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Research Article

Genetics of Multidrug Resistance

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Despite major advances in the treatment of cancer, resistance to multiple chemotherapeutic agents remains a major cause of treatment failure. Some tumors are initially resistant to many of the most active antineoplastic agents. Other tumors that are initially sensitive to cytotoxic agents often recur and frequently develop resistance to a broad range of chemotherapeutic agents including those used during the initial therapy. Both the intrinsic and acquired resistance to chemotherapeutic agents have been inherently difficult to study in the clinical setting. However, drug-resistant cell lines have provided a tool for studying the mechanisms underlying resistance to the drugs used in chemotherapy. Although drug-resistant cell lines are usually derived by selection for resistance to a single cytotoxic agent, they often develop cross-resistance to a broad spectrum of structurally and functionally unrelated compounds. This phenomenon has been termed multidrug resistance. Recently, an understanding of the biological basis of multidrug resistance has begun to emerge through the application of molecular genetics. This approach has led to the identification, isolation, and characterization of a gene family whose members include genes with the capacity to confer multidrug resistance on otherwise drug-sensitive cells. Molecular probes and antibodies developed as a consequence of genetic analysis promise a new and precise level of understanding of the role of this gene family in human physiology and tumor biology. This knowledge will in turn be crucial for the development of strategies for circumventing multidrug resistance in the clinical setting.

Multidrug-resistant cell lines

The isolation of drug-resistant human and rodent cell lines has been documented for a wide variety of agents (1). Cell lines selected for resistance to an anthracycline, a *Vinca* alkaloid, or actinomycin D are often cross-resistant to a broad spectrum of structurally and functionally unrelated compounds (2–7). Cross-resistance is almost always observed for the entire range of anthracyclines, *Vinca* alkaloids, and actinomycin D, although cell lines are usually most resistant to the agent used in the primary selection. Resistance to additional agents is often observed, but the spectrum of compounds and the amount of resistance varies from cell line to cell line. These observations suggest that although a common mechanism may be involved in the development of multidrug resistance, cell lines can exhibit specific differences in the genetic or physiological expression of the multidrug-resistant phenotype.

Two basic clues emerged that helped us to understand the genetic basis of multidrug resistance. First, Ling and coworkers analyzed the membrane polypeptides of a series of multidrug-resistant hamster cell lines. They demonstrated that dramatically increased levels of expression of a high molecular weight membrane glycoprotein, termed P-glycoprotein, was consistently associated with multidrug resistance (8). Other groups have confirmed and extended the original findings, noting that some multidrug-resistant cell lines overexpress more than one species of membrane glycoprotein that have molecular weights estimated to be in the range of 120–180 kD (9, 10). Second, the view that multidrug resistance was due to overexpression of a gene product was consistent with karyotypic abnormalities observed in multidrug-resistant cells. Highly multidrug-resistant cell lines often exhibit homogenous staining regions or double minute chromosomes (11–13). These chromosomal abnormalities have been demonstrated to be directly associated with gene amplification in a number of other experimental systems. Efforts to address the problem of multidrug resistance using molecular genetic approaches were initiated in the early 1980s based on these observations.

Gene amplification

Amplification of specific DNA sequences in multidrug-resistant cell lines was first analyzed in detail by Roninson et al. (14) using the technique of DNA renaturation in agarose gels. Amplified DNA sequences were detected in two independently derived multidrug-resistant hamster cell lines, the Adriamycin-derived LZ cell line (6) and the colchicine derived CH^RC5 cell line (4). 18 Bam H1 fragments with a total length of at least 150 kbp were amplified fifteen times or more in each cell line. Nine of the fragments that were shared by the two independently derived multidrug-resistant cell lines were found to be identical and likely to be part of the biologically relevant portion of the amplified DNA domain. These DNA sequences became the target for molecular cloning procedures.

