

Glutathione *S*-transferase π : a potential role in antitumor therapy

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Abstract: Glutathione *S*-transferase π (GST π) is a Phase II metabolic enzyme that is an important facilitator of cellular detoxification. Traditional dogma asserts that GST π functions to catalyze glutathione (GSH)-substrate conjugation to preserve the macromolecule upon exposure to oxidative stress, thus defending cells against various toxic compounds. Over the past 20 years, abnormal GST π expression has been linked to the occurrence of tumor resistance to chemotherapy drugs, demonstrating that this enzyme possesses functions beyond metabolism. This revelation reveals exciting possibilities in the realm of drug discovery, as GST π inhibitors and its prodrugs offer a feasible strategy in designing anticancer drugs with the primary purpose of reversing tumor resistance. In connection with the authors' current research, we provide a review on the biological function of GST π and current developments in GST π -targeting drugs, as well as the prospects of future strategies.

Keywords: tumor resistance, glutathione *S*-transferase pi, drug treatment

Introduction

In addition to combating a variety of noxious substances from the external environment, cell-detoxification mechanisms are capable of resisting the deleterious effects of certain endogenous substances (eg, ROS, a product generated from normal cellular metabolism), in order to maintain physiological homeostasis. Drug metabolism represents an important component of cellular detoxification and involves two enzymes: Phase I and Phase II drug-metabolism enzymes. The Glutathione *S*-transferase π (GST) family of enzymes is a group of typical Phase II detoxification enzymes and is found in many prokaryotes and eukaryotes.¹ Of these enzymes, GST π catalyzes the conjugation between GSH and its electrophilic substrates upon exposure to damaging free radicals. Besides metabolite detoxification, GST π also exhibits ligand-binding properties that initiate cellular apoptosis when triggered by cellular stress.² Further research has also demonstrated that GST π is expressed abundantly in tumor cells and associated closely to carcinogenesis, tumor formation, and chemotherapy resistance.^{3,4} Moreover, experiments involving drug-resistant cell lines have also demonstrated increased GST π expression.^{5,6} In multidrug-resistant HL60/VCR acute myelogenous leukemia cells, GST π is found to be expressed at higher levels than HL60.⁷ GST π is involved in facilitating tumor resistance and suppressing apoptosis in tumor cells via two mechanisms. First, GST π weakens the efficacy of chemotherapy drugs by promoting their in vitro extrusion. Second, GST π also functions as an MAPK-pathway inhibitor to prevent tumor-cell apoptosis.

A variety of anticancer drugs based on these principles have been synthesized in efforts to improve their therapeutic indices and reverse tumor resistance. Drugs that work through the GST system include GST π inhibitors and their respective prodrugs.

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The former work by exerting high GST π -inhibitory activity, while the latter comprise inactive compounds designed to target tumor tissue locally by undergoing GST π catalysis in the tumor to release cytotoxic metabolites.⁸ Development of therapies targeting GST π is a major field of research. As such, we believe that there is a need for more detailed studies outlining the diverse biological functions of GST π , in order to assist drug discovery and unlock more exciting possibilities in the realm of tumor treatments.

Structure

GSTs consist of the following three superfamilies: cytoplasmic (cGSTs), mitochondrial (κ GSTs), and microsomal (membrane-associated proteins in eicosanoid and glutathione metabolism [MAPEG]). Among these families, cGSTs are the most complex and most closely linked to the development of human diseases.^{9,10} The cGSTs are divided into seven subtypes according to similarities in amino-acid sequence, different structure of genes, and immunological cross-reactivity. These subtypes are α , π , μ , θ , ω , σ , and δ .¹¹ Among them, GST α is highly expressed in many normal cells.¹² However, recent studies have shown that GST α also takes part in promoting multidrug resistance in p53-mutated lung cancer cells.¹³ GST μ has been found to be able to act synergistically with MRP1 to decrease the effects of vincristine treatment.¹⁴ GST π is widespread in tumor cells, and is intricately involved with cellular carcinogenesis, tumor formation, and tumor-drug resistance.¹⁵ Current evidence supports the role of cGSTs in facilitating multidrug resistance across different types of tumors.^{16,17}

GST π is the most frequently and extensively studied of all the GSTs. Its encoding gene is located on chromosome 11 and is composed of seven exons. In humans, GST π often consists of two identical dimer subunits, with each subunit consisting of 210 amino acids and two binding sites, the G-site and the H-site (Figure 1). Different G- and H-site locations in the amino-acid residue of different GSTs exert different functions. GST π is able specifically to bind to GSH or GSH analogs via the G-site, which catalyzes the interaction

between GST amino-acid residues with GSH sulfhydryl and conventional electrophilic substances at the H-sites to promote catalytic action.¹⁸ Therefore, G-site modification often guides the development of specific GST π inhibitors.

