

Molecular Cytogenetic Characterization of Drug-resistant Leukemia Cell Lines by Comparative Genomic Hybridization and Fluorescence *in situ* Hybridization

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Resistance to chemotherapeutic drugs is one of the major difficulties encountered during cancer chemotherapy. To detect genomic aberrations underlying the acquired drug resistance, we examined three cultured human myelomonocytic leukemia cell sublines each resistant to adriamycin (ADR), 1- β -D-arabinofuranosylcytosine (ara-C), or vincristine (VCR), using comparative genomic hybridization (CGH), fluorescence *in situ* hybridization (FISH), RT-PCR, and western blot techniques. Chromosomes 7, 10 and 16 most conspicuously showed frequent aberrations among the resistant sublines as compared to the parental KY-821 cell line. In ADR-resistant cells, gains at 7q21, 16p12, 16p13.1-13.3, 16q11.1-q12.1, and losses at 7p22-pter, 7q36-qter, 10p12, 10p11.2-pter, 10q21-q25, 10q26-qter were notable. In ara-C-resistant cells, no remarkable gain or loss on chromosome 7, but losses at 10p14-pter, 10q26-qter and 16p11.2-p11.3 were observed. In VCR-resistant cells, gain at 7q21 and losses at 10p11-p13, 10p15 and 16p11.2-p13.3 were found. FISH identified amplified signals for the *MDR-1* gene located at 7q21.1 in ADR- and VCR- but not ara-C-resistant cells, and for the *MRP-1* gene located at 16p13.1 in ADR-resistant cells. These findings were validated at the mRNA and protein levels. Overlapping of the amplified *MRP-1* gene with *MDR-1* gene may play a critical part in the acquisition of resistance to ADR. Resistance to ara-C excluded *MDR-1* gene involvement and highlighted other key genes such as *MXR* gene. Several other genes putatively involved in the development of drug resistance might lie in other aberrated chromosomal regions.

Key words: Leukemia cell line — Drug resistance — CGH — *MDR-1* — *MRP-1*

Acquired drug resistance is a major difficulty during the treatment of many human cancers. *In vitro* molecular cytogenetic studies on cell lines provide an important approach for the characterization of the biologic mechanisms mediating this phenomenon.¹⁾ Several multidrug resistance mechanisms have been disclosed such as increased drug efflux, enhanced intracellular drug detoxification, alterations in nuclear targets, modifications of DNA repair systems, apoptotic regulatory systems and drug target regulation systems.²⁾

Cytogenetic analyses have identified several drug-resistance genes including multidrug resistance gene-1 (*MDR-1*), multidrug resistance-associated protein-1 (*MRP-1*) and canalicular multispecific organic anion transporter (*cMOAT* or *MRP-2*). The *MDR-1* gene is located at 7q21.1,^{3,4)} the *MRP-1* at 16p13.1,⁵⁾ and the *cMOAT* or *MRP-2* at 10q23-24^{6,7)} chromosomal regions. The *MDR-1* gene encodes a cell surface glycoprotein termed P-glycoprotein (P-gp)⁸⁾ that has been extensively studied and characterized. To date, considerable evidence has shown that *in vitro*-acquired resistance to multiple natural products is

mediated primarily by the P-gp, which acts as an energy-dependent drug efflux pump to reduce intracellular concentrations of drugs.⁹⁾ In addition, random chromosomal rearrangement leading to capture and activation of *MDR-1* gene has recently been proposed as a mechanism of acquired drug resistance.^{1,10)} The *MRP-1* gene encodes a 190 kD protein termed MRP-1 which belongs to the superfamily of ATP-binding cassette (ABC) transporters¹¹⁾ to which P-gp also belongs. Overexpression of MRP-1 resulted in resistance to various chemotherapeutic agents.¹²⁾ MRP-1 has been shown to transport a wide range of organic materials, such as glutathione (GSH) conjugates,⁵⁾ and other anionic conjugates.¹³⁾ Recently, several homologues of *MRP-1*, including *MRP-2* through *MRP-6*, have been identified.¹⁴⁻¹⁶⁾ The *cMOAT* or *MRP-2* protein was mainly expressed in the canalicular membrane of hepatocytes,^{6,14)} and was substantially overexpressed in several drug-resistant cell lines.¹⁵⁾

Several studies have successfully used comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH) techniques as adjuncts to the standard cytogenetic methods for the characterization of genetic aberrations associated with the acquisition of drug resistance.^{10,17-19)} Identification of such genetic aberrations

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provides clues as to the chromosomal regions which may harbor putative genes responsible for the drug resistance.

