Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance

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The human glutathione S-transferase, GSTs, possess both enzymatic and non-enzymatic functions and are involved in many important cellular processes, such as, phase II metabolism, stress response, cell proliferation, apoptosis, oncogenesis, tumor progression and drug resistance. The non-enzymatic functions of GSTs involve their interactions with cellular proteins, such as, JNK, TRAF, ASK, PKC, and TGM2, during which, either the interacting protein partner undergoes functional alteration or the GST protein itself is post-translationally modified and/or functionally altered. The majority of GST genes harbor polymorphisms that influence their transcription and/or function of their encoded proteins. This overview focuses on recent insights into the biology and pharmacogenetics of GSTs as a determinant of cancer drug resistance and response of cancer patients to therapy.

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Introduction

Human GSTs are categorized into cytosolic/nuclear, mitochondrial, and microsomal [1]. Seven classes of cytosolic GSTs have been identified, namely, alpha, mu, pi, sigma, omega, theta, and zeta, based on their sequence similarities, substrate specificity, and immuno-reactivity. Microsomal GSTs, are also designated membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEGs). Overexpression of one of the MAPEGs, MGST1, has been associated with resistance to chloroambucil, melphalan, and cisplatin [2]. The only confirmed mitochondrial GST in humans is GST-kappa, which is also present in peroxisomes [3,4]. Evidence to date indicates that nuclear GSTs are cytosolic GSTs that have translocated, by, as yet unkown mechanisms, to the nucleus. GSTs play a central role in phase II metabolism, in which they catalyze the conjugation of various substrates, with electrophilic moieties to glutathione (GSH; γ-L-glutamyl-L-cysteinyl glycine). The resulting more water-soluble conjugates are excreted via the MRP efflux pumps or undergo further metabolism to mercapturic acids [5,6]. Substrates of GSTs include a wide range of endogenous metabolites, xenobiotics and alkylating and free radical generating anti-cancer drugs. Some GSTs also function as glutathione peroxidases and/or cis–trans isomerases [7]. In addition to their enzymatic and related function, GSTs also possess non-enzymatic functions, in which they regulate a number of cellular processes that contribute to the intrinsic ability of cells to survive genotoxic, metabolic and oxidative stress. This overview summarizes some of the recent findings, with appropriate reference to earlier findings, on the structure and function of this important group of cellular proteins as they relate to tumor response to therapy and/or drug resistance.

GST polymorphisms and tumor drug resistance

Most human GSTs harbor polymorphisms, primarily, single nucleotide polymorphisms (SNPs) and less frequently, deletions (Table 1) and the relationship between these polymorphisms and clinical outcome in cancer therapy is, currently a major area of research focus, with the main focus being directed at GST classes alpha, mu, pi and theta. This section summarizes some of the more recent advances in this area.

GST-alpha

The alpha class GSTs, GSTA1-A5, are encoded by genes clustered within chromosome 6p12 (Table 1) and possess overlapping substrate specificities [6]. GSTA1, GSTA2 and GSTA3 are widely expressed in human tissues, predominantly, the liver [6]. By contrast, GSTA4 is rarely expressed and GSTA5 protein is normally undetectable.

The two human GSTA1 alleles, GSTA1*A and GSTA1*B, result from SNPs in the GSTA1 promoter region that result, under normal conditions, in higher transcriptional activity of the GSTA1*A gene [6]. GSTA*1 also catalyzes the glutathionylation of anti-cancer agents, notably, the nitrogen mustard analogues, chloroambucil, melphalan and thiotepa, more effectively than GSTA1*B [8,9]. Consistent with this, in a recent study, patients with the GSTA1*A/*A genotype had a significantly higher rate of elimination of busulfan than those with the heterozygous genotype [10]. GSTA1 transfection has also been shown to render small cell lung cancer cells more resistant to doxorubicin-induced apoptosis [11]. In breast cancer, patients with the homozygous

Table 1

Chromosomal location, substrate specificities and genetic polymorphisms of cytosolic GSTs

