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Targeting gamma-glutamyl transpeptidase: A pleiotropic enzyme involved in glutathione metabolism and in the control of redox homeostasis

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ABSTRACT

Gamma-glutamyl transpeptidase (GGT) is an enzyme located on the outer membrane of the cells where it regulates the metabolism of glutathione (GSH), the most abundant intracellular antioxidant thiol. GGT plays a key role in the control of redox homeostasis, by hydrolyzing extracellular GSH and providing the cell with the recovery of cysteine, which is necessary for de novo intracellular GSH and protein biosynthesis. Therefore, the upregulation of GGT confers to the cell greater resistance to oxidative stress and the advantage of growing fast. Indeed, GGT is upregulated in inflammatory conditions and in the progression of various human tumors and it is involved in many physiological disorders related to oxidative stress, such as cardiovascular disease and diabetes. Currently, increased GGT expression is considered a marker of liver damage, cancer, and low-grade chronic inflammation.

This review addresses the current knowledge on the structure-function relationship of GGT, focusing on human GGT, and provides information on the pleiotropic biological role and relevance of the enzyme as a target of drugs aimed at alleviating oxidative stress-related diseases. The development of new GGT inhibitors is critically discussed, as are the advantages and disadvantages of their potential use in clinics. Considering its pleiotropic activities and evolved functions, GGT is a potential "moonlighting protein".

1. Introduction

γ-Glutamyltranspeptidase (GGT, E.C.2.3.3.2) is a ubiquitous enzyme evolutionarily conserved from prokaryotes to multicellular eukaryotes such as plants and animals. It belongs to the superfamily of N-terminal nucleophilic hydrolases characterized by an N-terminal catalytic nucleophile capable of cleaving amide bonds [1]. In detail, GGT catalyzes the cleavage of the γ-glutamyl amide bond of a donor substrate and the transfer of the released γ-glutamyl moiety to acceptors such as water (hydrolysis) or amino acids and short peptides (transpeptidation) [2,3]. It is very likely that GGT enzyme originated as a simple γ-glutamyl hydrolase in archaea and ancient prokaryotes and only later developed transpeptidase activity in eubacteria and finally in eukaryotes [4,5]. The enzyme is a dimer encoded by a single gene and is synthesized as a unique polypeptide that autocleaves into a large and a small subunit [6–9]. Mammalian and plant GGTs are glycoproteins, and their molecular mass varies, due to the different degrees of protein glycosylation [9–12]. The primary structure of the small subunit is more conserved than that of the large subunit as contains most of the residues that form the active site, including the catalytic threonine responsible for autocatalytic cleavage and enzymatic activity [13,14]. The crystal structures of GGTs from bacterial sources [13,14], and humans [15] have revealed that the two subunits are tightly intertwined and their interactions are essential for enzymatic activity [13–15]. GGTs from mammals and plants are membrane-bound proteins with the active site directed toward the extracellular space [16–19], whereas the yeast homolog is localized in the vacuolar membrane [20]. In bacteria, GGT is a soluble protein, it is secreted into the extracellular medium in Gram-positive bacteria [21], while it is located in the periplasm in most Gram-negative species [12,22], with the exception of *Neisseria meningitidis*, where it is located on the inner membrane facing the cytoplasm [23].

Although GGT is widespread in most organisms, its physiological functions diversify among various life forms. The most common substrate of GGT is glutathione (GSH), one of the major intracellular antioxidant molecules, found in most prokaryotes and almost all eukaryotes.

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GSH is a tripeptide (γ-L-glutamyl-L-cysteinylglycine) biosynthesized in the cytosol and characterized by an unusual peptide bond between the amino group of cysteine and the γ-carboxyl group of glutamate [24]. Under oxidative stress conditions, GSH can be oxidized to GSSG, and both can be effluxed into the extracellular milieu through the multidrug resistance-associated proteins (MRP), belonging to the ABC transporter gene family [25,26]. GGT is able to specifically cleave the peculiar γ-glutamyl bond, initiating the degradation of extracellular GSH and allowing the cell to use its amino acid components for GSH and/or protein biosynthesis. Therefore, in organisms in which GSH acts as a key antioxidant molecule, including humans and other higher eukaryotes, GGT plays a critical role in GSH metabolism and in maintaining cellular redox homeostasis [27,28]. GGT mutant in *Arabidopsis thaliana* resulted in pleiotropic effects related to biotic and abiotic stress response, antioxidant metabolism, senescence, and photosynthesis [29]. In the yeast *Saccharomyces cerevisiae*, GGT is involved in GSH metabolism and allows the cell to utilize it as a source of cysteine and nitrogen under nutrients limitation [20,30]. Moreover, the expression of GGT in some pathogenic bacteria, including *Helicobacter pylori* and *N. meningitides*, has been associated with their pathogenicity [31–33]. For example, in *H. pylori*, the causative agent of gastritis, peptic ulcers, and cancer, GGT is considered a virulence factor that allows bacterial cells to utilize host GSH as a source of glutamate for growth advantage [31,34]. In *Campylobacter jejuni*, GGT activity was associated with the inhibition of lymphocyte proliferation and persistent bacterial colonization [35]. However, GSH is not found in all prokaryotes, particularly in Gram-positive bacteria, where GGT has distinct physiological roles [36,37]. For example, the protein CapD, belonging to the GGT family, by hydrolyzing the poly-gamma-glutamate from the bacterial peptidoglycan, confers a potent virulence to *Bacillus anthracis* [38]. In humans, GGT is expressed on the luminal surface of excretive and absorptive cells lining glands and ducts in the body, with the highest level of GGT activity in the kidney [2,6]. The expression of GGT is significantly increased in several human tumors, and following liver damage [6,8,39].

In the last decades, efforts have been devoted to uncovering the mechanisms by which GGT is involved in GSH metabolism and in the control of cellular redox homeostasis. However, there is a lack of comprehensive review on the development of GGT inhibitors and their potential clinical applications. Therefore, the aim of the present manuscript is to comprehensively review the role of GGT in the development of various diseases associated with altered GSH and redox metabolism and explore the potential of novel GGT inhibitors as adjuvants in therapies. This review begins with a brief description of the structure and function of human GGT (hGGT), followed by an overview of its physiological involvement in GSH metabolism and control of redox homeostasis, as well as of the pathological conditions related to its overexpression or deficiency. In the second part, the review covers the development of the most promising inhibitors of GGT activity and the latest advances in their application in *in vitro* and *in vivo* models of several diseases, as well as the advantages and disadvantages of their use.

