frontiers in ONCOLOGY

REVIEW ARTICLE
published: 24 October 2011
doi: 10.3389/fonc.2011.00039

Glutathione *S*-transferases in pediatric cancer

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The glutathione *S*-transferases (GSTs) are a family of ubiquitously expressed polymorphic enzymes important for detoxifying endogenous and exogenous compounds. In addition to their classic activity of detoxification by conjugation of compounds with glutathione, many other functions are now found to be associated with GSTs. The associations between GST polymorphisms/functions and human disease susceptibility or treatment outcome, mostly in adults, have been extensively studied and reviewed. This mini review focuses on studies related to GST epidemiology and functions related to pediatric cancer. Opportunities to exploit GST in pediatric cancer therapy are also discussed.

Keywords: glutathione *S*-transferase, pediatric cancer, epidemiology, drug resistance, therapeutic target, microsatellite

GENERAL INFORMATION ON GLUTATHIONE *S*-TRANSFERASES

THE GST FAMILY

Glutathione *S*-transferase (GST) isozymes were originally discovered in rat liver cytosol during the early 1960s (Booth et al., 1961; Coombes and Stakelum, 1961). GSTs are drug-metabolizing enzymes that catalyze conjugation of glutathione with carcinogens, drugs, toxins, or products of oxidative stress. Two distinct superfamilies of GST isozymes exist (**Table 1**). One superfamily comprises cytosolic, soluble, dimeric enzymes. At present, eight distinct classes have been identified in this superfamily: alpha, kappa, mu, omega, pi, sigma, theta, and zeta. The other superfamily is composed of microsomal, membrane bound, trimeric proteins. This family of proteins is also called MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism; Hayes and Strange, 2000). While GSTs are expressed in all tissues, specific isozyme distribution across different tissues in mammals is variable and complex (Hiley et al., 1989; Strange et al., 1989).

GST SUBSTRATES

Crystal structures have been determined for many soluble GSTs, often with bound substrates or products (Oakley, 2011). The "canonical fold" of a soluble GST subunit reveals an N-terminal α/β domain forming the GSH-binding site (G-site) and a second, α-helical domain forming most of the H-site that binds the electrophilic substrate (**Figure 1**). CDNB (1-chloro-2,4-dinitrobenzene) was initially adopted by biochemists as a "universal" GST substrate. However, it was soon realized that, although different transferases may exhibit overlapping substrate specificities, no common substrate exists that is metabolized by all GST isozymes (**Table 1**). Different amino acids in the substrate-binding site (H-site) of GST isozymes can account for substrate specificities. In view of the separate evolutionary histories of the cytosolic and MAPEG superfamilies, it is not surprising that they display marked differences in catalytic activities (Armstrong, 1997). Because its binding pocket for electrophiles is hydrophobic, Microsomal GST1 (MGST1) is uniquely suited among the GSTs to detoxify reactive intermediates of a more hydrophobic nature (Schaffert, 2011). The substrate specificity of individual GST isozymes suggests that each of them play a unique role in biotransformation of drugs. Genetic variations in these enzymes will therefore influence the response of individuals to environmental agents.

POLYMORPHISM AND EPIDEMIOLOGY

Genetic polymorphisms in the *GST* genes arise from nucleotide alterations that may change codons to generate unique alleles or even null genotypes. These amino acid substitutions cause steric changes in the substrate-binding site of the enzyme. As a consequence, the enzymatic activities of GST are significantly affected. Because different GST proteins differ in their ability to catalyze specific detoxification reactions, polymorphisms in *GST* will likely impact response to specific therapies. Many molecular epidemiological studies have tested associations between polymorphisms of *GST* genes and disease susceptibility. The results of such studies have often been conflicting. As a consequence, a number of meta-analyses have been carried out as part of the Human Genome Epidemiology (HuGE) Network, which provided evidence that *GST* polymorphisms can result in a small but significant increase in risk of some types of cancers or diseases (Hayes and Strange, 2000; Bolt and Their, 2006; Di Pietro et al., 2010; Economopoulos and Sergentanis, 2010; Josephy, 2010; McMahon et al., 2010). However, most meta-analyses suffered from a serious limitation: fail to distinguish between heterozygous and homozygous genotypes, which resulted in heterogeneity between studies. Now boundaries of the deletion polymorphisms have been cloned and analytical methods that assess copy number such as real-time PCR are now available (Timofeeva et al., 2009). These will be helpful in dissolving some of the heterogeneity observed in clinical evaluations.