Class	Chromosome	Subunits	Substrates	Alleles	Nucleotide/codon change
Alpha	6p12	GSTA1	BCNU, brostallicin busulfan, melphalan, thiotepa, chloroambucil nitrogen mustard	GSTA1*A	Promoter: -567T, -69C, -52G
				GSTA1*B	Promoter: -567G, -69T, -52A
		GSTA2*		GSTA2*A	P110, S112, K196, E210
				GSTA2*B	P110, S112, K196A210
				GSTA2*C	P110, T112, K196, E210
				GSTA2*D	P110, S112, N196, A210
				GSTA2*E	S110, S112, K196, E210
		GSTA3		None*	
		GSTA4	Thiopurines	None	
		GSTA5		None	
Mu	1q13	GSTM1	BCNU, brostallicin ethacrynic acid thiopurines	GSTM1*0	Deletion
				GSTM1*A	K173
				GSTM1*B	N173
				GSTM1*1x2	Duplicate
		GSTM2		None	
		GSTM3	BCNU	GSTM3*A	Reference intron 6
				GSTM3*B	Three base deletion in intron 6
		GSTM4		GSTM4*A	Y2517
				GSTM4*B	C2517
		GSTM5		None	
Pi	11q13	GSTP1	Brostallicin, cisplatin, chloroambucil, doxorubicin, ethacrynic acid, cyclophosphamide, ifosphamide, thiotepa	GSTP1*A	Ile105, Ala114
				GSTP1*B	Val105, Ala114
				GSTP1*C	Val105, Val114
				GSTP1*D	Ile105, Val114
Theta	22q11	GSTT1	BCNU	GSTT1*0	Deletion
				GSTT1*A	T104
				GSTT1*B	P104
			GSTT2	GSTT2*A	M139
				GSTT2*B	I139

Only substrates that are known anti-cancer or related drugs are listed.
* None reported to date.

GSTA1*B/1*B genotype had a better five-year survival than those with other more active GSTA1 genotypes [12].

The GSTA2 locus contains five GSTA2 allelic variants, GSTA2*A-E [13] (Table 1). GSTA2*A-D proteins are catalytically more active than GSTA2*E [14]. The effect of this differential enzymatic activity on drug resistance and/or patient survival, however, remains to be determined. To date, no polymorphism has been reported in the other GSTA subunits, namely, GSTA3-5. The functional role of GSTA4 remains unknown.

GSTM (GSTμ)

The genes of the Mu class of GSTs, GSTM1-M5, (Table 1) are located on chromosome 1p13 [15]. GSTM1 has four functional alleles, GSTM1*A-B, a null (deleted) allele, GSTM1*0 and GSTM1*1x2 [16]. GSTM1*A contains a lysine and GSTM1*B an asparagine at codon 173 [17] and, although, this does not change enzymatic activity [17], the effect on other properties of the proteins cannot be excluded. A unique GSTM1 variant dGSTM1*1x2, containing a duplicated GSTM1 gene was identified among a Saudi Arabian population [18,19].

Increasing evidence supports a relationship between GSTM1 polymorphism and treatment outcome. In pediatric acute lymphoblastic leukemia (ALL) [20] and in ovarian cancer [21], the GSTM1*0 genotype was associated with a longer disease-free survival and a higher response to chemotherapy, than GSTM1*A and GSTM1*B [20]. The mechanisms underlying the more favorable outcome associated with GSTM1*0 are not fully understood. It is likely, however, that GSTM1*0 patients respond better, in part, because of their inability to metabolize and inactivate anticancer agents. It should be noted, however, that, in a recent study in lung cancer, the GSTM1*0 genotype was associated with shorter patient survival rates [22].

To date, no polymorphisms have been reported for the GSTM2 gene. The GSTM3 locus harbors two alleles, GSTM3*A and GSTM3*B, which differ in three base deletions in intron 6 that generate a YY1 transcription factor binding site and result in differential expression of the two gene variants [23]. Compared to GSTM1 and GSTM2 [24,25], GSTM3 is more active in metabolizing the clinically active chloroethylnitrosourea anticancer agent, BCNU and, consistent with that, GSTM1 was shown to have no effect on BCNU resistance of human gliomas and lymphocytes [26,27]. In another study, in colorectal cancer, less advanced stage tumors and longer disease-free survival were associated with the GSTM3*A than the GSTM3*B genotype [28]. Based on the current available data, the effect GSTM polymorphisms on cancer susceptibility and treatment outcome is likely to be both tumor type and agent specific.

