THE MULTIPLE DRUG RESISTANCE GENE, MDRI: EXPRESSION AT THE PROTEIN AND RNA LEVELS

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A comparison of three different approaches to detect MDRI expression in myeloid leukemia cells was undertaken. With respect to the 4 different antibodies studied, a high proportion of false positive reactions were detected. Substantial discordance between MDRI expression as indicated by Northern blot analysis, PCR, and immunohistochemistry was found. These findings complicate the clinical interpretation of data derived from these methods.

Key Words: Monoclonal antibodies, Multiple drug resistance, Myeloid Leukemia, Northern blot analysis, Polymerase chain reaction, Southern blot analysis.

INTRODUCTION

Multidrug resistance may be an obstacle to successful treatment in acute myelogenous leukemia (AML) and blastic crisis of chronic myeloid leukemia. The mechanisms involved are complex, but it has been clearly shown that leukemia cells may become resistant to drugs by acquiring the ability to increase drug efflux by means of an activated energy-dependent membrane transport system. The protein involved in this process, a 170 Kd glycoprotein (P-gp) is coded by a multidrug resistance (MDR) gene. In man, two MDR genes (MDR1 and MDR3), sharing approximately 80% nucleotide homology, have been identified. To date only the MDR1 gene has been associated with the multidrug resistance phenotype. Here we report the study of P-gp in 16 patients with AML and 2 with myelodysplastic syndrome (MDS) using four MAbs (JSB1, HYB241, C219 and MRK16) and comparing these data with MDRI RNA levels.

MATERIALS AND METHODS

Sample collection

Peripheral blood or bone marrow were obtained from 16 patients with AML and two with MDS. Among the 16 patients with AML, 11 were studied at the time of initial presentation, 3 at first relapse and 2 in complete remission (CR). Acute leukemia was defined using French-American-British (FAB) criteria. Informed consent was obtained from each patient. The specimens were anticoagulated with heparin and immediately placed on ice. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (sp 1.077 g/cm³; Pharmacia, NJ).

RNA extraction and Northern blot analysis

RNA was prepared and analyzed as previously described in detail. In brief, the RNA was recovered from the guanidium isothiocyanate extraction by centrifugation through 5.8 M cesium chloride (CsCl); the pelleted RNA was dissolved in sterile distilled water and precipitated twice from 0.3 M sodium acetate with two volumes of ethanol at -20 °C. 5 μg of whole cell RNA was fractionated through a 1.2 % agarose gel, transferred to nylon mesh (Zetabind membrane, Cuno Inc), and hybridized with 32p-labelled MDR probe as previously described. The blots were stripped and reprobed with 32p-labelled beta-actin, to be certain that the absence of MDR1 transcripts were not due to degradation of the RNA. The MDR1 expression level was quantified by densometric scanning of the autoradiographs. For convenience, we have classified expression levels into four groups on the basis of the ratio of the signals for MDR1 and beta-actin RNA. A ratio of 0.1 or less was referred to as undetectable (−), 0.1 to 0.2 as low expression
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(‘+’), 0.2 to 0.6 as intermediate (‘++’), and 0.6 or more as high expression (‘+++’). RNA from the CEM cell line had an MDR1:RNA:beta-actin ratio of zero; and this served as negative control. The positive control was CEM/VLB (C/V), a drug resistant cell line, which had an MDR1:beta-actin ratio between 9 and 12.

**DNA preparation and Southern blot analysis**

DNA was purified by centrifugation through a 5.8 M CsCl gradient, dialysed in TE buffer, extracted twice with an equal volume of redistilled phenol and chloroform/isoamyl alcohol (24:1, vol/vol), precipitated in NaOAC/ethanol, and dissolved in TE buffer. 5 μg of genomic DNA was digested with EcoRI or HindIII, electrophoresed through 0.8 % – 1.0 % agarose gel, and blotted on nylon mesh (Zetabind, Cuno Inc). The DNA content in each lane and transfer efficiency was checked by staining the gel with ethidium bromide before and after blotting. The blotted membranes were sequentially washed in 2 × SSC at room temperature for 5 min and in 0.1 % SDS and 0.1 × SSC at 65 °C for 60 minutes, as recommended by the manufacturer (Cuno Inc). After hybridization with a nick-translated MDR1 cDNA probe, the blots were washed by the method of Church and Gilbert and exposed to X-ray film. The membranes were then sequentially stripped and rehybridized with beta-actin probe. The latter is a single copy gene DNA used to monitor the amount of DNA loaded.

