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THE ROLE OF THE *MDR*1 (P-GLYCOPROTEIN) GENE IN MULTIDRUG RESISTANCE *IN VITRO* AND *IN VIVO*

IGOR B. RONINSON*
Department of Genetics, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

Abstract—This review describes the studies that address the role of the *MDR*1 (P-glycoprotein) gene in multidrug resistance in cell lines selected *in vitro* and in clinical cancer. Molecular genetic studies have demonstrated that expression of P-glycoprotein, an efflux pump acting at diverse lipophilic compounds, is sufficient to provide resistance to a large number of lipophilic drugs in tissue culture. The *MDR*1 gene is expressed in several normal human tissues associated with secretory or barrier functions and in some bone marrow and blood cells, including hematopoietic progenitor cells. *MDR*1 expression in clinical cancer is often found in untreated tumors of different types. Several studies showed a correlation between *MDR*1 expression and tumor resistance to combination chemotherapy. *MDR*1 expression in untreated tumors may reflect their origin from *MDR*1-positive normal cells or cellular changes associated with neoplastic transformation or progression. *MDR*1 expression in some types of cancer may be a marker of a more aggressive subpopulation of tumor cells, possessing multiple mechanisms for resistance to treatment.

The phenomenon of the multidrug resistance of tumor cells in tissue culture and in clinical cancer has been a subject of extensive investigation in recent years [1]. Multidrug-resistant cell lines can be derived by *in vitro* selection with a single lipophilic cytotoxic drug. Such cells show cross-resistance to many other compounds with different mechanisms of cytotoxicity including *Vinca* alkaloids, colchicine, taxol, anthracyclines, epipodophyllotoxins, puromycin, actinomycin D, trimetrexate, gramicidin D and some other drugs. Multidrug-resistant cell lines selected with different drugs from this group usually show the same spectrum of cross-resistance, thus indicating that these cell lines share a common mechanism for resistance. Some of these lines, however, show preferential resistance to the agent that was actually used in their selection, suggesting that their primary resistance mechanism could have undergone some modifications. In the first descriptions of the multidrug-resistant phenotype, this phenomenon was associated with decreased intracellular accumulation of the drugs [2], resulting at least in part from an increased energy-dependent drug efflux [3]. This transport change was associated with increased expression of a 170-kD glycoprotein of unknown function, termed P-glycoprotein [4]. The importance of P-glycoprotein became apparent once it was identified as the product of the *MDR*1 gene and once the functional role of that gene in multidrug resistance was demonstrated by molecular biological studies. These *in vitro* studies and the results of the analyses of *MDR*1 expression in clinical cancer are summarized in the present review.

* Correspondence address: Department of Genetics (M/C 669), University of Illinois at Chicago, 808 South Wood St, Chicago, IL 60612, U.S.A. Tel. (312) 996-3486; FAX (312) 413-0353.

† Abbreviation: PCR, polymerase chain reaction.

The role of the MDR1 *gene in multidrug resistance*

The multidrug resistance (*MDR*) genes were first identified as transcribed DNA sequences of unknown nature that were commonly amplified in multidrug-resistant cell lines selected with different drugs [5–7]. Subsequently, *MDR* sequences were found to match with the independently isolated cDNA clones for P-glycoprotein [8], indicating that P-glycoprotein was a product of *MDR* genes [9]. *MDR* genes were found to belong to a small gene family that includes two members in human and three members in rodent cells [10–12]. Of the two human genes, only one, designated *MDR*1, was consistently expressed at a high level in different multidrug-resistant cell lines and amplified in many of these lines [10]. Furthermore, the levels of *MDR*1 mRNA and protein expression showed a good correlation with the levels of cellular drug resistance. The other gene, designated *MDR*2 [7] or *MDR*3 [13], was co-amplified with *MDR*1 in some cell lines but *MDR*2 mRNA was expressed at exceedingly low levels even in those cells where the *MDR*2 gene was amplified [10]. *MDR*1 and *MDR*2 were found to be closely linked in the genome, indicating that *MDR*2 could be a "passenger gene" fortuitously co-amplified with *MDR*1 in some multidrug-resistant cell lines [10]. In rodent (mouse and hamster) cells, two genes are closely related to human *MDR*1, suggesting that they arose by a recent duplication whereas the third rodent gene is more homologous to human *MDR*2 [14–16]. Either one or both of the two *MDR*1-like rodent genes, but not the MDR2 homolog, are overexpressed in different multidrug-resistant cell lines [16, 17].

