Review article

Class-Pi of glutathione S-transferases

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Abstract

Class-Pi of glutathione s-transferases (GST-Pi) is the specific form of GSTs that are known to participate particularly in the mechanisms of resistance to drugs and carcinogens. This class of the enzyme is referred to as class-P or class-Pi or class π. The accepted terminology in this review article is class-Pi. In this article following a brief description of identified molecular forms of GSTs, we focus on GST-Pi. We review new findings about the structure and regulation of GST-Pi gene. Then, the role of GST-Pi in liver damage, oxidative stress, carcinogenesis and drug resistance are discussed. Also, the presence of common genetic polymorphism, hypermethylation in GST-Pi gene and the consequences GST-Pi knock out is regarded.

Keywords: *Glutathione S-transferase Pi; Drug resistance; Carcinogenesis; Oxidative stress; Polymorphism*

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INTRODUCTION

The glutathione S-transferases (GST: E.C.2.5.1.18) are a multi-gene family of enzymes involved in drug biotransformation and xenobiotic metabolism and, in a few instances , activation of a wide variety of chemicals (Jakoby, 1978; Chasseaud, 1979). Since their discovery in the early 1970s, thousands of articles have been written on the structure, function, and toxicologic significance of GSTs and it is obviously beyond the scope of this article to provide a comprehensive review of the field. This review article will highlight the major features of GSTs, with a focus on Pi class of GSTs (GST-Pi) because this class of GST plays different roles particularly in carcinogenesis and drug resistance.

Structure and classification of GSTs: GSTs are soluble proteins with typical molecular masses of around 50 kDa, each composed of two polypeptide subunits. GSTs catalyze the transfer of the reduced glutathione (gamma-glutamyl-cysteinyl-glycine; GSH) to a substrate (R-X) containing a reactive electrophilic centre to form a polar S-glutathionylated reaction product (R-SG). Each soluble GST is a dimer of approximately 26 kDa subunits, typically forming a hydrophobic 50 kDa protein with an isoelectric point in the pH range of 4-5. The ability to form heterodimers greatly increases the diversity of the GSTs, but the functional significance of this mixing and matching of subunits has yet to be determined. Each GST subunit of the protein dimer contains an independent catalytic site composed of two components. The first is a binding site specific for GSH or a closely related homolog (the G site) formed from a conserved group of amino-acid residues in the amino-terminal domain of the polypeptide. The second component is a site that binds the hydrophobic substrate (the H site), which is much more structurally variable and is formed from residues in the carboxy-

terminal domain. Between the two domains is a short variable linker region of 5-10 residues. The GST proteins have evolved by gene duplication to perform a range of functional roles (Hayes *et al*., 2005). GSTs also have non-catalytic roles, binding flavonoid natural products in the cytosol prior to their deposition in the vacuole. Recent studies have also implicated GSTs as components of ultraviolet-inducible cell signaling pathways and as potential regulators of apoptosis (Dixon *et al*., 2002). Mammalian cytosolic GSTs are all dimeric with subunits of 199–244 amino acids in length. Based on amino acid sequence similarities, seven classes of cytosolic GST are recognized in mammalian species, designated Alpha, Mu, Pi, Sigma,Theta, Omega, and Zeta. In the case of pi and alpha GSTs, only subunits from the same class will dimerise. Within a class, however, the subunits can dimerize even if they are quite different in amino-acid sequence. Class Kappa GST is mammalian mitochondrial isoenzyme that are dimeric and comprise subunits of 226 amino acids. Mouse, rat, and human possess only a single Kappa GST. Other classes of cytosolic GST, namely Beta, Delta, Epsilon, Lambda, Phi, Tau, and the "U" class, have been identified in non-mammalian species. In rodents and humans, cytosolic GST isoenzymes within a class typically share >40% identity, and those between classes share <25% identity (Hayes *et al*., 2005).

By comparison with other major groups, such as mammals, plants and insects, relatively little is known about GSTs from fungi (Sheehan *et al*., 2001). Moreover GST expression in fungi may be related to mycotoxic production (Saxena *et al*., 1991; Allameh *et al*., 2002; Mohammadi *et al*., 2000).

Structure of GST-Pi protein: The GST-Pi class appears to contain only 1 to 2 distinct subunits in most species, and this is considerably less complicated than the mu and alpha class multi-familial (Sheehan *et al*., 2001; Eaton and Bammler, 1999). Pi-class GST are recognized by ethacrynic acid substrate specificity (Nuccetelli *et al*., 1998). The pi-class H subsite has been found to be comparatively open (Allardyce *et al*., 1999), perhaps explaining specificity towards the lesser hydrophobic substrates. The structural analysis indicated that the protein is comprised of 209 amino acid residues with the relative molecular mass of 23,224 KDa with no evidence of post-translational modifications. Comparison of the amino acid sequences between rat GST-Pi and human GST-Pi subunits suggests that they are the corresponding enzymes in these species. Human GST-Pi and rat GST-Pi both consist of 209 amino acids and differ in only 30 amino acids (85.6% homology). The difference in amino acid composition can explain the large difference in isoelectric point between rat enzyme subunit (pI 5.5) and human GST-Pi subunit (pI 6.9) (Kano *et al*., 1987).

