Chapter

STRUCTURE AND CATALYTIC PROPERTIES OF HUMAN GLUTATHIONE TRANSFERASE P1-1

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ABSTRACT

Glutathione transferases (EC 2.5.1.18, GSTs) catalyze the nucleophilic attack of glutathione (GSH) on the electrophilic centre of a number of electrophilic compounds helping to detoxify a diverse array of toxic xenobiotics including carcinogenic, and pharmacologically active compounds. In this review, detailed descriptions are given on the structure and catalytic properties of human glutathione transferase P1-1 (hGSTP1-1) an enzyme that ubiquitously expressed in human tissues and exhibits many biological functions and multiple roles. The detoxification properties of hGSTP1-1 have been a primary research focus for the last years. However, now it has become apparent that the noncatalytic functions of GSTP1-1 have expanded the biological roles of this enzyme in cell survival, cell death and stress signalling mechanism.

ABBREVIATIONS

ASK1	signal-regulating kinase 1
GSH,	glutathione;

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GST	glutathione transferase;
G-site	glutathione binding site;
H-site	hydrophobic binding site;
hGSTP1-1	human glutathione transferase P1-1;
JNK	c-jun N-terminal kinase;
TRAF2	tumor necrosis factor receptor-associated factor 2

1. INTRODUCTION

Glutathione transferases (EC 2.5.1.18, also known as glutathione S-transferases, GSTs) are multifunctional Phase II detoxification enzymes which protect cell from reactive endogenous or xenobiotic electrophile compounds [1-5].

GSTs catalyze the conjugation of GSH to the electrophilic center of these compounds thereby decreasing toxicity and increasing their solubility that help their excretion from the cell [6]. GSTs also exhibit more functions. For example, they are involved in several biosynthetic reactions, in GSH-dependent isomerization reactions, in reduction of toxic organic hydroperoxides, and in protection against oxidative stress [7-9]. In addition, GSTs are implicated in the intracellular transport and storage of hydrophobic molecules, such as haem, bilirubin, hormones, flavonoids, fatty acids and xenobiotics [8, 9].

GSTs are present in most aerobic organisms such as animals, plants, insects, parasites, yeast, fungi and bacteria [10-12] and can be divided into four families that include the soluble cytoplasmic GSTs, the microsomal bound GSTs, the mitochondrial and bacterial GSTs [13-15].

The soluble cytoplasmic family is widespread across all organisms and consists of a large number of enzymes that can be further divided into different classes. [16]. Human cytosolic GSTs can be grouped into seven classes on the basis of their amino acid sequence, which are: alpha, mu, pi, kappa, theta, omega and zeta [3]. Each class constitutes from different isoenzymes [17] that exhibit usually overlapping substrate specificities toward electrophilic compounds [1-4, 16, 18].

Human GSTP1-1 (hGSTP1-1), a member of the pi class, is ubiquitously expressed in human tissues and has attracted particular interest because of its multiple roles and functions. In the next sections of this chapter will be discussed the structural and catalytic properties of hGSTP1-1 and its roles in cell detoxification and stress signaling mechanism.

2. STRUCTURE AND FUNCTION OF HUMAN GSTP1-1 ISOENZYME

2.1. Catalytic and Structural Properties of Human GSTP1-1 Isoenzyme

hGSTP1-1 exhibits wide substrate specificity towards a range of electrophile substrates. The structure of these substrates are diverse and include important endogenous compounds, as well as drugs, xenobiotic chemicals and the products of the transformation of xenobiotics by other enzymes. Nucleophilic displacement of an alkyl or aryl halogen or a nitro-group is

catalysed effectively by hGSTP1-1 (Figure 1). Halogens or nitrogroups of these molecules are soft electrophiles and react readily with the GSH. Organic isothiocyanates, which are abundant in edible plants, also undergo conjugation with GSH by hGSTP1-1. Isothiocyanates, with benzyl-NCS and phenethyl-NCS appears to be the best substrates for hGSTP1-1 [19].