The functional role of the *MDR*1 gene in multidrug resistance was demonstrated by several types of gene transfer study. In the first type of experiment, genomic DNA from a multidrug-resistant subline of human KB carcinoma cells was used to transfer the multidrug-resistant phenotype to mouse NIH 3T3

cells. Both primary and secondary multidrug-resistant transfectants, selected with colchicine, were found to carry the human *MDR*1 (but not the *MDR*2) gene. The human gene was amplified and expressed in the transfected cell lines [18]. These experiments, however, could not rule out a possibility that some other gene, closely linked to *MDR*1, could be responsible for multidrug resistance in the transfectants.

The second set of studies was carried out using a full-length coding sequence of *MDR*1 cDNA, inserted in an expression vector. Transfection with the cDNA of the mouse [19] or human [20] *MDR*1 gene enabled the isolation of multidrug-resistant transfectants upon selection with cytotoxic drugs. In contrast, no multidrug-resistant colonies could be isolated under the same selective conditions from untransfected cells or from cells transfected with plasmids that did not express *MDR*1. Transfection with the *MDR*2 gene failed to confer resistance to any of the drugs associated with multidrug resistance [21].

In addition to increased *MDR*1 (P-glycoprotein) expression, many different biochemical changes had been associated with multidrug-resistant cells and most of these changes were suspected to play a role in multidrug resistance [1]. Thus, the *MDR*1 transfection studies, while demonstrating a functional role for the *MDR*1 gene, could not rule out the possibility that multidrug resistance *in vitro* is a multifactorial phenotype in which *MDR*1 expression is just one of the components. In particular, the use of cytotoxic selection in the isolation of *MDR*1 transfectants could have led to the simultaneous selection of some other changes complementing *MDR*1 in establishing the resistance. First, evidence that cytotoxic selection was not necessarily required for multidrug resistance came from the work of Guild *et al.* [22]. These investigators infected drug-sensitive NIH 3T3 cells with a recombinant retrovirus carrying a mouse *MDR*1 gene and then subcloned the infectants either with or without prior selection with cytotoxic drugs. Slightly elevated levels of drug resistance were found in most of the unselected infectants but these levels were lower than in any of the infectants selected with a cytotoxic drug. Although the unselected infectants showed lower levels of *MDR*1 mRNA than the drug-selected clones, it was also conceivable that some *MDR*1-independent mechanisms of resistance could have been selected by the drugs.

To determine if the drug selection of *MDR*1-expressing infectants leads to the emergence of any *MDR*1-independent mechanisms of resistance, we have carried out an experiment schematically illustrated in Fig. 1 [23]. Mouse NIH 3T3 cells were infected with a recombinant retrovirus carrying the human *MDR*1 gene. The surface of the infected cells was then labeled by indirect immunofluorescence with a human P-glycoprotein specific monoclonal antibody MRK16 [24] and infectants expressing the highest levels of P-glycoprotein were isolated by multiple rounds of flow sorting. Most of the infectant cell lines derived by flow sorting expressed relatively high levels of human P-glycoprotein which was associated with moderate amplification of the integrated proviral DNA. Another series of infectants was obtained by cytotoxic selection with vinblastine. The levels of resistance in both types of infectant showed an excellent correlation with the density of P-glycoprotein on the cell surface; expressed in relative terms as the ratio of immunoreactivity for P-glycoprotein to that for an unrelated cell surface antigen (Thy1.2). Cytotoxic selection conferred no additional increase in resistance relative to P-glycoprotein density [23]. These results indicate that P-glycoprotein expression on the cell surface is a sufficient determinant of the cellular level of multidrug resistance. It should be noted, however, that our results cannot rule out the contribution of some putative cellular factors to *MDR*1-mediated drug resistance, as long as such factors are present in excess relative to P-glycoprotein.