Structure and regulation of GST-Pi gene

Structure and regulatory mechanism of human GST-Pi gene: The GST-Pi is gene mapped to a relatively small region of chromosome11q13. Investigators sequenced 4261 bp which include the entire GST-Pi gene as well as over 1200 bp of the 5´ and 200 bp of the 3´ flanking regions (Morrow *et al*., 1989). The GST-Pi gene has 7 exons and 6 introns contained within approximately 2.8 kb. Primer extension experiments identified four possible transcription start points closely spaced between 29 and 33 nucleotides 5´ to the start of translation. According to Morrow *et al*. (1989), the first 200 nucleotides 5´ to the start point of transcription contain a G+C rich region (79%). Additionally, an intriguing A+T-rich region was found between nucleotide (nt) positions –505 and –413 which contained 17 AAAAT tandem repeats. The GST-Pi amino acid coding regions is located between nt position +30 and 2724. The gene contains 211 codons (including start and stop codon) on seven exons. Each of the splice sites contain GT/AG splice junction which are located on the six introns. As a first step toward understanding the differential regulation of GST-Pi expression, Morrow *et al*. (1989) isolated the human genomic GST-Pi gene. They determined the possible start points of transcription and examined the sequence of the GST-Pi promoter region. Analysis of the GST-Pi promoter region revealed four putative transcription regulatory motifs. These sequences include a "TATA" box 29 bp upstream from the major transcription start point (nt position -29), 2 Sp1 recognition sequences (GGGCGG, nt positions -46 to –41 and –56 to –51), and an AP-1 (heterodimer of c-fos and c-jun) recognition sequences (nt TGACTCA, positions- 69 to –63).

The regulation of GST-Pi is interesting because their expression is significantly increased under various disease condition such as human cancer, resistant to chemotherapeutic agents in cell lines and during hepatocarcinogenesis in experimental animals. Functional AP-1 and SP-1 response elements have also been identified in the 5´ regulatory region of human GST-P1 gene (Moffat *et al*., 1994). Regulation of expression of hGST-P1 may also be influenced by the methylation status of a CpG island in the regulatory region of the gene (Jhaveri and Morrow, 1998). It has been suggested that GST-Pi gene expression may be regulated at the level of mRNA, although, the molecular mechanisms that regulate the increase GST-Pi

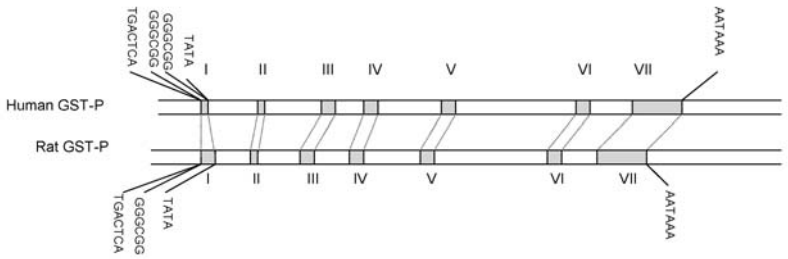

Fig 1. Organization of the human and rat GST-Pi genes (Adapted from Okuda *et al*., 1987).

mRNA expression are still not completely understood. One of the possible mechanisms to achieve over-expression of GST-Pi mRNA is through gene amplification (Shimizu *et al*., 1995). Changes to the level of CpG–site methylation appear to play a role in gene activation/suppression during carcinogenesis. In general, there is an inverse relationship between promoter CpG site methylation and the potential for transcription. Compton *et al.* (1999) also believe that GST-Pi mRNA expression is controlled at the transcriptional level. Another possible mechanism for transcriptional silencing is hypermethylation of CpG islands within promoter and 5´ regions of various genes (Laird and Jaenisch, 1996; Baylin *et al*., 1998). Hypermethylation of the GST-Pi gene in prostate cancer and adenocarcioma of the esophagus has been reported recently (Nakayama *et al*., 2004; Easds *et al*., 2001). Brabander *et al*. (2002) and others have found similar evidence of the presence of a wide spread oncogenic "field effect" in the normal esophagus of cancer patients in studies of gene expression and DNA methylation analysis (Lord *et al*., 2000, 2001; Easds *et al*., 2001; Brabander *et al*., 2001; Brabander *et al*., 2002; Easds *et al*., 2000a,b).

Structure and regulatory mechanism of GST-Pi Gene in rat: Following determination of complete sequence of human GST-Pi, genomic organization of the human gene has been compared to the sequence of the rat GST-Pi gene (Okuda *et al*., 1987). Organization of the human and rat GST-Pi genes have been presented in Figure 1. The sizes and relative positions of exons I-VII are shown. Motifs conserved in the promoter regions as well as the relative position of the polyadenylation signals of each gene are also indicated.

GST-Pi gene expression depends on at least two regulatory elements: a promoter and a far-upstream enhancer. GST-Pi enhancer I (GPEI) located at –2.5 kb, consists of imperfect TPA (12-O-tetradedacanolylphorbal-13-acetate) responsive element (TPE) like sequences that are palindromically oriented (Sakai *et al*.,1988). The AP-1 DNA- binding site, or TRE, contains the sequence 5´-TGACTA-3´ which shares some homology with the core consensus ARE (Antioxidant Responsive Element) sequence (5´-TGAC NNNG C-3) (Hayes and Polford., 1995). A striking feature of the ARE sequence is the presence of a conserved sequence GC immediately 3´ of the TRE–like sequence, suggesting that trans-acting factors other than AP-1 com-

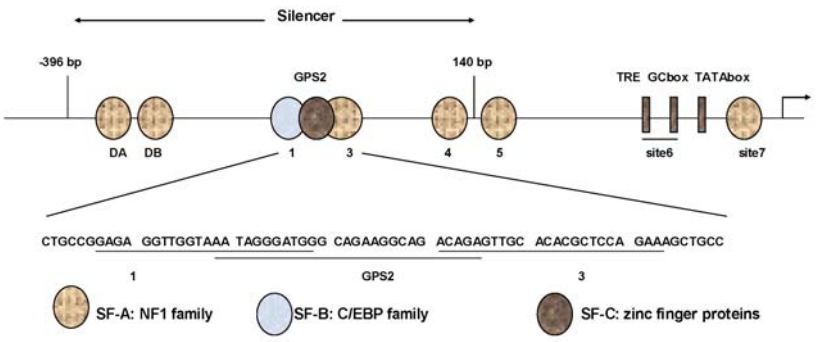

Figure 2. Schematic diagram of the promoter and silencer regions in the rat GST-Pi gene (Adapted from Imagawa *et al*., 2002).

