P-glycoprotein and Tumor Progression

Samuel Benchimol, Victor Ling*

Development of resistance to chemotherapy is a hallmark of many advanced human cancers. The molecular basis of clinical resistance is not well understood. Over the past 15 years, a number of different molecular mechanisms of resistance to anticancer drugs have been identified in many cell lines.

One mechanism involves the increased expression of the plasma membrane P-glycoprotein (Pgp), which is thought to function as an adenosine triphosphate-dependent efflux pump that reduces the cellular accumulation of a wide range of chemotherapeutic drugs (1,2). Studies (1-3) of biopsy samples from patients have demonstrated that elevated levels of Pgp can be detected in tumors of every histologic type. In addition, numerous studies have been conducted to determine whether or not Pgp expression is associated with clinical outcome. The conclusions of such studies have varied; however, the basis for these differences in conclusions may be attributed in part to differences in the techniques used for detecting Pgp in tumor samples. Nevertheless, several studies (3-7) on leukemias, lymphomas, and some childhood solid tumors have demonstrated a strong association between the detection of Pgp in tumor samples and poor response to chemotherapy. The observed association between Pgp expression and poor outcome in the clinical studies raises the question of how changes in Pgp expression are regulated in malignant cells. At present, mechanisms involved in the regulation of Pgp expression in human cancers and in normal human tissues are unknown.

In this issue of the Journal, Schneider et al. (8) address the question of whether or not Pgp expression was associated with p53 mutation and HER-2/neu expression in a number of human gynecological tumors. The rationale for their study was based on the hypothesis that, since previous experiments have shown that c-Ha-ras and mutant p53 appear to activate promoter activity of the human MDR1 (Pgp) gene using assays of expression of reporter gene plasmid constructs transfected into NIH 3T3 cells, it is possible that genes associated with oncogenic development may also activate concomitantly Pgp in human cancers. Such an association may provide an explanation for the frequent observation of elevated Pgp expression in many advanced cancers that have not been treated with chemotherapy. Although the question posed by the report by Schneider et al. is an important one, finding a definitive answer is far from simple.

It has been reported that p53 appears to be involved in the regulation of a wide variety of cellular processes (9,10). As far as its ability to regulate transcription is concerned, the central core region of wild-type p53 protein has sequence-specific DNA-binding activity, while the N-terminus of p53, when fused to the DNA-binding domain of yeast protein Gal4, can function as a transcriptional activator. As a result of these two activities, wild-type p53 protein can stimulate the expression of reporter genes that contain a p53-binding sequence in their promoters. In contrast, a large number of cellular and viral promoters that lack a p53-binding sequence, such as the MDR1 promoter, are repressed by wild-type p53 protein (11). It is interesting that p53 missense mutants have been shown to activate a number of promoters, including those present in the MDR1 and PCNA genes as well as in the long terminal repeat (LTR) of human immunodeficiency virus (12-16). Activation by mutant p53 is not dependent on the presence of a consensus p53-binding sequence. Indeed, the mechanisms by which mutant p53 can stimulate the expression of genes that do not contain p53-binding sites is not yet known. All of these studies have relied on transient expression-reporter gene assays, and it is pertinent to consider the physiological relevance of such assays in which constitutively overexpressed p53 protein acts on plasmid-borne extrachromosomal copies of a promoter-reporter gene. It has been demonstrated, for example, that certain promoters that are inhibited by p53 protein in transient transfection assays are not inhibited in their native state, implying that regulation of genes by p53 is subject to the natural chromatin state of the endogenous gene.

In light of these observations and of the enormous difficulty in extrapolating findings from transient expression-reporter gene assays to endogenous gene regulation, the results presented by Schneider et al. (8) are not entirely surprising and argue against the hypothesis that MDR1 gene expression is regulated by p53 protein. Schneider et al. found no association between p53 gene status and MDR1 expression in mammary, endometrial, and cervical tumors. Moreover, el Rouby et al. (17) reported a similar...
lack of association in B-cell chronic lymphocytic leukemia. If p53 were the sole regulator of MDR1 expression, one might reasonably have expected to see low levels of MDR1 protein in tumor cells containing wild-type p53 protein (p53-mediated repression), higher levels in tumor cells expressing no p53 protein (lack of p53-mediated repression), and even higher levels in tumor cells expressing missense mutant p53 protein (mutant p53-mediated stimulation).

Regulation of Pgp expression in tumor cells is likely to be complex. In cultured cell lines selected for drug resistance, elevated Pgp expression can be mediated via gene amplification, increased messenger RNA (mRNA) content, or increased protein levels (18). At these levels, Pgp expression appears to be highly responsive to modulation by various intrinsic and extrinsic factors.

Pgp expression is responsive to a variety of stimuli. For example, in renal carcinoma cell lines, an increase in Pgp mRNA expression was observed after heat shock or sodium arsenite treatment; this increase was thought to be mediated via an increase in transcriptional activity (19). Treatment of colon carcinoma cell lines with sodium butyrate or treatment of neuroblastoma cell lines with retinoic acid appears to result in an increased level of mRNA without an increase in transcriptional activity (20,21). Other investigators have observed an increase in Pgp protein expression in cell lines treated with repeated doses of x radiation. Such treatment did not result in an increase in the level of mRNA but rather increased the level of the protein (22). These are only a few examples of studies that have shown that Pgp expression is highly responsive to a variety of factors. It is likely therefore that in a complex heterogeneous tumor cell environment, different factors may affect Pgp expression.