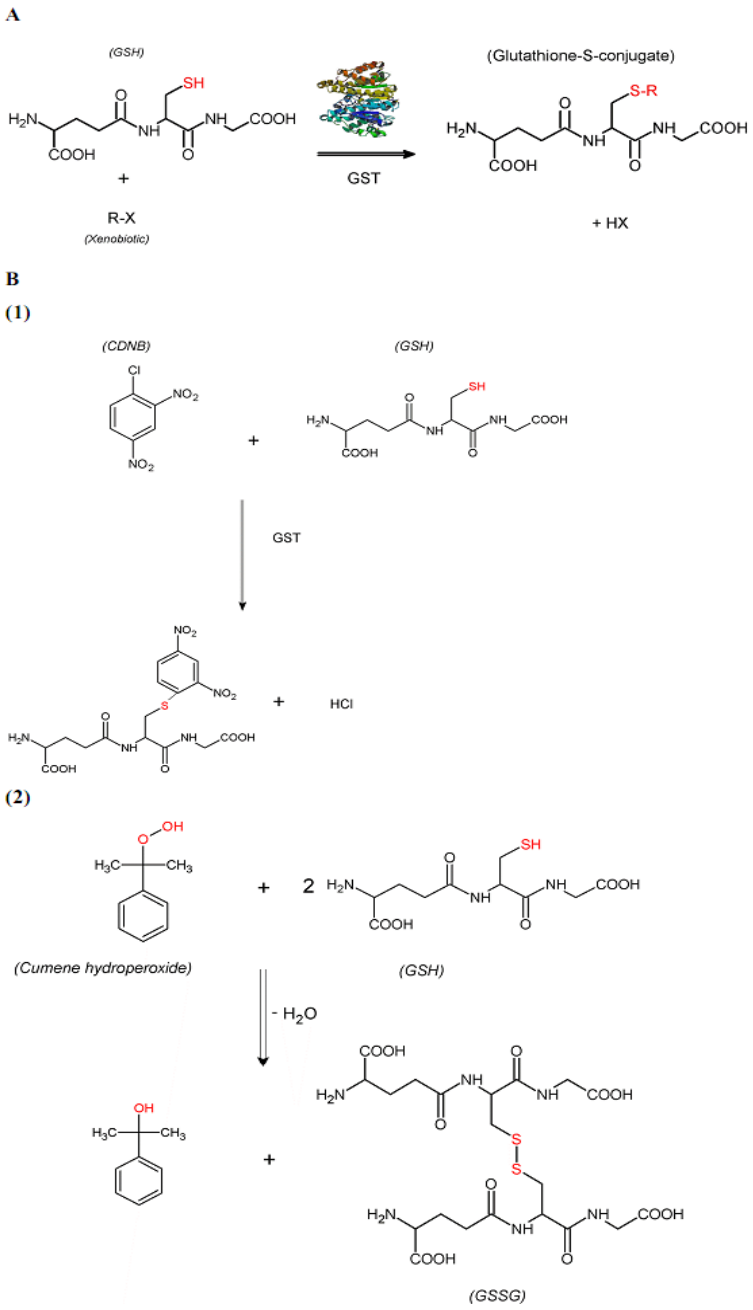

Figure 1. Glutathione conjugation to xenobiotic compounds catalyzed by hGSTP1-1. A: Glutathione conjugation to a generic xenobiotic (X) results in the formation of a glutathione-S-conjugate. B: Typical GST-catalyzed reactions. (1): nucleophilic aromatic substitution with 1-chloro-2,4-dinitrobenzene (CDNB), (2): hydroperoxide reduction with cumene hydroperoxide.

