clone coding for human MDR3 P-gp. The PCR fragment was cloned in the *Bam*HI-*Hin*dIII site of the vector pQE-9 and transformed into the bacterial *E. Coli* strain m15pREP4. The production of the recombinant protein in the bacteria was induced by IPTG, and monitored with SDS polyacrylamide gel electrophoresis. The N-terminal histidine tag containing protein was purified from the bacterial slurry with a Ni-NTA column and fast pressure liquid chromatography. BALB/c mice were immunized. Production of antibodies was monitored by an ELISA assay; 55 supernatants from the spleen cells of a positive mouse fused to the plasmocytoma cell line SP2/0 were found to be positive after screening in an ELISA assay. The hybridoma cells were cultured and 15 were subcloned to produce positive monoclonal hybridoma lines producing antibodies predominantly of the IgG_1 subclass (one IgM and one IgA). On Western blots culture supernatants recognized the recombinant protein. Monoclonals were purified by CM-Sephadex ionexchange columns and affinity chromatography (CNBr Sepharose-4B). Purified monoclonals were tested on Western blot. Enhanced chemiluminescence was used to visualize the P-gp antibody steptavidine HRP complex. Positive signals were found in five out of 14 monoclonals in cell lines expressing the human MDR3. Human MDR1 expressing cells did not react with the purified monclonals. Seven monoclonals were also used in FACScan experiments on the same cell lines. Two of them were positive from which one was also positive on Western blot. The expression of MDR1 and -3 in the tested cell lines (Nalm6, V01V01, FVBNC, KB V-1 and KB 3-1) was verified with MDR1 and MDR3 specific probes on Northern blots. On Western blots and in the FACscan the presence of P-gp was confirmed with C219 and or MRK 16. The expression of MDR3 in various histological subtypes of NHL is currently under investigation with the APAAP technique. Preliminary data indicate that expression is heterogeneous in each histological subtype. The role of MDR3 in relation to MDR1 expression will be discussed.

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The development of an assay for multidrug resistance using fine needle aspirates and the polymerase chain reaction (PCR)

JB Reed, M Carr, TWJ Lennard and B Westley

Departments of Surgery and Pathology, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK.

A new quantitative PCR assay has been developed for P-glycoprotein mRNA. It utilizes a synthetic mutated RNA as an internal standard and is designed for use on fine needle aspirates of tumors and recurrences. Using the Kunkel method of *in vitro* mutagenesis, a point mutation is introduced into the MDR cDNA sequence. Single stranded DNA is produced and an oligonucleotide with one nucleotide mismatch is annealed. The second strand of DNA is synthesized, producing a copy of the MDR gene containing a single point mutation which generates a new site of action for a restriction endonuclease (*Hin*dIII). This is then transcribed to produce synthetic mutated RNA. Known quantities of the mutant RNA are added to aliquots of cellular RNA from tumor aspirates which then undergo reverse transcription. The DNA produced is amplifed using PCR. Extensive testing and optimization of the PCR reaction has been carried out using a cell line (CaCo II) known to express MDR. PCR primers have been chosen to lie exactly symmetrically about the mutation site so that after digestion with *Hin*dIII, product from the synthetic mutated DNA is cut into two equal length fragments and the product of cellular origin remains intact. Thus two distinct bands appear on gel electrophoresis and image analysis of the relative amounts of the two bands allows quantification of MDR expression in the tumor sample. This technique has widespread applications in the clinical setting allowing the identification of patients likely to respond to chemotherapy and enabling drug regimens to be tailored more accurately.

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Quantitative *in vitro* determination of P-glycoprotein expression and function in tumor cells

B Sarkadi, L Homolya, Zs. Holló and M Müller

National Institute of Haematology, Blood Transfusion and Immunology, 1113 Budapest, Hungary.

In this report we describe a multiparameter *in vitro* analysis of the expression and function of P-glycoprotein (MDR1) in a series of cultured tumor cell lines and in leukocytes of leukemic patients. The expression of P-gp in MDR1 transfected or drug-selected cells with variable levels of drug resistance was assessed by quantitative immunoblotting, while MDR1 cell surface expression was followed by immunofluorescence and flow cytometry. The transport function of the P-gp was examined by measuring the extrusion of calcein AM with fluorometry and flow cytometry. In parallel experiments drug resistance was directly assessed in cytotoxicity assays. We demonstrate that a parameter calculated from calcein AM extrusion, measured in the absence and presence of an inhibitor of MDR transporter, e.g. verapamil, provides a quantitative measure for the maximum transport rate of the multidrug transporter. This parameter in most cases shows a good correlation with the amount of the expressed P-gp and with its appearance on the cell surface. However, in a human erythroleukemia cell line a mutant form of human MDR1 was found to be expressed and appearing on the cell surface in large quantities, without any drug-transport function. Data from the present experiment indicate that the calcein trapping functional assay is a highly sensitive method which directly reflects the level of MDR-dependent drug extrusion and a parameter calculated from this assay provides a good indication of the clinically expectable drug resistance.