FUNCTIONS OF GST

The classic activity of the GSTs is to detoxify reactive electrophiles by conjugation to glutathione (GSH), thereby reducing

Table 1 | Human GSTs and their biochemical properties.

Super-family	Class	Chromo-some	Gene	Protein	Substrate	Reference
Cytosolic	Alpha	6p12.2	*GSTA 1*	GSTA1-1	CDNB, NBD-Cl, D5AD, PGE2, cholesterol a-oxide, dibenzo(a,l)pyrene	Hayes and McLellan (1999), Dreij et al. (2002)
			GSTA 2	GSTA2-2	CDNB, NBD-Cl, CuOOH, PGD2	Hayes and McLellan (1999)
			GSTA 3	GSTA3-3	Δ5AD, dibenzo(a,l)pyrene	Johansson and Mannervik (2001), Dreij et al. (2002)
			GSTA 4	GSTA4-4	HNE, ETA	Hayes and McLellan (1999), Hubatsch et al. (1998), Balogh et al. (2011)
Cytosolic	Mu	1p13.3	*GSTM1*	GSTM1-1	CDNB, AFB1-epoxide, trans-4-phenyl-3-buten-2-one, tSO, adrenochrome, aflatoxin B1-8,9-epoxide	Hayes and McLellan (1999), Baez et al. (1997), Johnson et al. (1997)
			GSTM2	GSTM2-2	CDNB, DCNB, cyano DMNG, aminochrome, dopachrome, noradrenochrome	Hayes and McLellan (1999), Baez et al. (1997), Norrgard and Mannervik (2011)
			GSTM3	GSTM3-3	H2O2, PGH2	Hayes and McLellan (1999), Beuckmann et al. (2000)
			GSTM4	GSTM4-4	n.d.	
			GSTM5	GSTM5-5	n.d.	
Cytosolic	Pi	11q13	*GSTP1*	GSTP1-1	CDNB, acrolein, adenine propenal, thymine propenal, ETA, 4-vinylpyridine, BPDE, benzo(c)-phenanthrene, benzo(g)chrysene, BITC, actin, GSTP, peroxiredoxin VI	Hayes and McLellan (1999), Berhane et al. (1994), Coles et al. (2000), Hu et al. (1997a,b,c), Hu et al. (1998), Nakamura et al. (2000), Tew et al. (2011)
Cytosolic	Sigma	4q22.3	*GSTS1*	GSTS1-1	PGH2	Kanaoka et al. (1997)
Cytosolic	Theta	22q11.2	*GSTT1*	GSTT1-1	NBD-Cl, CuOOH, acrylamide, glycidamide, EO, CAA, DCM, DBE, EPNP, MB	Huang et al. (1997), Sherratt et al. (1997) Sherratt et al. (1997), Hayes and McLellan (1999), Doroshyenko et al. (2009)
			GSTT2	GSTT2-2	CuOOH, 1-menaphthyl sulfate	Hayes and McLellan (1999), Tan et al. (1996)
Cytosolic	Zeta	14q24.3	*GSTZ1*	GSTZ1-1	DCA, fluoroacetate, maleylacetoacetate	Tong et al. (1998a,b)
Cytosolic	Omega	10q24.3	*GSTO1*	GSTO1-1	MMA, dehydroascorbate, HED, *s*-(4-nitrophenacyl)glutathione	Tanaka-Kagawa et al. (2003), Board et al. (2000, 2008)
Mitochondria bound	Kappa	7q34-35	*GSTK1*	GSTK1-1	2-Hydroxychromene-2-carboxylate	Robinson et al. (2004)
Micro-somal	MAPEG	12p12.3	*MGST1*	MGST1	CDNB, NBD-Cl, 4-nitrobenzyl chloride, Couth	Hayes and McLellan (1999)
		4q28.3	*MGST2*	MGST2	CDNB, 5-HPETE, LTA4	Hayes and McLellan (1999), Jakobsson et al. (1996)
		1q23	*MGST3*	MGST3	5-HPETE, LTA4	Jakobsson et al. (1997)
		5	*LTC4S*	LTC4S	LTA4	Jakobsson et al. (1996)
		13q12	*FLAP*	FLAP	Binds to AA and MK-886	Mancini et al. (1993), Mancini et al. (2001)

