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Research Article

Therapeutic approach of glutathione/glutathione peroxidase-4 axis modulation in the light of ferroptosis

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Abstract

In the 21st century beginning, the evidence of a new type of programmed cell death, different from apoptosis, began to accumulate. In 2012, the ferroptosis concept was officially introduced. It refers to a kind of cell death that is associated with iron accumulation in the cell, impaired redox potential, and ROS increment with concomitant lipid peroxidation. Ferroptosis plays an important role in the pathophysiology of several organ damages such as tumors, neurodegenerative, ischemia-reperfusion, inflammatory diseases, and others. In ferroptosis, the leading mechanism is the glutathione (GSH) depletion and inactivation of Glutathione peroxidase-4 (GPX4), which strongly shifts the oxidative balance in the cell, leading to the activation of certain signalling pathways to induce oxidative death. The article aims to focus attention on the modulation of the GSH/GPX axis as a key factor in the treatment of these diseases.

Keywords

Ferroptosis, GSH, GPX4, ROS, Lipid peroxidation

Introduction

At the beginning of the 21st century, evidence of a new type of programmed cell death, different from apoptosis, began to accumulate (Dolma et al. 2003; Dixon et al. 2012; Li et al. 2020). One of the fundamental differences between this process and other types of cell death is the accumulation of iron in the cell. Therefore, in 2012, the concept of ferroptosis was officially introduced (Dixon et al. 2012). It refers to a type of cell death associated with iron accumulation in the cell, impaired redox potential, mitochondrial damage, and accumulation of ROS with concomitant lipid peroxidation.

The morphological changes, pathways, and related genes by which ferroptosis leads to cell destruction are different from other types of programmed cell death (Li et

al. 2020). In ferroptosis, the main mechanism causing cell death is the depletion of glutathione (GSH) and inactivation of glutathione peroxidase-4 (GPX4), which strongly shifts the oxidative balance in the cell, leading to the activation of different signalling pathways to induce oxidative death (Yang and Stockwell 2016).

In the last decade, it was proven that ferroptosis plays an important role in the pathophysiology of a number of organ damages and diseases such as: tumor development – gastric cancer, hepatocellular carcinoma, colorectal carcinoma, lung cancer, renal cell carcinoma, adrenocortical carcinoma, pancreatic carcinoma, breast cancer (Eling et al. 2015; Sun et al. 2016; Alvarez et al. 2017; Miess et al. 2018; Belavgeni et al. 2019; Lee et al. 2021); neurological diseases – neurodegenerative conditions, traumatic brain injury (Ahmad et al. 2014; Hambright et al. 2017); ischemic disorders – ischemia/reperfusion in the heart, brain, liver, kidneys (von Mässenhausen et al. 2018; Fang et al. 2019; Li et al. 2019); liver fibrosis (Wang et al. 2019); heart transplantation (Li et al. 2019); acute renal failure (Friedmann Angeli et al. 2014; Linkermann et al. 2014; Martin-Sanchez et al. 2017); antiviral immunity (Matsushita et al. 2015); inflammation (Li et al. 2018); infertility (Stockwell et al. 2020). These studies along with the discovery of natural and synthetic compounds that could affect the process may well prove to be a key step in the control of the diseases mentioned above (Fan et al. 2021).

The activators and inhibitors of ferroptosis could affect various critical steps and pathways of the process, but the central role is attributed to GSH and GPX4 bioavailability and activity (Table 1). Thus, an answer to speculations about the relationship between ferroptosis and other types of cell death and the role of ferroptosis in the development of cancer and inflammation could be provided only if we have more knowledge about the factors that affect GSH and GPX4 production.

GSH biosynthesis and bioavailability

GSH is a tripeptide (γ-L-glutamyl-L-cysteinylglycine), present in all mammalian tissues. Biosynthesis of GSH occurs in the cytosol via two steps. The first step involves the conjugation of cysteine with glutamate, a reaction catalyzed by glutamate-cysteine ligase (GCL). This is the rate-limiting enzyme in GSH synthesis. The second step is the addition of glycine, catalyzed by GSH synthetase (GS). The main sources of cysteine supply in the cells are System Xc- – an antiporter for glutamate and cysteine, and additionally, the transsulfuration pathway in the liver. In the cytosol of the cell, there are no enzymes capable to degrade GSH. (Fig. 1). In human cells GSH is distributed predominantly in the cytosol (80–85%), 10–15% is in the mitochondria and a small percentage is in the endoplasmic reticulum (Lu 2013).

