Journal of Pharmacological Sciences 155 (2024) 75–83

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Sulfur metabolism as a new therapeutic target of heart failure

Akiyuki Nishimura[a,b,c,**], Xiaokang Tang[a,b,c], Liuchenzi Zhou[a,b,c], Tomoya Ito[d], Yuri Kato[d], Motohiro Nishida[a,b,c,d,*]

[a] National Institute for Physiological Sciences, National Institutes of Natural Sciences (NINS), Okazaki, 444-8787, Japan
[b] Exploratory Research Center on Life and Living Systems, NINS, Okazaki, 444-8787, Japan
[c] SOKENDAI (The Graduate University for Advanced Studies), Okazaki, 444-8787, Japan
[d] Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan

ARTICLE INFO

Keywords:
Supersulfides
Sulfur catabolism
Heart failure
Redox
Mitochondria

ABSTRACT

Sulfur-based redox signaling has long attracted attention as critical mechanisms underlying the development of cardiac diseases and resultant heart failure. Especially, post-translational modifications of cysteine (Cys) thiols in proteins mediate oxidative stress-dependent cardiac remodeling including myocardial hypertrophy, senescence, and interstitial fibrosis. However, we recently revealed the existence of Cys persulfides and Cys polysulfides in cells and tissues, which show higher redox activities than Cys and substantially contribute to redox signaling and energy metabolism. We have established simple evaluation methods that can detect polysulfides in proteins and inorganic polysulfides in cells and revealed that polysulfides abundantly expressed in normal hearts are dramatically catabolized by exposure to ischemic/hypoxic and environmental electrophilic stress, which causes vulnerability of the heart to mechanical load. Accumulation of hydrogen sulfide, a nucleophilic catabolite of persulfides/polysulfides, may lead to reductive stress in ischemic hearts, and perturbation of polysulfide catabolism can improve chronic heart failure after myocardial infarction in mice. This review focuses on the (patho)physiological role of sulfur metabolism in hearts, and proposes that sulfur catabolism during ischemic/hypoxic stress has great potential as a new therapeutic strategy for the treatment of ischemic heart failure.

1. Introduction

The myocardium is an extremely robust cells and tissue that continuously repeat contraction and relaxation during our lifetime without cell proliferation and replacement. The myocardium has unique stress adaptation mechanisms that differ from those of other cell types. In particular, cardiomyocytes have well-developed cysteine (Cys)-based antioxidant systems such as thioredoxin and glutathione (GSH).[1] Cys, a nucleophilic sulfur-containing amino acid, is thought to generate the reducing power of cardiomyocytes. The human genome encodes about 214,000 Cys, of which 10–20% are redox-active thiol groups with a dissociated proton at physiological pH conditions.[2,3] These deprotonated Cys thiols mainly react with various reactive oxygen species (ROS), reactive nitrogen species (RNS), and electrophilic secondary metabolites (electrophiles) that are generated from the reaction of ROS and RNS with lipids and nucleic acids. Oxidative posttranslational modification with Cys thiols alters the structure and function of sensor proteins, which serve as signal mediators in redox signaling. Oxidative modification of various proteins including ATP synthase,[4] histone deacetylase 4 (HDAC4)[5,6] and GTP-binding proteins (G proteins) such as the α subunit of heterotrimeric $G_{i/o}$ family proteins ($G\alpha_{i/o}$)[7] and H-Ras[8] has been discovered to mediate the progression of cardiac remodeling and heart failure.[9] Oxidative modification has been widely investigated as a general concept of redox signaling in cardiovascular pathophysiology. However, the recent discovery of highly reactive sulfur metabolites containing catenated sulfur atoms (supersulfides) such as Cys persulfide (Cys-SSH) and Cys polysulfide has led to a review of the concepts of redox biology and its therapeutic applications. In fact, we have recently revealed that Cys persulfide/polysulfide catabolism in proteins by exposure to environmental electrophiles decreases the contractility of cardiomyocytes and increases the mortality and morbidity of heart failure in mice after pressure overload.[10,11] In addition, ischemic/hypoxic stress causes catabolism of endogenous

Peer review under responsibility of Japanese Pharmacological Society.
* Corresponding author. Department of Physiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan.
** Corresponding author. Division of Cardiocirculatory Signaling, National Institute for Physiological Sciences, National Institutes of Natural Sciences (NINS), Okazaki, 444-8787, Japan.
E-mail addresses: aki@nips.ac.jp (A. Nishimura), nishida@phar.kyushu-u.ac.jp (M. Nishida).

https://doi.org/10.1016/j.jphs.2024.04.005
Received 22 March 2024; Received in revised form 7 April 2024; Accepted 21 April 2024
Available online 23 April 2024
1347-8613/© 2024 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Abbreviations

3-MST	3-Mercaptopyruvate sulfurtransferase
CARSs	cysteinyl-tRNA synthetases
CBS	Cystathionine β-synthase
CSE	cystathionine γ-lyase
Cys	cysteine
Cys-SSH	cysteine persulfide
Drp1	dynamin-related protein 1
Ech-A	echinochrome A
ETHE1	ethylmalonic encephalopathy 1
FRET	Förster resonance energy transfer
GSH	reduced glutathione
GSSG	glutathione disulfide
GSSH	glutathione persulfide
GSSSG	glutathione trisulfide
H_2S/HS	hydrogen sulfide/hydrosulfide anion
HPE-IAM	β-(4-hydroxyphenyl)ethyl iodoacetamide
IAA-biotin	Iodoacetyl-biotin
IR	ischemia/reperfusion
LC-ESI-MS/MS	Liquid chromatography-electrospray ionization–tandem mass spectrometry
MeHg	methylmercury
MI	myocardial infarction
Na_2S_4	sodium tetrasulfide
RNS	reactive nitrogen species
ROS	reactive oxygen species
RSOH	sulfenic acid
RSSH	persulfide
RSSR	disulfide
PLP	pyridoxal-5′-phosphate
SQOR	sulfide quinone oxidoreductase
TAC	transverse aortic constriction
TME-IAM	*N*-iodoacetyl l-tyrosine methyl ester

supersulfides in hearts, leading to a worse prognosis of chronic heart failure after myocardial infarction.[12]

In this review, we first provide an overview of recent progress in sulfur biology including the definition and properties of supersulfides and the detection of supersulfides and protein polysulfidation. Next, we provide an overview of sulfur biology in cardiac remodeling and heart failure, and then highlight the roles of supersulfide catabolism by exposure to ischemic/hypoxic or electrophilic stresses in cardiac homeostasis and diseases together with our latest findings.

