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Invited editorial

Current status and future directions of research on multidrug resistance

The impact of contemporary biotechnology

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Clinical and experimental oncologists are intrigued by the fact that some cancers are drug-sensitive and thus curable, whereas other types are drug-resistant and respond poorly or only partially to chemotherapy. It is widely believed that if the biochemical and molecular basis of drug resistance is fully elucidated, it should become possible to devise new strategies for the circumvention of this resistance, hence increasing the number of cancers that can be cured.

The past decade has seen the successful application of genetic techniques to the dissection of the most important phenotypes of cancer cells. Thus, the malignant growth phenotype appears to be due to abnormal expression or altered function of proto-oncogenes and suppressor genes, the invasive and metastatic phenotypes appear to result from expression of protease genes and other genes regulated by proto-oncogenes, and drug resistance of cancer cells has been defined by the expression of specific genes involved in the metabolism and transport of anticancer drugs. In the case of drug resistance, the elucidation of the genes involved in resistance to chemotherapeutic agents has led to new and unexpected information about tumor physiology and may well open therapeutic options by virtue of reversing clinical drug resistance.

Cancers such as renal cell carcinomas are often resistant to more than one type of drug with varying structures and different mechanisms of action. This is termed multidrug resistance (MDR). The high frequency of this drug resistance, seen both in the clinical treatment of cancer and in tissue culture models, suggests that cancer cells can express genes which confer simultaneous resistance to different kinds of anticancer drugs. One such mechanism, recently shown to occur commonly in human cancers, is the expression of an ATP-dependent efflux pump, termed P-glycoprotein and encoded by the *MDR*1 gene, for a broad range of chemotherapeutic agents. Both function and modulation of P-glycoprotein have been profoundly studied [1–15], and I shall confine my personal annotation to this so-called classical pathway of MDR. Alternative resistance factors that have been biochemically and genetically defined comprise glutathione metabolism and topoisomerase enzymes.

How will the oncologist in the mid 1990's or in the early 2000's use the information on the nature of drug resistance? And how will the cancer patient benefit from this knowledge? I will briefly discuss three potential clinical scenarios including treatment strategies that can be adopted immediately, innovative therapies that will emerge within several years, and concepts that will require the development of a new generation of drugs.

P-glycoprotein determination to guide design of drug therapy

Because it is possible to measure *MDR*1 expression, we can currently design drug therapy based on this information. If tumors express significant amounts of *MDR*1, the treshhold levels having yet to be clearly defined, they are extremely unlikely to respond to the natural product agents handled by P-glycoprotein. Therefore, patients might be spared the unpleasant and unnecessary side effects of these types of cytotoxic reagents; there is abundance of retrospective clinical studies in neuroblastomas, childhood sarcomas, acute non-lyphocytic leukemias, etc., that claim to support this hypothesis. This strategy depends upon having accurate and reproducible methods of measuring *MDR*1 gene and gene product expression levels available in a clinical setting. The original procedures to obtain genomic MDR-fragments used in-gel renaturation technique (human), subtractive hybridization from a cDNA library (mouse), and an antibody against a protein product from a cDNA expression library (hamster). Derivatized molecular probes and monoclonal antibodies have currently been assessed in evaluating *MDR*1 expression. mRNA levels were determined using RNA slot blots, RNase protection assays, in situ hybridization, and Northern blot analysis, and protein was detected by Western blot and immunohistochemical tech-

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niques. These current methods involve obtaining quick-frozen tumor samples in large enough quantities to prepare RNA or to apply a panel of antibodies on frozen sections. This appears easy for many primary lesions, but hardly feasible for some recurrent tumors. The latter difficulty of sample size can be surmounted using new technology based on reverse transcription followed by polymerization chain reaction, which amplifies the RNA signal in a small number of tumor cells. With adequate quality control these measurements may allow the generation of a individual, patient-oriented drug-resistance database from which prognostic information can be gleaned.

Reversing, circumventing and overcoming multidrug resistance

Further clinical interest was instigated when it became apparent that inhibition of the underlying resistance factor, P-glycoprotein, may reverse drug resistance. It should be noted that many conventional chemosensitizers, such as calmodulin inhibitors or calcium antagonists, are potent pharmacologic compounds that do not allow the achievement of sufficient plasma levels in humans. Hence, pharmacologic development of second generation chemosensitizers with reduced toxic side effects is in progress, but was delayed by the difficulty and expense of introducing valid animal models.

