Molecular Cloning and Characterization of the Complementary DNA for the M, 85,000 Protein Overexpressed in Adriamycin-resistant Human Tumor Cells

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ABSTRACT

An M, 85,000 membrane protein was identified by a monoclonal antibody MRK20 raised against an Adriamycin-resistant subline of human myelogenous leukemia K562 (K562/ADM) cells. The M, 85,000 protein was found to be overexpressed in both innate and acquired Adriamycin-resistant tumor lines. A complementary DNA (cDNA) clone coding for the M, 85,000 protein was isolated by mixed oligonucleotide-primed polymerase chain reaction and further screening of a cDNA library from K562/ADM. Amino acid and nucleotide sequence analysis of the M, 85,000 protein revealed that this protein is identical with CD36, a cell surface adhesion molecule of endothelium, platelets, and monocytes. We constructed an expression vector utilizing two different promoters, SV40 and MMTV, and two cDNAs for the M, 85,000 protein that have different 3'-ends. DNA transfection experiments were carried out by the calcium phosphate method with a selectable marker using drug-sensitive human tumor lines KB3-1 and A2780 as recipient cells. We obtained transfected clones expressing the M, 85,000 protein stably or inducibly but found no resistance against Adriamycin or vincristine. Direct selection with Adriamycin-resistant subline of human myelogenous leukemia K562, termed K562/ADM, and an etoposide-resistant subline of K562, Keto (25), as well as cultured acridine-resistant human ovarian carcinoma cells A2780, as well as other drug-resistant cell lines. Among them, MRK16 and MRK17 recognize the cell surface epitopes of P-glycoprotein (21). MRK4 and MRK20 recognize an M, 85,000 membrane protein (22). MRK18 recognizes an M, 300,000 membrane protein (23). HOT12 and three other monoclonal antibodies recognize an M, 22,000 cytosolic calcium-binding protein (CP22/sorcin) (24).

The expression of the M, 85,000 protein was found in several acquired drug-resistant human cell lines such as K562/ADM, an Adriamycin-resistant human ovarian cancer cell line 2780AD (22), and an etoposide-resistant subline of K562, Keto (25), as well as cultured human leukemia and lymphoma cell lines that show innate drug resistance to Adriamycin (26). The expression of the M, 85,000 protein in K562/ADM cells was diminished when the cells were kept in media without Adriamycin, and the expression was increased when the cells were treated with Adriamycin (22). The M, 85,000 protein was also found to be expressed in normal monocytes, granulocytes, and endothelial cells (27), and the expression levels of the protein were found to be related to the stage of differentiation of hematopoietic cells as determined by flow cytometric analysis (28) and Northern analysis. These findings suggest that the M, 85,000 membrane protein is closely related to the resistant mechanism of Adriamycin; however, the primary structure and function of the protein have not yet been clarified. In the present study, we isolated the cDNA clones of the M, 85,000 protein and examined the possible involvement of the protein with drug resistance by cDNA transfection experiments.

INTRODUCTION

Chemotherapy of hematological malignancies often fails because of the emergence of drug-resistant tumor cells and, thus, the development of drug resistance. Great attention has recently been given to the study of multidrug resistance (1, 2). MDR (1) cell lines show cross-resistance to structurally unrelated drugs of different origins (3). The MDR cells also show lowered accumulation and enhanced efflux of drugs (4). The most frequently occurring change in MDR cells is the increased expression of a membrane protein (P-glycoprotein) with an Mr of 170,000–180,000 (5, 6). We and others (7–9) have isolated the saline without calcium and magnesium (pH 7.2); SSC, standard saline citrate (1 x SSC = 0.15 M NaCl-0.015 M sodium citrate, pH 7.2); FACS, fluorescence-activated cell sorting; ADM, Adriamycin.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MDR, multidrug resistance; cDNA, complementary DNA; IC50, concentration of drug required for 50% inhibition of tumor cell growth; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; PBS, 10 mM phosphate-buffered saline without calcium and magnesium (pH 7.2); SSC, standard saline citrate (1 x SSC = 0.15 M NaCl-0.015 M sodium citrate, pH 7.2); FACS, fluorescence-activated cell sorting; ADM, Adriamycin.