The in-gel renaturation technique was used to physically purify one of the Bam H1 fragments amplified in both cell lines. A 1.1-kbp fragment was isolated from the gel and then introduced into a plasmid vector (14). This probe revealed that the amount of amplification of the 1.1-kbp fragment in each cell line correlated with the amount of drug resistance. However, the 1.1-kbp probe was not directly expressed as a transcript in any of the multidrug-resistant cell lines that were tested. To identify the functional multidrug-resistant gene, Gros et al. (15) expanded the amplified domain by cloning in cosmid vectors and by isolating a contiguous 140 kbp of DNA amplified in both the LZ and CH^RC5 cell lines. The in-gel renaturation technique was then used to prove that a precise

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representation of the amplified domain of the drug-resistant cell line had been isolated in the cloned sequences.

Identification of an mRNA species and isolation of cDNA clones associated with multidrug resistance

Several approaches were taken to identify mRNA sequences and to isolate the corresponding cDNA clones that were likely to encode the gene product responsible for multidrug resistance. Within the cloned, amplified domain of cell line LZ, Gros et al. (15) identified sequences that hybridized to an mRNA species of ~ 5 kb. This mRNA species was expressed at dramatically increased levels in multidrug-resistant cell lines. Using probes from the amplified domain of the LZ Chinese hamster multidrug-resistant cell line, Gros et al. (16) isolated two homologous but distinct cDNAs, λDR11 and λDR29, from the cDNA library of a mouse pre–B cell line that was not drug resistant. Hybridization of the cDNAs to cloned genomic DNA sequences demonstrated that the size of the transcription unit for each mRNA was in excess of 75 kbp.

Using a similar approach with amplified human genomic sequences that were isolated by cross hybridization to the cloned hamster sequences (17), Chen et al. (18) have isolated a series of overlapping cDNA clones from a human multidrug-resistant KB carcinoma cell line library. These cDNAs also identify a series of highly amplified DNA sequences in multidrug-resistant cells and hybridize to a 4.5-kb mRNA species overexpressed in multidrug-resistant cell lines (19).

After the development of antibodies against the P-glycoprotein, Ling and co-workers screened a λGT11 expression library of a CH^RC5-derived Chinese hamster multidrug-resistant cell line (20). A 600-bp cDNA was isolated that also hybridized to DNA fragments that were amplified in multidrug-resistant cell lines and to a 4.5-kbp mRNA species overexpressed in multidrug-resistant cell lines.

Borst and co-workers took a complementary approach to the isolation of cDNA clones from the CH^RC5 multidrug-resistant cell line (21). A differential hybridization technique was used to identify six distinct classes of cDNA clones corresponding to mRNA species that are expressed at increased levels in CH^RC5. Among the cDNA classes isolated were cDNAs that encoded the P-glycoprotein. The mRNA for the P-glycoprotein is the only member of this group that is consistently expressed at increased levels in a variety of multidrug-resistant cell lines (22). Using a similar technique, Melera and co-workers have isolated a cDNA from a vincristine-selected cell line that also hybridizes to a 4.5-kb mRNA overexpressed in multidrug-resistant cell lines (23).

Because of the multiplicity of methods by which different groups have isolated genes belonging to the multidrug-resistance gene family, several designations have been applied, including multidrug resistance (*mdr*) and P-glycoprotein (pgp). For the purpose of this paper, we have adopted the term *mdr* P-glycoprotein genes to refer to this gene group.

