Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4538–4542, June 1986
Medical Sciences

# Isolation of human *mdr* DNA sequences amplified in multidrug-resistant KB carcinoma cells

**(colchicine/Adriamycin/vinblastine/gene amplification)**

IGOR B. RONINSON*, JANICE E. CHIN*, KYUNGHEE CHOI*, PHILIPPE GROS†, DAVID E. HOUSMAN†, ANTONIO FOJO‡, DING-WU SHEN‡, MICHAEL M. GOTTESMAN‡, AND IRA PASTAN‡

*Center for Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL 60612; †Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ‡Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892

*Contributed by Ira Pastan, February 11, 1986*

ABSTRACT **The ability of tumor cells to develop simultaneous resistance to structurally different cytotoxic drugs constitutes a major problem in cancer chemotherapy. It was previously demonstrated that multidrug-resistant Chinese hamster cell lines contain an amplified, transcriptionally active DNA sequence designated *mdr*. This report presents evidence that multidrug-resistant sublines of human KB carcinoma cells, selected for resistance to either colchicine, vinblastine, or Adriamycin (doxorubicin), display amplification of two different DNA sequences homologous to the hamster *mdr* gene. Segments of the human *mdr* DNA sequences, designated *mdr1* and *mdr2*, have been cloned. *mdr1* sequences were amplified in all of the highly drug-resistant sublines and were expressed as a poly(A)$^+$ RNA species of 4.5 kilobases that was detected in the resistant cells but not in the parental cell line. No expression of *mdr2* sequences was detected. *mdr2* sequences were coamplified with *mdr1* in some of the multidrug-resistant sublines and, in two independently derived cell lines, underwent very similar rearrangements. The data suggest that the *mdr1* gene is involved in multidrug resistance in human cells.**

Selection of mammalian cells for resistance to plant alkaloids or antitumor antibiotics frequently results in the development of cross-resistance to other drugs unrelated in their structure and mode of action to the original selective agent. The phenomenon of multidrug resistance constitutes a major problem in cancer chemotherapy because it involves resistance to some of the most commonly used anticancer drugs, such as anthracyclines, *Vinca* alkaloids, epipodophyllotoxins, and actinomycin D. Multidrug resistance has been shown in most cases to result from decreased intracellular drug accumulation, apparently as a result of alterations in the plasma membrane (1–3). In many multidrug-resistant cell lines, the resistance was found to correlate with overexpression of a 170-kDa membrane glycoprotein and, in some cases, a 19- to 21-kDa cytosolic protein (1–6).

Two different multidrug-resistant Chinese hamster cell lines selected for resistance to either colchicine or Adriamycin (doxorubicin) have amplified a common region of DNA (7, 8). This region was found to contain a transcription unit, presently designated *mdr*, that encodes an mRNA of ≈5 kilobases (kb). Expression of this mRNA correlates with multidrug resistance in hamster cells (8). Riordan *et al.* (9) reported that the gene encoding a 170-kDa membrane glycoprotein (P-glycoprotein) is also amplified in several multidrug-resistant mammalian cell lines. The relationship between *mdr* and the P-glycoprotein gene is still unknown.

To analyze the mechanism of multidrug resistance in human tumor cells, we have isolated sublines of the human KB carcinoma cell line, selected for resistance to colchicine, vinblastine, or Adriamycin. These sublines display decreased accumulation of various drugs with increased drug efflux and several biochemical changes (10–13). By use of the in-gel DNA-renaturation technique (14), some of the multidrug-resistant sublines of KB cells were shown to contain amplified DNA sequences, and karyotypic analysis revealed double minute chromosomes (15). In the present study, we report that multidrug-resistant KB cell lines have amplified two related DNA sequences that are homologous to the Chinese hamster *mdr* gene. One of these sequences (*mdr1*) is amplified in all sublines selected for a high degree of resistance to colchicine, vinblastine, or Adriamycin and is expressed in multidrug-resistant cells.

## MATERIALS AND METHODS

**DNA Extraction and Southern Hybridization.** DNA from the cell lines was extracted as described (15, 16) and was digested with 5 units of restriction enzymes as recommended by the supplier. In most experiments, the concentration of digested DNA was determined by the diphenylamine reaction (17). Five micrograms of each DNA digest was electrophoresed in an agarose gel and transferred onto a Biodyne membrane (Pall, East Hills, NY) by the procedure of Southern (18). Gel-purified inserts of the appropriate plasmid clones were labeled with $^{32}$P to a specific activity of 1–3 × $10^9$ dpm/μg by "oligolabeling" (19) and used as probes at a concentration 2–4 × $10^5$ dpm/cm$^2$. Hybridization was performed under conditions recommended by the manufacturer. After hybridization, the membranes were washed with 4× SSC (1× SSC is 0.15 M NaCl/15 mM trisodium citrate, pH 7.0)/0.5% $NaDodSO_4$ at 65°C (low stringency) or 0.1× SSC/0.5% $NaDodSO_4$ at 65°C (high stringency) and autoradiographed. To confirm the absence of plasmid contamination in human DNA preparations, each blot was rehybridized with $^{32}$P-labeled pSP64 plasmid vector. For quantitation of gene copy number, the intensities of bands in autoradiograms were estimated by densitometer tracing and computation of the areas of the corresponding peaks.