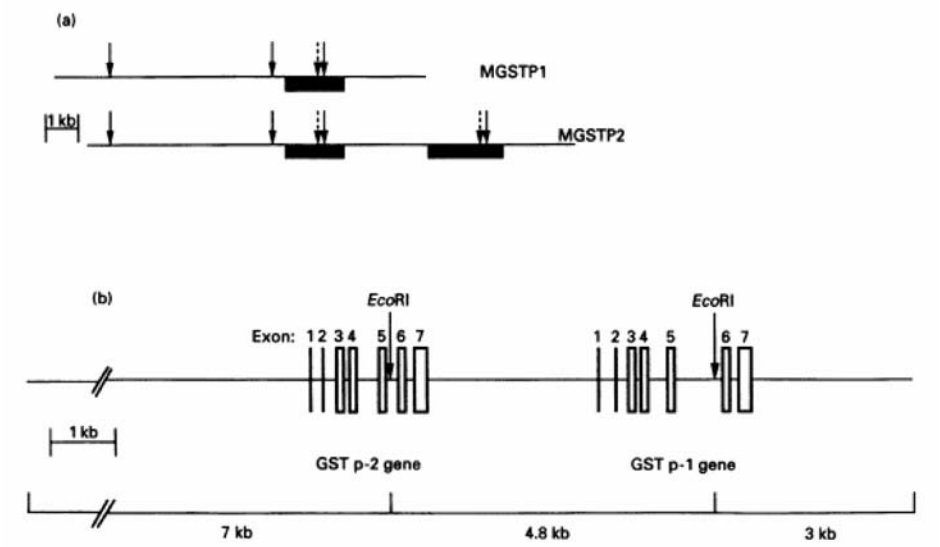

Figure 3. Organization of the murine GST-Pi locus (Adapted from Bammler *et al*., 1994).

[a] Restriction maps of the phage clones MGSTP1 and MGSTP2, containing one and two mouse Pi-class GST genes respectively. Solid and dashed arrows represent *HindIII and EcoRI* sites; solid box indicate regions complementary to the pobe MPCR2.2. [b]Restriction mapand intron/exon arrangement of the two mouse Pi-class GST genes, GST P1, and GST P2, are contained in clone MGSTP2 exons are shown as boxes numbered 1-7. Introns sizes were determined by DNA sequencing.

plex are involved in ARE-mediated regulation of gene expression (Hayes and Polford, 1995). The upstream half-site (5´-GTCAGTCA-3´) binds factors that are electrophoretically distinct from AP-1, whereas the downstream half site (5´-TGATTCAG-3´) can bind AP-1. Both of the half-sites of GPEI are required for AP-1-independent transcriptional activation (Diccianni *et al*., 1992).

In addition to GPEI, the regulatory region of the rat GST-Pi gene has another TRE-like sequence located 61 bp upstream from the cap site, which is designated as the Maf recognition element (MARE) (Sakai *et al*., 1995). Both Jun and Maf can bind to this element and activate the gene. The core sequence of GPEI, GPEII specific binding proteins expressed in HeLa and CBRH7919 cells may play an important role in the high transcriptional level of the rGSTPi gene in tumor cells (Liu *et al*., 2002). It is still controversial whether AP-1 complex specifically binds to GPEI for the dramatic induction of GST-Pi *in vivo*, because most of studies were carried out *in vitro* cultured cell system.

In the 5´–flanking region of the rat GST-Pi gene, the negative regulatory elements located around 400 bp upstream from the cap site are also significant, and these elements would aid the repressive mechanism of the GST-Pi gene in normal rat liver (Imagawa *et al*., 1991). One of the binding factors, a member of the CCAAT/enhancer binding protein (C/EBP) family, binds to GST-Pi silencer-1 (GPS-1) as silencer factor-B (SF-B). The ratio of C/EBP-alpha to C/EBP-beta is an important factor for GST-Pi silencer activity and a decrease in the ratio reduces the silencer activity and consequently increase the GST-Pi expression. Furthermore, Osada *et al*. (1997) demonstrated that the nuclear factor-1 (NF-1) family bound to several other silencer elements, closely located to GPS-1, and contributed to negative regulation of GST-Pi gene expression (Osada *et al*., 1997). This factor was identified as silencer factor-A (SF-A). Recently, zinc finger proteins as candidate silence factor, that bind to GST-Pi silencer 2 (GPS2), were isolated. These proteins include BTEB2, EZF, EKLF and TIEG1 and other factors containing multiple zinc factor motifs (TFIIIA and MZFP) (Imagawa *et al*., 2002). The promoter and silencer regions in the rat GST-Pi gene is shown in Figure 2.

The SF-C binding site as well as SF-A and SF-B are shown. GPS2, the SF-C binding site, partially overlaps with GPS1 and GPS3, which are bound by the C/EBP family and the NF-1 family, respectively. Some zinc finger proteins bind to GPS2.

Structure and regulation of GST-Pi gene in mice: The amino acid sequences deduced from the mouse Pi-class GST cDNA reported by Hatayama *et al*. (1990) and the rat Pi- class gene (Okuda *et al*., 1987) share 92% identity. Unlike, other species characterized to date, researchers found that the mouse have two functional GST-Pi genes (Bammler *et al*., 1994). Organization of the murine GST-Pi locus is shown in Figure 3. Both genes, GST-P-1 and GST-P2, were

sequenced completely shown to contain seven exons interrupted by six introns (Bammler *et al*., 1994). GST-P1 is about 2.7 Kb in length, whereas, GST-P2 is about 2.1 Kb long because of a smaller-sized intron 5 (Bammler *et al*., 1994). Mouse GST-P1 and GST-P2 gene Sequences are identical with rat GST-Pi and human GST- gene (Table 1).