Evolutionary relationships and function of P-glycoproteins

The amino acid sequences of mammalian P-glycoproteins were deduced from the sequences of cDNA clones [25]. P-glycoproteins are about 1280 amino acids long and consist of two halves that share a high degree of sequence similarity. Each half of the protein includes a hydrophobic region with six predicted transmembrane segments and a relatively hydrophilic region which contains consensus sequences of nucleotide-binding domains. Various combinations of similarly organized transmembrane regions and homologous nucleotide-binding domains are found in a large superfamily of pro- and eukaryotic membrane proteins, associated with various transport processes [25]. Proteins encoded by some recently discovered genes of lower eukaryotes share homology with mammalian P-glycoproteins throughout their entire length and these genes should be properly regarded as members of the *MDR* family. These include the product of the yeast *ste*6 gene involved in the efflux of *a*-pheromone [26], *pfMDR*1 gene of malarial *Plasmodium* associated with chloroquine resistance [27] and some other protozoan *MDR* homologs. Outside the *MDR* family, the highest levels of homology to P-glycoproteins are found in bacterial proteins such as HlyB or NdvA that are involved in the active efflux of their substrates, high molecular weight proteins or carbohydrates [25]. Proteins of the HlyB group include a hydrophobic region with six transmembrane segments and a nucleotide-binding domain, thus resembling one half of P-glycoprotein. Some recent eukaryotic additions to the *MDR* superfamily include the product of the gene responsible for cystic fibrosis, whose architectural organization is almost identical to that of P-glycoprotein [28], and genes of the major histocompatibility complex involved in peptide transport; these genes resemble HlyB in their structural organization [29, 30].

The sequence similarity between the two halves of P-glycoprotein suggested initially that this protein may have arisen by duplication of an ancestral gene [31]. This hypothesis, however, was not confirmed by the analysis of the exon/intron structure of *MDR*1 [32]. When the two halves of the protein-coding sequence were aligned with each other, only two