Modified from Hayes and Strange (2000). 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; AA, arachidonic acid; BPDE, benzo(a)pyrene diolepoxide; BITC, benzyl isothiocyanate; CAA, chloroacetaldehyde; CDNB, 1-chloro-2,4-dinitrobenzene; CuOOH, cumene hydro-peroxide; DBE, dibromoethane; DCA, dichloroacetic acid; DCM, dichloromethane; DCNB, 1,2-dichloro-4-nitrobenzene; ?5AD, delta(5)-androstene-3,17(20)-dione; EO, ethylene oxide; EPNP, 1,2-epoxy-3-(p-nitrophenoxy)propane; ETA, ethacrynic acid; HED, hydroxyethyl disulfide; HNE, 4-hydroxynonenal; LTA4, leukotriene A4; MB, methyl bromide; MMA, mono-methylarsonic acid; n.d., not determined; tSO, trans-stilbene oxide.

the likelihood of deleterious interactions between such reactive species and essential cellular components like proteins and nucleic acids. Knockout and transgenic mouse models were generated for many GST family members which helped to reveal the physiological function of GST isozymes (Elsby et al., 2003; Henderson and Wolf, 2011). Based on these and other model systems, many other activities are now associated with GSTs, including regulation of signaling pathways and anti-apoptotic activity by GSTP (Tew et al., 2011), anti- and pro-inflammatory functions of sigma-class GSTs (Flanagan and Smythe, 2011), activities of MGST1 related to mitochondria (Aniya and Imaizumi, 2011), regulation of the cardiac muscle ryanodine receptor (Dulhunty et al., 2011), and functions associated with asthma (Minelli et al., 2010).

GSTs and signaling pathway regulation

It is becoming apparent that GSTP family members participate in the maintenance of cellular redox homeostasis through a variety of mechanisms (Tew et al., 2011). GSTP1, for example, displays an anti-apoptotic activity based on protein–protein interactions with c-Jun N-terminal kinase (JNK; Adler et al., 1999; **Figure 2**).

GSTP1 was implicated in mediating *S*-glutathionylation of specific clusters of target proteins and in reactions that play a negative regulatory role in some kinase pathways through other protein–protein interactions. GSTP1 has also been implicated in regulating tumor necrosis factor-alpha (TNF-alpha) signaling primarily through a physical association with tumor necrosis factor receptor-associated factor 2 (TRAF2; Wu et al., 2006). In addition, physical interactions between the HPV16 E7 viral factor and GSTP1 were found to improve survival capabilities of host cells (Mileo et al., 2009). GSTA1 is also capable of suppressing JNK signaling caused by inflammatory cytokines or oxidative stress, likely because of the homology between GST A and P family members, (Romero et al., 2006). It was also reported that GSTM1 binds and inhibits the activity of Apoptosis Signaling Kinase-1 (ASK1; Cho et al., 2001). The GSTM1:ASK1 interaction dissociates under oxidative stress or heat shock, leading to activation of ASK1 and apoptosis (Dorion et al., 2002).

FIGURE 1 | A cartoon diagram of GSTP, a representative of a soluble GST enzyme. The N-terminal GSH-binding site (G-site, in yellow) and the C-terminal H-site (in purple) that binds the electrophilic substrate (in orange) are shown.

MGST1 and its dual function in mitochondria

Microsomal GST1 protects cells (and mitochondria) from oxidative stress by both conjugation and glutathione peroxidase functions (Johansson et al., 2010; Aniya and Imaizumi, 2011; Schaffert, 2011). MCF7 cells overexpressing MGST1 showed protection against agents that induce peroxidation. Mitochondria in these cells were shown to be protected from oxidative insult as measured by calcium loading capacity and respiration. MGST1 also induced cellular resistance against cisplatin in these cells (Johansson et al., 2010). In addition to these protective effects, MGST1 also has deleterious effects that contribute to oxidative stress-induced liver injury. Preliminary studies indicated that mitochondrial MGST1 was activated by reactive oxygen species (ROS). This activation led to its aggregation and induction of non-classical mitochondrial permeability transition (MPT). MPT induces mitochondrial dysfunction which results in apoptosis and necrosis. Therefore, depending on the context, MGST1 activation can either exhibit a protective or toxic effect on the liver and possibly other tissues (Schaffert, 2011).