Table 1. Sequence identities of the mouse GST-P1 gene with mouse GST-P2, rat GST-P and human GST-p genes (Adapted from Bammler *et al*., 1994).

	number of neucleotides(bp)			
	GST-P1	GSP-2	GST-P	GST-π
Exon 1*	1(100%)	1(100%)	1(100%)	1(100%)
Intron 1	242	226(80%)	209(69%)	288(38%)
Exon 2	36	36(86%)	36(94%)	36(86%)
Intron 2	188	212(75%)	185(60%)	293(36%)
Exon 3	107	107(99%)	107(92%)	107(78%)
Intron 3	98	98(100%)	101(83%)	114(48%)
Exon 4	88	88(98%)	88(94%)	88(81%)
Intron 4	312	309(94%)	329(75%)	365(40%)
Exon 5	104	104(93%)	104(88%)	104(85%)
Intron 5	761	168(14%)†	716(75%)	862(37%)
Exon 6	108	108(100%)	108(96%)	108(87%)
Intron 6	162	168(96%)	156(80%)	177(54%)
Exon 7*	186	186(100%)	186(95%)	186(95%)

* Only the number of coding nucleotides of exons are given.•
† The low identity is due to the different introns sizes of GST-P1 and GST-P2

The values given in parentheses represent identity comparison with the mouse GST-P1 sequence. The sequence for rat GST-P and human GST-p (GSTP1-1) were taken fom Okuda *et al.* (1987) and Marrow *et al.* (1989) respectively.

The nucleotide sequence at all splice junctions is consistent with the GT/AG role described by Breathnach and Chambon (1981) showing that the exonic segment within each gene transcript can be spliced accurately to form functional mRNA. The polyadenylation signal, AATAAA, occurs in the seventh exon of the two genes, which shows that both of gene transcripts can be processed to mature mRNA. A TATAA sequences and a possible CCAAT element can be found in the 5´ flanking regions of both genes (Bammler *et al*., 1994). According to Maniatis *et al.* (1987), these elements, which are present in most eukaryotic promoters, are associated with the accurate initiation and promotion of transcription. Furthermore, a GC box, which precisely matches the consensus sequence 5´-(G/T) GGGCGG(G/A)(G/A) (C/T)-3´ for the binding site of transcription factor SP1, forms part of the promoter of the two genes (Kadonaga and Tjian,1986). GST-P2 has an additional GC box, 5´-GGGGCGGCAT-3´, which differs from a perfect SP-1 binding site in only one nucleotide, and is missing in GST-P1. Immediately upstream of the GC boxes in both genes is the sequence 5´-TGAGTCAG-3´ which corresponds to the consensus sequence found in promoters of genes that are responsive to phorbal esters [e.g. phorbol 12-myristate 13-acetate (PMA; TPA)]. This motif is called a PMA-responsive element (PRE) (Bammler *et al*., 1994). Diccianni and co-workers (1992) have shown that a PRE is involved in the high level of transcription of GST-Pi observed during the rat hepatocarcinogenesis. In addition, the 5´ untranslated regions of both gene exhibit various substitutions and deletion. The overall differences between the 5´ non-coding regions of the two mouse genes suggest that they are regulated differently. The number of Pi-class GST genes in the mouse genome was estimated by Southern blot analysis. Four *Eco*R1 fragments of a mouse DNA, approximately 2.5 kb, 4.8 kb, 6.8 kb and 9 kb in size, hybridize to probe an approximately 2.2 kb PCR product. On the basis of the restriction map of clone MGST-P2, this suggests that there are at least three Pi-class GST-related sequences in the mouse genom. Clone MGST-P2 accommodates three of these hybridizing fragments, approximately 4.8 kb, 6.8 kb and 9 kb in length (Bammler *et al*., 1994). However, the approximately 2.5 kb band is not present in any of positive clones isolated (Bammler *et al*., 1994). This fragment requires further characterization to establish whether it is a pseudo gene or a further functional pi-class GST gene. Pi-class GST pseudo genes have been found in rat and human (Okuda *et al*., 1987; Board *et al*., 1992).

Tissue distribution of GST-Pi: In human, GST-Pi was first detected in placenta (Satoh *et al*., 1985) as the main isoform of GSTs. In normal placenta tissues, GST-Pi comparises of 36% of total GST activity (Steisslinger and Pfleiderer., 1988) and 67% (Zusterzeel *et al*., 1999) of the total GST concentration. Further investigations showed its presence in kidney, testis, lung, pancreas, small intestine, skin, erythrocyte and brain (Satoh *et al*., 1985; Sies *et al*.,1990). Analysis of GST activity shows distinct tissue specificity with high levels in placenta and low levels found in liver tissue (Kano *et al*., 1987; Moscow *et al*., 1989).

GST-Pi in the adult human liver is only expressed in the biliary epithelium, while the enzyme is expressed strongly in biliary epithelium including the gallbladder (Howie *et al*., 1989). Large quantities of the isoenzymes can be determined in human bile (Howie *et al*., 1989). Likewise, heterogeneity of GST isoenzyme distribution has been shown in mall bowel epithelium. GST-Pi is present in cell linings of both villie and crypts (Hayes *et al*., 1989). In the colon, the enzyme is present in the pancreas epithelium (Hayes *et*

al., 1990). GST-Pi has been identified in the centroacinar cells and ducts but neither in the exocrine acini nor in the islets of Langerhans (Hayes *et al*., 1990). In rats GST-Pi is the predominant form of GST in the small intestine but it is not expressed in adult liver except in the bile ducts, but is well expressed in fetal liver (Guthenberg *et al*., 1986; Hiley *et al*., 1988).

Since 1984, attention has focused on GST-Pi, identified as a good marker for rat hepatic preneoplastic and neoplastic lesions during chemical carcinogenesis. Molecular forms expressed in mouse liver differ considerably from those in rat liver. As described above, rat GST-Pi is not expressed in adult liver, but GST-Pi in mouse is a major form in adult male mouse liver and though present at significant levels as a minor form in adult female liver.

GST-Pi expression in liver damage: Homogenates of normal human liver obtained at necropsy contain extremely low concentration of Pi class GST (Howie *et al*., 1990) and immunohistochemical examination has shown that this GST is confined to the cells of the biliary epithelium (Batist *et al*., 1987; Strange *et al*., 1989). In alcoholic liver disease, however, sinusoidal macrophages, kupffer cells, and hepatocytes express GST-Pi (Harrison *et al*., 1990a, 1990b), Biliary epithelial cells secrete GST-Pi in bile possibility as a mechanism to transport potentially harmful toxins from the cell.