4 K. Noguchi, Y. Sugimoto, and T. Tsuruo, manuscript in preparation.

MATERIALS AND METHODS

Cell Culture and Assay of Drug Sensitivity. A human myelogenous leukemia K562 cell subline resistant to Adriamycin (K562/ADM) was established in our laboratory as described previously (6). The human ovarian cancer cell line A2780 was kindly provided by Drs. R. F. Ozols and T. C. Hamilton (National Cancer Institute, Bethesda, MD) (29). Human KB carcinoma cell subline KB3–1 was found by Drs. I. Pastan and M. M. Gottesman (National Cancer Institute; 30). These tumor cells were grown in RPMI-1640 medium supplemented with 5% fetal bovine serum and 100 mg/ml kanamycin in a humidified atmosphere of 5% CO2. The sensitivity of tumor cell lines or transfectants to inherently, without expressing detectable amounts of P-glycoprotein (16). Drug-resistant mechanisms other than P-glycoprotein are important problems remaining to be solved. Indeed, several experimental results have shown that ADM-resistant cells possess certain alterations other than P-glycoprotein, which may contribute to ADM resistance. For example, an increase in glutathione S-transferase was reported in an ADM-resistant subline of human breast carcinoma MCF-7 (17). An increased amount of cellular glutathione was observed in an ADM-resistant variant of human ovarian carcinoma A2780 (18), and a decrease in DNA topoisomerase II activity was reported in ADM-resistant murine P388 leukemia cells (19, 20).

We previously established an Adriamycin-resistant subline of human myelogenous leukemia K562, termed K562/ADM (6), and reported the characterization of monoclonal antibodies against the proteins overexpressed in K562/ADM cells as well as other drug-resistant cell lines. Among them, MRK16 and MRK17 recognize the cell surface epitopes of P-glycoprotein (21). MRK4 and MRK20 recognize an M, 85,000 membrane protein (22). MRK18 recognizes an M, 300,000 membrane protein (23). HOT12 and three other monoclonal antibodies recognize an M, 22,000 cytosolic calcium-binding protein (CP22/sorcin) (24).

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Adriamycin or vincristine was evaluated by the inhibition of cell growth after incubation at 37°C for 5 days with various concentrations of drugs, as described previously (6). The number of tumor cells was counted in a Coulter Counter, and the IC50 was determined.

**Determination of Partial Amino Acid Sequence.** Purification of the M, 85,000 protein was carried out by immunoaffinity column chromatography with MRK20 essentially according to the methods used for the purification of P-glycoprotein (13). After immunoaffinity chromatography, we purified the protein on SDS-polyacrylamide gel electrophoresis. The purified M, 85,000 protein band was excised from the gel and digested with lysylendopeptidase at 37°C for 20 h in a solution containing 50 mM Tris-HCl, pH 8.3-0.1% SDS. The resultant peptides were separated by reversed-phase high performance liquid chromatography with a linear gradient of 0-80% aqueous acetonitrile containing 0.1% trifluoroacetic acid. Amino acid sequences of the peptides were determined by a gas-phase sequenator (Applied Biosystems model 470A) equipped with an on-line phenylthiobetainodion amino acid analyzer.

**Mixed Oligonucleotides.** Two sets of mixed oligonucleotides were used according to the two 6-amino acids sequences within peptide 16. One is 5'-CARGAYGCGNGARGAYA-3' (17-mer 64 mix) which codes QDAE DN (except the third letter of the last N), and the other is 5'-GTYGGNTTRCCNCGNTA-3' (17-mer 256 mix) which is complementary to the nucleotide sequence coding for QPENGAI (except the third letter of the last I).

**Oligonucleotide Hybridization.** Mixed oligonucleotide 5'-GTYGGNTTRCCNCGNTA-3' (17-mer 256 mix) was synthesized with an Applied Biosystems DNA synthesizer. Polyadenylated mRNA (10 μg) from K562 or K562/ADM was subjected to electrophoresis on a 1% formaldehyde-denatured agarose gel and transferred to a nitrocellulose membrane (31). The baked filter was incubated for 4 h at 65°C in a solution containing 6× SSC 0.5% SDS-0.1% Ficoll 400-0.1% polyvinylpyrrolidone-0.1% bovine serum albumin-0.05% sodium pyrophosphate-100 μg/ml denatured salmon sperm DNA and then hybridized with 32P-labeled oligonucleotides in a solution containing 6× SSC; 0.02% Ficoll 400-0.02% polyvinylpyrrolidone-0.02% bovine serum albumin-0.05% sodium pyrophosphate-100 μg/ml Escherichia coli tRNA at 37°C for 20 h. Filters were washed 4 times (30 min each) at 48°C with 6× SSC-0.05% sodium pyrophosphate and then autoradiographed.

