Glutathione-degrading enzymes in the complex landscape of tumors (Review)

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Abstract. Glutathione (GSH)-degrading enzymes are essential for starting the first stages of GSH degradation. These enzymes include extracellular γ-glutamyl transpeptidase (GGT) and intracellular GSH-specific γ-glutamylcyclotransferase 1 (ChaC1) and 2. These enzymes are essential for cellular activities, such as immune response, differentiation, proliferation, homeostasis regulation and programmed cell death. Tumor tissue frequently exhibits abnormal expression of GSH-degrading enzymes, which has a key impact on the development and spread of malignancies. The present review summarizes gene and protein structure, catalytic activity and regulation of GSH-degrading enzymes, their vital roles in tumor development (including regulation of oxidative and endoplasmic reticulum stress, control of programmed cell death, promotion of inflammation and tumorigenesis and modulation of drug resistance in tumor cells) and potential role as diagnostic biomarkers and therapeutic targets.

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Abbreviations: ACM, a novel-248 ATF/CRE modifier; AP-1, activator protein 1; AP-2, activating protein 2; ATF, activating transcription factor; C/EBP-β, CCAAT/enhancer binding protein β; ChaC1, cation transport regulator homolog glutathione specific γ-glutamylcyclotransferase 1; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor-2α; EpRE, electrophile response element; ER, endoplasmic reticulum; GBM, glioblastoma multiforme; GCN2, general control nonderepressible 2; GGT, γ-glutamyl transpeptidase; GP5, GGT promoter 5; HCC, hepatocellular carcinoma; hESC, human embryonic stem cell; HNE, 4-hydroxynonenal; ISR, integrated stress response; IκB, inhibitor of κB; KIRC, kidney renal clear cell carcinoma; L2, alveolar type II; Pa, Pseudomonas aeruginosa; ROS, reactive oxygen species; Sp1, specific protein 1; UPR, unfolded protein response

Key words: GSH degrading enzyme, GGT, ChaC1, tumor

Contents

1. Introduction

Glutathione (GSH), also known as γ-glutamylcysteylglycine, is the most common small molecular weight thiol molecule generated in living cells (1). It is extensively present in all eukaryotes and is particularly concentrated in the liver. GSH manifests in two forms, mercaptan reduction (GSH) and disulfide oxidation (GSSG). GSH is the more common form, with a concentration >100 times higher than that of GSSG (2). Synthesized from glutamic acid, cysteine, and glycine, GSH serves a pivotal role in the pathway using niacinamide adenine dinucleotide to establish a reducing environment key for cellular function. A previous study (3) underscored the crucial involvement of GSH in diverse cellular processes, including immunological function, cell proliferation, differentiation and

programmed cell death. As a regulator agent in key signal transduction pathways, GSH is involved in maintaining cellular homeostasis. The dysregulation of GSH expression strongly correlates with onset and progression of numerous types of disease, including tumors (4), liver disease (5), diabetes (6) and neurodegenerative disease (7,8). Tumor cells require elevated GSH levels to combat reactive oxygen species (ROS) and detoxify carcinogens. Thus, decreasing intracellular GSH renders tumor cells more susceptible to oxidative stress and chemotherapeutic drugs (9).

While existing studies (2,4) predominantly focused on the anabolic aspects of GSH metabolism, the catabolic process of GSH has received limited attention (10,11). Previously, the cytoplasm was hypothesized to have no role in GSH catabolism. However, the identification of novel GSH degradation pathways in the cytosol (12,13) underscores the importance of exploring GSH degradation. GSH-degrading enzymes are essential for maintaining GSH homeostasis in cells. Dysregulation of these enzymes significantly impacts GSH homeostasis, leading to pathological changes. Such dysregulation is frequently observed in tumor tissue and has been shown to play an essential role in tumor development (14,15). Since different GSH-degrading enzymes are oriented to either intracellular or extracellular GSH pools, intracellular degrading enzymes directly decrease intracellular levels of GSH. By contrast, extracellular degrading enzyme produces cysteine, providing an additional rate-limiting amino acid for resynthesis of intracellular GSH (16). Therefore, different GSH-degrading enzymes exhibit different effects on cancer, either promoting or suppressing it, and their specific functions varies according to the type of tissue and tumor. In addition, the levels of GSH degradation products glutamic acid, cysteine and glycine serve as growth factors, proliferation stimulators and signal transducers of tumor cells (17,18). Thus, understanding of GSH-degrading enzymes and their roles in cancer is imperative for developing more effective therapeutic interventions.

The present review summarizes the crucial role of enzymes in GSH degradation in tumors, as well as their potential as biomarkers and targets for tumor therapy and their potential directions for clinical translation in tumor therapies.

2. Extracellular and intracellular GSH-degrading enzymes

Initiation steps in the mammalian GSH degradation pathway fall into two categories, intracellular and extracellular degradation (Fig. 1) (10). The first category is classical GSH degradation, which commences with extracellular enzyme γ-glutamyl transpeptidase (GGT) (11). Intracellular GSH is released into the extracellular space via multidrug-resistant protein 1-mediated transporter (19). Once outside the cell, GSH is hydrolyzed to Cys-Gly and glutamate by plasma membrane-bound GGT, marking the initial step in extracellular GSH degradation. Discoveries in the cytoplasmic cation transport regulator homolog (ChaC) family of γ-glutamylcyclotransferases have expanded the understanding of GSH degradation (12,13). This family, including ChaC1 and ChaC2, directly breaks down GSH within the cell into Cys-Gly and 5-oxoproline (11,20).

GGT: Classical perspective. GGT, a core component of the γ-glutamyl cycle (21), has long been associated with GSH degradation, predating the discovery of the ChaC family. Initially considered the sole enzyme capable of degrading GSH, GGT hydrolyzes the γ-glutamyl bond of extracellular reduced and oxidized GSH (22). This results in cleaved glutamate, cysteine and glycine while facilitating the transfer of γ-glutamyl moiety of GSH to either water (hydrolysis) or substrates such as peptides (transpeptidation). Consequently, GGT is classified as a bisubstrate enzyme (23).

GGT is a glycosylated heterodimer protein formed by the non-covalent combination of a heavy chain subunit (relative molecular mass, 50,000-62,000) and a light chain subunit (relative molecular mass 22,000-30,000; Fig. 2) (24). The human GGT family members are synthesized and cleaved by an autocatalytic processing reaction. They have a conserved 'sandwich-like' three-dimensional domain with four layers of αββα folds (23). The catalytic site of GGT consists of two successive regions: Well-characterized donor site, specifying the substrates to which donor γ-glutamyl groups bind, and the acceptor site, about which little is currently known regarding the involved residues (24).

Anchored in the plasma membrane by the N-terminus of the heavy chain across the membrane segment, GGT protein, under physiological conditions, is typically confined to the plasma membrane. It is distributed on the apical surface of epithelial and endothelial cells in glands and lumens. A unique characteristic of GGT is that it is located on the extracellular surface of mammalian cells with a catalytic active site oriented to the extracellular environment (11). The kidney expresses GGT at the highest levels, while notable expression is also found in bile canaliculi of hepatocytes, ducts within the pancreas, the apical surface of the intestinal epithelium and the luminal surface epithelium of many reproductive organs (25).

GGT gene family and proteins. A GGT gene family exists in the human genome (Table I), suggesting that regulating GGT activity may be associated with activating different GGT genes rather than identifying distinct gene loci (26).

The human genome sequence contains 13 GGT homologs, the most active of which is GGT. The GGT gene was first discovered on human chromosome 22 at q11.1-q11.2 (27), although it was also subsequently discovered on additional autosomes (26). In addition, two homologs, GGTLC1 (previously GGTL6, GGTLA4) and GGTLC2, which may only encode the light-chain portion of GGT, as well as at least three other homologs, exhibit activity: GGT5 (formerly GGL, GGTLA1/GGT-rel), GGT6 (formerly rat GGT6 homologous) and GGT7 (formerly GGTL3, GGT4) (28). While GGT5 and GGT1 share 40% of the amino acid sequence (22), GGT5 is not as active in hydrolyzing GSH, GSSG and leukotriene C4 as GGT1 is (29). Despite the absence of verified protein-coding activity, GGT6 and GGT7 exhibit aberrant expression in conditions such as tumors (30-32) and pancreatic disease (33). Furthermore, proteins expressed by the human GGT2 gene share 94% of the amino acid sequence encoded by GGT1 (34), even though GGT2 only encodes inactive pro-peptides. GGT2 also exhibits abnormal