**Cloning Procedures.** Restriction mapping and subcloning into the pSP64 plasmid vector were performed by standard procedures (20). Plasmid DNA was isolated by alkaline lysis (21). Genomic libraries were constructed by digesting 0.5 μg of KB-C3 DNA to completion with either *Eco*RI or *Hin*dIII, followed by ligation into the single *Eco*RI site of the phage vector λgt11 (22) or the single *Hin*dIII site of phage Charon 28 (23). The insertion-cloning strategy provided for selective cloning of the fragments smaller than 7 kb (for *Eco*RI) or 11 kb (for *Hin*dIII). Plaques ($10^5$) of each library were screened with the gel-purified insert of pDR4.7 hamster *mdr* clone by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase(s).

plaque hybridization (24). Positive phages were plaque-purified and phage DNA was isolated (25), and the inserts were recloned in pSP64. To locate highly repeated sequences, cloned DNA was digested with several restriction enzymes and hybridized by the procedure of Southern with $^{32}$P-labeled total DNA ($0.35 \times 10^5$ dpm/cm$^2$) from human peripheral blood cells.

**RNA Isolation and Analysis.** Poly(A)$^+$ RNA was extracted as described (26). One microgram of each RNA preparation was electrophoresed in a glyoxal/1.5% agarose gel (27), transferred onto GeneScreen*Plus* membranes, and hybridized with the appropriate $^{32}$P-labeled probes at $3 \times 10^5$ dpm/cm$^2$. Hybridization was done at 42°C in 1 M NaCl/10% dextran sulfate/1% NaDodSO$_4$/50% (vol/vol) formamide containing denatured salmon sperm DNA at 100 $\mu$g/ml. The membranes were washed with 0.1× SSC/0.5% NaDodSO$_4$ at 65°C and autoradiographed. Sizes of RNA species were determined relative to the positions of 28S and 18S rRNA. To control for variation in sample size, the filters were rehybridized with a dihydrofolate reductase cDNA clone (28).

## RESULTS

**Amplification of Human *mdr* DNA Sequences in Multidrug-Resistant Cell Lines.** The Chinese hamster *mdr* DNA sequences used in this study were derived from the cosmid clone cosDR3A, containing a 5′ segment of the hamster *mdr* gene (8). After digestion with the restriction enzymes *Xba* I and *Kpn* I, individual restriction fragments of 1.5–6 kb were subcloned in a pSP64 plasmid vector (Fig. 1*a*) The subclones were then used as probes for hybridization with restriction digests of human genomic DNA. Most probes, when used under conditions of low hybridization stringency, produced either no hybridization signal or a continuous smear suggesting hybridization with human repetitive DNA sequences (data not shown). Only one of the cosDR3A subclones, containing a 4.7-kb *Xba* I fragment and designated pDR4.7 (Fig. 1*a*), gave rise to distinct bands when hybridized to human DNA under low-stringency conditions (Fig. 1 *b* and *c*). This subclone, containing the relatively conserved DNA sequences, was employed as a probe in subsequent experiments.

To determine whether the *mdr* gene was amplified in multidrug-resistant human cells, DNA extracted from the parental KB-3-1 cells, the multidrug-resistant sublines, and a revertant (KB-C1-R1) described in Table 1 was digested with *Eco*RI or *Hin*dIII, electrophoresed in agarose gels, and hybridized to the pDR4.7 probe (8). pDR4.7 hybridized to two *Eco*RI fragments (13.5 and 4.5 kb, Fig. 1*b*) and to two *Hin*dIII fragments (10.5 and 4.4 kb, Fig. 3) in KB-3-1 DNA. All of these fragments were amplified in colchicine-selected sublines KB-8-5-11, KB-8-5-11-24, KB-C3, and KB-C4 but not in the revertant subline KB-C1-R1. Unlike the colchicine-selected sublines, the subline KB-V1, selected in vinblastine, showed amplification of only the 13.5-kb *Eco*RI and 4.4-kb *Hin*dIII bands. In the Adriamycin-resistant cells KB-A1 and KB-A2, these two bands were also amplified. KB-A1 contained a new amplified band of 7 kb in the *Eco*RI digest and of 6.5 kb in the *Hin*dIII digest. The same bands were present in KB-V1, but they were not amplified. No bands of this size were detected in the parental KB-3-1 DNA, suggesting they arose as a result of DNA rearrangement.

