How Cancer Cells Evade Chemotherapy: Sixteenth Richard and Hinda Rosenthal Foundation Award Lecture

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Introduction: The Problem of Multidrug Resistance in Cancer

Ira Pastan and I began our research with a clinical problem that has concerned oncologists since chemotherapy became generally useful for the treatment of cancer. There are approximately 1,000,000 new patients with cancer in the United States each year. About 50% of the patients who go to their doctors with cancer can be cured by surgery and radiation therapy since their tumors have not spread. Of the remaining 50%, about 10% are curable with systemic chemotherapy, including children with leukemia and sarcomas and adults with testicular cancer and choriocarcinoma. However, the majority of metastatic cancers are not currently curable by chemotherapy or any other kind of therapy. These fall into two categories: cancers that are intrinsically resistant to chemotherapy (i.e., there is no significant response to chemotherapy); and those cancers which respond initially to chemotherapy but then acquire resistance during the course of therapy. Thus we set out to learn what were the causes of such drug resistance and to use this information to improve the therapy of cancer.

In this lecture, I will be focusing on one cause of multidrug resistance, that due to the product of the MDR1 gene, or P-glycoprotein, which is an energy dependent multidrug efflux pump (reviewed in Refs. 1–3). I hope to provide convincing arguments that the study of this mechanism of drug resistance is clinically relevant and that the application of principles derived from this work will improve chemotherapy of cancer.

Genetic Analysis and Phenotype of Multidrug Resistant Cells

We have used a genetic approach to analyze the problem of multidrug resistance and clone the major gene responsible for this resistance. The strategy was to take a large population of cells which are sensitive to chemotherapy but which include among them a rare cell which is resistant. By selecting in specific anticancer drugs such as doxorubicin, vinblastine, or other drugs such as colchicine, one can develop a population of cells which is highly drug resistant by killing the sensitive cells and allowing the rare resistant cells in the population to multiply (4, 5). We believe that a similar process is responsible for the acquisition of drug resistance in cancers from patients whose tumors have acquired drug resistance during chemotherapy. Normally these selections are done in multiple steps and care is taken at each step of selection to be sure that the pattern of resistance that is observed is the same for all the cells so that one does not introduce spurious mutations during this analysis.

If one selects with these kinds of drugs, a pattern of resistance develops to a wide variety of natural product anticancer drugs, such as Vinca alkaloids, epipodophyllotoxins, actinomycin D, anthracyclines, mithramycin, and the new and exciting chemotherapeutic drug taxol. One problem, of course, is that as we develop new drugs, many of them turn out to be affected by the resistance mechanism developed as a part of the MDR2 phenotype. MDR cells of the classical kind described here are sensitive to a variety of drugs which tend to be much more water soluble. Many antimetabolites are included in this group such as methotrexate, 5-fluorouracil, cis-platinum, and cyclophosphamide. Resistance to these agents must be due to other causes and, in fact, many scientists have been actively searching for those causes of resistance as a way to understand why chemotherapy is not more effective.

Since MDR cells are resistant to a great many drugs, it is reasonable to ask if they have any chemistry or pharmacology in common that we can understand as the means by which cells become resistant to them. Analysis of the structure of these drugs shows that there is no obvious strong chemical similarity among them. They do, however, have some physical properties in common. All the drugs are relatively hydrophobic, which means they dissolve in lipid bilayers and they have partition coefficients between hydrophobic and aqueous solvents that favor solubility in the hydrophobic phase. These are amphipathic compounds, which in addition to their lipid solubility are also somewhat water soluble. Another feature which was thought to be common to these drugs was that many of them were cationic. In fact, the anthracyclines and the Vinca drugs are cationic and are weak bases which are positively charged at neutral pH. But the epipodophyllotoxins and taxol are uncharged at neutral pH. Of the many drugs known to be affected by the MDR phenotype about two-thirds of them are positively charged and about one-third are not. Therefore that feature is not so important. What is critical is that they cannot be anionic, since negatively charged drugs do not appear to be substrates for this multidrug transport system.