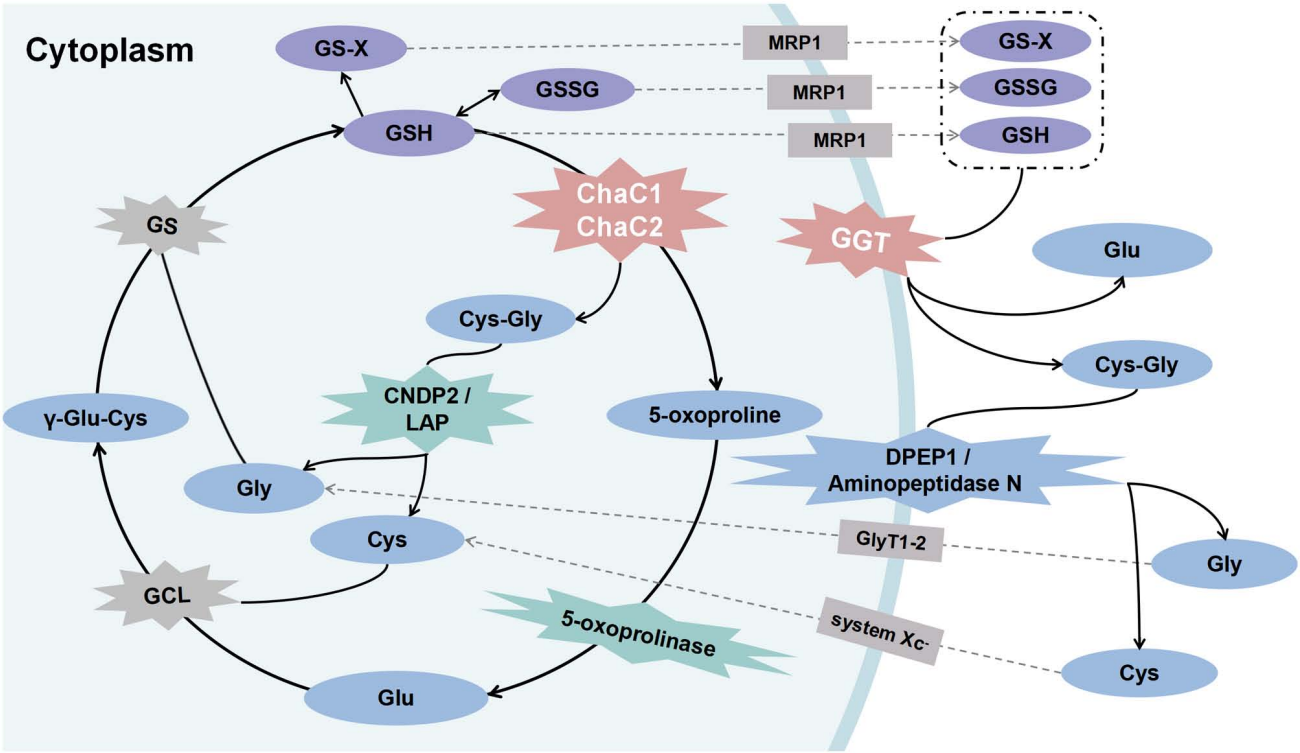

Figure 1. Role of GSH-degrading enzymes in mammals. The classical GSH degradation pathway occurs extracellularly. Intracellular GSH, GSSG and GS-X are released from cells into the extracellular space via MRP1 transporter. GGT, located on the plasma membrane, hydrolyzes them to Cys-Gly and Glu, serving as the initial step in extracellular GSH degradation. Cys-Gly undergoes catalysis to Gly and Cys by DPEP1 or minopeptidase N. ChaC1 and ChaC2 hydrolyze GSH to Cys-Gly and Glu or their cyclized form of 5-oxo-proline directly in the cell. Among these, 5-oxo-proline is hydrolyzed to Glu by 5-oxoprolinase. Cys-Gly, induced by cytoplasmic Cys-Gly peptidase LAP or CNDP2, is hydrolyzed to Gly and Cys, completing the degradation of GSH. ChaC1, glutathione-specific γ-glutamylcyclotransferase 1; CNDP2, carnosine dipeptidase 2; DPEP1, M19 metallopeptidase dipeptidase 1; GCL, glutamate cysteine ligase; GGT, γ-glutamyl transpeptidase; GlyT1-2, glycine transporter 1 and 2; GS, glutathione synthetase; LAP, leucyl aminopeptidase; MRP1, multidrug resistance protein 1.

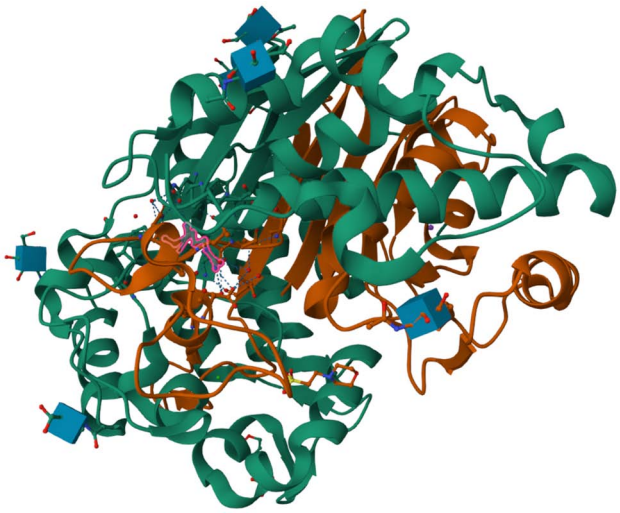

Figure 2. Ribbon drawing of the human GGT1 heterodimer. The heavy and light chain subunits are shown in green and red, respectively. The binding site is displayed in pink. The GGT heterodimer has a stacked αββα-core. Image obtained from US Data Center for the Global Protein Data Bank, Sequence Annotations in 3D: 4Z9O (rcsb.org/) GGT, γ-glutamyl transpeptidase.

expression in tumors (30) and upregulating GGT2 can overcome H_2O_2-induced apoptosis (35). These findings suggest that GGTs play potential roles in disease physiology and pathology.

Catalytic activity of GGT. As a member of the N-terminal nucleophilic hydrolase superfamily (Ntn), GGT uses a highly conserved catalytic mechanism. An N-terminal Thr residue is essential for substrate priming in human GGT (22), with the substrate binding site featuring a key Thr side chain. This facilitates conversion of the γ-glutamyl bond of GSH into an acyl bond, releasing Cys-Gly and glutamate (11).

GGT gene expression. While the human GGT gene is not fully characterized, evidence suggests its existence in multiple copies within the human genome (36). As GGT mRNAs share a common coding sequence and have 59 untranslated regions (UTRs), its structural complexity is evident (37). Understanding of the human GGT promoter remains limited (38).

A number of promoters control human GGT transcription, and the resulting transcripts undergo selective splicing in untranslated regions and coding sequences (37). The initiation of GGT mRNA transcription involves cis-reactive elements, including the cis-regulatory element (TRE) and the binding element for activator protein 1 (AP-1) (38). TRE, also known as 12-O-tetracylacylphobolol 13 acetic acid reactive element, incorporates binding sites for activating protein 2 (AP-2) and specific protein 1 (Sp1; Fig. 3).

A study (39) on HeLa cells highlighted that phorbol 12-myric acid 13-acetic acid enhance the expression of human GGT, pinpointing its binding site at the AP-1 binding site 2,214/2,225 nucleotides upstream from the transcription

Table I. GGT-homologous sequences.

Gene[a]	Previous names	Functional protein	Abnormal expression
GGT1	Gene 6; GGT type I	Functional protein	Dysregulated in various tumors
GGT2	Clone F15; Gene 3 (L10396); GGT type II	Inactive propeptide, 94% homologous to part of GGT1	Low expression in glioblastoma multiforme
GGT3P	Clone F11; GGT3	Pseudogene	Not reported
GGT4P	Gene 12 (L10398); clone F30	Pseudogene	Not reported
GGT5	GGL, γ-glutamyl leukotrienase; GGTLA1/GGT-rel; GGT5 precursor; GGTLA1	Functional protein, 40% homologous to GGT1, exhibits <1/46 activity of GGT1 in hydrolyzing GSH, GSSG and leukotriene C4	High expression of GGT5 is beneficial to the prognosis of hepatocellular carcinoma
GGT6	Rat GGT6 homolog	Not characterized	Overexpressed in low-grade glioma
GGT7	GGTL3, GGT4, GGTL5; GC20M032896	Not characterized	Low expression in gastric cancer; high expression may lead to poor overall survival in hepatocellular carcinoma; GGT7 polymorphic loci rs6119534 and rs11546155 are associated with risk of pancreatic disease
GGT8P	/	Pseudogene	Not reported
GGTLC1	GGTL6; GGTLA4; GGTLA4	Encode only the light chain part of GGT	Not reported
GGTLC2	Gene 1 (L10394); GGTL4; GGTL4	Encode only the light chain part of GGT	Not reported
GGTLC3	γ-glutamyl transferase light chain 3; LOC728226	May encode only the light chain of GGT	Not reported
GGTLC4P	γ-glutamyl transferase light chain 4 pseudogene	Pseudogene	Not reported
GGTLC5P	γ-glutamyl transferase light chain 5 pseudogene	Pseudogene	Not reported

[a]Heisterkamp *et al* (2008) combed a summary of the human γ-glutamyl transferase gene family (27). GGT, γ-glutamyl transpeptidase; GGTLA, γ-glutamyl transferase-like activity.

start site. More research is necessary to understand the transcriptional mechanism of the human GGT gene and promoters.

Although rat and human GGT promoters may have similar structures, the human promoter is more complex. As the rat GGT gene is single-copy, replicating unique genes after species transfer between rats and humans likely results in multiple human GGT genes (40). Therefore, rat GGT may provide insight into human GGT expression and regulation.

In rats, GGT expression is controlled by a tandem P1-P5 promoter, facilitated by variable splicing. This yields transcripts sharing the same coding region and diverse 5'-UTRs (38). These unique promoters have high tissue stage-specificity (38).

GGT regulation. Upregulation of GGT activity is primarily dependent on the Ras protein and its downstream effectors, which include extracellular signal-regulated kinase 1/2 (ERK1/2), p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT and c-Jun N-terminal kinase (JNK) signaling pathways (Fig. 3) (41). The Ras protein is a key regulator of signaling pathways key for normal cell proliferation. Malignant phenotype of tumor cells is caused by the Ras gene, often mutated or active in tumor cells, and leads to aberrant tumor cell proliferation, programmed cell death, invasion and angiogenesis (42).

Through the electrophile response element (EpRE), ERK1/2 and p38MAPK signaling pathways actively contribute to upregulation of GGT promoter 5 (GP5) activity in response to 4-hydroxynonenal (HNE) (43). Following activation by redox signaling downstream of the MAPK signal pathway, the EpRE binding protein, also an oxidative stress-associated transcription factor, induces the dissociation of Nrf2 from Keap1 via redox modification and/or phosphorylation of Nrf2. Subsequently, Nrf2 translocates from cytoplasm to the nucleus, forming heterodimers with other proteins to bind to