The different patterns of amplification of the two types of bands hybridizing to the hamster *mdr* probe in different sublines suggested that they might correspond to two different related DNA sequences, possibly different members of a multigene family, rather than to two different parts of one contiguous hybridizing region. DNA sequences corresponding to the 13.5-kb *Eco*RI and 4.4-kb *Hin*dIII fragments were designated *mdr1*, and the sequences corresponding to the 4.5-kb *Eco*RI and 10.5-kb *Hin*dIII fragments were designated *mdr2*.

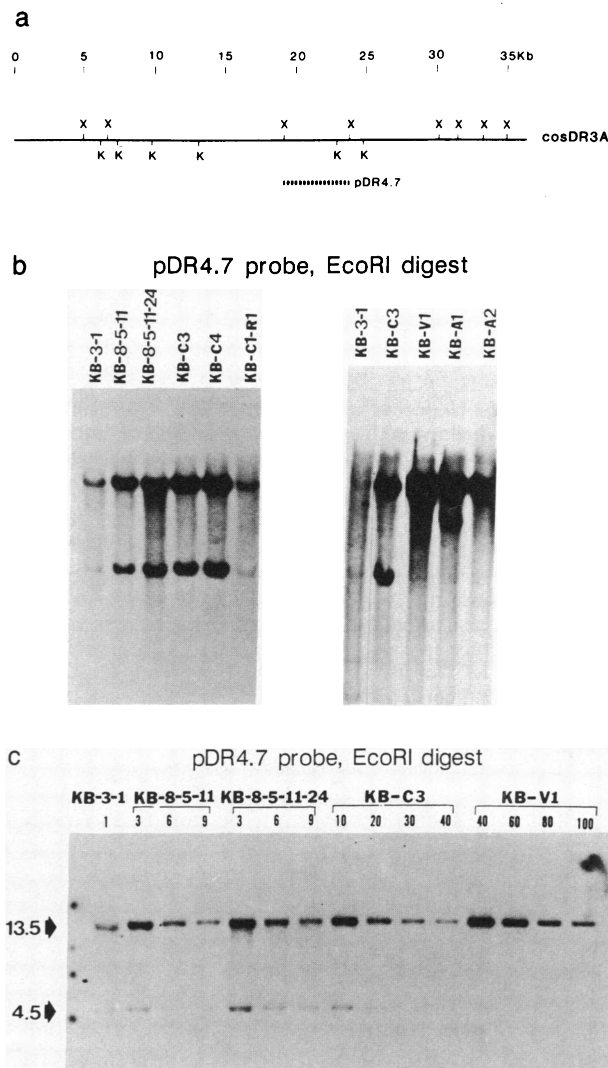


FIG. 1. (*a*) Restriction endonuclease map of the cosmid clone cosDR3A, containing a 5′ portion of the hamster *mdr* gene (8). Position of the 4.7-kb *Xba* I fragment (pDR4.7), containing DNA sequences hybridizing to human DNA, is indicated. Restriction sites: X, *Xba* I; *K*, *Kpn* I. (*b*) Southern hybridization of pDR4.7 with *Eco*RI-digested DNA (5 $\mu$g per lane) from multidrug-resistant KB cells. See Table 1 for characterization of the sublines. The filter was hybridized with the pDR4.7 probe under conditions of low hybridization stringency. Fragment sizes (in kb) are indicated. Several cross-hybridizing bands of KB-3-1 DNA detectable in the right panel were not seen in most experiments. (*c*) Estimation of the degree of amplification of *mdr* DNA sequences in multidrug-resistant KB cells. pDR4.7 was used as a probe for hybridization with *Eco*RI-digested genomic DNA. The intensity of the signal from serial dilutions of DNA from different sublines was compared to the signal from 5 $\mu$g of parental KB-3-1 DNA. The reciprocal of the dilution factor is indicated above each lane. DNA concentrations were determined by the diphenylamine reaction, and the signal intensity was estimated by densitometry.