We previously developed three cultured human myelomonocytic leukemia cell sublines from the parental KY-821 cell line,^{20–23} each resistant to adriamycin (ADR), 1- β -D-arabinofuranosylcytosine (ara-C), or vincristine (VCR). The ADR-resistant subline was also cross-resistant to VCR and Actinomycin D, and VCR-resistant subline was cross-resistant to ara-C and Actinomycin D.²¹ The aim of the present study was to characterize the nature of the acquired resistance in these sublines at the molecular cytogenetic level and to provide further insights into the mechanisms underlying drug resistance. Genetic aberrations were determined by CGH and confirmed by FISH. The expression of potential genes at the mRNA and protein levels was detected by means of reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot analyses.

MATERIALS AND METHODS

Cell lines and measurement of drug resistance The three cell lines mentioned above were used. The degrees of resistance and cross-resistance to various drugs were measured using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described previously.²⁴ The ADR-, ara-C-, or VCR-resistant cells were seeded into the wells of 96-well plates (Corning, Inc., New York, NY) at 2×10^4 cells/100 μ l of culture medium containing various concentrations of the corresponding drug and incubated at 37°C in a CO₂ incubator for 72 h. Each drug and concentration were tested in triplicate. The absorption rates were measured using a fluorometer (Bio-Rad Laboratories, Inc., Hercules, CA). The IC₅₀ was defined as the concentration of drugs that inhibited cell growth by 50% compared with untreated cells as determined by linear regression analysis.

DNA preparation Genomic DNA was extracted from 1×10^6 cells each of ADR-, ara-C-, and VCR-resistant cells. High-molecular-weight DNA was extracted according to standard silica-gel membrane extraction procedures using a QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany).

CGH CGH was performed essentially as described by Kallioniemi *et al.*²⁵ Normal human genomic DNA (control DNA) was labeled with Spectrum Red-deoxyuridine triphosphate (dUTP) and cell line DNA was labeled with Spectrum Green-dUTP by standard nick translation reaction (Vysis, Inc., Downers, IL). For comparison between the two cell lines, DNA from the parental KY-821 cells was labeled with Spectrum Red-dUTP and that from the resistant cells with Spectrum Green-dUTP. The amount of DNase was adjusted for each sample so that the probe-fragment-size distribution after labeling was 600–2000 bp.

Each hybridization was performed on normal metaphase spreads (purchased from Vysis) using 200 ng of labeled tumor DNA, 100 ng of labeled normal control DNA and 10 μ g of human Cot-1 DNA in a moist chamber at 37°C for 72 h. After hybridization, the preparations were counterstained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI-II; Vysis). For each cell line, at least 8 to 10 metaphases were analyzed. Image capture and quantitative analysis of the ratio of green-to-red fluorescence intensities along each chromosome were done using a Leica Digital Image Analysis System (Q-FISH/Q-CGH Software Package; Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK). A chromosomal region was considered to have an increased DNA copy number if the average green-to-red fluorescence ratio exceeded the 1.15 threshold (a gain), an amplified copy number if the ratio exceeded the 1.5 threshold, and a decreased copy number if the ratio was below the 0.90 threshold (a loss). These threshold levels were based on the average green-to-red fluorescence ratio levels and 95% confidence intervals derived from control experiments using normal-normal genomic DNA co-hybridizations. Telomeric and pericentromeric regions, as well as chromosomes X and Y were excluded from the analysis. In addition, chromosomes 1p, 16p, 17p, 19, 22, were meticulously analyzed because these regions had been previously found to be prone to false-positive CGH results.²⁶ In each case, the averaged data from 3 repeated experiments was used for the analysis.

FISH FISH was performed using a BAC-derived probe²⁷ with *MDR-1* or *MRP-1* sequences in the pBAC-Lac vector and labeled with Spectrum Orange by nick translation. This was identified as a red fluorescence signal. As internal controls, centromeric probes, CEP 7 and CEP 16 labeled with Spectrum Green (Vysis) were used to identify chromosome 7 and 16, respectively. Hybridization was performed for 16 h at 37°C. Slides were washed and stained with DAPI. Detection and analysis were performed using the Leica Q-FISH system.