2. Human GGT: structural properties and mechanism of catalysis

In humans, GGT is located on the outer cell membrane and it is typically expressed in the epithelium, most commonly on the luminal surfaces of secretory cells, particularly in the bile ducts, renal proximal tubules, liver, and pancreas [2,17]. hGGT is a heterodimer and the degree of glycosylation varies in different tissues [6]. The molecular mass of the large subunit ranges to 48–66 kD due to the presence of six potential N-glycosylation sites, whereas the small subunit displays a single glycosylation site and ranges to 22–30 kDa [40,41]. An altered N-glycosylation pattern of hGGT is observed in malignant compared to non-malignant tissues, and can be exploited for early tumor detection [10,40,41]. The enzyme is synthesized as an inactive polypeptide or zymogen, which is auto-cleaved into a large subunit containing residues 1–380 and a small subunit containing residues 381–569 [15]. The autoproteolytic cleavage is favored by the highly conserved Thr-381, which also acts as a nucleophile for the enzymatic activity of the mature enzyme. Briefly, the hydroxyl group of Thr-381 in the proenzyme attacks the carbonyl group of the preceding residue (Gly380 in hGGT), to form a tetrahedral intermediate, which is finally cleaved to give Thr-381 as the new N-terminal residue [15]. In hGGT, glycosylation is required for proenzyme maturation, although little is known about the role of this modification in self-processing. Analysis of the crystal structure of glutamate-bound mature hGGT confirmed that it has a stacked αββα-core structure, similar to bacterial GGTs [15]. The N-terminus of the large subunit (residues 5–26) is structured as a hydrophobic single-pass transmembrane domain that anchors the heterodimer to the cell membrane and allows the facing of the catalytic site to the extracellular space, where hydrolysis of GSH occurs via a ping-pong mechanism [15]. Specifically, the catalytic Thr381 in the active site of hGGT acts as a nucleophile and attacks the δ-carbon of the glutamate moiety, leading to the formation of a tetrahedral intermediate (γ-glutamyl enzyme complex) stabilized by two conserved glycines (Gly473 and Gly474). The positioning of the donor substrate in the active site is stabilized by hydrogen bonds between glutamate and key neighboring residues (Arg107, Ser451, Ser452, and Asn401). After hydrolysis, the cysteinyl-glycine dipeptide is released and cleaved by cell surface dipeptidases into cysteine and glycine, while the outgoing γ-glutamyl group can be transferred to a second substrate (the acceptor), probably binding the acceptor site via the conserved residues Lys562 and Tyr403 (Fig. 1).

3. GGT and GSH cycle

The human genome displays at least eight genes encoding GGT-like proteins, but only *ggt1* and *ggt5* produce functional enzymes [42]. In both cases, the mature enzyme is a membrane-bound glyco-heterodimer, which hydrolyzes the γ-glutamyl group of GSH, as the main reaction under physiological conditions [42,43]. However, hGGT1 and hGGT5 are endowed with different substrate specificities, for example, the K_m value for GSH was found to be the same (11 μM) for both hGGT1 and hGGT5, while the K_m values for GSSG differ (9 μM for hGGT1 and 43 μM for hGGT5) [44]. More evidence indicated that hGGT5 can convert leukotriene C4 to leukotriene D4, thus playing a key role in inflammation [43]. On the other hand, GGT2 also found as a full protein, does not localize to the plasma membrane, and fails to autocleave, therefore it is inactive and rapidly degraded within the cell [45]. The transcriptional regulation of hGGT1 is tissue-specific with multiple mRNAs differing in their 5′ untranslated region. The hGGT1 promoter contains binding sites for several transcription factors, like AP-1, AP-2, CREB, GRE, NF-κB, and Sp1, activated by oxidative stress, therefore hGGT1 expression is redox-regulated [46].

In the past, the activity of GGT was proposed by Alton Meister to be involved in the γ-glutamyl cycle, considered responsible for the transport of γ-glutamyl amino acids across the plasma membrane [2]. This theory was widely accepted and has influenced amino acid biochemistry for decades. However, subsequent evidence has significantly weakened the concept of a γ-glutamyl cycle because the reaction of transpeptidation can only occur at concentrations (mM) of the acceptor not compatible with the physiological levels of amino acids in the extracellular space [24]. Currently, it is clear that the main physiological role of hGGT is the hydrolysis of extracellular GSH, which is essential to maintaining cysteine levels in the body [27,46]. The biosynthesis of GSH occurs in the cytosol through the action of two enzymes, glutamate-cysteine ligase, and GSH synthase, which finally adds glycine to obtain GSH. Under physiological conditions, the reduced form of GSH is present in the cells with a concentration 10- to 100-fold higher than the oxidized species (GSSG) [24,47]. Under oxidative stress, GSH is

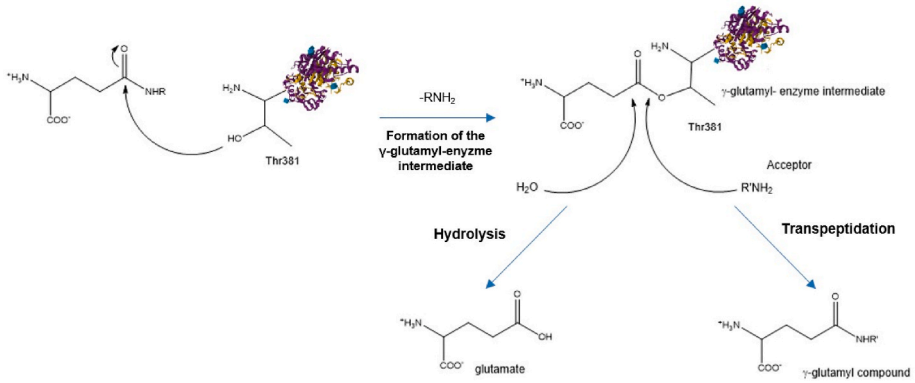

Fig. 1. Mechanism of hydrolysis and transpeptidation of hGGT.

converted by GSH-dependent peroxidases (GPx) into GSSG upon its reaction with hydrogen peroxide (H_2O_2). Then GSSG can be reduced to GSH by GSH reductase (GR) [24,47]. After intracellular biosynthesis, some of the GSH is delivered into specific intracellular organelles, like mitochondria and endoplasmic reticulum, and most quickly delivered to extracellular space, mainly in blood, bile, and lung lining fluid, by membrane transporters MRP family members, like Mrp1, Mrp2, and the cystic fibrosis transmembrane regulator CFTR [25,26]. GSH is rapidly turning over even under normal unstressed conditions and it is rapidly released from cells, at a rate depending on cell types [48,49]. For example, the flow rate of renal GSH was estimated about at 4.1 μmol/h/renal cells to tubules [49]. The extracellular GSH is then hydrolyzed by membrane-bound GGT to glutamate and cysteinyl-glycine, which can be cleaved by membrane-bound dipeptidases to glycine and cysteine, finally transported into the cell by neutral amino acid transporters, such as the alanine-serine-cysteine transport system (ASCT) [50]. In the cell, these amino acids serve as a source for the de novo synthesis of GSH, which contributes to the maintenance of intracellular redox homeostasis and tissue protection against oxidative stress, with the help of GR and GPx (Fig. 2). The involvement of GGT in attenuating oxidative stress has been demonstrated in GGT-deficient mice, which were found to be more susceptible to oxidative stress and lung injury compared to wild-type mice [51,52]. GGT1-deficient mice showed significantly higher GSH concentrations in plasma (175 μM) and urine (15.4 μM) compared with wild-type mice (27.6 μM and 6.2 μM, in plasma and urine respectively) and displayed growth retardation and cysteine deficiency, indicating that GGT1 is essential to hydrolyze extracellular GSH and to provide cells with a continuous source of cysteine [53]. This is also confirmed by the finding that the phenotype of GGT-knockout mice can be rescued by supplementing their drinking water with N-acetyl-cysteine (NAC) [53]. On the other hand, GGT5-deficient mice appeared phenotypically normal, as GGT5 in mice is unable to cleave GSH [54]. In humans, GGT deficiency is a rare autosomal recessive disease and leads to disruptive GSH homeostasis, reproductive defects, mental retardation, and cataract [55]. Usually, patients with GGT deficiency have glutathionuria [55], however, the phenotype is milder compared to mice and varies in symptoms, due to the presence of two functional GGT genes [42,44]. When accumulating extracellularly *in vivo*, cysteine is quite stable and transported inside the cell by ASCT under physiological conditions. Under oxidative stress, the GSSG produced by oxidation of GSH can be delivered outside cells by MRP, where it can be cleaved by GSSG to give cystine, the oxidized form of cysteine [46]. Cystine is present in human serum and interstitial fluid at 66 μM, five times the concentration of cysteine and it is transported into the cell via the sodium-independent cystine/glutamate antiporter xc^- composed of a light chain, xCT, and a heavy chain, 4F2 heavy chain (4F2hc) [56]. However, xCT has a rather restricted expression pattern *in vivo* with the highest levels in the central nervous system and parts of the immune system. Moreover, an elevated expression of xCT has been