2. Recent progress in sulfur biology

2.1. Discovery of biological activity of pharmacological administration of H_2S

Hydrogen sulfide (H_2S) is a colorless gas with a distinctive odor known as rotten egg smell and acutely toxic to humans at high concentrations (100 ppm and higher).[13] However, H_2S is detected in various cells, and its biological activity has been widely investigated over the last few decades of the past century.[14] Abe and Kimura first identified that H_2S is detected in the brain and acts as a neurotransmitter.[15] Then, numerous numbers of papers reported the involvement of H_2S in many physiological and pathological processes in animals including inflammation,[16] autophagy,[17] neuromodulation, cardioprotection,[18] and cancer.[19,20] In addition, H_2S has also been linked with a wide variety of plant physiology including development,[21,22] photosynthesis[23] and stress tolerance.[24] More recently, the anti-aging effects of H_2S have been attracting a great deal of attention since it was reported to extend the average life span of yeast, nematodes, flies and mice.[25–27]

2.2. Definition and biochemical feature of supersulfides

Although the physiological and pathological roles of H_2S have been widely reported,[28,29] it is unclear whether H_2S is acting directly in all cases. The biological effects of H_2S are mainly considered due to nucleophilicity like cysteine thiol.[8,30–35] However, reactive kinetics of nucleophilicity by H_2S suggests that it is insufficient to directly react with oxidants and electrophiles.[8,35] These results raise our speculation that intermediates from H_2S are bona fide nucleophilic substances. Nucleophilic HS^- can produce persulfide (RSSH) by reacting with oxidized forms of thiols such as disulfide (RSSR) or sulfenic acid (RSOH) (Equations (1) and (2)).[36,37] Recent innovations in mass spectrometry analysis identified that diverse and abundant reactive persulfide and polysulfide species including cysteine per/polysulfides and glutathione per/polysulfides are observed in tissues and cells, which are shown to play pivotal roles in the regulation of redox signaling.[38–42] Supersulfides are currently defined as persulfides and polysulfides with catenated sulfane sulfur (RSS_nR, $n > 1$, R = hydrogen or alkyl). Supersulfides include not only small molecules such as cysteine and glutathione but also the thiol group of proteins. Supersulfides in proteins (protein polysulfidation) can modulate protein structure and function as a post-translational modification.[41,43] Catenated sulfane sulfur atoms in supersulfides have unique redox properties. Due to the α-effect, the increased reactivity of α-nucleophiles due to adjacent unpaired electrons, supersulfides have more nucleophilic power than the corresponding thiol.[44] Most supersulfides are deprotonated under physiological pH conditions and can easily react with oxidants and electrophiles. Supersulfides have both nucleophilic and electrophilic sulfur in a single molecule and behave as either nucleophile or electrophile depending on the situation.

$$HS^- + RSSR \rightleftharpoons RSSH + RS^- \quad (1)$$

$$HS^- + RSOH \rightleftharpoons RSSH + OH^- \quad (2)$$

Supersulfides highly exist in various living organisms. Quantitative mass spectrometry analysis revealed that glutathione persulfide (GSSH) is present at about 150 μM in the brain and 50 μM in the heart and liver in mice, which corresponds to 2–5% of GSH.[38] In vegetables, onion, broccoli, Chinese chive and garlic contain large amounts of polysulfides.[45] Some gut bacteria such as *Lachnospiraceae* and *Ruminococcaceae* families highly produce Cys-SSH.[46] Supersulfides would have fundamental roles in sulfur-related biological processes in all living organisms.[1,47,48]

2.3. Biosynthesis and metabolism of supersulfides and H_2S

Three enzymes were initially reported to catalyze H_2S formation in mammals. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are enzymes for the transsulfuration pathway (de novo biosynthetic pathway of cysteine in mammals). CBS catalyzes the formation of cystathionine from homocysteine and serine, and the resulting cystathionine is converted into cysteine by CSE. Both CBS and CSE are pyridoxal-5′-phosphate (PLP)-dependent enzymes that generate H_2S from cysteine and homocysteine.[18,28] 3-Mercaptopyruvate sulfurtransferase (3-MST) generates H_2S from 3-mercaptopyruvate, which is produced from cysteine and α-ketoglutarate by cysteine aminotransferase.[49] Expression of CBS is mainly found in the brain, kidney and liver, whereas CSE is predominantly present in vascular systems.[50]

Although CBS and CSE have been identified as H_2S-generating enzymes as described above, recent innovations in mass spectrometry

analysis identified that these enzymes are able to produce Cys-SSH from cystine as a substrate (Fig. 1).[38] Product analysis indicated that CSE and CBS undergo a CS lyase-like reaction to directly form Cys-SSH from cystine. Cysteinyl-tRNA synthetases (CARSs) are enzymes that catalyze the covalent attachment of cysteine to cysteinyl-tRNA for protein translation. There are two CARS genes in mammals: a cytosolic CARS1 and a mitochondrial CARS2. The Cys–SSH–producing activity of CARSs has been identified as a moonlighting function.[42] Two cysteines would be involved in Cys-SSH formation by CARSs. In this reaction, sulfur is cleaved from the donor cysteine and transferred to the acceptor cysteine thiol. The amount of Cys-SSH and GSSH in the liver and lung of CARS2 heterozygous mice is decreased by about 50%, whereas CBS/CSE/3-MST triple KO mice show no changes in the formation of these supersulfides, indicating that CARS2 has a critical role in supersulfide formation.[42,51] CARSs can generate persulfidated cysteinyl-tRNA and Cys-SSH is directly incorporated into nascent polypeptides during translation.[42] In addition to this co-translational process, protein polysulfidation can be also posttranslationally regulated by the reaction with other supersulfides or electrophiles as described below.

