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Editorial

Drug resistance markers: Are they bad or good?

Two papers presented in this issue examine the relationship between the tumor levels of putative drug resistance parameters and prognosis of breast and ovarian cancers [1, 2]. Common to both studies are the measurements of total glutathione *S*-transferase (GST) activity in bulk tumor specimens. The rationale for these measurements is provided by a body of experimental data that have linked the expression of isozymes of GST with resistance to some antineoplastic [3, 4]. GSTs catalyze the conjugation with glutathione of a number of toxic electrophiles including some anticancer drugs [5]. Such conjugates are generally less toxic to the cell and can be exported by specific glutathione-conjugate efflux pumps [6]. Additionally, increased expression of some isozymes, particularly the pi class isozyme of GST, is associated with neoplastic transformation and unfavorable clinical behavior of malignant tumors in some studies [7, 8].

Both papers in this issue report that, in contrast to expectation, GST levels are significantly higher in tumors with more favorable clinical characteristics. Indeed, if GST is a significant mediator of drug resistance and determinant of response to chemotherapy, then these results are unanticipated. These are interesting papers whose paradoxical results underscore the complexities and pifalls of attempting to use experimental parameters identified *in vitro* for the evaluation of clinical response and outcome. Closer examination of the data collected in the Ferrandina et al. and Buser et al. papers in the context of a broader experimental background may help put these studies in perspective.

Both studies have assayed bulk tumor for total GST activity. This is potentially problematic. First, there is likely significant variability in the proportions of tumor *versus* normal tissue in the specimens examined. Hence, measurements of GST will be confounded by variable tissue contributions and true differences in GST activities between tumors may be over- or underestimated. Secondly, GSTs are actually a superfamily of different isozymes (alpha, mu, pi, theta, and microsomal) and each isozyme has different biochemical characteristics and substrate specificities. In both studies only total GST activity was measured and no attempt was made to quantitate the expression of different GST isozymes. This is a critical omission because the isozymes of GST vary considerably in their anticancer drug substrate specificities and in their utility as markers of malignant potential. Previously in node negative breast cancer, unfavorable prognosis has been positively correlated with increase pi class GST (GSTP1-1) expression [7] although at least two other classes of GST (alpha and mu) are represented in normal and malignant breast tissues [9–12]. Thus, examination of specific isoforms of GST in the Ferrandina et al. study could have significantly altered the conclusions [2]. Furthermore, the relatively high level of expression GSTs in normal breast epithelial cells also emphasizes the potential problem with assaying heterogenous specimens.

Previous studies reported an inverse correlation with GST pi expression and hormone receptor levels in breast cancer cell lines and in breast tumors [7]. While Buser et al. [1] report a trend towards increased total GST activity in estrogen receptor negative breast cancers, Ferrandina et al. [2] found no such correlation between total GST activity and hormone receptor status in ovarian cancer patients, suggesting possible tissue specific differences in regulation of GST expression. While the Gilbert et al. study [7] reported an inverse correlation between GST pi and hormone receptor levels the current two studies did not examine specific GST isozyme expression in tumors. Furthermore, since hormone receptor levels are associated with a good prognosis in early stage breast cancer, the finding by Buser et al. [2] that GST activity was greater in hormone receptor negative early stage breast cancer patients and yet was associated with a better prognosis is unexpected.

The Ferrandina et al. paper [2] includes an analysis of ovarian cancer response to chemotherapy [1] while the Buser et al. [2] paper does not discuss the possible relationship between antineoplastic drug or hormone therapy and GST expression. Once again, the absence of GST isozyme data represents a particularly significant omission since GST isozymes differ considerably in their reactivities with the various antineoplastic drugs and metabolites. For example, increased levels of the alpha class GST are especially associated with alkylating agent resistance [5]. With respect to three drugs frequently used in the treatment of ovarian cancer, cisplatin, doxorubicin, and cyclophosphamide, only cyclophosphamide and some of its metabolites have been shown to participate in conjugation reactions catalyzed by alpha, mu or pi class GSTs [13]. Although some gene transfer experiments have suggested a causal relationship between increased GSTP1-1 expression and low level doxorubicin and cisplatin resistance [14, 15], others have failed to confirm this relationship [16–18]. Moreover, there are no reactions with doxorubicin or cisplatin known to be catalyzed by GSTs. Thus, there is no clear biochemical basis for any claims of GST-mediated resistance to doxorubicin or cisplatin. For cisplatin, the level of intracellular thiols, especially glutathione, are

probably more important determinants of resistance than are GST levels. In view of these considerations, the results of Ferrandina et al. [2] may not be surprising – increased GST activity would not necessarily be expected to be associated with resistance to cisplatin-based chemotherapy.

An intriguing possibility is raised by both papers – namely, that increased total GST levels may be associated with more favorable response to therapy or less aggressive tumor behavior, suggesting that the regulation of GST expression may be linked with biological behavior of tumor cells. However, in both papers, the mean differences between GST levels in bulk tumor specimens from patients with favorable *versus* unfavorable clinical characteristics are small. The range and overlap of GST levels in these tumor groups are large and the overall sample sizes are relatively small. In order to assess the prognostic significance of GST and to clarify the biological significance of altered GST levels, more refined measurements, including identification of the isozymes expressed, are needed.

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