Expression of glutathione S-transferases in normal and malignant pancreas: an immunohistochemical study

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Abstract

The glutathione S-transferases (GSTs) are a family of detoxification and metabolising enzymes, which have been linked with the susceptibility of tissues to environmental carcinogens and resistance of tumours to chemotherapy. Environmental carcinogens have been implicated in the pathogenesis of pancreatic carcinoma, which is also a tumour characterised by marked chemotherapeutic drug resistance. In this study 26 pancreatic adenocarcinoma and 12 normal pancreatic samples were examined immunohistochemically for expression of pi (acidic), alpha (basic), and mu (neutral) GST. Fourteen (54%) of the tumours expressed pi GST alone, two (8%) expressed both pi and alpha GST, and two (8%) showed immunoreactivity with alpha GST alone. In the normal pancreas the intralobular ducts and centroacinar cells expressed pi GST alone whereas the large ducts expressed both pi and alpha GST. The acinar cells showed immunoreactivity only with anti-alpha GST. Mu GST was not expressed by normal or malignant pancreas. Expression of pi GST by pancreatic carcinoma may be a marker of the malignant phenotype and be induced during neoplastic transformation. Alternatively it could possibly reflect cell of origin, suggesting that the tumour arises from the centroacinar cells or intralobular ducts, or both rather than the large ducts.

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Glutathione S-transferases (GSTs) are a multi-gene family of proteins that function as enzymes of detoxification.^{1,2} By catalysing the conjugation of potentially mutagenic electrophilic compounds with reduced glutathione cells may be protected from toxic damage. Inactivation of chemical carcinogens in this way may protect normal cells from insult as well as preventing already mutated cells from further damage and so maintaining the malignant phenotype. Some cytotoxic drugs are also metabolised by GSTs potentially leading to tumour drug resistance.³

Human cytosolic GST isoenzymes have been classified on the basis of functional and structural relations; the alpha class tends to have alkaline (>8.0) isoelectric points while the pH values for the mu class are near neutral (7-8) and the pi class enzymes are acidic (<7.0).¹ An additional class of GSTs, designated theta, has recently been purified from human liver.⁴ The proteins within a class are probably members of a family that evolved from a single gene suggesting that each class provides an essential function to the survival of the whole organism.

Pi GSTs unlike the alpha enzymes, are expressed by a variety of human tumours^{3,5-10} and at higher values than corresponding normal tissues.^{5,6} Those tissues such as stomach and lung particularly exposed to environmental toxins have higher pi enzyme values suggesting a role in normal cellular protection.¹⁰ Both pi and alpha GSTs have been implicated in chemotherapeutic drug resistance. Adriamycin resistant MCF-7 breast carcinoma cells express higher values of pi GST than sensitive cells.¹³ In yeast, transfection with human alpha GST confers resistance to both adriamycin and chlorambucil. Resistance to the latter also occurs in cell lines expressing high values of alpha GST.^{14,15}

Adenocarcinoma of the pancreas is a tumour with considerable intrinsic drug resistance and a resulting poor prognosis. Chemical carcinogens may be an important aetiological factor; this is suggested by an increased incidence in those working in the chemical industry and by the best animal model of the human tumour, hamster ductal adenocarcinoma, being induced by nitrosamines.^{16,17} Recent studies have also shown a high incidence of mutations in the K-ras proto-oncogene at codon 12, which is consistent with carcinogen involvement.¹⁸ The GSTs, particularly pi, might therefore participate in the initiation or maintenance, or both, of the malignant phenotype as well as drug resistance and this is supported by an immunohistochemical study of GST in the hamster pancreas.¹⁹

We have therefore assessed the distribution of the three cytosolic GSTs immunohistochemically in human pancreatic adenocarcinoma and normal pancreas.

Methods

CASE MATERIAL

Formalin fixed paraffin embedded tissue from 25 cases of pancreatic carcinoma was used in this study. Clinical information was available on all patients from medical records. The male to female ratio was 12:15 with an age range of 33-78 years (median 61). Normal pancreas was available for analysis in a further five cases, the tissue having been obtained from Whipple's resections performed for diseases other than pancreatic carcinoma. Normal pancreas was also present adjacent to neoplastic tissue in 7/25 of the cases studied.

PREPARATION OF ANTISERUM SAMPLES

Antiserum samples to the alpha, pi, and mu GST enzymes were raised in rabbits as previously described.²⁰ Briefly, alpha and mu enzymes were

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purified from human necropsy liver and pi GST from the spleen of a patient with chronic lymphocytic leukaemia. These antigens were homologous as judged by SDS-PAGE and isoelectric focusing. Two hundred µg of protein in Freund's complete adjuvant was injected into male New Zealand white rabbits initially and subsequently monthly in Freund's incomplete adjuvant. The antiserum samples specificity was assessed using an enzyme linked immunosorbent assay (ELISA) technique and western blotting.²⁰

IMMUNOHISTOCHEMISTRY

Three µm paraffin wax embedded sections were cut from tissue blocks, dewaxed in xylene, and rehydrated through graded alcohols. Endogenous peroxidase was blocked with 0.5% hydrogen peroxide in water for 10 minutes. Sections were incubated with the primary polyclonal antibodies pi GST (1/400), alpha GST (1/500), and mu GST (1/200) overnight at 4°C. Immunohistochemical staining was carried out using an indirect immunoperoxidase method and 3,3' diaminobenzidine (Sigma) was used as

the chromagen. The sections were counterstained with haematoxylin, dehydrated, and mounted. Human kidney was used as a positive control for alpha and pi GST.