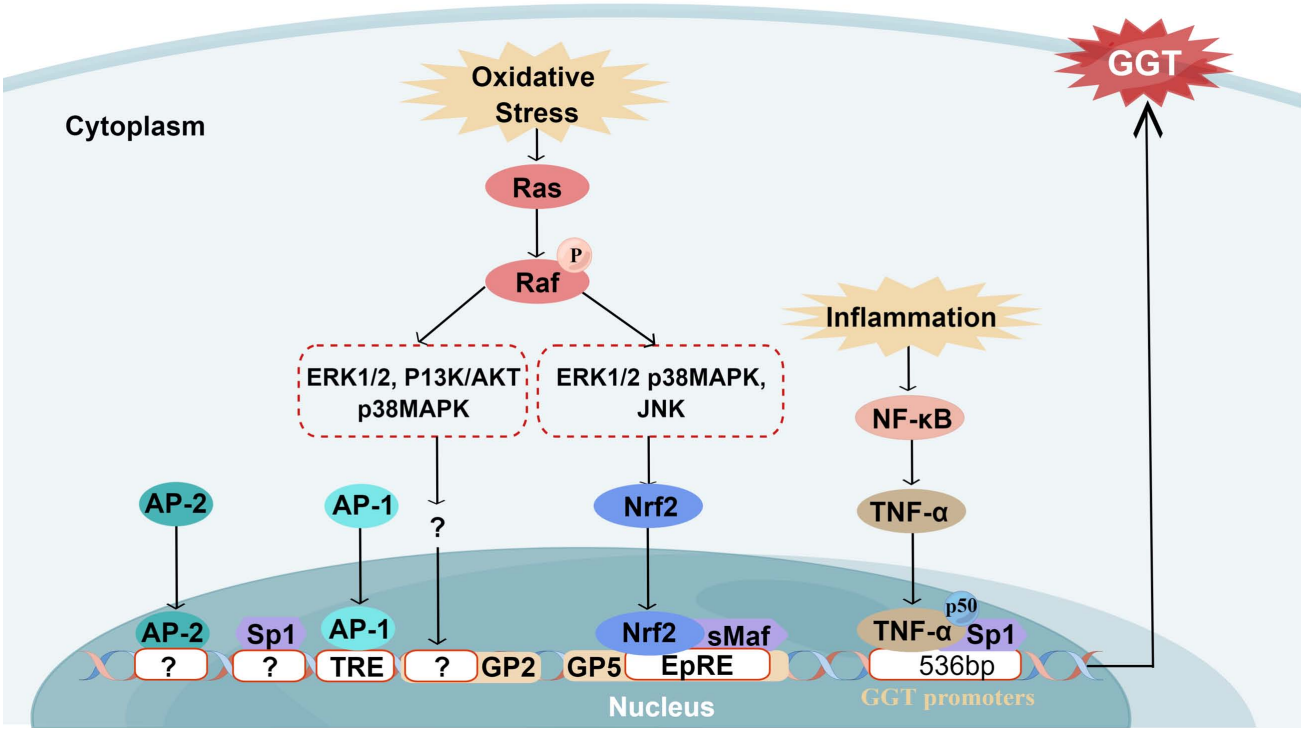

Figure 3. Regulation of GGT. The regulation and expression of GGT remain incompletely characterized. GGT mRNA transcription is co-triggered by multiple potential cis-reactive elements, similar to rat GGT promoters. The proximal region of the GGT promoter contains the binding sites for TRE (often called AP-1 binding elements), AP-2, and Sp1. Ras protein and its downstream oxidative stress effectors, such as ERK1/2, p38MAPK, PI3K/AKT and JNK signaling pathways, serve a key role in upregulating GGT. The P13K/AKT, ERK1/2 and p38MAPK signaling pathways activate Nrf2 via EpRE and sMaf. Nrf2 then transfers from cytoplasm to the nucleus, forming heterodimers with other proteins to bind to EpRE, participating in the upregulation of GP5 activity. Activated H-Ras is also implicated in inducing GP2 activation via downstream ERK1/2, p38MAPK and JNK. Inflammatory conditions activate the NF-κB pathway, initiating downstream TNF-α transcription and transfer to the 536 bp site of the nuclear GGT proximal promoter. The site contains a p50, TNF-α and Sp1 binding site, thereby promoting GGT expression. AP2, activator protein 2; ERK1/2, extracellular signal-regulated kinase 1/2; EpRE, electrophile response element; GGT, γ-glutamyl transpeptidase; GP2, GGT promoter 2; sMaf, small musculoaponeurotic fibrosarcoma; Sp1, specific protein 1; TRE, cis-regulatory element. Drawn with Figdraw (figdraw.com).

EpRE. This leads to the amplification of GGT transcription. In alveolar type II (L2) cells, EpRE motif in the proximal region of GP5 EpRE induces the expression of the major GGT transcript mRNA V-2 in the lung (38). However, pretreatment with ERK1/2 pathway inhibitor (PD98059) or p38MAPK inhibitor (SB203580) partly decreases the expression of GGT mRNA V-2 induced by HNE in L2 cells (38). Furthermore, activated Ras is also implicated in inducing activation of GP2 and increasing expression of GGT transcriptional products and protein in colon cancer cells treated with naphthoquinone during acute oxidative stress (Fig. 3) (41).

Moreover, inflammatory conditions significantly enhance GGT levels by activating the NF-κB pathway. NF-κB, the core transcription factor in the NF-κB signaling pathway, is a dimer family formed by p50/p105/NF-κB1, p52/p100/NF-κB2, c-Rel, p65/RelA and RelB (44). It regulates the expression of chemokines, cytokines, transcription factors and regulatory proteins, playing a crucial role in inflammation and immunity (44). Upon cell stimulation by an external signal, the NF-κB dimer is released from its inhibitor (IκB) and freely transferred into the nucleus. When the NF-κB pathway is activated, it triggers production of pro-inflammatory proteins downstream, such as tumor necrosis factor-α (TNF-α), which in turn causes inflammatory responses and pain. Furthermore, by serving as NF-κB activators, these inflammatory cytokines intensify inflammation by further triggering the NF-κB pathway (45). In the 536 bp site of the proximal promoter of GGT, a binding site exists between p50, TNF-α and Sp1, regulated by the activation of the NF-κB signaling pathway, thereby promoting expression of GGT and inducing inflammatory response (Fig. 3) (46). Moreover, it has been reported (46) that inhibitors of the NF-κB pathway can effectively block the trans-activation of GGT promoters at different levels. For example, remicade, a clinically used anti-TNF-α antibody targeting the p50 and p65 NF-κB subtype of small interfering RNA and curcumin, a well-characterized natural NF-κB inhibitor that is also a dominant negative inhibitor of κBα (IκBα), can inhibit GGT activation through distinct mechanisms. This suggests the involvement of the NF-κB pathway in regulating GGT expression. Therefore, inflammatory conditions may increase GGT synthesis, potentially acting as a cellular protective mechanism under increased oxidative stress or promoting inflammatory progression. Further research is necessary to explore these possibilities.

ChaC1/ChaC2: Additional perspective. In addition to the well-established extracellular GSH degradation mechanism, studies have shown an additional intracellular hydrolysis pathway for GSH degradation (47,48). ChaC protein features a BtrG/γ-GCT fold and distinctive β-barrels surrounded by α-helices (Fig. 4) (13). Mammals exhibit two isoforms of ChaC: Mammalian pro-apoptotic factor ChaC1 (formerly MGC4504) and its homologous counterpart ChaC2. Conversely, only one

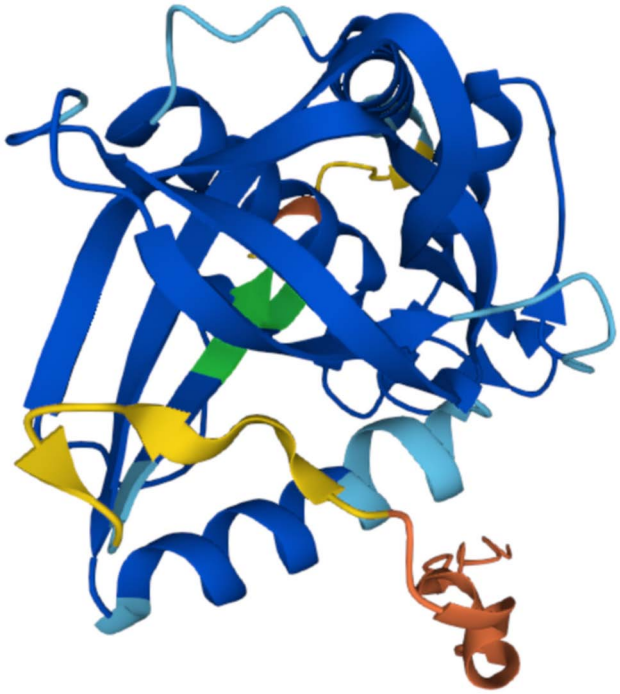

Figure 4. Ribbon drawing of human ChaC glutathione specific γ-glutamylcyclotransferase 1 protein. Very high confident areas are shown in dark blue, Confident areas are shown in light blue, Low confident areas are shown in yellow; very low confident areas are shown in red. The binding site is shown in green. Figure obtained from UniProt, 2018 (uniprot.org/). ChaC, glutathione-specific γ-glutamylcyclotransferase.

ChaC member is present in lower eukaryotes, especially in unicellular eukaryotes (47).

Gene and protein structure of ChaC1/ChaC2. The human ChaC1 gene is on chromosome 15q15.1 and comprises three exons, encoding a protein with 222 amino acid residues and a molecular weight of ~25 kDa (49). ChaC1, 30% identical to mammalian and prokaryotic genes, serves a crucial role in basic physiology (12).

ChaC2 is on chromosome 2p16.2 and encodes a protein with 184 amino acid residues and a molecular weight of ~20.9 kDa (20,47,50). A phylogenetic study (47) highlighted that ChaC2 evolved earlier than ChaC1 and shares key structural similarities with ChaC proteins of lower eukaryotes. Human ChaC2 and ChaC1 share 50% of their protein identity (47). ChaC2 typically exists in dimer crystals with a unique flexible loop 2 structure, with an open conformation that can facilitate close contact with crystallographically adjacent ChaC2 molecules. Additionally, ChaC2 E74Q/E83Q active site mutants exhibit a closed conformation, regulating the degradation activity of ChaC2 to GSH (20).

Catalytic activity of ChaC1/ChaC2. ChaC1, an inducible enzyme, can be expressed under specific stresses or pathological conditions (12,47). It selectively hydrolyzes GSH, producing Cys-Gly and 5-oxoproline (a cyclized form of glutamate) (11,51), thereby accelerating formation of the cellular oxidative environment. The Michaelis constant of ChaC1 for GSH is ~2.2±0.4 mM (47), comparable with the concentration of intracellular GSH (1-10 mM) under physiological conditions. ChaC1 often forms dimers or tetramers, with dimerization being key for regulating enzyme activity and substrate specificity. Under stress or tumor growth, there is an increased need for enzyme breakdown, which leads to formation of dimers or longer oligomers of ChaC1 (20).

ChaC2 is constitutively expressed and exhibits catalytic efficiency for GSH 10-20 times weaker than that of ChaC1 (47). The lower activity of ChaC2 may be partly attributed to flexible loop 2, acting as a gating function to achieve specificity for GSH binding and regulate a constant GSH degradation rate. In addition, the Glu74 and Glu83 residues of ChaC2 are key for directing the conformation of the enzyme and regulating enzyme activity (20).

Expression and regulation of ChaC1/ChaC2. Various signals, including endoplasmic reticulum (ER) and oxidative stress and viral infection, activate ChaC1 promoters in different cell types, cellular processes and diseases through the unfolded protein response (UPR) (12,48,52). ChaC1 is downstream of the protein kinase R-like ER kinase (PERK)/eukaryotic initiation factor-2α (eIF2α)/activating transcription factor (ATF) 4/ATF3/CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) pathway, serving as a pro-apoptotic and pro-ferroptosis component downstream of UPR. ATF4, ATF3, CHOP and C/EBP-β upregulate ChaC1 transcription (Fig. 5). The activation of ChaC1 by UPR primarily relies on ATF4, while the involvement of CHOP, C/EBP-β, and ATF3 is indirect (12). A direct relationship between ATF4 and regulatory elements within the ChaC1 promoter has been identified. A-267 ATF/cAMP response element (CRE) in conjunction with a novel-248 ATF/CRE modifier (ACM), serving as a binding site for ATF4 and ATF3 transcription factors, regulates the activity of the basic ChaC1 promoter. Among these elements, ATF3 predominantly regulates the basal and stress-induced expression of ChaC1 through ATF/CRE, while ATF4 primarily regulates stress-induced ChaC1 expression through ATF/CRE and ACM (48). Additionally, conserved-209 CEBP-ATF response element has a limited impact on regulating human ChaC1 transcription (48,53).