Biological function

GST π in metabolite detoxification and antioxidation

The classical view holds that as a dimeric isoenzyme, GST π can conjugate GSH with substrate molecules in efforts to promote clearance of active ionic substances.¹⁹ However, tumor cells also utilize GST π to form a GSH-X complex between antitumor drugs and GSH, proceeding to excrete the complex out of the cell by Pgp and MRP. Pgp, encoded by *MDR1*, is often found to be highly expressed in tumor cells.^{6,20} What is more, GST π and Pgp or MRPs (eg, MRP2) are synergistic in driving the development of multidrug resistance in tumor cells²¹ (Figure 2). Studies have documented high GST π expression in various tumor cells, such as cancers of the gastrointestinal tract,⁶ pancreas,²² breast,²³ liver,²⁴ lymphoma,²⁵ as well as melanoma.²⁶

Recent literature has characterized GSH and other related metabolic enzymes as vital in protecting cells from ROS²⁷ through oxidation and reduction (redox) mechanisms.²⁸ GSH carries a cysteine residue with an active thiol group and is responsible for maintaining thiol equilibrium. Meanwhile, it can also modulate the activities of many signaling molecules and redox-sensitive transcription factors through *S*-glutathionylation, a form of posttranslational modification that combines cysteine residues with GSH.²⁹ GST π serves as a general *S*-glutathionylase enzyme and promotes *S*-glutathionylation. Its enzymatic function is based on two aspects: its catalytic activity and the auto-*S*-glutathionylation of GST π by itself on Cys47 and Cys10, both of which disturb the subsequent interaction with c-Jun NH₂-terminal kinase (JNK), resulting in the formation of a GST π multimer³⁰ (Figure 3). Besides GST π , other members of the GSH-redox system, such as glutamate cysteine ligase,³¹ glutathione peroxidase, and glutathione reductase, also play significant roles in this process.³²

GST π in regulation of MAPK pathway

Besides metabolite detoxification, GST π also exhibits ligand-binding properties that allow the enzyme to interact covalently and noncovalently with compounds, resulting in inhibition of conjugation activity.¹⁸ GST π can induce cellular apoptosis in the setting of cellular stress by activating MAPK, MKK4, downstream JNK-signal components, and p38 kinase. Normal cells have low basal JNK activity to maintain optimal cellular growth conditions. However, in the presence

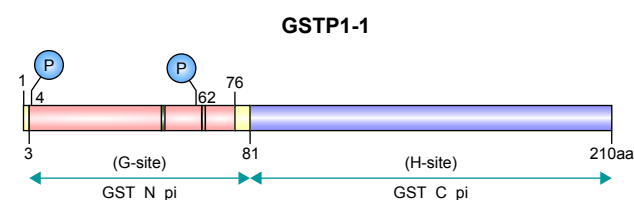


Figure 1 The two binding sites (G and H) of GST π .

Note: The G-site represents a GSH-binding site and the H-site a substrate-binding site.

Abbreviation: GST, glutathione S-transferase.

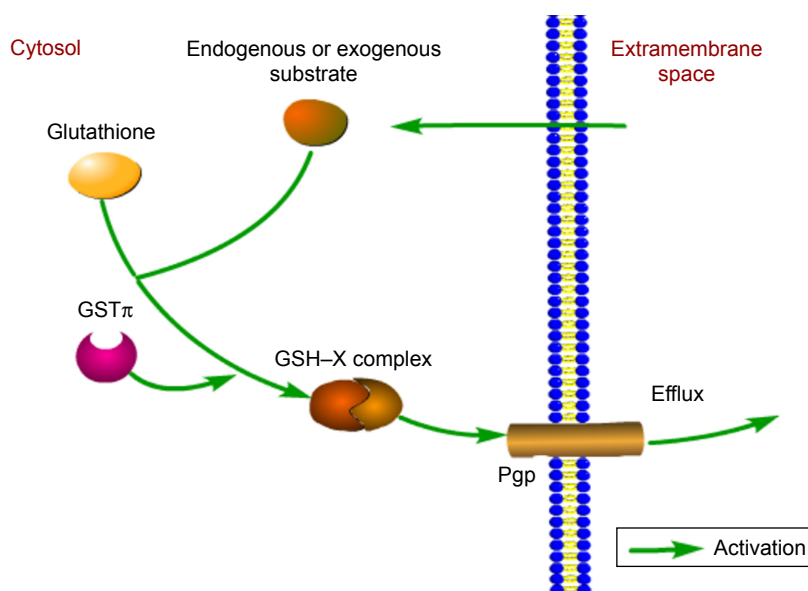


Figure 2 Involvement of GST π in the detoxification of exogenous and endogenous substrates.

Note: In the process, Pgp or MRPs (eg, MRP2) give assistance to GST π to excrete the complex out of the cell.