The degree of amplification of *mdr* sequences in different multidrug-resistant sublines was estimated by comparing the intensity of hybridization signals from serially diluted *Eco*RI digests of different cellular DNAs (Fig. 1*c*). Estimates of the copy number of *mdr* sequences in different sublines are given in Table 1. Among the sublines selected for a 40- to 1750-fold greater resistance to colchicine, there is a parallel increase in drug resistance and in the number of *mdr* sequences. The

Table 1. Copy number of *mdr* sequences in KB sublines

| Cell line | Relative resistance | | | Relative amplification | |
|---|---|---|---|---|---|
| | Colchicine | Adriamycin | Vinblastine | *mdr1* | *mdr2* |
| KB-3-1 | 1 | 1 | 1 | 1 | 1 |
| KB-8-5-11 | 40 | 23 | 51 | 7–8 | 7–8 |
| KB-8-5-11-24 | 128 | 26 | 20 | 9 | 9 |
| KB-C3 | 487 | 141 | 206 | 20 | 20 |
| KB-C4 | 1750 | 254 | 159 | 30 | 30 |
| KB-C1-R1 | 6 | 3 | 4 | 1 | 1 |
| KB-V1 | 171 | 422 | 213 | 100 | 1* |
| KB-A1 | 19 | 97 | 43 | 70 | 30* |
| KB-A2 | ND | 140 | ND | 80 | 1 |

Derivation and characterization of multidrug-resistant sublines of KB cells have been described (10–13). KB-8-5-11, KB-8-5-11-24, KB-C3, and KB-C4 are subclones selected in medium containing colchicine at 0.1, 1, 3, and 4 μg/ml, respectively. KB-C1-R1 is a revertant of colchicine-resistant KB-C1 cells, cloned from a population growing in the absence of colchicine. KB-V1 was selected in multiple steps and is resistant to vinblastine at 1 μg/ml. KB-A1 and KB-A2 are resistant to Adriamycin at 1 μg/ml and 2 μg/ml, respectively. Since the drug-resistance phenotype is unstable, cell lines are maintained in medium with selecting concentrations of drug. Relative resistance is expressed as the $LD_{10}$ of the resistant line divided by the $LD_{10}$ of the parental KB-3-1 cells (10). Amplification is the copy number of the corresponding DNA sequences in multidrug-resistant cell lines relative to the parental KB-3-1 cells. ND, not determined.

**mdr2* DNA sequences are rearranged (see text).

*mdr1* and *mdr2* sequences appear to be amplified to a similar degree in these cells. The loss of amplified *mdr* sequences in a revertant of a multidrug-resistant cell line (KB-C1-R1) provides strong additional evidence that *mdr* gene amplification underlies multidrug resistance in the highly resistant cells.

**Cloning of Human *mdr1* and *mdr2* DNA Sequences.** To investigate the nature of the human *mdr* genes, we have cloned the 4.4-kb *Hin*dIII fragment of *mdr1* and the 4.5-kb *Eco*RI fragment of *mdr2* from the DNA of subline KB-C3. The *Eco*RI library was constructed by insertion into the *Eco*RI site of the λgt11 phage vector (22), and the *Hin*dIII library was made by insertion into the *Hin*dIII site of Charon 28 (23). Both libraries were screened by plaque hybridization (24) with the Chinese hamster pDR4.7 probe. A clone containing the 4.4-kb fragment of *mdr1* was isolated from the *Hin*dIII library, and a clone containing the 4.5-kb fragment of *mdr2* was isolated from the *Eco*RI library. Both inserts were subsequently recloned in the plasmid vector pSP64 (20), giving rise to plasmid clones designated pHDR4.4 and pHDR4.5, respectively. Restriction maps of these clones are shown in Fig. 2*a*. Subfragments of pHDR4.4 and pHDR4.5 free of highly repeated sequences were identified by their failure to hybridize to $^{32}$P-labeled human genomic DNA (data not shown). Hybridization with the pDR4.7 hamster probe (not shown) permitted localization of the conserved sequences within the clones (Fig. 2*a*). The regions of pHDR4.4 and pHDR4.5 hybridizing with the hamster probe were also found to cross-hybridize with each other under conditions of low stringency (Fig. 2*b*).

We subcloned those repeat-free fragments of both clones that hybridized to pDR4.7 into the plasmid pSP64 (Fig. 2*a*). The clone containing a 0.8-kb *Pvu* II fragment of pHDR4.4, inserted into the *Sma* I site of the vector, was designated pMDR1. The clone containing a 1.0-kb *Pst* I fragment of pHDR4.5, inserted into the *Pst* I site of the vector, was designated pMDR2.