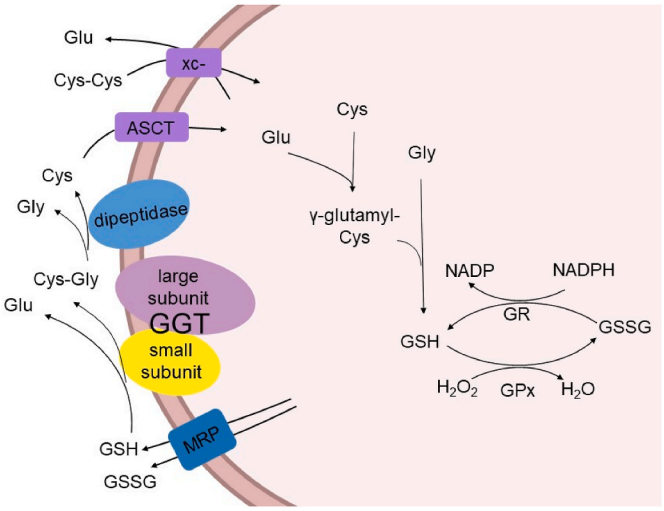

Fig. 2. GGT and GSH metabolism in cancer cells. The biosynthesis of GSH occurs in the cytosol starting from the formation of γ-glutamyl-cysteine, followed by the addition of glycine to give GSH. Intracellular GSH can be oxidized in GSSG to balance the redox environment. In turn, GSSG can be reduced to GSH by GR, and GSH can scavenge H_2O_2 through GPx. Both GSH and GSSG can be delivered out of the cytosol by MRP transporters. Membrane-bound GGT hydrolyzes the extracellular GSH in glutamate and cysteinyl glycine. This latter can be further cleaved into glycine and cysteine, which is subsequently assimilated by the cell through ASCT. GGT can also hydrolyze extracellular GSSG and give cystine, which can be taken up by cells expressing xc^-. Once inside the cells, cystine is reduced to cysteine and serves as a source for the de novo synthesis of GSH and proteins.

reported in cancer cells thus favoring cystine transport [56]. Once inside the cell, most of the cystine is reduced to cysteine and used for the biosynthesis of GSH and proteins, especially in rapidly dividing neoplastic cells [57]. On the contrary, cysteine is very unstable in the extracellular environment of cultured cells, where it is easily oxidized to cystine, which becomes essential to maintain the cellular cysteine pool for GSH synthesis in cancer cells in these conditions [58]. Besides, maintaining cellular redox homeostasis, cysteine and GSH have been reported to play a key role in a plethora of biological processes, including the prevention of ferroptosis, an iron-dependent form of cell death caused by excessive lipid peroxidation and inhibited by GPx4 [59,60].

4. GGT role in detoxification and in serum

Besides GSH and GSSG, hGGT can cleave other substrates with γ-glutamyl moieties in the extracellular space, such as GSHS-conjugates,

and the GSH xenobiotic adducts. Also, synthetic γ-glutamyl compounds can be substrates for hGGT, and γ-glutamyl anticancer prodrugs have been suggested to be selectively cleaved/activated by GGT-expressing cancer cells [8,46]. In the body, many xenobiotics and relative metabolites are first conjugated to GSH by the enzyme GSH *S*-transferase (GST) and then transported to the liver and kidney, where they are cleaved by hGGT to undergo a series of reactions leading to the formation of mercapturic acids, which are eventually excreted in the urine and bile [61]. By catalyzing the conjugation of GSH to electrophilic drug metabolites, GST prevents the drugs from binding to DNA and other nucleophilic cellular components [62]. These GSH-conjugates are transported out of the cell by the MRP transporters and contribute to depleting intracellular GSH [25,26]. Therefore, GGT by hydrolyzing the γ-glutamyl moiety of xenobiotics, previously conjugated to GSH plays a key role in cellular detoxification [48,49]. Compounds that are targeted in this manner include pesticides, herbicides, carcinogens, and even some chemotherapeutics [48,49]. Studies in GGT-knockout mice and in culture have shown that the chemotherapy drug, cisplatin, is metabolized to a nephrotoxin through this pathway [63]. For decades, elevated levels of hGGT in serum, compared to reference levels of healthy individuals, have been considered only a hepatobiliary marker, resulting from the passive release of the enzyme from dead cells originating from liver injury, induced, for example by alcohol and drug abuse [49,64]. Besides the hydrophilic form of GGT present in the serum of healthy individuals (<60U/L), increased circulating hGGT levels have been associated with a remarkable array of chronic conditions and diseases, which include nonalcoholic fatty liver disease, vascular and nonvascular diseases, pancreatitis, type II diabetes, atherosclerosis, and hypertension [65–68]. High serum hGGT levels have been shown to be positively associated with body mass index, LDL cholesterol, triglycerides, and blood glucose, which are the major indicators of obesity, metabolic syndrome, and insulin resistance [69,70]. Large increases in serum hGGT levels have been observed in rare families presenting the dominant genetic condition known as “GGTemia”, in which specific mutations disrupt the GGT1 transmembrane domain [71], and thus enzymes retain their activities but are no longer anchored in the plasma membrane and are released from the cells. However, subjects of these families do not present any symptoms, due to the presence of a hGGT1 wild-type copy. Other studies showed that hGGT circulates in the blood under at least four different molecular forms, and distinct patterns of plasma hGGT fractions have been described for selected liver diseases [72]. A few recent studies have also suggested GGT as an independent prognostic indicator of hepatocarcinoma [73]. In most cancer patients, except those with liver tumors, the level of GGT in serum does not increase unless the tumor (GGT-positive or GGT-negative) has metastasized to the liver [6]. Recently, the finding of GGT activity in exosomes isolated from serum has been predicted to be useful for the differential diagnosis of prostate cancer and renal carcinoma [74,75].