**Mixed Oligonucleotide-primed PCR.** A single-strand cDNA template was synthesized in a reaction mixture (50 μl) containing 50 μM Tris-HCl, pH 8.3-7.5 μM KCl-3 μM MgCl2-10 μM dithiothreitol-500 μM each dNTP, oligonucleotide triphosphate, 50 μg/ml oligodeoxythymidylate, 100 μg/ml mRNA from K562/ADM, 1000 units/ml human placenta RNase inhibitor, and 10,000 units/ml SuperScript reverse transcriptase. After the synthesis reaction at 37°C for 1 h, the cDNA was purified by phenol extraction. Two mixed oligonucleotides, 5'-CAGGACGCGGAGAACAACAGTCTTCTCTGCGACCC-AACGGCGCCAT-3' and 5'-CAGCAGCAGGAGAACAACAGTCTTCTCTGCGACCC-AACGGCGCCAT-3', were used as PCR primers. Single-strand cDNA (20 ng) from K562/ADM was amplified by 30 cycles of PCR (32). The amplified cDNA fragment (50 base pairs) was cloned into Smal-digested pUC13 and sequenced by the dideoxy method using the Sequenase version 2.0 kit.

**Isolation of cDNA Clones.** A cDNA library was constructed in bacteriophage vector λgt11 starting with mRNA from K562/ADM (33, 34). Recombinant phages were plated and screened with a synthetic 50-mer oligonucleotide 5'-CAGGACGCGGAGAACAACAGTCTTCTCTGCGACCC-AACGGCGCCAT-3'. Thirteen cDNA clones were isolated and subcloned into pUC13 vector. Complete sequences of two cDNA clones (pR85-13 and pR85-21) were determined by dideoxy methods as described above.

**DNA Transfection.** Two mammalian expression vectors, pEUK-C1 (35) and pMAMneo (36), were used for the cDNA transfection experiments. Two cDNA inserts, containing the whole coding region for the M, 85,000 protein, were obtained from pR85-13 and pR85-21. The cDNA inserts were cloned into the XhoI sites of expression vectors using EcoR I-XhoI linker. For a control study, MDR1 cDNA obtained from human placenta mRNA was subcloned into pEUK-C1.

**Transfections were carried out using the calcium phosphate coprecipitation technique (37). An expression construct (20 μg) was used per 100-mm Petri dish, and 2 μg of a G418-resistant plasmid pMC1-neoPolyA was added when the pEUK-C1-derived construct was used. Twelve dishes of drug-sensitive recipient cells (6×10⁵ cells/100-mm dish) were transfected with expression construct plasmid DNA. After selection in medium containing 600 μg/ml G418 for 10 days, 10-30 G418-resistant colonies were isolated, and the other G418-resistant cells (derived from 10²-10⁶ colonies) were pooled to examine the emergence of drug-resistant cells in the transfectants.

**Protein Expression Analysis.** The expression of the M, 85,000 protein in the transfectants was screened by an enzyme-linked immunosorbent assay. Tumor cells plated in 96-well plates (10⁴ cells/well) were fixed in a solution containing 0.05% glutaraldehyde and washed with radioimmunoassay buffer (PBS containing 2% bovine serum albumin and 0.02% sodium azide). The tumor cells were treated with MRK20 (20 μg/ml) in radioimmunoassay buffer, washed three times with PBS, and then incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Cappel, Malvern, PA). The binding of antibody was estimated by the hydrolysis of p-nitrophenylphosphate.

A quantitative immunofluorescence staining assay was also carried out to examine the cross-reactivity of the transfectant to anti-CD36 antibody, OKM5 (Ortho Diagnostic Systems, Raritan, NJ). Cells (5×10⁵) were treated with MRK20 (10 μg/ml) or OKM5 (diluted 1:2), washed two times in PBS, and then incubated with fluorescein-conjugated F(ab')2 fragment of rabbit anti-mouse immunoglobulins (Zymed, diluted 1:20). The cells were washed three times, and the fluorescence staining was detected using FACSort (FACS system: Beckton-Dickinson, Sunnydale, CA).

**RESULTS**

**Determination of Partial Amino Acid Sequence.** Membrane preparation from K562/ADM was solubilized and applied to MRK20 affinity chromatography. The eluted protein was loaded and subjected to electrophoresis on an SDS-polyacrylamide gel. The band showing the M, 85,000 protein was cut out of the gel. The purified M, 85,000 protein was NH₂-terminally blocked. Therefore, the protein was digested with lysylendopeptidase to determine the sequence of peptides fragments. Two peptides were successfully purified and their amino acid sequences were determined. The sequence of peptide 12 was EGRPVYISLPHFLYASPDVSEP. The fluorescence staining was detected using FACSort (FACS system: Beckton-Dickinson, Sunnydale, CA).