**Rearrangement of *mdr2* DNA Sequences.** To determine whether the rearranged bands in KB-V1 and KB-A1 DNA correspond to *mdr1* or *mdr2*, DNA from different sublines

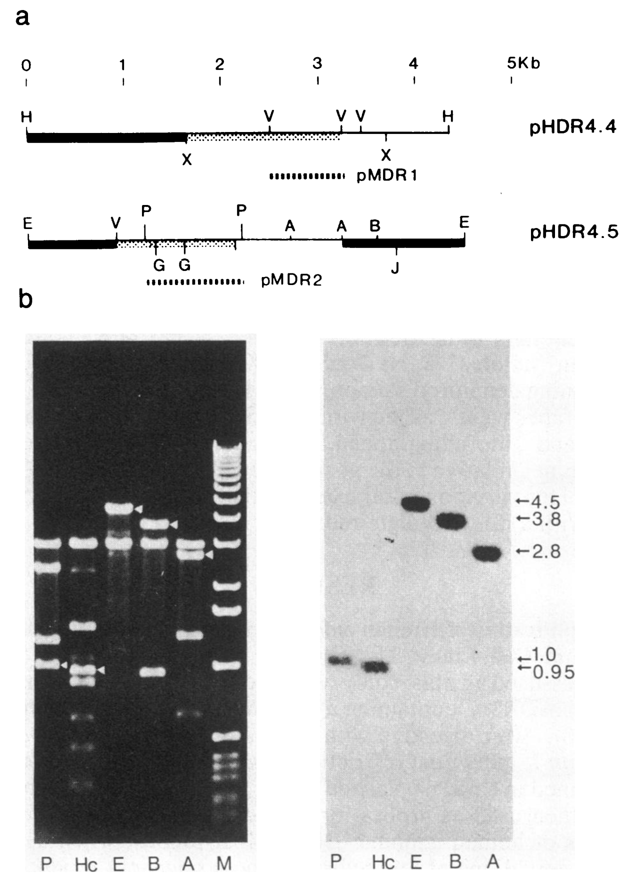


FIG. 2. (*a*) Restriction maps of the clones pHDR4.4 and pHDR4.5, containing human DNA sequences hybridizing to the hamster pDR4.7 probe. Solid bars indicate the fragments containing highly repeated sequences. Dotted bars indicate the DNA sequences hybridizing to the pDR4.7 clone. Positions of the pMDR1 and pMDR2 subclones are indicated. Restriction sites: A, *Ava* I; B, *Bam*HI; E, *Eco*RI; G, *Bgl* II; H, *Hin*dIII; J, *Hae* II; P, *Pst* I; V, *Pvu* II; X, *Xba* I. (*b*) Ethidium bromide staining pattern of pHDR4.5 digested with various restriction enzymes (*Left*) and hybridization with the 0.8-kb *Pvu* II fragment of pHDR4.4 under low-stringency conditions (*Right*). pHDR4.5 DNA was digested with *Eco*RI alone (E) or in combination with *Pst* I (P), *Hinc*II (Hc), *Bam*HI (B), or *Ava* I (A). A 1-kb "ladder" (Bethesda Research Laboratories) was used as size standards (M). The sizes of cross-hybridizing fragments (in kb) are indicated.

was digested with *Hin*dIII and hybridized to either the hamster pDR4.7 probe under conditions of low hybridization stringency or to the human pMDR1 or pMDR2 probes at high hybridization stringency (Fig. 3). The rearranged bands in both KB-A1 and KB-V1 sublines correspond to *mdr2*. Both types of cells also contain *mdr2*-hybridizing bands of the same size as in the parental cell line, 10.5 kb, suggesting that only one allele of the *mdr2* locus was rearranged. The mobility of the rearranged bands is also identical in KB-V1 and KB-A1 DNA digested with *Eco*RI or *Stu* I (Fig. 4; 7.0-kb *Eco*RI band and 3.9-kb *Stu* I band) as well as with several other restriction enzymes (data not shown), thus indicating that a similar or identical rearrangement may have occurred in both independently selected sublines. This rearrangement has been mapped to the 0.9-kb *Bam*HI–*Eco*RI fragment of pHDR4.5 (the rightmost region in Fig. 2*a*). However, although the rearranged sequences are amplified in KB-A1, they do not appear amplified in KB-V1 cells. Further, in the subline KB-A2, the rearranged *mdr2* band is no longer detectable. In the *Eco*RI digest of KB-A1 DNA (Fig. 4), in addition to the rearranged and amplified 7-kb and parental 4.5-kb *mdr2* bands, two other weak bands, which may

