Glutathione S-transferases in human liver cancer

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Abstract

An immunohistochemical study of glutathione S-transferase (GST) expression in hepatocellular carcinoma and cholangiocarcinoma is described. Unlike most animal models of hepatic malignancy pi class GST was not consistently overexpressed in hepatocellular carcinoma. This tumour type either predominantly expressed alpha class GST or failed to express GST. By contrast, cholangiocarcinoma always expressed pi class GST, presumably reflecting the tissue of origin, since in human biliary epithelium pi class GST is the predominant GST. The variable expression of pi class GST which was observed in hepatocellular carcinoma may reflect transformation of hepatocytes damaged by toxins, since this GST can be induced after a chemical insult such as alcohol. As well as indicating the biochemical heterogeneity of hepatocellular carcinoma with respect to GST, this study indicates the need for further study of the nature of inherent drug resistance in these tumour types.

The glutathione S-transferases (GST) are a complex supergene family of enzymes identified in a wide variety of tissues. These enzymes are believed to be important in detoxification, conjugating lipophilic electrophiles including a variety of carcinogens with glutathione. They also bind various non-substrate ligands including bile acids and bilirubin.^{1,2}

The mammalian cytosolic GST isoenzymes are dimeric and can be divided into three principal gene families: alpha class, mu class, and pi class. In humans these families encode proteins with basic, near neutral, and acidic isoelectric points respectively. An additional membrane bound GST has been identified and named microsomal GST.^{3,4}

We recently reported the distribution of both cytosolic and microsomal GST in the normal human liver.⁵ Pi class GST is restricted to biliary epithelium whereas the other isoenzymes are found predominantly in hepatocytes. In many animal models of hepatocellular carcinoma there

is overexpression of pi class GST.^{6,7} This has led to the suggestion that pi class GST is a marker of preneoplasia and neoplastic transformation. However, biochemical studies on human hepatocellular carcinoma have not shown a uniform rise in pi class GST in all patients.

The purpose of the present study was to identify the distribution of GST isoenzymes in human hepatocellular carcinoma and compare it with cholangiocarcinoma and adjacent normal liver tissue where this was available.

Methods

Antisera to the cytosolic alpha, mu, and pi class enzymes and microsomal GST were raised in rabbits according to methods previously described.^{9,10} Specificity of the antisera from serum subsequently collected was determined by western blot analysis and radioimmunoassay.

Twelve cases of hepatocellular carcinoma and eight of cholangiocarcinoma were included in the present study. Wedge or needle biopsy specimens, fixed in formalin and embedded in paraffin, were obtained from archives in the Department of Pathology, University of Edinburgh. In four of the cases hepatocellular carcinoma adjacent to normal liver tissue was available for examination.

IMMUNOSTAINING

Tissue sections of 2 µm were treated with 1% (vol/vol) hydrogen peroxide in methanol for 20 minutes to block endogenous peroxidase activity. Sections were washed in Tris buffered saline (pH 7.6) and treated with 20% normal goat serum (diluted in Tris buffered saline pH 7.6) for 15 minutes before being incubated with primary antiserum to GST (30 minutes 1:200 dilution at 20°C). Normal rabbit serum (1:200) was used in place of the primary antibody for the negative controls. Incubation for 30 minutes with 1/200 biotinylated goat antirabbit antiserum (Dako, UK)¹¹ was followed by streptavidin-peroxidase conjugate for 15 minutes (Dako, UK). Visualisation of antibody binding was by treatment with diaminobenzidine solution (5 mg/10 ml Tris buffered saline 0.1 mol/l imidazole, 10 µl hydrogen peroxide) for 10 minutes. Sections were rinsed in tap water, counterstained with Meyer's haematoxylin, and mounted before being examined by light microscopy.

Results

NORMAL HEPATIC TISSUE

Consistent with our previous report⁵ alpha class GST stained strongly in hepatocytes in the normal hepatic tissue, obtained in four cases adjacent to the hepatomas, with only occasional

TABLE 1 GST expression in hepatocellular carcinoma

Patient No	alpha	pi	mu	microsomal
1	-	-	-	+
2	-	++	+	++
3	++	-	-	++
4	+/++	++	+	+
5	++	+	++	++
6	+	-	-	-
7	-	-	-	-
8	+	-	-	+
9	-	-	+/-	+/-
10	-	-	-	-
11	-	-	-	-
12	+	-	+/++	+

- = negative or trace, + = weakly positive, ++ = strongly positive.

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Figure 1: Staining for alpha class GST of hepatocellular carcinoma on left (strong but heterogeneous) and normal liver right (arrowed).

Figure 3: Staining for mu class GST in hepatocellular carcinoma showing weak positivity in all cells.

cells being stained in larger bile ducts. Pi class GST was present in biliary epithelium but not in normal hepatocytes. Mu class GST was present in all samples, moderately staining in three, and strongly staining in the other. Similarly, microsomal GST stained moderately in three and strongly in the remaining sample.