Abbreviation: GST, glutathione S-transferase.

of oxidative or nitrosative stress, GST π can form homodimers that alter the reduced states of cysteine residues in its structure, resulting in JNK dissociation from the GST π -JNK heterocomplex and causing subsequent activation of the

c-Jun protein. Ultimately, these series of reactions will activate the apoptotic pathways^{33,34} (Figure 4).

Further research indicates that GST π can influence the MAPK pathway both through JNK and TRAF2 modulation.

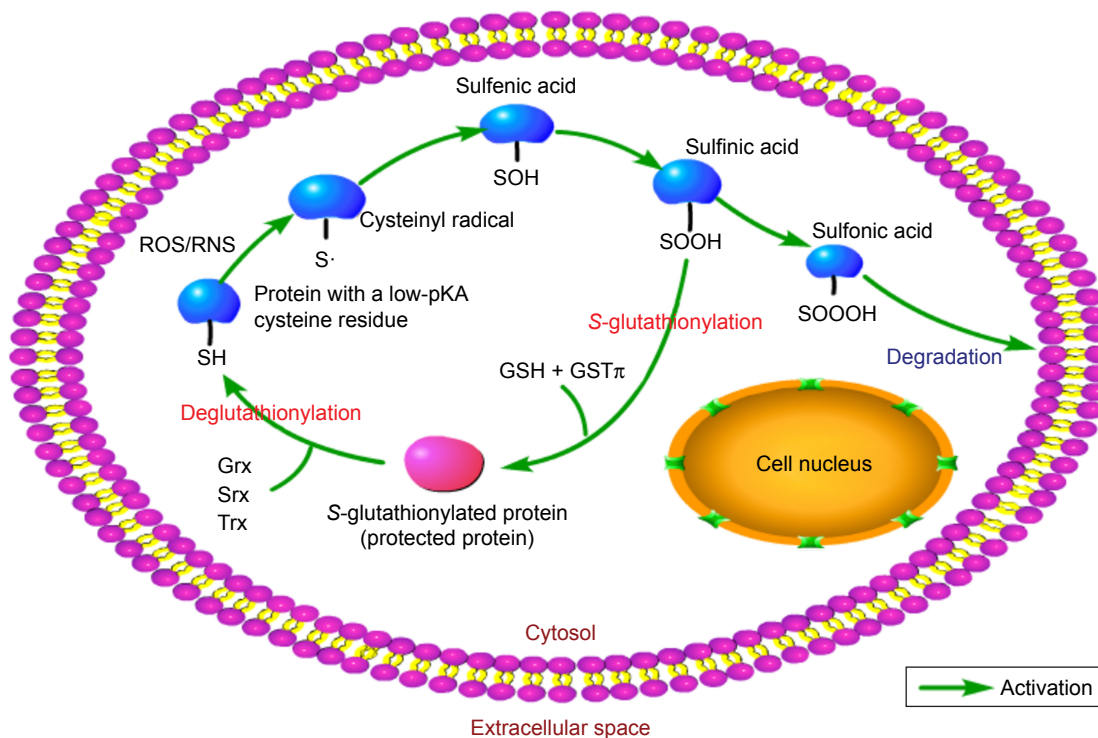


Figure 3 The process of S-glutathionylation.

Notes: In this process, low-pKA cysteine-residue proteins are targeted upon exposure to nitrosative or oxidative stress. The cysteine residues are oxidized to produce protein sulfinic (P=OOH) and sulfenic (P=OH) acids. GSH functions as a thiol donor to facilitate GST π -mediated S-glutathionylation, protecting the target protein from further damage. This reaction is also influenced by enzymes involved in deglutathionylation, such as sulfiredoxin (Srx), glutaredoxin (Grx), and thioredoxin (Trx).

Abbreviation: GST, glutathione S-transferase.

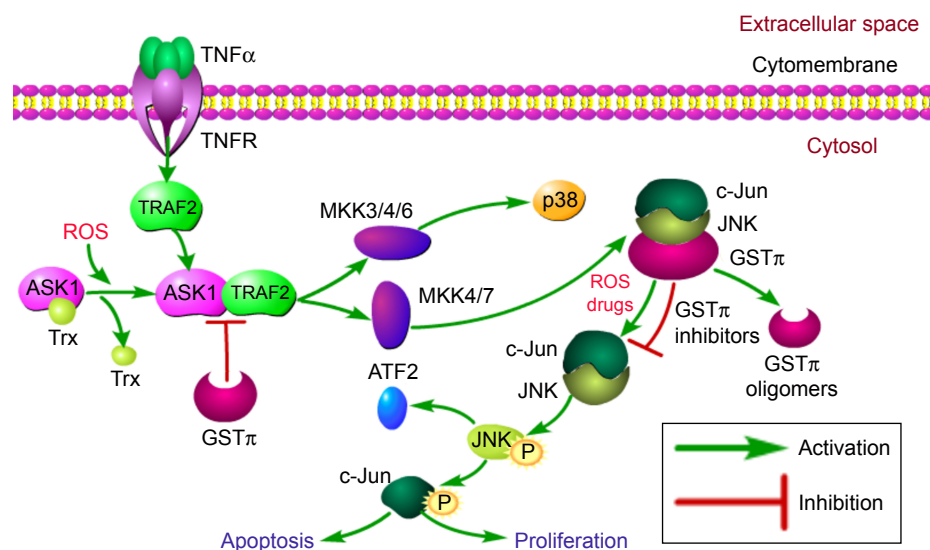


Figure 4 Ligand-binding properties of JNK and TRAF2.