GST-Pi in malignant tissues: The higher frequencies of over-expression of GST-Pi have been reported in malignancies by several research groups, although their biological importance has not been well undrestood (Tsuchida and Sato, 1992; Dogru-abbasoglu *et al*., 2002; Schipper *et al*., 1996). Over-expression of GST-Pi in Esophagous Squamous Cell Carcinoma (ESCC) biopsies was found to be associated with increased total GST activity (Mohammadzadeh *et al*., 2003; Dogru-abbasoglu *et al*., 2002; Howie *et al*., 1990; Moorghen *et al*., 1991; Peters *et al*., 1993).

GST-Pi expression in malignant tissues as well as plasma in human colorectal and gastric cancers believed to be increase depending on the stages of tumor (Tsuchida *et al*., 1989; Dogru-abbasoglu *et al*., 2002). Elevated Serum GST-Pi levels in patients with malignancies is assumed to originate from tumor cells because elevated expression of GST-Pi in tumor tissue was indicated in tumor tissues by either RNA blot hybridization or immunohistochemical techniques (Dogru-abbasoglu *et al*., 2002; Niitsu *et al*., 1989; Fan *et al*., 1995).

Over expression of GST-Pi in human malignant tissues may play an important role in prognosis and resistance to chemotherapeutic agents (Howie *et al*., 1990; Kantor *et al*., 1997; Monden *et al*., 1997; Hengstler *et al*., 1998; Sutoh *et al*., 2000; De Bruin *et al*., 2000; Schipper *et al*., 1997).

Tsuchida *et al*. (1989) reported that GST-Pi content in esophageal tissues is increased about 6-fold over the levels found in normal mucosa, and that the content was significantly greater in highly differentiated mucosa. But no evidence for GST-Pi mRNA level being related to the degree of differentiation of individual esophageal cancers was observed. The RNA transcript levels of GST-Pi in esophageal cancerous tissues was found to be higher than that in normal tissues of 80% of patients and the mean GST-Pi mRNA value in tumor tissues was significantly elevated as compared to that in background mucosa (Ishioka *et al*., 1991). More recently, this fact was approved in iranian patients by measuring GST Pi mRNA using PCR-ELISA technique (Rasmi *et al*., 2006).

Peters *et al*. (1993) found that the ratio of mean GST-Pi levels in tumor to normal esophagus was 0.9, a value that is not apparently correlated with the data of Moscow *et al*. (1989) on GST-Pi expression at mRNA levels. Whilst, according to Ishioka and coworkers (1991), correlation between GST-Pi mRNA levels and clinical stages or histologic characteristic was not noticable. Niitsu *et al*. (1989) reported that increased serum GST-Pi levels were observed in 61.3%, 53.3% and 79.9% of patients with cancers of stomach, esophagus and colon respectively.

There are contradictory reports on GST-Pi expression in Barrette's esophagus and esophageal adenocarcinoma (ADC) tissues. According to Compton *et al*. (1999) GST-Pi mRNA expression decreases in metaplastic Barrett's and ADC tissues compared to normal sqaumous esophagus tissue, however, Ishioka *et al*. (1991) reported elevated GST-Pi mRNA expression in ADC of the esophagus. According to Brabander *et al*. (2002) GST-Pi mRNA expression levels offer more promise than GST-Pi protein levels as a biomarker for following-up disease progression in individuals with Barrett's esophagus. Studies by Peters *et al*. (1993) demonstrated that Barrett's metaplasia has significantly lower GST-Pi enzyme activity and content as compared to normal esophageal tissue. These data suggested that the difference is controlled at the level of GST-Pi transcription or mRNA abundance (Compton *et al*., 1999).

In case of leukemia, GST-Pi mRNA was markedly decreased in B-cell from most patients with chronic lymphoblastic leukemia (CLL), when compared to

normal B-cells (Marie *et al*., 1995). According to Stammler *et al.* (1995) the expression of GST-Pi in acute lymphoblastic leukemia (ALL) is independent of cell cycle.

GST-Pi and oxidative stress: As a consequence of aerobic growth, organisms are exposed to damaging reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (Veal *et al*., 2002). They also demonstrated for the first time that GSTs are involved in the cellular resistance to oxidative stress in a eukaryote and also suggest that GSTs play important role in the response of yeast to anti-mitotic drugs.

Generally, studies have correlated high GST levels with increased resistance to oxidative stress. The 1-cys peroxiredoxin, Prx VI, defends against cellular membrane damage by reducing phospholipid hydroperoxides to their respective alcohols. Reduction of these substrates results in oxidation of Cys-47 in Prx VI to sulfenic acid. It has been proposed that GSTPi physically interacts with Prx VI, a process that leads to recovery of peroxiredoxin enzyme activity through glutathionylation of the oxidized protein (Manevich *et al*., 2004).Through this process, GSTPi may indirectly combat oxidative stress by restoring the activity of oxidized Prx VI. Adler *et al*. (1999), demonstrated a novel function for GST-Pi as an inhibitor of the stress-induced jun N-terminal kinase (JNK). In the absence of GST-Pi, the constitutive activation of jun N-terminal kinase results in activation of the transcription factor c-jun, which is known to be involved in the regulation of many genes involved in both cytoprotection and apoptosis, and which contains AP-1 sites in their promoters (Henderson *et al*., 2000). GST-Pi inhibits JNK through ligand binding with the N-terminal fragment of the kinase (Gate *et al*., 2003). However, GST-Pi may play a role in the pulmonary defense against oxidative stress caused by various pollutants including Diesel exhaust particles (Koike *et al*., 2002).