GST IN PEDIATRIC CANCER

EPIDEMIOLOGY

As in adult cancers, a large number of epidemiological studies have tested possible associations between GST polymorphic variants as well as deletions with susceptibility of pediatric cancers (**Table 2**). GSTM1 is subject to a deletion polymorphism that is found in as many as 75% of Caucasians (Zhong et al., 1991;

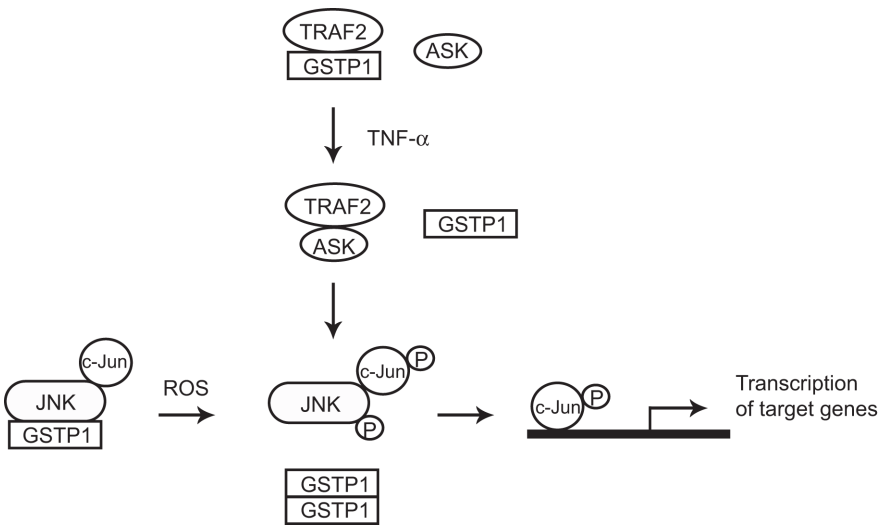

FIGURE 2 | Cartoon model of how GSTP1–JNK and GSTP1–TRAF2 interactions modulate JNK and TNF-α signaling pathways. In non-stressed cells, GSTP1 form complexes with JNK and TRAF2 respectively. GSTP1–JNK interaction keeps JNK at low activity, while GSTP1–TRAF2 interaction competes against TRAF2–ASK1 binding which also leads to low JNK activity. In cells exposed to ROS or TNF-α, GSTP1 dissociates from the complexes and accumulates as oligomers, with resultant activation of JNK and downstream signaling pathways.

Table 2 | Case–control studies on association of GST polymorphism with susceptibility or risk of relapse of childhood cancers.

Reference	Tumor type	Population	Region	Age (year; Median)	Genotype	No. of cases	No. of controls	Odds ratio	95% Confidence interval	*p* Value	Treatment applied
SUSCEPTIBILITY											
Krajinovic et al. (1999)	ALL	French Canadian	Canada	1–21 (8)	*GSTM1* null	174	304	1.8	1.2–2.6	0.004	NA
					GSTT1 null	176	274	0.9	0.5–1.5	0.8	
Pakakasama et al. (2005)	ALL	Thai	Thailand	0.83–14.75(6.25)	*GSTM1* null	107	320	1.7	1.0–2.7	0.04	NA
					GSTT1 null	107	320	1.4	0.9–2.2	0.12	
					GSTM1 and *GSTTT1* null	107	320	1.7	1.1–2.9	0.02	
Joseph et al. (2004)	ALL	Indian	India	0–14 (NA)	*GSTM1* null	118	118	2.1	1.21–3.67	0.009	NA
					GSTT1 null	118	118	1.82	0.8–4.16	0.16	
Ashton et al. (2007)	NB	White	Australia	0–13.51 (1.26)	*GSTM1* null	89	116	1.6	1.02–2.49	0.04	Standard protocol (see Reference)
			New Zealand		*GSTT1* null	88	117	0.67	0.37–1.21	0.185	
					GSTP1 V105 homozygote	88	203	1.16	0.64–2.13	0.620	
Davies et al. (2000)	AML/MDS	White	U.S.	NA (NA)	*GSTM1* null	232	153	2.0	1.3–3.1	0.001	NA
					GSTT1 null	232	153	1.6	0.9–2.9	0.12	
Krajinovic et al. (2002)	ALL	French Canadian	Canada	NA (4.9)	*GSTP1* V105	278	301	1.5	1.1–2.0	0.02	NA
					GSTP1 V105 *and GSTT1* null	278	301	2.1	1.3–3.4	0.003	
Gatedee et al. (2007)	ALL	Thai	Thailand	0.83–14.75 (5)	*GSTP1* V105	100	100	0.92	0.52–1.62	0.886	Risk-adapted chemotherapy regimens modified total XII protocol (see Reference)
RISK OF RELAPSE											
Stanulla et al. (2000)	ALL	NA	Germany Austria Switzerland	0–18 (NA)	*GSTM1* null	64	64	0.5	0.23–1.07	0.078	ALL-BFM 86 and 90 trials (see Reference)
					GSTT1 null	64	64	0.36	0.13–0.99	0.048	
					GSTP1 V105 homozygote	64	64	0.33	0.09–1.23	0.099	

