ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 4/2023
735–744
https://doi.org/10.18388/abp.2020_6756

Review

Emerging relationship between hydrogen sulfide and ferroptosis: A literature review

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Gaseous hydrogen sulfide (H_2S) can function as a signaling molecule similar to nitric oxide or carbon monoxide under physiological conditions, ultimately exerting anti-inflammatory, anti-apoptotic, and antioxidant activities. Many studies have investigated the role of H_2S in a variety of biological contexts, and both endogenous H_2S and H_2S donors have been leveraged as tools for fundamental biomedical research, and it has been suggested that they may provide value for the design of novel therapeutic strategies in the years to come. Ferroptotic cell death is a distinct programmed cell death resulting from excessive lipid peroxidation in an iron-dependent manner, and is characterized by high levels of iron accumulation, reactive oxygen species (ROS) production, and peroxidation of cellular lipids. Several recent studies have revealed a close relationship between ferroproteins and their precursors, H_2S, and the enzymes that produce them. This review summarizes the current information pertaining to the relationship between ferroptosis and H_2S, with a particular focus on the underlying mechanisms and biological applications of this knowledge.

Keywords: Hydrogen sulfide, ferroptosis, iron, reactive oxygen species, lipid peroxidation

Received: 27 March, 2023; revised: 25 September, 2023; accepted: 03 October, 2023; available on-line: 07 December, 2023

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Acknowledgements of Financial Support: This study was supported by the National Natural Science Foundation of China (82172441), the Scientific Research Project of Gusu School of Nanjing Medical University (GSKY20210244), the Clinical Medical Science and Technology Development Fund of Jiangsu University (JLY2021048), and the Suzhou Key Clinical Diagnosis and Treatment Technology Project (LCZX202024).
Abbreviations: H_2S, Hydrogen sulfide; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; RCD, regulated cell death; GPX4, glutathione peroxidase 4; PLP, pyridoxal 5′-phosphate; CNS, central nervous system; Hcy, homocysteine ; CAT, cysteine aminotransferase; 3MP, 3-mercaptopyruvate; NADPH, nicotinamide adenine dinucleotide phosphate; NADH, nicotinamide adenine dinucleotide; GSH, glutathione; xCT, Xc-system; 5-FU, 5-fluorouracil; VSMC, vascular smooth muscle cell; HHP, high hydrostatic pressure; Nrf2, nuclear factor erythroid 2-related factor 2; COPD, chronic obstructive pulmonary disease; PFC, prefrontal cortex; ALI, acute lung injury; Fpn1, ferroportin 1; TfR1, transferrin receptor 1; miRNAs, MicroRNAs

INTRODUCTION

Hydrogen sulfide (H_2S) is a gas that can serve as a signaling intermediary and that, in mammals, it is produced by several enzymes including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur-transferase (3-MST) (Wang, 2012). Many recent studies have sought to elucidate the biological roles of H_2S and have demonstrated that its endogenous production is related to processes ranging from vasodilation, microbial antibiotic resistance, oxidative stress, and mitochondrial function to inflammatory activity, neuroprotection, and endoplasmic reticulum stress (Kozich *et al.*, 2019). H_2S functions as a reductant at physiological concentrations, providing protection against inflammation, oxidative stress, and apoptotic cell death (Kimura *et al.*, 2010). In plasma, endogenously derived H_2S levels are negatively correlated with oxidative stress and aging (Hine *et al.*, 2015). In addition, owing to the essential roles of H_2S in various physiological and pathological processes, its practical applications have been explored. For example, a novel fluorescent probe, Rho-HS, was developed to detect H_2S and can be used for biological imaging (Guo *et al.*, 2022). Furthermore, a surface-filled H_2S-releasing silk fibroin (SF) hydrogel was developed to achieve small-dose local administration and avoid volatile and toxic side effects (Chen *et al.*, 2022). These investigations shed light on the potential clinical application of H_2S. The production and functionality of H_2S have been identified as promising targets for the treatment of a range of conditions owing to its significant role in regulating cell death and interactions with the cell death pathway.

Multiple cell death pathways contribute to the occurrence and progression of various diseases, and H_2S plays a vital role in regulating or suppressing cell death, including necroptosis, pyroptosis, and apoptosis (Rodkin *et al.*, 2023) (Table 1). For instance, H_2S upregulates the expression of Bcl-2 and P62 (Chen et al., 2022; Guo *et al.*, 2014) or downregulates NF-κB to reduce apoptosis in organ ischemia/reperfusion (Kuo *et al.*, 2013; Weirather *et al.*, 2014). Moreover, H_2S can reduce the expression of caspase-1 to exert an anti-pyroptosis effect on bone-derived macrophages and aortic endothelium (Castelblanco *et al.*, 2018; Xi *et al.*, 2016).

Ferroptosis is a subtype of regulated cell death (RCD) with features distinct from those of necrosis, autophagy, or apoptosis (Hadian & Stockwell, 2020; Jiang *et al.*, 2021). It often results from iron-dependent accumulation of toxic concentrations of lipid peroxides within cells (Rodkin *et al.*, 2023; Stockwell *et al.*, 2017), characterized by the production of high levels of lipid peroxides, accumulation of iron, and downregulation of glutathione peroxidase 4 (GPX4) (Fuhrmann & Brüne, 2022; Mahoney-Sánchez *et al.*, 2021). These characteristics offer markers that can be used to specifically detect RCD (Hadian & Stockwell, 2020). Ferroptotic cells exhibit a rounded, small morphology with intercellular separation, an intact crenulated plasma membrane, and the absence of nuclear condensation. Mitochondria in these cells often have ruptured outer membranes, increased membrane density, and shrunken or absent cristae (Liang

Table 1. Mechanisms of H_2S on different cell death in disease models.