Role of GST-Pi in chemical carcinogenesis: The covalent binding of electrophiles derived from carcinogen to macromolecules, especially DNA, has been considered as an initial step in chemical carcinogenesis. Such electrophilic compounds are known to be detoxified by enzymatic or in some cases, spontaneous conjugation with GSH (Coles and Ketterer, 1990; Chasseaud, 1979). Studies revealed the involvement of specific GST forms in the conjugation of particular carcinogens including GST-Pi (Shigeki and Kiyomi, 1992). In addition to the conjugation mechanism, GST-Pi also possess selenium-independent glutathione GSH peroxidase activity toward lipid or DNA hydroperoxides (Prohaska *et al*., 1977; Tan *et al*., 1986).

GST-Pi induction by xenobiotic compounds: Immunohistochemical staining revealed that very small GST-Pi positive foci or even single cells, appear 1 or 2 weeks following administration of single dose of carcinogen to rat (Sato, 1988) or in one model even within 48 h (Moore *et al*.,1987). The numbers of these cells increased with increasing doses of initiator e.g. diethylnitrosamine and are not induced by promoters of liver carcinogenesis such as phenobarbital (Moore *et al*., 1987). Thus, GST-Pi positive single cells are considered to be "initiated cells", indicating a clonal origin of GST-Pi positive foci and hepatomas. Unlike the majority of drug metabolizing enzymes, GST-Pi is not inducible by administration of a large variety of hepatocarcinogenic promoters or modulators such as 3-methyl cholanthrene, α-hexa-cholrocyclohexane, carbon tetrachloride, cyproterone acetate, phenobarbital, and polychlorinated biphenyls or even by hepatoacarcinogens such as diethyl nitrosamine, 2-acetylaminefluorene, 3´-methyl-4-dimethylaminbenzene, aflatoxin B1 (Fatemi *et al*., 2006; Sato *et al*., 1984; Ito *et al*., 1989).

However, GST-Pi prove to be slightly induced by the antioxidants butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Tatematsu *et al*., 1985, 1988) and by ethoxyquine in periportal areas (Thumavit *et al*., 1985; Manson *et al*., 1987; Allameh *et al*., 1988a, 1988b).

Recently, analysis of single GST-Pi positive cells suggested that a specific genetic change at the initiation step is not necessary for the establishment of GST-P positive cells and the formation of single GST-Pi positive cells may not depend on DNA replication, indicating no requirement of mutation (Grasl-Kraupp *et al*., 2002). Therefore, it is likely that GST-Pi gene expression is induced through the common actions of carcinogenic rather than a specific genetic change. Satoh *et al.* (2002) reported that the endoplasmic reticulum was severely damaged in the liver by diethynitrosamin (DEN) prior to formation of single GST-Pi positive cells suggesting that an epigenetic mechanism rather than genetic mechanism accounts for GST-Pi expression in hepatocytes.

Role of GST-Pi in multi drug resistance (MDR): Drug resistance to chemotherapeutic agents is a major obstacle in human cancer chemotherapy (Van Zanden *et al*., 2004). Many anticancer drugs as well as carcinogens have been considered to be detoxified by

conjugation with GSH (Tew, 1989; Arrick and Nathan, 1984; Waxman, 1990; Hayes and Wolf, 1990). Thus, several alkylating agents or their metabolites have been shown to be conjugated with GSH catalysed by cytosolic GST, and some agents also by microsomal GST.

Pharmacological studies have suggested that GST-Pi contributed to anticancer drug metabolism that attenuates cellular sensitivity to a drug (Maeda *et al*., 1993; Goto *et al*., 1999; Awasthi *et al*., 1996). Generally, two proteins have been reported to be capable of conferring MDR in human cancers, including the170 kDa P-glycoprotein and the 190 kDa MDR-associated protein (MRP), encoded by mdr-1 (Pu *et al*., 1996) and MRP genes (Narasaki *et al*., 1996), respectively. Both MDR-1 and MRP proteins belong to the ATP-binding cassette transporter superfamily and act as energy-dependent efflux pumps that decrease the intracellular accumulation of cytotoxic agents. Previous studies have shown that most of the naturally occruing anti-cancer drugs currently used, are excellent substrates for MDR-1 or MRP (Narasaki *et al*., 1996; Chin *et al*., 1993). Morrow *et al.* (2000) examined the effect of regulatable GST-Pi expression in MRP2-rich HepG2 cells on 4-nitroquinoline 1-oxide (4NQO)-induced cytotoxicity and genotoxicity. They concluded that MRP2-mediated efflux of the GSH conjugate of 4NQO and/or another toxic derivative of 4NQO is required to support GST-Pi associated protection against 4NQO toxicities in HepG2 cells. Morever, the ability of chemopreventive agents such as beta-lapachone, emodin, sanguinarine, capsaicin, trans-anethole and silymarin to decrease GSTPi gene expression mechanisms could to reducing the incidence of GSH-related drug resistance in human leukemia (Duvoix *et al*., 2004). An increase in GST-Pi activity was reported after acquisition of doxorubicin resistance by a human breast cancer cell line MCF7 (Batist *et al*., 1986). Since then, the relationship between drug resistance and the expression of GST has been studied extensively in many cancer cell lines. The results indicate a two-to five fold increase of activity mainly due to the Pi class in cell line resistant to alkylating agents, doxorubicin, or *cis*–platinum compared with the respective sensitive cell lines. GST-Pi are expressed in many cell lines resistant to structurally unrelated drugs is analogous to the expression of rat GST-Pi in hepatocarcinogenesis induced by genotoxic carcinogens (Satoh *et al*., 1989). However, transfection of MCF-7 cells with GST-Pi cDNA did not result in resistance to doxorubicin, irrespective of the expression of amounts of GST-Pi comparable to those in resistant cells (Moscow *et al*., 1989). MCF-7 cells transfected with cDNA encoding the B1 subunit in the Alpha class, again, did not demonstrate resistance to doxorubicin or chlorambucil indicating that GST-Pi expression may not be involved in the resistance, but that other mechanisms, including an increase in p-glycoprotein and GSH proxidase, may be responsible in this case (Fairchild *et al*., 1990). Human GST-Pi and human GST-A transfected into *S. crevisiae* (Black *et al*., 1990) resulted in a significant reduction in the cytotoxic effects of chlormobucil and doxorubicin, a member of the anthracycline family of antibiotic anticancer drugs.