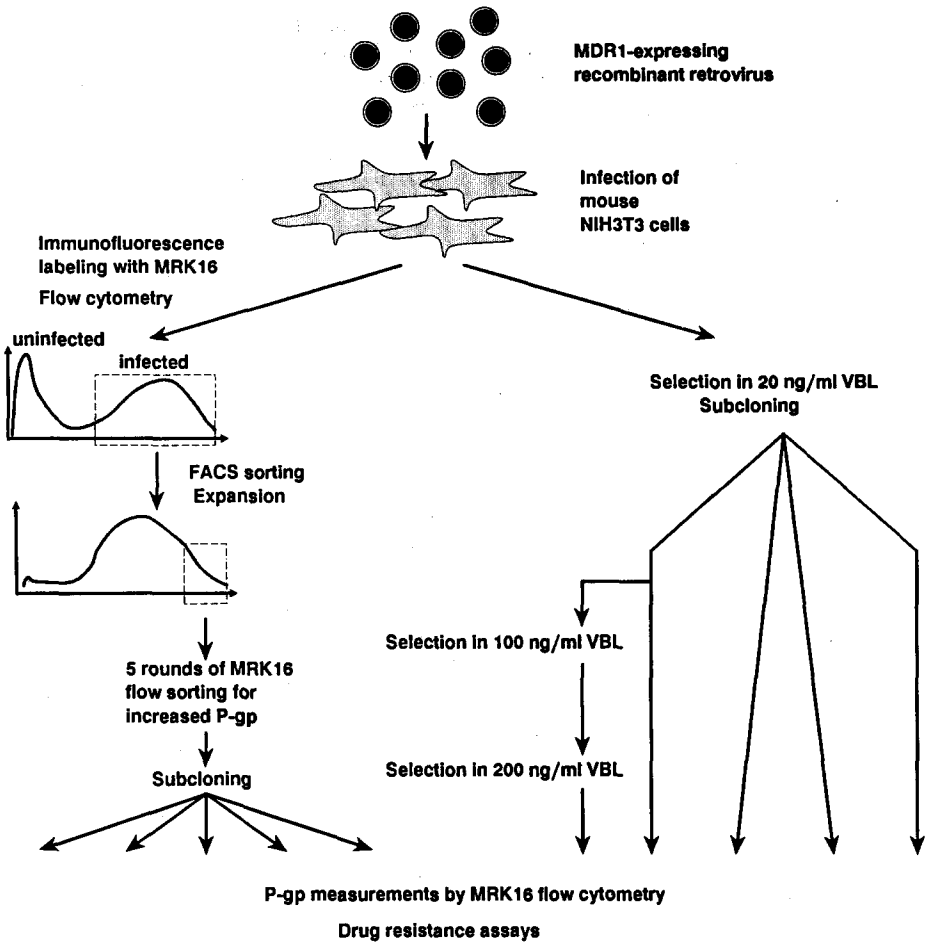

Fig. 1. Cytotoxic and non-cytotoxic selection of multidrug-resistant infectants. (See text and Ref. 23 for details.)

intron pairs, located in the coding sequence for the nucleotide-binding domains, were found at identical or nearly identical positions. All other intron pairs were mismatched in this alignment and, in most cases, interrupted the corresponding codons in different phases. This misalignment and especially the shifts in the intron phase, are almost impossible to reconcile with a common origin for these parts of the gene, as discussed in detail elsewhere [32]. We have suggested therefore that the original *MDR* gene arose not by internal duplication, but rather by fusion of genes for two related but independently evolved proteins of the HlyB type.

The homology of P-glycoprotein with proteins involved in active membrane transport suggested that P-glycoprotein functions as the ATP-dependent efflux pump postulated by Danö [3]. This function for P-glycoprotein is in agreement with the results of biochemical studies that demonstrated the ability of P-glycoprotein to bind its substrates directly [33] and to hydrolyse ATP [34]. In addition, P-glycoprotein-containing membrane vesicles from multidrug-resistant cells were shown to carry out active drug transport [33]. The function of P-glycoprotein was also confirmed by the results of mutational analysis indicating that multidrug resistance requires the function of both ATPase sites of P-glycoprotein (Ref. 35; B. Morse and I.B.R., unpublished data), and that the patterns of cross-resistance in multidrug-resistant cells can be altered by point mutations in the *MDR*1 gene (see below). Although ATP-dependent transport in a "pure" biochemical system, such as liposomes reconstituted with purified P-glycoprotein, has not yet been demonstrated, the available evidence leaves hardly any doubt in the efflux pump function of this protein.

Mutational changes in the substrate specificity of P-glycoprotein

As described above, expression of *MDR*1-encoded P-glycoprotein is sufficient for multidrug resistance. Nevertheless, it is known that multidrug-resistant cell lines, selected with different drugs, are sometimes more resistant to the drug that was used in their selection than to the other agents. Such preferential resistance has been used as an argument that multidrug resistance in many cell lines is likely to be multifactorial. We have investigated the mechanisms responsible for the emergence of preferential

resistance to colchicine in colchicine-selected multidrug-resistant derivatives of human KB carcinoma cells [10]. We have found that preferential resistance to colchicine, accompanied by a simultaneous decrease in the resistance to vinblastine, occurred simultaneously with a cluster of point mutations in the *MDR*1 gene, leading to a Gly-185 → Val-185 substitution in P-glycoprotein sequence [36]. These mutations arose in a cell line where the wild-type *MDR*1 gene was amplified, but in the cell line isolated at the next step of colchicine selection, all the amplified copies of *MDR*1 already carried the mutations. Gene transfer studies utilizing *MDR*1 cDNA encoding either the wild-type glycine or the mutant valine residue at position 185 have demonstrated that the mutations were indeed responsible for the altered pattern of cross-resistance. Transfectants expressing the mutant P-glycoprotein showed a strong increase in their resistance to colchicine and etoposide along with a significant decrease in their resistance to *Vinca* alkaloids and actinomycin D, relative to transfectants expressing a similar amount of the wild-type P-glycoprotein [36, 37]. These changes in cellular resistance were paralleled by a decreased accumulation of colchicine and increased accumulation of vinblastine in cells expressing the mutant protein [37].