Amino acid starvation can induce the expression of ChaC1 (Fig. 5). The amino acid starvation response activates ATF4 via the general control nonderepressible 2 (GCN2)/eIF2a/ATF4/ATF3 pathway (54). Both ER stress and amino acid starvation induce stress synergistically by activating ATF4 and ChaC1 is one of the downstream targets of ATF4. In regulating ChaC1 expression, C/EBP-β has been observed to recruit ATF4 to the ChaC1 promoter in response to ER stress (48). However, the precise C/EBP-β response element on the ChaC1 promoter remains unclear (48). Further studies are necessary to elucidate the detailed mechanism of C/EBP-β-mediated ATF4 recruitment and its impact on ChaC1 expression. These findings highlight the intricate nature of ChaC1 transcriptional regulation and underscore the importance of maintaining appropriate redox balance in cells (48). The mechanism governing ChaC1 protein expression requires further characterization.

Understanding of the regulation mechanism of ChaC2 is limited. A previous study (47) suggested that ChaC2 is expressed at higher basal levels under physiological conditions than ChaC1. However, under cellular stress such as ER

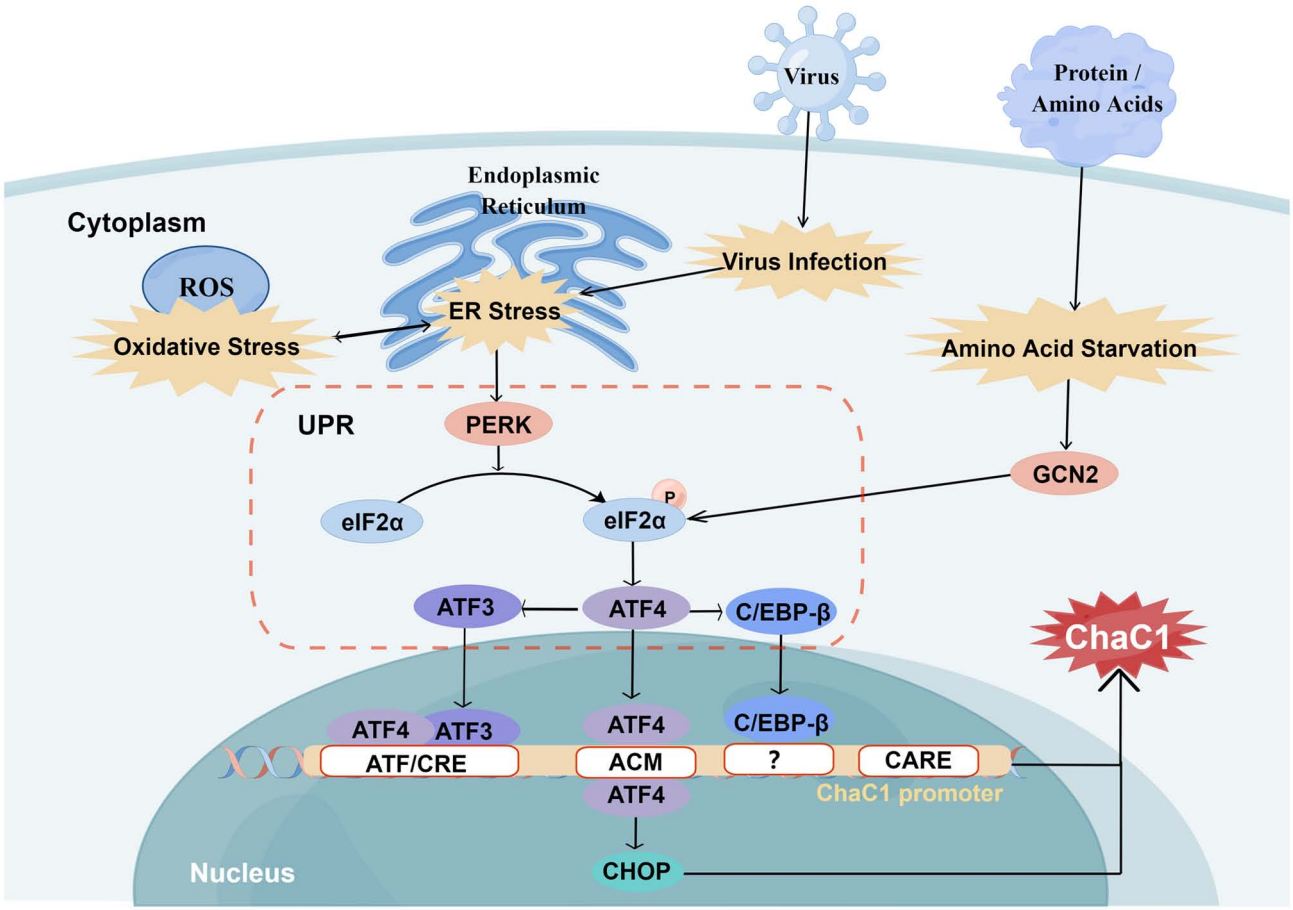

Figure 5. Regulation of ChaC1. Oxidative and ER stress, and viral infection induce PERK/eIF2α/ATF4/ATF3/CHOP cascade activation via UPR. ATF3 may primarily regulate basal ChaC1 expression via ATF/CRE, while ATF4 may mainly regulate stress-induced ChaC1 expression via ATF/CRE and ACM. In response to ER stress, C/EBP-β recruits ATF4 to the ChaC1 promoter, but the precise C/EBP-β response element on the ChaC1 promoter remains unclear. CARE serves a secondary role in regulating human ChaC1 transcription. Amino acid starvation induces ChaC1 expression, activating ATF4 via the GCN2/eIF2a/ATF4/ATF3 pathway. ACM, a novel-248 ATF/CRE modifier; ATF/CRE, activating transcription factor/cAMP response element; CARE, conserved-209 CEBP-ATF response element; C/EBP-β, CCAAT/enhancer binding protein β; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor-2α; ER, endoplasmic reticulum; GCN2, general control nonderepressible 2; PERK, protein kinase R-like ER kinase; UPR, unfolded protein response. Figure constructed using Figdraw (figdraw.com).

stress or amino acid starvation, ChaC1 is upregulated, while ChaC2 expression remains unaffected (47). Thus, ChaC2 serves as a constitutively expressed protein for basal hydrolysis of GSH, acting as a steward for slow and continuous GSH turnover (47).

3. GSH-degrading enzymes in tumorigenesis and progression

Tumor cells enhance GGT expression across the entire cell membrane, facilitating the acquisition of additional cysteine and cystine from GSH in blood and interstitial fluid to replenish intracellular GSH levels (22). Consequently, aberrant GGT expression is observed in various types of cancer, including ovarian (55), renal cell (56), lung (57), stomach (58) and pancreatic cancer (59). Elevated GGT expression is generally associated with poor prognosis, as patients with high levels of GGT in tumors exhibit shorter overall and progression-free survival (60). However, some breast tumor tissues exhibit GGT loss (61).

Studies (54,62,63) of the ChaC family have yielded conflicting findings regarding ChaC expression and its role in tumor tissues, emphasizing the complexity of GSH regulation and function. ChaC1, as a tumor-influencing factor, enhances ER stress, contributing to necroptosis and ferroptosis of multiple cancers, including metastatic melanoma (64), breast (54), prostate (65) and primary liver cancer (66), Burkitt's Lymphoma (67), head and neck squamous cell carcinoma (68), glioblastoma multiforme (GBM) (69), oral squamous cell carcinoma, T lymphoblastic leukemia Molt4 cells and colitis-associated carcinogenesis (15). Decreased ChaC1 expression is an indicator of poor prognosis in kidney renal clear cell carcinoma (KIRC) (70) and certain types of gastric cancer (71,72). Conversely, reports suggest that ChaC1 overexpression may be associated with tumor cell dedifferentiation, proliferation, invasion and migration, leading to lower patient survival rates (73,74). ChaC1 serves as a reliable indicator for poor prognosis of certain types of gastric cancer (63) and melanoma (75,76), as well as an independent indicator for elevated risk for female germ line tumors (including breast and ovarian cancer) (77). Therefore, different effects of ChaC1 may be linked to the specific functions of GSH in different types of tumor tissues.

ChaC2 may be implicated in numerous vital physiological functions, including DNA replication and repair,

cell cycle regulation, RNA and damaged DNA binding, oocyte meiosis and maturation (50). Its important physiological role was initially discerned in undifferentiated human embryonic stem cells (hESCs), where ChaC2 is prominently expressed and maintains cell self-renewal and pluripotency by modulating GSH homeostasis. Conversely, downregulation of ChaC2 decelerates the cell cycle progression of hESCs and triggers cell death (78), underscoring its pivotal role in regulating human growth and development. In pathological functions, ChaC2 exhibits a multifaceted role in tumor tissue. Generally acting as a tumor suppressor, ChaC2 decreases GSH levels in tumor cells, instigates mitochondrial apoptosis and autophagy via UPR and hinders tumor cell proliferation and migration *in vitro* and *in vivo* (79). Therefore, ChaC2 expression is commonly downregulated in tumor tissues, such as gastric and colorectal cancer (79). ChaC2 may exert a tissue-specific function, promoting the survival of tumor cells in specific contexts (50,81,82). The expression of ChaC2 increases with progression of lymph node metastasis and the stage of breast cancer, aligning with findings of GGT loss (61) and increased ChaC1 expression (77) in breast cancer cells. The increased expression of ChaC2 is associated with the high expression of p53 (50), and ChaC2 is the upstream regulator of the main antioxidant regulator Nrf2 (78). In addition mutated p53 regulates NRF2-dependent antioxidant responses that are critical for supporting cancer cell survival (83). Therefore, increased ChaC2 expression may be related to regulating the binding of p53 mutants to Nrf2. When transcription and activity of GSH synthase is increased, the GSH level of the tissue is increased (80). The GSH overexpression then foster the survival and proliferation of tumor cells. Additionally, increased ChaC2 expression targets the Cadherin 1 gene (encoding E-cadherin) mutation, resulting in E-cadherin loss, increased epithelial-mesenchymal transformation and lymph node metastasis, ultimately contributing to the low differentiation of breast cancer (50). Consequently, overall ChaC2 expression is associated with lymph node metastasis and stage progression of breast cancer. ChaC2 promotes lung adenocarcinoma growth by elevating ROS levels and activating MAPK signaling pathways (81).