H_2S is oxidized back to glutathione persulfide (GSSH) from GSH by sulfide quinone oxidoreductase (SQOR) in mitochondria.[52,53] GSSH is further oxidized by sulfur dioxygenase such as ethylmalonic encephalopathy 1 (ETHE1) to sulfite. Subsequently, sulfite is oxidized to sulfate or thiosulfate by sulfide-detoxifying enzymes such as rhodanese.[54,55]

The supersulfide transport system in multiple cells and organs is still largely unknown. When intracellular supersulfides are increased in cardiomyocytes and hepatocytes, these are released to the extracellular side in a time-dependent manner.[56] It is speculated that cells have transporters for supersulfide influx and efflux. ATP-binding cassette transporter Atm1 from *Saccharomyces cerevisiae* transports glutathione trisulfide (GSSSG) and glutathione disulfide (GSSG) but not GSH.[57] We recently identified that the cystine/glutamate transporter SLC7A11 involves the extracellular efflux of Cys-SSH.[56]

3. Methods for detecting supersulfides and protein polysulfidation

3.1. Quantitative mass spectrometry analysis

Liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) is one of the most powerful methods to directly detect and quantify each supersulfides from biological samples. In this assay, the terminal SH group of supersulfides has to be alkylated by alkylating reagents. Proper alkylating reagents and labeling conditions are required for the stabilization of the catenated sulfur chain. The hydroxyl anion attacks the catenated sulfur chain in supersulfides, cleaving it into thiolates and sulfenic acids, and this alkaline hydrolysis reaction is enhanced by several alkylating reagents such as *N*-ethylmaleimide, monobromobimane and iodoacetamide.[58–61] β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) which is commercially available is widely used as an alkylating reagent because the hydroxyphenyl moiety of HPE-IAM has potent stabilizing effects for diverse supersulfides.[58] More recently, *N*-iodoacetyl l-tyrosine methyl ester (TME-IAM), which is developed from HPE-IAM, and *N*-*t*-butyl-iodoacetamide have been reported as more potent alkylating reagents.[60,62] Apart from the quantitative method of target products using stable isotope-labeled standards, the method to quantify total supersulfide and reactive supersulfide contents using LC-ESI-MS/MS has also been developed.[45]

3.2. Fluorescence probes

Fluorescence imaging is a useful technique to analyze the spatiotemporal dynamics of supersulfides in cells and tissues. Several chemical-based fluorescence probes that respond to sulfane sulfur have been developed. The detailed mechanism of action of these fluorescence probes is reviewed in Ref. 63. In this review, we introduce three fluorescence probes for supersulfide detection. First, SSP4 is a commercially available fluorescence probe. The original SSP fluorescence probes for sulfane sulfur were developed by Xian's group.[64] SSP4 is non-fluorescent in the basal state. SSP4 contains two thiol groups at which sulfane sulfur

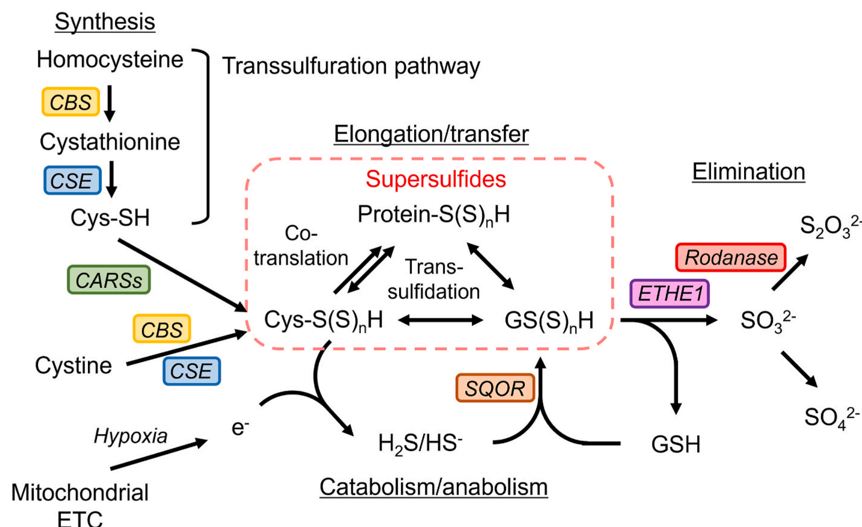

Fig. 1. Pathways of supersulfide synthesis and metabolism in mammals. Cysteine (Cys-SH) that is synthesized through the transsulfuration pathway serves as substrates to yield cysteine per/polysulfide (Cys-S(S)$_n$H) by cysteinyl-tRNA synthetases (CARSs). Cys-SSH is also synthesized from cystine by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE). Cys-SSH is co-translationally incorporated into nascent polypeptides for protein per/polysulfidation (Protein-S(S)$_n$H). The sulfane sulfur is transferred and elongated during multiple supersulfides in a non-enzymatic *trans*-sulfidation. Leaked electrons from the mitochondrial electron transport chain (ETC) lead to the catabolism of Cys-S(S)$_n$H, generating hydrogen sulfide/hydrosulfide anion (H_2S/HS^-). Sulfide quinone oxidoreductase (SQOR) mediates the oxidation of H_2S using glutathione (GSH), yielding glutathione per/polysulfide (GS(S)$_n$H). GS(S)$_n$H is oxidized by ethylmalonic encephalopathy 1 (ETHE1) to sulfite (SO_3^{2-}). SO_3^{2-} is further oxidized to sulfate (SO_4^{2-}) or thiosulfate ($S_2O_3^{2-}$) by sulfide-detoxifying enzymes such as rhodanese.

reacts to cleave and release fluorescein. This cleavage reaction is highly selective to sulfane sulfur and does not occur with ROS and H_2S. Because SSP4 irreversibly reacts with sulfane sulfur, SSP4 should not be used to analyze the dynamical changes of supersulfides in the cells. Second, Hanaoka's group developed SSip-1 which is a reversible fluorescence probe to analyze intracellular supersulfide dynamics.[65] SSip-1 is also commercially available. SSip-1 has weak fluorescence before the reaction with sulfane sulfur because of Förster resonance energy transfer (FRET)-based fluorescence quenching. Sulfane sulfur reversibly alters SSip-1 conformation to decrease FRET, generating bright fluorescence. Using the SSip-1 probe, we recently analyzed supersulfide in mouse heart slices.[12] Third, QS10 which is developed by Umezawa et al. is also a FRET-based reversible fluorescence probe for supersulfide dynamics.[66] QS10 fluorescence is not quenched in the basal state. So, QS10 FRET analysis by measuring donor and acceptor fluorescence enables more quantitative and real-time monitoring of supersulfide dynamics in living cells. SSP4 and SSip-1 emit green fluorescence, whereas QS10 was developed as a FRET pair at wavelengths similar to Cy3 and Cy5. Therefore, QS10 enables to perform simultaneous imaging with different probes with green fluorescence. SSip-1 and QS10 preferentially associate with mitochondrial membrane. These probes show good retention in the cell, but imaging is limited to supersulfide change in mitochondria. SSP4 shows more diffused distribution in the cell reflecting the global distribution of supersulfides. However, SSP4 is easily leaked in several types of cells such as neonatal rat cardiomyocytes. It would be necessary to select an appropriate probe for the experiment's purpose.