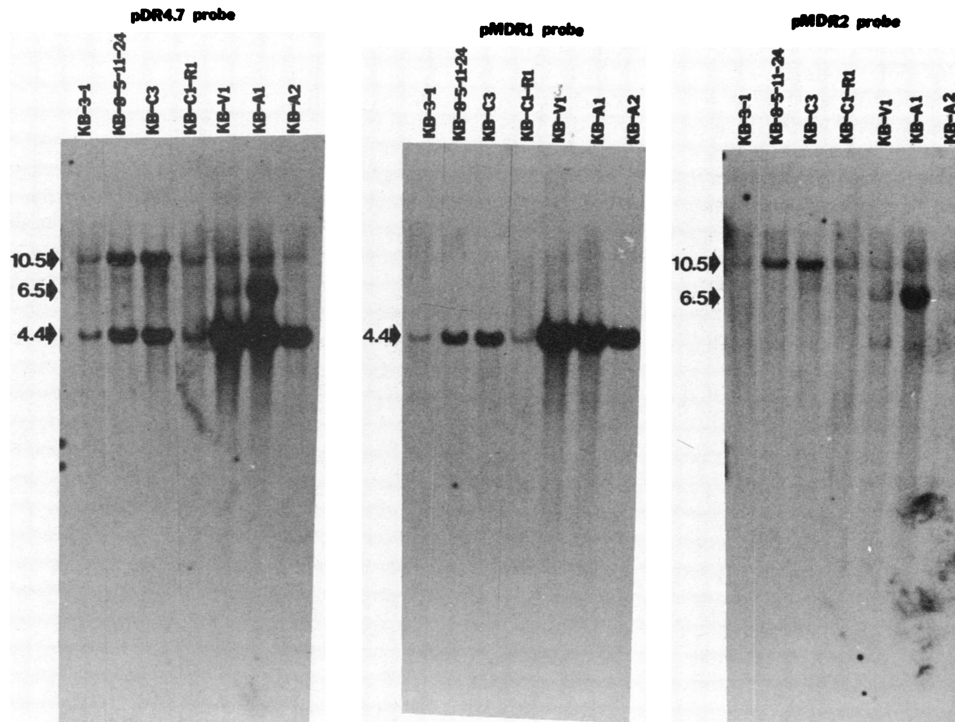


FIG. 3. Hybridization of cloned *mdr* probes with *Hin*dIII-digested genomic DNA. Hybridization with the gel-purified insert of the plasmid pDR4.7 was done under conditions of low stringency. The same blot was then rehybridized with gel-purified inserts of the plasmids pMDR1 and pMDR2 under high-stringency conditions. Ethidium bromide staining (not shown) indicated that the lane containing KB-A2 DNA was underloaded, accounting for the lower intensity of the bands. Fragment sizes (in kb) are indicated.

correspond to additional rearrangements occurring in the course of amplification, are detected.

**Transcription of *mdr1* DNA.** To determine whether the evolutionarily conserved regions of *mdr1* and *mdr2* contained transcribed sequences, pMDR1 and pMDR2 were used as probes for RNA blot hybridization (29) with poly(A)$^+$ RNA extracted from the parental KB-3-1 and multidrug-resistant KB-C2.5 cells (the immediate precursor of the KB-C3 subline in colchicine selection; refs. 12 and 13). Under conditions of high hybridization stringency, pMDR1 hybridized to a single mRNA species of 4.5 kb that is highly expressed in the drug-resistant cells (Fig. 5). This mRNA was not detected in the parental KB-3-1 cells. No *mdr2*-specific RNA was found using either pMDR2 (Fig. 5) or other repeat-free subfragments of pHDR4.5 as probes or by using RNA from other multidrug-resistant sublines of KB cells (data not shown).

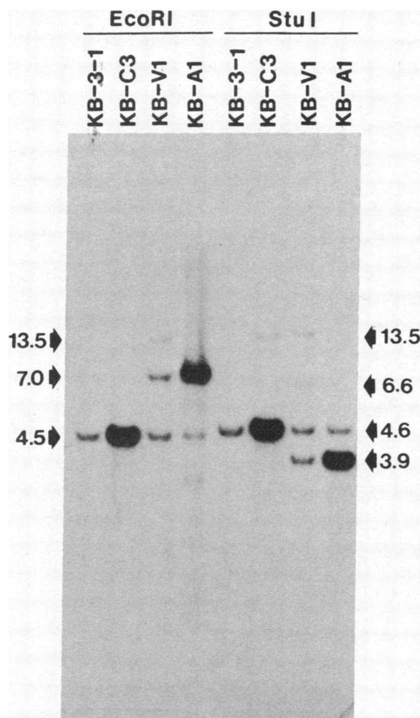


FIG. 4. Rearrangement of *mdr2* DNA sequences in KB-A1 and KB-V1 cells. Genomic DNA was digested with *Eco*RI or *Stu* I and hybridized with the gel-purified insert of the plasmid pMDR2 under conditions of high stringency. Fragment sizes (in kb) are indicated. The 13.5-kb band in the *Eco*RI digest and the 13.5- and 6.6-kb bands in the *Stu* I digest arise from cross-hybridization of pMDR2 with the amplified *mdr1* DNA sequences.