Anderer et al. (2000)	ALL	NA	Germany Austria Switzerland	0–18 (NA)	*GSTM1* null *GSTT1* null *GSTP1* V105 homozygote	45 45 45	90 90 90	1.13 0.18 0.84	0.52–2.46 0.02–1.53 0.14–4.93	0.764 0.117 0.851	ALL-BFM 86 and 90 trials (see Reference)
Takanashi et al. (2003)	ALL	Japanese	Japan	1.5–15 (NA)	*GSTM1* null *GSTT1* null *GSTM1* and *GSTTT1* null	12 12 12	70 70 70	NA NA NA	NA NA NA	0.68 0.22 0.027	ALL protocol (see Reference)
Chen et al. (1997)	ALL	Black and white Black White	U.S.	NA (NA)	*GSTM1* null *GSTT1* null *GSTM1* and *GSTTT1* null *GSTM1* and *GSTTT1* null	197 197 34 163	416 416 203 213	1.2 1.12 7.36 0.75	0.87– 0.74– 2.61– 0.35–	0.19 0.34 0.0005 0.68	Extended intensified chemotherapy (see Reference)
Davies et al. (2002)	ALL	White Black	U.S.	Mostly 1-10 (NA)	*GSTM1* and *GSTTT1* null *GSTM1* and *GSTTT1* null	616 35	532 201	NA NA	NA NA	1 1	CCG protocols (see Reference)
Balta et al. (2003)	ALL/ANLL	Turkey	Turkey	0.58–17 (6.8)	*GSTM1* null *GSTT1* null *GSTP1* V105 homozygote	139 139 136	185 185 185	1.03 0.9 0.75	0.66–1.61 0.53–1.53 0.24–2.34	NA NA NA	NA
Zielinska et al. (2004)	ALL/AML/ NHL/RMS/ PNET/CNST et al	Polish	Poland	3.5–12.92 (7.54)	*GSTM1* null *GSTT1* null *GSTP1* V105 *GSTP1* I105 A114	234 234 234 234	460 460 460 460	1.54 1.2 5.7 3.29	0.84–2.83 0.6–2.39 2.4–13.8 0.73–14.67	0.16 0.7 0.0001 0.03	Polish Paediatric Oncology Study Group recommended protocol (see Reference)

NA, not available.

Huang et al., 2009). Multiple case-controlled studies have indicated statistically significant associations of the GSTM1 null genotype with increased risk of childhood cancers. Groups from different regions in the world found that GSTM1 null genotype related to increased risk of acute leukemia (ALL and AML) in various populations (Krajinovic et al., 1999; Davies et al., 2000; Joseph et al., 2004; Pakakasama et al., 2005; Marino et al., 2009). Ashton et al. (2007) found that GSTM1 null genotype is associated with high risk of neuroblastoma in Australia and New Zealand. GSTP1 V105 was found to relate to increased susceptibility to childhood leukemia in Canada (Krajinovic et al., 2002). However, another group found that there was no statistically significant association between GSTP1 polymorphism and susceptibility to ALL in Thai children (Gatedee et al., 2007). Interestingly, one group reported that GSTO1 A140D and GSTO2 N142D polymorphism were both significantly associated with susceptibility to ALL in Thailand (Pongstaporn et al., 2009). These conflicting results were thought to be related to differences in ethnicity and age of the patients, treatment, and follow-up periods included in these studies.