Cell death	Character	Disease model	Mechanism	Reference
Necroptosis	Following the activation of the tumor necrosis receptor (TNFR1) by TNFα	Diabetic cardiomyopathy	Alleviated myocardial oxidative stress, necroptosis and NLRP3	(Gong *et al.*, 2022)
		Diabetic vascular complications.	p38 MAPK signaling pathway	(Lin *et al.*, 2021)
		Atherosclerosis	Attenuated the increased RIP3 expression	(Lin *et al.*, 2018)
Pyroptosis	A type of cell death culminating in the loss of plasma membrane integrity and induced by activation of so-called inflammasome sensors. These include the Nod-like receptor (NLR) family, the DNA receptor Absent in Melanoma 2 (AIM2) and the Pyrin receptor	Ischemia-reperfusion (I/R)--induced acute kidney injury (AKI)	Inhibited the NLRP3/Caspase-1 axis	(Ni *et al.*, 2021)
		Chronic obstructive pulmonary disease (COPD)	Targeted the TLR4/NF-κB pathway	(L. Wang *et al.*, 2022)
		Nephrotoxicity caused by U exposure.	Upregulation of PI3K/AKT/mTOR pathway	(Hu *et al.*, 2023)
Apoptosis	The release of cytochrome *c* from mitochondria, regulated by a balance between proapoptotic and antiapoptotic proteins of the BCL-2 family, initiator caspases (caspase-8, -9 and -10) and effector caspases (caspase-3, -6 and -7).	Asthma	Reduced the airway inflammatory infiltrate	(Mendes *et al.*, 2019)
		Lipopolysaccharide (LPS)--induced memory impairment (MI)	Decreased c-Jun and caspase-3 levels	(Kshirsagar *et al.*, 2021)
		Myocardial reconstruction	Blocked ER stress-autophagy axis	(Li *et al.*, 2020)
		Experimental glaucoma model	Suppressed Bax, caspase-3 and p53 activations	(Erisgin *et al.*, 2019)
		Methotrexate (MTX) hepatotoxicity	Modulated IL-6/STAT3 pathway	(Fouad *et al.*, 2020)

et al., 2019). Many diseases have been linked to ferroptosis, including osteoporosis and cancer, and this form of RCD also arises in the context of reproductive system functionality and organ damage (Proneth & Conrad, 2019; Stockwell *et al.*, 2020). Ferroptosis is also associated with the efficacy of a range of therapeutic modalities, such as chemotherapy, radiotherapy, and immune checkpoint blockade treatments (Lei *et al.*, 2020). Several studies have reported the anti-ferroptosis effects of H_2S in diseases such as cardiovascular disease (Wang *et al.*, 2023; Wu *et al.*, 2023), air inflammation (Zhang *et al.*, 2020), intracerebral hemorrhage (Zhang *et al.*, 2020) and acute lung injury (ALI) (Li *et al.*, 2022). For instance, H_2S reduces ferroptosis by regulating the Nrf2-PPAR-ferritinophagy signaling pathway in PM-induced emphysema (Wang *et al.*, 2022). It also protects DOX-induced cardiotoxicity from ferroptosis by targeting the OPA3-NFS1 axis (Wang *et al.*, 2023). Overall, its functional pathways are diverse and complex.

This review provides a summary of the role of H_2S metabolism in mammals and the signaling pathways associated with H_2S-mediated ferroptosis.

ENDOGENOUS MAMMALIAN H_2S PRODUCTION

In mammals, H_2S is generated via enzymatic and nonenzymatic mechanisms (Fig. 1). Both CSE and CBS are cytosolic pyridoxal 5'-phosphate (PLP)-dependent, whereas 3-MST is a mitochondrial enzyme that functions independently of PLP (Cao *et al.*, 2019).

CBS is formed by tetramerization subunit proteins of 63 kDa in size and generates H_2S *via* the catalytic processing of L-homocysteine and L-cysteine, yielding H_2S and L-cystathionine through a mechanism comparable to its role as a component in the trans-sulfuration pathway. When L-cysteine is present, CBS mediates a β-replacement reaction to generate H_2S, while producing L-serine. An up to 23-fold higher rate of H_2S generation was observed when l-homocysteine was available compared to when only L-cysteine was available. CBS is thought to be the main producer of H_2S in the central nervous system (CNS) and is expressed throughout the brain, with the highest levels of expression in the cerebral cortex, cerebellum, and hippocampus (Zuhra *et al.*, 2020). CBS expression is also detectable in lymphocytes and organs, such as the liver, kidneys, pancreatic islets, uterus, and placenta (Kaneko *et al.*, 2006; Patel *et al.*, 2009).

Similar to CBS, CSE catalyzes the processing of homocysteine (Hcy) to produce H_2S, α-ketobutyrate, and ammonia, in addition to catalyzing the processing of L-cysteine to yield H_2S, along with ammonia and pyruvate. When L-cysteine and Hcy are present at physiological levels, CSE catalyzes the α- and β-elimination of L-cysteine to generate approximately 70% H_2S, whereas the α,γ-elimination of Hcy catalyzed by this enzyme generates only 29% of the total H_2S. However, when Hcy levels are elevated to concentrations similar to those observed in the context of hyperhomocysteinemia (HHcy), such α,γ-elimination generates approximately 90% CSE-derived H_2S, indicating that relative Hcy and L-cysteine concentrations within mammalian cells are pivotal determinants of the primary substrate utilized by this enzyme (Chiku *et al.*, 2009).