5. Role of GGT in tumor progression and drug resistance

GGT was proposed to play a role in tumor progression in 1985 when the enzyme was found to be over-expressed in pre-neoplastic liver foci of rats treated with chemical carcinogens [76]. Previous studies had observed an increase in mRNA levels and GGT activity in the liver of adult animals in response to the administration or intake of alcohol, xenobiotics, and carcinogens [48,49]. In general, drugs can induce two peaks of GGT activity: the former is reversible, as GGT returns to basal levels after stopping the drug administration; the latter is irreversible and independent of the discontinuation of the drug [9,49]. In tumor development, the reversible increase of GGT activity seems to correspond to the initiation step, when GGT-positive foci and hyperplastic nodules in the liver can be reversed by redifferentiation of the cells. The late irreversible increase in GGT activity, instead, seems to be associated with carcinogenesis [9,46]. Therefore, it was proposed that the expression of GGT could provide the cells with a selective growth advantage during the promotion phase of carcinogenesis [46]. The hypothesis was that GGT, by allowing salvage of the extracellular GSH, could enable the cells to preserve their intracellular GSH levels, thus preventing the toxicity of the carcinogenic compounds. This hypothesis was confirmed by *in vivo* experiments, in which GGT-positive tumors in nude mice were observed to display an increased growth rate compared to GGT-negative counterparts. On the other hand, GGT-deficient mice, which accumulated GSH in the extracellular environment were found to be resistant to the nephrotoxic effects of cisplatin [63]. Subsequent studies from several laboratories suggested that the same mechanism could also confer hGGT-positive tumors with resistance to pro-oxidant anticancer therapy, including platinum-based compounds, alkylating agents, anthracyclins, and radiation. This was confirmed in human patients, in which increased expression of hGGT has been commonly found in both solid and hematologic tumors, as well as in metastases [46,77,78]. In these tumors, GGT is expressed over the entire cell membrane, and provides cells with access to additional cysteine and cystine originating from GSH and GSSG hydrolysis in the blood and interstitial fluid [79]. The cysteine and cystine concentrations in the serum and interstitial fluid are normally sufficient to enable cells to maintain intracellular GSH levels. However, under redox stress, for example in tumor cells in which the high metabolic rate results in enhanced reactive oxygen species (ROS) production, increased amounts of GSH are synthesized and cysteine becomes rate-limiting for GSH synthesis [80]. Therefore, the increased re-synthesis of intracellular GSH could explain the increased resistance of hGGT-positive cancers to pro-oxidant and alkylating agents, enabling the tumors to maintain their redox balance during the fight against ROS and to escape cell death pathways triggered by oxidative stress. For example, in leukemia cells, the drug-resistant lines displayed 3-fold higher hGGT activity and 3-fold more intracellular GSH levels, allowing cells to metabolize more extracellular GSH and take up more cysteine [46]. However, it is worth considering that tumors with high GGT activity may also express high levels of membrane exporters for anticancer agents, further contributing to the development of drug resistance [46].

When studying the role played by GGT in cancer cells grown in culture media, the situation becomes more complex. Generally, in culture media exposed to the high oxygen concentration in air, cysteine is readily oxidized to cystine aided by contaminated Cu^{2+} and Fe^{3+}, therefore cells in culture become dependent on exogenous cystine for proliferation and viability [56,58]. However, the expression of xCT to import cystine is restricted to specific cells, other cells such as lymphocytes cannot survive by themselves in conventional cultures. On the other hand, xCT expression is induced in several cancer cell lines, and its regulation results in fueling cancer cell proliferation [56,58]. Moreover, in cultured cells, hGGT has been observed to exert pro-oxidant effects in the extracellular environment, due to the high reactivity of cysteinyl-glycine, produced by the hydrolysis of GSH [81]. Due to the lower pKa of the sulfydrìl group, cysteinyl-glycine is mainly present in the dissociated form at physiological pH, and it can reduce Fe^{3+} and Cu^{2+} present in culture media, producing H_2O_2 by Fenton reaction. This reaction may be responsible for the formation of mixed disulfides in cell surface proteins and may promote cellular oxidative damage, which favors the progress of preneoplastic foci to malignancy [77,81,82]. Indeed, as H_2O_2 diffuses inside cells through aquaporins [83,84], it can also affect intracellular targets, and promote the resistance phenotype of hGGT-positive cancer cells. These prooxidant effects have been also observed to increase the uptake of antioxidants [85,86]. For example, ascorbic acid has been observed to be oxidized to dehydroascorbic acid, and efficiently transported inside the cells, where it is then reduced back to Vitamin C by cellular reductases [85]. In melanoma cell lines high levels of hGGT and H_2O_2 have been also found to correlate with NF-kB activation [87]. It has been suggested the prooxidant effects of GGT account for NF-kB translocation into the nucleus, but not for DNA binding and consequent gene activation, which instead need the maintenance of a reduced cysteine residue in the NF-kB DNA binding domain

[88]. The increased levels of extracellular cysteine-glycine due to high hGGT activity have been further associated with the formation of adducts with cisplatin, trapped by sort of an 'extracellular detoxification' mechanism, which prevents the entry into the cells and cytotoxicity [77,89].

6. GGT, chronic inflammatory conditions, and oxidative stress-related diseases

Due to the pivotal role played by GGT in modulating redox homeostasis within the cell and in its surroundings, it is not surprising that the alteration of its expression and activity has been associated with the pathogenesis of several diseases characterized by oxidative stress, such as atherosclerosis, endothelial dysfunction, lung airway inflammation, asthma, ischemia-reperfusion injury [90]. For example, in rat renal tissues exposed to ischemia/reperfusion, GGT activity was markedly increased immediately after reperfusion [91]. Chronic low-level activation of inflammatory cells, a status often associated with cardiovascular diseases, cancer, and a number of other chronic conditions has been associated with a minor increase in plasma hGGT levels [90]. Interestingly, hGGT enzyme has been found in the subcellular compartments of inflammatory cells, such as neutrophils and mononuclear cells, and to be released following activation [90]. In addition, hGGT activity has been found to be associated with the peroxidation of isolated low-density lipoprotein in serum [92], and its accumulation was observed within the core of atherosclerotic plaques [93]. Recent studies have finally reported that macrophages characterized by a pro-inflammatory phenotype release a particular form of hGGT in the blood, which contributes to intra-plaque accumulation and thus to the progression of atherosclerosis [94]. Another important function attributed to GGT has been the conversion of nitrosylated GSH (GSNO) to *S*-nitrocysteinglycine, which can release NO in the presence of metal ions [95]. This property is particularly important for the functioning of blood vessels, as demonstrated in rat aorta [96]. Indeed, since GGT is expressed on the luminal face of the endothelium of arterial vessels, it can physiologically mediate the release of bioactive NO from circulating GSNO, thus promoting a vaso-relaxant effect on the vessel wall smooth muscle tone. GGT activity has also been observed to play a key role in lung dysfunctions, being the enzyme localized in the alveolar epithelium of the lung parenchyma, on the luminal surface of type II and Clara cells. Lung epithelial cells are protected by a thin layer of extracellular fluid, enriched by GSH normally present approximately at 400 μM, a concentration 140-fold higher than in the plasma. In the lungs of GGT-deficient GGTenu mice, GSH was found to be 10-fold higher than in WT animals, and this resulted in the protection of airway epithelium from the pathological changes induced in an IL-13-induced allergic inflammation model [97]. It is very likely that extracellular GSH can serve as a scavenger of free radicals produced by neutrophils during airway inflammation. Indeed, loss of GGT activity in the mutant GGTenu1 mouse is protective against asthma, whereas normal mice are susceptible to asthma [97]. Another cause of chronic inflammation of the lung is cystic fibrosis (CF), in which the genetic deficiency of the plasma membrane protein CFTR exposes airway epithelium to recurrent infections, due to the decrease in GSH efflux, thus leading to parenchymal damage, fibrosis, and respiratory failure [98]. In CF patients, the increasing hGGT levels in epithelial lung fluid have been demonstrated to be the result of GGT secretion from neutrophils accumulating in airways during severe inflammatory conditions [98]. Inhalation of GSH has long been practiced in CF patients to counteract the oxidative injury consequent to recurrent lung inflammation [99], however, the benefits of such therapies have been generally negligible, likely due to the increased levels of hGGT, which promptly catabolizes inhaled GSH, thus preventing its potential antioxidant effects. This hypothesis was confirmed by comparing the effects of GSH inhalation in patients with lower levels vs. patients with higher levels of hGGT in bronchial-alveolar lavage fluid. Indeed, GSH inhalation was found to induce decreased levels of pro-inflammatory cytokines and to enhance respiratory function only in subjects with lower fluidic hGGT. Therefore, GSH inhalation should not be recommended as an adjuvant therapy in CF. Rather, a promising alternative might be represented by GGT inhibitors or GSNO inhalation, whose metabolism by hGGT in the extracellular fluid would induce the local release of bioactive NO, thus modulating IL-8 expression in CF bronchial epithelial cells [98].