Genetic transfer of multidrug resistance

The ability to transfer the multidrug-resistant phenotype to a drug-sensitive cell via a defined DNA sequence constitutes the most direct demonstration that the gene responsible for multidrug resistance has indeed been identified. Early attempts to transfer the multidrug-resistant phenotype were carried out by transfection of total genomic DNA from resistant cell lines into drug-sensitive recipient cells (24, 25). However, the power of these experiments was limited by the inability to definitively demonstrate that the resulting drug resistance was due to the transfected DNA rather than the amplification of endogenous DNA sequences. The availability of molecular probes made possible direct analysis of the outcome of gene transfer experiments. Using species-specific restriction length polymorphisms to distinguish amplification of transfected DNA from endogenous genes, the transfer of the multidrug-resistant phenotype was documented with chromosome- (26) and DNA- (27, 28) mediated gene transfer. In each case the transfer of the multidrug-resistant phenotype was correlated with the transfer of multiple copies of the DNA from the donor cell line, which hybridized to the cloned *mdr* P-glycoprotein sequences, and the overexpression of the 5-kb mRNA previously shown to be overexpressed in multidrug-resistant cell lines. Deuchars et al. (28) were able to document that increased expression of P-glycoprotein was of donor origin using a species-specific MAb. Further, the P-glycoprotein sequences were transfected independently of the group of six genes previously shown to be coamplified in multidrug-resistant cell lines (21).

Transfer of multidrug resistance by cloned DNA sequences

The use of cloned genes permitted a definitive demonstration that increased expression of a single gene was capable of causing multidrug resistance. Gros et al. (16) showed that the mouse *mdr* cDNA, λDR11, engineered for expression under control of the SV40 promoter, conveyed resistance to either Adriamycin or colchicine when introduced into drug-sensitive cells. Each of the drug-resistant colonies that resulted from the transfection was resistant to Adriamycin, colchicine, and vinblastine, contained ~ 10–15 copies of the expression vector integrated into the genome, and transcribed high levels of mRNA from the expression vector. Similar results were subsequently obtained with a human *mdr* cDNA as described by Ueda et al. (19). These observations have been confirmed and extended with λDR11 being placed in retroviral expression vectors with the β-actin promotor (29) or histone H4 promoter (30) transcribing the *mdr* cDNA. The latter studies have indicated that the expression vector is preferentially amplified rather than the endogenous gene when selective pressure is increased on the multidrug-resistant clones. Furthermore, it has been possible to transfer the drug-resistance phenotype with λDR11 by transfection and retroviral infection without prior drug selection. These results indicate that increased expression of the normal *mdr* P-glycoprotein gene can confer multidrug resistance.

Sequence analysis

Analysis of the nucleic acid sequence of the mouse and human *mdr* cDNAs suggest that the P-glycoprotein is encoded by the *mdr* gene (18, 31). The predicted amino acid sequence of the mouse and human *mdr* polypeptide indicates that the primary structure contains 1,276 and 1,280 amino acids, respectively, which would give the polypeptide a calculated molecular weight of ~ 140 kD. The predicted molecular weight of the nonglycosylated P-glycoprotein is 136,000. The structural model of the *mdr* polypeptide that was suggested by the hydrophobicity analysis indicates that a highly charged intracellular amino-terminal sequence is followed by three transmembrane loops that cross the membrane six times and place the first of two putative ATP binding sites on the intracellular side of the membrane (Fig. 1). This structure is repeated, placing a second putative ATP binding site and the carboxy terminus of the polypeptide on the intracellular side of the cell

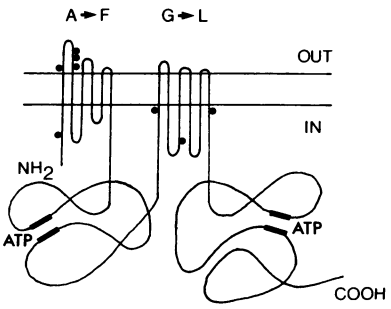

Figure 1. Schematic representation of the putative structure of the *mdr* P-glycoprotein and its orientation in the cell membrane. Possible NH_2-linked glycosylation sites (*closed circles*), transmembrane domains A–L, ATP-binding folds, and amino and carboxyl termini are indicated. From Gros et al. 1986. *Cell.* 47:371–380, with permission.

membrane. A cluster of four potential extracellular NH_2-linked glycosylation sites are present near the amino terminus of the polypeptide. Finally, an interspecies comparison of the predicted amino acid sequence of the mouse and human, with the partial hamster cDNA isolated with antibody to the P-glycoprotein (32), indicates that they represent the same or closely related molecules with a high degree of evolutionary conservation.