GSH/GPX4 axis and ferroptosis

GSH is a peptide that neutralizes thiyl radicals and thiol toxicity in the presence of oxygen (Kajarabille and Latunde-Dada 2019). The depletion or inhibition of GSH suppresses the conversion of lipid peroxides into lipid alcohols, which could initiate the process of ferroptosis. GSH is a cofactor that acts on glutathione peroxidases. In the presence of iron, hydroxyl radicals are generated from hydrogen peroxide in the process of cellular oxygen metabolism via the Fenton and Haber-Weiss reactions. GPX4 catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to water or the corresponding alcohols, by conversion of GSH into oxidized glutathione (GSSG). Thus, GPX4 reduces the cytotoxic lipid peroxides (L-OOH) to the corresponding alcohols (L-OH) Fig. 2 (Yang et al. 2014). GPX4 is isolated for the first time in 1982 by Ursini and colleagues (Stockwell et al. 2020). GSSG, generated during the reduction of hydroperoxides by GPX4,

Table 1. Drugs and substances that affect the GSH/GPX4 axis associated with sensitization or promotion of ferroptosis.

Mechanism	Inhibitors of GSH/GPX4 axis	Reference
Inhibition of System Xc^- and blockage of glutathione synthesis	Erastin	Dolma et al. 2003
	Sorafenib	Wu et al. 2019b
	Sulfasalazine	Sugiyama et al. 2020
	Buthionine sulfoximine	Friedmann Angeli et al. 2014
	Glutamate	Gao et al. 2016
	activators of p53 pathway	Xie et al. 2016
	inhibitors of the sulphur-transfer pathway	Hao et al. 2018
	NADPH inhibitors	Wang et al. 2021
	Nrf2 inhibitors	Jaramillo and Zhang 2013
	TGF-β1	Kim et al. 2020
	Toxic bile acid	Yang et al. 2010
Direct inactivation of GPX4	RSL3	Yang and Stockwell 2008
	DPI7	Yang et al. 2014
	Altretamine	Woo et al. 2015
	MVA pathway inhibitors	Yu et al. 2017
	Acetaminophen	Lőrincz et al. 2015
Degradation of GPX4	FIN56	Shimada et al. 2016
	Chaperone-mediated autophagy	Wu et al. 2019a
Iindirect inhibition of GPX4 and direct lipid peroxidation	$FINO_2$	Abrams et al. 2016

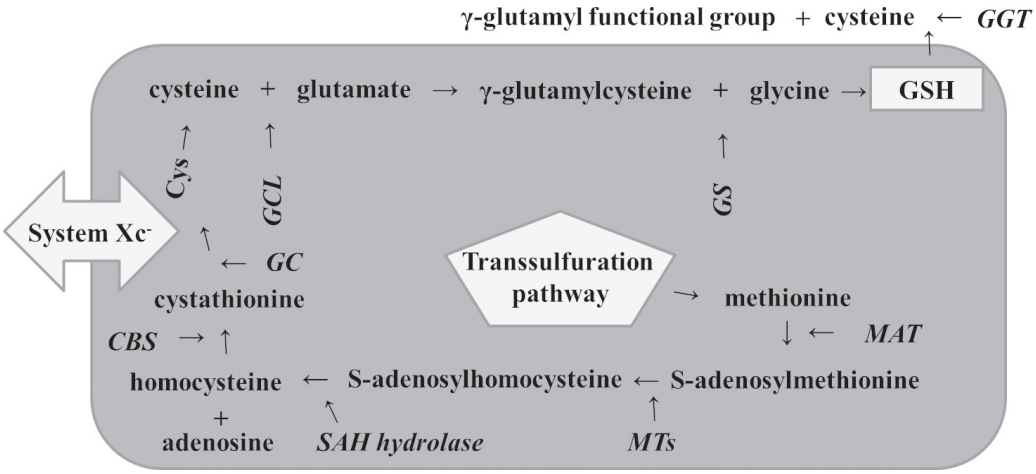

Figure 1. GSH biosynthesis. Cys – Cysteine; GCL – glutamate-cysteine ligase; GS – GSH synthase; GGT – γ-glutamyltranspeptidase; MAT – methionine adenosyltransferase; MTs – methyltransferases; SAHH – Sadenosylhomocysteine hydrolase; CBS – cystathionine β synthase; GC – γ-cystathionase.