Mitochondrially localized 3-MST is an H_2S-generating enzyme that was most recently discovered. Following the initial cysteine aminotransferase (CAT)-mediated conversion of L-cysteine to 3-mercaptopyruvate (3MP), 3-MST transfers sulfur atoms from 3MP to 3-MST, resulting in persulfide generation. When a reductant such as thioredoxin is present, H_2S is released from the persulfide (Nagahara *et al.*, 1998). Regulation of CAT activity ultimately shapes the ability of 3-MST to generate H_2S. CAT inhib-

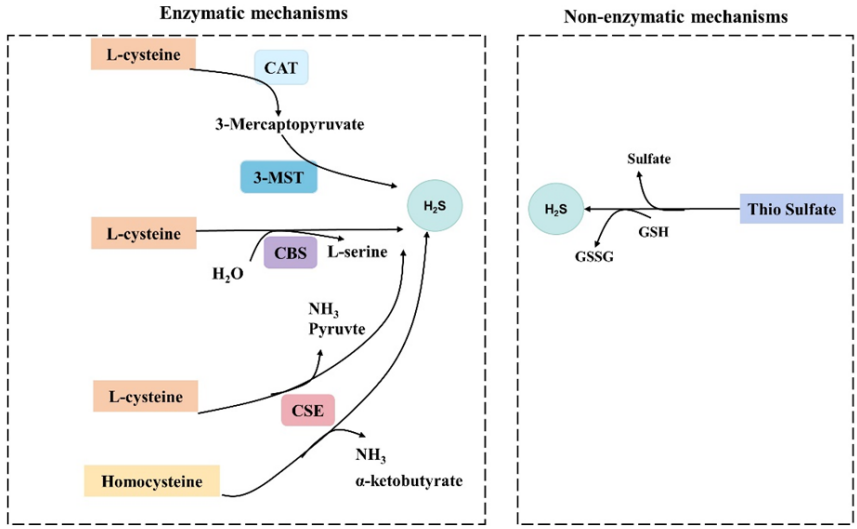

Figure 1. Endogenous generation mechanism of H_2S in cells.
The enzymatic and nonenzymatic generation pathway was presented in the above figure, in which the enzyme CBS, CSE and 3-MST play pivotal role in producing H_2S in enzymatic generation pathway, with L-cysteine being the main substrate. CAT, Cysteine aminotransferase; CBS, Cyststhionine β-synthase; CSE, Cyststhionine γ-lyase; GSH, glutathione; GSSG, glutathione disulfide; 3-MST, 3-mercaptopyruvate sulfurtransferase.

itors, such as aspartate, largely suppress the biosynthesis of H_2S and can therefore be leveraged as tools for pharmacological studies to explore the functional importance of H_2S generated by this enzyme (Shibuya *et al.*, 2009).

Some endogenous H_2S is also generated via the nonenzymatic chemical reduction of sulfane sulfur through the action of the ubiquitous cellular electron donor nicotinamide adenine dinucleotide (NADH), its reduced state nicotinamide adenine dinucleotide phosphate (NADPH), or other reductive equivalents (Searcy & Lee, 1998). Under these conditions, the reduction of reactive sulfur species present in polysulfides, persulfides, and thiosulfate can give rise to a range of metabolites, including H_2S (Olson *et al.*, 2013).

ENDOGENOUS H_2S AND FERROPTOSIS

In mammalian cells, the transsulfuration pathway produces H_2S as a byproduct of Hcy and cysteine processing by CSE, CBS, and 3-MST. The functional Xc-system subunit xCT (SLC7A11) is responsible for the import of cystine into cells, where it is converted into cysteine, which functions as the rate-limiting substrate necessary for the biosynthesis of glutathione (GSH) (Liu *et al.*, 2020; Stockwell *et al.*, 2017). GSH functions as a substrate for GPX4, which mitigates ferroptosis by reducing membrane phospholipid hydroperoxides to produce lipid alcohols. When xCT expression or activity is suppressed, immunotherapy- or radiotherapy-induced ferroptosis is enhanced (Lang *et al.*, 2019). The commonly utilized xCT inhibitor erastin (Era) can also activate ferroptosis, although long-term treatment with Era may contribute to enhanced cellular resistance to ferroptotic death. Moreover, Era treatment for extended periods has been shown to improve CBS and CSE expression, independently regulating ferroptosis (Liu *et al.*, 2020). Disrupting xCT activity increases both CSE and CBS expression yet significantly reduces endogenous H_2S production. The supplemental addition of the H_2S donor GYY4137 [GYY, morpholin-4-ium-4-methoxyphenyl (morpholino) phosphinodithioate] reversed the loss of resistance to 5-fluorouracil (5-FU), which emerges upon the inhibition of xCT, highlighting the functional importance of H_2S as a mediator of xCT-associated chemoresistance activity (Chen *et al.*, 2021). Interestingly, supplementation with H_2S increased the protein levels of xCT without influencing its mRNA expression, suggesting that H_2S has a post-transcriptional regulatory effect on xCT. The H_2S axis maintains xCT stability through persulfidation of OTUB1 at cysteine 91 (Chen *et al.*, 2021). Correspondingly, xCT and the trans-sulfuration pathway have been identified as key ferroptotic regulators in cancer cells, functioning primarily by producing cysteine as a precursor for the production of the critical antioxidant glutathione (GSH). When cells are treated with both Era and AOAA, which inhibit CBS, the levels of GSH present within cells are markedly reduced, thus contributing to higher rates of ferroptosis associated with increased PTGS2 expression at the mRNA level and higher levels of MDA, which is a lipid peroxidation end-product (Marrocco *et al.*, 2017).