## DISCUSSION

We have used a segment of a Chinese hamster *mdr* gene known to be amplified and expressed in multidrug-resistant hamster cells to isolate segments of two homologous human DNA regions, designated *mdr1* and *mdr2*, from multidrug-resistant KB cells. *mdr1* sequences are expressed as a 4.5-kb poly(A)$^+$ RNA in multidrug-resistant cells. We have not detected expression of *mdr2* DNA sequences, nor is the *mdr2*

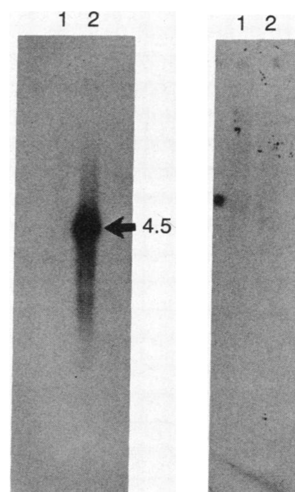


FIG. 5. Analysis of *mdr1* and *mdr2* RNA expression by blot hybridization. Poly(A)$^+$ RNA was extracted from the parental drug-sensitive KB-3-1 cells (lanes 1) and from the colchicine-resistant subline KB-C2.5 (lanes 2). One microgram of each RNA preparation was electrophoresed in a glyoxal/1.5% agarose gel. The filters were hybridized with pMDR1 (*Left*) or pMDR2 (*Right*) probe under high-stringency conditions. The size of the RNA band was determined relative to the positions of 28S and 18S rRNA.

gene always amplified in multidrug-resistant cell lines. Amplification of the human *mdr1* gene during selection for increased drug resistance and the loss of amplified *mdr1* DNA in a revertant cell line strongly indicate that *mdr1* or a closely linked amplified gene is responsible for multidrug resistance. It appears likely that *mdr1* rather than another gene represents the essential part of the amplified region, since *mdr* DNA sequences were found to be amplified and overexpressed in all multidrug-resistant human, hamster (7), or mouse (unpublished data) cell lines so far tested. Finally, the transfer of the human *mdr1* gene but not the *mdr2* gene is linked to the acquisition of multidrug resistance in mouse NIH 3T3 cells transfected with DNA from multidrug-resistant human KB cells (unpublished data).

The correlation between the degree of *mdr1* DNA amplification and relative drug resistance varies among different independently selected sublines of KB cells (Table 1). In addition, the copy number of *mdr* genes is reduced to apparently single-copy level in the revertant cell line KB-C1-R1, even though this cell line maintains a 3- to 6-fold level of relative drug resistance. We have recently found (30) that the KB-C1-R1 cell line, as well as the KB sublines corresponding to the first two steps of selection with colchicine (10), have elevated expression of *mdr1* RNA in the absence of *mdr1* gene amplification, suggesting that transcriptional activation of *mdr1* may precede its amplification during the development of resistance.

The existence of two differentially amplified human DNA sequences that cross-hybridize with the hamster *mdr* gene suggests that human *mdr* DNA sequences may comprise a multigene family. Different cross-hybridizing *mdr* sequences have also been observed in hamster and mouse DNA (ref. 8 and unpublished data). Riordan *et al.* (9) have also suggested that the gene for hamster P-glycoprotein, which may be identical with *mdr*, is a member of a multigene family. If *mdr1* and *mdr2* are linked in the genome, amplification of *mdr2* DNA sequences in multidrug-resistant cell lines could be explained as coamplification of DNA sequences flanking the essential *mdr1* gene. By digesting human DNA with infrequently cutting restriction enzymes and utilizing pulsed-field gradient gel electrophoresis (31) for separation of very large DNA fragments, we have found that *mdr1* and *mdr2* are linked within 350 kb of DNA in KB-C3 cells (unpublished data).

DNA rearrangements have been frequently associated with gene amplification (8, 32). The rearrangement of *mdr* DNA sequences in KB-A1 and KB-V1 cells is unusual, however, in that the rearranged bands appear to be identical or nearly identical in these independently derived cell lines.