RT-PCR Total RNA was extracted using an RNeasy Mini Kit (QIAGEN) and tested for quality and quantity. One microgram of RNA was reverse-transcribed and transcripts were assayed by RT-PCR using the following gene-specific oligonucleotide primers: *MDR-1* primers corresponding to nucleotides 1045–1064 (5'-CTTATGCTCTGGCC TTCTGG-3') and 1523–1542 (5'-CCTGACTACCA-CACCAATG-3') respectively, which yielded a 500-bp product; *MRP-1* primers corresponding to nucleotides 3761–3779 (5'-CTGAGAAGGAGGCGCCCTG-3') and 4357–4375 (5'-CTGTCCGGATGGTGGACTG-3') respectively, which yielded a 613-bp product; and *cMOAT/MRP2* primers corresponding to nucleotides 4072–4091 (5'-CTGCCTTTCAGAATCTTAG-3') and 4312–4294 (5'-CCCAAGTTGCAGGCTGGCC-3'), which yielded a

241-bp product. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with specific sequences (5'-ACCACAGTC-CATGCCATCAC-3'), and (5'-TCCACCACCCTGTTG-CTGTA-3') yielding a 450-bp product was used as control. **Western blot** Proteins were extracted from 1×10⁷ cells/ml harvested from each subline after lysis with TNE buffer (10 mM Tris, pH 8.0, 1% NP-40, 0.15 M NaCl, 1 mM EDTA). Protein from each subline (50 µg) was resolved on 12% sodium dodecyl sulfate (SDS) polyacrylamide gel and transferred onto the polyvinylidene difluoride (PVDF) membranes. After blocking, the membranes were treated with appropriate dilutions of monoclonal antibody to P-gp (Fujirebio Diagnostic, Inc., Fairfield, NJ), anti-MRP (Nichirei Co., Tokyo), or anti-cMOAT (Alexis Corp., San Diego, CA). Then the membranes were washed and treated with 1:500 dilution of horseradish peroxidase-conjugated secondary antibody for 1 h. The membrane was finally reacted with 3,3-diaminobenzidine tetrahydrochloride with metal enhancer (Sigma Chemical Co., St. Louis, MO).

RESULTS

The patterns of resistance of the three leukemia cell sublines are presented in Table I. The ADR-resistant subline was also cross-resistant to VCR and Actinomycin D, and the VCR-resistant subline was cross-resistant to ara-C

and Actinomycin D. The ADR-, ara-C-, and VCR-resistant cells showed 400-, 2600-, and 2 000 000-fold resistance respectively as compared with the parental KY-821 cells. **CGH** A summary of all gains and losses of DNA copy numbers in the parental KY-821 leukemia cell line and the drug-resistant sublines is presented in Table II. The most common DNA copy number gains in ADR-resistant cells were observed on chromosomes 7q21-q22, 8p22-p23, 16p12, 16p13.1-13.3, 16q11.1-q12.1, 20q13.1-q13.3, and the most frequent losses were noted at 7p22-pter, 7q36-qter, 8q21.2-q21.3, 8q22, 8q24.2-qter, 9q34, 10p12, 10p11.2-pter, 10q21-q25, 10q26-qter, 14q11.1-q11.2, 14q22-q31. In ara-C-resistant cells, chromosomes 4p14, and 4q21-24 showed the most frequent gains and chromosomes 1p33-pter, 8p23-pter, 8q24.2-qter, 10p14-pter, 10q26-qter, 14p11.2-pter, 16p11.2-p11.3, 16p13.1-pter, 17p12-pter, 17q22-q25, 18p11.2-p11.3, 21p11.2-pter, 22qcen-qter showed frequent losses. In VCR-resistant cell line, the most common gains were at chromosome 1qcen-q12, 1q23-q31, 1q42, 2p23-p24, 2q32-q33, 5q11.1-q35, 7q21, 8pcen-p12, 8p21-p23, 8qcen-q24.1, 14q21-q32, 21q21-qter and the most frequent losses were at 10p11-p13, 10p15, 16p11.2-p13.3, 19p13.1-13.2, 19p13.4, 20p11.2, 20p13-pter. High-level amplification was not detected in any of the resistant cell lines. The most conspicuous chromosomes that showed frequent aberrations among the resistant cell sublines as compared to the parental KY-821 cells appeared

Table I. The IC₅₀ Values and Fold Resistances of the ADR-, Ara-C-, and VCR-resistant Sublines Compared to KY-821 Parental Cells

Drug	KY-821 (mol/liter)	Resistant sublines		
		ADR	Ara-C	VCR
Adriamycin	8.9×10 ⁻⁸	3.6×10 ⁻⁵ (400)	7.6×10 ⁻⁸ (0.8)	4.3×10 ⁻⁶ (48)
Vincristine	3.1×10 ⁻¹²	2.6×10 ⁻¹⁰ (84)	2.9×10 ⁻¹² (0.9)	6.4×10 ⁻⁶ (2×10 ⁶)
Arabinofuranosyl cytosine	3.3×10 ⁻⁸	3.9×10 ⁻⁸ (1.2)	8.6×10 ⁻⁵ (2600)	6.4×10 ⁻⁶ (194)
Actinomycin D	6.3×10 ⁻¹¹	5.6×10 ⁻⁹ (89)	5.6×10 ⁻¹¹ (0.9)	3.8×10 ⁻⁸ (600)