GSTP (GSTπ)

The GST pi class, the most highly expressed in human cancer, is encoded by a single gene, mapped to chromosome 11q13 (Table 1) [29,30]. The GSTP1 protein catalyzes the glutathione-conjugation of several anti-cancer agents [31–33]. Consistent with this, in many solid tumors and leukemias, high tumor GSTP1 expression has been associated with drug resistance, failure of therapy and poor patient survival [34,35]. In gliomas, the nuclear localization of GSTP1 complements its high expression as a determinant of poor survival [36].

The GSTP1 locus is polymorphic with four different alleles, GSTP1*A, GSTP1*B, GSTP1*C and GSTP1*D, arising from nucleotide transitions that change codons 105 from Ile to Val and codon 114 from Ala to Val [37–39]. Increasing evidence demonstrates that the different GSTP1 allelic proteins differ significantly in their ability to metabolize anti-cancer agents [32,40,41]. For example, thiotepa and chloroambucil are preferentially metabolized by GSTP1*A [40,41]. Consistent with this, an earlier report showed GSTP*1C to be more protective against cisplatin and carboplatin than the other two GSTP1 variants [32]. In pediatric astrocytomas, the GSTP1*C has been shown to be involved and to be associated with an increase in microsatellite instability [42].

Because of the differential drug metabolizing properties of the different GSTP1 allelic proteins, GSTP1 pharmacogenetics is receiving increasing attention for its role in outcome of cancer chemotherapy. Thus, in acute and chronic myeloid leukemias, glioma, multiple myeloma, Hodgkin's lymphoma, and cancers of the bladder, colorectum, esophagus, stomach, testicles and many other cancers, patients with the Val105 polymorphism, present in GSTP1*B and GSTP1*C, had a better response and survived longer than those without this allele [41,43–46]. The Val105 alleles also protect against therapy-associated toxicities, such as, cisplatin-induced hearing impairment [44]. Paradoxically, the Val105-containing GSTP1 variants have been associated with chemotherapy-induced AML [45] and in multiple myeloma, the Val114 polymorphism was associated with a significantly worse survival [46]. Although, not directly related to cancer therapy, it is interesting that a recent study, found that mothers with the Ala113 polymorphism, present in GSTP1*A, had an increased risk of having children born with autistic disorder, suggesting a potential role for GSTP1 in neurodevelopment [47].

Despite the overwhelming number of studies demonstrating a strong association between GSTP1 polymorphism and both treatment outcome and patient survival, it should be noted that a few studies [48] have found no statistically significant association between this polymorphism and clinical outcome.

The strong association between GSTP1, tumor resistance and aggressive tumor growth has led to efforts to develop small molecule GSTP1-targeted agents as novel anti-cancer therapeutics and as agents with which to overcome tumor drug resistance [49,50]. Despite initial encouraging Phase I and II clinical trials [51], the recent preliminary results of a Phase III trial of TLK286, a first generation GSTP1-activated glutathione prodrug with GSTP1 inhibitory activity, have shown no clinical advantage, and indeed, in some cases appeared to enhance tumor growth [52].

GSTT (GST-θ)

The human theta class of GSTs is comprised of two subunits, GSTT1 and GSTT2, both of which are located on chromosome 22q11 (Table 1) [53,54]. GSTT1 has two functional alleles, GSTT1*A, GSTT1*B and a null allele, GSTT1*0 [52,55,56]. GSTT1*A and GSTT1*B differ in an amino acid residue at codon 104, which in GSTT1*A is threonine and in GSTT1*B, a proline [57]. The resulting proteins display a 2-fold difference in enzymatic activity [57]. GSTT2 also has two alleles, GSTT2*A and GSTT2*B, that although differ in amino acid residue 139 (methionine in 2*A and isoleucine in 2*B), are similar in enzyme activity [58]. In pediatric astrocytomas, the GSTP1*C has been shown to be involved and to be associated with an increase in microsatellite instability [42]. In addition to the two genes, a pseudogene, designated GSTT2P, was identified and shown to have an exon-2/intron-2 splice site abnormality and a premature translation stop signal at codon 196 [58].