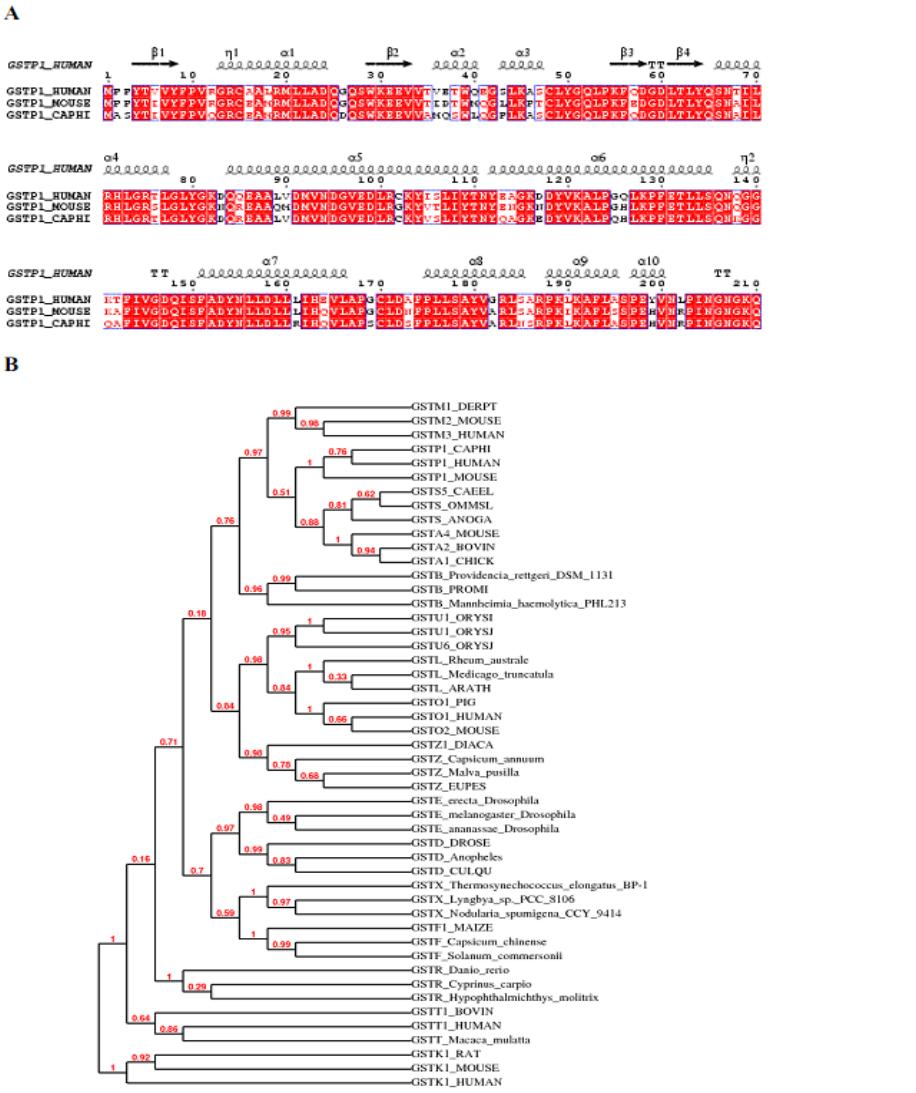

Figure 2. A: Sequence alignment of hGSTP1-1 with homologues from *Mus musculus* and *Capra hircus* produced using ESPript (http://espript.ibcp.fr/ESPript/ESPript/) [53]. Human GSTP1-1 numbering is shown above the alignment. Secondary structure elements of hGSTP1-1 are shown above the alignments. Alpha helices and beta strands are represented as helices and arrows, respectively, and beta turns are marked with TT. Conserved areas are shown shaded. A column is framed, if more than 70% of its residues are similar according to physico-chemical properties. The accession numbers of GSTP1-1 sequences that were used were: P09211, Q9TTY8, P19157. B: Phylogenetic analysis. The phylogenetic tree was created using representative members from all known GST classes (α, β, δ, ε, ζ, θ, κ, λ, μ, π, ρ, σ, τ, φ, χ, ω) and hGSTP1-1. The cladogram shows the groups which were formed after alignment of the protein sequences using ClustalW (Thompson et al. 1994) [54]. The accession numbers of GST sequences that were used were: alpha, (Q08392, O18879, P24472); beta, (P15214, D4C334, A7JQU5); delta, (B0W6B0, Q9GNE9, B4HHD9); epsilon, (B3NMR7, Q7KK90, B3MBB5); phi, (A5YWI8, Q5DUH0, P12653), kappa, (Q9DCM2, P24473, Q9Y2Q3); lambda, (B7FHT3, Q9LZ06, B5M1W3); mu, (P21266, P15626, P46419); omega, (Q9N1F5, P78417, Q8K2Q2); pi, (P09211, Q9TTY8, P19157); ro, (A7XZW2, Q0GZP3, Q1L907); sigma, (Q09596, P46088, P46428); theta, (E2RD21, P30711, Q2NL00); tau, (Q10CE7, A2XMN2, Q06398), chi, (A0ZF61, A0YYY7, Q8DMB4); and zeta, (P57108, Q84VH0, Q8H1P7, P28342).