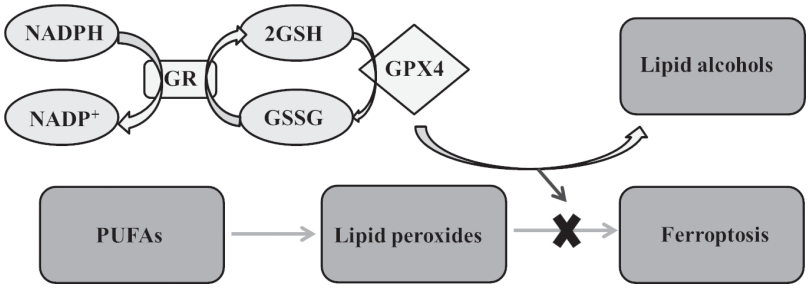

Figure 2. GSH/GPX4 axis (pathway). GPX4 converts GSH into oxidized glutathione (GSSG) and reduces the cytotoxic lipid peroxides (L-OOH) generated from membrane polyunsaturated fatty acids (PUFAs) to the corresponding alcohols (L-OH). The oxidized glutathione generated during the reduction of hydroperoxides by GPX4, is recycled by glutathione reductase (GR) and NADPH.

is potentially toxic to the cells, but the cells normally contain highly active glutathione reductase, which maintains most of the GSH in the reduced form (Forman et al. 2009). Additionally, GSH reduces ROS and reactive nitrogen under the action of glutathione peroxidases (Lu 2013). GSH participates in the detoxification of xenobiotics and regulates cell proliferation, apoptosis, immune function, and fibrogenesis (Lu 2013). GPX4 is the first-discovered central inhibitor of ferroptosis (Yang et al. 2014).

Any suppression in GSH/GPX4 pathway could initiate the ferroptosis process. When intracellular GSH levels drop below a critical threshold, the GSH-dependent GPX4 cannot function, which could cause a fatal increase in ROS and cell death (Dixon et al. 2012). There are four mechanisms that could cause such depletion (Li et al. 2020).

The first mechanism is the direct reduction of GSH levels, due to inhibition of the system Xc-. System Xc- is an amino acid exchanger widely expressed in membrane phospholipid bilayers. It is part of the antioxidant mechanisms in the cells. The system is composed of two subunits, SLC7A11 and SLC3A2. The subunit SLC7A11 is of greater importance, being a target of many regulatory pathways (Lu 2013). The role of Xc^- system is to transport the amino acids cysteine in and glutamate out of the cell in a ratio of 1:1. The cysteine that is taken up is involved in the synthesis of GSH (Fig. 1). Inhibition of the activity of the system Xc^- decreases the synthesis of GSH, hence the activity of GPX4. Substances such as Erastin (Dolma et al. 2003), Sulfasalazine (Sugiyama et al. 2020), Sorafenib (Wu et al. 2019b), Buthionine sulfoximine (Friedmann Angeli et al. 2014) and down-regulators of SLC7A11 subunit expression (Stockwell et al. 2020), cause a reduction in cell antioxidant capacity, accumulation of lipid ROS, and finally initiation of ferroptosis (Xie et al. 2016). In a study by Kim et al. (2020), the suppression of SLC7A11 subunit expression has been shown to enhance the lipid peroxidation in hepatocellular carcinoma cells. Additionally, glutamate and transferrin can regulate cell ferroptosis via the glutamine decomposition pathway and the transferrin receptors on the surface of cells (Gao et al. 2016). Ferroptosis may also contribute to glutamate excitotoxicity of neurons (Magtanong and Dixon 2018). L-buthionine sulphoximine directly inhibits GCL (Friedmann Angeli et al. 2014). The trans-sulfuration pathway provides a compensatory source of cysteine when the uptake pattern is inhibited (Hao et al. 2018). Through the sulphur-transfer pathway, methionine can be converted into cysteine (Fig. 1). Homocysteine can also be transferred into methionine or cysteine in case of cysteine insufficiency and Vitamin B12 is essential for this conversion (Jacques et al. 1996). Cysteine is mostly used for the synthesis of GSH and other anti-oxidative peptides. The oxidative stress promotes methionine-to-cysteine conversion and GSH synthesis through the sulfurtransfer pathways (Sbodio et al. 2019). The metabolite γ-glutamylcysteine (if GS activity is decreased) could be converted to 5-oxoproline,