The nature of the protein encoded by the *mdr* gene(s) is still unknown. Several lines of evidence suggest that *mdr* DNA sequences may be identical or related to the gene for the 170-kDa membrane glycoprotein (P-glycoprotein), a cDNA clone of which was recently isolated from Chinese hamster cells (9). Both *mdr* and the P-glycoprotein gene encode a 4.5-kb mRNA. Both genes were found to be amplified in two independently derived sets of multidrug-resistant Chinese hamster cells (7, 9), as well as in a multidrug-resistant human leukemia cell line (9, 30). The sublines of human KB cells used in the present study were found by immunoblotting assay using an anti-P-glycoprotein monoclonal antibody (33) to have an increased amount of P-glycoprotein in their membranes (13).

It is not known whether *mdr* genes are involved in the development of multidrug resistance by human tumor cells both *in vitro* and *in vivo* and whether the same genes are responsible for tumor resistance acquired in the course of chemotherapy as well as for the initial lack of response to drug treatment observed in some untreated tumors. The availability of cloned probes that detect transcription of *mdr* DNA in human cells makes it possible now to investigate the expression of these sequences in clinical samples of multidrug-resistant tumors.

We thank Rena Soffir and Carol Cardarelli for excellent technical assistance. This work was aided in part by the University of Illinois Biomedical Research Support Grant and Grant CA40333 from the National Cancer Institute.

1. Biedler, J. L., Chang, T., Meyers, M. B., Peterson, R. H. F. & Spengler, B. A. (1983) *Cancer Treat. Rep.* **67**, 859–868.
2. Ling, V., Kartner, N., Sudo, T., Siminovitch, L. & Riordan, J. R. (1983) *Cancer Treat. Rep.* **67**, 869–875.
3. Beck, W. T., Muellen, T. J. & Tanzer, L. R. (1979) *Cancer Res.* **39**, 2070–2076.
4. Kartner, N., Riordan, J. R. & Ling, V. (1983) *Science* **221**, 1285–1289.
5. Debenham, P. G., Kartner, N., Siminovitch, L., Riordan, J. R. & Ling, V. (1982) *Mol. Cell. Biol.* **2**, 881–889.
6. Robertson, S. M., Ling, V. & Stanners, C. P. (1984) *Mol. Cell. Biol.* **4**, 500–506.
7. Roninson, I. B., Abelson, H. T., Housman, D. E., Howell, N. & Varshavsky, A. (1984) *Nature (London)* **309**, 626–628.
8. Gros, P., Croop, J. M., Roninson, I. B., Varshavsky, A. & Housman, D. E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 337–341.
9. Riordan, J. R., Deuchars, K., Kartner, N., Alon, N., Trent, J. & Ling, V. (1985) *Nature (London)* **316**, 817–819.
10. Akiyama, S.-i., Fojo, A., Hanover, J. A., Pastan, I. & Gottesman, M. M. (1985) *Somatic Cell Mol. Genet.* **11**, 117–126.
11. Fojo, A., Akiyama, S.-i., Gottesman, M. M. & Pastan, I. (1985) *Cancer Res.* **45**, 3002–3007.
12. Richert, N., Akiyama, S., Shen, D.-w., Gottesman, M. M. & Pastan, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2330–2333.
13. Shen, D.-w., Cardarelli, C., Hwang, J., Richert, N., Ishii, S. Pastan, I. & Gottesman, M. M. (1986) *J. Biol. Chem.*, in press.
14. Roninson, I. B. (1983) *Nucleic Acids Res.* **11**, 5413–5431.
15. Fojo, A. T., Whang-Peng, J., Gottesman, M. M. & Pastan, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7661–7665.
16. Gross-Bellard, M., Oudet, P. & Chambon, P. (1978) *Eur. J. Biochem.* **36**, 32–38.
17. Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
19. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
20. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
21. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1520.
22. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
23. Rimm, D. L., Horness, D., Kucera, J. & Blattner, F. R. (1980) *Gene* **12**, 301–309.
24. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
25. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
26. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5306.
27. McMaster, G. K. & Carmichael, G. C. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4839.
28. Mulligan, R. C. & Berg, P. (1980) *Science* **209**, 1422–1427.
29. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
30. Shen, D.-w., Fojo, A., Chin, J. E., Roninson, I. B., Richert, N., Pastan, I. & Gottesman, M. M. (1986) *Science*, in press.
31. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **37**, 67–75.
32. Federspiel, N. A., Beverly, S. M., Schilling, J. W. & Schimke, R. T. (1984) *J. Biol. Chem.* **259**, 9127–9141.
33. Kartner, N., Evernden-Porelle, D., Bradley, G. & Ling, V. (1985) *Nature (London)* **316**, 820–823.