3.3. Biotin switch assay for protein polysulfidation

The thiol group of cysteine has various posttranslational modifications including polysulfidation, oxidation, S-nitrosylation, S-glutathionylation and palmitoylation.[9,67] Biotin switch assay is widely used to identify proteins having a posttranslational modification of your interest. This assay was first developed to detect S-nitrosylated proteins.[68,69] Free thiol groups are first blocked with an alkylating reagent, and then S-nitrosothiol groups are cleaved by ascorbate. Newly generated thiol groups are labeled with a biotin-containing alkylating reagent. According to the biochemical feature of posttranslational modification of your interest, a proper biotin switch strategy is required. Because the biochemical features of cysteine thiol and polysulfidated cysteine thiol are similar, the specific isolation of polysulfidated proteins is difficult and a wide variety of methods have been reported. The detail of the biotin switch assay reported is reviewed in Refs. 63,70. As described above, some alkylating reagents and labeling conditions accelerate the cleavage of the catenated sulfane sulfur.[42,58,61] We tested various biotin switch methods and alkylating reagents and developed a modified iodoacetyl-biotin (IAA-biotin) method that was originally reported by Doka et al.[71] in our recent work (Fig. 2).[11,56] Alkylating labeling using IAA-biotin with tyrosine most effectively detected the changes in protein polysulfidation in Na_2S_4-treated cells (Fig. 2).

4. Sulfur biology in cardiac remodeling and heart failure

4.1. Cardiac remodeling and heart failure

Heart disease including heart failure is one of the leading causes of death worldwide. Heart failure refers to the cardiac inability to pump enough blood to the body. Heart failure prevalence was estimated at more than 37.7 million in the world in 2016, and this number is predicted to continuously grow in the coming decades.[72,73] The morbidity and mortality associated with heart failure are still high with a 5-year

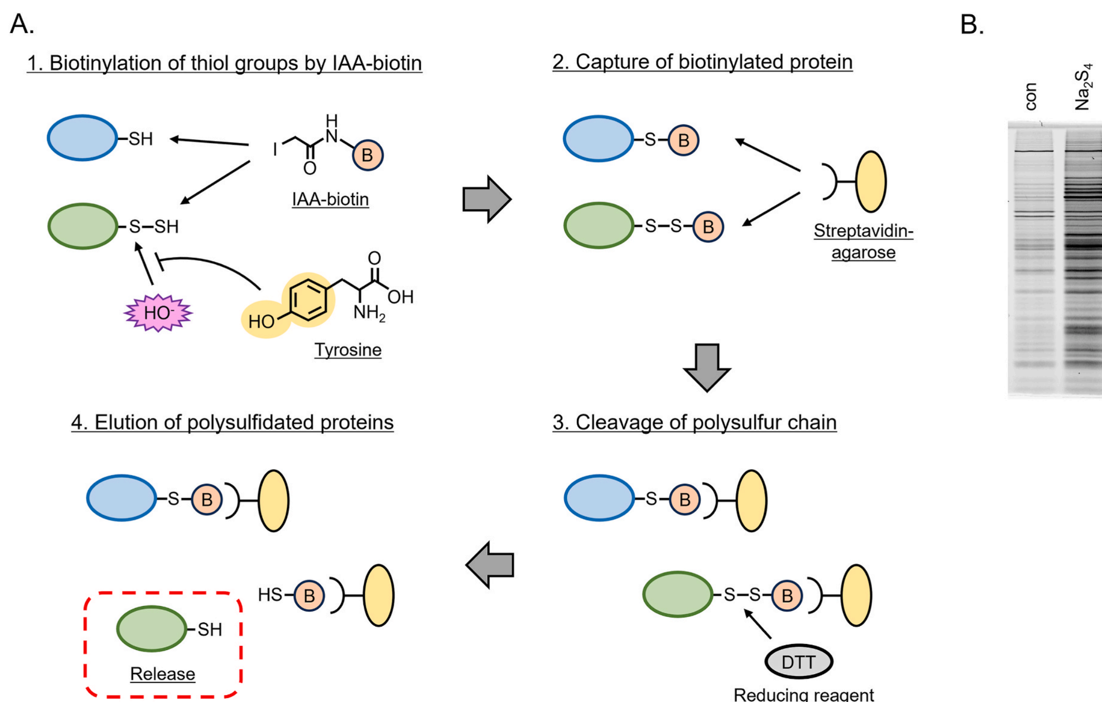

Fig. 2. Detection of polysulfidated proteins by IAA-biotin switch assay. (A) A schematic diagram of IAA-biotin switch assay. The SH groups of cysteine and polysulfidated cysteine are biotinylated using iodoacetyl-biotin (IAA-biotin) in the presence of tyrosine. The hydroxyphenyl residue of tyrosine protects polysulfides from the degradation triggered by the hydroxyl anion (HO^-). Biotinylated proteins are captured by streptavidin-agarose, then the polysulfur chain is cleaved by the reducing reagent such as dithiothreitol (DTT), and polysulfidated proteins are finally released from the resin. (B) Detection of polysulfidated proteins. Neonatal rat cardiomyocytes were treated without or with Na_2S_4. Polysulfidated proteins were collected using IAA-biotin assay. Polysulfidated proteins were separated by SDS-PAGE and detected. Na_2S_4 treatment drastically increased polysulfidated proteins.

survival rate after hospitalization of around 50%,[74] which has improved only slightly in the 21st century compared with other serious diseases such as cancer.[75] Therefore, a novel concept and potential target for heart failure therapy would be required.