GGT serves as an extracellular degradation enzyme of GSH, catalyzing enzymatic degradation products to enter cells and providing an additional cysteine source for intracellular GSH synthesis. Intracellular degrading enzymes ChaC1 and ChaC2 play a key role in downregulating intracellular GSH levels, exerting a counteractive effect to regulate intracellular GSH homeostasis. The functions of GGT and ChaC1/ChaC2 include regulation of oxidative and ER stress (38,84), modulation of programmed cell death (85), promotion of inflammation and cell drug resistance (24,86) (Fig. 6).

GSH degradation products such as glutamic acid, glycine and cysteine also play key roles in the metabolic network of the tumor environment. For example, glutamate regulates proliferation, migration and survival of neuroprogenitor cells and immature neurons. While the ability to proliferate and migrate uncontrollably is characteristic of tumor cells, glutamate has been shown to serve as a growth factor and signaling medium in certain types of tumor tissues in both an autocrine and paracrine manner (17). Glycine is involved in cell transformation and tumorigenesis via cleavage into one-carbon metabolism (18). Therefore, exploring the function of GSH-degrading enzymes in tumors may clarify the role of the complex metabolic network of tumors.

Regulating oxidative and ER stress. GSH-degrading enzymes key central to coordinating cellular metabolism by regulating amino acid availability under physiological conditions (11). The modulation of the cellular stress environment relies on regulation of GSH metabolism (4). Tumor cells, in their quest for survival and proliferation, generate abnormally high levels of oxidative stress, partly due to increased cellular redox buffer GSH (87). Therefore, the role of GSH-degrading enzymes is key to regulate the cell stress environment.

The GSH degradation pathway initiated by extracellular GGT effectively controls the intracellular oxidative stress environment (38). GGT deficiency leads to oxidative stress and cellular vulnerability to oxidative damage. Animal studies (88-90) have indicated that GGT knockout mice exhibit 20% of the plasma cysteine concentration in wild-type mice. GGT knockout mice experience increased accumulation of DNA oxidative damage, decreased intracellular GSH levels, elevated oxidative stress and death by 10 weeks of age due to cysteine deficiency (88). Patients with partial GGT homozygous deletions report glutathionuria and neurodevelopmental disorder (91). Hence, maintaining regular GGT expression is essential for cellular GSH homeostasis and protecting cells against oxidative stress.

Cells overexpressing GGT gain an advantage in environments with physiological and limited cysteine concentrations by efficiently utilizing extracellular GSH as a source of cysteine (92). Tumor cells with elevated intracellular GSH levels often induce overexpression of GGT. It has been reported (22) that both GSH depletion and GGT inhibition significantly enhance cytotoxicity under oxidative stress in tumor cells. Tumor cells with high GGT expression demonstrate notable oxidative stress tolerance without DNA damage, while clones with low GGT expression exhibit increased sensitivity to oxidative stress and apoptosis (93). As a marker of oxidative stress, GGT expression in advanced tumor cells surpasses that in early tumor cells. The increased oxidative stress and impaired immune responses may be key for promoting cancer progression to advanced stages and may be induced by inflammatory mediators within the tumor (94). Therefore, upregulation of GGT in tumor cells provides a potential mechanism to resist oxidative stress and foster tumor progression.

Simultaneously, changes in the tumor microenvironment generate persistent ER stress signals in various types of tumor (95) such as colorectal (96), pancreatic cancer (97) and so on. This state has a dual effect on tumor cells, on one hand controlling several tumor-promoting features, on the other hand dynamically reprogramming immune cells and inducing tumor cells autophagy, apoptosis and ferroptosis (95). ChaC1, a component of the UPR pathway, is a target of ferroptosis induced by ER stress signals (12). Thus, ChaC1 is as a key regulator of tumor development, metastasis and responses to chemotherapy, targeted therapy and immunotherapy.

Previous research (78) has also revealed a broader range of functions for ChaC2 than previously understood. ChaC2 inhibits ChaC1-mediated GSH degradation,

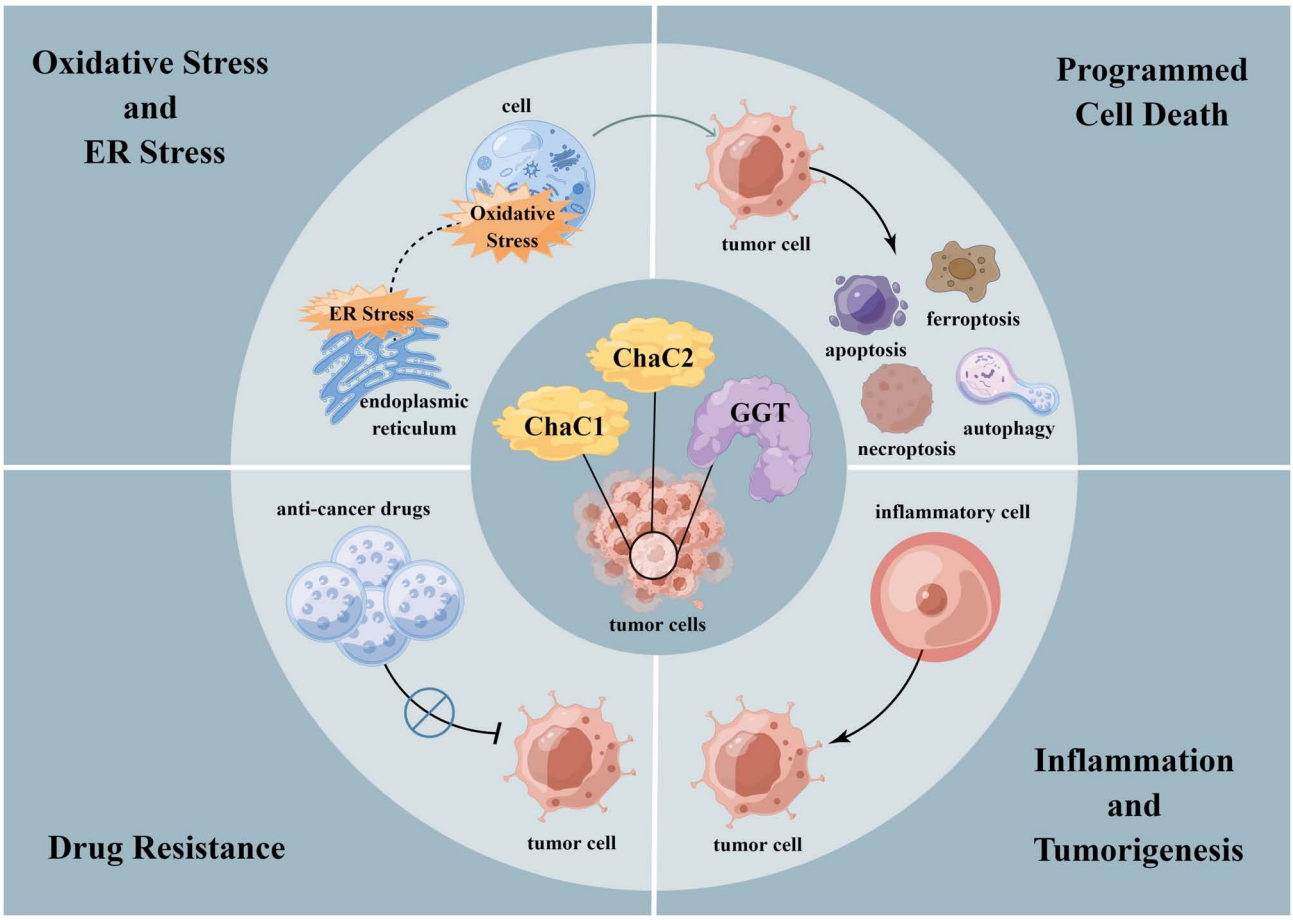

Figure 6. Functions of glutathione-degrading enzymes in tumors. The functions of GGT and ChaC1/ChaC2 include regulating oxidative and ER stress and programmed cell death, promoting inflammation and cell drug resistance. ChaC1, glutathione-specific γ-glutamylcyclotransferase 1; ER, endoplasmic reticulum; GGT, γ-glutamyl transpeptidase. Figure constructed with Figdraw (figdraw.com).

indicating competition with ChaC1 to maintain GSH homeostasis (78). ChaC2 directly regulates GSH production via a ChaC1-independent pathway (78). ChaC2 enhances GSH production by upregulating Nrf2, a key regulator of antioxidation, and its downstream glutamate-cysteine ligase (78). These diverse actions underscore the importance of ChaC2 in maintaining cellular redox homeostasis and antioxidation mechanisms.

Extensive crosstalk exists between oxidative and ER stress as oxidative stress can disrupt redox homeostasis in the ER, triggering ER stress (95). GGT and ChaC1 may be key factors in this mechanism. A study (98) demonstrated that GGT1 and GGT7 stimulate induction of ER stress-related protein, CHOP-10 and immunoglobulin heavy chain binding protein BiP, indicating specific roles for these GGT protein subtypes in ER stress response. This suggests possible crosstalk between GGTDelta1, GGTDelta7 and ChaC1 via the ER stress/CHOP pathway. A recent study (99) proposed that the ATF4/CHOP/ChaC1 signaling pathway might be vital for apoptosis induced by crosstalk between oxidative and ER stress. Under extreme heat stress, cells produce a large amount of ROS, leading to oxidative stress and protein misfolding in the ER, resulting in ER stress and triggering ChaC1-associated UPR. Moreover, induction of ChaC1 serves an essential regulatory role in ER stress-mediated apoptosis of cancer cells induced by the anticancer monosaccharide xylitol, leading to secondary induction of oxidative stress in treated cells and apoptosis (100). This evidence collectively demonstrates the key role of GGT and ChaC1 in mediating crosstalk between oxidative and ER stress.

Modulating programmed cell death. Programmed cell death, encompassing apoptosis, ferroptosis, necrotic apoptosis and autophagy, is instigated by a series of intracellular processes (101). GGT- and ChaC1/ChaC2-mediated intracellular GSH depletion can concurrently or sequentially initiate multiple forms of programmed cell death. Studies (101,102) have indicated that the GSH/GSSG redox status serves as a vital indicator of tumor programmed cell death, consistently associating programmed cell death with a decrease in the GSH/GSSG ratio. Therefore, targeting GGT and ChaC1/ChaC2 to modulate programmed cell death holds implications for tumor therapy.