Human GSTP1-1 also exhibits sulphonamidase activity, catalyzing the GSH-mediated hydrolysis of sulphonamide bonds. Such reactions are of interest as potential tumour-directed prodrug activation strategies (e.g. chimaeric sulphonamide derivatives of bombesin) [20].

In the cell, the formation of organic hydroperoxides is due to the attack of reactive oxygen species (ROS) on organic compounds. GSTs participate in oxidative stress defence mechanisms by catalyzing GSH-dependent reactions that inactivate such products by conjugation or reduction to the corresponding non-toxic alcohols (Figure 1B). For example, hGSTP1-1 is involved in the deactivation process of lipid peroxidation products such as oxidised DNA-bases, lipid hydroperoxides and their derivatives such as hydroxyalkenals, malondialdehydes and propenals [21, 22]. In addition, hGSTP1-1 can react directly with ROS via the modification of a reactive SH-group. This reaction leads to enzyme inactivation and disulphide bond formation that can be reversed by GSH.

The phylogeny of GST enzymes is complex. It is widely accepted that GST activity has emerged independently at least four different times throughout evolution, producing four different GST families - cytoplasmic, microsomal, mitochondrial, and bacterial [23]. The phylogenetic relationship of hGSTP1-1 with other GSTs from all known classes (α, β, δ, ε, ζ, θ, κ, λ, μ, π, ρ, σ, τ, φ, χ, ω) and a dendrogram generated by multiple amino acid sequence alignment is shown in Figure 2. Members of the classes mu, sigma and alpha form a large group, in which hGSTP1-1 is included. Their phylogeny probably reflects parallel evolutionary paths for these classes.

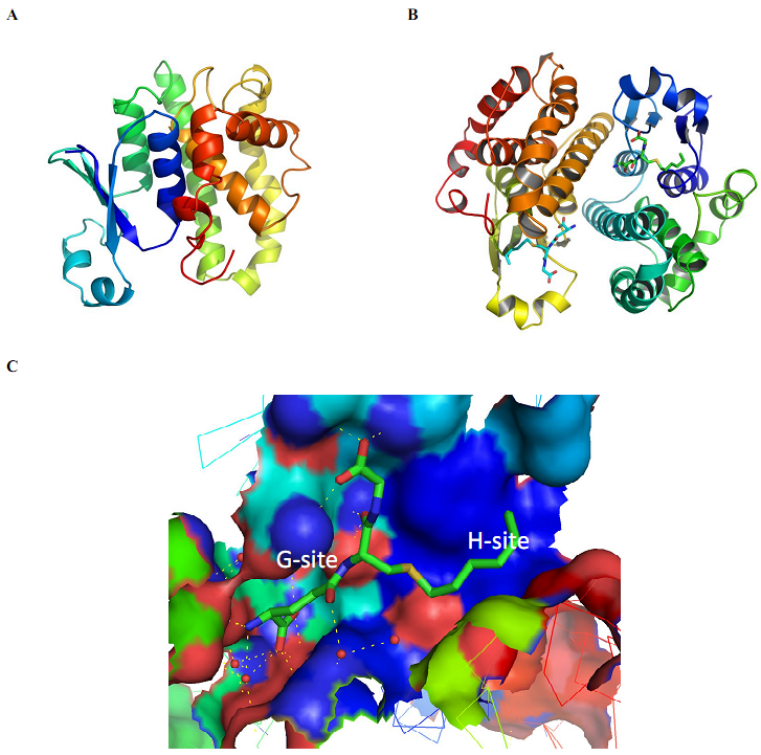

Figure 3. A cartoon representation of the hGSTP1-1 monomer (A), dimer (B) and the substrate binding site (C). The bound inhibitor S-hexyl-GSH is shown in B and C in a stick representation. The figures were produced using PyMol [55].