Cardiac remodeling refers to alterations in the size, shape, or structure of either atria or ventricles, which can result in the disruption of cardiac architecture.[76,77] This will lead to increased stiffness, deterioration of systolic or diastolic function, and a higher likelihood of arrhythmia. Cardiac remodeling is generally an adverse sign and is closely related to the progression of heart failure.

4.2. The role of supersulfides and H_2S on cardiac remodeling and heart failure

Starting with the report about the identification of H_2S as an endogenous neuromodulator and vasorelaxant,[15,78,79] the cardioprotective effects of H_2S in animal models have been intensively studied over the past few decades.[18] However, most evidence is from the pharmacological effects of the exogenous administration of H_2S or its donor reagent, and it is mostly unclear how H_2S mediates cardioprotective effects and whether H_2S or its metabolite directly catalyzes the biological process. The discovery and biochemical characterization of supersulfides raises the probability that supersulfides act as molecular entities for cardioprotective effects. Recently, several studies including ours have focused on the involvement of supersulfides in cardiac homeostasis and disease progression.

Myocardial ischemia/reperfusion (IR) injury contributes to adverse cardiac events after ischemia and cardiac surgery and is one critical cause of heart failure in the clinical field.[80,81] Myocardial IR injury occurs when the blood supply to the heart is blocked for a short time (ischemia) and then restored (reperfusion). Rapid reentry of oxygen induces ROS burst in mitochondria, leading to myocardial cell death and tissue damage. Growing evidence shows that the pharmacological administration of H_2S or its donating agents improves cardiac function after IR injury.[48,82–86] At high concentrations, H_2S is toxic by inhibiting cytochrome C oxidase of the electron transport chain 87,88. Cardioprotective effect of NaHS is biphasic, and high dose of NaHS loses cardioprotective effects.[83] The mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channel has a pivotal role in cardioprotective action.[89,90] H_2S treatment increases K_{ATP} channel currents.[91,92] Additionally, H_2S treatment induces polysulfidation of K_{ATP} channel subunit, Sulphonylurea 2B at Cys24 and Cys1455.[93] Polysulfidation of mitoK_{ATP} would be involved in the cardioprotective effect against IR injury. Recently, it has been reported that cardiac function is maintained after an ex-vivo IR injury model by exogenous donation of RSSH rather than H_2S.[94]

Aberrantly increased afterload and hormone stimulation result in pathological cardiac hypertrophy and fibrosis. Administration of H_2S and its donating agents ameliorates cardiac function and remodeling induced by the transverse aortic constriction (TAC) model[95,96] and the chronic infusion of Ang II[97,98] and isoprenaline.[99,100] H_2S improves cardiac hypertrophy by decreasing oxidative stress, and the antioxidant effect of H_2S depends on the PI3K-Akt signaling pathway.[95,96]

4.3. Changes in supersulfide catabolism under the ischemic heart

O_2 as a terminal electron acceptor plays a fundamental role in the regulation of a circuit of electron flow and ATP production in the electron transport chain (ETC) in mammalian mitochondria. Bacteria that live in anaerobic conditions use a wide variety of substrates for energy production including sulfate (SO_4^{2-}), nitrate (NO_3^-), ferric iron (Fe^{3+}) and carbon dioxide (CO_2).[101] Mammalian can use fumarate as an electron acceptor in an alternative pathway of electron flow in mitochondria under limited oxygen conditions.[102] Cys-SSH is highly accumulated in mitochondria and acts as a terminal electron acceptor in ETC like O_2.[42] After receiving electrons in ETC, Cys-SSH is converted (catabolized) into Cys-SH and HS^-. Depletion of Cys-SSH in CARS KO cells decreases the oxygen consumption rate under normoxia, indicating the critical role of supersulfide-mediated respiration in mitochondrial homeostasis.[42]

Under limited oxygen availability, supersulfides would be more pivotal in energy metabolism and biological processes. Supersulfide and H_2S imaging analysis show a decrease of supersulfide and an increase of H_2S in cardiomyocytes under hypoxia. This change in supersulfide dynamics is also observed in the ischemic heart slice of the mouse MI model.[12] Supersulfides preferentially receive electrons and are catabolized to H_2S in ETC under hypoxia. Echinochrome A (Ech-A), a natural pigment isolated from sea urchins, has been proven in multiple clinical trials including cardiac ischemia diseases.[103] Consistent with the recovery of cardiac function, supersulfide catabolism is improved by the administration of Ech-A in the MI model mouse.[12] Supersulfide-H_2S metabolic cycle is also involved in the ischemic tolerance of the brain 104. Accumulation of H_2S under hypoxia induces brain injury by inhibiting the activity of the ETC complex, and the expression level of SQOR that catalyzes the resynthesis of supersulfides from H_2S determines the ischemic tolerance.[104] On the other hand, Cys-SSH and its substrate Cys-SH are increased in the heart immediately after ischemia (30 min after ischemia).[105] This would be a compensatory mechanism for energy production using supersulfides instead of O_2 under hypoxia. Actually, the administration of supersulfides such as Cys–SSS–Cys and diallyl trisulfide improves cardiac function and remodeling of the mouse heart disease model.[105,106]