The deduced amino acid sequence contains the consensus sequence for two potential ATP-binding sites (33). This consensus sequence is present in a class of closely related ATP-binding proteins that contain a structurally and functionally related subunit and share a common evolutionary origin. This domain serves to couple ATP hydrolysis with a variety of cellular functions in bacteria (34). A predicted ATP-binding site in the *mdr* polypeptide would be consistent with models that invoke an energy dependent process to explain drug resistance.

A high degree of symmetry is apparent between the proximal and distal halves of the *mdr* polypeptide. Analysis of the predicted amino acid sequence indicates an extensive region of homology between the two halves of the polypeptide. The most conserved portion of the duplication lies within the putative intracellular domains, which include the potential ATP binding sites where 77% of the amino acids are either identical or highly conserved substitutions. Duplication of an ancestral gene is most likely responsible for the two highly homologous halves of the *mdr* polypeptide.

The predicted amino acid sequence of a region of the multidrug resistance polypeptide has a strong homology to the amino acid sequences of several bacterial transport proteins. The bacterial proteins include a series of permeases that shuttle small oligopeptides (oppD), maltose (malK), histidine (hisP), ribose (rbsA), and phosphate (pstB) into the cell across the periplasmic membrane. These proteins are each members of multicomponent, energy-dependent membrane transport systems (35). Homology to the *mdr* polypeptide occurs in the predicted intracytoplasmic domains that include the potential ATP binding sites and represent almost 40% of the *mdr* polypeptide. An even greater degree of sequence relatedness is observed between homolysin B (HlyB) and the *mdr* polypeptide. Hemolysin B transports the hemolysin toxin out of the cell across the outer bacterial membrane. The homology between the *mdr* polypeptide and hemolysin B is even more striking than with the permeases and extends over a much longer distance, including several of the hydrophobic domains spanning ~ 70% of the *mdr* polypeptide. The strong homology between the *mdr* polypeptide and the bacterial transport proteins suggests that these molecules all share a highly conserved functional unit involved in membrane transport (Fig. 2).

Mechanistic basis for multidrug resistance

The homology of *mdr* P-glycoprotein genes to bacterial transport protein genes provides a framework with which it is possible to interpret data on the mechanistic basis of multidrug resistance. Multidrug-resistant cells demonstrate decreased intracellular concentrations of cytotoxic drug when compared with drug-sensitive cells. The mechanism by which drug-resistant cells achieve a lower intracellular drug level is not well understood, however the strongest evidence suggests that an energy-dependent drug efflux system is responsible for the multidrug resistance phenotype (35–38). When multidrug-resistant cell lines are grown in the presence of metabolic inhibitors that deplete intracellular ATP, the drug-resistant cells accumulate the cytotoxic agents to levels approaching the parental drug-sensitive cell line. When the inhibitors of ATP production are removed there is a rapid efflux of cytotoxic agent out of the cell. These findings are consistent with a model in which the *mdr* P-glycoprotein acts to pump drug out of the cell in an energy dependent manner.

A simple model of this sort, however, may not explain the broad spectrum of drugs with which the multidrug resistance system can interact. An important problem which must be addressed is competition between the *mdr* P-glycoprotein and the high affinity binding sites for drug within the cell. To effectively remove drug once it has entered the cell compartment in which the target is located, the *mdr* P-glycoprotein system must bind a wide variety of substrates with equal or higher affinity than the primary target of the drug. The binding of the *mdr* P-glycoprotein to photoactive, radioactive analogues of vinblastine in a variety of multidrug-resistant cell lines further supports the role of this protein in the development of multidrug resistance but does not delineate the mechanism of this interaction (39, 40). A second model has been proposed by analogy to the bacterial transport systems. In these systems, transport is achieved by the substrate binding to a high affinity carrier protein which is then recognized by a group of proteins, including a polypeptide homologous to the *mdr* P-glycoprotein, which transports the substrate across the cell membrane in an energy dependent manner (35). It has been suggested that the *mdr* polypeptide interacts with a series of high affinity binding proteins for each drug or family of drugs in a manner analogous to the bacterial transport systems. A third model that we have proposed suggests that interaction of drug with the *mdr* P-glycoprotein occurs in a cell compartment distinct from the drug target. If drug entry into the cell is preceded by a