Polymorphism in human GST-Pi: Polymorphism has been described in many genes in GST families. To date, most attention has focused on allelism in the mu, theta and pi families (Hayes and Strange, 2000; Rebbeck, 1997). Four allelic variants have been identified for the human GSTP1 gene: GSTP1*A (the most common allele), GSTP1 *B , GSTP1*C and GSTP1*D (Table 2, Ali-Osman *et al*., 1997; Watson *et al*., 1998). Polymorphisms in exon 5 (Ile 105 Val) of GST P1 *B and exon 6 (Ala 114 Val) of GSTP1*C were first reported by Board *et al*. (1989).

GSTP1 *A is the wild type variant. This variant has Ile amino acid at position 105 in exon 5. GSTP1 *B results from A_{313} G substitution at exon 5 that leads to a replacement of isoleucine (Ile) by valine (Val) at this position of the GSTPi protein. The affected codon is in

Table 2. Genetic variation in GST-Pi

	Nucleotide in gene at	
Allele	**Variable position(s)**	**Protein affected**
*GSTP1*A*	313A, 341C, 555C	Ile105, Ala114, Ser185
*GSTP1*B*	313G, 341C, 555T	Val105, Ala114, Ser185
*GSTP1*C*	313G, 341T, 555T	Val105, Val114, Ser185
*GSTP1*D*	313A, 341T	Ile105, Val114

electrophile binding site of GSTPi enzyme and the enzymes encoded by this affected codon have lower activity toward the universal GST substrate i.e 1-chloro-2,4-dinitrobenzene in *Escherichia coli* (Ali-Osman *et al*., 1997). In contrast, recent studies have showed that valine variants were more efficient toward different substrates than either homozygous Ile/Ile or heterozygous Ile/Val. Hu *et al.* (1997) showed that Val alleles were more efficient than heterozygous Ile/Val or homozygous Ile/Ile in the detoxification of the ultimate carcinogen of benzo(a)pyrene, the (+)–anti-enantiomer of benzo(a) pyrene, the(+)- anti-enantiomer of benzo(a) pyrene diol epoxide.

Zimniak *et al*. (1994) found that the GSTP1 *B enzyme containing 105 Val had higher rather than lower activity for ethacrynic acid and bromo-sulfophthalein conjugation, suggesting that the differences in activity reflect differences in binding of the electrophilic substrate to the hydrophobic binding site of GSTP1. Biochemical studies have demonstrated a lower thermal stability of GSTP1 Val-105 compared to GSTP1 Ile-105 (Zimniak *et al*., 1994; Johansson *et al*., 1998) and also lower conjugating activity in Val homozygote compared with Ile homozygote, with heterozygote displaying intermediate activity (Watson *et al*., 1998). Because of GST-Pi potential role in detoxifying carcinogenic compound, it is plausible that individuals with inferior variants may be at increased risk of cancer form exposure to chemicals detoxified by GSTP1 enzyme.

Some studies have shown an increased risk of prostate, oral, laryngeal, pharyngeal, bladder and esophageal (squamous-cell) cancers among individuals with the homozygous [Ile/Ile] (Cao *et al*., 2005; Harries *et al*., 1997; Morita *et al*., 1998; Morita *et al*., 1999). Vijayalakshmi *et al.* (2005) indicates that the GSTP1 Ile/Val genotype may decrease risk of prostate cancer in the South Indian population. Increased risk for oral cancer was observed in individuals who were homozygous for any combination of GSTP1 polymorphic alleles (Park *et al*., 2000). Yuan *et al*. (2003) suggested that gene mutation of GST-Pi exon5 is one of the potential vulnerable factors in leukemogenesis of the Chinese children. GSTP1 genotypes are important determinants of lung function in childhood (Carroll *et al*., 2005). Genotypes of combining GSTP1 mutant/GSTM1(-) or GSTP1 mutant/GSTT1(-) led to high risk of lung cancer (Chen *et al*., 2006). More rapid decline in lung function is seen in individuals with GSTP1 val(105)/val(105) (Carroll *et al*., 2005). However, other studies suggest that polymorphisms within GSTP1 do not play a major role in the development of bronchial asthma (Nickel *et al*., 2005; Oh *et al*., 2005). Some of the studies also showed no association between GSTP1 and the following cancers : lung, laryngeal, colorectal, prostate and esophageal (Agalliu *et al*., 2006; Saarikoski *et al*., 1998; Jourenkova-Mironova *et al*., 1999; Welfare *et al*., 1999; To-Figueras, 1999; Lin *et al*., 1998). These different results in various cancers may be explained by different profiles of exposure to carcinogen, although the conflicting results could be due to many reasons, such as study design, power of the study, bias, and confounding factors. Thus always larger studies will be needed to confirm these preliminary findings.

It has been shown that different polymorphisms influence the risk of malignancies and the outcome after chemotherapy. Individuals with Val at GST-Pi codon 105 may respond better to chemotherapy given for their primary cancer because of lower GST-Pi activity and increased chemotherapy-induced cytotoxicity in target tumor tissue. GSTPi polymorphisms are modifiers of response to chemotherapy in patients with metastatic colorectal cancer (Stoehlmacher *et al*., 2002) and those with multiple myeloma (Dasgupta *et al*., 2003).

It also influences risk of therapy-related acute myeloid leukemia in patients successfully treated for breast cancer, non-Hodgkin's lymphoma, ovarian cancer, and Hodgkin's disease (Allan *et al*., 2001). Indeed, codon 105 Val homozygote have a significantly better prognosis than codon 105 Ile homezygotes treated with cyclophosphamide and adriamycin (both GSTP1 substrates) for breast cancer (Sweeney *et al*., 2000).

It has been suggested that the polymorphic expression of GST-P1 did significantly influence the long-term remission rate after cyclophosphamide treatment of steroid-sensitive nephrotic syndrome in children (Vester *et al*., 2005). GST-P1 polymorphism seems to be related to enhanced susceptibility to further relapses.In HBV infection, inheritance of the GSTP1-Val(105) involves a host genetic factor that is relevant to disease progression (Mohamadzadeh *et al*., 2005).