Table II. DNA Copy Number Changes in ADR-, Ara-C-, and VCR-resistant KY821 Leukemia Cell Lines

Cell line	Changes
ADR-resistant	Gain: 2p11.2, 7q21-q22, 8p22-p23, 16p12, 16p13.1-p13.3, 16q11.1-q12.1, 20q13.1-q13.3. Loss: 7p22-pter, 7q36-qter, 8q21.2-q21.3, 8q22, 8q24.2-qter, 9q34, 10p11.2-pter, 10p12, 10q21-q25, 10q26-qter, 14q11.1-q11.2, 14q22-q31.
Ara-C-resistant	Gain: 4p14, 4q21-q24. Loss: 1p33-pter, 8p23-pter, 8q24.2-qter, 10p14-pter, 10q26-qter, 14p11.2-pter, 16p11.2-p11.3, 16p13.1-pter, 17p12-pter, 17q22-q25, 18p11.2-p11.3, 21p11.2-pter, 22qcen-qter.
VCR-resistant	Gain: 1qcen-q12, 1q23-q31, 1q42, 2p23-p24, 2q32-q33, 5q11.1-q35, 7q21, 8pcen-p12, 8p21-p23, 8qcen-q24.1, 14q21-q32, 21q21-qter. Loss: 10p11-p13, 10p15, 16p11.2-p13.3, 19p13.1-13.2, 19p13.4, 20p11.2, 20p13-pter.

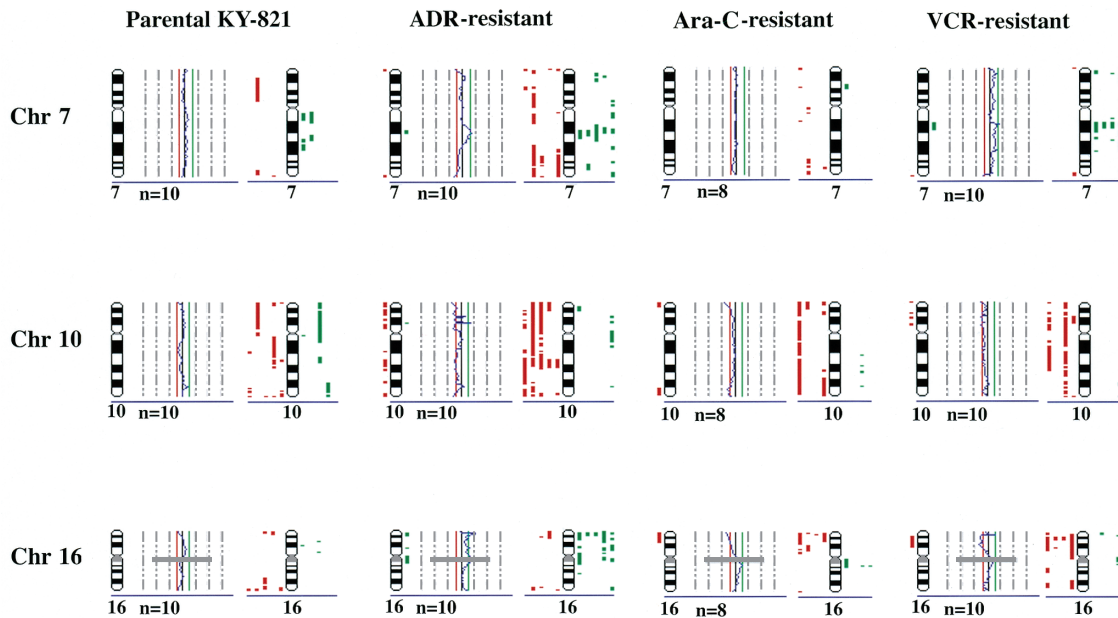


Fig. 1. Summary of the CGH profiles (intensity profile and multi-gain/loss profile) of chromosomes 7, 10, and 16 in the parental KY-821 cells versus normal reference DNA, and in the drug-resistant sublines versus parental KY-821 DNA. Gains of DNA copy number are shown to the right of the chromosome ideograms (green bars), and losses are shown to the left (red bars). The mean fluorescence intensity ratios for “*n*” number of chromosomes are shown in blue.

to be chromosomes 7, 10 and 16. In parental KY-821 cells, no appreciable gain or loss of DNA copy number was observed on chromosome 7, 10 or 16 (Fig. 1). In ADR-resistant cells, gains of 7q21, 16p12, 16p13.1-13.3, 16q11.1-q12.1, and losses of 7p22-pter, 7q36-qter, 10p12, 10p11.2-pter, 10q21-q25, 10q26-qter were notable. In ara-C-resistant cells, no remarkable gain or loss on chromosome 7, but losses of 10p14-pter, 10q26-qter and 16p11.2-p11.3 were observed. In VCR-resistant cells, gain of 7q21 and losses of 10p11-p13, 10p15 and 16p11.2-p13.3 were found (Fig. 1).