4.4. Mitochondrial quality control and cardiac protection by supersulfides

The proper balance of mitochondrial fission and fusion cycle has a pivotal role in maintaining mitochondrial quality and functions. Aberrant fission/fusion balance of mitochondria leads to the progression of heart failure. In particular, mitochondrial hyperfission is observed in various heart failure models and triggers pathological cardiac remodeling such as myocardial senescence. Mitochondrial fission is regulated by dynamin-related protein 1 (Drp1), a large GTPase. Mitochondrial hyperfission due to abnormal activation of Drp1 has attracted attention as a cause of cardiomyocyte dysfunction and heart failure.[1,107] Drp1 inhibition ameliorates cardiac dysfunction in diabetic cardiomyopathy and chronic heart failure models.[108] Genetic Drp1 Cys452F mutation causes cardiomyopathy in the python mouse.[109] In addition to phosphorylation, Drp1 activity is regulated by posttranslational modification of redox-active cysteine (Cys644) at the C-terminus. S-nitrosylation and sulfenylation enhance Drp1 activation,[110,111] whereas covalent modification of 15-deoxy-Δ12,14-prostaglandin J2 leads to Drp1 inactivation.[112] This redox-active Cys644 is polysulfidated in a basal state and its polysulfidation is dynamically changed in response to intracellular supersulfide levels.[42] Polysulfidation negatively regulates Drp1 activation by inhibiting the interaction with its guanine nucleotide exchange factor, filamin A (Fig. 3).[10,107] MeHg which is an environmental electrophilic neurotoxicant is reported as a potent risk factor for cardiac events.[113–115] Exposure to low-dose of MeHg leads to mitochondrial hyperfission of cardiomyocytes through Drp1 depolysulfidation, which exacerbates cardiac dysfunction induced by pressure overload. NaHS administration recovers Drp1 polysulfidation and mitochondrial quality, improving cardiac function after pressure overload. Exposure to cigarette smoke extract also induced Drp1 depolysulfidation, leading to mitochondrial hyperfission and impairment of cardiac contractility.[11] Moreover, Drp1 polysulfidation by CSE also improves isoprenaline-induced mitochondrial dysfunction and cardiomyocyte death.[100] This evidence indicates the relationship between supersulfide metabolism and mitochondrial quality followed by cardiac homeostasis through protein polysulfidation such as Drp1 (Fig. 3).

5. Conclusion and future direction

The relationship between the disturbance of redox homeostasis and heart failure progression has been mainly focused on oxidative stress.

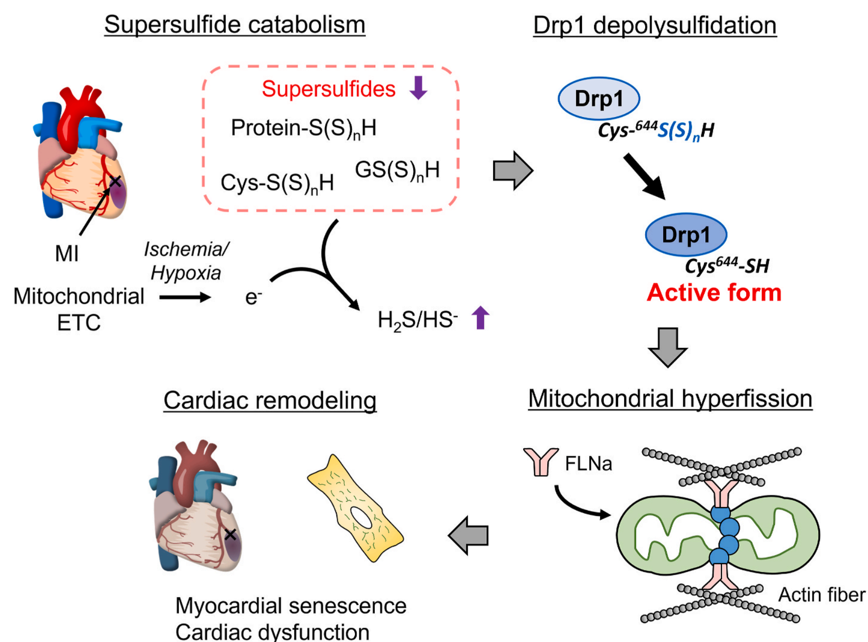

Fig. 3. Supersulfide catabolism-associated mitochondrial hyperfission and cardiac dysfunction. Myocardial infarction (MI) promotes ischemic cardiac remodeling. Under limited oxygen conditions, leaked electrons from the mitochondrial electron transport chain (ETC) promote the catabolism of supersulfides to H_2S. Drp1 activity is negatively regulated by its polysulfidation at Cys644, and supersulfide catabolism leads to Drp1 depolysulfidation. Depolysulfidated Drp1 can be associated with its guanine nucleotide exchange factor filamin A (FLNa), resulting in Drp1 activation-mediated mitochondrial hyperfission. Mitochondrial hyperfission triggers maladaptive cardiac remodeling such as myocardial senescence and the progression of heart failure.

Aberrant oxidative stress over the buffering capacity by antioxidant defense systems has a critical role in the development and progression of clinical and experimental heart failure.[116–119] Although various studies targeting anti-oxidative stress therapy in experimental heart failure models show promising results, clinical trials of anti-oxidative stress therapy have almost failed.[120–122] These facts may reflect the importance of therapeutic and drug pharmaceutical strategies that focus not only on oxidative stress but also on supersulfides and sulfur metabolism. Since the innovation to quantitively analyze supersulfides and sulfur metabolites, the role of supersulfides in various biological processes including the cardiovascular system is becoming clear. On the other hand, the reactivity of supersulfides is very complex and intractable. Failure to select an appropriate analytical method may lead to incorrect interpretations. For further development of supersulfide research, it is desirable to develop more accurate and tractable techniques in the future. Future studies focusing on supersulfide-related redox biology would contribute to our understanding of a novel therapeutic strategy for heart failure.

CRediT authorship contribution statement

Akiyuki Nishimura: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Xiaokang Tang:** Writing – original draft. **Liuchenzi Zhou:** Writing – original draft. **Tomoya Ito:** Writing – review & editing. **Yuri Kato:** Writing – review & editing. **Motohiro Nishida:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

Acknowledgements

This work was supported by JST CREST Grant Number JPMJCR2024 (20348438 to M.N. and A.N.), Grant-in-Aid for Scientific Research on Innovative Areas(A) "Sulfur biology" (21H05269 and 21H05258 to M. N.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, JSPS KAKENHI (22H02772 and 22K19395 to M.N.), Joint Research of the Exploratory Research Center on Life and Living Systems (ExCELLS) (ExCELLS program No,23EX601), the Sumitomo foundation grant for basic science research project (to A.N.), Naito Foundation (to M.N.) and Smoking Research Foundation (to M.N.).

