Multidrug Resistance During Chemical Carcinogenesis: A Mechanism Revealed? 

Michael M. Gottesman 

The classic Solt-Farber model of chemical carcinogenesis in rat liver involves sequential treatment of rats with diethylnitrosamine, partial hepatectomy, and 2-acetylamino)fluorene (AAF). After several weeks, the rats develop multiple preneoplastic hepatic nodules, and some of these become frank hepatocarcinomas after several months. Cells derived from the nodules appear to be resistant to a variety of toxic agents. In humans, this may translate into the well-known intrinsic multidrug resistance of hepatocarcinomas. In this issue of the journal, Burt and Thorgeirsson show that treatment with certain chemical carcinogens increases RNA levels for at least two proteins involved in cellular handling of toxic materials: (a) the multidrug transporter P-glycoprotein, an energy-dependent efflux system for natural product cytotoxic drugs that is the product of the MDR-1 gene (also known as PGY1); and (b) cytochrome P-450 isoform d, a component of the system that oxidizes potentially harmful xenobiotics. Does this coordinate increase in levels of RNA for two potential detoxifying systems account for the multidrug resistance of chemically induced tumors and perhaps other cancers as well, and does it reflect a global regulatory mechanism that controls expression of detoxifying systems? 

Although the induction of various components of the cytochrome P-450 system in the liver by specific xenobiotics has been well studied, agents that increase MDR-1 gene expression are less well understood. Thorgeirsson et al. and Fairchild et al. have previously reported that MDR-1 RNA levels are elevated in preneoplastic and neoplastic nodules during Solt-Farber carcinogenesis in rat liver. This stable elevation apparently follows more transient but substantial increases in MDR-1 RNA levels in liver following treatment either with AAF or partial hepatectomy.

What is the evidence that expression of MDR-1 RNA contributes to the multidrug resistance of hepatic tumors? The MDR-1 protein product P-glycoprotein has been localized to the luminal surface of several epithelial cell types, including hepatocytes, intestinal mucosal epithelial cells, and kidney proximal tubule cells. In tissue culture cells, expression of a cloned MDR-1 gene results in simultaneous resistance to drugs such as doxorubicin, vinca alkaloids, dactinomycin, colchicine, taxol, and epipodophyllotoxins because of rapid efflux of these drugs from cells expressing the transporter. Therefore, it seems reasonable to speculate that expression of the multidrug transporter in the liver, kidney, and intestine is responsible for the excretion of these cytotoxic drugs, as well as unknown endogenous materials, into bile and urine and into the lumen of the lower gastrointestinal tract. Furthermore, the elevated expression of the transporter in tumors derived from epithelial cells of the liver, kidney, and intestine suggests that the drug resistance of these tumors is at least partially attributable to the multidrug transporter (Goldstein L, Gottesman MM, Pastan I; unpublished data).

If the MDR-1 gene product accumulates to much higher levels during chemical carcinogenesis in the liver, perhaps this system helps account for the known resistance of developing hepatic tumors to carcinogens. Unfortunately, many hepatic carcinogens, including AAF, which has been shown to lead to increased MDR-1 RNA levels; Burt and Thorgeirsson, do not appear to be transported by P-glycoprotein, since multidrug-resistant cultured cells with high levels of the transporter are not resistant to AAF. Hence, although elevated MDR-1 gene expression may contribute to the known resistance of certain hepatic tumors to chemotherapy including doxorubicin, the vinca alkaloids, and epipodophyllotoxins, it cannot account for the resistance of hepatic nodules to most chemical carcinogens. Other mechanisms must be invoked to account for this resistance. These include metabolic detoxification and, perhaps, novel transport systems including the MDR-2 (also known as mdr3) gene product. The MDR-2 RNA is expressed at low levels in the liver, but levels of expression during chemical carcinogenesis have not yet been determined.

If the multidrug transporter is not responsible for carcinogen resistance in hepatic nodules, why is it induced by such diverse agents as AAF, aflatoxin B1, isosafrole, phenothiazine, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)? Burt and Thorgeirsson and Cowan et al. speculate that, as a detoxifying transport system, the MDR-1 transporter might be coordinately regulated in the liver and other tissues with other known detoxifying systems such as UDP-glucuronosyltransferase, glutathione transferase, and cytochrome P-450 isoforms. Thus, in the simplest model, an intracellular signal generated by the interaction of these compounds with the cell could result in alterations in RNA levels for several different detoxifying systems. One intriguing observation made by Burt and Thorgeirsson is that DBA/2 (D2) mice, which lack the Ah receptor needed for cytochrome P-450 response to TCDD and other polycyclic hydrocarbons, also fail to show elevated MDR-1 RNA levels after TCDD, AAF, or aflatoxin B treatment. This result suggests that induction of the MDR-1 RNA does lie along the same pathway involved in cytochrome P-450 d induction, but the observation does not prove that a single signal is responsible for this induction.