FISH Since chromosomes 7, 10 and 16 are known to harbor genes involved in the acquisition of multidrug resistance, i.e., *MDR-1*, *cMOAT/MRP-2* and *MRP-1* respectively, we used FISH to test whether the gains or losses at 7q21 and 16p13 might be due to the amplifications or deletions of *MDR-1* and *MRP-1* genes. FISH confirmed amplification of the 7q21.1 locus for *MDR-1* gene. Fig. 2 depicts representative FISH images. The percentage of KY-821 parental cell nuclei with four *MDR-1* signals (red) was 97.4% (Fig. 2A). CEP7 internal control probe (green) also indicated four signals in chromosome 7 in 97.4% of the cells. ADR-resistant cell nuclei with three *MDR-1* signals (red) and a homogeneous staining region (HSR) were detected in 92.9% of the cells (Fig. 2B). CEP7 internal control probe (green) also indicated three

signals in chromosome 7 in almost all of the cells. VCR-resistant cell nuclei with three *MDR-1* signals (red) and an HSR were seen in 96.2% of the cells (Fig. 2C). CEP7 probe (green) also indicated three signals in chromosome 7 in almost all of the cells. FISH also demonstrated the amplification or deletion of *MRP-1* at 16p13.1. The percentage of parental KY-821 cell nuclei with three *MRP-1* signals (red) was 94% (Fig. 2D). CEP16 internal control probe (green) also indicated three signals in chromosome 16 in 97.5% of the cells. ADR-resistant cell nuclei showed four signals for *MRP-1* (red) in 98% of the cells (Fig. 2E). CEP16 control probe (green) indicated three signals for chromosome 16 in 97.3% of the cells. Ara-C-resistant cell nuclei showed three *MRP-1* signals (red) in almost all the nuclei (Fig. 2F). CEP16 control probe (green) indicated five signals for chromosome 16 in 85.2% and four signals in 10.2% of the nuclei. VCR-resistant nuclei showed two *MRP-1* signals (red) in 75.7%, three signals in 17.1%, and four signals in 6.3% of the cells (Fig. 2G). CEP16 control probe (green) indicated three signals in 2.7%, four signals in 83.8%, and five signals in 6.3% of the cells.

RT-PCR The *MDR-1* mRNA (500 bp band) was found in ADR- and VCR-resistant cell sublines (Fig. 3). However, parental KY-821 cells and ara-C-resistant cells did not express *MDR-1* mRNA. Expression of *MRP-1* mRNA (613 bp band) was not found in parental KY-821 cells or

