At low stringency (Fig. 6A), pMDR1 hybridized to mRNA in both human and mouse cell lines, whereas at higher stringency (Fig. 6B), *mdr*1 mRNA was no longer detectable in mouse cells but was still seen in human cells and in the transformants, with no reduction in signal intensity. These data demonstrate that the transferred human *mdr*1 gene is expressed in the NIH 3T3 primary and secondary multidrug-resistant transformants.

DISCUSSION

A DNA segment encoding multidrug resistance in human KB carcinoma cells was successfully transferred into mouse NIH 3T3 cells. This segment contains the *mdr*1 gene, previously found to be amplified and overexpressed in various multidrug-resistant human cell lines (23, 24). Previous studies by Debenham et al. (8), Robertson et al. (20), and Gudkov et al. (14) suggested that the gene encoding multidrug resistance in Chinese hamster ovary (CHO) cells could be transferred from hamster to mouse L cells by DNA-mediated gene transfer and that this transfer correlates with increased expression of a 170-kilodalton membrane glycoprotein (8, 20). However, the nature of the gene(s) transferred in these experiments has not yet been demonstrated at the DNA level. In our studies, in which a two-step protocol which virtually eliminates the background of spontaneous recipient mouse cell multidrug resistance was used, the acquisition of multidrug resistance by the recipient mouse cells was shown to be associated with transfer, expression, and amplification of the human *mdr*1 gene.

The requirement for high-molecular-weight DNA in these studies and the association of at least 75 kb of *Alu*-containing sequences with the transferred DNA in both primary and secondary transformants suggest that the gene(s) that we transferred is quite large. This explains the relatively low frequency of genomic transformation (approximately 4×10^{-7}), despite the fact that the *mdr*1 gene was amplified at least 20-fold in both donor cells and primary transformants (KB-C1.5 and NIH-T1-C1). Gros et al. (13) have recently reported that the hamster *mdr* gene spans at least 80 kb of genomic DNA. In similar studies (K. Ueda, I. Pastan, and M. M. Gottesman, unpublished observations), we have found that the human *mdr*1 gene is carried on cosmid vectors containing at least 120 kb of genomic DNA.

The *mdr*2 sequences are coamplified with *mdr*1 in the donor KB-C1.5 cell line (23). When infrequently cutting restriction enzymes and pulsed-field gradient gel electrophoresis were used, these sequences were found to be linked within 350 kb of the amplified region (K. Choi and I. B. Roninson, unpublished data). However, the *mdr*2 sequences were not transferred into multidrug-resistant NIH 3T3 cell recipients in our experiments (Fig. 3C). This result, as well as our estimate that approximately 75 kb of *Alu* repeat-containing human DNA fragments is present in secondary transformants (Fig. 4), suggests that the size of the transferred DNA segment is comparable to the size of the *mdr*1 gene. These estimates, however, do not rule out the possibility of the transfer of another closely linked gene.

As we have previously shown for a series of human cell lines manifesting various degrees of multidrug resistance

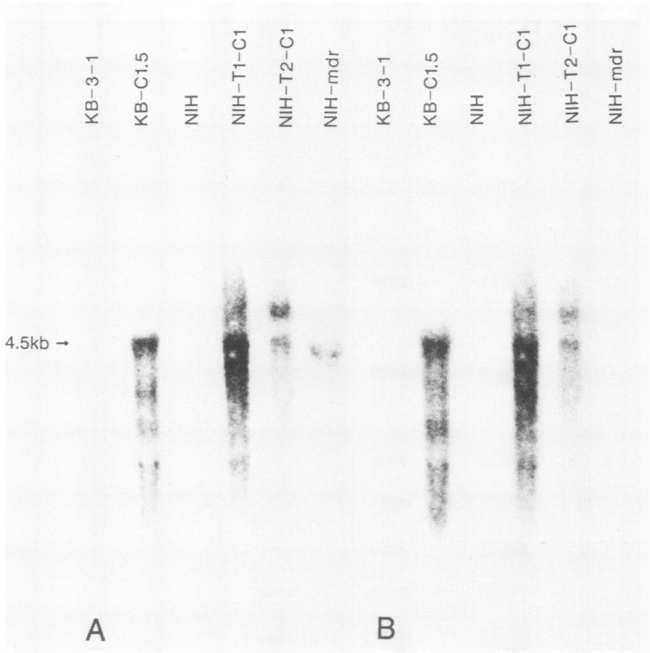

FIG. 6. Comparison of mouse and human *mdr*1 mRNA sequences under various hybridization conditions. (A) Low stringency. Northern blots were hybridized with the pMDR1 0.8-kb *Pvu*II insert as described in Materials and Methods and washed at 50°C. (B) High stringency. The blot shown in panel A was washed at 70°C before autoradiography, and an autoradiogram was prepared. The lanes contained total RNA as follows: KB-3-1, 10 μg; KB-C1.5, 10 μg; NIH, 10 μg; NIH-T1-C1, 5 μg; NIH-T2-Cl, 5 μg; NIH-mdr (control), 25 μg. See the legends to Fig. 5 and 3 for explanations of NIH and NIH-mdr, respectively.

(24), resistance of the transformants correlates well with levels of a 4.5-kb mRNA detected by an *mdr*1 probe. This mRNA is not detected with a nick-translated *mdr*1 DNA probe in parental KB-3-1 or NIH 3T3 cells and appears initially at low levels and then at higher levels as selection becomes more stringent in primary and secondary transformants. The major 4.5-kb mRNA species detected in the transformants is similar in size to and hybridizes at the same stringency as the mRNA found in the human KB multidrug-resistant donor cells, whereas the mRNA found in mouse multidrug-resistant cells is slightly smaller in size and does not hybridize with the human probe under low-stringency conditions. We do not know if the human mRNA encodes a mutant protein or whether increased expression of a wild-type 4.5-kb mRNA is sufficient to encode multidrug resistance. The answer to this question must await cloning and sequencing of *mdr*1 genes from multidrug-sensitive and -resistant cell lines.

The protein product of the 4.5-kb mRNA is also not known. One possible candidate is a high-molecular-weight glycoprotein (p170) which has been found on the surface of a variety of multidrug-resistant cells (3, 4, 15), including multidrug-resistant sublines of KB-3-1 cells (25; unpublished data). Consistent with this possibility is the published evidence that a partial cDNA clone of CHO cell p170 recognizes amplified sequences in both rodent and human cells and hybridizes on Northern blots with a 4.5-kb mRNA (19) and that p170 is expressed on the surface of L cells transformed with DNA from multidrug-resistant CHO cells (8). We have also found that p170 is expressed on the surface of NIH 3T3 primary and secondary transformants (data not shown), but at this time we cannot distinguish human p170 from mouse p170. In addition, multidrug-resistant KB cells contain increased amounts of a vinblastine-binding protein that comigrates with p170 (7). Efforts are under way to determine the protein product of the *mdr*1 gene.

These gene transfer experiments indicate that the *mdr*1 gene is likely to be responsible for the development of the multidrug resistance phenotype. Determination of the protein product of this gene and its mode of regulation should eventually allow the development of strategies to circumvent multidrug resistance in clinical situations in which multidrug-resistant tumors arise during the course of chemotherapy.

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