**Oligonucleotide Hybridization.** The mixed oligonucleotide (17-mer 256 mix, antisense) hybridized with a 2.2-kilobase mRNA band overexpressed in K562/ADM cells (Fig. 1). This oligonucleotide also hybridized with other mRNA bands with higher molecular weights. The 2.2-kilobase mRNA band appears to be the mRNA for the overexpressed M, 85,000 protein because the mRNA is overexpressed in K562/ADM cells. This oligonucleotide, however, is not specific for ADRIAMYCIN-RESISTANT TUMOR.

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Fig. 1. Northern blot analysis of mRNA from Adriamycin-sensitive and resistant cell lines. Polyadenylated mRNA (10 μg) was electrophoresed in a formaldehyde-agarose gel, transferred to a nitrocellulose filter, and hybridized with 32P-labeled synthetic mixed oligonucleotides 5'-GTYGGNTTRCCNCGNTA-3' (17-mer 256 mix). Lane 1, K562; Lane 2, K562/ADM.
the putative M, 85,000 protein mRNA. Therefore, we prepared more a specific probe by mixed oligonucleotide-primer PCR.

Mixed Oligonucleotide-primed PCR. We synthesized another mixed oligonucleotide (sense) for PCR. PCR was carried out using cDNA from K562/ADM or K562. We successfully obtained a 50-base pair double-strand cDNA only when K562/ADM cDNA was used as the template. The cDNA band was excised from an agarose gel. The cDNA was cloned into SmaI-digested pUC13 vector and sequenced. Four independently obtained clones showed the same cDNA sequence (Fig. 2). This sequence was in good accordance with the possible nucleotide sequence deduced from the amino acid sequence of peptide 16. Therefore, the 50-base pair cDNA is a fragment of the M, 85,000 protein cDNA.

Isolation and Analysis of cDNA Clones. We synthesized a 50-mer oligonucleotide probe identical with a sense nucleotide sequence of the PCR product and screened a K562/ADM cDNA library. Thirteen positive cDNA clones were isolated and subcloned into the EcoRI site of the pUC13 vector. We determined the partial nucleotide sequences of the 13 cDNA clones and found that two types of 3'-end sequence exist in the M, 85,000 protein cDNA clones. Two cDNA clones, pR85-13 and pR85-21, were proven to possess different 3'-end structures and to be the longest cDNAs among the cDNA subgroups.

Fig. 3 shows the schematic structure of the two cDNA clones. Both cDNA clones possess the same open reading frame of 1413 nucleotides, corresponding to 471 amino acids. The nucleotide- and amino acid sequences were identical with CD36, a cell surface adhesive protein (38). Therefore, the M, 85,000 protein overexpressed in human Adriamycin-resistant cells is CD36. The putative amino acid sequence, however, was not identical with the determined amino acid sequence of peptide 16. The sequence of peptide 16 is EDVTQDAEDNVTSLQGNTNAYLTYGKARCCNAAYGGCNAT (GenBank, M98398). The other clone, pR85-21, possessed an entirely different 3'-noncoding region starting from the termination codon (GenBank, M98399).

DNA and RNA Blot Analysis. We previously reported the amplification of MDR1 gene in K562/ADM cells (9). To examine the possible amplification of the gene encoding the M, 85,000 protein, Southern blot analysis was performed (Fig. 4A). As shown in the figure, the gene was not amplified in K562/ADM cells. RNA blot analysis (Fig. 4B) showed the overexpression of the cloned gene in K562/ADM cells.

DNA Transfection Experiment. We introduced the two types of cDNA clones coding for the M, 85,000 protein with different 3'-noncoding regions to mammalian expression vectors. One is pEUK-C1 with an SV40-derived promoter which allows constitutive expression of downstream cDNA (35). The other is pMAMneo which has a long terminal repeat of mouse mammary tumor virus which is a dexamethasone-inducible promoter (36). These expression constructs were introduced into a human ovarian cancer cell line, A2780, which does not express a detectable amount of P-glycoprotein. A G418-resistant clonal cell line was transfected with MDR1 cDNA, and showed reactivity with MRK20 is summarized in Table 1. No clones showed significant resistance to Adriamycin. In earlier reports, transfectants introduced with MDR1 cDNA showed only low levels of resistance at low frequency (approximately 10^-3) when cells were not treated with any anticancer agents (39, 40). Therefore, we transfected the cDNAs into A2780 and KB3-1, pooled the G418-resistant cells, and treated with any anticancer agents (39, 40). Therefore, we transfected the cDNAs into A2780 and KB3-1, pooled the G418-resistant cells.