Of the different GSTT1 alleles, the GSTT1*0 has emerged as the most important predictor of cancer risk and therapeutic response, both alone and in combination with other GST polymorphic variants. For example, individuals with the GSTT1*0 genotype are at a significantly higher risk of developing bladder cancer, meningioma, acute myeloid leukemia and squamous cell carcinoma than those with at least one active GSTT1

allele [59]. Although, relatively few, a number of reports have examined the relationship between GSTT1 polymorphism and clinical outcome. In AML, breast and ovarian cancer, patients with the GSTT1*0 genotype had a higher response to chemotherapy and a longer relapse-free survival than patients with the active gene [21,60]. Conversely, in follicular non-Hodgkin's Lymphoma, patients with a GSTT1 (or a GSTM1) deletion had a significantly worse event-free survival, compared with patients with the undeleted genotype. Together, these studies demonstrate that the GSTT1 gene status is an important determinant of response to therapy, presumably, a result of the decreased ability to metabolize/detoxify the anticancer agents used in the therapy.

Multigene GST polymorphisms in tumor drug resistance

Recent studies have shown that the simultaneous analysis of polymorphisms of multiple classes of GSTs may correlate with therapeutic outcome than the individual gene studies. This polygenetic approach should better address the fact that various classes of cytosolic GSTs share overlapping substrate specificities and therefore, absence of one GST isoform can be compensated by increased expression of other GSTs. Another rationale is that cancer patients are frequently treated with a combination of anticancer agents that are differentially metabolized by different GSTs. The polygenic approach is yielding new and interesting results, particularly, in studies of the GST null genotypes, GSTM1*0 and GSTT1*0. For example, the combined GSTM1*0/GSTT1*0 or the GSTM1*0/GSTP1Val105 genotype was associated with higher response rates and better survival in ovarian cancer [21,61]. In brain tumors, patients with the GSTP1*A and GSTM1*0 combination survived longer than other groups [62] and, following, nitrosourea therapy, the GSTP1*A and GSTM1*0 combination was a better predictor of adverse events secondary to chemotherapy [62]. The positive results, as well as, the limitations of candidate gene analyses has led to the current trend in pharmacogenomics to emphasize the analysis of large numbers of genes related to specific pathways and even in the whole genome. For the GSTs, this can be approached using linkage disequilibrium-based analyses that allows the use of a small subset of tagging single nucleotide polymorphisms to address the diversity in large regions of the genome.

GST protein–protein interactions, cell signaling, apoptosis and drug sensitivity

The best-characterized non-enzymatic function of the GSTs is their interaction with other cellular proteins, resulting in significant functional alteration of the binding partners or post-translational modification and functional alteration of the GSTP1 protein itself. These interactions underlie the role of GSTs as regulators of cell signaling in response to stress, growth factors and DNA damage, and in cell proliferation, cell death and other processes that ultimately lead to tumor growth and drug resistance.

Jun N-terminal kinase, JNK

JNK, a member of the family of stress-activated Ser/Thr kinases, is the best-characterized GST interacting protein. Under normal conditions, GSTP1 binds to and inhibits JNK resulting in suppression of JNK downstream signaling and apoptosis. Under oxidative or other stress, GSTP1 undergoes oligomerization and disassociation from JNK, leading to increased apoptosis [63,64]. Indirectly, GSTP1 increases the activity of other stress-activated kinases, such as, ERK and p38 [63]. In human neuroblastoma cells, GSTP1 has been shown to associate with JNK and treatment with the topoisomerase inhibitor, etoposide, induced dissociation of the JNK-GSTP1 complex and increased the level of apoptosis [65]. Whether the GSTP1 polymorphic variant proteins differ in their regulation of JNK signaling remains to be determined.