References

1. Nishimura A, Tanaka T, Kato Y, et al. Cardiac robustness regulated by reactive sulfur species. *J Clin Biochem Nutr*. 2022;70:1–6. https://doi.org/10.3164/JCBN.21-84.
2. Jones DP. Redox theory of aging. *Redox Biol*. 2015;5:71–79. https://doi.org/10.1016/J.REDOX.2015.03.004.
3. Go YM, Jones DP. Thiol/disulfide redox states in signaling and sensing. *Crit Rev Biochem Mol Biol*. 2013;48:173–181. https://doi.org/10.3109/10409238.2013.764840.
4. Wang SB, Foster DB, Rucker J, et al. Redox regulation of mitochondrial ATP synthase: implications for cardiac resynchronization therapy. *Circ Res*. 2011;109: 750–757. https://doi.org/10.1161/CIRCRESAHA.111.246124.
5. Ago T, Liu T, Zhai P, et al. A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. *Cell*. 2008;133:978–993. https://doi.org/10.1016/j.cell.2008.04.041.
6. Oka SI, Ago T, Kitazono T, et al. The role of redox modulation of class II histone deacetylases in mediating pathological cardiac hypertrophy. *J Mol Med*. 2009;87: 785–791.
7. Nishida M, Maruyama Y, Tanaka R, et al. Gαi and Gαo are target proteins of reactive oxygen species, 2000 *Nature*. 2000;408(6811):492–495. https://doi.org/10.1038/35044120, 408.
8. Nishida M, Sawa T, Kitajima N, et al. Hydrogen sulfide anion regulates redox signaling via electrophile sulfhydration. *Nat Chem Biol*. 2012;8:714–724. https://doi.org/10.1038/NCHEMBIO.1018.
9. Chung HS, Wang SB, Venkatraman V, et al. Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. *Circ Res*. 2013; 112:382–392. https://doi.org/10.1161/CIRCRESAHA.112.268680.
10. Nishimura A, Shimoda K, Tanaka T, et al. Depolysulfidation of Drp1 induced by low-dose methylmercury exposure increases cardiac vulnerability to hemodynamic overload. *Sci Signal*. 2019;12. https://doi.org/10.1126/SCISIGNAL.AAW1920.
11. Nishimura A, Zhou L, Kato Y, et al. Supersulfide prevents cigarette smoke extract-induced mitochondria hyperfission and cardiomyocyte early senescence by