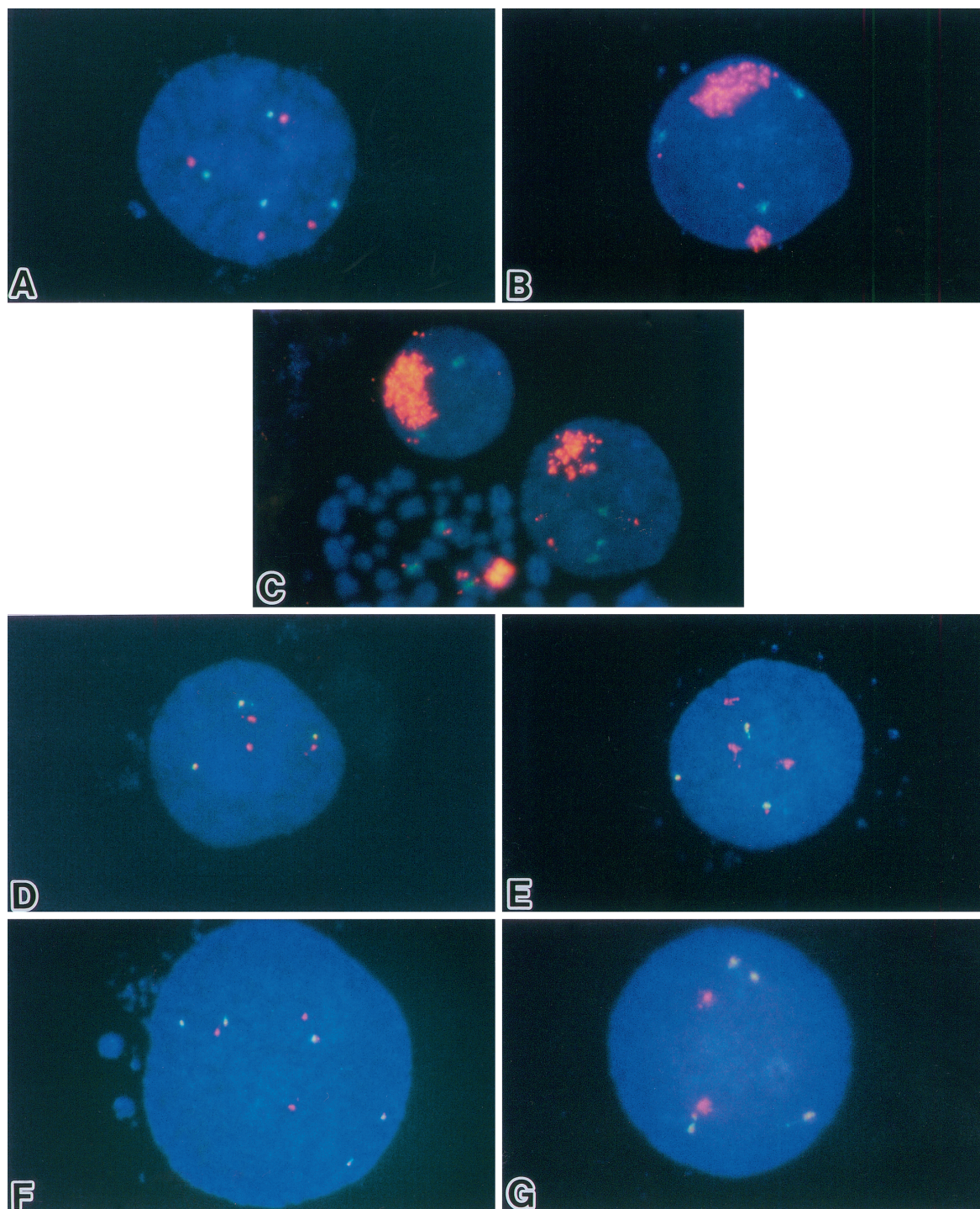


Fig. 2. Fluorescence *in situ* hybridization of interphase nuclei from the cell lines. (A) Interphase nuclei from parental KY-821 cells showing 4 copies of 7q21.1 signals for the *MDR-1* gene (red). (B) Interphase nuclei from ADR-resistant cells showing 3 copies and 2 homogeneously stained regions of 7q21.1 signals for the *MDR-1* gene (red). (C) Interphase nuclei from VCR-resistant cells showing 3 copies and 2 homogeneously stained regions of 7q21.1 signals for the *MDR-1* gene (red). (D) Interphase nuclei from parental KY-821 cells showing 3 copies of 16p13.1 signals for the *MRP-1* gene. (E) Interphase nuclei from ADR-resistant cells showing 4 copies of 16p13.1 signals for the *MRP-1* gene (red). (F) Interphase nuclei from ara-C-resistant cells showing 3 copies of 16p13.1 signals for the *MRP-1* gene (red). (G) Interphase nuclei from VCR-resistant cells showing 2 copies of 16p13.1 signals for the *MRP-1* gene (red).

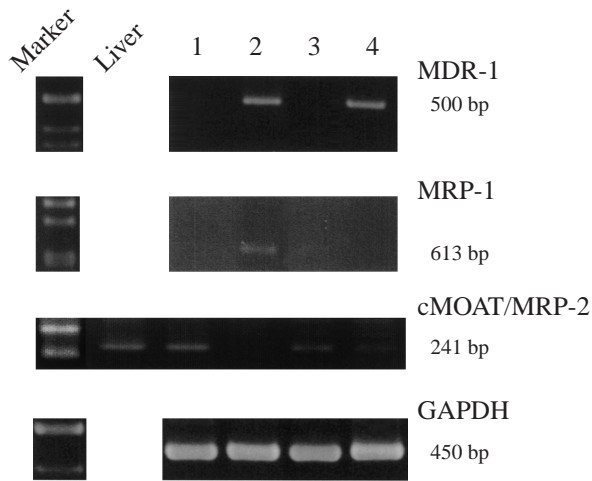


Fig. 3. Expression of *MDR-1* mRNA by RT-PCR showed 500 bp bands for the *MDR-1* gene. Expressions of *MRP-1* and *cMOAT/MRP-2* mRNAs by RT-PCR showed 613 bp bands for the *MRP-1* gene in ADR-resistant subline and 241 bp bands for the *cMOAT/MRP-2* gene in parental KY-821 cells and ara-C- and VCR-resistant sublines (lane 1, parental KY-821 cells; lane 2, ADR-resistant cells; lane 3, ara-C-resistant cells; lane 4, VCR-resistant cells). Note that RNA from liver cells was used as a positive control for the *cMOAT/MRP2* gene.