GSTP1-1 occurs as homodimer (Figure 3) with subunit molecular mass of 23,355.8 Da and a theoretical pI of 5.43. Each subunit of GSTP1-1 is composed of 210 amino acid residues and has one active site. The active site is suggested to consist of a GSH binding region (the G-site) and a nonspecific hydrophobic region (the H-site) that accommodates the electrophilic substrates. The G-site exhibits high specificity for GSH and includes the active site residue (Tyr8) that interacts with and activates the sulfhydryl group of GSH to generate the catalytically active thiolate anion. The H-site is not a conserved pocket and many electrophilic compounds are able to bind to it in a non-specific binding mode.

Each monomer of hGSTP1-1 constitutes two distinct domains; a smaller thioredoxin-like N-terminal domain and a larger helical C-terminal domain. It is known that all soluble GSTs have very similar polypeptide folds; however, each class exhibits unique features, particularly at the C-terminus [24]. The N-terminal small domain is an α/β structure with the folding topology βαβαββα. At the end of helix α3 begins a short linker that connects the N- and C-terminal domains. The C-terminal domain is made exclusively of helices. The active site of hGSTP1-1 appears as a large and open cavity. The G-site is located in a polar region of the N-terminal domain, formed by the beginning of helices H1, H2 and H3.

Coulombic surface analysis (Figure 4) indicates that the G-site in hGSTP1-1 exhibits positive electrostatic potential. This positive electrostatic potential of the G-site may contribute to high affinity GSH binding and –SH ionisation. The involvement of positively charged residues in the electrostatic field regulation has also been observed in other GSTs [25]. The H-site of hGSTP1-1 is formed by residues from the C-terminal domain and is hydrophobic in nature. Positive charged residues make the approach to the H-site basic. These residues form a positively charged region at the H-site, which presumably enable the enzyme to bind negatively charged substrates.

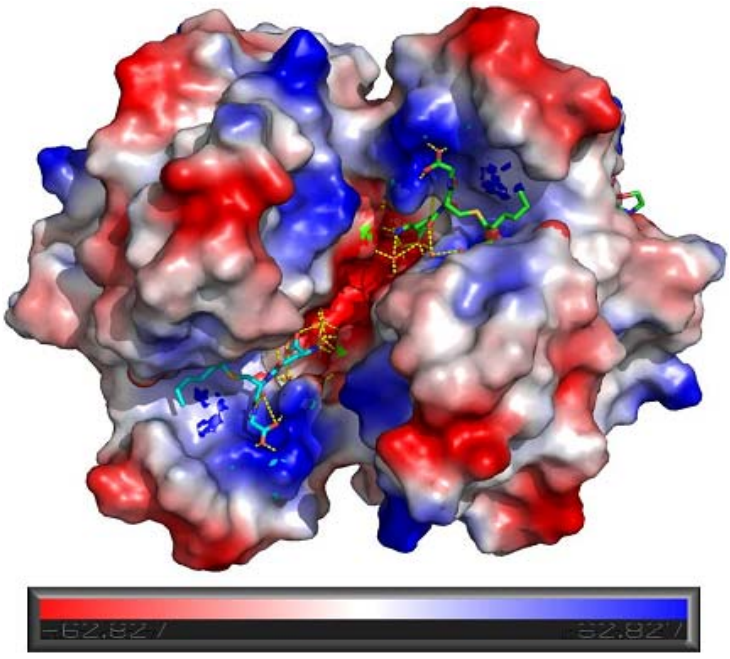

Figure 4. Coulombic surface analysis of hGSTP1-1. The bound inhibitor S-hexyl-glutathione is shown in a stick representation. The analysis was carried out using PyMol [56].

Helix H2 (Figure 2A), at the N-terminal domain, exhibits high flexibility in hGSTP1-1 and influences the catalytic activity of the enzyme. It is not clear how the mobility of this helix influences the rate of catalysis, although it is well established that the flexibility of helix H2 modulates GSH binding and product release. A plot of the crystallographic B-factors along the polypeptide chain can give an indication of the relative flexibility of the protein portions [26-28]. As shown in Figure 5, hGSTP1-1 displays a well defined flexibility pattern. Several important regions which including the helix H2 and helix H6 (Figure 2A and Figure 5) undergo large conformational changes. Comparison of the plots obtainded in the free, GSH-hGSTP1-1 and S-hexyl-GSH- hGSTP1-1 complexes it is evident that the flexibility of helix H6 is restricted in the GSH-hGSTP1-1 and S-hexyl-GSH-hGSTP1-1 complexes, indicating an induced fit mode of substrate binding and catalysis. An enzyme self-preservation mechanism of hGSTP1-1 was associated with an intersubunit communication [29].