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Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bldg. 37, Rm. 2E22, Bethesda, MD 20892.
Another confounding issue is the mechanism by which MDR-1 RNA is increased. Recent data from our laboratory (Marino P, Pastan I, Gottesman MM: unpublished data) suggest that there is little or no increase in transcription of MDR-1 RNA after partial hepatectomy in the rat, although large increases in steady-state levels of MDR-1 RNA are seen. This result, if it can be generalized to the increased MDR-1 RNA levels seen after treatment with chemical carcinogens and in hepatic nodules, rules out stimulation of transcription as the sole mechanism responsible for elevated MDR-1 RNA levels. If increased MDR-1 RNA levels in the liver are caused by mRNA stabilization, then there are two possibilities. (a) The postulated coordinate regulation of different detoxifying systems may involve both transcriptional and posttranscriptional controls. (b) The mRNA stabilization may be the major mechanism for activating other detoxifying systems as well. One implication of this analysis is that the search for common nucleotide sequences and factors that coordinately regulate expression of detoxifying systems should not be restricted to the promoter region of the regulated genes; it should involve other sequences or the structure of the RNA itself.

The transient increase in MDR-1 RNA induced in the liver by hepatotoxicity and chemical carcinogens may be explained by activation of a “detoxifying pathway,” but the stable high level of expression of MDR-1 RNA in preneoplastic and neoplastic nodules (5) is less easily understood. One distinct possibility is that chemical carcinogens, by killing cells that fail to express stable high levels of various detoxifying systems, select for clones of cells that are stable high expressers. A second possibility is that the process of malignant transformation itself activates the putative detoxifying pathway. Recently, Burt et al. (12) have found that transformation of rat liver epithelial cells with v-H-ras or v-raf results in a multidrug-resistant phenotype. These cells have increased expression of the MDR-1 gene and of glutathione-S-transferase, an enzyme that detoxifies hydrophobic xenobiotics by conjugation with glutathione. Expression of v-H-ras in other cell types, such as fibroblasts and colon and kidney cells, does not result in multidrug resistance (Gottesman MM: unpublished data); nor do all oncogenes produce this effect (Burt RK, Thorgeirsson SS: unpublished data), so this is not a general phenomenon related to oncogene expression. It is possible that v-H-ras and v-raf induce a program of growth and/or differentiation that is unique to liver cells and involves the activation of detoxifying pathways.

Is there any evidence that malignant transformation per se can result in elevated levels of MDR-1 RNA? In tumors such as kidney and colon cancers, which are intrinsically resistant to chemotherapy, investigators have observed high MDR-1 RNA levels that seem to be directly related to the extent of differentiation of the tumor; i.e., the most highly differentiated tumors seem to express the most MDR-1 RNA (Kakehi Y, Pastan I, Gottesman MM, et al.: unpublished data) (Fojo AT: unpublished data). Hence, if it is assumed that less-differentiated tumors are more malignant, then there is a negative correlation between MDR-1 expression and transformation. However, for tumors of the hematopoietic system, in which the cells of origin do not show elevated MDR-1 RNA levels, occasional but perhaps highly significant elevations of MDR-1 RNA are seen prior to clinical treatment with chemotherapeutic agents. Examples include (a) adult acute nonlymphocytic leukemia, in which a minority of wbc's, at first presentation of disease, showed elevated MDR-1 RNA levels, and (b) chronic myelogenous leukemia in blast crisis, in which a majority of blasts showed MDR-1 RNA elevations despite the fact that the chronic phase of this disease involves low or unmeasurable MDR-1 RNA levels (Goldstein L, Gottesman MM, Pastan I: unpublished data). These intriguing observations, taken together with the data of Burt et al. (12), suggest that the transformation process may result, in some cases, in multidrug resistance.

It is too early to assess the clinical relevance of these results, but the implications are promising. The relative contribution of various detoxifying mechanisms to the clinical resistance of human cancer cells must be established. Studies to measure the expression in clinical samples of RNA and protein for the MDR-1 system as well as other detoxifying systems are in progress. These data will establish which tumors express which detoxifying systems so that specific therapy to counteract or bypass these systems can be designed. If detoxifying systems are coordinately regulated in human tumors as they apparently are in rat liver, then the control system itself may be a target for the next generation of agents to reverse multidrug resistance. The development of molecular tools for the analysis of drug resistance has spawned a new era of cancer chemotherapy.

References