The amino acid residue 185 was located within the first hydrophobic region of P-glycoprotein and, therefore, was likely to be involved in P-glycoprotein–drug interactions. The amino acid substitution at this position could change the specificity of P-glycoprotein mediated efflux by altering either the initial drug binding to P-glycoprotein or the release of the bound drug to the outside of the cell. To resolve these alternatives, we have compared the binding of photoactive drug analogs to the wild-type and mutant P-glycoproteins [37]. We found that the mutant protein bound a photoactive analog of colchicine more strongly and a vinblastine analog less strongly than did the wild-type P-glycoprotein. Since the mutant protein was more efficient in the efflux of colchicine and less efficient in the efflux of vinblastine, this result suggested that the mutation alters the relative efficiency of disassociation of the drugs from P-glycoprotein and their release to the outside of the cell, rather than the initial drug binding.

The Gly-185 → Val-185 substitution in colchicine-selected KB cells is so far the only established mechanism for preferential resistance to the selective agent in multidrug-resistant human cells. We have tried to find other cases of point mutations in the coding sequence of *MDR*1 by using the techniques of RNAase protection and chemical mismatch cleavage to scan *MDR*1 cDNA from eight independently derived multidrug-resistant human cell lines for sequence variations. Although we have found several polymorphic variations in the *MDR*1 cDNA sequence, none of them resulted from mutations associated with drug selection (C.-j. Chen, C. Zelnick and I.B.R., unpublished data).

Preferential resistance to the selective agent may be especially common in multidrug-resistant rodent cell lines. In these cells, a different mechanism for preferential resistance is likely to be operational. As indicated above, hamster and mouse genomes contain two genes that appear to have arisen from the duplication of the primordial *MDR*1 gene. Expression of either one of these genes is sufficient for multidrug resistance. Devault and Gros [38] have shown that mouse cell lines transfected with different *MDR*1-homologous genes show different patterns of cross-resistance. It is likely, therefore, that differences in the pattern of cross-resistance of multidrug-resistant rodent cell lines are likely to reflect differential expression of these genes. This possibility is also in agreement with the observed correlations between the expression of different members of the *MDR* gene family and the patterns of cross-resistance in the derivatives of mouse J774.2 cells [39], although there is some discrepancy between the specific correlations observed in the two studies [38, 39].

MDR1 *expression in normal solid tissues and bone marrow*

High levels of *MDR*1 expression have been found using immunohistochemical assays in several normal human tissues including the adrenal cortex; lumenal surfaces of the colon, jejunum, kidney and liver; placental trophoblasts; and endothelial cells of the brain and testes [40, 41]. These patterns suggest that P-glycoprotein, a pleiotropic transport protein, may be utilized for different purposes by different types of cells. Its tissue-specific functions may include excretion of toxic or potentially toxic substances, maintenance of homeostatic levels of steroid hormones in the adrenal gland cells and maintenance of blood–brain, blood–testis, and placental barriers. In addition to these major sites, some other tissues also showed heterogeneous patterns of immunohistochemical staining for P-glycoprotein [41, 42].

Initial immunohistochemical assays for P-glycoprotein expression in peripheral blood and bone marrow cells produced negative results. Studies by Neyfakh *et al.* [43] demonstrated, however, that some subsets of normal lymphocytes show increased efflux of P-glycoprotein-transported fluorescent dyes such as the mitochondrial dye rhodamine 123 (Rh-123). The efflux of these dyes was sensitive to P-glycoprotein inhibitors, suggesting that it could be mediated by P-glycoprotein [43]. In the bone marrow, decreased staining with Rh-123 had been shown to be a property of hematopoietic stem cells; this observation, however, was interpreted as indicating a low number or activity of mitochondria in the stem cells [44]. We have analysed Rh-123 staining of human bone marrow cells and found that it was determined not by the initial dye accumulation, as would have been expected from differences in the mitochondrial index, but rather by an efflux process sensitive to P-glycoprotein inhibitors [45]. Furthermore, using double- and triple-labeling flow cytometric assays, we found that the efflux of Rh-123 and several other dyes was directly correlated with the expression of P-glycoprotein in bone marrow cells. *In vitro* assays for hematopoiesis demonstrated P-glycoprotein expression and dye efflux in practically all the hematopoietic progenitor cells. The highest levels of P-glycoprotein among the progenitors were

associated with cells displaying physical properties and the antigenic phenotype of pluripotent stem cells ($CD34^{++}$, $HLA\text{-}DR^{low}$, $CD33^{-}$). The function of P-glycoprotein in hematopoietic progenitor cells of the bone marrow remains to be determined [45].

