Does P-Glycoprotein Predict Response to Chemotherapy, and If So, Is There a Reliable Way to Detect It?

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The development of resistance to cancer chemotherapeutic drugs remains a major obstacle in the treatment of cancer patients. Medical oncologists are all too familiar with the phenomenon of tumor recurrence following an initial clinical response to chemotherapeutic drugs. Determining mechanisms of drug resistance is critical to the development of rational therapeutic strategies to overcome or prevent drug resistance. One mechanism of resistance which has been well characterized is that of multidrug resistance (MDR) (*1*). Tumor cells that have the MDR phenotype are generally resistant to such natural products as the vinca alkaloids, anthracyclines, and podophylotoxins. This form of drug resistance has consistently been associated with increased expression of a 170-kilodalton molecular weight plasma membrane protein termed the P-glycoprotein (*2*). P-glycoprotein functions as an energy-dependent drug efflux pump that reduces intracellular drug accumulation, thereby conferring resistance to many different drugs.

P-glycoprotein is encoded by a small family of closely related genes, and of the two genes in humans, only the mdr1 gene (also known as PGY1) causes the MDR phenotype (*3*). In order to evaluate fully the role of P-glycoprotein in clinical drug resistance, it is important to develop assays that are specific for the mdr1 protein and yet sensitive enough to detect the minute concentrations of protein present in small tissue specimens. An added confounding factor is the fact that P-glycoprotein is expressed in certain normal tissues; thus assays not only must be specific and sensitive, but also must be able to discriminate between normal and neoplastic tissue. Initial studies analyzing the role of P-glycoprotein in human tumor specimens utilized biochemical analyses of bulk tumor tissue. While these initial analyses were considered to be specific for P-glycoprotein, they were relatively insensitive and did not discriminate between normal and neoplastic tissue.

In this issue of the Journal, Verrelle and colleagues (*4*) analyze the expression of P-glycoprotein in locally advanced breast cancer, using an immunohistochemical means of detection. These investigators used the monoclonal antibody C494, which is considered specific for the mdr1 protein (*5*). This study of 20 patients found a surprisingly high incidence of P-glycoprotein expression in tumor cells from patients with primary, locally advanced breast carcinoma. Eighty-five percent of the patients expressed P-glycoprotein in at least some of their tumor cells. Using a semiquantitative scaling system that considered both the number of positive cells and the intensity of staining, they demonstrated a significant association between response to chemotherapy and time to treatment failure (ie, disease progression or recurrence) in seven patients who had a high degree of staining in the majority of tumor cells. While this semiquantitative approach found a subset of patients with a poor prognosis, a number of factors must be considered in order to prudently interpret their data.

One primary concern is the small number of patients in this study. Performing a subset analysis on a total of 20 patients is generally fraught with a number of pitfalls. For example, it is difficult to achieve equivalence between two small patient subsets regarding other pertinent prognostic factors (such as proliferative capacity of the tumor) that might influence therapeutic outcome. Obviously, more patients will need to be analyzed prospectively in future studies to determine the role of P-glycoprotein in predicting relapse following chemotherapy.

A second concern is the very high incidence of P-glycoprotein expression detected in these patients. Not only does this result differ from the relatively low incidence of P-glycoprotein expression in breast cancers described by other investigators, who used different antibodies (*6,7*) or other methods such as the detection of RNA (*8,9*), but it also raises the issue of the signal-to-background ratio in attempting to develop a quantitative immunohistochemical assay. If one analyzes the tumor specimens as being either positive or negative for P-glycoprotein expression, then only three patients are in the negative category and P-glycoprotein is rendered useless as a prognostic factor. Nonetheless, Verrelle and colleagues have demonstrated that patients in whom the majority of tumor cells strongly express P-glycoprotein do less well following treatment with chemotherapy than patients in whom P-glycoprotein detection in tumor cells is weak or absent. Attention to the refinement of immunohistochemical techniques in order to increase the signal-to-background ratio will be critical to achieving quantitation of P-glycoprotein in future studies.

A similar study of P-glycoprotein in locally advanced breast cancer was recently reported by Ro et al (*10*). Using the C-219 monoclonal antibody and different fixative techniques, these investigators observed the frequent expression of P-glycoprotein in tumors following pre-operative chemotherapy. Such expression was significantly associated with a poor response to chemotherapy.

Taken together, these two studies of P-glycoprotein expression in locally advanced breast cancer (*4,10*) suggest the prospect of predicting therapy failures by pretreatment testing for P-glycoprotein expression. Positive results would provide a rationale for alternative chemotherapy or for the circumvention of MDR by using chemosensitizers such as verapamil (*11*).