GST AND DRUG RESISTANCE

Glutathione *S*-transferases have been implicated in the development of resistance toward chemotherapeutic agents which lead to relapse in pediatric cancers (**Table 2**). GSTT1 null genotype has been shown to confer a reduced risk of relapse in childhood ALL in several case studies (Anderer et al., 2000; Stanulla et al., 2000). However, the GSTT1 null genotype was also shown to be associated with an increased risk of death after chemotherapy in childhood AML (Davies et al., 2001). As to the GSTM1–GSTT1 double-null genotype, one group reported an increased risk of early relapse of ALL with double-null genotype (Takanashi et al., 2003) while a subsequent study reported the opposite result (Kham et al., 2004). Yet other studies showed that the double-null genotype was not associated with risk of relapse or treatment outcome in ALL (Chen et al., 1997; Davies et al., 2002; Balta et al., 2003; Jazbec et al., 2003). GSTP1 expression was significantly increased in relapsed AML, and GSTP1 V105 was found to be related to increased relapse rate of childhood leukemia (Sauerbrey et al., 1994; Beck et al., 1996; Zielinska et al., 2004).

Several studies using microarray technology have identified glutathione metabolism pathway to reflect tumor resistance to chemotherapy in Ewing's sarcoma. The authors found that the expression of MGST1 clearly predict Ewing's sarcoma prognosis and to be associated with doxorubicin chemosensitivity (Townsend and Tew, 2003; Schaefer et al., 2008; Scotlandi et al., 2009). In a separate microarray analysis for target genes of EWS/FLI, the master regulator of Ewing's sarcoma, GSTM4 was found to be upregulated by EWS/FLI. Reduction of GSTM4 levels resulted in an increased sensitivity of Ewing's sarcoma cells to chemotherapeutic agents (etoposide and fenretinide). This suggested a role for GSTM4 in drug resistance. In support of this hypothesis, patients with Ewing's sarcoma whose tumors had higher levels of GSTM4 expression had worse outcomes than those with lower expression levels (Luo et al., 2009).

GSTM1 has been reported to be a significant risk factor for hematologic relapse in childhood ALL. Transduction of GSTM1 into T-acute lymphoblastic leukemia cells selectively decreased cellular sensitivity to dexamethasone in a manner that was independent of glutathione conjugation, but was due to apoptosis inhibition. Interestingly, p38-MAPK and Bim activation were suppressed, and NF-kappaB p50 was activated, in these GSTM1 expressing cells. The authors proposed that GSTM1 is a novel regulator of dexamethasone-induced apoptosis, and causes dexamethasone resistance by suppression of Bim through dual mechanisms of downregulation of p38-MAPK and up-regulation of NF-kappaB p50 (Hosono et al., 2010). Consistently, GSTM1 null genotype was associated with a reduced risk of relapse in ALL (Stanulla et al., 2000). Association of GSTM1 with resistance to adriamycin and cisplatin was also found in childhood hepatoblastoma (Bader et al., 1998).

Drug resistance is a common problem in the treatment of childhood rhabdomyosarcoma (RMS). To identify causes of drug resistance in this disease, Seitz et al. (2010) performed gene expression analysis of tumors from mice transplanted with embryonal or alveolar RMS cells and treated with vincristine. The authors found 2314 differentially expressed genes between the groups in alveolar RMS and 1387 in embryonal RMS. Pathway analysis revealed a cluster of five overexpressed genes of the GST family in animals treated with vincristine, suggesting a cause for drug resistance. *In vitro* experiments confirmed up-regulation of GST activity following incubation with doxorubicin and topotecan in RMS cell lines. Incubation with GST inhibitors resulted in a decreased cell viability. The authors concluded that reversal of drug resistance in childhood RMS may be achieved by GST inhibitors, at least in part. Thus, the GST family represents a promising target for further treatment strategies in childhood RMS (Seitz et al., 2010).