MDR1 *expression in clinical cancer*

Since the role of the *MDR*1 gene in multidrug resistance *in vitro* was established, detection of *MDR*1 expression in clinical tumor samples has been viewed as a potential diagnostic test for the prediction of tumor response to chemotherapeutic drugs. Analysis of the role of P-glycoprotein in tumor drug resistance has been linked to the expectation that P-glycoprotein inhibitors, many of which have little or no intrinsic toxicity [46], may be therapeutically useful for reversing P-glycoprotein-mediated resistance. Many investigators utilized *MDR*1 cDNA probes or anti-P-glycoprotein antibodies to analyse the expression of this gene in various tumors [47–49]. In some cases, *MDR*1 (P-glycoprotein) expression in tumors became detectable or increased only after chemotherapeutic treatment but, in many other instances, fairly high levels of *MDR*1 expression were found in untreated tumors intrinsically unresponsive to chemotherapy. These results suggested that *MDR*1 expression could be associated with both the acquired and the intrinsic types of clinical drug resistance.

P-glycoprotein expression in some intrinsically resistant tumors could be explained by the derivation of these tumors from cells that normally express *MDR*1. Thus, a correlation between the degree of differentiation and *MDR*1 expression has been demonstrated in renal cell carcinomas [50], presumably reflecting high expression of this gene in normal cells of proximal tubules of the kidney from which these tumors arise [51]. Other tumors derived from tissues that normally express P-glycoprotein, such as the adrenal cortex or the colon, also frequently express high levels of *MDR*1. In the cases of untreated tumors derived from tissues with low normal levels of P-glycoprotein expression (carcinomas of the lung, ovary or breast; sarcomas) *MDR*1 activation could possibly be a consequence of neoplastic transformation. It should be noted, however, that *MDR*1 expression in some normal tissues that are largely P-glycoprotein-negative may be confined to a specific subpopulation of cells [41, 42] and it is possible that *MDR*1-positive tumors of such tissues may originate from this subpopulation. For example, a much greater incidence of high *MDR*1 expression in untreated chronic myeloid leukemia has been associated with the blast crisis rather than with the chronic phase [48, 52], suggesting at first glance an association of *MDR*1 expression with increased malignancy. On the other hand, since the levels of P-glycoprotein in normal hematopoietic progenitors appear to be inversely correlated with the degree of differentiation [45], increased *MDR*1 expression in blast crisis cells may reflect the correspondingly lower degree of differentiation in these cells. This hypothesis is also in agreement with the results of List *et al.* [53] who found a positive correlation between the levels of CD34, the marker of hematopoietic stem cells, and P-glycoprotein expression in myelodysplastic syndromes and associated leukemias which parallels the findings in normal hematopoietic cells [45].

There is other evidence suggesting that *MDR*1 expression in some types of cancer may be associated with neoplastic transformation and tumor progression. Thus, increased expression of a *MDR* gene was detected in regenerating rat liver and in chemically induced hepatocarcinogenesis [54], as well as in oncogene-transformed hepatocytes [55]. In human solid tumors, *MDR*1 expression is often heterogeneous and in some tumor types it is preferentially associated with areas of apparent invasive growth [56, 57]. In untreated colon carcinomas, the presence of P-glycoprotein at the invasive edge of the tumor shows statistically significant correlation with small vessel invasion and lymph node metastases [57], suggesting that P-glycoprotein is a marker of the more malignant subpopulations of tumor cells. It appears, therefore, that *MDR*1 expression in untreated tumors may reflect the parameters of both differentiation and malignant progression.

A number of recent studies addressed the issue of a correlation between *MDR*1 expression in individual tumors and the patient's response to chemotherapy. Several reports utilizing standard hybridization or immunohistochemical assays indicate that *MDR*1 expression in myelomas, leukemias, lymphomas and renal cell, breast and esophageal carcinomas is preferentially associated with tumors that show drug resistance either clinically [58–61] or in *in vitro* clonogenic assays [62, 63]. The strongest association between P-glycoprotein expression, on the one hand, and lack of response to chemotherapy and poor survival, on the other hand, was described in childhood soft tissue sarcomas [64].