The reason for multidrug resistance is that the drugs fail to accumulate in the cells that are resistant. The reason for failure of accumulation is that these cells express a pump at the cell surface which prevents the drug from accumulating within the cell. Juliani and Ling (6) first detected this protein in the cell membranes of MDR cells and named it P-glycoprotein. P-glycoprotein has a molecular weight of about 170,000; it is both phosphorylated and glycosylated. Tsuruo (7) and his coworkers were able to show several years ago that the activity of this system could be inhibited by many different agents, including the calcium channel blocker verapamil, the antiarrhythmic quinidine, the antihypertensive reserpine, and a large number of other drugs. We have been interested in the mechanism by which this inhibition works, and it appears that most of these agents are nontoxic substrates for the pump system. The way they work appears to be by simply swamping the ability of the pump to handle toxic agents, because the nontoxic ones are busy keeping it pumping. This is a kind of competitive inhibition, although it is not entirely clear that all of these agents are purely competitive in the kinetic sense (8, 9).

Initially, we were interested in cloning the human gene responsible for this multidrug resistance. In order to do that, we used the human cell lines that we had developed, which were highly drug resistant (4, 5). We confirmed an observation that had been made many times...
before in other laboratories, particularly by the pioneering work of Biedler and Riehm (10), who had shown that highly resistant cells of this type tended to have amplified genes that were cytogenetically detectable as double minute chromosomes or homogeneously staining regions. Minute and double minute chromosomes are extrachromosomal pieces of DNA which carry amplified genes, and homogeneously staining regions are amplified segments of DNA residing within chromosomes. In our cell lines, which were derived from a drug sensitive human KB adenocarcinoma cell line, there were amplified genes present as extrachromosomal elements, seen as minute and double minute chromosomes.

In order to clone the gene responsible for MDR we took advantage of the fact that we had amplified this gene on extrachromosomal pieces of DNA (11). The cloning was done in collaboration with Roninson (12) who had developed a technique called in-gel renaturation for detecting and cloning amplified genes. As a result of our collaborative work with him, Ueda in our laboratory was able to clone a full-length cDNA for the gene responsible for multidrug resistance, which we called the MDR1 gene (13–16). We also cloned a fragment of a second, closely related gene called MDR2 which was highly homologous but not identical to MDR1, was frequently coamplified with MDR1 since it mapped adjacent to MDR1 on chromosome 7, but, as demonstrated by Borst’s group, was not a drug resistance gene (17). mdr genes have also been cloned in the laboratories of Melera, Ling, and Housman (18–21).

One of the questions we have addressed is the origin of the extrachromosomal elements which carry the MDR1 gene (11, 22). As illustrated in Fig. 1, we have considered three basic models to explain the origin of these minute and double minute chromosomes. One model, which Wahl (23) and others have suggested, is that there is an excision process in which either a small piece of DNA, including the drug resistance gene and flanking sequences (episomal excision), or a larger piece of DNA (direct excision) is excised. In the case of the small piece, the excised fragment would be too small to see cytogenetically. In order to develop minute and double minute chromosomes which are cytogenetically visible, these would have to multimerize or acquire other bits of DNA until they were large enough to see in the microscope (approximately 1000 kbp). In the case of excision of a larger piece of DNA, there might need to be deletion of some of this DNA to result in the relatively small double minute chromosomes which we and others have observed in highly selected cell lines. The third model has been supported by work of Stark et al. (24), which suggests there is an event which occurs in situ in the chromosome, such as an unequal crossing-over event that duplicates the region around the gene of interest. Such a duplication, if repeated, might result in a homogeneously staining region. This region could be excised, resulting in an extrachromosomal element which could undergo internal rearrangements and deletions to give a double minute chromosome.