A second polymorphism, a C $\longrightarrow$ T transition at nucleotide 341 results in a Ala 114 Val amino acid substitution. GSTP1*C has the same codon 313 as GSTP1*B, but also has T base in codon 341 that changes Ala to Val. The codon 341 change has been identified as a variant allele by itself, designed as GSTP1*D, B and C allele variants appear to be functional, as the catalytic efficiency (K_{cat} /K_m) of both variant enzymes for CDNB is about 3-4 fold lower than the wild type protein protein, and catalytic differences between these variants have been shown for the carcinogenic (+)-anti-benzo(a)pyrene diol epoxide

(BPDE), such that the variant enzyme(105 Val) exhibits a V_{max} 3-4 fold higher than the V_{max} for the common (105 Ile) form (Hu *et al*., 1997; Sundberg *et al*., 1998).

Lu *et al*. (2006) suggested that GSTP1 exon 6 variant genotypes may be associated with improved survival among patients with stage III and IV non-small cell lung carcinoma. Increased risk for oral cancer was observed in individuals who were homozygous for any combination of GSTP1 polymorphic alleles (i.e. *B, *C, and/or *D alleles in both Caucasians and African-Americans (Park *et al*., 2000).

The alterations in restriction end nuclease sites caused by the nucleotide transitions in the GSTP1 gene variants provide a simple, rapid, and specific technique for determining the GSTP1 gene variants in cells and tissues (Ali-Osman *et al*.,1997). The method is suitable for screening large number of specimens. Genotype analysis is best achieved by restriction mapping, single strand conformational analysis and nucleotide sequence determination. In the absence of variant specific antibodies, GSTP1 phenotype is best determined by RT-PCR of the polymorphic region, followed by restriction site mapping and/or sequencing of the resultant cDNA (Shen *et al*., 1995).

Hypermethylation in human GST-Pi gene: Transcriptional silencing by CpG-island hypermethylation now is believed to be an important mechanism of tumorigenesis. The expression of GST-Pi and the methylation status of the promoter area of GST-Pi were investigated in some cancers. Hypermethylation of *GSTP1* is found in the great majority of prostate cancers and in a considerable fraction of high-grade prostate intraepithelial neoplasias (HGPIN), a likely precursor (Nakayama *et al*., 2004). Therefore, *GSTP1* hypermethylation assays are being developed for prostate cancer detection. The great promise of these assays tends to obscure how strange *GSTP1* hypermethylation is in. In other cancers, the GSH transferase, GSTP1 is typically upregulated, often contributing to drug resistance. Moreover, the consistency with which this gene and a number of others are hypermethylated in prostate cancer is singular (Schulz and Hatina, 2006). Concurrent hypermethylation of multiple tumor-related genes including GST-Pi is detected frequently in gastric carcinoma and adjacent normal tissues (Leung *et al*., 2001). Also, loss of GST-Pi expression is clustered in a subset of gastric carcinomas with Epstein-Barr virus incorporation, and the methylation of the promoter of the GSTP1 gene is correlated with this loss of GST-Pi expression (Kim *et al*., 2005).

Consequences of GST-Pi knockout (KO): Mice lacking both GSTP1 and GSTP2 have been generated (Henderson *et al*., 1998). Under normal conditions, the double gene knockout on 129 MF1 or C57/BL6 backgrounds had no obvious phenotype. At a biochemical level, the mutant mice demonstrated a complete lack of transferase activity toward ethacrynic acid in the liver (Henderson *et al*., 1998). Although GSTP1-1 is quantitatively the principal transferase in male mouse liver, Western blotting technique failed to demonstrate compensatory increases in expression of hepatic GSTA1/2, GSTA3, and GSTM1 subunits in the double gene KO animals (Henderson *et al*., 1998). However, livers from GSTP1/P2-/- mice have been reported to contain a higher activator protein-1 activity than livers from GSTP1/P2+/+ mice (Elsby *et al*., 2003), a finding that is consistent with the hypothesis that class Pi GST inhibits JNK (Ruscoe *et al*., 2001; Adler *et al*., 1999). In a skin tumorigenesis regimen, GSTP1/P2-/- mice yield approximately threefold more papillomas using 7, 12-dimethylbenzanthracene as initiator and TPA as promoter (Henderson *et al*., 1998), demonstrating a role for GSTP1-1 in xenobiotic defense. Surprisingly, GSTP1/P2-/- mice are more resistant than wild-type mice to liver toxicity caused by the analgesic acetaminophen, and this is attributed to faster regeneration of hepatic GSH in the double gene KO animals (Henderson *et al*., 2000).

It was proposed that while Pi-class GST does not catalyze the conjugation of Acetaminophen with GSH, it contributes to oxidative stress by facilitating redox-cycling of the drug metabolite NAPQI, possibly through formation of labile *ipso* adducts with intracellular thiol groups (Henderson *et al*., 2000). It is postulated that the absence of Pi class GST lessens the ability of NAPQI to redox-cycle and thus deplete GSH.

CONCLUSION

Remarkable progress has been made in the late decade in defining the different roles of GST-Pi. The discovery of certain metabolites of andegenous molecules as substrates for GST-Pi suggest a possible physiological role of GST-Pi in protecting against chronic disease that arise from oxidative tissue damage.

The finding of over expression of GST-Pi in many cancer tissues as well as in drug resistant cell line, suggests that elevated GST-Pi expression may be of direct relevance not only to acquired resistance, but also in natural resistance. The relationship between hypermethylation and GST-Pi polymorphism with some cancers has provided a evidence that clarify the important

role of GST-Pi in carcinogenesis. However, the presence of a "natural knock-out experiment" in the human population, in the form of common homozygous deletion polymorphisms in GST-Pi provides unique opportunities to assess the importance of this enzyme in the human population. Further studies are clearly needed to obtain a better understanding for use of GST-Pi as marker of malignancies.

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