The association of GSTs with risk of relapse and drug resistance may not be a straightforward reflection of their ability to participate in detoxification reactions. Greater understanding of the numerous factors affecting GST expression and activity, as well as GST functions, may reveal further connections between GST and individual responses to disease and drugs.

REGULATION OF GST

Most cases of Ewing's sarcoma express the EWS/FLI fusion oncoprotein (Turc-Carel et al., 1988). EWS/FLI functions as an aberrant transcription factor that mediates the transformed phenotype through the deregulation of several key target genes (Kinsey et al., 2006; Owen and Lessnick, 2006; Smith et al., 2006; Tirado et al., 2006; Luo et al., 2009). Furthermore, EWS/FLI has been shown to transcriptionally activate some of its gene targets through GGAA-containing microsatellite promoter elements (Gangwal et al., 2008). Interestingly, *GSTM4* contains a GGAA-microsatellite in its promoter. *In vitro* and *in vivo* studies revealed that EWS/FLI binds to the microsatellite and up-regulates *GSTM4* via this element. Other work has shown that the ability of EWS/FLI to activate gene expression through GGAA-microsatellite response elements is proportional to the length of the microsatellite, suggesting that microsatellite polymorphisms might affect target gene expression (Gangwal et al., 2010). Indeed, this hypothesis was supported by the finding that the number of GGAA repeats present in the *NR0B1* promoter positively correlated with the level of NR0B1 mRNA expression in Ewing's cells (Garcia-Aragoncillo et al., 2008). Further work will be needed to determine if a similar relationship

exists for the *GSTM4* gene, and if such a relationship correlates with drug resistance in Ewing's sarcoma.

The GGAA-microsatellite is not shared by other GST family members, however. Other GST promoters contain response elements such as an antioxidant response element and a xenobiotic response element, as well as putative binding sites for transcription factors such as AP-1, MAF, Nrf1, Jun, Fos, and NF-kappaB. Such complex response elements suggest a mechanism for differential regulation of GST isozymes across tumor types in response to differing toxic insults.

GST AS TARGETS IN PEDIATRIC CANCER TREATMENT

The design and discovery of compounds that bind GST isozymes and modulate their biological activity has become an important aim in cancer research because GST isozymes are overexpressed in many cancer cell lines (Tew et al., 1996), and induce drug resistance by inactivating many chemotherapeutic compounds via GSH conjugation (Tew et al., 1997). There are a number of candidate GST-targeted drugs at various stages of preclinical development (Tew et al., 1997; Ruzza and Quintieri, 2009; Wondrak, 2009; Sau et al., 2010). Ethacrynic acid, an inhibitor that lacks class specificity for GSTs, represented a first attempt in this direction; however, its low affinity and deleterious side effects have discouraged its use in clinical practice (Tew et al., 1997). More recently, GSH peptidomimetic compounds have been designed, including TER 199 (**Figure 3**). However, many GSH derivatives are actively extruded from cancer cells by specific export pumps, such as the multidrug resistance protein, and so are unlikely to be highly efficacious (Muller et al., 1994; Morgan et al., 1996).

A new class of GST inhibitors, called NBD derivatives, has been designed recently (Ricci et al., 2005). A representative of this class is NBDHEX, which interacts with GSTP1 and triggers apoptosis in human tumor cells through dissociation of the JNK–GSTP1 complex (Turella et al., 2005; **Figure 3**). Osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma cell lines were all found to be sensitive to NBDHEX *in vitro* and in xenograft models (Scotlandi et al., 2009; Pasello et al., 2011). Importantly, NBDHEX was not extruded from tumor cells by multidrug resistance protein pumps (Ricci et al., 2005; Turella et al., 2005, 2006). NBDHEX had synergistic effect with doxorubicin, vincristine, cisplatin in an *in vitro* study. *In vivo* studies confirmed the cytostatic efficacy of NBDHEX and its synergy with vincristine in Ewing's sarcoma cells, and also its effect against the metastatic spread of osteosarcoma cells (Pasello et al., 2011). Although NBDHEX is still under preclinical *in vivo* evaluation, it may be an interesting new therapeutic option for patients who are not highly responsive to conventional regimens.