Fig. 2. Nucleotide sequence of 50-mer cDNA obtained from reverse transcriptase-PCR. Lane 1, amino acid sequence of the M, 85,000 protein (partial sequence of peptide 16); lane 2, deduced nucleotide sequence of the amino acid sequence of lane 1; lane 3, nucleotide sequence of 50-mer cDNA.

1; GlnAspAlaGluAspThrValSerPheLeuGlnProAsnGlyAlaAlaLe
2; CANGAYGCNGARGAYAAYACNGTNTCNTTYCTNCRARCCNAAYGGCNAT
3; CAGGAACGGGAGGAGCAACAAGCTCTTTCTTCTCTGAGCCCAACGGCCTCAT

( R = A, G; Y = C, T; N = A, G, C, T )

Fig. 3. Schematic profile of the M, 85,000 protein cDNAs. A. structure of cDNAs. M, 85,000 protein cDNA; pR85-13 : TAAGT----ATGTAACAAAAAA
pR85-13 : TAAGTAAAGGATAGACAAAAAA
pR85-21 : TAAGATCTCCTCAAGCACAA

Fig. 4. DNA and RNA blot analysis of the M, 85,000 protein cDNA and its transcript. A. DNA (10 µg) from K562 or K562/ADM was digested with EcoRI, fractionated on a 1% agarose gel, transferred to a nitrocellulose, and hybridized with 32P-labeled p85-13 cDNA insert. Lane 1, K562; lane 2, K562/ADM. B. polyadenylated mRNA (5 µg) from K562 or K562/ADM was electrophoresed on a 1% formaldehyde agarose gel, transferred to a nitrocellulose, and hybridized with 32P-labeled p85-13 cDNA insert. Lane 1, K562; lane 2, K562/ADM.
and then selected with Adriamycin or vincristine. The concentrations of drugs used for the selection were as follows: for A2780, 7.5 ng/ml Adriamycin or 1.8 ng/ml vincristine; for KB3-1, 10 ng/ml Adriamycin or 1.8 ng/ml vincristine. These concentrations are approximately 5-fold higher than the IC_{50} values of the recipient cells. In this study, an MDR1 cDNA clone introduced into pEUK-C1 was used as a positive control. The numbers of drug-resistant colonies are summarized in Table 2. Again, no drug-resistant colonies were found when the cells were transfected with the cDNA constructs for the M_r 85,000 protein (Table 2).

Fig. 5 shows the reactivity of MRK20 and anti-CD36 antibody OKM5 to K562/ADM and the transfectants (24-2-1 and 6-3) expressing the M_r 85,000 protein. FACS analysis clearly demonstrated that both antibodies react with the transfectants expressing the M_r 85,000 protein. This result indicates that the M_r 85,000 protein cDNA cloned in this study is the cDNA for CD36.

DISCUSSION

In previous studies, we identified an M_r 85,000 membrane protein overexpressed in K562/ADM cells (22) and found that the protein is expressed in several acquired drug-resistant human cell lines (22, 25) as well as cultured human leukemia and lymphoma cell lines which show innate Adriamycin resistance (26). In the present study, we successfully isolated cDNA clones of the protein and determined that the M_r 85,000 protein is identical with a membrane adhesive protein, CD36 (38, 41). The protein, however, did not confer drug-resistant phenotype when the cDNA was introduced into drug-sensitive human cell lines.

Many researchers have reported the changes in protein expression in drug-resistant cell lines. In these studies, several membrane adhesive proteins have been investigated. A cellular adhesion molecule CD56 (Leu 19) was found to be strongly up-regulated in an Adriamycin-resistant subline of human multiple myeloma 8226 (42). On the other hand, expression of epithelial cell adhesion molecule SQM1 was decreased in human squamous carcinoma cells resistant to methotrexate and cisplatin (43, 44). Involvement of these adhesive proteins in drug resistance, however, was not clarified. Some of them may be explained by the coinduction of mRNA expression with the MDR1 gene. Some may be attributed to changes during cell differentiation, and some may be truly related to drug resistance. Indeed, an unknown but non-P-glycoprotein-mediated mechanism for drug resistance was found to precede P-glycoprotein expression during in vitro selection for Adriamycin resistance (45). Many efforts should be made to find and analyze the drug-resistant mechanisms other than MDR1.