Notes: Under physiological conditions, cells possess low JNK activity. JNK is sequestered in the form of the GST π -JNK protein complex. GST π dissociates from the GST π -JNK complex upon oxidative stress induced by drug administration, resulting in the accumulation of GST π oligomers. This results in c-Jun phosphorylation and/or ATF2 activation, and this progress further impacts downstream events as well. Meanwhile, TRAF2 recruitment to the plasma membrane stimulates the production of ROS. Subsequent oxidation of the previously inactive ASK1-Trx complex causes dissociation of the ASK1 molecule. TRAF2 then binds to ASK1 to activate the ASK1-JNK signaling cascade. Nevertheless, on account of high expression in tumor cells, GST π acts as an endogenous negative regulatory switch by forming complexes with JNK, preventing TRAF2-ASK1 interactions and ultimately inhibiting tumor-cell apoptosis and proliferation.

Abbreviation: GST, glutathione S-transferase.

In-depth analysis of biological information tools has revealed that the TRAF family is strongly linked to GST π . Of all the TRAF members, TRAF2 is expressed most abundantly and has been subjected to intense research.³⁵ Following the activation of TNFR through TNF α binding, TRAF2 is recruited to the plasma membrane, resulting in the production of ROS. ROS generation leads to oxidation of the ASK1 inhibitor thioredoxin, separating and activating ASK1 from the inactive ASK1-thioredoxin complex. ASK1 goes on to bind to TRAF2, which in turn activates downstream-signaling cascades, including the MKK3/4/6-p38 and MKK4/7-JNK signaling pathways^{36,37} (Figure 4). Further evidence from steady-state fluorescence analysis confirms that direct binding between TRAF2 and GST π also exists.³⁸⁻⁴² In tumor cells, GST π can suppress JNK activity and block the interaction between TRAF2 and ASK1 to inhibit tumor-cell apoptosis. Consequently, regarding the formation of multidrug resistance in tumor cells, besides acting as a detoxification enzyme through excretion of drugs to decrease pharmacological efficacy, GST π can also act as a MAPK-pathway inhibitor to improve tumor-cell survival. GST π also represents a scaffold protein to unite different members across signaling pathways.

Other functions

GST π also functions as a chaperone protein that regulates common but significant cellular functions. It interacts with

several key cellular proteins, including TGM2⁴³ and FANCC.⁴⁴ Research has found STAT3 to be an active factor in signal transduction and transcription. STAT3 overexpression is a key molecule that drives the progression of hepatocellular carcinoma, and its activation may be critical in initiating oncogenesis.^{33,45,46} There are studies illustrating that GST π interaction with STAT3 can inhibit the STAT3-signaling pathway, curb aberrant cell-cycle progression, and decrease cell proliferation.³⁶ Furthermore, GST π can participate in nonhomologous end-joining DNA repair by inhibiting DNA-dependent protein kinase.⁴⁷ In conclusion, these findings mentioned show that seemingly disparate functions of GST π in fact work on several aspects of tumor carcinogenesis, making it an ideal molecule for further antitumor therapy research.

Gene variants and polymorphisms

The study of pharmacogenomics in recent decades has proved that genetic polymorphism of drug-metabolizing enzymes is an important mediating factor in determining individual drug responses. Genetic polymorphism is due to a single-nucleotide mutation in the genomic sequence that fundamentally changes how a person responds to chemotherapeutic drugs. Polymorphisms affecting GST π have been documented to exert significant modulatory effects on the biological cascade of carcinogenesis and have been discussed vigorously in the literature.⁴⁸

The human *GSTP1* gene exists as two functionally different variants, with both variants having documented nucleotide transitions from isoleucine to valine at codon 105 (A→G) and alanine to Val at 114 (C→T). This results in four alleles of *GSTP1*: wild-type *GSTP1**A (Ile105 + Ala114), *GSTP1**B (Val105 + Ala114), *GSTP1**C (Val105 + Val114), and *GSTP1**D (Ile105 + Val114).⁴⁹ Besides *GSTP1*, cytosolic GSTs demonstrate clinically significant gene polymorphism (Table 1). These changes occur in active sites, leading to a decrease in encoded protein activity, decreased excretion of foreign substances, and suboptimal catalytic efficiency.