Hcy is an amino acid that contains sulfur and is important for methionine metabolism, gene methylation, phospholipid biosynthesis, neurotransmission, and nucleic acid biosynthesis (Shen *et al.*, 2020). Given its essential role as a mediator of Hcy transsulfuration, inhibition of CBS increases the concentration of Hcy in hepatocellular carcinoma cells, triggering ferroptosis (Wang *et al.*, 2018). Patients with excessively high levels of methionine, vitamin B12, or folic acid deficiencies can have high serum Hcy concentrations in the form of HHcy. This condition, in turn, contributes to the development of abnormal bone metabolism and mitochondrial dysfunction through oxidative stress and modification of collagen cross-linking (Levasseur, 2009). Hcy may contribute to more severe oxidative stress in nucleus pulposus cells and aggravate the induction of ferroptosis, resulting in increased lipid peroxidation, ROS production, and reduced GPX4 expression. Fer-1 is a ferroptosis inhibitor that protects nucleus pulposus cells from degeneration (Zhang *et al.*, 2020). It also reduced Hcy-induced injury

in ovarian granulosa cells in a dose-dependent manner (Shi *et al.*, 2022). Vascular smooth muscle cells (VSMCs) may become dysfunctional due to high hydrostatic pressure (HHP) levels. HHP exposure induces ferroptosis in VSMC, accompanied by high levels of ROS production, lipid peroxidation, iron accumulation, downregulation of CSE/H2S, and suppression of GSH production (Jin *et al.*, 2022).

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a vital mediator of ferroptosis induction, and H_2S has repeatedly been shown to activate antioxidant activity mediated by Nrf2 in respiratory diseases, including rhinitis, sinusitis, conjunctivitis, acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), and asthma (Han *et al.*, 2011; Khattak *et al.*, 2021). Wang et al. demonstrated that H_2S was capable of inhibiting ferroptosis and ferritinophagy, while this activity was impaired in mice or cells lacking Nrf2 expression, and on the other hand, the anti-ferroptosis capability of H_2S was enhanced when Nrf2 was overexpressed because the ability of H_2S to protect against injury is regulated by Nrf2 activation and PPAR-γ signaling (Y. Wang *et al.*, 2022). These results showed that the upstream of the whole protective pathway is Nrf2 because the activation of PPAR-γ and the inhibitory effects of H_2S on ferritinophagy and ferroptosis were significantly restricted when Nrf2 was knocked out, both in vivo and in vitro.

The deubiquitinating enzyme OTUB1 stabilizes proteins through specific mechanisms (Wiener *et al.*, 2012), and OTUB1 overexpression stabilizes xCT in oncogenic settings, thereby interfering with the ability of xCT to regulate ferroptotic pathways (Koppula *et al.*, 2021; Yasuhara *et al.*, 1997). Endogenous H_2S production is closely linked to interactions between xCT and OTUB1, with H_2S stabilizing xCT *via* the persulfidation of cysteine 91 of OTUB1 (Chen *et al.*, 2021).

H_2S DONORS AND FERROPTOSIS

Inorganic sulfurized salts, including NaHS and sodium sulfide (Na_2S), are among the earliest known H_2S donors and are commonly used as H_2S equivalents in research. Currently, multiple H_2S donors have been applied in various disease models and achieved remarkable effect via different signal pathways (Table 2).

C2C12 cells treated with exogenously administered NaHS could remediate abnormal redox conditions induced in response to RSL3 through the upregulation of GPX1 and GPX4, thereby normalizing lipid metabolism and mitochondrial function while also increasing acetyl-CoA levels. NaHS also inhibits the expression and acetylation of ALOX12, thus protecting C2C12 cells from ferroptosis via the CSE/H_2S signalling pathway (Wang, Yu, *et al.*, 2021). Increased GSH content and GPX4 activity took place in response to NaHS treatment. Decreases in ROS, lipid ROS, and MDA levels have been reported in both BV2 cells and in the prefrontal cortex (PFC) of diabetic mice administered with NaHS. Mechanistically, these effects were found to be mediated by the increased expression of SLC7A11 protein in these BV2 cells or in the PFC, indicating that NaHS may act in an antidepressant-like manner *via* the upregulation of SLC7A11. NaHS can also improve PFC cysteine levels in a murine model of type 1 diabetes, resulting in upregulation of GPX4 (Wang, Wang, *et al.*, 2021), indicating that NaHS may exert its protective effect by upregulating the protein expression of SLC7A11 and cysteine levels.

In plants, bacteria, and some fungi, H_2S is converted to cysteine through sulfur assimilation. In contrast, studies have also found that the addition of Na_2S as a donor of H_2S could promote the generation of cysteine in mammalian cells in the presence of cystine. Since H_2S exists as HS^- in a neutral solution, disulfide compounds such as cystine can react with HS^- in the culture medium as well as in the cell. This study demonstrated that after the addition of Na_2S solution to the culture medium, HS^- was transiently generated and disappeared immediately through the reaction between HS^- and cystine to form cysteine persulfide and polysulfides (see Fig. 10 in Koike *et al.*, 2017). A significant increase in cystine concentration was observed after treatment with Na_2S. The mechanism by which Na_2S protects SH-SY5Y cells from MG cytotoxicity is *via* the activation of Nrf2, but not HS^- itself. However, this mechanism has not been verified in ferroptosis, and further investigation is needed to elucidate its molecular mechanism.

The slow-acting H_2S donor GYY4137 can release this gas for several hours (Chen *et al.*, 2016). Ferroptosis is an important component of sepsis-associated ALI, and GYY4137 has been reported to protect against ALI by alleviating oxidative stress through the removal of ROS. GYY4137 also markedly suppressed COX-2 and NOX1 expression while increasing sepsis-induced GPX4 and SLC7A11 expression, thereby inhibiting the occurrence of ferroptosis in sepsis-induced ALI (Li *et al.*, 2022).

H_2S AND IRON METABOLISM

The metabolism of iron is integral to the induction and progression of ferroptotic pathways, whereas H_2S has a complex relationship with iron metabolism. Iron is

Table 2. Summarization of current H2S donors application in different disease models.