Apoptosis-signal-regulating kinase 1, ASK1

ASK1 was shown to bind to GSTM1 and to inhibit oxidative stress-induced ASK1-dependent apoptosis [66]. Overexpression of GSTM1 has subsequently been shown to repress ASK1 activity and ASK1-induced apoptosis in hepatocytes [67]. The role that the GSTM1-ASK1 interaction plays as a mediator of tumor drug resistance is presently unknown but the diminished ASK1 activity following the interaction with GSTM1 suggests that this may be associated with decreased apoptotic response that could contribute to drug resistance. Similar to GSTM1, GSTP1 overexpression suppresses ASK1-induced apoptosis and protects cells against chemical-induced damage [67]. Most recently, GSTP1 has been shown to interact with an ASK1 upstream regulator, tumor necrosis factor receptor-associated factor-2 (TRAF2), which is activated and recruited to TNF receptor leading to ASK-1/JNK/p38 activation [68]. Unlike the interaction between JNK and GSTP1, however, GSTP1 interacts with TRAF2 under both un-stressed conditions and following TNF-α stimulation in human embryonic kidney cells and human cervical cancer cells [68]. Indirectly these observations of the GSTP1-ASK1 interaction can affect cell survival and apoptotic response, which in turn, can lead to drug resistance.

Fanconia anemia group C protein, FANCC

FANCC, the only known cytoplasmic protein of the group of proteins encoded by the Fanconi anemia genes is a GSTP1-interacting protein and the interaction plays an important role in mediating cellular response to therapy. Cells from Fanconi anemia patients, deficient in FANCC, are highly sensitive to DNA cross-linking agents, including, anticancer agents, and transfection of the normal FA gene into mutant cells reverses this hypersensitivity and increases cell viability [69]. In addition, FANCC is involved in cellular protection

against apoptosis [69]. In hematopoietic cells, overexpression of both GSTP1 and FANCC prevents the formation of inactivating disulfide bonds and increases enzymatic activity of the GSTP1 protein during apoptosis [70] Although, it remains to be confirmed, these observations, taken together, suggest that the interaction between GSTP1 and FANCC could contribute to drug resistance by protecting cells against the induction of apoptosis following treatment with chemotherapeutic agents.