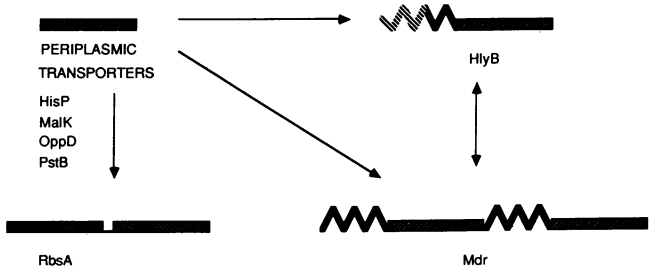

Figure 2. Possible evolution of conserved domain involved in energy dependent transport.

period in which drug is in the cell membrane, then the removal of drug from the cell can occur under conditions in which the drug molecule has not had the opportunity to interact with the target before an energy-dependent efflux from the cell.

The functional aspects of the *mdr* P-glycoprotein must also be able to account for the ability of a wide range of compounds to reverse the multidrug-resistant phenotype. These compounds include calcium channel blockers and calmodulin antagonists (41), quinidine (42), chloroquine (43), synthetic isoprenoids (44), tamoxifen and other triparanol analogues (45), the nonionic detergent Tween (46), cyclosporin A (47), and inactive analogues of daunomycin (48). The most extensive studies have focused on the calcium transport blockers. Tsuruo and co-workers first showed that verapamil increased the accumulation of vincristine in vitro in a vincristine-resistant P388 cell line by decreasing drug efflux, and that it enhanced the cytotoxic effects of vincristine both in vitro and in vivo (41). The reversal of drug resistance does not appear to be related to calcium fluxes (49). The photoactive calcium channel blocker, azidopine, has been shown to bind to the *mdr* P-glycoprotein (50, 51). Binding was inhibited by vinblastine, actinomycin D, Adriamycin and colchicine, and a variety of calcium channel blockers. This would suggest that agents that reverse multidrug resistance inhibit the efflux of cytotoxic agents by competitively or sterically inhibiting the binding of the drug to the *mdr* P-glycoprotein.

Some multidrug-resistant cell lines have also been shown to display collateral sensitivity to a range of compounds (52). These compounds are cytotoxic to multidrug-resistant cell lines at doses that do not kill the parental cell lines. While this response of multidrug-resistant cell lines is not yet explained mechanistically, like the reversal of the multidrug-resistant phenotype described above, collateral sensitivity may represent a means of circumventing the multidrug-resistance phenotype in the clinical setting.

Expression of the multidrug-resistant gene in normal tissues

The high degree of evolutionary conservation of genes in the *mdr* P-glycoprotein gene family between rodents and primates suggests that the function of this gene family has been under strong selective pressure during evolution. Although it is possible that the *mdr* P-glycoprotein genes normally function to remove toxic metabolites from cells, it is also possible that these gene products are involved in other physiological processes. One approach to this question is to determine the distribution in tissues of *mdr* P-glycoprotein gene expression. Fojo et al. (53) have analyzed total RNA in slot blots from a variety of human tissues with a probe from an *mdr* cDNA. The highest levels of expression were found in both the medulla and cortex of the adrenal gland. The kidney, colon, liver, lung, jejunum, and rectum had intermediate levels of expression, whereas the majority of tissues, including the brain, prostate, skin, muscle, heart, spleen, bone marrow, lymphocytes, stomach, esophagous, ovary, kidney cortex, and spinal cord, had low levels of expression. Interestingly, the amount of *mdr* expression was found to vary from sample to sample. Both regenerating rat liver after hepatectomy and hyperplastic liver nodules induced by carcinogens have also been shown to have high levels of *mdr* expression (54, 55). Of particular physiological significance is the physical location of the *mdr* P-glycoprotein. In a number of tissues, immunocytochemistry localized the *mdr* P-glycoprotein to the luminal surface of tissues involved in secretory functions. Analysis of human tissue indicated that the *mdr* P-glycoprotein is localized diffusely on the surface of cells in the cortex and medulla of the adrenal, on the biliary front of hepatocytes and the apical surface of the epithelial cells in the small biliary ductules in the liver, in the small ductules in the pancreas, in the proximal tubules of the kidney, and in the colon and jejunum (56). No detectable antibody labeling was found in the lung, stomach, salivary gland, cerebral cortex, cerebellum, spinal cord, ovary uterus, skin, spleen, or placenta.