On the other hand, reducing the rate of drug excretion has the benefit of improving an individual's sensitivity to chemotherapeutic drugs, enhancing their curative effects.^{50–52}

There are a great number of reviews that have summarized the associations between individual GST π variability and the drug sensitivity of malignant tumors.^{53–55} However, data are scarce regarding the relationship between GST π and clinical response to chemotherapy. Khrunin et al showed that 104 patients with ovarian cancer with mutant-type GST π possessed longer progression-free survival compared to wild-type GST π .⁵⁶ These results highlight the possibility that

Table 1 Genetic polymorphisms of cytosolic GSTs

Class	Chromosome	Gene	Exon count	Alleles	Nucleotide variability	Reference(s)
α	6p12.2	<i>GSTA1</i>	7	<i>GSTA1</i> *A <i>GSTA1</i> *B	Wild-type Promoter point mutation	116
		<i>GSTA2</i>	8	<i>GSTA2</i> *A	Pro110;Ser112;Lys196;Glu210	117, 118
				<i>GSTA2</i> *B	Pro110, Ser112, Lys196, Ala210	
				<i>GSTA2</i> *C	Pro110;Thr112;Lys196;Glu210	
				<i>GSTA2</i> *D <i>GSTA2</i> *E	Pro110;Ser112;Asn196;Glu210 Ser110;Ser112;Lys196;Glu210	
μ	1p13.3	<i>GSTM1</i>	8	<i>GSTM1</i> *A <i>GSTM1</i> *B <i>GSTM1</i> null <i>GSTM1</i> *1.2	Lys173 Asn173 Gene deletion Duplication	119, 120
		<i>GSTM3</i>	9	<i>GSTM3</i> *A <i>GSTM3</i> *B	Wild-type Protein unchanged	
		<i>GSTM4</i>	10	<i>GSTM4</i> *A <i>GSTM4</i> *B	Tyr2517 T2517C frameshift	
π	11q13.2	<i>GSTP1</i>	7	<i>GSTP1</i> *A <i>GSTP1</i> *B <i>GSTP1</i> *C <i>GSTP1</i> *D	Ile105;Ala114 Val105;Ala114 Val105;Val114 Ile105;Val114	124–128
θ	22q11.23	<i>GSTT1</i>	6	<i>GSTT1</i> *A <i>GSTT1</i> *B <i>GSTT1</i> *null	Thr104 Pro104 Gene deletion	124 129
				<i>GSTT2</i> *A <i>GSTT2</i> *B	Met139 Ile139	130, 131
ω	10q25.1	<i>GSTO1</i>	7	<i>GSTO1</i> *A <i>GSTO1</i> *B <i>GSTO1</i> *C <i>GSTO1</i> *D	Ala140;Glu155 Ala140 Asp140;Glu155 Asp140	132, 133
				<i>GSTO2</i> *A <i>GSTO2</i> *B	Asn142 Asp142	
δ	14q24.3	<i>GSTZ1</i>	12	<i>GSTZ1</i> *A <i>GSTZ1</i> *B <i>GSTZ1</i> *C <i>GSTZ1</i> *D	Lys32;Arg42;Thr82 Lys32;Gly42;Thr82 Glu32;Gly42;Thr82 Glu32;Gly42;Met82	136–138

Abbreviation: GST: Glutathione S-transferase.

genetic variation may have significant effects on susceptibility toward cancer. In future, precise genetic polymorphic screening tests may play a more central role in determining chemotherapeutic treatment regimens for patients.

GST π inhibitors

GST π inhibitors reverse tumor resistance by means of suppressing GST π activity and improving the chemotherapeutic drug sensitivity of tumor cells. Ethacrynic acid (EA) is a classic GST π inhibitor.⁵⁷ However, due its aspecific pharmacological properties in targeting GST π , the newer GST π inhibitors TLK117/TLK199 and NBDHEX may prove to be more promising. Antitumor agents targeting GST π in context are listed in Table 2.

EA and its analogs

EA represents the first clinical application of GST π inhibitors. Previously, it was widely used for decades as a diuretic in clinical research. EA works to halt GST π activity through a number of mechanisms. First, it is able to bind directly to substrate-binding sites of isozymes to inhibit GST π . Second, it is able to induce the combination of α,β -unsaturated ketones and GSH through the nucleophilic addition reaction, depleting GSH and reducing the amount of GSH available to combine with chemotherapeutic agents, thus producing an overall GST π -inhibitory effect by sensitizing a cell to chemotherapeutic agents.⁵⁷ However, the clinical applications of EA have been limited, due to its diuretic properties and lack of enzyme specificity, with long-term intake possibly risking water and salt imbalance.¹⁸