Disease model	H2S donor	Mechanism	Reference
Myocardial fibrosis	AP39	PINK1/Parkin pathway	(Yang *et al.*, 2023)
Hypertension	NaHS	Upregulated the VSMC GSH content and cystathionine gamma-lyase (CSE)/hydrogen sulfide (H2S)	(Ruxi Jin *et al.*, 2022)
Intracerebral hemorrhage (ICH)	NaHS	Improved GPX4 and SLC7A11 via the CBS/H2S system	(Y. Yu *et al.*, 2023)
Retinal degenerative diseases.	NaHS	AMPK- and p62-dependent non-canonical NRF2-KEAP1 pathway	(M. Yu *et al.*, 2023)
Sepsis-induced cardiomyopathy (SIC)	NaHS	Inhibited the phosphorylation of BECN1 and increased expression levels of SLC7A11 and GPX4	(Cao *et al.*, 2022)
Beryllium disease	NaHS	Decreased the accumulation of Fe2+and lipid peroxides	(Liu *et al.*, 2023)
Sepsis-induced acute lung injury (ALI)	GYY4137	Stimulated autophagy by blocking mTOR signaling	(Jianhua Li *et al.*, 2022)

involved in H_2S production *via* non-enzymatic processes. Meanwhile, the uptake, transport, and accumulation of iron is regulated by H_2S. The interactions between H_2S and iron shape the cellular homeostasis of H_2S and define the relevant signaling crosstalk.

In addition to the enzymatic mechanisms mentioned above, non-enzymatic H_2S in eukaryotes may come from a cysteine reaction with iron and vitamin B6 or from the reduction of elemental sulfur (Kolluru *et al.*, 2013; Yang *et al.*, 2019). Nucleophilic attack by vitamin B6 on cysteine forms a cysteine-aldimine, which reacts with free or heme-bound iron to yield a quinonoid containing cysteine. Subsequently, iron ions remove the thiol group from H_2S, and the resulting de-sulfureted aldimine is hydrolyzed to produce vitamin B6, ammonia, and pyruvate.

Free iron (Fe^{2+}) is taken up by the cell through ion channels or transporters, such as L-type Ca^{2+} channels on cellular membranes. H_2S is also an endogenous gaseous ATP-sensitive K+ (KATP) channel opener that induces vasodilation (Arif *et al.*, 2022). H_2S and iron may interact to suppress L-type Ca^{2+} channels via S-sulfhydration, as shown in mouse pancreatic β-cells (Tang *et al.*, 2013). The influx of iron into cells through L-type Ca^{2+} channels has been demonstrated in cardiomyocytes (Arif *et al.*, 2022). The inhibition of these processes by H_2S decreases the amount of iron entering the cell. Ferroportin 1 (Fpn1) and transferrin receptor 1 (TfR1) are essential regulators of iron homeostasis in most cells. Injecting NaHS *in vivo* significantly increases serum iron levels and transferrin saturation while significantly downregulating TfR1 and Fpn1 at the protein level (Zhang *et al.*, 2019). Under the influence of ferric ions, cysteine catalyzes the generation of H_2S through a protective mechanism that mitigates excessive iron concentrations by reacting with iron and generating acid-labile iron sulfide, such that excessive iron cannot cause further oxidative stress (Tang *et al.*, 2023; Yang *et al.*, 2019).

Recently, it was found that CBS is essential for iron homeostasis, and the hepatic, splenic, cardiac, and serum iron levels in CBS-deficient mice are elevated together with oxidative stress-related marker levels; mice homozygous for the knockout of CBS failed to survive for more than 4 weeks (Zhou *et al.*, 2018). Moreover, hepcidin can accelerate the degradation of Fpn1 by interacting with it, thereby reducing intracellular iron release into the systemic circulation. Upregulation of hepcidin in both the liver and systemic circulation has been reported to lead to decreased Nrf2 expression. Elevated hepcidin levels resulted in a marked decrease in Fpn1 expression and exacerbated iron retention, causing severe liver damage. An adenoviral vector was used to restore CBS expression, and this attempt was able to reverse the damaged phenotypes (Zhou *et al.*, 2018). Inflammatory cytokines such as IL-6 promote the upregulation of hepcidin via JAK-STAT3 signaling through interactions with activin B and the SMAD1/5/8 pathway. In contrast, H_2S suppresses the upregulation of hepcidin *via* JAK-STAT signaling *via* the suppression of STAT3 phosphorylation, thereby reducing IL-6 production (Zhang *et al.*, 2019). Similar findings were also reported by Xin and others who demonstrated that H_2S promoted the expression of SIRT1, stabilizing SIRT1-STAT3 interactions to reduce the acetylation of STAT3, thereby decreasing hepcidin levels (Xin *et al.*, 2016). Collectively, these results indicate that H_2S reduces hepcidin levels by acting upstream of STAT3, which provides an opportunity for further investigation. A better understanding of the mechanism underlying the regulation of STAT3 *via* H_2S may shed light on its clinical potential.

H_2S AND LIPID PEROXIDATION

Lipid peroxidation is a hallmark of ferroptosis, which results from the generation of excessive levels of ROS due to increased intracellular iron concentrations, together with the simultaneous depletion of GSH. The antioxidant enzyme GPX4 is responsible for lipid peroxide neutralization and can utilize GSH as a cofactor to preserve membrane fluidity, thus shielding the membrane against peroxidation-related damage (Su *et al.*, 2019). Inhibition of GPX4 contributes to high levels of ROS production, and its overexpression can conversely reduce ROS generation and prevent ferroptosis induction. Iron metabolism-related ROS generation is a major cause of lipid peroxidation, given that iron is required for numerous physiological processes, and excessive iron levels ultimately disrupt normal cellular function through the resultant oxidative damage, contributing to cell death (Su *et al.*, 2019).

H_2S has been reported to exhibit concentration-dependent pro- or antioxidant activities, such that plasma lipid peroxidation is suppressed and enhanced by low (10 μM) and high (1000 μM) concentrations of NaHS (Sun *et al.*, 2012). Sun and others found that the use of NaHS to pretreat neonatal rat cardiomyocytes resulted in the generation of lower concentrations of ROS during hypoxia/reoxygenation, and that H_2S suppressed the activity of mitochondrial complex IV while simultaneously enhancing superoxide dismutase activity (Sun *et al.*, 2012). *In vivo*, Wu and others reported that 50 μmol/kg NaHS per day for 16 weeks with a high-fat diet prevented the increase in diet-induced lipid oxidation (Wu *et al.*, 2009), suggesting that NaHS has protective effects against lipid oxidation. H_2S is traditionally known for its toxic effects on living organisms. Current studies are still focused on cell and animal experiments, and relevant data and safety for clinical use are needed.