The *mdr* P-glycoprotein has also been localized to the apical surface of the secretory epithelium of the gravid uterus in the mouse and is apparently induced by the physiologic changes of pregnancy (unpublished observations). Expression of the *mdr* P-glycoprotein gene increases dramatically during pregnancy from the low levels of the nonpregnant uterus. In situ RNA hybridization confirms that the increased expression is strictly localized to the secretory epithelium. These studies suggest that the *mdr* P-glycoprotein may play a role in some aspect of normal secretory functions. However, if this hypothesis proves correct, the normal substrates for the *mdr* P-glycoprotein are as yet unknown.

How many members are there in the mdr P-glycoprotein gene family?

The complex expression pattern suggests that the *mdr* P-glycoprotein gene family may perform different biological functions in different tissues. Establishing the number and characteristics of the members of the gene family take on added significance in this context. These issues become especially important if the product of one member of the gene family is more effective than another in conferring drug resistance on cells. Although the data are not yet complete, several members of the gene family from different species have been identified. Gros et al. (16) initially isolated two homologous but distinct cDNAs, λDR11 and λDR29, with the same probes from a drug-sensitive mouse pre–B cell cDNA library. Sequence analysis (21, unpublished observations) indicates 73% nucleic acid and 85% predicted amino acid homology between the two cDNAs but also shows that they are encoded by separate genes. Using a P-glycoprotein probe, Endicott et al. (57) isolated partial cDNAs from a drug-sensitive Chinese hamster ovary cDNA library which could be classified into two distinct gene groups, pgp1 and pgp2. A high degree of both nucleic acid and predicted amino acid homology is present between the two genes. A comparison of the 3′ untranslated regions and coding regions indicated that the mouse cDNA, λDR11, is more closely related to pgp2 whereas the human cDNA isolated by Chen et al. (18) is more closely related to pgp1. Van der Bliek et al. (58) have isolated several partial *mdr* P-glycoprotein cDNAs from a human liver cDNA library which are distinct from pgp1 and pgp2, which indicates the presence of a third *mdr* P-glycoprotein gene. Two alternative splices were identified, one inserting seven amino acids and the other deleting 43 amino acids. The predicted amino acid sequence of the mouse cDNA, λDR29, isolated by Gros et al. (16) shows a high degree of homology to this third class of *mdr* P-glycoprotein genes (unpublished observations). These preliminary sequence observations indicate that each of the three *mdr* P-glycoprotein genes encodes a protein with a similar structure (see above) with ~ 80–85% overall amino acid homology which is greatest around the putative ATP binding sites and most divergent in the proximal regions of the internal duplication.

Clinical implications of recent findings on multidrug resistance

The advent of immunological and molecular probes for the gene responsible for multidrug resistance in vitro has made it possible to analyze human tumors for the expression of this gene. Previous observations have indicated that some human tumors display resistance to multiple drugs; the pattern of resistance, however, did not necessarily correspond to the multidrug-resistant phenotype (59). Nevertheless, several preliminary reports have indicated that the P-glycoprotein can be found overexpressed in drug-resistant tumors. However, it remains unclear whether the overexpression of the P-glycoprotein is responsible for the failure of current chemotherapeutic regimens.