7. GGT inhibitors: structure and mechanism of inhibition

In summary, the overexpression of hGGT has been correlated with drug resistance in several tumors and with inflammatory conditions associated with different pathologies [46,90]. Therefore, inhibitors of hGGT have been proposed, for example, as adjuvants in chemotherapeutic strategies directed toward hGGT-positive tumors [46]. However, these compounds can also be useful for the therapy of GGT-negative tumors, as by inhibiting hGGT activity in the kidney, they can induce the excretion of GSH in the urine and the rapid decrease of cysteine levels in the blood, thus leading to a general depletion of intracellular GSH in cells [46]. Currently, hGGT inhibitors are being developed for clinical use to sensitize tumors to chemotherapy [46], as well as to ameliorate different pathologies associated with altered hGGT activity [8,90].

The first compounds discovered to inhibit GGT included the glutamine analog Acivicin (L-(aS,5S)-a-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) and the amino acid derivatives, 6-diazo-5-oxo-L-norleucine (DON) and Azaserine (O-diazoacetyl-L-serine) (Fig. 3). These inhibitors have been widely used for *in vitro* and *in vivo* experiments on GGTs [100–102]. Acivicin has been shown to display a protective effect on cisplatin-induced nephrotoxicity and suppress GGT-dependent oxidative damage in ischemic rat kidneys [103]. Benlloch and colleagues showed that inhibiting GGT with acivicin in a model system of melanoma metastasis to the liver in the presence of normal extracellular levels of cysteine, resulted in a 50% reduction in the GSH concentration in the tumors [104]. Acivicin decreased GGT activity to nondetectable levels and reduced GSH resynthesis, without significantly affecting the rate of GSH efflux [104]. However, acivicin has also been reported to inhibit some glutamine amido-transferases, including imidazole glycerol phosphate synthase and guanine monophosphate synthetase, and to inactivate enzymes involved in the biosynthesis of purine and pyrimidine, and amino acids, thus resulting in potent cytotoxicity. In addition, Acivicin was reported to cause bone marrow suppression [100]. DON is a γ-glutamyl diazocompound, acting as an irreversible inhibitor of hGGT, and binding to the small subunit of the enzyme. However, it also lacks specificity as it inhibits enzymes like glutaminases and L-asparagine synthetase and has significant toxic side effects [102]. Serine–borate is another inhibitor of GGT, which is 8-fold more potent in inhibiting GGT1 than GGT5 [44], and it has been shown to abolish the protection exerted by extracellular GSH in alveolar macrophages exposed to hyperoxia-induced by GGT [105]. Another class of less toxic inhibitors of hGGT, has been derived from benzene-sulfonamide, and called OU749 (Fig. 3). Experimental evidence suggests that these inhibitors are uncompetitive and species-specific since they inhibit hGGT, but not mouse, rat, and pig enzymes [106]. The development of more potent and specific inhibitors of hGGT has been hampered for a long time by the lack of structural information regarding substrate/enzyme binding. However, more recently, the determination of the structures of hGGT and apo-hGGT has opened the way to knowledge towards the design of clinically useful hGGT inhibitors [15,107]. Indeed, based on the structural features of the hGGT active site, novel γ-glutamyl analogs have been designed and identified as hGGT inhibitors. For example, a series of γ- (monophenyl) phosphono glutamate derivatives have been recently developed and tested for GGT inhibitory activity [108] Among them, 2-amino-4-(3-(carboxymethyl) phenyl(methyl) phosphono) butanoic acid (GGsTOP) seems to be one of the most promising (Fig. 3). Indeed, GGsTop is an electrophilic phosphonate phenyl ester, endowed

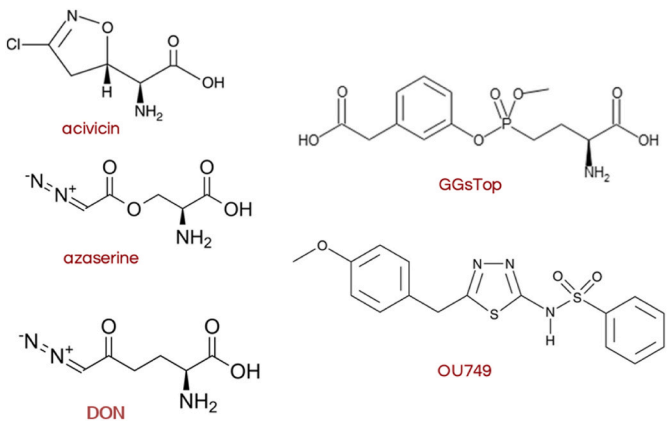

Fig. 3. Synthetic GGT inhibitors. Molecular structure of the most studied synthetic GGT inhibitors: acivicin, azaserine, DON, GGsTop, and OU749.

with 100-fold higher inhibitory activity toward hGGT compared to acivicin; it does not inhibit the asparagine synthetase and does not show cytotoxicity towards human fibroblasts and hepatic stellate cells up to 1 mM [108]. Therefore, GGsTop is considered a highly potent and non-toxic hGGT inhibitor to be used for further *in vivo* as well as *in vitro* studies. For these reasons, GGsTop is currently the most studied hGGT inhibitor.

More recently novel analogs of 2-amino-4-boronobutanoic acid have been designed and evaluated as inhibitors of hGGT. However, all the analogs showed much lower inhibitor potency compared to the original molecule [109]. The inhibition mechanisms of acivicin and azaserine were elucidated by X-ray crystallographic studies of GGT and previously reviewed [9]. Briefly, the molecular structure of acivicin and azaserine complexes compared with the ligand-free enzyme highlighted the formation of an adduct between the oxygen atom of the catalytic Thr and GGT, very similar to the transition state of the catalytic reaction. On the other hand, the proposed mechanism of hGGT inactivation by DON indicates that Thr381 attacks the carbonyl carbon of DON and forms a tetrahedral adduct stabilized by the interaction with Gly473 and Gly474 of hGGT, resulting in the formation of a covalent bond between Thr381 and DON [102]. The inhibition mechanism supposed for GGsTOP also implies that the inhibitor binds covalently to the catalytic Thr, and electrostatically interacts with the Lys562 in the active site of hGGT [108]. In conclusion, acivicin, azaserine, DON, and GGsTop are considered irreversible inhibitors of hGGT. On the contrary, the mechanism of action of OU749 seems to be different, because the inhibitor was predicted to occupy the acceptor binding site [106]. The discovery of OU749 has therefore been considered promising for the design of species-selective inhibitors of hGGT directed to the acceptor binding site [110].