In the past few years is has become apparent that the use of recombinant DNA technology to engineer animals for the specific testing of new classes of pharmacologic agents can speed the development of new drugs. The general principle is that proteins with which drugs interact can be introduced into transgenic mice and used to predict the activity of the drugs in certain disease states. Using recombinant DNA technology, a transgenic mouse has been engineered with bone marrow cell expressing functional human P-glycoprotein at levels equal to those found in drug-resistant tumors such as renal cell carcinoma in humans. Assessment of the number of peripheral leukocytes before and a few days after administration of chemotherapy in conjunction with chemosensitization provides a rapid, screening model for testing the efficacy and toxicity of new chemosensitizers and combinations of chemosensitizers that reverse MDR in an intact animal.

We are now faced with the emergence of an array of clinical investigations all with the same aim of attempting to intensify classical chemotherapy by means of inhibiting the underlying resistance mechanism. So far, hematologic neoplasms and lymphomas have been considered appropriate diseases for the initiation of clinical evaluation. These are tumors in which many active chemotherapeutic agents are handled and subsequently expelled by P-glycoprotein so that alteration in drug efflux via chemosensitization should indeed have an impact on response. These studies have recently been extended to include solid malignancies such as renal cell carcinomas.

Suffice it to say here that there is a variety of "reversing" agents that apparently act as false substrates for the multidrug transporter whereas true inhibitors of P-glycoprotein await further characterization. However, all these modulators have been identified empirically, and rational drug design needs to be brought to the problem, as this will ultimately provide for the best therapeutic index. Molecular modeling may in particular hold promise for defining the *MDR*1 "pharmacophore", i.e., the essential molecule necessary to inhibit P-glycoprotein function. Whether this structure will compete for drug-binding sites or bind allosterically is yet unknown, but this approach is an active research area mainly adopting photoaffinity labeling, peptide/amino-acid analysis, and site-directed mutagenesis techniques.

An alternate method of chemosensitization relies on circumventing rather than reversing the activity of P-glycoprotein. Delivery of drugs via liposomes or via conjugation to antibodies may bypass the plasma membrane, directly discharging drug into the cytoplasm, from which MDR-purging of drug may be less efficient.

Another attempt to eliminate the activity of the multidrug transporter is based on overcoming MDR by selectively killing cells that express P-glycoprotein on their surfaces. This result can be experimentally achieved by molcular targeting via antisense oligonucleotides or via bacterial toxins chemically linked to or recombinantly attached to certain anti-P-glycoprotein antibodies.

Use and regulation of *MDR*1 gene expression

Bone marrow suppression is a salient feature of many kinds of classical chemotherapy and thus the dose-limiting factor. It was recently demonstrated that bone marrow cells expressing a human drug-resistance gene have a selective advantage in the presence of anticancer drugs in vivo. This is the kind of selective system which is needed for the development of gene therapy in which an unselected gene can be introduced into bone marrow or any other organ by selecting for expression of the drug-resistance gene. Moreover, the transfection of the *MDR*1 gene to bone marrow cells using a Harvey sarcoma virus LTR or other constructs as promoter may have a direct impact on new strategies of chemotherapy. Current studies revealed that the human *MDR*1 gene can make bone marrow up to tenfold resistant to chemotherapeutic drugs. This result, if transferrable to clinical situations, may offer new therapeutic options. It has become an article of faith for many oncologists that many more human cancers could be cured if it was only possible to give higher drug doses. Dose escalation studies afforded by virtue of *MDR*1 bone marrow protection are in progress and may determine whether substantially higher doses of these drugs will destroy tumors which otherwise would have been incurable.

Even more futuristic is an approach providing for gene regulation systems. The biochemical basis of tissue-specific expression of the *MDR*1 gene is just beginning to be understood. But the information available indicates that regulation of its expression is complex and occurs at several different levels including initiation of transcription and processing of the mRNA encoding P-glycoprotein. Each of these steps involves the interaction of a specific set

of proteins with DNA or RNA and affords a site at which drugs can inhibit the process. Just as cyclosporin acts specifically to inhibit the expression of cells of the immune system, new drugs that inhibit *MDR1* gene expression will be discovered, evaluated, and eventually added to the armamentarium of the oncologist.

Within the next few years, it should be possible to use our knowledge of drug resistance mechanisms to design more effective antitumorous regimens based either on the predictive value of *MDR1* gene expression, or on agents that specifically inhibit P-glycoprotein, or on the application of gene-therapeutic principles using the *MDR1* gene as a selectable marker. However, it must be stated that although there appears to be a promising present breakthrough in research on MDR, mainly instigated by the progess of contemporary biotechnology, there is still a long way to go into the clinical future.

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