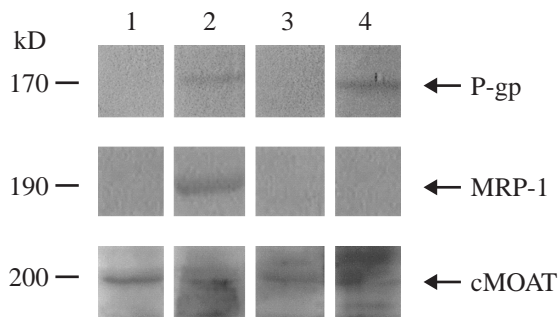


Fig. 4. Western blot analysis showing single bands of about 170 kD reacted with the primary antibody to P-gp in lanes 2 and 4 (upper panel), a single band of 190 kD reacted with anti-MRP-1 antibody in lane 2 (middle panel), and single bands of 200 kD reacted with anti-cMOAT antibody with different intensities in lanes 1 to 4 (lower panel). Lane 1, parental KY-821 cells; lane 2, ADR-resistant cells; lane 3, ara-C-resistant cells; lane 4, VCR-resistant cells.

ara-C- and VCR-resistant sublines, but was present in ADR-resistant cells. The *cMOAT/MRP-2* mRNA (241 bp band) was expressed in parental KY-821 cells and faintly in ara-C- and VCR-resistant cells, but not in ADR-resistant cells (Fig. 4). GAPDH mRNA was used as an internal control.

Western blot The *MDR-1* encoding protein, P-gp, was detected as a 170 kD band in ADR- and VCR-resistant cells at the expected position. But the parental KY-821 cells and ara-C-resistant cells did not show the P-gp band (Fig. 4). The MRP-1 protein band at 180–190 kD was not detected in parental KY-821 cells or ara-C- and VCR-resistant sublines, but was detected in ADR-resistant cells. The cMOAT protein was detected as a 200 kD band in parental KY-821 cells, and was absent or markedly decreased in the drug-resistant sublines.

DISCUSSION

In our study, the most conspicuous chromosomes that showed frequent aberrations among the drug-resistant sublines appeared to be chromosomes 7, 10 and 16. These chromosomes harbor multidrug resistance-related *MDR-1* (7q21.1), *MRP-1* (16p13.1) and *cMOAT/MRP-2* (10q23-24) genes, respectively. As expected, we observed amplification of *MDR-1* and overexpression of its encoded protein, P-gp, in ADR- and VCR-resistant sublines by FISH, RT-PCR, and western blot. Furthermore, we found overlapping amplified *MRP-1* in ADR-resistant cells and deletion of *MRP-1* in ara-C-resistant cells.

In this study, we confirmed the value of P-gp as a marker of the multidrug resistance phenotype and ADR or VCR resistance. Previous studies have shown amplification of *MDR-1* and its encoded protein, P-gp, in a large number of drug-resistant cell lines.²⁸⁾ In fact, the P-gp level was often low prior to treatment, but was frequently increased after chemotherapy.²⁹⁾ Our observations in ADR-resistant cells provide additional insight into the overlapping of the multidrug resistance phenotype identified by P-gp with that identified by *MRP-1*, resulting in a complex phenotype. In a study on a panel of human cancer cell lines not selected for drug resistance, but characterized for *MDR-1* encoded P-gp expression, a concomitant expression of different multidrug resistance phenotypes (including *MDR-1* and *MRP-1*) was observed in 64% of the cell lines and was, in general, associated with relatively high levels of drug resistance, supporting the view that overlapping phenotypes contribute to multidrug resistance.²⁹⁾

We found frequent gains at 4q21-24 in ara-C-resistant cells by CGH. This chromosomal region has been found to be amplified in mitoxantrone-resistant (MXR) cell lines, and molecular cloning of cDNAs which were highly overexpressed in MXR cells demonstrated homology to ABC transporter genes.³⁰⁾ The *MXR* gene encodes a half-transporter molecule that undergoes heterodimerization to form a complete transporter.³¹⁾ In addition, inactive, alternatively spliced forms of deoxycytidine kinase have been identified in ara-C-resistant acute myeloid leukemia cells.³²⁾ The gene encoding this enzyme resides on chromosome 4q13.3-q21.1, a region close to the *MXR* gene. It

was suggested that alternatively spliced deoxycytidine kinase monomers might form heterodimers with the wild-type monomers of the enzyme leading to the reduced expression of the active enzyme, as seen in ara-C-resistant cells. These data may point to *MXR* or partners in close proximity to *MXR* as potential mediators involved in acquisition of resistance to ara-C. Further investigations seem justified.