which can cause severe metabolic acidosis, hemolytic anemia and central nervous system damage (Lu 2013). NADPH also plays a central role in the ferroptosis process. NADPH deficiency will lead to a decrease of GSH, promoting the accumulation of lipid ROS (Wang et al. 2021). NADPH oxidase and agents down-regulating SLC7A11 subunit expression act as stimulators of ferroptosis by promotion of ROS (Xie et al. 2016). The nuclear factor erythroid 2 (Nrf2) can protect cells and tissues from a variety of toxicants and carcinogens, but may also protect cancer cells from chemotherapeutic agents and facilitate cancer progression (Jaramillo and Zhang 2013). Nrf2 is able to activate a number of antioxidant genes, including those encoding the subunit SLC7A11 of system Xc^-. (Jaramillo and Zhang 2013). Any factor which can affect the activity of Nrf2, like toxic bile acid, leads to decreased expression of GSH synthetic enzymes and reduced GSH levels (Yang et al. 2010). In contrast, zinc supplementation has been shown to increase GSH levels in ARPE-19 cells by activation of Nrf2-mediated pathway (Ha et al. 2006).

The second mechanism is direct inhibition of GPX4 activity. This could be achieved by substances such as Ras-selective lethal small molecule 3 (RSL3) and DPI7 (Yang et al. 2014). RSL3 activates nonapoptotic and iron-dependent oxidative cell death (Yang and Stockwell 2008). RSL3 targets enzymes that possess a nucleophile active site, such as selenocysteine. When testing 7α-cholesterol hydroperoxide rate of reduction to the corresponding alcohol (7α-cholesterol-OH), a specific substrate only for GPX4 (Kriska and Girotti 2005), no reduction after treatment with RSL3, or with DPI7 was observed, indicating that GPX4 was being inhibited (Yang et al. 2014). An additional mechanism that contributes to the oxidative stress in RAS-activation pathway is the up-regulation of transferrin receptor 1 and down-regulation of ferritin (Yang and Stockwell 2008). GPX4 is a selenoprotein with selenocysteine in its active center. Mevalonate (MVA) pathway by isopentenyl pyrophosphate (IPP) is a regulator of selenoprotein synthesis (Hao et al. 2018). There is data to support the fact that statins lower cholesterol levels through inhibition of a specific enzyme, responsible for MVA formation (Friedmann Angeli and Conrad 2018). As a conclusion, inhibitors of the MVA pathway such as statins (simvastatin) could affect selenocysteine insertion and GPX4 synthesis (Yu et al. 2017). Another factor that has the ability to inhibit GPX4 activity is Paracetamol (Acetaminophen). Acetaminophen administration results in a 60% reduction in GPX4 activity. Moreover, one of the derivatives of acetaminophen has been shown to cause major GSH depletion (Lőrincz et al. 2015). Woo et al. (2015) experimentally confirmed that altretamine is an inhibitor of GPX4 activity. Finally, insufficient delivery of selenium to cells, essential for GPX4 synthesis, could sensitize the cell for ferroptosis (Yang et al. 2014; Friedmann Angeli and Conrad 2018).