Schoenlein in our laboratory has analyzed one series of KB cells which Akiyama, Cardarelli, and Shen had selected in increasing concentrations of colchicine and has found direct evidence that in this cell series an extrachromosomal element began as a small piece of DNA (890 kilobases) and increased in size by successive dimerization (25). The analysis depended upon the use of pulsed field gradient gel electrophoresis of irradiated DNA from the resistant cells, which was able to resolve extrachromosomal DNA fragments of 890, 1780, and 3560 kbp in cell lines of increasing drug resistance. In addition, use of the restriction enzyme Not1, which cuts in only a single site in the 890-kbp extrachromosomal DNA fragment, was able to reduce all of the amplified segments to a single 890-kbp unit length, proving that they were multimers of this unit. These data strongly support the first model illustrated in Fig. 1 for the development of double minute chromosomes in our colchicine selected multidrug resistant cells. However, preliminary analysis of a different multidrug resistant line selected in vinblastine indicates that the earliest steps in gene amplification involve a large segment of DNA, which becomes smaller, but more numerous, with drug selection, consistent with models 2 or 3.

Once we had obtained a full-length cDNA for the MDR1 gene, we were able to sequence it and discovered that it encoded a protein of 1280 amino acids (Fig. 2) (26). The computer analysis of the amino acid sequence predicts 12 transmembrane regions, of which 6 are in the amino terminus and 6 are in the carboxyl terminus. There are also two domains, which, in analogy to many other proteins which have since appeared in the data base, appear to encode ATP sites. The predicted structure of this protein yields a model which has become quite familiar looking. It is one of the first examples of what has turned out to be a large family of energy dependent transport proteins. In addition to the transmembrane regions and the ATP sites, there is one large extracellular loop, the first extracellular loop, which is potentially glycosylated at three positions. The filled in balls represent amino acid differences between a mouse mdr gene, which Gros et al. (21) had cloned, and the human gene, which we cloned. As can be seen, there are not too many differences between mouse and human P-glycoproteins, but these differences are clustered in the first extracellular region, in the amino and carboxyl terminus, and in the region we call the linker region, which separates the two halves of the protein that are otherwise 43% identical (for the human P-glycoprotein) at the amino acid level. This type of analysis, showing little divergence between mice and humans, tells us that the bulk of this protein is probably essential for function.

The presence of the two ATP sites defines P-glycoprotein as a member of the ATP binding cassette superfamily of proteins (27). This superfamily has many members, including bacterial transporters, and eukaryotic transporters, such as analogues of the MDR1 gene in protozoans, and many other types of transporters in mammalian cells.
These transporters handle nutrients, proteins and peptides, carbohydrates, drugs, pigments, and many other substrates, both at the cell surface and in interior membranes. There is a very interesting MDR1 analogue in yeast called STE 6 cloned independently by Thorner (28) and Varshavsky (29) which transports a peptide which is a hydrophobic sex signalling peptide. Introduction of the murine or human MDR1 gene into yeast can compensate for mutants in the STE 6 gene (30). In addition, there are members of this family that are responsible for transport of proteins for antigen presentation into the endoplasmic reticulum (31), there is a major peroxisomal pump protein (32), and there are other interesting members of the family, including the cystic fibrosis transmembrane regulator (33).

**Mechanism of Action of P-Glycoprotein**

To improve cancer treatment, it is obviously important to understand the mechanism by which P-glycoprotein works. In addition, to understand how the ATP binding cassette superfamily of protein functions, it is necessary to figure out how the energy is transduced in at least one family member. Thus we and others have begun an analysis using biochemistry and genetics to study P-glycoprotein. We have done three different kinds of analyses: (a) photoaffinity labeling experiments with substrate drugs and drug analogues; (b) structure-function analyses of mutations either introduced into P-glycoprotein or discovered in P-glycoprotein after cellular selections; and (c) purification and reconstitution of P-glycoprotein. The first analysis using photoaffinity analogues has established which parts of P-glycoprotein interact directly with substrates. Current work in our laboratory (34-35) and in the laboratories of Akiyama, Horwitz, Greenberger, and Seamon (36-39) indicates that there are roughly equivalent photoaffinity labeling sites in both halves of P-glycoprotein near transmembrane 5 and 6 and 11 and 12, in both the amino terminal half and the carboxyl terminal half. This result suggests that these labeled sites come together in some way in the whole molecule to form a central pore through which the drugs are moving.