Although gains of genetic materials have been implicated in the development of drug resistance, losses of genetic materials also appear to be important in this process. In our study, losses at 10p11.2-p14 in ADR- and ara-C-resistant cells might include several zinc finger proteins located at this region, which is consistent with a shut-off of the transcription factors. Losses at 10q21-q25 in ADR-resistant cells and at 16p11.2-p13.3 in ara-C- and VCR-resistant cells might involve *cMOAT/MRP2* and *MRP-1* genes, respectively, undermining a role for these genes in such resistant phenotypes. Alternatively, losses at 16p11.2-p13.3 in VCR-resistant cells could suggest the involvement of the *axin* gene. A possible mechanism may be that mutations in *axin*, which is a downstream component of *Wnt* signaling, abrogate *APC-GSK-3 β -axin* activity, leading to the accumulation of free cytosolic β -catenin and induction of *Wnt-1*-mediated *β -catenin/Tcf* transcription which inhibit chemotherapy-induced apoptosis by preventing cytochrome *c* release and subsequent caspase 9 activation.³³⁾ This hypothesis remains to be tested. Interestingly, CGH demonstrated loss of *cMOAT* region in ADR-resistant but not in ara-C- and VCR-resistant cells, and expression of *cMOAT* mRNA and protein was diminished in each drug-resistant subline compared with the parental KY-821 cells. On the other hand, our three drug-resistant sublines showed remarkable sensitivity to SN-38 agent, an activated form of irinotecan hydrochloride which is an inhibitor of topoisomerase I (unpublished data). Because SN-38 is excluded through *cMOAT* protein in liver cells *in vitro*,³⁴⁾ diminished expression of *cMOAT* might contribute to high sensitivity to SN-38. This finding indicated that additional alterations of genes related to drug sensitivity may occur during the acquisition of resistance to some types of chemotherapeutic agents, and CGH is an effective method to search for such changes at the DNA level. However, this method has the following limitations which should be taken into account: (1) genetic aberration(s) found in drug-resistant cells is not necessarily responsible for drug resistance, (2) CGH can detect changes of DNA

copy number at specific chromosomal loci, but cannot detect mutations, and (3) although CGH can detect genetic aberrations, difficulties still remain in terms of identifying specific gene(s) related to a disease.

Importantly, losses at 8q21-q24 observed in ADR- and ara-C-resistant cells may prove novel and might well contribute to the resistance phenotype. Chromosomal region 8q22 contains a p53-dependent damage-inducible nuclear protein 1 gene which promotes cell death induced by DNA double-strand breaks.³⁵⁾ The 8q23.1 region harbors a p53-inducible ribonucleotide reductase small subunit 2 homologue. Induction of this gene in p53-deficient cells caused G2/M arrest and prevented cell death in response to adriamycin.³⁶⁾ Also, the 8q23 region contains an oxidation resistance 1 (*OXRI*) gene which confers protection against oxidative damage. Cells deficient in repair and protective mechanisms have elevated levels of spontaneous mutations. For example, mutations in genes affecting the cell's ability to repair oxidative damage, such as *BRCA1* and *ATM*, have been shown to predispose patients to cancer.³⁷⁾ Finally, losses of DNA copy number observed in the drug-resistant sublines might have occurred as a result of translocation of genetic materials leading to increased expression of responsive genes, as has been shown in several studies.^{1, 10)}

In conclusion, the acquisition of resistance to ADR and VCR was proved to be mediated by amplification of *MDR-1* gene and overexpression of its encoding protein, P-gp. Amplified *MRP-1* gene overlapped the amplification of *MDR-1* gene in ADR-resistant cells. Resistance to ara-C excluded the involvement of *MDR-1* gene and highlighted several other key genes, such as the *MXR* gene. Other chromosomal aberrations identified by CGH might well contain putative genes involved in the acquisition of drug resistance. Characterization of drug-resistant cell lines by CGH and FISH provides information useful in the development of new drugs or the design of strategies to reverse drug resistance.

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