The third mechanism is faster degradation of GPX4. The substance FIN56 possesses such an effect (Shimada et al. 2016). The substance serves as a ferroptosis inducer and was derived from a compound, which triggers cell death by influencing the biosynthesis of long-chain fatty acids (Dixon et al. 2015). Except promoting GPX4 degradation, FIN56 additionally binds to the enzyme squalene synthase, which leads to the depletion of the endogenous antioxidant coenzyme Q10 (CoQ10) (Shimada et al. 2016). CoQ10 is an integral part of the mitochondrial electron transport chain, but also functions outside of mitochondria to suppress lipid peroxidation by capturing the free radicals formed in the process. Nowadays it is known that NADPH-FSP1-CoQ10 pathway acts in parallel to GSH-GPX4 to suppress ferroptosis (Bersuker et al. 2019). In the last years, another GPX4-independent ferroptosis-blocking pathway involving the gene GTP cyclohydrolase 1 (GCH1) was identified (Kraft et al. 2020). The enzyme is the rate-limiting step in the production of the metabolite tetrahydrobiopterin (BH4). BH4 was found to suppress ferroptosis through aiding the formation of reduced CoQ10 (Kraft et al. 2020). Chaperone and Nuclear Receptor Coactivator4 (NCOA4)-mediated autophagy also promotes the degradation of GPX4, as a result of increased levels of lysosomal-associated membrane protein 2a and lipid peroxidation (Wu et al. 2019a; Yoshida et al. 2019; Cui et al. 2020).

The fourth mechanism is a combination of both indirect inhibition of GPX4 and direct oxidation of iron, causing lipid peroxidation. Such effects possesses the endoperoxide 1,2-dioxolane ($FINO_2$). The name comes from "ferroptosis-inducing compounds" (Gaschler et al. 2018). $FINO_2$ is organic peroxide that has similar effects to artemisinin. An interesting feature of $FINO_2$ is its thermal stability to at least 150 °C (Abrams et al. 2016).

Conversely, we know today of several important positive modulators of the GSH/GPX4 axis and inhibitors of ferroptosis. Such substances are the hormones insulin and cortisol (Lu et al. 1992). Research has shown that they increased GSH of cultured hepatocytes, while melatonin (Urata et al. 1999) induced the expression of GCS in ECV304 human vascular endothelial cells. Curcumin elevates the level of cellular GSH and induces de novo synthesis of GSH in hepatic stellate cells (Zheng et al. 2007). Lipid peroxidation, one the main factors for the initiation of ferroptosis, can occur via enzymatic and non-enzymatic pathways (Capelletti et al. 2020). Lipid autoxidation inhibitors (e.g., radical trapping antioxidants), such as vitamin E, provide a potent protective effect and have the ability to suppress the formation and propagation of oxidized lipids and to suppress GSH depletion (Yang and Stockwell 2016). Another group of substances that exhibits vast potential to fight lipid peroxidation and GSH depletion comprises lipoxygenase inhibitors such as ferrostatin-1 (Fer-1) and liproxstatin-1 (Lip-1) (Dixon et al. 2012; Friedmann Angeli et al. 2014; Xie et al. 2016). Other types of substances that inhibit ferroptosis, without impacting directly the GSH/GPX4 axis are iron chelators such as DFOM, SRC kinase inhibitors and MEK1/2 inhibitors (Stockwell et al. 2020).

Therapeutic potential of GSH and GPX4 modulation

Ferroptosis has been reported to be implicated in various disorders, including cancer, neurodegenerative diseases, liver fibrosis, ischemia/reperfusion injuries, and kidney

failure. Thus, manipulation of the process might be beneficial in achieving therapeutic effects: the activation of ferroptosis with specific inducers could result in a destruction of certain tumor cells, while the inhibition with specific ferrostatines may be useful to protect the organs from the damages mentioned above.

For example, in antitubercular therapy, the combination of Isoniazid (INH) and Rifampicin (RMP) can activate hepatic stellate cells through generation of NADPH oxidase-related oxidative stress, leading to the development of liver fibrosis (Biswas et al. 2020). Ferrostatines could counteract the process. Hambright et al. (2017) created a novel forebrain neuron specific, tamoxifen inducible GPX4 knockout mouse model to observe cognitive impairment and hippocampal neurodegeneration. Ferroptosis is a major contributor to ischemic organ injuries -associated cell death, and inhibition of ferroptosis has been reported to significantly relieve such injuries in experimental models (Stockwell et al. 2020). Cigarette smoke induced ferroptosis in lung epithelial cells in a patient suffering from chronic obstructive pulmonary disease (Yoshida et al. 2019). The process was triggered by NCOA4-mediated ferritin selective autophagy (ferritinophagy) (Gao et al. 2016). Sun et al. (2015) demonstrated that phosphorylated HSPB1 acts as a negative regulator of ferroptosis by reducing cellular iron uptake and lipid ROS production.