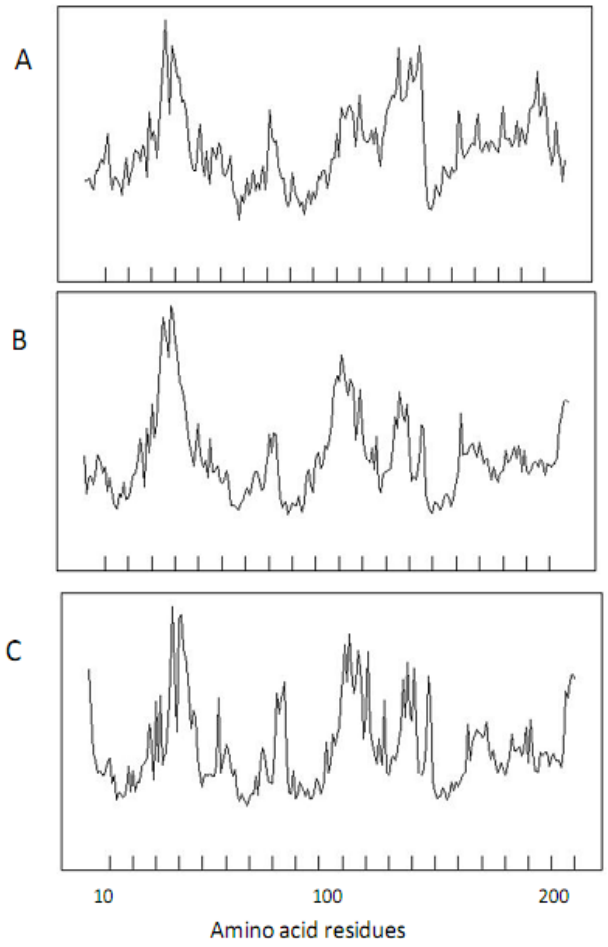

Figure 5. The flexibility of hGSTP1-1. Plot of the crystallographic B-factors along the polypeptide chain obtained from the crystal structure of free hGSTP1-1 (A); hGSTP1-1 in complex with GSH (B); hGSTP1-1 in complex with S-hexyl-GSH (C). The plots were produced using the WHAT IF software package (Vriend 1990) [56]. The height at each residue position indicates the average B-factor of all atoms in the residue.

Recent investigation has indicated that when a subunit is inactivated by a chemical or a physical factor, the second subunit triggers a different conformation to protect itself. Molecular Dynamics simulations suggested that the helix H2 has an important role in this phenomenon [29].

Analyses of crystal structures of hGSTP1-1 [30-32] have provided insights into the major interactions between subunits in the homodimers. Their subunit interfaces have three types of interactions: polar contacts, hydrogen bonds as well as hydrophobic interactions including a lock-and-key motif. In the lock-and-key motif an aromatic residue from N-terminal domain I in one subunit is wedged into a hydrophobic pocket formed by helices H4 and H5 in C-terminal domain of the other subunit. The lock-and-key motif is a common feature of pi, mu, and alpha class GSTs and the key residue is either phenylalanine or tyrosine (Tyr50 in hGSTP1-1). To investigate the importance of the key residue for dimerization and stability in hGSTP1-1, mutagenesis has been used [33]. Tyr50 in hGSTP1-1 plays both a structural role as the key residue and a functional role as part of the flexible helix H2. Hegazy et al., (2004) [34] reported that the mutant Tyr50Ala exhibits decreased k_{cat} value about 1,300-fold in comparison with the wild-type enzyme.