A second kind of analysis that has been initiated is a mutational analysis. Four mutations in P-glycoprotein have been described which affect the substrate specificity of the transporter. Two of those mutations are in the first intracytoplasmic loop. The best characterized mutation is a conversion of a glycine to valine at position 185 in the first intracytoplasmic loop discovered by Roninson in one of our colchicine-selected cell lines (40). This mutation results in a multidrug transporter with improved pumping of colchicine and etoposide and decreased ability to pump vinblastine and actinomycin D. In addition, we have recently described a mutation which converts an asparagine to a serine at residue 183, which in combination with the Val185 mutation increases transport of actinomycin D (41). Gros et al. (42) have described a mutation in the TM11 region which also affects the specificity of transport and Melera’s group (43) has described a double mutation in TM6 which increases relative resistance to actinomycin D. The distribution of these four mutations throughout the transporter emphasizes our previous conclusion that both halves of P-glycoprotein are involved in forming a transport channel.

Another way to learn about mechanism is to study the kinetics and physiology of transport. Working with Stein, who has recently taken a sabbatical year in our laboratory, we have reanalyzed our own data and the literature, beginning with the pioneering work of Dano and Skovsgaard, on the kinetics of drug entry into and efflux from multidrug resistant cells. When the uptake of a radioactive MDR drug, such as colchicine or vinblastine, is measured in drug sensitive cells, the uptake rate is high, drug accumulation increases rapidly, and then accumulation levels off as drug binds to intracellular targets. In a multidrug resistant cell, the initial rate of uptake is frequently dramatically reduced, as much as 5- or 10-fold, and the final accumulation of drug is also reduced. Evidence that P-glycoprotein is an efflux pump

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3 K. Kuchler, J. Thorner, and M. M. Gottesman, unpublished data.
4 L. M. Greenberger. Major photoaffinity drug binding sites for iodaryl azidoprazosin in P-glycoprotein are immediately C-terminal to transmembrane 6 and 12, submitted for publication.
6 W. Stein, I. Pastan, and M. M. Gottesman. The multidrug transporter as an osmotic engine, submitted for publication.
arose by blocking the supply of energy (ATP) in the cell by addition to the medium of sodium azide and 2-deoxyglucose. If you block energy in this way, the drug resistant cell becomes indistinguishable from a drug sensitive cell, with the same initial rate of drug uptake and final drug accumulation as a sensitive cell. However, if you remove the azide and 2-deoxyglucose from the medium and add glucose to allow the regeneration of ATP, there is a rapid efflux of drug that has accumulated within the cell in the absence of a functional multidrug transporter, until the level of intracellular drug falls to a steady state level no different from that in a MDR cell line for which the energy supply was never interrupted. Thus, the P-glycoprotein pump is fully capable of both reducing influx and increasing efflux.

To explain these kinetic data and account for the photoaffinity labeling results and molecular analysis, we have generated a working model illustrated in Fig. 3. This model has two major features: (a) based on the biochemical and structure-function data, we hypothesize that the two halves of P-glycoprotein come together to form a single channel for drug transport; (b) we propose that the major function of the multidrug transporter is to detect drug within the plasma membrane and extrude it from the cell. In this way, drugs entering the cell by passive diffusion will be removed from the membrane before they can enter the cytoplasm (hence, decreased influx), and drugs which have entered the cell either because they have eluded the transporter or, in the example given above, because the transporter was not energized will be removed either from the inner leaflet of the plasma membrane or the cytoplasm of the cell.