From this study, we conclude that the M_r 85,000 protein is identical with CD36. However, the amino acid sequence of the purified protein had one amino acid change, namely, the purified protein was 102 D; however, the deduced amino acid sequence from our cDNAs and from CD36 cDNA shows that residue 102 is N. Two cDNA clones possessed the same sequence; therefore, it is unlikely that the contradiction is caused by a mistake in the course of cDNA cloning and sequencing. Change from asparagine to aspartic acid can occur by chemical degradation of the amino acid, and this may be the reason for the misreading of the amino acid sequence. Another possible explanation is that the amino acid change occurs because of a single nucleotide mutation between A and T. These results suggest the possibility that K562/ADM cells express two types of molecules, because of a mutation in one allele. In this study we used only the 102 N-type cDNA for the CD36 expression experiments. If 102 D-type mRNA truly exists in the cells, the function of this mutant molecule is also to be determined.

According to results of our transfection experiments, introduction of the M_r 85,000 protein cDNA did not affect the sensitivity to Adriamycin and vincristine in drug-sensitive human tumor lines. There remains, however, some possibility that the M_r 85,000 protein is implicated in drug resistance. First, some additional changes, such as expression of other unknown proteins, may be necessary for the M_r 85,000 protein to participate in the drug resistance mechanism. To clarify this point, we need to transfect the cloned cDNA into various

Table 1 Sensitivity of A2780 transfectants to Adriamycin

<table>
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<th>cDNA</th>
<th>IC_{50} to Adriamycin (ng/ml)</th>
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<td>Dexamethasone 1 µm</td>
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<td>Clone 3</td>
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a 13R, the reverse orientation.

Table 2 Drug-resistant clones obtained after transfection of pEUK-C1-derived constructs

<table>
<thead>
<tr>
<th>Cells</th>
<th>cDNA</th>
<th>No. of resistant colonies</th>
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<tr>
<td>A2780</td>
<td>pr85-13</td>
<td>0</td>
</tr>
<tr>
<td>A2780</td>
<td>pr85-21</td>
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</tr>
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<td>A2780</td>
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<td>MDR1</td>
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a ADM, 7.5 ng/ml for A2780 and 10 ng/ml for KB3-1.
b Vincristine (VCR), 1.8 ng/ml for the both cell lines.
cell lines. Second, the M₅₈,85,000 protein may interact with P-glycoprotein and modulate the function of P-glycoprotein. This possibility must also be analyzed. Third, there remains the possibility that the M₅₈,85,000 protein may play an indirect role in multidrug resistance. To test this possibility, antisense oligonucleotides can be used to disrupt the M₅₈,85,000 protein expression.

The expression of the M₅₈,85,000 protein was found in two Adriamycin-resistant human tumor cell lines, K562/ADM and 2780AD (22), and an etoposide-resistant cell line, Keto (25). The expression of the protein has also been reported in cultured human leukemia and lymphoma cell lines such as K562, HEL, P31/FUJ, and KOPM-28 (26). Of these cell lines, K562/ADM, 2780AD, KYO-1, and HEL also express P-glycoprotein. The expression of P-glycoprotein in K562/ADM was increased after treatment with sodium butyrate, a differentiating agent (46). The expression levels of the M₅₈,85,000 protein in HEL are also related to the stage of differentiation (28). These results suggest the possibility that some common mechanisms may exist in the expression of P-glycoprotein and the M₅₈,85,000 protein. However, Keto, P31/FUJ, and KOPM-28 show a drug-resistant phenotype without P-glycoprotein expression. Recently, MRP, another potential drug transporter, was cloned from an Adriamycin-resistant human lung cancer cell line, H69AR (47). The existence of other drug transporters may provide additional or alternative mechanisms for drug resistance. Efforts must continue to identify and characterize new genes involved in drug resistance in tumor cells.

Apart from molecular biological analysis, we have sometimes found that drug-resistant leukemia/lymphoma cells tend to form homotypic clusters. Our K562/ADM cells grow in the medium with clusters of 10⁻³ to 10³ cells. Aclarubicin-resistant variants of murine lymphoblastoma L5178Y also form clusters. This preliminary observation may imply some important connections between cell adhesion molecules and drug resistance.

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Molecular Cloning and Characterization of the Complementary DNA for the Mr 85,000 Protein Overexpressed in Adriamycin-resistant Human Tumor Cells

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