Regulating apoptosis. Apoptosis, the quintessential programmed cell death process, primarily relies on the caspase family for initiation and is typically characterized by membrane contraction, chromatin concentration and apoptotic body formation (103). In tumor cells, the apoptosis pathway is often impeded by various mechanisms, many of which contribute to intrinsic resistance to chemotherapy, the most

prevalent anticancer therapy (104). Reducing GSH impairs cellular antioxidant regulation, increasing ROS production, thereby accelerating mitochondrial damage and apoptosis induction (101). Consequently, inhibiting GGT1 in tumor cells facilitates induction of apoptotic phenotypes (105). Simultaneously, research (106) on another member of the GGT family has shown that low GGT7 expression may elevate cell ROS levels, inhibiting apoptosis and fostering tumor proliferation. This suggests variations in regulation of ROS levels within the GGT family. ChaC1 overexpression can augment apoptosis by activating caspase-3/9, degradation of poly (ADP ribose) polymerase, induction of autophagy, ROS generation, increased intracellular calcium and loss of mitochondrial membrane potential (69).

Regulating ferroptosis. Ferroptosis, a distinct iron-dependent form of cell death, arises from lethal accumulation of lipid peroxides (107). In tumor cells, evasion of ferroptosis mediated by oncogenes or carcinogenic signaling contributes to tumor onset, progression, metastasis and resistance to treatment. Simultaneously, some tumor cells, owing to specific mutations, elevated ROS levels and other unique biological features, exhibit ferroptosis susceptibility, with their survival hinging on the ferroptosis defense system (108). For example, ferroptosis resistance is conferred by frequently occurring PI3K activating mutations or loss of phosphatase and tensin homolog deleted on chromosome 10 function in human cancer such as lung adenocarcinoma (109) and breast cancer (110) and so on. Conversely, inhibition of the PI3K/AKT/mTOR signaling axis sensitizes cancer cells to ferroptosis induction (111). Consequently, targeting ferroptosis regulation holds implications for cancer immunotherapy and tumor suppression.

Regarding ferroptosis regulation, GGT-activated extracellular GSH catabolism produces iron-derived ROS, inducing lipid peroxidation via NF-κB pathway activation (112). GGT-mediated GSH catabolism via lipid peroxidation enhances NF-κB DNA binding capacity in tumor cells (113). GGT increased intracellular GSH levels (114), restores the reduced GSH/GSSG ratio and reactivates GSH peroxidase 4, a core ferroptosis regulator. Therefore, elevated GGT expression increases tumor cell resistance to ferroptosis, safeguarding cells from ROS and lipid peroxidation, thus driving tumor cell proliferation, metastasis and chemotherapy drug resistance (85,107). In addition, GGT activates the mTORC1 pathway and inhibits integrated stress response (ISR) by modulating cystine-GSH crosstalk. This inhibition of ferroptosis promotes cancer development and other cysteine-deficient diseases (115). Inhibiting GGT impairs GSH ability to restore mTORC1 signaling and ISR, inducing ferroptosis. This implies that the role of GGT in inducing capacity of GSH to release cysteine, rather than GSH itself, modulates the mTORC1 pathway, ISR and ferroptosis. By contrast, ChaC1 induces ferroptosis in tumor cells by activating the GCN2/EIF2α/ATF4 pathway to intensify cystine depletion (116). ChaC1 overexpression depletes GSH, initiating and executing ferroptosis. The deletion of ATF4, an upstream factor of ChaC1, in embryonic fibroblasts, results in a ferric oxide-dependent death phenotype, emphasizing the role of ATF4 as a downstream molecule of the eIF2α/ATF4 pathway (117). Hence, ferroptosis control is associated with ChaC1 expression.

Regulating necroptosis. Necroptosis, a regulated form of cell death primarily dependent on receptor-interacting protein kinase 3 and mixed lineage kinase domain-like, is characterized by widespread cytoplasm and organelle swelling, plasma membrane rupture and release of cell components into the microenvironment (118). This pro-inflammatory form of cell death holds implication for combating pathogen infection, inflammatory progression (119), and therefore may also contribute to early prevention of inflammatory cancer transformation.

Understanding of the impact of GGT and ChaC1/ChaC2 on necroptosis is limited. GGT, one of the virulence factors of *Helicobacter pylori*, has been demonstrated to induce necroptosis in gastric epithelial cells (119). In the early stage of infection, necroptosis may serve a protective role in the mucosa by triggering an immune response (119). However, as the disease progresses, uncontrolled necroptosis exacerbates mucosal inflammation and contributes to the transformation of inflammation into cancer (119). Additionally, ChaC1, by stimulating the GCN2/eIF2α/ATF4 pathway, enhances necroptosis induced by cystine deprivation (54).

Potential regulation of autophagy. Autophagy, a highly conserved cellular degradation process, involves breaking down cytoplasmic components and damaged organelles via lysosomes, recycling resulting macromolecules to shield cells from diverse stressful conditions. Traditionally viewed as a cytoprotective mechanism, autophagy, when excessive, can also instigate cell death and contribute to tumor suppression (120). GSH is implicated in inducing autophagy, where low GSH levels serve as a signal activating autophagy as an adaptive stress response (101). Inhibition of GGT (105) and elevated expression of ChaC1/ChaC2 (69,79) are associated with autophagy phenotype. Nevertheless, evidence (79,121) elucidating the precise mechanisms and interactions between GGT and ChaC1/ChaC2 and initiation and promotion of autophagy remains limited.

Promoting inflammation. Chronic non-specific inflammation is pivotal in tumorigenesis (122) and is a primary environmental factor contributing to the onset and metastasis of specific types of cancer such as non-small cell lung cancer and colorectal cancer (122,123). Various blood tests, either individually or in combination, reflecting local or systemic inflammation, are valuable prognostic indicators for multiple tumor types (124).

GGT serves as a well-established inflammatory marker associated with inflammatory environments and malignancy (125,126). Combining serum albumin and GGT levels serves as an inflammatory indicator for assessing the prognosis of hepatocellular carcinoma (HCC). Patients with elevated GGT and decreased albumin expression exhibit poorer prognosis, revealing significant differences in tumor characteristics, including larger maximum tumor diameters, more tumor nodules and potential for macroscopic vascular invasion and higher serum tumor marker levels (124).

Furthermore, although there is limited research on the effect of human GGT on the colonization of *H. pylori*, it is known that *H. pylori* GGT is a bacterial virulence factor that contributes to the colonization of *H. pylori* in human stomach parietal cells, hence inducing inflammation and gastric parietal cell carcinogenesis (127). In *H. pylori* infection, stimulation

of *H. pylori* GGT accelerates the decrease of GSH levels in gastric epithelial cells, thereby exacerbating ROS production, leading to DNA damage and playing a key role in the emergence of chronic gastritis and gastric cancer (127). *H. pylori* GGT is also key for the tolerogenic effect of dendritic cells in *H. pylori* infection, ensuring bacterial persistence and cross-protection from chronic inflammation and autoimmune diseases by promoting *H. pylori* to reprogram dendritic cells into tolerogenic phenotypes (128). More research is needed to determine whether human GGT functions similarly in *H. pylori*-infected gastric parietal cells.

Human ChaC1 has potential ability to promote inflammation (129). ChaC1 is highly expressed in gastric cancer associated with *H. pylori* infection (129). Infection with *H. pylori* triggers ChaC1 overexpression in gastric epithelial cells, leading to GSH degradation and ROS accumulation, suppressing nucleotide alterations in TP53 that induce tumor suppressor gene expression (130). Overexpression of ChaC1 in *H. pylori*-infected parietal cells may also lead to *H. pylori*-induced somatic mutation, thereby promoting the development of gastric cancer (131). In summary, high expression of human ChaC1 is involved in inducing development of gastric cancer.

ChaC1 expression varies in normal and cystic fibrosis bronchial epithelial cells, with low ChaC1 expression hypothesized to play a significant role in regulating the chronic inflammatory response induced by *Pseudomonas aeruginosa* (Pa) (132). When exposed to Pa and its virulence components, normal bronchial epithelial cells preferentially produce ChaC1. Conversely, low ChaC1 expression is associated with increased secretion of inflammatory markers interleukin-8, interleukin-6 and prostaglandin E2 in the presence of lipopolysaccharide and flagellin stimulation (132). Low ChaC1 expression also promotes increased phosphorylation of NF-κB p65, possibly contributing to the exacerbation of characteristic inflammation in the lungs of patients with cystic fibrosis following Pa infection (132). Cystic fibrosis itself is a risk factor for various cancers, including lung cancer (133).

Drug resistance. High GGT and low ChaC1 expression in cancer cells are pivotal factors in developing drug-resistant phenotypes in tumors (86). GGT expression provides cells with an additional supply of cysteine, while low ChaC1 expression hinders degradation of intracellular GSH. Both factors contribute to GSH consumption in tumor cells during anticancer chemotherapy, leading to drug resistance. Maintaining high intracellular GSH expression preserves redox status, allowing cells to respond to proliferative and differentiation signals in tissue after toxin injury (22) and rapidly supplement GSH during pro-oxidant anticancer therapy.

Chemotherapy-resistant tumors often exhibit high GGT expression, exemplified by cisplatin resistance (134). Platinum (II) class antitumor drugs such as cisplatin and oxaliplatin, widely used in cancer chemotherapy, target DNA damage and overall cytotoxicity in tumor cells, resulting in cell death (135). GGT-related detoxification of Pt(II) medication is a key mechanism of drug resistance (136). Cisplatin can strongly bind to mercaptan metabolites produced by GGT-mediated GSH cleavage, reducing Pt ion entry into cells and inactivating Pt drugs outside the cell (136). GGT-transfected cells show decreased DNA platinization, resulting in decreased sensitivity to cisplatin and lower susceptibility to DNA damage (114). GGT-transfected HeLa cells exposed to cisplatin exhibit a 10-fold increase in cisplatin resistance (134). Systematic inhibition of GGT expression is conducive to suppressing nephrotoxic side effects of cisplatin without diminishing its intracellular toxicity to tumor cells (137). These findings underscore the key role of high GGT expression in promoting drug-resistant phenotypes in tumor cells.

By contrast with GGT, high ChaC1 expression, combined with drugs such as bortezomib and docetaxel, inhibits tumor cell viability by blocking cell cycle progression from the G1 phase to the mitotic S phase. Additionally, high ChaC1 expression increases tumor cell sensitivity to anti-tumor drugs by inducing ER stress and ferroptosis (65,69,138). GSH depletion triggered by the Nrf2/ATF3/4/ChaC1 pathway appears to be the primary factor inducing death in drug-resistant tumor cells (138), positioning ChaC1 as a key target for certain potential anti-tumor drugs such as busatol (139) and glaucocalyxin A (140). Low expression of ChaC1 appears in the drug-resistant phenotype of tumor cells (141), which may be related to enhanced intracellular GSH levels.