HEPATOCELLULAR CARCINOMA TISSUE (Table I)

Alpha class GST stained strongly in only three of the 12 samples (Fig 1), moderately in two samples, and showed patchy moderate staining in two further specimens and weak or negative in the remaining five. Pi class GST stained strongly in two specimens, moderately in one, and was weak or negative in the remaining nine (Fig 2). Mu class GST stained strongly in two, moderately in three (Fig 3) and was weak or negative in the remaining seven. Microsomal GST stained strongly in three specimens, moderately in four, and showed a trace or was negative in the remaining five.

CHOLANGIOCARCINOMA (Table II)

In all eight of the cholangiocarcinomas studied pi class GST was readily shown and usually stained uniformly (Fig 4). Although alpha class was present in all cases, it was generally weak or present in scattered cells (Fig 5). Microsomal GST was identified in four tumours and was absent in four. Mu class GST expression was weak, but was seen in most cases.

Discussion

We have shown that, whereas cholangiocarcinomas seem to retain a similar pattern of GST isoenzyme expression to that found in normal bile ducts, the expression of all the GST families in hepatocellular carcinomas is variable. In at least six cases of hepatocellular carcinoma there was a decrease in alpha class GST and in three cases malignant hepatocytes expressed pi class GST (unlike normal hepatocytes). The presence of pronounced pi class GST expression in only one quarter of cases is perhaps surprising in view of the frequent overexpression of this isoenzyme in many human tumours including lung,¹² kidney,¹³ and pancreas.¹⁴

In most animal models of hepatocarcinogenesis there is uniform de novo expression of pi class GST by neoplastic hepatocytes. Pi class GST therefore has been regarded as a marker of preneoplasia,¹⁵ and its overexpression is thought to be involved in the acquisition of drug resistance. However, this thesis can be criticised on several grounds. Firstly, nitrosamine induced preneoplastic nodules in rat liver do not overexpress pi class GST when rats are fed hepatic peroxisome proliferators such as clofibrate.¹⁶ Secondly, although aflatoxin B1 induced preneoplastic hepatocyte nodules in rats overexpress pi class GST, it is the induction of an alpha class GST which confers resistance against the cytotoxic effects of aflatoxin B1.¹⁷ Thirdly, hepatocytes in human liver biopsy specimens from patients with alcoholic liver disease express pi class GST.¹⁸ Alcoholic liver disease is not usually

Figure 2: Staining for pi class GST in hepatocellular carcinoma showing positive staining in bile ducts (arrowed) but negative staining of malignant liver cells on right.

Figure 4: Staining for pi class GST in cholangiocarcinoma showing strongly positive staining of epithelial cells in duct-like formations. Stromal cells are also stained.

Figure 5: Staining for alpha class GST in cholangiocarcinoma showing focal positivity in malignant duct-like structures.

regarded as 'preneoplastic,' although a proportion of patients with alcoholic cirrhosis subsequently develop hepatocellular carcinoma.¹⁹ The biological importance of pi class GST expression therefore remains uncertain.

In the rat pi class GST expression in hepatomas may reflect oncogene activation since it has recently been reported that the AP1 binding sites exist at the 5' end of the pi class GST gene.²¹ However, this consensus sequence does not seem to exist in the pi class human gene. Alternative explanations for occasional GST pi class expression in hepatocellular carcinoma may be dedifferentiation, since fetal hepatocytes have been shown to express pi class GST,²⁰ or that the expression of pi class GST by hepatocytes results from a stress response mechanism. GST pi class has been shown to be induced by interferon.²²

In 40% of the population from which this study was drawn mu class GST is not expressed in leucocytes,²³ reflecting the known polymorphism of this enzyme first described in Australia.²⁴ Patients who have the null polymorphism for this isoenzyme are more susceptible to lung cancer induced by cigarette smoke²⁵ and may be more likely to develop chronic liver disease.²⁶ The demonstration of mu class GST in seven of 12 hepatocellular carcinomas and four of eight cholangiocarcinomas does not suggest any protective effect of this enzyme, but assessment of the importance of this mu class GST polymorphism would require a much larger series. Although we have been able to phenotype subjects by immunohistochemistry,^{5,18} mu class enzyme expression is variable between subjects since many are heterozygous for the null polymorphism. Genotyping is therefore a more precise and accurate screening investigation.

The expression of GST isoenzymes in liver tissue adjacent to hepatocellular carcinomas

seems to be normal. There is no consistent pattern of expression within hepatomas, although in general the expression of all the GST isoenzymes is reduced. This finding is contrary to the results reported in animal models of hepatocellular carcinoma where pi class GST and other classes are reported to be increased. In cholangiocarcinoma retention of pi, the predominant biliary GST isoenzyme, occurs and may explain the high concentration of pi class GST identified in the bile of patients with cholangiocarcinoma.²⁷

Whether the prognosis of patients in whom GST expression is maintained in the hepatocellular carcinoma is improved compared with those in which GST expression is absent deserves further study.

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TABLE II GST expression in cholangiocarcinoma

Patient No	alpha	pi	mu	microsomal
1	-/+ H	++	-	-
2	++ H	++	+	+ H
3	F+	++	-	+
4	-/+ H	++	-	-
5	F+	++ H	-	-
6	F+	++	+	+
7	F+	+ H	NA	-
8	F+	++	+	+

- = absent or trace, + = weakly positive, ++ = strongly positive, H = heterogeneous, F = focal, NA = not available.

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