Mutant p53 is often identified indirectly on the basis of overexpression in immunohistochemical assays or by single-strand conformation polymorphism (SSCP) DNA analysis directed at exons 5 to 8 where most mutations reside. Conversely, lack of immunohistochemical staining and/or absence of abnormal band patterns by limited SSCP analysis is used to identify wild-type p53.

Conclusions derived from the assays described above must be considered provisional until they are confirmed by complete sequence analysis. Tumors containing wild-type p53 alleles present a further challenge. Is the gene expressed, and is the p53 protein functional? For example, in cervical carcinomas, many of which contain and express human papillomavirus (HPV) sequences, the HPV E6 protein binds p53 protein and promotes its degradation through a ubiquitin-dependent pathway. Hence, HPV E6 protein effectively reduces the intracellular level of p53 protein and, in so doing, interferes with its function. A number of other viral (e.g., simian virus 40 large T antigen and adenovirus E1B) and cellular proteins (e.g., Mdm2) have been shown to bind wild-type p53 protein, resulting in functional inactivation (23). It would be of interest to determine the HPV status and the functional state of p53 protein in the eight cervical tumors studied by Schneider et al. (8), seven of which expressed MDR1 protein.

It is noteworthy that Schneider et al. (8) observed a statistically significant association between the expression of the HER-2/neu gene and Pgp in mammary carcinomas. The basis for this connection is not known; however, it would be of interest to determine if both the HER-2/neu and Pgp genes are regulated by a common mechanism. Schneider et al. found the coexpression of Pgp and HER-2/neu gene in a subgroup of aggressive, locally advanced, inoperable mammary carcinomas; however, they did not find a statistically significant association in the operable tumors. This finding is consistent with Pgp overexpression being associated with an advanced tumor phenotype. A similar conclusion has been made by Weinstein et al. (24) in human colon cancer and by Bradley et al. (25) in rat liver cancer.

One may speculate as to what role Pgp might play in highly malignant cancers. There is little controversy that an elevated expression of Pgp provides the tumor cells with a growth advantage during treatment with lipophilic anticancer drugs. However, since Pgp is able to transport a wide variety of other substrates, including hormones, peptides, and ions (1,2), it is possible that the export of some as yet unidentified Pgp substrates may provide a growth advantage to malignant cells expressing high levels of Pgp. For example, it is possible that Pgp may be implicated in the secretion of autocrine growth factors or factors involved in stimulating angiogenesis. Current studies (4,5,7,26) to inactivate Pgp function using chemosensitizing agents (multidrug resistance-reversing agents) may provide further insights into the postulated dual role of Pgp in chemotherapy and in tumor progression.

References

(13) Zastawny RL, Salvino R, Chen J, et al: The core promoter region of the P-glycoprotein gene is sufficient to confer differential responsiveness to wild-type and mutant p53. Oncogene 8:1529-1535, 1993


Manuscript received April 28, 1994; accepted April 29, 1994.

Have You Seen our Videotapes?

I Need a Friend

Or Would You Like to Schedule a Speaker?

A videotape, Finding Strength: A Look at the Pediatric Branch, is available to health care professionals and families interested in learning more about programs and research protocols at the Branch. Also available are two videotapes for health care professionals working with HIV-infected children and their families: Conducting an HIV Parent Support Group and I Need a Friend: Kids Talk About the AIDS Virus.

If you plan to show the tape at a meeting of health professionals or other interested persons, we would be happy to arrange for a staff member to attend the showing and conduct a follow-up question and answer session. To schedule a presentation or order tapes, call Molly Matthews at (301) 951-1104.

For patient referral or information, contact:

Philip Pizzo, M.D.
Chief, Pediatric Branch
National Cancer Institute
Bethesda, Maryland 20892
(301) 402-0696
Business Professionals!

Save time and money with the new 1994 U.S. Industrial Outlook

Your **ONE-STOP SOURCE** for authoritative analyses and statistical data on U.S. manufacturing and services industries, with emphasis on high technology and trade.

**New in this edition:** Coverage of trade finance and educational training.

Fill out the order form below to order your copy of the '94 U.S. Industrial Outlook today.

Order Processing Code: 7150-M

Superintendent of Documents Order Form

Charge your order. It's easy!

To fax your orders (202) 512-2250

☐ YES, please send me ______ copies of the **1994 U.S. Industrial Outlook**, S/N 003-009-00635-0, at $39.00 each ($48.75 foreign), so I can find out about trends, projected growth rates, trade and much more for 1994 and beyond for manufacturing, services, and high-technology industries.

The total cost of my order is $_______ . Price includes regular domestic postage and handling and is subject to change.

Please choose method of payment:

☐ Check payable to the Superintendent of Documents

☐ GPO Deposit Account ________

☐ VISA ☐ MasterCard ________

Expiration date ________

Thank you for your order!

Authorizing signature 1/94

Mail to: Superintendent of Documents

P.O. Box 371954, Pittsburgh, PA 15250-7954