We have called this model the "hydrophobic vacuum cleaner" model of P-glycoprotein action, and there are three lines of evidence that support this kind of model. The first line of evidence, already discussed, is that the drugs themselves are amphipathic and highly lipid soluble and therefore will concentrate in cellular membranes. Second, the kinetic data are most consistent with a model in which drug is removed directly from the membrane. Finally, there is direct evidence that the amount of certain MDR drugs in the plasma membrane of MDR cells is reduced relative to the amount of drug in drug sensitive cells. This evidence was obtained in a collaboration with Yossef Raviv (44) who used energy transfer from daunorubicin to the highly hydrophobic membrane probe iodinated naphthalene azide (INA) to demonstrate reduced amounts of daunorubicin in the membranes of MDR cells. In addition, studies by Kessel, working with the highly fluorescent MDR substrate rhodamine 123, have demonstrated decreased lipid association of this agent in drug resistant as compared with drug sensitive cells (45).

To truly understand the mechanism of action of P-glycoprotein, it will be necessary to purify and reconstitute this protein to allow the direct analysis of the biochemical effects of specific mutations and covalent modifications of the transporter. We have initiated these studies with Ambudkar who has had extensive experience in reconstituting transporters and channels of bacterial origin. We have used two sources of P-glycoprotein, a highly multidrug resistant cell line called KB-V1 and another interesting system which is an insect cell line, SF9, which can be infected with a MDR baculovirus to yield large amounts of functional protein. This baculovirus system developed in our laboratory by Germain (46) makes about three times as much P-glycoprotein as the KB-V1 system, but the protein is not glycosylated and we chose to do our initial studies on the mammalian system. Preliminary data suggest that the baculovirus system will prove to be useful in the future as an enriched source of active P-glycoprotein (47).

P-glycoprotein is extracted from cellular membranes by octyl glucoside and can be purified by column chromatography (48). This protein can be further purified by reconstitution into proteoliposomes formed from Escherichia coli lipids with the addition of cholesterol and lecithin. Our recent preparations are approximately 80–90% pure. As shown in Fig. 4, the reconstituted protein shows a drug-dependent ATPase activity. Basal and drug stimulated activity is inhibited by vanadate, a known inhibitor of the transport process (8). The drugs verapamil and vinblastine, two excellent substrates for the transporter, give 4–5-fold stimulation of the ATPase which is also inhibited by vanadate, but the hydrophobic anticancer drug camptothecin, which is not an MDR substrate, gives no stimulation. Interestingly, octyl glucoside solubilized P-glycoprotein retains some ATPase activity but it is no longer drug stimulated, suggesting either that the detergent inhibits this activity or that there is a requirement for a lipid bilayer for proper folding or interaction of the multidrug transporter with drugs. These studies represent a beginning to the biochemical analysis of P-glycoprotein. We need to purify the protein to complete homogeneity, and we need to reconstitute a vesicle system which is capable of drug transport.

Function of P-Glycoprotein in Normal Tissues and Cancer

P-glycoprotein is normally expressed in a variety of different tissues, and we need to understand what the normal function of P-glycoprotein is to predict what will happen when we reverse drug resistance by systemic pharmacotherapy of tumor cells. Immunohistochemical studies of Willingham and Thiebaut using antibody MRK16, originally isolated in Tsuruo's laboratory, showed expression of P-glycoprotein in epithelial cells of the liver, kidney, pancreatic ductules, and small and large intestine (49, 50). These are all epithelia lining a luminal surface. We also see expression in capillaries in the
There are likely to be other specific sites of localization which include lymphocytes, the trophoblast of human and mouse placenta, and endometrial glands in the pregnant uterus (51--53). There are likely to be other specific sites of localization which will be discovered as immunohistochemical tools improve.