8. Natural products endowed with GGT inhibitory activity

Marine-derived sulfur-containing amino acids have been recently reported to inhibit hGGT activity [111]. These molecules are thiohistidines endowed with key properties in the scavenging of peroxides [112,113], and include: 2-thiohistidine and its tri-methylated derivative ergothioneine, mainly produced by some fungi and cyanobacteria; 5-thiohistidine and its methylated forms, ovothiols (Fig. 4), found in most marine invertebrates, bacteria, and microalgae [114–117]. The position of the thiol group on the imidazole ring of histidine makes ovothiols the most acidic natural thiols and confers their unique glutathione peroxidase-like activity [118–120]. A deep phylogenetic analysis of the genes responsible for ovothiol biosynthesis has pointed to a widespread occurrence of these natural products, from microorganisms to multicellular eukaryotes [121,122].

Ovothiols have indeed recently attracted the research community's interest for their therapeutic potential [113]. Ovothiol, isolated from sea urchin eggs, and oxidized into disulfide form, is known to induce mixed cell death of apoptosis and autophagy in HepG2 and leukemia HG3 cell lines [111]. This effect has been correlated to ovothiol ability to inhibit hGGT activity in hGGT-positive cancer cells, like HepG2 and HG3 [111]. Indeed, ovothiol and its precursor 5-thiohistidine in disulfide have been found to be at least 10-fold more potent in inhibiting hGGT activity compared to DON and ergothioneine. However, ovothiol, 5-thiohistidine, and ergothioneine act as reversible inhibitors of GGT following a non-competitive-like behavior [111]. Both these properties make these inhibitors very promising and advantageous compared to others. For example, reversible inhibitors can be easily modulated *in vivo* compared to irreversible ones. Moreover, non-competitive inhibitors of hGGT can be more advantageous *in vivo* as they are more efficient in inhibition when the physiological substrate GSH rises in concentration, differently from competitive inhibitors, which become less potent when GSH concentration increases, following hGGT inhibition [123]. The finding that

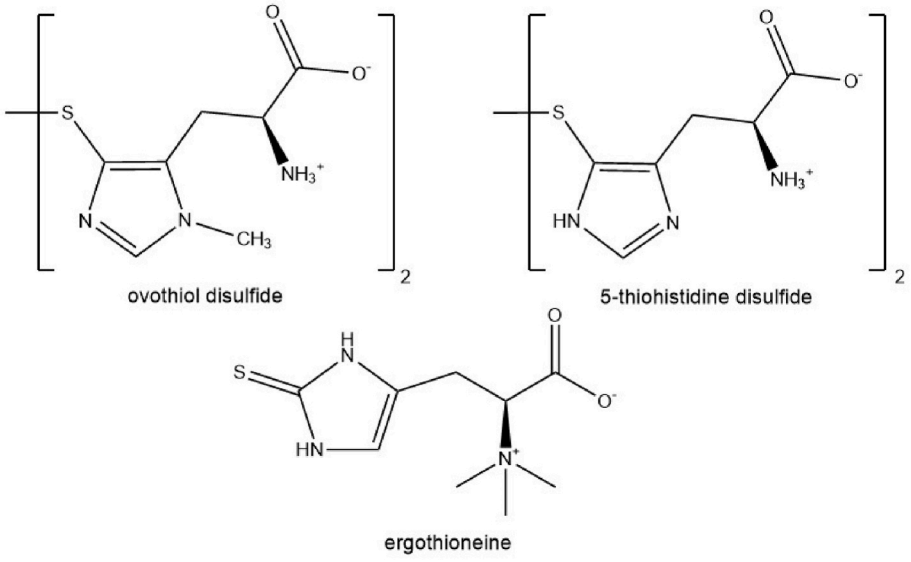

Fig. 4. Natural sulfur-containing histidine derivatives displaying GGT inhibitory activity. Molecular structure of ovothiol and 5-thiohistidine in disulfide form and ergothioneine in thionic form.

ovothiol can inhibit hGGT and synergically induce autophagy in hGGT-positive tumoral cells also points to an interesting correlation between hGGT inhibition and the induction of an autophagic mechanism. While this hypothesis needs further confirmation, the fact that GGT inhibition blocks cysteine supply to cells [46] and that another GGT inhibitor, like DON induce autophagy [111] supports the hypothesis that cells can undergo a sort of starvation leading to suicide [111]. Molecular simulations by docking analysis predicted that ovothiol and 5-thiohistidine in their disulfide forms fit into the binding pocket of hGGT, and interact with the same key residues in the donor and the acceptor binding site, responsible for the interactions of hGGT with GSH [124]. The predicted small distance between the –OH group of the catalytic Thr381 and the sulfur atom of ovothiol (2.62 Å) and 5-thiohistidine (2.36 Å), compared to ergothioneine (3.03 Å) may explain the stronger inhibition of hGGT by 5-thiohistidine derivatives. Moreover, the additional interactions of ovothiol and 5-thiohistidine in disulfides with one or two residues in the acceptor site may explain the stronger inhibitory activity of both molecules compared to DON. Based on these observations, it has been proposed that the initial nucleophilic attack by Thr381 to one of the oxidized sulfur atoms of ovothiol and 5-thiohistidine, leads to the formation of a reversible ovoS–enzyme complex [124]. On the contrary, ovothiols have been reported to exhibit no toxicity towards non-proliferating cells [125] and to exert protective activities in non-tumoral cells [126,127]. Ovothiol, for example, is known to exert protective activity in an *in vitro* model of endothelial dysfunction associated with diabetes, through intracellular NO and GSH modulation [126], and in *ex-vivo* inflamed skin tissues, also promoting the translocation of Nrf2 into the nucleus in keratinocytes [127]. It is interesting to highlight that, ovothiol bioavailability in endothelial cells, showed that ovothiols added to culture in disulfide enter inside the cells [126], where they are likely reduced by redox exchange with GSH [118–120]. On the contrary, in hGGT-positive tumoral cells, like HepG2 cell lines, ovothiol was not detectable inside the cells [125], but most remained outside in the culture media, supporting the idea of its trapping by hGGT and consequent inhibition also after 24 h of treatment [111]. Currently, natural ovothiols, purified from sea urchin eggs, do not represent an eco-sustainable source for extensive testing and transfer to clinical trials [125]. However, 5-thiohistidine and ovothiols derivatives, named iso-ovothiols, can be prepared by chemical synthesis [128] and their biological activities need to be tested for further potential drug development or dietary supplementation. On the other hand, microalgae, especially diatoms, which are capable of growing fast in the laboratory, may represent an optimal alternative natural source for ovothiol biosynthesis and purification [117].