Hcy has been suggested to increase the severity of oxidative stress owing to the generation of free radicals through the autooxidation of excessive Hcy concentrations (Malinowska *et al.*, 2012). Yan et al. further reported that H_2S protected against HHcy-induced cellular injury through its antioxidant activity, leading to a 55.8% decrease in the overproduction of $O^{2-\bullet}$ induced by Hcy (Yan *et al.*, 2006). Chang et al. found that the administration of H_2S decreased total plasma Hcy concentration and lipid peroxidation. CBS- and CSE-knocked out mice exhibit lower GSH levels and are more susceptible to oxidative stress, further confirming the link between stress and endogenous H_2S (Chang *et al.*, 2008).

Lipid metabolism is closely related to the conversion of carbohydrates into fat. The cytoplasmic hydrolysis of lipids is the first step in metabolism, resulting in the production of glycerol and fatty acids. The two compounds are then glycolyzed and β-oxidated, and H_2S regulates glycolysis and fatty acid metabolism through protein S-sulfhydration (Cheung & Lau, 2018). Activated glycolysis and the pentose phosphate pathway can increase antioxidant activity (D'Alessandro *et al.*, 2014). Exposure of clonal HepG2 hepatocyte cells to NaHS (–10–100 μM) reduced glucose consumption and glycogen levels (Zhang *et al.*, 2013). In addition, the CSE/ H_2S pathway has recently been shown to play a key role in the regulation of glucose synthesis through pyruvate carboxylase S-sulfhydration in gluconeogenesis (Ju *et al.*, 2015).

Further details regarding the association between H_2S and ferroptotic activity are shown in Fig. 2. The interaction pathway between H_2S and ferroptosis is shown in Fig. 3.

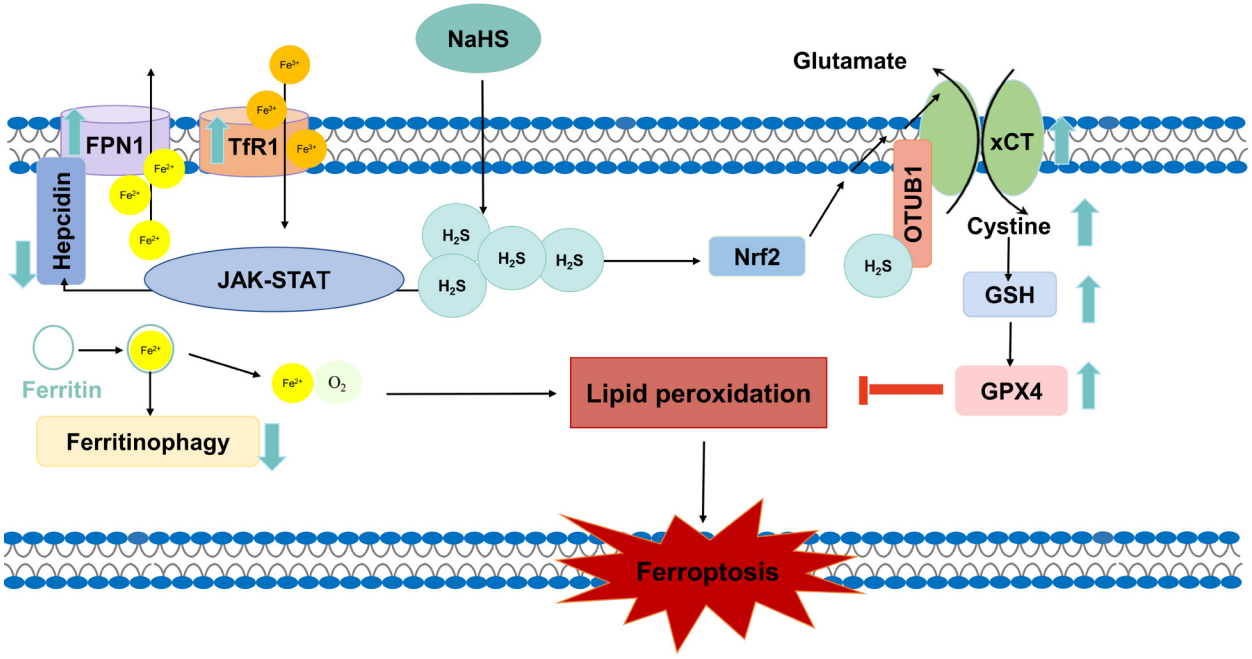

Figure 2. The effect of H2S on ferroptosis.
Cystine is imported by the Xc-system and converted into cysteine, which in turn serves as a GSH precursor. GSH is a GPX4 substrate. Both cysteine and Hcy are also substrates for the production of H_2S, and the H_2S equivalent NaHS can increase GSH levels and GPX4 activity while suppressing the activity of the lipoxygenase ALOX12, thus remediating dysregulated lipid metabolism. Overly high Fe^{2+} concentrations can contribute to ROS generation *via* the Fenton reaction, whereas HHcy can drive enhanced ROS production and aberrant mitochondrial functionality. HHP promoted ROS generation by suppressing CSE/H2S expression and reducing GSH generation. TfR1 and FPN are important regulators of cellular iron homeostasis and NaHS can suppress their downregulation at the protein level. Hepcidin interacts with Fpn1 to facilitate its degradation, whereas H_2S reduces hepcidin expression *via* JAK-STAT signaling. The deubiquitinase OTUB1 is capable of stabilizing the xCT Xc-system subunit via cysteine 91 persulfidation. The xCT inhibitor Era, when used to treat cells for an extended period of time, upregulates CBS expression and enhances the resistance of cells to ferroptosis. In contrast, the CBS inhibitor AOAA, when administered together with Era, downregulated cellular GSH levels and enhanced ferroptotic death.