Zhao et al attempted to modify EA using thiazole derivatives of uric acid to strengthen its GST π -inhibitory effects. The team demonstrated that these derivatives had higher GST π -inhibitory activity in comparison to unmodified EA when administered to acute myeloid leukemia parental cells (HL60).⁵⁸ In addition, the combination of EA and GSH has also been proven to possess superior inhibitory activity

over EA alone and is able functionally to inhibit many GST isoenzymes. However, this compound also possesses limited clinical viability, given its tendency toward dissociation by γ -glutamyltransferase.⁵⁹ Burg et al synthesized modified peptidomimetic glutathione analogs of these EA–GSH compounds, which were hypothesized to be stabler against peptidase-mediated dissolution. Unfortunately, these analogs instead reduced its GST π -inhibitory activity, despite demonstrating increased resilience toward γ -glutamyltransferase compared to the unmodified EA–GSH compounds.⁵⁷ Taken together, EA and its analogs still represent novel avenues of research in the search for more efficacious antitumor drugs.

TLK117 and TLK199

Telintra (ezatiostat hydrochloride, TER199, TLK199) is a small-peptide, glutathione-analog molecule and was developed by Telik. Upon entering the body, TLK199 undergoes esterase hydrolysis, which releases TLK117, its activated form that has anti-GST π activity. TLK199 is able to enhance the potency of various antineoplastic agents against various tumor cell lines. The agent is also able to inhibit MRAP1 and prevent the combination of GST π and JNK, resulting in high JNK production that triggers tumor-cell apoptosis.⁶⁰

Furthermore, clinical studies have found TLK199 to be able to promote the maturation of hematopoietic progenitor cells, induce cancer-cell death, and inhibit myeloproliferative diseases.^{61–63} In 2013, TLK199 successfully passed a US Food and Drug Administration audit and was approved to treat low-to intermediate-risk myelodysplastic syndrome. Long-term observation studies have highlighted the ability of TLK199 to enhance bone-marrow maturation and cellularity.⁶⁴

NBDHEX and its analogs

NBDHEX (6-[7-nitro-2,1,3-benzoxadiazol-4-ylthio]hexanol) is a recently developed compound designed as a

Table 2 Antitumor agents targeting GST π in context

Drugs	Selected examples	Functional significance	References
GST π inhibitors	EA and its analogs	Inhibiting detoxification activity, mainly by binding to substrate-binding sites of GST π	139, 140
	TLK117/TLK199	Promoting tumor-cell apoptosis by preventing combination of GST π and JNK and then activating MAPK pathway	62, 141
	NBDHEX and its analogs	Catalyzed by GST π to release nitrogen-mustard segment to induce tumor-cell apoptosis	142–145
GST π prodrugs	GSH or GSH derivatives (TLK286)	Catalyzed by GST π to release high-concentration NO to kill tumor cells directly	146–148
	NO prodrugs (JS-K)		149

Abbreviations: GST, glutathione S-transferase; EA, ethacrynic acid; NO, nitric oxide.

“mechanism-based inhibitor” that exerts potent effects on GST π . Since its first reports by the Tor Vergata University of Rome,⁶⁵ numerous preclinical studies have shown that NBDHEX exerts high GST π -inhibitory activity across a wide range of tumor types. Pasello et al reported that this agent effectively reversed cisplatin resistance in osteosarcoma, alluding toward potentially improved clinical outcomes when using a combination of NBDHEX and cisplatin.⁶⁶ NBDHEX GST π -inhibitory effects have also been observed in HL60 cells and their chemotherapy-resistant phenotype HL60/DNR.⁶⁷ Other cell lines that have demonstrated NBDHEX sensitivity include Ewing sarcoma,⁶⁸ the human mesothelioma cell lines MPP89, MMB1, MSTO211H, and Mero48a,⁶⁹ melanoma cell lines Me501 and A375,⁴² and the non-small-cell lung cancer cell line H69AR.⁷⁰ Further research indicates that aside from inherent antimelanoma activity, NBDHEX also has the ability to enhance the function of temozolomide, with the two able to work synergistically to suppress tumor growth.⁷¹

NBDHEX employs several methods in combating malignant cells. First, it is able to accumulate specifically in tumor cells, remaining unaffected by MRP while maintaining good cell-membrane permeability. Second, this agent can decompose the GST π -JNK complex and promote activation of the apoptosis pathway.^{42,65,67,70–72} Further research has also suggested that TRAF2 plays a key role in facilitating NBDHEX-mediated apoptosis. NBDHEX simultaneously activates JNK and TRAF2, interfering with the effect of GST π via two different pathways, leading to cell-cycle arrest and cell death.³⁴ Intriguingly, researchers have provided further evidence to demonstrate that NBDHEX can also act as an autophagy inhibitor in tumor cells.⁴⁰