9. Therapeutic potential of GGT inhibitors

The therapeutic potential of the above-described GGT inhibitors has been tested in different *in vivo* models of pathologies characterized by increased levels of GGT. Initial pretreatment of animals with the GGT inhibitor acivicin was reported to prevent ischemia-induced lipid peroxidation and morphological alterations in the kidney [103]. Unfortunately, due to its cytotoxicity, the use of this compound was abandoned during the transfer to clinical trials [110]. More recent studies have been performed on the most promising GGT inhibitors GGsTop and OU749. Regarding GGsTop, the pretreatment of animals exposed to 24 h reperfusion after 45 min ischemia induced by artery clamping of the kidney, was shown to prevent GSH loss as well as the production of superoxide and lipid peroxidation [91]. Comparable results were obtained in isolated-perfused Langendorff rat hearts exposed to a 40 min ischemia-30 min reperfusion cycle, where the pretreatment with GGsTop, indirectly prevented the production of O^{2-}, the consequent lipid peroxidation, and the excessive norepinephrine release in the coronary perfusate, caused by the overexpression of GGT during ischemia [129]. These observations indicate that enhanced GGT activity contributes to renal and cardiac damage after ischemia/reperfusion, and that GGT inhibitors have the potential to prevent myocardial and renal ischemia/reperfusion injury *in vivo* [91,129]. Since GGT is also involved in the inflammatory response of asthma [97], GGsTop has also been inhaled in the airway to inhibit GGT activity in lung lining fluid [130]. This treatment was associated with an increase in the extracellular fluid GSH, known to protect lung airway epithelial cells against oxidant injury associated with inflammation in asthma [130]. In addition, as GGT was observed to be also expressed in mouse periodontal ligament tissue, the treatment with GGsTOP has also been tested in this model and associated with greater proliferation and migration of human periodontal ligaments cells, and with a transient increase of cellular H_2O_2 compared to untreated cells [131]. This apparent contradictory effect of GGsTOP can be explained by the accumulation of the extracellular GSH following GGT inhibition, with a consequent decrease in cysteine recovery inducing an unbalance in redox homeostasis. The treatment with GGsTOP has also been associated with increased levels of collagen I and alpha-smooth muscle actin (α-SMA), which were instead inhibited in human ligament cells co-cultured with NAC, a precursor of GSH biosynthesis [131]. GGsTop has also been reported to protect hepatic ischemia-reperfusion injury in rats when injected into the liver at 1 mg/kg body weight [132]. In this case, the treatment with GGsTop has been associated with increased GSH levels and prevented the formation of free radicals in the hepatic tissue thus protecting against liver injury. GGsTop has also been recently reported to display a therapeutic effect on stomatitis in mice, by promoting collagen production in the oral mucosa and reducing the ulcerated area and an early recovery compared to untreated mice affected by oral mucositis [133]. More recently, the GGsTop injection has been reported to ameliorate ischemia-reoxygenation injury in rats affected by hepatic steatosis [134], thus confirming the involvement of GGT in liver injury. In addition, recent studies carried out on myeloid-derived immunosuppressor cells have indicated that the granulocyte colony-stimulating factor (G-CSF) can enhance the immunosuppressive function of the cells through the upregulation of GGT [135]. The treatment with GGsTop has been observed to prevent G–CSF–induced tumor growth, without affecting the promotion of myelopoiesis [135]. These results suggest that targeting hGGT can ameliorate G–CSF–induced immunosuppressive functions and prevent the tumor-promoting effect of G-CSF. Regarding the therapeutic potential of OU749, the inhibitor has been recently encapsulated in a supramolecular Pt prodrug nano-assembly delivery system for the synergistic chemotherapy of cisplatin-resistant cancer [136]. Indeed, the detoxification of cisplatin drugs is already one of the most important problems to solve in chemotherapy. These nano-assemblies have been proven to be efficiently taken up by cisplatin-resistant cancer cells and to release the drug in the intracellular environment. This strategy has allowed the efficient suppression of hGGT activity, depleting intracellular GSH and augmenting H_2O_2 via the reduction of the Pt prodrug, thus demonstrating that through hGGT inhibition and redox unbalance, the detoxification and anti-apoptotic mechanisms of Pt drugs can be overcome. The therapeutic efficacy of these nano-assemblies has been validated on a cisplatin-resistant human non-small cell lung cancer model [136].

The therapeutic potential of marine-derived ovothiol has so far been tested in mice models of liver fibrosis [137], a process caused by chronic hepatic injury, and characterized by an excessive accumulation of extracellular matrix proteins. The effect of ovothiol in disulfide form was investigated *in vivo* on mice affected by liver fibrosis and injected with a 50 mg/kg dose of the natural product three times a week for two months. Treatment with ovothiol in disulfide caused a significant reduction of collagen fibers accumulated in the extracellular matrix and a significant decrease of transaminase activities in the serum compared to fibrotic mice treated with vehicle solution. At the molecular level, this antifibrotic effect was associated with the decrease of fibrogenic markers, such as the transforming growth factor (TGF-β), α-SMA, the tissue metalloproteinases inhibitor (TIMP-1), and the inhibition of GGT. Briefly, ovothiol in disulfide inhibited membrane-bound GGT activity,

thus affecting GSH metabolism and redox homeostasis and leading to the reduction of the key fibrotic markers responsible for the degradation of the collagen fibers in the extracellular matrix (Fig. 5). This study has highlighted the involvement of the membrane-bound GGT form in the evolution of liver fibrosis, thus pointing to this enzyme as a potential target of therapeutic strategies directed to ameliorate liver fibrosis. Finally, it has confirmed *in vivo* the inhibitory action of ovothiol on GGT, also demonstrated by *in vitro* studies in hGGT-positive cells [111].

10. On pleiotropy of GGT

The interest in GGT has increased over the years due to its pleiotropic functions, potential applications as a biomarker, and as a drug target in clinics. Indeed, GGT plays a key role in crucial cellular events such as chronic inflammation and cancer, thanks to its involvement in regulating the metabolism of GSH and the balance of redox homeostasis. Regarding hGGT, the enzyme has been reported to display multiple biological roles: 1. it controls redox homeostasis, by regulating the intracellular and extracellular levels of GSH [8,27,46]; 2. it contributes to the re-uptake of amino acids, like cysteine and glutamate, thus regulating GSH and protein synthesis [2,3,28,46]; 3. it is involved in the detoxification of previously γ-glutamylated drugs and xenobiotics [48,49]; 4. when overexpressed, GGT can promote oxidative stress resistance in cancer cells [46,77]. In addition, hGGT is present in different glycosylated forms in different tissues and types of tumors, likely accounting for different regulated mechanisms [40,46]. The presence of hGGT has also been documented in subcellular compartments of inflammatory cells, like neutrophils and mononuclear cells and mature hGGT is also known to be released in the extracellular environment as a "sort of cytokine" during inflammation [90]. The multiple functions and subcellular and extracellular localizations of GGT pose the question of whether GGT can be considered a moonlighting protein. Moonlighting proteins are usually enzymes, that can perform more than one function in different contexts, cell types, tissues, or during evolution and cancer progression [138]. Most of the currently known moonlighting proteins have evolutionarily originated from ancient enzymes, as this increases the chance of the enzymes developing secondary functions. Under this aspect, GGT is evolutionarily conserved in most organisms, including ancient prokaryotes, and GGTs isolated from extremophilic microorganisms, considered the first bacteria to colonize the earth, display only the ability to hydrolyze GSH. Only later in evolution, in eubacteria and eukarya, GGT enzymes evolved the second function to transfer the γ-glutamyl group to amino acids and short peptides [4,5]. Actually, this feature represents a gain of function in evolution, whose physiological significance has not yet been understood. Altogether, these features put the enzyme at the center of an interesting debate about the known and yet unknown functions.