Results

NORMAL PANCREAS

The centroacinar cells, intralobular ducts, and larger ducts stained with the pi GST antibody but there was no staining of the acinar tissue or islets (Fig 1 upper panel). Conversely alpha GT was detected only in the acinar cells and larger ducts (Fig 1 lower panel). The acini staining was not uniform but the centroacinar cells and intralobular ducts seemed consistently free of immunoreactivity. There was no immunoreactivity with the mu GST within the pancreas. All staining was confined to the cytoplasm. The pattern of staining described above was also seen in the normal pancreas present adjacent to tumour tissue.

DUCTAL ADENOCARCINOMA

Sixteen of 26 (61%) cases of tumour showed immunoreactivity with pi GST (Fig 2). The staining was predominantly cytoplasmic with only occasional additional nuclear staining. The extent of staining was variable affecting over 50% of the tumour cells in seven cases with very focal staining in four tumours. When immunoreactivity occurred it tended to affect groups of cells suggesting clonality. Only 4/26 (15%) tumours expressed alpha GST, which was weak and focal. In two of these the tumour had also stained with the pi GST. Mu GST was not expressed by any of the tumours (Table).

Discussion

The expression of pi GST in most pancreatic adenocarcinoma is in agreement with that seen in a wide variety of other tumours including colon, lung, cervix, and brain.^{8 21 22} Overexpression of pi GST in malignant tissue compared with corresponding normal tissue combined with its expression in preneoplastic and neoplastic lesions in the cervix¹¹ and colon²³ have been used to argue that pi GST is a 'tumour marker'.²¹ It is not a universal tumour marker, however, in that some tumours (for example, liver²⁴) do not express pi GST. In addition pi GST has been shown to be induced in other disease states such as alcoholic hepatitis²⁵ so that overexpression cannot be equated solely with neoplastic transformation.

Of equal interest is the consistent absence of immunoreactivity for alpha GST in most pancreatic tumours. This has also been reported in human hepatocellular carcinoma, breast carcinoma, rat chemically induced preneoplastic liver nodules, and poorly differentiated cell lines.^{24 26-28} Mu GST, unlike pi and alpha, is not expressed by almost half of the normal population because of a genetic polymorphism.²³ The biological significance of our findings in pancreatic cancer awaits a greater understanding of the normal cellular function of the different isoenzymes; thus it may be that a reduced level of

Figure 1: (upper): staining with anti-pi GST confined to intralobular ducts and centroacinar cells (arrowed) within normal pancreas (original magnification $\times 128$); (lower): strong cytoplasmic immunoreactivity with polyclonal antibody to alpha GST confined to exocrine acini (A) and large pancreatic ducts (D). No staining is seen in the islets of Langerhan (I) (original magnification $\times 128$).

Figure 2:
Strong cytoplasmic immunoreactivity with polyclonal antibody to pi GST in a well differentiated pancreatic carcinoma (original magnification $\times 320$).

alpha GST is more important than increased expression of the pi isoenzyme.

To determine the significance of GST isoenzyme expression it is important to compare findings in neoplastic *v* normal tissue as has been shown with pi GST in colon cancer.⁵⁶ A major obstacle to comparing protein expression in normal pancreas *v* tumour tissue is that the cell of origin of pancreatic adenocarcinoma is unknown. In our study it was notable that the centroacinar cells of the normal pancreas only expressed pi GST whereas the larger duct stained with both alpha and pi GST. Identical findings have been reported by others in the normal pancreas and Hayes *et al* have also shown weak expression of mu GST in the islet cells.^{22 29} The absence, however, of a recognised preneoplastic lesion in human pancreas makes it impossible to follow changes in GST expression during carcinogenesis, which would resolve the dilemma as to whether pi GT is induced during carcinogenesis. In the animal model of nitrosamine induced pancreatic carcinoma in the hamster centroacinar cells form pseudoductular structures, which are a precursor of ductal adenocarcinoma.^{30 31} In this model pi and alpha GST increase during early ductal/ductular proliferation and in dysplastic lesions followed by a fall in alpha GST during transition to carcinoma.¹⁹ The distribution of GST isoenzymes in normal human pancreas together with pi GST expression in pancreatic tumours is compatible with a similar mechanism of neoplastic transformation in the pancreas in humans.

Expression of pi GST in pancreatic adenocarcinoma could thus reflect tumour cell origin rather than simply a role for this isoenzyme in pancreatic carcinogenesis or multidrug resistance. Further understanding of the biochemistry

of the different classes of GST and the origin of the pancreatic tumour cell are needed to evaluate this hypothesis further.

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Expression of glutathione S-transferase (GST) isoenzymes in normal and malignant pancreas

GST isoenzyme	Normal pancreas (n=12)	Adenocarcinoma (n=26)
Pi	Centroacinar cells Intralobular ducts	14 (54%)
Alpha	Acinar cells	2 (8%)
Pi and alpha	Large ducts	2 (8%)
Mu	-	-

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