We have used a highly sensitive PCR technique to study *MDR*1 mRNA expression in more than 300 different tumors and normal tissues of types that rarely show *MDR*1 expression when less sensitive procedures are used [49]. These assays showed that low levels of *MDR*1 mRNA, detectable by PCR, were present in most but not all of the tumors. In contrast to the absence of *MDR*1 mRNA in a sizable minority of the tested tumors and tumor-derived cell lines, almost no *MDR*1-negative samples were found among normal tissues. This result suggested that most normal tissues express *MDR*1 in some cells but *MDR*1-negative tumors may represent a clonal outgrowth of *MDR*1-negative individual normal cells [49].

The incidence of tumors that did not express any detectable *MDR*1 mRNA appeared to correlate with the overall frequency of initial response to chemotherapy for different tumor types. Thus, six out of six Ewing's sarcomas showed no detectable *MDR*1 mRNA whereas osteosarcomas or non-small cell lung carcinomas had a low (10–20%) incidence of *MDR*1-negative tumors, and acute non-lymphocytic leukemias showed approximately 50% incidence of *MDR*1-negative samples. These results are in general agreement with the incidence of initial response in these types of tumors [49]. To determine if the low levels of *MDR*1 mRNA expression, detectable by PCR, were predictive of resistance to chemotherapy

in individual tumors, we analysed the correlation between the levels of *MDR*1 mRNA and response to chemotherapy in more than 100 individual ovarian and lung carcinoma patients, 56 of whom were subsequently treated with various chemotherapeutic regimens and their response to treatment evaluated [T. A. Holzmayer, D. D. von Hoff and I. B. R., unpublished data]. The results of this study showed a highly significant correlation between the absence of PCR-detectable *MDR*1 expression and subsequent (partial) response to chemotherapy. Within individual tumor types, this correlation was apparent in the ovarian and small cell lung cancers. Interestingly, there was no correlation with response when the lowest detectable levels of *MDR*1 mRNA were considered as negative. In the light of the above mentioned immunohistochemical and *in situ* hybridization studies showing heterogeneity of *MDR*1 expression in solid tumors, it seems most likely that very low levels of *MDR*1 expression, correlating with the resistance to chemotherapy, represent *MDR*1 expression in a small subpopulation of cells within the tumor.

Surprisingly, *MDR*1 expression in our study and others [58–61, 64] showed a correlation with resistance to combination chemotherapy regimens that included drugs resistance to which should not have been affected by P-glycoprotein. These results suggest that P-glycoprotein expression in some types of tumors marks a population of cells that possess multiple changes making them resistant to various cytotoxic agents. The relative contribution of P-glycoprotein to this postulated multifactorial intrinsic resistance remains unknown.

Thus, *MDR*1 (P-glycoprotein) expression appears to play different roles in multidrug-resistant cell lines selected *in vitro* and in clinically resistant human tumors. In the first case, *MDR*1 expression appears to be the major and perhaps the only determinant of the multidrug-resistant phenotype. This is not surprising, considering that multidrug-resistant cell lines represent genetic mutants arising at a low frequency. Since a single mutation, leading to increased *MDR*1 expression, is sufficient to make cells resistant to their selective agent, the probability of an additional resistance-associated genetic change occurring in the same cells is rather low. In contrast, tumors growing *in vivo* represent a heterogeneous dynamic population [65] in which pleiotropic changes affecting various parameters of cell growth are constantly arising. *MDR*1 expression in at least some types of tumor may be just one of the traits of a subpopulation of tumor cells possessing a complex of features associated with resistance to different types of treatment and more aggressive behavior. Although *MDR*1 expression is emerging as an important diagnostic and prognostic marker for such tumors, much better understanding of the origin and the nature of *MDR*1-expressing tumor cells is required to evaluate the feasibility of using P-glycoprotein inhibitors to overcome multidrug resistance in different types of clinical cancer.

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