Despite NBDHEX's promising anticancer activity, consideration should be given to the relatively low GST π -target selectivity and poor water-solubility.⁷³ In efforts to counter this limitation, a study group designed, synthesized, and screened 40 new NBDHEX analogs.⁷⁴ The group added one or two oxygen atoms on the hydroxyl chain of the NBD bone, forming two NBDHEX analogs, MC3181 and MC3165, that were able to display higher selectivity and better external activity by forming a stable σ -complex with the active site of GST π . After extensive experiments, MC3181 was deemed the more promising compound of the two, due to it having a 50-fold rise in aqueous solubility and higher selectivity toward GST π . This novel compound yielded positive results when administered to several distinct human melanoma cell lines, particularly when used in *BRAF*^{V600E}-mutation melanoma cells.⁷⁵ Moreover, both intravenous and

oral treatment of MC3181 in animals with different types of human melanoma xenografts resulted in astonishing curative effects and a satisfactory safety profile.⁷⁵ In summary, we conclude that NBDHEX and its analogs may serve as potential treatment strategies in the management of patients with melanoma.

Prodrugs of GST π

Both conventional chemotherapeutic and targeted agents have well-established toxicity profiles, with a wide range of adverse effects, eg, bone-marrow suppression, gastrointestinal toxicity, immunosuppression, gastrointestinal toxicity, hepatotoxicity, and cardiotoxicity.⁷⁶ GST π prodrugs appear to have a more favorable adverse-event profile, given that they are ingested as an inactive compound and undergo breakdown to release cytotoxic metabolites only in the presence of high concentrations of enzymes that occur in the proximity of tumor cells, thereby reducing collateral damage to healthy cells.² Additionally, tumor cells generally have heightened expression of GST π , providing ideal conditions for GST π -activated prodrugs. There are two primary classes of GST π prodrugs. The first of these are GSH or GSH derivatives, such as canfosfamide (Telcyta, TER286, TLK286), which have cytotoxic drug segments. When catalyzed by GST π , the prodrug releases cytotoxic compounds. The other type, such as JS-K, consists of a similar structure, but without GST analogs. Upon being catalyzed by GST π , it forms an intermediate with GSH and releases its cytotoxic drug segments. Currently, prodrugs under research comprise TLK286, purine analogs,⁷⁷ sulfonamides,⁷⁸ and brostallicin.

TLK286

L- γ -Glutamyl-3-(bis[bis(2-chloroethyl)amino-phosphinyl]oxy)ethylsulfonyl-L-alanyl-2-phenyl-[2R]-glycine hydrochloride salt (TLK286) represents the most promising GST π -prodrug candidate. It can generate a GSH analog and a phosphorodiamidate, the latter an active, alkylating agent.⁵³ Following activation, the former competitively inhibits molecules that stimulate drug resistance, while the nitrogen-mustard segment induces apoptotic activity by influencing the activities of MAPK, p38 kinase, JNK, MKK4, and caspase 3.^{53,79–81}

In vitro studies have revealed that the GSTP1-null cell lines show a different degree of resistance in response to TLK286 compared with GSTP1^{+/+} cells. These differences were abrogated by cotransfecting these cells with GST π . Similar findings were reflected in in vivo analyses with nude mice. These data support the rationale that tumors with

elevated GST π expression are more sensitive to the cytotoxic effects of TLK286.⁸²

The promising mechanism of action of TLK286 and its positive preclinical data have sparked a series of clinical trials where the prodrugs have been applied alone or in combination with other standard chemotherapeutic agents. Completed Phase II and III clinical trials have indicated that the agent has a nonoverlapping toxicity profile and synergistic effects with carboplatin, paclitaxel, and anthracycline, has no cross-drug resistance and is well tolerated, with patients mostly reporting fatigue and nausea.^{83–88}

A completed Phase I/IIA multicenter dose-ranging clinical trial that sought to assess the safety and efficacy of TLK286 found that the compound was highly efficacious. Patients who underwent TLK286 maintenance treatment experienced prolonged median survival of 16.8 months compared to the 8.8 months experienced by those who did not receive the agent.⁸⁹ These clinical trials provide sound scientific evidence that supports the therapeutic efficacy of TLK286 in managing different types of malignancies.

Nitric oxide (NO) prodrugs

Another class of prodrugs are the NO prodrugs. These medications work by binding to intracellular GST π and undergoing GST π -mediated catalysis. This process releases NO molecules that go on to exert antitumor activity. NO is an ephemeral but pleiotropic molecule. It has been shown to have the capacity to affect several vital functions of the body.⁹⁰ As such, this molecule has been investigated keenly for its role in carcinogenesis, tumor progression, invasion, angiogenesis, and other key biological processes.⁹¹ However, available experimental evidence suggests that NO is a double-edged sword when used to manage tumor diseases. NO itself is a source of cytotoxic molecules, and its deleterious effects are enhanced by its ability to concentrate locally around tumor cells and the tumor microenvironment.⁹² At modest concentrations, NO exerts a protumorigenic response that may benefit tumor growth and survival. Nevertheless, at fairly high concentrations, NO takes on antitumor-agent properties to accelerate tumor-cell death and to inhibit tumor-cell angiogenesis.⁹³ Based on these observations, it is clear that NO has a role to play in combating tumor-cell resistance.