11. Future challenges

Several questions remain to be addressed on hGGT pleiotropy, especially regarding the diversified biological roles of the inactive precursor, the membrane-bound hGGT, and the mature enzyme released in the blood and extracellular fluids. For example, in mice affected by liver fibrosis, the increase in the expression of the GGT inactive precursor compared to the mature protein, likely indicates that liver damage can prevent the GGT maturation process [137]. In fibrotic mice treated with the natural GGT inhibitor, on the other hand, the increase in the mature protein compared to the immature precursor suggests that the small thiol may favor the autocleavage process. Curiously, the activity of GGT increased in a highly variable manner following the induction of the fibrotic process and damage to the liver in mice [137], reflecting the presence of mixed populations of the active and inactive forms of the enzyme. Future analyses need to be performed to further confirm these speculations. However, the significant expression of the inactive precursor in treated and untreated mice tissues indicated that the liver stores a certain amount of inactive zymogen, probably to regulate its maturation when a higher activity of GGT is required. This may suggest

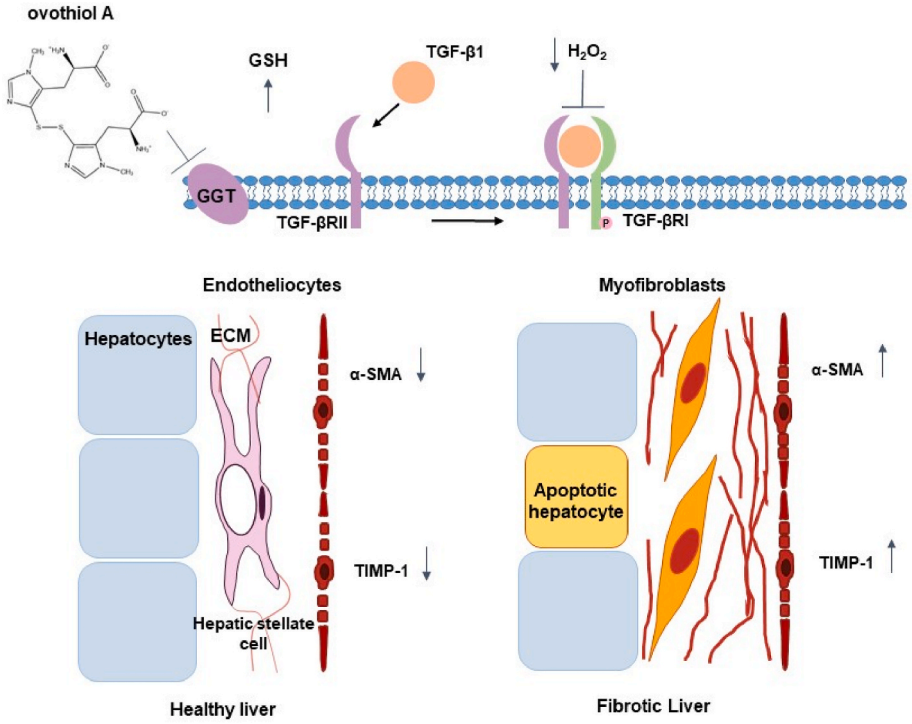

Fig. 5. Mechanism of action of the natural ovothiol in mice affected by liver fibrosis. Ovothiol in disulfide inhibits hGGT bound to the outer membrane of the liver tissue, consequently, the levels of GSH increase in the extracellular space, where TGF-β is secreted. The increased levels of extracellular GSH can induce the reduction of H_2O_2, thus inhibiting TGF-β-signaling, and consequently the protein expression of α-SMA and TIMP-1. Lastly, the down-regulation of TIMP-1 induces metalloprotease activity and degradation of ECM.

the importance of finely regulating the maturation process. Only mature GGT has been observed to be released in the serum, with no significant difference in the amount of protein between treated and untreated mice [137]. This confirms that membrane-bound GGT is exclusively present in the mature form and that the inactive precursor remains in the intracellular milieu. However, whether the anchoring of the transmembrane domain in the cellular membrane induces the maturation process or the maturation process is, instead, necessary for the anchoring to the membrane is not clear yet. Other intriguing aspects to deepen in the future would be the involvement of hGGT in the adaption mechanisms of cells to nutrient starvation and autophagy induction [111], as well as the co-regulation of cystine transport and hGGT expression, which could work in tandem, with GGT initiating the release of cystine from extracellular GSSG, and xc-system providing the uptake of cystine into the cell [46]. Also, the correlation existing between hGGT, MRP expression, and drug resistance would be worth studying [46,139]. Altogether, these questions need further studies to be answered, and the recent development of novel and less toxic GGT inhibitors, like GGsTop, OU749, and marine-inspired iso-ovothiols, may help to address most of them in the near future.

12. Conclusions

hGGT represents a potential enzymatic target for the therapy of a wide variety of pathologies, such as several types of tumors, liver fibrosis, ischemia/reperfusion-induced renal injury, asthma, and airway inflammation. Therefore, the development of hGGT inhibitors with high specificity and low toxicity has attracted the attention of the research community for their potential clinical applications. Different glutamine analogs, binding to the donor site and acting as irreversible inhibitors, have been developed over the years, however, most of them have been abandoned in clinical trials due to toxicity. On the other hand, glutamate derivatives have been proven to be a valid therapeutic alternative, being more selective and less toxic. Another class of uncompetitive inhibitors binding to the acceptor site of the enzyme resulted in less toxicity compared to glutamine analogs. More recently, marine-derived natural products have been identified as reversible non-competitive inhibitors of hGGT. Moreover, a reversible mode of action for GGT inhibitors could provide the additional advantage of a rapid inhibition rate, as well as being easier to modulate *in vivo*, thus preserving physiological GGT activity and resulting in lower toxicity. Further studies will be necessary to confirm the proposed mechanism of inhibition of hGGT by the most promising inhibitors. In particular, site-directed mutagenesis of hGGT followed by enzyme kinetics studies and crystal structure determinations of enzyme/inhibitors complexes will provide proof for the involvement of key residues in the inhibitory activity, and will shed new light on the mechanism of inhibition of these compounds for their potential use as new drugs to adjuvant the treatment of GGT-dependent pathologies. It is worth mentioning that the induction of GGT expression is redox-sensitive and can be associated with the overexpression of other proteins and enzymes regulated by the same transcription factors [140]. Therefore, the use of GGT inhibitors can be considered of help in more complex therapeutic strategies. Future *in vitro* and *in vivo* studies in models of liver, kidney, and lung dysfunction will help to deepen the efficacy of novel GGT inhibitors as adjuvants in the therapeutic strategies directed toward inflammatory and oxidative stress-related diseases.

Authors' contributions

I.C. conceived the review, wrote, and revised the final manuscript, and supervised the work. A.M. prepared the figures and wrote the initial draft of the manuscript. The authors gave final approval for publication.

Declaration of competing interest

The authors declare no conflict of interest.

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Abbreviations

ASCT	alanine-serine-cysteine transport system
CFTR	cystic fibrosis transmembrane regulator
GGT	γ-Glutamyl transpeptidase
GSH	glutathione
GSSG	oxidized glutathione
GSNO	nitrosylated glutathione
GPx	GSH-dependent peroxidase
GR	GSH reductase
H_2O_2	hydrogen peroxide
MRP	multidrug resistance-associated proteins
NAC	N-acetyl-cysteine
ROS	Reactive oxygen species
α-SMA	α-Smooth muscle actin
TGF-β	Transforming growth factor
TIMP-1	Tissue metalloproteinases inhibitor
G-CSF	granulocyte colony-stimulating factor

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