What do these localizations suggest about function? On the basis of these localization studies, one can speculate that P-glycoprotein is probably involved in transepithelial transport of toxic endogenous metabolites and xenobiotics and may serve as a barrier in the intestine, e.g., to prevent uptake of certain natural product drugs. Horio et al. (54, 55) have been able to model this situation by making epithelial monolayers of Madin-Darby canine kidney cells and demonstrating transport across the monolayers in a way entirely consistent with P-glycoprotein acting as a transepithelial transporter. The recent demonstration that P-glycoprotein also has volume regulated chloride channel activity which appears to be distinct from its drug transport activity (56, 57) raises the possibility that it is involved in volume regulation, especially in cells of the gastrointestinal tract which must deal with a wide range of osmotic conditions. Localization in brain and testis capillaries and in the placenta may reflect a barrier function for P-glycoprotein serving to keep toxic compounds out of the brain, out of the fetus, and out of the germ cells. Finally, location in steroid producing cells in the adrenal and in the pregnant uterus suggests that P-glycoprotein has a function either in secreting steroids or, even more likely, in protecting the membranes of cells that are secreting steroids from the high local concentration of steroid in these membranes. This speculation comes from the conclusion that P-glycoprotein is a pump that is able to remove hydrophobic materials from the membrane.

What have we learned about expression of P-glycoprotein in cancer? Fojo and Goldstein in our laboratory have accumulated a large number of different human cancers, prepared RNA from those cancers, and measured MDR1 RNA levels to see how commonly P-glycoprotein is expressed in human cancer (58, 59). Our experience with over 500 different human cancers indicates that expression of P-glycoprotein is quite common. We estimate that about 50% of the patients who have cancers in the United States will have some expression of P-glycoprotein at a level which is probably significant some time during the course of disease.

What cancers express P-glycoprotein? Expression is seen in cancers derived from tissues which normally express the MDR1 gene, namely cancers of the liver, colon, kidney, pancreas, and adrenal. In these tissues, especially in renal cell carcinoma, it appears to act as a marker of differentiation. The more highly differentiated renal cell cancers have more P-glycoprotein than do the less differentiated ones. Expression is also seen during the course of chemotherapy with MDR drugs in leukemias, lymphomas, breast and ovarian cancers, and many other cancers. These cancers may initially respond to chemotherapy, but when the cancers relapse, they frequently express more P-glycoprotein. Finally, the most intriguing category includes cancers which are not derived from tissues that normally express P-glycoprotein, or at least not large amounts, but in which P-glycoprotein expression increases during the development of the cancer. One of the most interesting examples of this phenomenon is chronic myelogenous leukemia, which, when it goes into blast crisis, expresses more P-glycoprotein irrespective of the previous treatment history (60). Acute nonlymphocytic leukemias also show this phenomenon (61). Many acute nonlymphocytic leukemias are apparently CD34+, and that may account for why they are positive, but some are not. Recently, there has been some work of Chan and Ling, suggesting that in sarcomas and neuroblastomas in childhood, expression of P-glycoprotein at any detectable level at all, even at very low levels in a small percentage of cells, is a very negative prognostic indicator (62, 63). This result suggests that P-glycoprotein is more than a marker of drug resistance but is also a marker of biological maliciousness of cancers.

To examine the basis of increased MDR1 gene expression in developing malignancy, Chin (64) in our laboratory has established a model system in which he cotransfected a construction consisting of an MDR1 promoter cloned upstream from a chloramphenicol acetyltransferase (CAT) gene together with a variety of oncogenes that were known to be activated in cancer, or tumor suppressor genes that were known to be inactivated very commonly in human cancer. Two such cotransfected genes that appear to activate the MDR1 promoter are ras and mutant p53. As we have published, ras in increasing doses stimulates the MDR1 promoter as shown by a dramatic increase in conversion of chloramphenicol to the acetylated form in cotransfected NIH 3T3 cells. Cotransfected mutant p53 gives the same phenomenon. If wild-type p53 (a tumor suppressor) is cotransfected with mutant p53 and the MDR1 promoter-CAT construction, the stimulation is blocked in a dose dependent manner. These data argue that some of the expression of P-glycoprotein as a predictor of poor response in cancer may be a measure of the classes of oncogenes that are turned on during tumor progression.