The side effects of certain drugs provide another aspect to the relation between the GSH/GPX4 axis and ferroptosis. It is known that aminoglycosides are one of the leading causes of drug-induced oto- and nephrotoxicity. Zheng et al. (2020) found that Lip-1 significantly attenuated neomycin-induced hair cells damage in neonatal mouse cochlear explants and suggest that ferroptosis inhibition may be a new clinical intervention to prevent hearing loss. The treatment of animals with gentamicin is related to apoptosis as well as necrosis of tubular epithelial cells predominantly in the proximal segment (Randjelović et al. 2017). Gentamicin causes phospholipidosis – an excessive accumulation of phospholipids in the lysosomes of various tissues (Smith 2011). Tamoxifen and acetaminophen are also associated with the induction of phospholipidosis (Nioi et al. 2007), which could be an evidence that gentamicin cytotoxicity is partly due to induction of ferroptosis. Statins, which inhibit MVA-derived CoQ10 through blocking the enzyme HMG CoA reductase, also sensitize cells to ferroptosis (Yu et al. 2017).

However, the paramount clinical significance of the process and the GSH/GPX4 axis concerns anti-cancer therapy. The ATP binding cassette (ABC)-family transporter multidrug resistance protein 1 (MRP1) causes multidrug resistance in tumor cells. Disruption of MRP1 prevents glutathione efflux from the cell and strongly inhibits ferroptosis (Cao et al. 2019). As a consequence, high ferroptosis sensitivity and traditional chemoresistance often co-occur (Stockwell et al. 2020). These connections suggest that induction of ferroptosis might be a promising cancer therapeutic approach in multidrug resistance tumors. In epithelial cancer cells Yes-associated protein (YAP) activation can promote epithelial-mesenchymal transition and metastasis (Johnson and Halder 2014). On the other hand, YAP possesses ferroptosis-potentiating activity, because of ACSL4 and transferrin receptor transcription. Both factors are critical in the initiation of ferroptosis (Doll et al. 2017). Therefore, metastasis-prone cancer cells are highly sensitive to ferroptosis. The anti-programmed cell death 1 receptor ligand (PDL1) immune checkpoint blockade could promote a cancer cell ferroptotic response through the down regulation of SLC7A11 expression, a consequence of CD8+-T-cell-secreted IFNγ (Stockwell et al. 2020). A combination of anti-PDL1 treatment with ferroptosis induction has a synergistic anticancer effect in mouse models (Stockwell and Jiang 2019). Ferroptosis agonists augment and ferroptosis antagonists limit radiotherapy efficacy in tumor models, which proves that ferroptosis is also partially responsible for the anticancer effect of radiation therapy (Lang et al. 2019). L-buthionine sulphoximine, on the other hand, blocks cellular resistance to chemotherapy and could be used to induce experimental glutathione deficiency (Friedmann Angeli et al. 2014).

Conclusion

Thanks to the efforts of a number of researchers, the modulation of GSH/GPX4 axis in the light of ferroptosis, as well as the factors that influence it, are beginning to be revealed. GSH/GPX4 axis is the major pathway that controls the sensitivity of cells to ferroptosis, despite the fact that two others also exist. GSH/GPH4 axis and ferroptosis have a prominent role in cancer development and treatment, especially in mesenchymal and de-differentiated tumors. The induction of ferroptosis might be a promising therapeutic approach, especially for the treatment of metastatic cancers, even with multiple drug resistance. Additionally, the initiation of ferroptosis and GSH depletion are associated with neurodegenerative diseases, liver fibrosis, ischemia/reperfusion injuries, kidney failure and many other inflammatory or stress conditions. The manipulation of the process might be beneficial in achieving therapeutic effects: the activation of ferroptosis with specific inducers could result in destruction of certain tumor cells, while the inhibition with specific ferrostatines may be useful to protect the organs from the damages mentioned above.

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Competing interests

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