H_2S AND FERROPTOSIS-ASSOCIATED MicroRNAs

MicroRNAs (miRNAs) are short RNAs of ~22 nucleotides in length that lack coding ability and function by suppressing translation and/or promoting the degradation of complementary target mRNAs. Inhibiting miR-30b-5p expression reportedly results in FPN1 and SLC7A11 upregulation in trophoblasts, while the upregulation of this mRNA under hypoxic conditions leads to the downregulation of these two genes, causing trophoblast ferroptosis (Zhang *et al.*, 2020). Members of the miR-30 family have also been confirmed to target CSE expression, thereby directly regulating the generation of H_2S (Shen *et al.*, 2015). By targeting GPX4, miR-15a can control ferroptotic induction in colorectal cancer cells, whereas overexpression of miR-15a suppresses GPX4 expression *in vitro* and *in vivo,* resulting in elevated ROS generation, intracellular Fe^{2+} accumulation, and increased MDA levels (Liu *et al.*, 2022). Fan et al. found that miR-15a overexpression resulted in elevated GPX4 protein levels, with a concomitant decrease in ferroptotic induction associated with fewer severe myocardial inju-

Table 3. An overview of H_2S and ferroptosis-associated proteins and miRNAs

Protein or miRNA	Relation with H_2S	Relation with ferroptosis	Disease involved
Nrf2	Enhances the anti-ferritinophagy and anti--ferroptotic effects of H_2S	Anti-ferroptotic	COPD
OTUB1	H_2S maintains the stability of xCT through persulfidation of OTUB1 at cysteine 91	Stabilizes xCT	Colorectal cancer
TfR1	NaHS inhibits the downregulation of TfR1 protein expression *in vivo*	Regulates cellular iron balance	LPS
FPN1	NaHS inhibits the downregulation of Fpn1 protein expression *in vivo*	Regulates cellular iron balance	LPS
Hepcidin	H_2S reduces hepcidin expression	Accelerates the degradation of FPN1	Anemia of inflammation
MiR-30b-5p	Regulates H_2S production	Downregulates SLC7A11 and FPN1	Myocardial ischemia/reperfusion injury
MiR-15a	H_2S upregulates miR-15a	Positively regulates ferroptosis *via* directly targeting GPX4	Broiler thymus
MiR-194	Decreasing H_2S can downregulate miR-194	Activates the Nrf2/HO-1 signaling pathway	Cerebral injuries.