4. Role of GSH-degrading enzymes in medicine

Promising tumor biomarkers. Early tumor screening and diagnosis are key for effective treatment and favorable prognosis. Reliable and specific tumor biomarkers are key for accurate screening and diagnosis. The aberrant expression of GSH-degrading enzymes is associated with the prognosis of certain cancers such as gastric adenocarcinoma (72), breast cancer (74) and so on, making them promising tumor biomarkers.

GGT as a biomarker. GGT has been extensively studied and is widely used as a biomarker in clinical practice (142-144). Serum GGT activity is a rapid, reliable and cost-effective method to assess liver function (145). Therefore, GGT has the potential to serve as a tumor biomarker. Elevated GGT expression can indicate the early-stage risk of tumor development, as suggested by an epidemiological study linking high GGT expression with increased risk of prostate cancer development (145). Moreover, high GGT expression indicates poor prognosis in various types of tumors, including renal cell carcinoma and prostate and urothelial cancer (145). The Cancer antigen 19-9/GGT ratio serves as an independent prognostic predictor following radical resection in ampulla carcinoma (146). GGT6 and GGT2 are novel synergistic prognostic biomarkers for low-grade glioma and GBM, potentially aiding early detection (30).

GGT probes have been developed to detect GGT activity accurately for tumor imaging. However, the imaging process often requires organic solvents, posing risks of damage to the enzyme and body. A water-soluble fluorescent probe, TCF-GGT (147), has shown promise by producing red fluorescence during GGT catalytic hydrolysis without interference from the background. This water-soluble compound holds clinical value due to its practical imaging, quick metabolic cycle and excellent water solubility. Due to shallow tissue penetration of many GGT-targeted fluorescent probes, their

clinical application is limited. A novel positron emission tomography imaging probe, ($[^{18}F]GCPA)_2$, has been designed to sensitively and precisely monitor GGT levels in living subjects because of the high sensitivity and intense tissue penetration of positron emission tomography (148). In addition, Cy-GSH, a zero-crosstalk ratio near-infrared GGT fluorescent probe, has also been designed to visualize deep cancer *in vivo*. The probe accurately visualizes tumors and metastases in mice, suggesting that it could be a convenient tool for fluorescence-guided cancer surgery (149). More clinically applicable GGT-targeted probes for visualizing deep cancer need further studies, which may contribute to the early diagnosis of clinical tumors.

ChaC1/ChaC2 as biomarkers. ChaC1, a GSH-degrading enzyme, is biomarker for certain types of tumors. *In vitro*, ChaC1 induces cell death in KIRC cell lines, signifying its potential as an effective marker for poor prognosis in KIRC (70). High ChaC1 expression also serves as a biomarker for adverse outcomes in gastric adenocarcinoma (72), corpus endometrial (73) and breast cancer (74) and uveal melanoma (75). Notably, a positive correlation exists between ChaC1 expression and immune infiltrating cells in corpus endometrial carcinoma (73). In uveal melanoma (75,150), ChaC1 is associated with poor overall, progression-free and disease-specific survival and progression-free interval, making it a promising indicator of unfavorable tumor prognosis. In aggressive breast tumor subtypes such as triple-negative breast cancer, ChaC1 exhibits significant upregulation (151). In addition, malignant breast cancer tissue with active lymph node metastases and high proliferation rates demonstrates elevated levels of ChaC1, supporting its potential for defining tumor progression and metastasis (Table II).

The abnormal expression of ChaC2 as a tumor biomarker has garnered recent attention (50,81). As an independent marker of poor prognosis for certain types of tumors such as breast cancer (50) and hepatocellular carcinoma (82), ChaC2 can monitor early tumor occurrence and treatment effects. Low expression of ChaC2 independently indicates poor prognosis in gastrointestinal tumors. ChaC2 induces mitochondrial apoptosis and autophagy via UPR, serving a pivotal role as a tumor suppressor gene in the onset, proliferation and metastasis of gastric and colorectal cancer (79). Immunohistochemistry and western blot analysis reveal ChaC2 downregulation in most tumor tissue, with ChaC2 expression positively correlating with 3-year survival rate (79). However, recent findings indicate that high ChaC2 expression is inversely correlated with overall survival in patients with breast cancer (50). Elevated ChaC2 expression is also associated with poor prognosis in HCC (82). This underscores the tissue-specific effect of ChaC2 on tumors, necessitating further study (Table II).

Further, the previous study (152) integrates traditional predictors and ChaC1, a novel biomarker, to develop a prognostic score for patients with tumors. The score can be used as a reference for clinical chemotherapy decision-making (152). In evaluating patients with primary breast cancer, including ChaC1 mRNA expression levels in the scoring model led to changes in chemotherapy decisions in 16% of patients (152). In addition, ChaC1 has been included in prognostic models of renal cell carcinoma (153) and glioblastoma (154). Including ChaC1 in tumor prognosis predictions may guide personalized treatment options. ChaC1/ChaC2 may be promising targets for precision medicine.

While studies (50,73,74,151,82) have reported ChaC1/ChaC2 as tumor biomarkers, the development of ChaC1/ChaC2 tracer fluorescent probes remains unexplored. Fluorescent probes are the foundation for tumor-specific imaging in clinical applications. Therefore, developing optical probes to track ChaC1/ChaC2 *in vivo* or *in vitro* is key for fully realizing the clinical potential of ChaC1/ChaC2 as tumor markers.

Therapeutic targets for tumors: Drug selection and emerging drug development. Exploration of GSH-degrading enzyme modulators presents a promising avenue for impeding cancer progression, overcoming tumor resistance to pro-oxidative therapy and preserving tumor sensitivity to chemotherapy (Table III).

Diminishing GGT expression is beneficial for tumor treatment. Classical GGT inhibitors, including glutamate analogs such as acivicin, 6-diazo-5-oxo-L-norleucine, and azaserine (155), have shown clinical toxicity against embryonic cells (156). γ phosphonoglutamate analogs such as GGsTop and derivatives of the lead compound N-[5-(4-methoxybenzyl)-1,3,4-thiadiazol-2-yl]benzenesulfonamide, OU749, represent another class of GGT inhibitors (22). GGsTop targets GGT, can reduce the immunosuppressive function of anti-tumor drugs when used in combination with anti-tumor drugs (157). OU749, a species-specific uncompetitive GGT inhibitor, exhibits low toxicity and a broad therapeutic window for humans.

The development of ChaC1 modulators introduces innovative approaches to cancer treatment. Various potential ChaC1 modulators have been reported, targeting tumor cell cycle arrest and apoptosis (68,158). Metformin, by regulating the Loc100506691-miR-26a-5p-miR-330-5p-CHAC1 axis, induces cell cycle arrest in the G2/M phase, inhibiting cancer cell proliferation (71). Nisin, an apoptotic bacteriocin, induces ChaC1 activation, calcium influx and cell cycle arrest in the G2 phase, leading to increased apoptosis and decreased cell proliferation *in vitro* and *in vivo* (68). Atovaquone, an antiparasitic agent, induces eIF2a phosphorylation at serine 51, amplifying the eIF2α/ATF/CHOP/ChaC1 signaling pathway under ER stress (158).

Natural extracts and their bioactive compounds have recently gained recognition as potential lead molecules in drug discovery for cancer treatment (159,160). They offer an alternative to chemical synthetic drugs, potentially minimizing toxic side effects and holding promise for enhancing clinical anti-tumor therapy. GGT and ChaC1 modulators derived from natural extracts have been explored. Ovothiol, a marine-derived 5(N)-methyl-thiohistidine, is a more effective non-competitive-like GGT inhibitor than traditional counterparts such as acivicin, 6-diazo-5-oxo-norleucine and azaserine (105,161). Ovothiol (105) induces apoptosis and autophagy in GGT-overexpressing cells. The anticancer monosaccharide xylitol (100) and natural Chinese herbal extracts, including dihydroartemisinin (66), artesunate (67), glaucocalyxin A (140), and tanshinone IIA (162), upregulate Prostaglandin-Endoperoxide Synthase 2, p53 and ChaC1 expression. This amplifies the ATF4/CHOP/ChaC1 cascade

Table II. Tumors with altered expression of GGT and ChaC1/ChaC2.

First author/s (year)	Tumor	Expression	(Refs.)
Chen, *et al*, 2023; Hayashima, *et al*, 2022; Chen, *et al*, 2017; Xu, *et al*, 2022	Glioma	Increased ChaC1; increased GGT	(69,85,154,180)
Wen, *et al*, 2017	Nasopharyngeal carcinoma	Increased GGT	(181)
Wang, *et al*, 2022; Mujawar, *et al*, 2020	Oral squamous cell carcinoma	Decreased ChaC1; increased GGT	(140,182)
Mizushima, *et al*, 2016	Head and neck squamous cell carcinoma	Increased GGT	(183)
Lee, *et al*, 2021	Laryngeal cancer	Increased GGT	(184)
Gu, *et al*, 2022	Thyroid cancer	Increased GGT	(185)
Foddis, *et al*, 2022	Malignant pleural mesothelioma	Increased GGT	(186)
Peng, *et al*, 2023; Lee, *et al*, 2021	Lung cancer	Increased GGT and ChaC2	(81,184)
Huang *et al*, 2017; Choi, *et al*, 2017	Esophagus cancer	Increased GGT	(187,188)
Tian, *et al*, 2021; Tian, *et al*, 2022	Hepatocellular carcinoma	Increased GGT and ChaC2	(32,82)
Chen, *et al*, 2021	Intrahepatic cholangiocarcinoma	Increased GGT	(189)
Catalano, *et al*, 2023; Liao, *et al*, 2023	Pancreatic cancer	Increase GGT	(190,191)
Wu, *et al*, 2021; Zhang, *et al*, 2022; Tseng, *et al*, 2021; Liu, *et al*, 2017 Ogawa, *et al*, 2019; Hong, *et al*, 2021; Yang, *et al*, 2019	Gastric cancer	Decreased ChaC1 and ChaC2; increased GGT and ChaC1	(62,63,71,79, 131,192,193)
Xiao *et al*, 2022	Stomach adenocarcinoma	Decreased ChaC1	(72)
Liu, *et al*, 2017; Hong, *et al*, 2021; Hong, *et al*, 2020	Colorectal cancer	Decreased ChaC2; increased GGT	(79,192,194)
Li *et al*, 2021; Yang, 2022; Horie, *et al*, 2020	Renal clear cell carcinoma	Decreased ChaC1; increased GGT	(70,195,196)
Nguyen, *et al*, 2019; Chand, *et al*, 2022; Mehta, *et al*, 2022; Goebel, *et al*, 2012; Mehta *et al*, 2022; Pankevičiūtė-Bukauskienė, *et al*, 2023; Seol, *et al*, 2021	Breast cancer	Increased GGT and ChaC1 and 2	(20,50,74, 77, 151,197,198)
Goebel *et al*, 2012; Shi, *et al*, 2018	Ovarian cancer	Increased GGT and ChaC1	(77,199)
Liu, *et al*, 2022	Uterine corpus endometrial carcinoma	Increased ChaC1	(73)
Schwameis, *et al*, 2016	Uterine leiomyosarcoma	Increased GGT	(200)
Polterauer, *et al*, 2011	Cervical cancer	Increased GGT	(201)
He, *et al*, 2021; Kawakami, *et al*, 2017	Prostate cancer	Decreased ChaC1; increased GGT	(65,202)
Su *et al*, 2021	Bladder cancer	Increased GGT	(203)
Takemura, *et al*, 2019	Urothelial carcinoma	Increased GGT	(204)
Liu, *et al*, 2022	Cutaneous melanoma	Increased ChaC1	(76)
Liu, *et al*, 2019; Jin, *et al*, 2021; Song *et al*, 2021	Uveal melanoma	Increased ChaC1	(75,150)
	Acute myeloid leukemia	Decreased ChaC1	(205)