Using Western blot analysis with antibodies to the P-glycoprotein, Bell et al. (60) was the first to note high levels of the P-glycoprotein in two out of five ovarian carcinomas that were unresponsive to therapy. The P-glycoprotein was not detected in normal ovarian tissue. In one of the tumors the amount of P-glycoprotein increased after further exposure to chemotherapeutic agents. Ma et al. (61) identified two patients with acute nonlymphoblastic leukemia whose initial circulating myeloblasts were negative, but during relapse had a progressive increase in their percentage and in the intensity of staining with an antibody to the P-glycoprotein. Recently, Gerlach et al. (62) detected the P-glycoprotein by Western blot analysis in 6 of 11 sarcomas, while 25 other tumors of different types and a survey of normal human tissue were negative.

Analysis of RNA from a variety of human tumors suggests that mRNA species homologous to *mdr* P-glycoprotein may be expressed at high levels upon presentation and can increase during therapy (53). Expression of the *mdr* gene in a number of pheochromocytomas, adrenocortical carcinomas, and neuroblastomas indicated that the majority expressed high amounts of *mdr* mRNA but not necessarily the same amount as, or more than, the normal adrenal gland. Indeed, 5 out of 18 of these tumors expressed low levels of *mdr* RNA rather than the high levels expected from adrenal tissue. Levels of *mdr* RNA did increase sixfold in one pheochromocytoma that had become unresponsive to therapy, which suggests an acquired, increased level of expression. Moderate levels of *mdr* expression were found in seven of eight colon tumors and one of four rhabdomyosarcomas while the other samples indicated low expression. Analysis of RNA from 10 patients with leukemia indicated that all of the presentation samples and five of the six relapse samples had low levels of expression of *mdr* mRNA. One of the relapse samples had levels which were 10-fold higher, which raises the possibility that this increase had been acquired during therapy. These data indicate that it is possible that the intrinsic or acquired resistance found in some tumors could be due to increased levels of *mdr* P-glycoprotein expression.

Two preliminary clinical studies have described attempts to treat patients who were refractory to multiple chemotherapeutic agents by using strategies that reverse the multidrug-resistance phenotype in vitro. Both regimens used calcium channel blockers. In four of five children with acute lymphoblastic leukemia that was refractory to standard therapy, the combination of diltiazem and vincristine resulted in a cytolytic effect as evidenced by either a decrease in the circulating blast count or in the number of blasts in the bone marrow (63). One child had a massive cytolytic response. Two children had reversible atrioventricular node conduction defects attributed to diltiazem toxicity. In a separate study, eight patients with refractory ovarian cancer were treated with verapamil and Adriamycin (64). There were no objective responses to this therapy. Significant toxicity developed, however, in two patients with complete heart block and in four patients with congestive heart failure. The noncardiologic toxicities of vincristine and Adriamycin did not appear to be enhanced by the calcium antagonists in either study. However, the studies suggest that agents that are less cardiotoxic at the levels required to reverse multidrug resistance are necessary.

The role of the *mdr* P-glycoprotein in the resistance of tumors to chemotherapy is likely to be complex. A multigene family is present whose members may each have a unique function and differences in the ability to convey drug resistance. This suggests that a complete understanding of each family member will be necessary to determine the significance of this gene family in human tumor biology. The sensitivity and specificity of the probes used to detect the *mdr* P-glycoprotein will be critical. A systematic analysis of the level of expression of each family member in tumor specimens at diagnosis and relapse will be necessary. It will be important to determine the criteria necessary to decide whether the overexpression of these gene products is in fact responsible for the failure of chemotherapy. These criteria would include high levels of endogenous expression or increased expression of the *mdr* P-glycoprotein mRNA after exposure to cytotoxic agents. Resistance to the drugs in the multidrug-resistant phenotype, increased sensitivity to collaterally sensitive drugs, and circumvention of drug resistance by agents that reverse the multidrug-resistant phenotype would also need to be considered as possible criteria. Ultimately, the goal will be to target specific therapies to individual patients who will benefit from the circumvention of the phenomenon of multidrug resistance.

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