2.2. Functional Roles of Human GSTP1-1 Isoenzyme

hGSTP1-1 exhibits many cellular functions, therefore has multiple roles in cell survival [35]. hGSTP1-1 is the GST isoenzyme which has the broadest distribution among tissues [6, 35]. Although hGSTP1 is classified as a cytosolic GST, it has been demonstrated that it can be associated with the plasma membrane of the small cell lung cancer cell lines such as H69 and H69AR and human embryonic kidney (HEK) 293 and MCF-7 cell lines. Ali-Osman and his co-workers in 1997 isolated three human Pi gene variants from normal cells and malignant gliomas and designated them as hGSTP1*A, hGSTP1*B and hGSTP1*C. These allelic variants differ at either a single or at two codon positions [Ile104 (GSTP1*A), Val104 (GSTP1*B), Val104/Val113 (GSTP1*C)] [36]. It has been found that polymorphisms of GSTs genes are associated with patient response to chemotherapy [30, 37]. For example the alloenzyme GSTP1*B has been found to correlate with longer survival of patients with colorectal cancer who had a mixed chemotherapy [38].

An essential role of hGSTP1-1 is the regulation of stress signaling pathways [39] and the glutathionylation of cellular proteins [40]. Through protein-protein interactions, hGSTP1-1 can sequester c-jun N-terminal kinase (JNK), a key enzyme in the apoptotic cascade [41], and act as a negative regulator of this stress kinase. Furthermore, hGSTP1-1 is involved in the forward S-glutathionylation reaction, a post-translational modification that regulates the function/activity of a number of proteins. In addition it is able to form complexes with other proteins that participate in redox regulation [24].

GSTP1-1 has also been reported to associate with tumor necrosis factor receptor-associated factor 2 (TRAF2) and inhibit TRAF2-induced activation of both JNK and p38-MAP kinase [42]. GSTP1-1 inhibited TRAF2-enhanced apoptosis signal-regulating kinase 1 (ASK1) autophosphorylation and TRAF2-ASK1-induced cell apoptosis.

hGSTP1-1 has also protection role mainly against the cytotoxic effects of some alkylating agents, and their metabolites [30]. In contrast with its protective role, overexpression of hGSTP1-1 in a variety of malignancies is associated with a poor prognosis due to failure of

chemotherapy. Human GSTP1-1 is frequently overexpressed in various tumors and is the predominant GST isozyme in a wide range of sensitive and resistant cancer cells [43]. In particular, the enzyme is overexpressed in several different human cancer such as lung [32], colon [44], stomach [33, 45], kidney [46] ovary [29], mouth [47], testis [48]. In addition has been observed overexpression of hGSTP1-1 in patients with glioma [49], while negative regulation of hGSTP1-1 expression has been found in breast lesions and prostate cancer [50]. Human GSTP1-1 overexpression has been linked to multidrug resistance to chemotherapeutic agents including cisplatin, adriamycin, etoposide, thiotepa, chlorambucil, and ethacrynic acid. This can be contributed to hGSTP1-1 conjugation ability but also to the "nonenzymatic" antiapoptotic activity of hGSTP1-1 through its interaction with JNK [41]. Oxidative stress and treatment with GSTP1-1 inhibitors induce GSTP1-1 oligomerization and release of stress-activated kinases, defying apoptosis [39, 51]. Another inhibitor of GSTP1-1, the 15-deoxy-$^{\Delta 12,14}$-prostaglandin J_2 cause an impairment of GSTP1-1 protective effect on cell survival [52] through irreversible oligomerization specifically involving Cys101 [35]. Other agents that cause reversible oligomerization in different patterns are ethacrynic acid, 4-hydroxy-2-nonenal, H_2O_2 and diamide [39].

CONCLUSION

The human glutathione transferase pi gene is a polymorphic gene encoding active, functionally different GSTP1-1 variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases. Human GSTP1-1 is capable of catalyzing several different reactions and substrates and exhibit wide substrate specificity. Structural analysis showed that hGSTP1-1 shares the same overall fold and domain organization of other cytosolic GSTs. Human GSTP1-1 can modulate cell-signalling pathways that control cancer cell survival, cell death by direct protein-protein interactions and stress signalling mechanism.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Education, Lifelong Learning and Religious Affairs for the financial assistance provided. This work was partly supported by the grant programs HERACLITUS II and THALES, co-funded by the European Social Fund and National Resources. The grants HERACLITUS II and THALIS.-"Gluathione transferases: multifunctional molecular tools in red and green biotechnology" fall under the Operational Programme "Education and Lifelong Learning".

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