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Expression of glutatione S-transferase B_1, B_2, Mu and Pi in breast cancers and their relationship to oestrogen receptor status

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Summary The concentrations of glutathione S-transferase (GST) B_1 and B_2 (Alpha), Pi and Mu have been measured by radioimmunoassay in cytosols from 28 oestrogen receptor (ER) rich and 30 ER-poor breast tumours. GST B_1, B_2 and Pi was detected in all 58 breast tumour cytosols whilst GST Mu was found in only 28. Of the GSTs, Pi was expressed most strongly in all cytosols and the concentration was significantly higher in ER-poor tumour cytosols than in ER-rich tumours ($P<0.01$). As with GST Pi, the highest levels of GST B_1 and GST B_2 were found in ER-poor tumour cytosols; the levels of GST B_1 and GST B_2 were positively correlated ($r=0.66$, $P<0.001$). No quantitative or qualitative association was found between ER status and GST Mu which was expressed in 46% of ER-rich and 50% of ER-poor tumour cytosols. No relationship could be found between GST expression and age, menopausal status, lymph node involvement or tumour T stage in the subgroup of patients in whom this information was available. These data suggest that a common mechanism is responsible for GST induction in ER-poor tumours and that the nulled Mu phenotype has no increased susceptibility to developing breast cancer.

The glutathione S-transferases (GST) are a family of dimeric enzymes which are found in the cell cytoplasm. They have been implicated in the detoxification of a wide range of xenobiotics and chemotherapeutic agents (Jakoby, 1978; Mannervik, 1985; Buller *et al.*, 1987). Three immunologically distinct major classes of GST occur in humans Alpha (that comprises B_1 and B_2 subunits), Mu and Pi (Mannervik *et al.*, 1985; Stockman *et al.*, 1985). Although present in most human cells, the expression of the various GST isoenzymes may vary between different types of tissue (Strange *et al.*, 1984). Approximately half the population do not express GST Mu and appear more susceptible to developing lung cancer if they smoke heavily (Seidegard *et al.*, 1986). GST Pi has been associated with pre-neoplastic and neoplastic change and may be present in many human tumours and cell lines (Ilio *et al.*, 1986; Kodate *et al.*, 1986; Shiratori *et al.*, 1987; Shea *et al.*, 1988; Mannervik *et al.*, 1987).

Using a cDNA probe, over-expression of GST Pi RNA has been found in a multidrug-resistant cell line of breast cancer and also in a small series of 21 breast cancers (Moscow *et al.*, 1988). In the same study an inverse correlation was found between expression of GST Pi and the oestrogen receptor levels in the tumour. The expression of the Alpha and Mu classes of GST was not measured.

In the present study we have used specific radioimmunoassays to measure the expression, at the protein level, of GST Pi, Mu, B_1 and B_2 in cytosols from a larger group of 58 breast cancers.

Methods

Patients

Fifty-eight women with histologically proven invasive breast cancer were studied. Apart from four patients (three treated with tamoxifen and one with chemotherapy), none had received prior therapy for their malignancy.

Tumour was obtained from the primary cancer in 53 cases and from an axillary lymph node invaded with cancer in the remaining five individuals. The tumours were transported on ice to a cold room and then stored in liquid nitrogen until assayed.

Cytosol preparation

All procedures were performed at 0–4°C. Tumour was dissected from surrounding fat and connective tissue, finely cut with scissors and homogenised in 20 mmol l^{-1} Tris buffer, pH 7.5 (w/v 1:10), using a Silverson homogeniser at maximum speed for 20 s, then 15 with 1 min interval for cooling. The homogenate was then centrifuged at 105,000 *g* for 1 h and the resultant supernatant was used as a cytosol.

Oestrogen receptors

Oestrogen receptors were measured in an adjacent portion of tumour by saturation analysis (Hawkins *et al.*, 1981). Tumour cytosol was incubated overnight at 4°C with ^{3}H-17 β-oestradiol. Separation of free and bound steroid was by addition of dextran-coated charcoal; the bound fraction was measured by liquid scintillation counting. Concentration of receptors was determined by Scatchard analysis (Scatchard, 1949). Activities below 20 pmol mg^{-1} cytosol protein were designated oestrogen receptor poor (ER-poor) and those with activities in excess of 20 pmol mg^{-1} cytosol protein as ER-rich.

Measurement of GST concentrations

Specific radioimmunoassays, described in detail previously (Beckett & Hayes, 1984; Hussey *et al.*, 1987; Howie *et al.*, 1988), were used to determine the concentration of GST Pi, GST Mu, GST B_1 and GST B_2 isoenzymes in the 58 breast cytosols. The standards used for the B_1, B_2 and Mu assays were purified from human liver obtained from transplant donors and for GST Pi from fresh human placenta.

The cytosol, if required, was diluted in assay diluent consisting of 25 mmol l^{-1} sodium phosphate buffer pH 7.6 bovine serum albumin (1 g l^{-1}) and sodium azide (0.2 g l^{-1}) before analysis. Within-assay coefficients of variation of less than 10% was achieved in all assays.