An example of an NO prodrug is *O*₂-(2,4-dinitrophenyl)1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1,2-diolate (JS-K), devised by Keefer et al from the National Cancer Institute.⁹⁴ The antineoplastic properties of JS-K rest on two modes of action: first, it can combine with GST π and then be

activated to release NO at a high concentration to directly kill tumor cells; second, it binds to cellular GST/GSH, depleting its intracellular content to weaken the efflux of chemotherapy drugs in tumor cells.⁹⁵ Furthermore, other literature suggests that JS-K inhibits angiogenesis,⁹⁶ induces cell apoptosis (a process related to PARP, caspase 8 and 9 cleavage, and cell differentiation),^{95,97} destroys double-stranded DNA,^{95,97–99} and is also able to interfere with the cell cycle and its respective signaling pathways.^{92,100–102} These mechanisms are highly codependent, and function in an integrated manner to exert antitumor effects.

Remarkably, flow-cytometry findings have shown that JS-K can improve the formation of acidic vesicle organelles, underscoring its ability to induce autophagy.¹⁰² Furthermore, electron-microscopy observations have indicated that JS-K induces autophagic death in cells.¹⁰² Nevertheless, JS-K was able to spare surrounding healthy mammary epithelial cells. JS-K has been shown to be effective in several types of cancers, of which leukemia and myeloma appear to be the most susceptible.^{92,96,100,103} In addition, it is also efficacious in the treatment of solid tumors, such as breast cancer,^{102,104} lung cancer,^{97,103,105} glioma,^{103,106} prostate cancer,¹⁰⁷ kidney cancer,¹⁰⁸ bladder cancer,¹⁰⁹ colon cancer,¹¹⁰ and hepatocellular carcinoma.¹¹¹ Furthermore, JS-K is tolerated well by healthy tissue. These data indicate that further investigation into JS-K as an alternative chemotherapeutic agent is much needed.^{102–104,109}

It is worth noting that JS-K works synergistically with chemotherapy drugs, such as cytarabine,⁹⁸ bortezomib,⁹⁵ cisplatin, and arsenic.¹¹² JS-K acts as a dose-sparing agent when used with typical chemotherapeutic agents and is able to alleviate the severity of adverse effects as a consequence. While JS-K has been shown to be advantageous in treating cancer, its clinical use has been hindered with reports of poor solubility. Structural modification of JS-K is able to prolong its half-life, and combining JS-K with special nanoparticles can greatly improve its solubility and stability,^{103,113} further improving its prospects for clinical applications. JS-K and many other NO prodrugs represent an innovative biological approach in the development of anticancer therapeutics.

Conclusion

Multidrug resistance to chemotherapy drugs is one of the main obstacles in human cancer chemotherapy and has prompted intense research into discovering novel and innovative mechanisms that can overcome this barrier.^{6,7,20} There is an intimate connection between abnormal GST expression and multidrug resistance, unequivocally implicating GST π ,

a member of the GST family in tumor-drug resistance.⁴ GST π inhibitors and prodrugs are crucial agents that can help reverse multidrug resistance in tumors and increase the therapeutic index of anticancer drugs, which collectively decreases the physical and economic burden of cancer patients. Nevertheless, while the rapid growth of research on development of medication based on GST π inhibition has resulted in clinical studies on several compounds, the drugs that make it to commercial consumption are few. Realistically, fundamental issues that stand in the way of large-scale drug production include the vast number of biological GST family members, with each subtype having their own structural and functional differences, in addition to the existence of several genetic modifiers. The situation is further compounded by the presence of posttranslational modifying factors, such as kinase activities and S-glutathionylation. More in-depth research that clarifies the roles of these components of the GST-detoxification system are much needed, in order to produce compounds that have minimal side effects and high GST π selectivity. While modulation of the GSH-antioxidant system has provided promising preclinical results, some of these compounds demonstrate unacceptable toxicity profiles (eg, buthionine sulfoximine). Having said that, GSH-based medication has also been successfully employed to protect against cisplatin induced nephrotoxicity.^{114,115} The ongoing development of chemical genomics, computer-aided drug design, and more extensive molecular and cellular biology research will serve to be extremely useful in contributing toward the preclinical and clinical development of more efficient GST π -targeting drugs.

Acknowledgments

This study was supported by the Jiangsu Provincial Medical Youth Talent (grant number QNRC2016653); the National Nature Science Foundation of Jiangsu Province (grant number BK20151016); the National Natural Science Foundation of China (grant number 81602441); and the Jiangsu Provincial Key Research Development Program (grant number BE2016794); the Postgraduate Research & Practice Innovation Program of Jiangsu Province (grant number KYCX17_1296).

Disclosure

The authors report no conflicts of interest in this work.

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