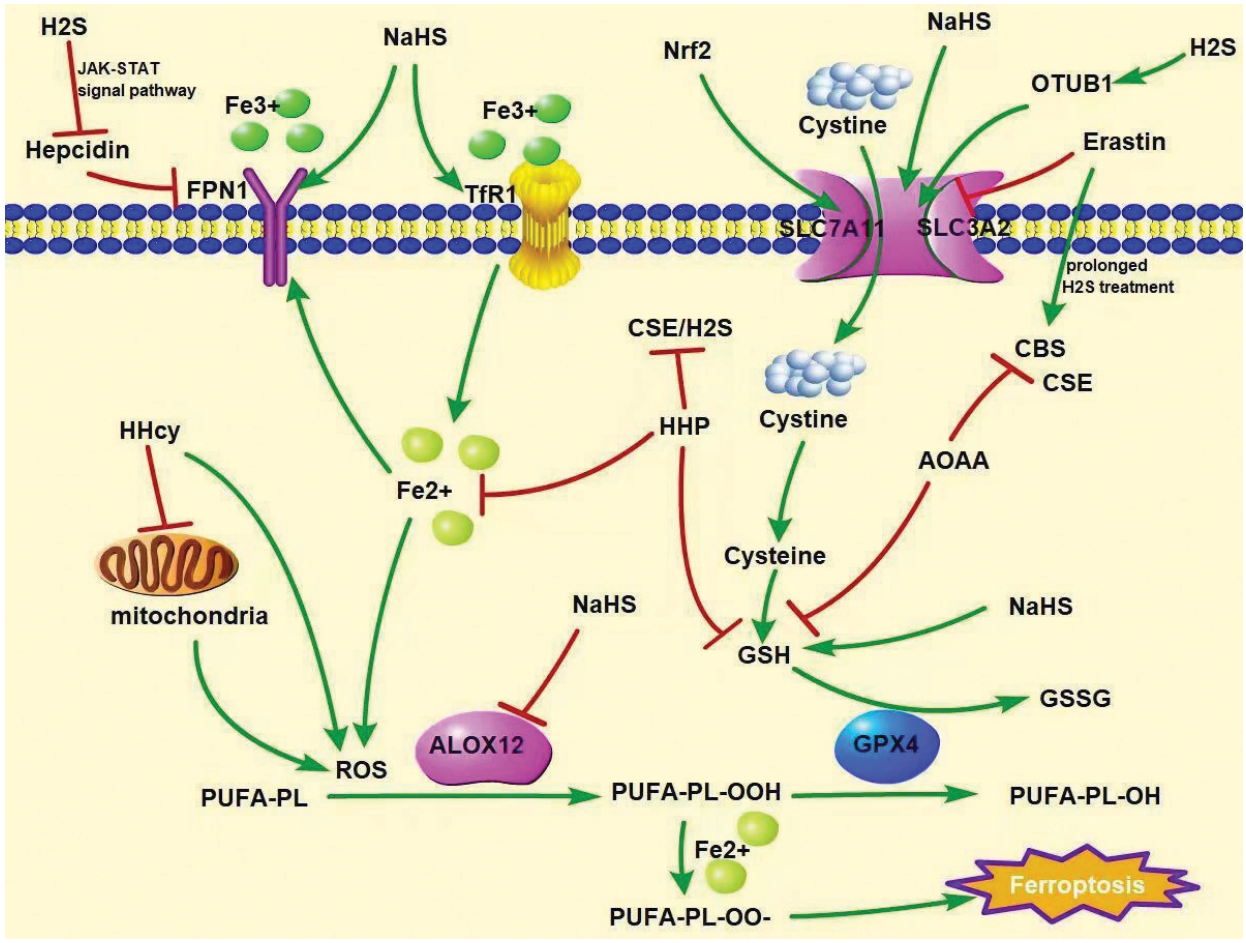

Figure 3. The pathway of H2S interacting with ferroptosis.
NaHS is a donor of H_2S, and once entered cells, it regulates ferroptosis-associated proteins to exert protective effect. First, it can be converted into cystine, increasing the content of GSH and upregulating the activity of GPX4, resulting in reduced ferroptosis *via* antioxidant effect. At the same, it maintains the stability of xCT through persulfidation of OTUB1 at cysteine 91. Second, it inhibits ferroptosis and ferritinophagy via upregulating Nrf2. It reduces the expression of hepcidin *via* the JAK-STAT pathway, preventing the downregulation of FPN1. Thirdly, NaHS itself upregulates the expression of FPN1 and TfR1 with or without inflammation, further regulating the metabolism balance of iron *in vivo*.

ries (Fan *et al.*, 2021). Exposure to H_2S also results in miR-15a upregulation (Xueyuan *et al.*, 2021). Additional evidence suggests that miR-194 can mitigate the severity of ischemia-reperfusion injury through the inhibition of Bach1 and introduction of signaling *via* the Nrf2 /HO-1 pathway. Therefore, miR-194 inhibition reversed these effects by reducing HO-1 and Nrf2 protein levels (Li *et al.*, 2021). Reduction in H_2S production reduces miR-194 expression and drives the deposition and realignment of collagen under diabetic conditions to attenuate fibrosis and renovascular constriction (John *et al.*, 2017). The proteins and miRNAs associated with ferroptosis are summarized in Table 3.

CLINICAL POTENTIAL APPLICATION

In cancer, the clearance of H_2S and the regulation of the enzymes involved in its pathways are essential for cancer therapy. A biocompatible fusiform iron oxide-hydroxide nano-spindles nano system (FeOOH NSs) was designed, which could be applied to magnetic resonance imaging (MRI) to monitor ferroptosis. It can efficiently scavenge endogenous H_2S by a reduction reaction to inhibit the growth of CT26 colon cancer cells (PMID:32789963). The biosafety of this nano-system has been verified in animal models for three months, indicating its potential clinical translation. In addition, zinc oxide-coated virus-like silica nanoparticles (VZnO) were also tested to establish an H_2S-responding nano-system to scavenge H_2S to alleviate ferroptosis in colorectal cancer (Pan *et al.*, 2021). Additionally, fluorescent probes that can be used for selective detection of hydrogen sulfide have been developed to explore the biological and pathological effects of H_2S during ferroptosis (Di *et al.*, 2021; Guo *et al.*, 2022). The two-photon fluorescent probe (PSP) exhibited excellent photostability and two-photon imaging performance, and elevated levels of H_2S were observed during ferroptosis in tumors (Di *et al.*, 2021). Needless to say, these tools will enable a more extensive expansion of H_2S-related technologies and knowledge for broad therapeutic applications in the near future.

FUTURE DIRECTIONS

In recent decades, a broad range of physiological functions of H_2S have been gradually recognized. Accumulating evidence has indicated that H_2S plays a role in various types of cell death. However, it is still not clear whether the suppression of cell death occurs simultaneously across cell death or is more potent toward one type of cell death than the others. As a relatively new

area, the interactive mechanisms between H_2S and ferroptosis need to be further explored, and the implications of H_2S and ferroptosis interactions in various diseases, such as chronic organ fibrosis, diabetes-associated diseases, stroke, and other degenerative diseases, have not been studied. More experiments are needed to develop a safety profile and treatment regimens for H_2S in various disease models and human subjects. Currently, the time and dosage of H_2S in animal experiments are being investigated, but the results are inconsistent (Wang *et al*., 2022; Zhang *et al*., 2023). In addition, newly developed nanoparticles or fluorescent probes have not been applied in clinical trials and require further validation and biosafety tests. Minimizing H_2S-induced toxicities remains a challenge and H_2S associated biomarkers need to be identified for the better clinical use of H_2S.

CONCLUSION

In summary, H_2S functions as a key gastronomist closely tied to ferroptosis with the substrates, enzymes, and donors involved in the production of H_2S, all of which are related to ferroptosis. The metabolism of iron, process of lipid peroxidation, and inactivation of antioxidation systems all participate in the process of ferroptosis. H_2S can also regulate the uptake, transport, and accumulation of iron, and reduce lipid oxidation by enhancing superoxide dismutase activity and regulating glucose production. miRNAs are involved in nearly every biological process, and several miRNAs have been found to be involved in both H_2S metabolism and ferroptosis. As the interplay between H_2S and ferroptosis has only been explored in recent years, much work remains to be done to adequately understand the relationship between them. For example, additional research exploring the production, storage, and release of H_2S is needed, as are efforts to understand how H_2S affects mammals *in vivo* under a range of settings and how it shapes the process of ferroptosis.

Declarations

Acknowledgements. Not applicable.

Declaration of conflict of interest: None.

Availability of data and materials: Not applicable because this was a review article.

Author contributions: XG, KL, and CL: literature reading, data collection, and paper drafting. CL: Data analysis and revision of the paper. All the authors have read and approved the final version of the manuscript. Ethics approval and consent to participate: Not applicable because this was a review article.

Patient consent for publication: Not applicable because this is a review article.

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