Protein measurement

The dye binding technique described by Bradford (1976), adapted for use on a Cobas Fara (Roche Diagnostic Ltd, Welwyn Garden City, UK) centrifugal analyser was used for total protein estimation.

Results

The median levels of each of the GST isoenzymes in the breast cytosols are shown in Table I. GST Pi, B_1 and B_2 were detected in all breast tumour cytosols, whereas GST Mu was found in only 28 (48%) of the 58 cytosols. GST Pi was expressed most strongly with concentrations ranging from 30 to 1,110 μg $.g^{-1}$ cytosolic protein. For GST B_1 and B_2 the

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Table I Median GST levels

	Median GST concentrations (μg GST g^{-1} protein)			
	B_1	*B_2*	*Pi*	*Mu*
All tumours ($n = 58$)	4.5	0.86	222	118
ER-rich tumours ($n = 28$)	3.6	0.98	198	183
ER-poor tumours ($n = 30$)	4.7	0.82	306	107

For GST Mu, data is given for only the 28 tumours that expressed the protein.

concentrations ranged from 0.6 to 48 μg g^{-1} cytosolic protein and 0.04 to 5.3 μg g^{-1} cytosolic protein, respectively (Figure 1). The levels of B_1 and B_2 were positively correlated ($r = 0.66$, $P<0.001$) but GST Pi did not correlate with the expression of other GST.

The concentrations of GST in the cytosols subdivided according to the ER status as shown in Figure 1. Levels of GST Pi were significantly higher in ER-poor tumours ($P<0.01$, Mann–Whitney U test). Indeed, the tumours with the 11 highest GST Pi values were in the ER-poor tumours. As with GST Pi, the highest levels of GST B_1 and GST B_2 were observed in ER-poor tumours with the eight highest GST B_1 values being found in the ER-poor tumour group. However, when compared as groups the differences in GST B_1 and GST B_2 between ER-rich and ER-poor tumours did not reach statistical significance. No significant correlation could be found in the quantitative levels of oestrogen receptors and the expression of any of the GST classes.

No association was found between GST Mu and ER status. GST Mu was present in 15 of the 30 ER-poor tumours and 13 of the 28 ER-rich tumours. The same pattern of GST expression between groups was observed if a level of <5 pmol mg^{-1} was used to define ER status.

There was no relationship between levels of GSTs and the age or menopausal status of the patients from whom the tumour was derived. Furthermore, no obvious association was apparent between GST and lymph node involvement or tumour T stage in the subgroup of patients in whom this information was available. Similarly, there was no indication that tumour cellularity, central necrosis or a special histological type was associated with increased expression of GSTs.

The frequency with which GST Mu was expressed in the breast cancers was not significantly different from the frequency of expression of GST Mu found using lymphocytes obtained from a group of 42 laboratory volunteers where 23 (55%) volunteers expressed the enzyme (test of confidence limits for a proportion; Snedecor & Cochran, 1974).

Discussion

Our results concerning the elevated expression of GST Pi in ER-poor breast cancer are compatible with the data of Moscow *et al.* (1988), who showed increased mRNA levels for GST Pi in a similar group of tumours. This suggests that raised mRNA levels in ER-poor tumours results in an increased production of GST Pi protein. In addition, we have shown that both B_1 and B_2 GST appear to be over-expressed in some ER-poor tumours.

Although the role of GST Pi in conferring drug resistance has been recently questioned (Yusa *et al.*, 1988) an association between GST Pi expression and acquisition of drug resistance has been widely reported (for review see Hayes & Wolf, 1988). It has been suggested that the increased levels of GST Pi in ER-poor tumours will confer a poorer prognosis because such patients will have a lesser chance of responding to chemotherapeutic agents (Moscow *et al.*, 1988). However,