**Probes for Northern and Southern blot analyses**

Three different probes were used to assess the expression of the MDR1 gene by Northern and Southern blot analysis: 1) a full-length MDR1 cDNA (pHAMDR1/A; a gift from Dr Gottesman M M, NIH, NCI, Bethesda, MD.), 2) pHDR5A, which carries a 1.38 kb EcoRI fragment from the middle third portion of the human MDR1 gene (a gift from Goldstein L J, NCI), and 3) a 1.2 kb BamHI-EcoRI fragment 5′MDR1 cDNA prepared from the full length cDNA. This region of MDR1 is only 50 % to 60 % homologous to the other member of human MDR family, MDR2 (also called MDR3). It does not include the highly homologous nucleotide-binding consensus sequences of MDR genes and therefore should not cross-hybridize with MDR2 under high-stringency conditions. The probes were labelled by the random primer method as previously described. The probes for Northern blot gave similar hybridization signals.

**Polymerase Chain Reaction (PCR)**

MDR expression was measured using PCR in 11 patients. The PCR data on these patients was kindly supplied by Dr Roninson (Department of Genetics, University of Chicago, Illinois).

**PCR procedure**

PCR was carried out with cDNA derived from 50ng of RNA, one unit of AmpliTaq polymerase and reaction kits (Perkin-Elmer/Cetus in a final volume of 50 μl. Thirty-five cycles, each consisting of 30 seconds of denaturation at 94 °C, 30 seconds of primer annealing at 55 °C and 2 minutes of extension/synthesis at 72 °C. The primer used for PCR was sense-strand primer CCCATCATGTGGAAAGCTTCTGCTTGAG (residues 2596–2615) and the anti-sense-strand primer TCTCTCTCGCTTGGTGAG (residues 2733–2752) which yield a 167-bp product. Each primer was added at 37.5 pmol per reaction.

**Immunocytochemical methods**

After density cut centrifugation, the cells were washed twice in phosphate buffered saline (PBS) at 1,500 rpm for five minutes each at 4 °C. The cell concentration was then adjusted to 5 × 10⁶/ml. Two drops of the cell suspension were placed on alcaic blue-coated coverslips after filtering through a No. 200 wire mesh and kept in a moist chamber for 10 minutes. The excess cell suspension was poured off and the coverslips immersed in freshly prepared mixture of acetone and chloroform (50:50) for 30 minutes at 4 °C followed by 0.5 % hydrogen peroxide (Sigma Chemicals Co., St. Louis #H-1009) in methanol for 20 minutes, with two washes in PBS between each step. The coverslips were then placed horizontally on rubber stoppers and the excess PBS wiped off carefully from the edges of the cell pellet. 100 μl of normal rabbit serum (Dako #X902) (diluted 1:10 with PBS containing 1 % w/v bovine serum albumin) was then placed on the coverslips, care being taken to cover the entire cell pellet. After incubation in a moist chamber for 30 minutes, the excess serum was poured off and the edges of the cell pellet were wiped with blotting paper.

The appropriate dilutions of the primary antibody (detailed below) were added and the incubation continued for 2 hours. Isotype-specific IgG was used as a negative control. CEM and the CEM/VLB cells (kindly provided by St. Jude Hospital, TN) were processed as described above and served as negative and positive controls respectively. After three washes in PBS containing 3 %, 2 % and 1 % normal human serum (NHS) (Gibco Laboratories, NY) respectively, the link antibody was applied (Rabbit anti-mouse immunoglobins...