What is the evidence that the P-glycoprotein that is expressed in cancers is affecting the clinical drug resistance of those cancers? There are basically three lines of evidence that support the involvement of P-glycoprotein in clinical drug resistance. First, we know that the levels of expression we see in human cancers are sufficient to confer drug resistance in vitro and in vivo in an MDR1 transgenic mouse system that will be discussed below. Second, if we take tumors that are MDR1 positive and grow them as explants ex vivo, those tumors in fact behave just like cell lines that have been selected in tissue culture using anti-cancer drugs (65). They are multidrug resistant, and you can reverse resistance with MDR reversing agents. Finally, clinical trials of myeloma and lymphoma, which were pioneered at the University of Arizona by Salmon, Dalton, List, and their colleagues, have shown that in highly drug resistant myelomas one can, at least in a significant number of cases, reverse resistance by using MDR reversing agents like verapamil (66) and, more recently, cyclosporin A. There have also been a number of other studies, including a well-designed cross-over study at the National Cancer Institute led by Wilson, Wittes, Bates, and Fojo, which shows clearly that there is a significant component of drug resistance due to P-glycoprotein expression in lymphoma and that resistance can be reversed in certain instances by R-ferapamil.

The way we originally studied the question of in vivo relevance of expression of P-glycoprotein was by developing a transgenic mouse system in which the human MDR1 gene was expressed in a drug sensitive tissue in the mouse (67--69). The human MDR1 gene under control of an actin promoter was introduced into fertilized eggs and resulted in one line of transgenic mice in which the expression of the MDR1 gene is in the bone marrow. These animals are resistant to chemotherapy with MDR anticancer drugs, such as daunorubicin, doxorubicin, vinblastine, vincristine, etoposide, teniposide, and taxol, which reduce the peripheral WBC of nontransgenic littermates but have little or no effect on the WBC of the transgenic mice. Their WBC do drop in response to non-MDR drugs such as 5-fluorouracil, methotrexate, and cis-platinum. These mice are otherwise healthy with no obvious disturbance of hematopoiesis and a normal life span, but after intensive inbreeding (approximately 20 generations), the level of expression of the MDR1 gene in bone marrow has declined, presumably because the actin promoter is not bone marrow specific and its activity has been suppressed.
How was this system exploited? One way in which it can be used is to test the in vivo efficacy of drugs known to reverse the MDR phenotype in vitro (68). There are a great many drugs capable of reversing drug resistance, including verapamil and its analogues, other calcium channel blockers, nontoxic analogues or chemotherapeutic drugs, hydrophobic cephalosporins, cyclosporin A and its analogues, quinidine and its optical isomer quinine, reserpine, and many other agents. These agents can be used to treat the MDR1 transgenic mouse along with cytotoxic drugs. Verapamil in combination with daunorubicin reduces the WBC of these transgenic mice. This model can also be used to show that combinations of agents may be synergistic in reversing drug resistance (i.e., verapamil and cyclosporin A), that antibodies to P-glycoprotein can sensitize the transgenic mice to MDR drugs, and that liposome encapsulation of doxorubicin seems to circumvent, albeit incompletely, the resistance conferred by the MDR1 gene. The question still remains if this chemosensitization phenomenon will work in patients, and what the side effects of inhibiting P-glycoprotein in normal tissues will be. There are a number of clinical trials currently beginning, and this accumulated experience will tell us something about the efficacy and side effects of inhibition of the multidrug transporter in humans.