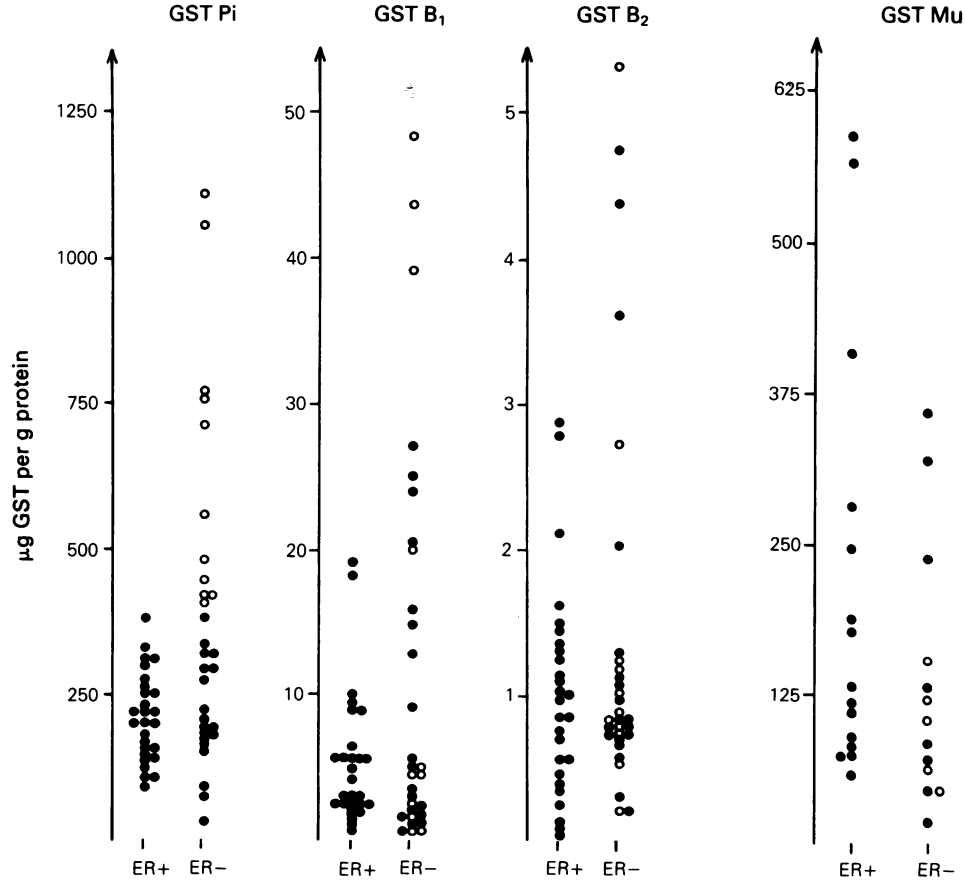

Figure 1 Concentrations of GST class Alpha (B_1 and B_2) and Pi and mu in oestrogen receptor rich (ER +) and poor (ER −) breast tumours (●). Tumours in which the concentration of GST Pi exceeded 400 μg g^{-1} cytosolic protein are shown by O.

it should be noted that ER-poor tumours often have a high proliferation rate (Meyer *et al.*, 1977) and this can be associated with rapid response to chemotherapy (Stoll, 1986). Additionally, there is no consensus on whether response rates to chemotherapy differ significantly in ER-rich and ER-poor tumours (Hawkins *et al.*, 1980; Carle *et al.*, 1984). In rat mammary tumours increases in the Alpha class GST are also associated with resistance to chemotherapeutic agents (Buller *et al.*, 1987). In our study 11 ER-poor tumours over-expressed GST Pi while six and four ER-poor tumour over-expressed GST B_1 and GST B_2, respectively. However, we have no clinical evidence that these increased Alpha class and Pi class GST levels are associated with chemotherapeutic insensitivity.

Aquisition of multi drug resistance in MCF-7 breast cancer cells is associated not only with an increase in GST Pi expression but also with a loss of hormone sensitivity. Our data show that both GST Pi and Alpha class GST expression is inversely linked to ER status. These observations are of interest since ER-poor tumours as a group are associated with worse prognosis than those which are ER-rich (McGuire *et al.*, 1975*a*; Nicolson *et al.*, 1981; Hawkins *et al.*, 1987; Courdi *et al.*, 1988). Whether this results from ER-rich cancers being inherently more indolent or more likely to respond to steroid hormone deprivation therapy is not clear (Howell *et al.*, 1984; Saez *et al.*, 1983; Adami *et al.*, 1985). However, it also has to be emphasised that whereas ER-poor tumours rarely respond to hormone manipulation (McGuire *et al.*, 1975*b*; Le Clerq & Heuson, 1977), the present results showed that only a small proportion of ER-poor tumours have elevated levels of GST. In this respect the observation that ER-negative breast cancers can be divided into sub-groups on the basis of other tumour characteristics such as epidermal growth factor receptors, vimentin and p53 expression is relevant (Cattoretti *et al.*, 1988). In the case of EGF receptors, this may be of prognostic value (Sainsbury *et al.*, 1987) but it is not known if there is an association between GST Pi expression and EGF receptor levels. It will be of interest to know whether patients having tumour with over-expression of GST will return more quickly with recurrent disease than those with lower GST, irrespective of ER status.

GST Pi maps to chromosome 11 (Moscow *et al.*, 1988), which has an increased incidence of deletions in ER-poor tumour cells when compared to ER-rich tumours (Ali *et al.*, 1987). These observations have led to the suggestion of a link between over-expression of GST Pi and a specific deletion on chromosome 11. The Alpha class of GST, however, map to chromosome 6 (Board & Webb, 1987) and we have shown that both GST B_1 and GST B_2 are also over-expressed in some ER-poor tumours. These data suggest that there may be a common mechanism, rather than specific chromosomal deletions, that leads to over-expression of the GST in certain breast cancers.

The incidence of GST Mu expression in both the ER-rich and ER-poor breast tumours was not significantly different from the incidence of GST Mu expression found in lymphocytes from the normal population (Seidegard *et al.*, 1986; Hussey *et al.*, 1987). This suggests a lack of association between expression of GST Mu and breast cancer.

In summary, glutathione S-transferases B_1, B_2 and Pi have been found in all cytosols of breast cancer whereas Mu was detected in only about one-half. High levels of GST B_1, B_2 and Pi were limited to ER-poor tumours, the clinical significance of which merits further study.

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