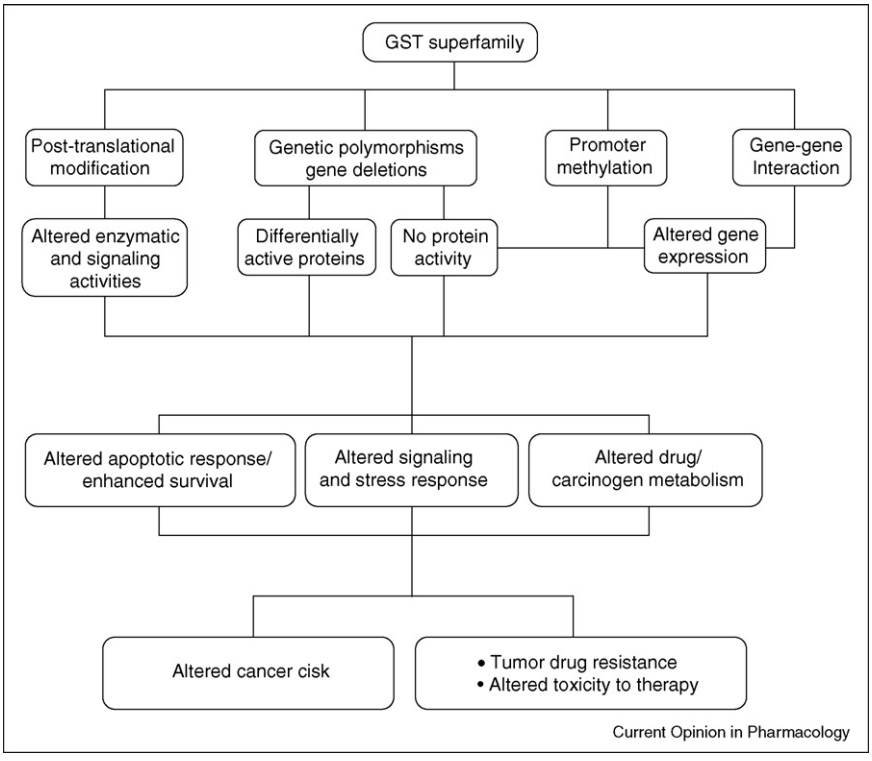
Tissue transglutaminase 2, TGM2

There is now conclusive evidence [71,72] that TGM2, a member of a family of proteins that catalyze the calcium-dependent post-translational crosslinking of a variety of cellular proteins, is a direct binding partner of GSTP1. TGM2 is involved in physiological cellular processes, such as apoptosis and in the pathophysiology of a number of neurological diseases, including Parkinson's disease and Alzheimer's disease, and is also frequently overexpressed in human cancers. In neural cells, the TGM2/GSTP1 interaction regulates apoptotic response to oxidative stress [71]. In our laboratory, we showed that, in glioma cells, the GSTP1/TGM2 complex is formed in the absence of apoptotic stimuli and protects the cells against DNA damage-induced apoptosis [72]. The implication of these observations on the role of this interaction in tumor drug resistance, however, remains to be established.

Serine/threonine kinases (PKA, PKC) and tyrosine kinase, epidermal growth factor receptor (EGFR)

Recent studies in our laboratory have established the human GSTP1 protein as a substrate for phosphorylation by the serine/threonine kinases, PKA and PKC [73] and by EGFR [74]. Phosphorylation of GSTP1 by these kinases is GSH-dependent and increases the catalytic activity of GSTP1. These data suggest the GSTP1 protein is likely to exist in a hyper-phosphorylated/hyper-active state in tumors with aberrant activation of the PKA/PKC and/or EGFR pathways and with high levels of glutathione [73,74]. Our data also demonstrate that the resistance of cells to cisplatin in enhanced following activation of PKC in a GSTP1-overexpressing glioblastoma cell line but not in the isogenic counterpart without GSTP1. These findings suggest that the phosphorylation of GSTP1 by PKC could represent a novel resistance mechanism and add to the complexity of the

Figure 1



GST structure, regulation and function in relationship to cancer risk and tumor drug resistance. Genetic polymorphisms result in differentially active GST proteins while gene deletions and promoter methylation result in the lack of, or altered protein expression. Post-translational modifications, for example, serine/threonine or tyrosine phosphorylation, alter protein stability and/or function, such as, in Phase II drug/carcinogen metabolism and signaling. Interaction of GST genes with each other and/or with other genes can alter the effect of or compensate for the absence of expression of a specific GST. Interactions of GST proteins with other proteins can impact protein function. Each of these can affect downstream cellular processes, such as, Phase II drug/carcinogen metabolism and detoxifications, signaling, stress response, apoptosis and cell survival ultimately leading to altered cancer risk, response to therapy and the severity of therapy-related normal tissue toxicities.

biology of GSTP1 and of its role in cancer biology, prognosis and therapeutic outcome.

Conclusion

Cumulative evidence over the past two decades has established the importance of GSTs as determinants of therapeutic response and patient survival in cancer. The best-characterized mechanism underlying this role of GSTs is their ability to metabolize and inactivate anticancer agents. Increasingly, however, other aspects of GST biology, such as their regulation and their non-enzymatic functions are being shown to be major components of the mechanisms underlying the role of GSTs in cancer (Figure 1). Single gene, and more recently, multigene analyses of GSTs are also providing important insights into their involvement in tumor progression and clinical outcome of cancer therapy. The inconsistencies in some of the results of the correlative studies conducted to date on the role of GSTs in cancer reflect both the complexity of this role of GSTs and the somewhat simplistic approaches taken in many of the studies. The evidence to date suggests that several factors should be taken into consideration in the interpretation of the results of studies of the role of GSTs in clinical outcome in cancer. These include not only the level of GST expression in the tumor and the GST genotype of the patient but also other factors, such as, the extent of GST phosphorylation, the levels of expression and/or activity of kinases, such as, PKA PKC, and EGFR, that phosphorylate the GSTs, the nature of the treatment regimen and the tumor type. Other relevant factors include the status of other GSTP1 interacting proteins, the methylation status of the GST gene and mutational status of genes, such as, p53 that can transcriptionally activate GST genes. There is intense ongoing research in all these areas of GST biology in cancer. The results should provide better insights into the clinical relevance and role of this important superfamily of genes in cancer. This should facilitate a more rational incorporation of the results of their expression and polymorphisms in both the management of the cancer patient, and the development of novel GST-targeted cancer therapeutics.

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