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These studies also demonstrate that the immunohistochemical detection of P-glycoprotein holds particular promise for clinical applications, since it is a practical, readily performed procedure. Solid tumors, such as breast cancer, tend to be a mixture of tumor cells and normal cells (stroma). Assays that measure P-glycoprotein from bulk tumor specimens are unable to differentiate between the presence of a few cells with a high degree of P-glycoprotein expression and the existence of a low level of P-glycoprotein expression in a majority of tumor cells. These two situations may have vastly different outcomes in terms of response to chemotherapy. The primary advantages of immunohistochemistry in the clinical setting include the abilities (*a*) to detect P-glycoprotein in a single cell, allowing for small sample size, (*b*) to determine the subcellular distribution of P-glycoprotein, and (*c*) to discriminate P-glycoprotein expression in normal cells from that in neoplastic cells. These features are especially important if one considers the heterogeneous nature of solid tumors.

The primary concerns regarding immunohistochemical methods are 1) the issue of false positives and negatives, and 2) the inability to detect low levels of P-glycoprotein that may be clinically relevant. False positives surely exist (eg, the cross-reactivities of C-219 with striated musculature and with the mdr3 isoform of P-glycoprotein). However, a number of monoclonal antibodies that react with different epitopes of the mdr1 P-glycoprotein now exist. Presumably a panel of antibodies may be superior to the use of a single antibody. Alternatively, competitive immunohistochemical staining may be performed with epitope-specific peptides to ensure specificity of detection (*5*). Regarding the detection of low levels of protein, the refinements by Chan et al (*12*) and Grogan et al (*13*), among others, have suggested that detection of low levels of protein is feasible provided proper controls are used (*14*).

While the results of Verrelle et al (*4*) are certainly preliminary, the study design is appropriate in addressing the question of the clinical relevance of P-glycoprotein expression in breast cancer. The authors also demonstrate that the immunohistochemical method of P-glycoprotein detection holds great promise for practical application. Further studies in this area are warranted and eagerly awaited.

References

(*1*) Gerlach JH, Kartner N, Bell DR, et al: Multidrug resistance. Cancer Surv 5:25–46, 1986

(*2*) Kartner N, Riordan JR, Ling V: Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. Science 221:1285–1288, 1983

(*3*) Ueda K, Cardarelli C, Gottesman MM, et al: Expression of a full-length cDNA for the human MDR1 gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci USA 84:3004–3008, 1987

(*4*) Verrelle P, Meissonnier F, Fonck Y, et al: Clinical relevance of immunohistochemical detection of multidrug resistance P-glycoprotein in breast carcinoma. J Natl Cancer Inst 83:111–116, 1991

(*5*) Georges E, Bradley G, Gariepy J, et al: Detection of P-glycoprotein isoforms by gene-specific monoclonal antibodies. Proc Natl Acad Sci USA 87:152–156, 1990

(*6*) Sugawara I, Kataoka I, Morishita Y, et al: Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK16. Cancer Res 48:1926–1929, 1988

(*7*) Schneider J, Bak M, Efferth TH, et al: P-glycoprotein expression in treated and untreated human breast cancer. Br J Cancer 60:815–818, 1989

(*8*) Ozols RF, ed: Drug Resistance in Cancer Therapy: Cancer Treatment and Research Series. Boston: Kluwer Academic Publishers, 1989

(*9*) Merkel DE, Fuqua SA, McGuire WL, et al: P-glycoprotein in breast cancer. Cancer Treatment Res 48:97–105, 1989

(*10*) Ro J, Sahin A, Ro JY, et al: Immunohistochemical analysis of P-glycoprotein expression correlated with chemotherapy resistance in locally advanced breast cancer. Hum Pathol 21:787–791, 1990

(*11*) Dalton WS, Grogan TM, Meltzer PS, et al: Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: Detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J Clin Oncol 7:415–424, 1989

(*12*) Chan HSL, Bradley G, Thorner P, et al: A sensitive method for immunocytochemical detection of P-glycoprotein in multidrug-resistant human ovarian carcinoma cell lines. Lab Invest 59:870–875, 1988

(*13*) Grogan TM, Dalton WS, Rybski J, et al: Optimization of immunocytochemical P-glycoprotein assessment in multidrug resistant plasma cell myeloma using 3 antibodies. Lab Invest. In press

(*14*) Dalton WS, Grogan TM, Rybski JA, et al: Immunohistochemical detection and quantitation of P-glycoprotein in multiple drug-resistant human myeloma cells: Association with level of drug resistance and drug accumulation. Blood 73:747–752, 1989