GGT, γ-glutamyl transpeptidase; ChaC1, glutathione-specific γ-glutamylcyclotransferase 1.

Table III. List of the classical and promising therapeutics affecting GGT or ChaC1/ChaC2.

First author (year)	Functional category	Therapy	Source	Mechanism/pathways	(Refs.)
Lyons, *et al*, 1990	GGT inhibitor	Glutamine analogues (acivicin, 6-diazo-5-oxo-norleucine and azaserine)	Fermentation products of *Streptomyces*	Glutamine analogs are competitive inhibitors that directly modify active site nucleophiles. Due to neurotoxicity, they are no longer used in the clinic	(161)
Han, *et al*, 2007; Watanabe, *et al*, 2017		GGsTop	Chemical synthesis	A phosphonate-basedpotent, non-toxic, highly selective and irreversible GGT inhibitor. Human GGT recognizes the negative charge of GGsTop instead of the C-terminal carboxy group of glutathione by a positively charged critical residue located in the Cys-Gly binding site	(206,207)
King, *et al*, 2009		OU749	Chemical synthesis	Species-specifically non-competitively inhibiting human GGT. OU749 binds to the covalent E-γ-glutamyl complex, the F form of the enzyme	(208)
Azouz, *et al*, 2020		Amlodipine	Chemical synthesis; fully substituted dialkyl 1,4-dihydropyridine-3,5-dicarboxylate derivative	Currently unclear	(164)
Brancaccio, *et al*, 2019		Ovothiols	Marine metazoans	Inhibit membrane-bound GGT of human cells non-competitively and reduce proliferation in GGT-positive cell lines with simultaneous occurrence of a non-protective/cytotoxic form of autophagy, indicating inhibition of GGT activity is likely involved in the modulation of autophagic mechanisms	(105)
Joo, *et al*, 2012	ChaC1 activator	Nisin	Bacterium *Lactococcus lactis*	Currently unclear	(68)
Stevens, *et al*, 2019		Atovaquone	Chemical synthesis of hydroxynaphthoquinone or analog of ubiquinone	Increased EIF2α/ATF4/ChaC1 pathway activity	(158)
Tomonobu, *et al*, 2020		Xylitol	Fruits and vegetables	Induction of CHAC1 by xylitol triggers endoplasmic reticulum stress	(100)
Wang, *et al*, 2019		Artesunate	*Artemisia apiacea*	Increased EIF2α/ATF4/ChaC1 pathway activity	(67)

Table III. Continued.

First author (year)	Functional category	Therapy	Source	Mechanism/pathways	(Refs.)
Wang, *et al*, 2021		Dihydroartemisinin	*Artemisia apiacea*	Increased EIF2α/ATF4/ChaC1 pathway activity	(66)
Wang, *et al*, 2022		Glaucocalyxin A	*Rabdosia japonica*	Increased EIF2α/ATF4/ChaC1 pathway activity	(140)
Guan, *et al*, 2020		Tanshinone IIA	*Salvia miltiorrhiza Bunge*	Currently unclear	(162)
Chen, *et al*, 2021		Omega-3 fatty acids docosahexaenoic acid or eicosapentaenoic acid	Marine metazoans	Increased EIF2α/ATF4/ChaC1 pathway activity	(138)
Tseng, *et al*, 2021	Negatively regulating CHAC1 expression factor	Metformin	*Galega officinalis*	Regulating the Loc100506691-miR-26a-5p-miR-330-5p-ChaC1 axis signaling induces cell cycle arrest in G2/M phase, inhibiting cancer cell proliferation	(71)
Zhai *et al*, 2022	ChaC2 activator	Naringin	Citrus fruit	Upregulating CHAC2 via activation of the Nrf2 signaling pathway	(209)

GGT, γ-glutamyl transpeptidase; ChaC1, glutathione-specific γ-glutamylcyclotransferase 1; EIF2α, eukaryotic initiation factor-2α; ATF4, activating transcription factor 4; miR, microRNA; GGsTop, 2-amino-4-[(3-(carboxymethyl)phenoxy)(methoyl)phosphoryl]butanoic acid.

under ER stress, resulting in decreased intracellular GSH and cysteine, increased intracellular ROS, redox homeostasis disruption, secondary oxidative stress in cancer cells and selective ferroptosis of cancer cells (66,100).

In clinical treatment of tumors, a key goal is to decrease toxicity and resistance of anti-tumor drugs by regulating low expression of GGT (22) and high expression of ChaC1 (65). Blocking nephrotoxicity induced by the anti-tumor drug cisplatin is achieved by inhibiting GGT (137). The unique renal sulfhydryl acid metabolic pathway, involving degradation of GSH by GGT, contributes to cisplatin nephrotoxicity (163). Supramolecular Pt prodrug nano-assemblies inhibiting c-glutamyl transferase prove beneficial for overcoming tumor resistance (136). GGT inhibitor amlodipine, through its anti-inflammatory effect, suppresses p38 MAPK-triggered pro-inflammatory signaling, decreasing expression of TNF-α and other downstream targets while upregulating expression of the transcription factor Nrf2 and the antioxidant protein heme oxygenase-1 (164). Amlodipine diminishes the pro-apoptotic effector/anti-apoptotic protein expression ratio induced by cisplatin, preventing inflammation, oxidative stress and apoptotic damage (165).

Combining ChaC1 overexpression with chemotherapy is advantageous in decreasing cell drug resistance. Temozolomide, for example, induces binding of ChaC1 to the Notch3 protein, inhibiting activation of Notch3. This weakens the Notch3-mediated downstream signaling pathway, inducing glioma cell death and indicating that ChaC1 can influence the cytotoxicity of tumor cells induced by temozolomide (69). The omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid serve a role in reducing the resistance of tumor cells to bortezomib by activating the serine synthesis pathway, mitochondrial folate cycle, methionine cycle-associated GSH synthesis and ChaC1-mediated GSH degradation in tumor cells, ultimately promoting GSH degradation (138).

5. Conclusion

Recent years have seen notable advancements in understanding the role of the intracellular degrading enzyme ChaC family in regulating cellular GSH levels (165,166). Notably, coordinated actions of GGT and ChaC1/ChaC2 have been identified in modulating GSH levels both within and outside the cell (11). These changes in intracellular GSH levels affect key biological processes, including signal transduction, cell survival, proliferation (167) and various forms of cell death (168,169).

The present review underscores the pivotal role of GSH-degrading enzymes, specifically GGT and ChaC1, in cancer development. GGT, via degradation of extracellular GSH, provides cysteine for intracellular GSH synthesis and regeneration, elevating intracellular GSH levels (170). Consequently, GGT can modulate the oxidative stress state of cancer cells, inhibiting programmed cell death (170). GSH regeneration also counteracts the depletion induced by cancer chemotherapeutic agents, potentially leading to development of cancer drug resistance (171). By contrast, intracellular degradation enzyme ChaC1, differentially expressed in tumor cells, serves as a pro-apoptotic molecule by downregulating intracellular GSH levels under amino acid linkage and ER stress (99). ChaC1 induces various forms of cell death, enhancing ER stress and influencing the tumor microenvironment (70,151). Moreover, ChaC1 has a mitigating effect on cell resistance to cancer drugs (69). Together, GGT and ChaC1 regulate both intracellular and extracellular GSH degradation, emerging as promising prognostic markers and therapeutic targets for specific types of tumor such as liver cancer (172,173), breast cancer (74) and so on. Leveraging these enzymes for targeted anti-tumor therapy holds the advantage of precision targeting, minimal damage to healthy cells and reduced toxic side effects, thereby enhancing and reversing tumor drug resistance.

Despite advancements, unanswered questions persist regarding GSH-degrading enzymes. The determinants of functional and regulatory changes in GGT and ChaC1/ChaC2 across different malignancies or settings remain elusive. Although external stimuli induce ChaC1/ChaC2 gene and GGT expression (15,174,175), the specific regulatory mechanisms governing these inductions remain unclear. Additionally, crosstalk effect of different pathways in GGT and ChaC1/ChaC2 regulation requires further investigation. Furthermore, while the roles of GGT and ChaC1/ChaC2 in tumor tissue are known, these enzymes represent only the initial steps in GSH degradation. The interaction between GGT, ChaC1/ChaC2 and downstream enzymes in the complete degradation of GSH, Cys-Gly degrading enzymes and 5-oxo-proline (176-179) remains unclear. The development of tumor treatment strategies targeting GSH-degrading enzymes faces challenges, including different roles of these enzymes in different types of tumor and their impact on the immune response or tumor microenvironment.

In conclusion, GGT and the ChaC family of GSH degrading enzymes, both intracellularly and extracellularly, are key for maintaining GSH homeostasis and serve key roles in normal cellular processes and tumor-related stress conditions. A more comprehensive understanding of these mechanisms may clarify the potential of GSH-degrading enzymes as targets for tumor diagnosis and therapeutic interventions.

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Authors' contributions

TZ and CY wrote and revised the manuscript. CY, DH and WS conceived and designed the review. XZ, SL, LQ, SZ and CZ performed the literature review. DH and WS revised the

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Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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