Gene Therapy Using the Multidrug Resistance Gene

The ability to confer a multidrug resistance phenotype on mouse bone marrow using a human MDR1 cDNA suggested that the MDR1 gene could be used in vivo as a selectable marker for gene therapy. We were able to demonstrate that transplantation of the MDR1 expressing bone marrow from a multidrug resistant mouse to a sensitive mouse conferred drug resistance on the recipient animal, thereby proving that the MDR phenotype was due to expression in the bone marrow and not some other tissue in the original transgenic mouse, and also that expression of the MDR1 cDNA was likely occurring in the stem cells of the donor animal (70). This result suggested that it might be possible to take a patient with a drug sensitive bone marrow, introduce the MDR1 gene into bone marrow ex vivo, make that bone marrow multidrug resistant, and return it to the patient so that increased chemotherapy might be tolerated. The general approach to this is to take a retrovirus carrying the MDR1 gene (71) and introduce it into bone marrow cells. These studies were done with mouse bone marrow in collaboration with MacLachlin and Anderson (72) and demonstrated that these bone marrow cells become multidrug resistant in vitro. More recently, we have asked whether these bone marrow cells continue to have a selective advantage if they are reintroduced into mice. In two published studies, one in collaboration with Bank at Columbia University (73), and the other in collaboration with Nienhuis at the NIH (74), stable, selectable expression of the MDR1 gene transferred to mouse bone marrow and selected with taxol in vivo has been demonstrated. The challenge of the transplanted animals with taxol results in both an increased average copy number of the MDR1 gene in the peripheral WBC population of these mice and an increase in the percentage of peripheral WBC expressing human P-glycoprotein. These studies provide support for the exciting possibility that it may be possible to protect normal human bone marrow during intensive chemotherapy, and clinical trials to test this idea will be initiated within the coming year.

Conclusions

There are several major conclusions which have come from these studies on multidrug resistance: (a) multidrug resistance is commonly associated with expression of the MDR1 gene in human cancers; (b) most commonly, MDR is caused by a drug efflux pump which can remove drug from the plasma membrane and also transport drug out of the cell once it has entered the cell; (c) amplification of the MDR1 gene which thus far has been observed mostly in tissue culture models may proceed via sequential dimerization of extrachromosomal elements; (d) expression of specific oncogenes, including ras and mutant p53, may activate expression of the MDR1 gene; (e) drugs that inhibit the transporter can reverse drug resistance by allowing cytotoxic drugs to accumulate in the cell, and this is the basis of current clinical trials to study this phenomenon in patients; and finally (f) perhaps in the not too distant future, gene therapy may be used to make the bone marrow drug resistant and permit high dose chemotherapy.

Acknowledgments

I am deeply honored to be the recipient of this year’s Richard and Hinda Rosenthal Award for Cancer Research. I accept this award on behalf not only of myself, but of my scientific colleagues, including my long time friend and collaborator Ira Pastan, current and past members of my laboratory, all of the scientists who contributed directly to this work, and the many cancer researchers who have worked so hard to develop preclinical models to improve therapy of cancer. In learning about the many charitable activities of the Rosenthal family, I discovered that the first Rosenthal Awards for Cancer Research were administered by the American Association for the Advancement of Science. The first recipient of the AAAS Award for Cancer Research supported by the Rosenthal family was Dr. Lloyd Law for his pioneering work on the development and use of the L1210 mouse leukemia model for studying the drug treatment of cancer and the basis of resistance to chemotherapy. When Dr. Law retired 2 years ago as Chief of the Laboratory of Cell Biology at the National Cancer Institute, I became his successor, apparently in more ways than one.

This historical note allows me to acknowledge with considerable gratitude the enormous importance of Dr. Law’s ground breaking studies in the 1940s and 1950s and to point out that much of the exciting science which was presented this year at the annual American Association for Cancer Research meeting owes its origin to pioneers like Lloyd Law. I would also like to thank the National Cancer Institute for many years of unwavering support for basic research on the causes of chemotherapy resistance in cancer, which we hope will soon bear fruit clinically, and finally I would like to thank the Rosenthal family for the encouragement they have given to two generations of cancer researchers.

References


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