Another approach is to design prodrugs that exploit the high GST expression levels found in drug resistant tumors and cells. Prodrugs would be preferentially activated by GST in malignant cells, thus sparing normal tissues and enhancing the therapeutic index. JS-K is a member of the *O*2-aryl diazeniumdiolate compound family which was designed to release nitric oxide (NO) when activated by GSTs (Shami et al., 2006). JS-K has shown promise as a novel cancer therapeutic agent in a number of studies. For instance, JS-K selectively induces programmed cell death in breast cancer cells while sparing normal mammary epithelial cells under the same conditions (McMurtry et al., 2011; **Figure 3**). The selective anti-tumor activity of JS-K warrants its further investigation in pediatric cancers.

CONCLUSION

Many epidemiological studies have investigated possible associations between GST polymorphisms with risk of pediatric cancers. Some studies have indicated increased risk for specific genotypes, such as the *GSTT1* and *GSTM1* deletions, while other studies have not confirmed this association. These conflicting results need to be interpreted with caution. Many GST activities other than detoxification have been discovered and may contribute to different GST activities among individuals. GSTs have also been implicated in the development of resistance toward chemotherapeutic drugs. Although further studies are required to reveal the underlying mechanisms, drugs targeting GSTs have been designed to overcome resistance to conventional therapeutic agents. Little

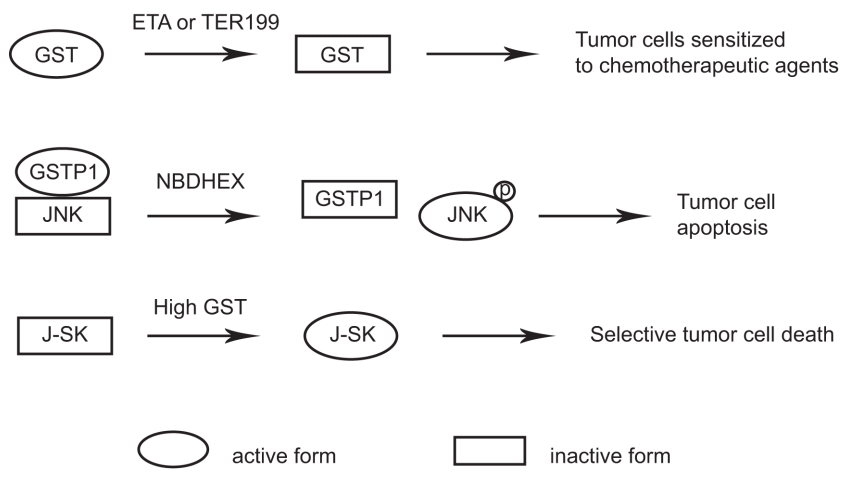

FIGURE 3 | GST as targets in pediatric cancer treatment. GST non-specific inhibitors such as ETA or TER199 decrease the GST activity in tumors and sensitize tumor cells to chemotherapeutic agents. NBDHEX treatment of tumor cells interrupts GSTP1–JNK interaction which releases and activates JNK and results in tumor cell apoptosis. Prodrugs such as J-SK is activated specifically in tumors with high GST activity and selectively kills tumor cells.

progress has been made in understanding how GST expression and activity is regulated. Such studies will allow an understanding of the upstream players in the glutathione metabolism pathway and provide potential new approaches for the treatment of pediatric cancers.

ACKNOWLEDGMENTS

Stephen L. Lessnick is supported by the NIH (R21 CA138295, R01 CA140394), the Terri Anna Perine Sarcoma Fund, and the University of Utah Department of Pediatrics and Huntsman Cancer Institute/Huntsman Cancer Foundation. Joshua D. Schiffman is supported by Damon Runyon Clinical Investigator Award, Alex's Lemonade Stand Foundation Epidemiology Award, and the Eunice Kennedy Shriver Children's Health Research Career Development Award NICHD 5K12HD001410. Wen Luo is supported by the Alex's Lemonade Stand Foundation and the Primary Children's Medical Center Foundation. We also acknowledge NIH support to the Huntsman Cancer Institute (P30 CA042014).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 July 2011; accepted: 03 October 2011; published online: 24 October 2011.

Citation: Luo W, Kinsey M, Schiffman JD and Lessnick SL (2011) Glutathione S-transferases in pediatric cancer. Front. Oncol. 1:39. doi: 10.3389/fonc.2011.00039

This article was submitted to Frontiers in Pediatric Oncology, a specialty of Frontiers in Oncology.