inhibiting Drp1-filamin complex formation. *J Pharmacol Sci*. 2024;154:127–135. https://doi.org/10.1016/J.JPHS.2023.12.008.
12. Tang X, Nishimura A, Ariyoshi K, et al. Echinochrome prevents sulfide catabolism-associated chronic heart failure after myocardial infarction in mice. *Mar Drugs*. 2023;21. https://doi.org/10.3390/MD21010052.
13. Reiffenstein RJ, Hulbert WC, Roth SH. Toxicology of hydrogen sulfide. *Annu Rev Pharmacol Toxicol*. 1992;32:109–134. https://doi.org/10.1146/ANNUREV.PA.32.040192.000545.
14. Aroca A, Gotor C, Bassham DC, Romero LC. Hydrogen sulfide: from a toxic molecule to a key molecule of cell life. *Antioxidants*. 2020;9:1–24. https://doi.org/10.3390/ANTIOX9070621.
15. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci*. 1996;16:1066–1071. https://doi.org/10.1523/JNEUROSCI.16-03-01066.1996.
16. Citi V, Martelli A, Brancaleone V, et al. Anti-inflammatory and antiviral roles of hydrogen sulfide: rationale for considering H2 S donors in COVID-19 therapy. *Br J Pharmacol*. 2020;177:4931–4941. https://doi.org/10.1111/BPH.15230.
17. Aroca A, Gotor C. Hydrogen sulfide: a key role in autophagy regulation from plants to mammalians. *Antioxidants*. 2022;11. https://doi.org/10.3390/ANTIOX11020327.
18. Kolluru GK, Shackelford RE, Shen X, et al. Sulfide regulation of cardiovascular function in health and disease. *Nat Rev Cardiol*. 2023;20:109–125. https://doi.org/10.1038/S41569-022-00741-6.
19. Hellmich MR, Szabo C. Hydrogen sulfide and cancer. *Handb Exp Pharmacol*. 2015;230:233–241. https://doi.org/10.1007/978-3-319-18144-8_12.
20. Khattak S, Rauf MA, Khan NH, et al. Hydrogen sulfide biology and its role in cancer. *Molecules*. 2022;27. https://doi.org/10.3390/MOLECULES27113389.
21. Liu H, Wang J, Liu J, et al. Hydrogen sulfide (H2S) signaling in plant development and stress responses. *aBIOTECH*. 2021;2:32–63. https://doi.org/10.1007/S42994-021-00035-4.
22. Corpas FJ, Palma JM. H2S signaling in plants and applications in agriculture. *J Adv Res*. 2020;24:131–137. https://doi.org/10.1016/J.JARE.2020.03.011.
23. Chen J, Wu FH, Wang WH, et al. Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. *J Exp Bot*. 2011;62:4481–4493. https://doi.org/10.1093/JXB/ERR145.
24. Aroca A, Zhang J, Xie Y, et al. Hydrogen sulfide signaling in plant adaptations to adverse conditions: molecular mechanisms. *J Exp Bot*. 2021;72:5893–5904. https://doi.org/10.1093/JXB/ERAB239.
25. Hine C, Harputlugil E, Zhang Y, et al. Endogenous hydrogen sulfide production is essential for dietary restriction benefits. *Cell*. 2015;160:132–144. https://doi.org/10.1016/J.CELL.2014.11.048.
26. Zivanovic J, Kouroussis E, Kohl JB, et al. Selective persulfide detection reveals evolutionarily conserved antiaging effects of S-sulfhydration. *Cell Metabol*. 2019;30:1152–1170.e13. https://doi.org/10.1016/J.CMET.2019.10.007.
27. Wilkie SE, Borland G, Carter RN, et al. Hydrogen sulfide in ageing, longevity and disease. *Biochem J*. 2021;478:3485–3504. https://doi.org/10.1042/BCJ20210517.
28. Kolluru GK, Shen X, Bir SC, Kevil CG. Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric Oxide*. 2013;35:5–20. https://doi.org/10.1016/J.NIOX.2013.07.002.
29. Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation–a tale of three gases. *Pharmacol Ther*. 2009;123:386–400. https://doi.org/10.1016/J.PHARMTHERA.2009.05.005.
30. Whiteman M, Armstrong JS, Chu SH, et al. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite "scavenger". *J Neurochem*. 2004;90:765–768. https://doi.org/10.1111/J.1471-4159.2004.02617.X.
31. Kimura Y, Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *Faseb J*. 2004;18:1165–1167. https://doi.org/10.1096/FJ.04-1815FJE.
32. Whiteman M, Cheung NS, Zhu YZ, et al. Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain? *Biochem Biophys Res Commun*. 2005;326:794–798. https://doi.org/10.1016/J.BBRC.2004.11.110.
33. Yonezawa D, Sekiguchi F, Miyamoto M, et al. A protective role of hydrogen sulfide against oxidative stress in rat gastric mucosal epithelium. *Toxicology*. 2007;241:11–18. https://doi.org/10.1016/J.TOX.2007.07.020.
34. Laggner H, Muellner MK, Schreier S, et al. Hydrogen sulphide: a novel physiological inhibitor of LDL atherogenic modification by HOCl. *Free Radic Res*. 2007;41:741–747. https://doi.org/10.1080/10715760701263265.
35. Jha S, Calvert JW, Duranski MR, et al. Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling. *Am J Physiol Heart Circ Physiol*. 2008;295. https://doi.org/10.1152/AJPHEART.00377.2008.
36. Cuevasanta E, Lange M, Bonanata J, et al. Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide. *J Biol Chem*. 2015;290:26866–26880. https://doi.org/10.1074/JBC.M115.672816.
37. Wedmann R, Onderka C, Wei S, et al. Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. *Chem Sci*. 2016;7:3414–3426. https://doi.org/10.1039/C5SC04818D.
38. Ida T, Sawa T, Ihara H, et al. Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc Natl Acad Sci U S A*. 2014;111:7606–7611. https://doi.org/10.1073/PNAS.1321232111/-/DCSUPPLEMENTAL/SAPP.PDF.
39. Ono K, Akaike T, Sawa T, et al. Redox chemistry and chemical biology of H2S, hydropersulfides, and derived species: implications of their possible biological activity and utility. *Free Radic Biol Med*. 2014;77:82–94. https://doi.org/10.1016/J.FREERADBIOMED.2014.09.007.
40. Jung M, Kasamatsu S, Matsunaga T, et al. Protein polysulfidation-dependent persulfide dioxygenase activity of ethylmalonic encephalopathy protein 1. *Biochem Biophys Res Commun*. 2016;480:180–186. https://doi.org/10.1016/J.BBRC.2016.10.022.
41. Kasamatsu S, Nishimura A, Morita M, et al. Redox signaling regulated by cysteine persulfide and protein polysulfidation. *Molecules*. 2016;21. https://doi.org/10.3390/MOLECULES21121721.
42. Akaike T, Ida T, Wei FY, et al. Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat Commun*. 2017;8. https://doi.org/10.1038/S41467-017-01311-Y.
43. Petrovic D, Kouroussis E, Vignane T, Filipovic MR. The role of protein persulfidation in brain aging and neurodegeneration. *Front Aging Neurosci*. 2021;13. https://doi.org/10.3389/FNAGI.2021.674135.
44. Edwards JO, Pearson RG. The factors determining nucleophilic reactivities. *J Am Chem Soc*. 1962;84:16–24. https://doi.org/10.1021/ja00860a005.
45. Kasamatsu S, Kinno A, Hishiyama JI, et al. Development of methods for quantitative determination of the total and reactive polysulfides: reactive polysulfide profiling in vegetables. *Food Chem*. 2023;413, 135610. https://doi.org/10.1016/J.FOODCHEM.2023.135610.
46. Uchiyama J, Akiyama M, Hase K, et al. Gut microbiota reinforce host antioxidant capacity via the generation of reactive sulfur species. *Cell Rep*. 2022;38. https://doi.org/10.1016/J.CELREP.2022.110479.
47. Kolluru GK, Shen X, Kevil CG. Reactive sulfur species: a new redox player in cardiovascular pathophysiology. *Arterioscler Thromb Vasc Biol*. 2020;40:874–884. https://doi.org/10.1161/ATVBAHA.120.314084.
48. Li Z, Polhemus DJ, Lefer DJ. Evolution of hydrogen sulfide therapeutics to treat cardiovascular disease. *Circ Res*. 2018;123:590–600. https://doi.org/10.1161/CIRCRESAHA.118.311134.
49. Shibuya N, Tanaka M, Yoshida M, et al. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxidants Redox Signal*. 2009;11:703–714. https://doi.org/10.1089/ARS.2008.2253.
50. Yang G, Wang R. H2S and blood vessels: an overview. *Handb Exp Pharmacol*. 2015;230:85–110. https://doi.org/10.1007/978-3-319-18144-8_4.
51. Zainol Abidin QH, Ida T, Morita M, et al. Synthesis of sulfides and persulfides is not impeded by disruption of three canonical enzymes in sulfur metabolism. *Antioxidants*. 2023;12. https://doi.org/10.3390/ANTIOX12040868.
52. Jackson MR, Melideo SL, Jorns MS. Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry*. 2012;51:6804–6815. https://doi.org/10.1021/BI300778T.
53. Jackson MR, Melideo SL, Jorns MS. Role of human sulfide: quinone oxidoreductase in H2S metabolism. *Methods Enzymol*. 2015;554:255–270. https://doi.org/10.1016/BS.MIE.2014.11.037.
54. Levitt MD, Furne J, Springfield J, et al. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J Clin Invest*. 1999;104:1107–1114. https://doi.org/10.1172/JCI7712.
55. Kimura H. Hydrogen sulfide and polysulfides as biological mediators. *Molecules*. 2014;19:16146–16157. https://doi.org/10.3390/MOLECULES191016146.
56. Akiyama M, Unoki T, Aoki H, et al. Cystine-dependent antiporters buffer against excess intracellular reactive sulfur species-induced stress. *Redox Biol*. 2022;57. https://doi.org/10.1016/J.REDOX.2022.102514.
57. Schaedler TA, Thornton JD, Kruse I, et al. A conserved mitochondrial ATP-binding cassette transporter exports glutathione polysulfide for cytosolic metal cofactor assembly. *J Biol Chem*. 2014;289:23264–23274. https://doi.org/10.1074/JBC.M114.553438.
58. Hamid HA, Tanaka A, Ida T, et al. Polysulfide stabilization by tyrosine and hydroxyphenyl-containing derivatives that is important for a reactive sulfur metabolomics analysis. *Redox Biol*. 2019;21. https://doi.org/10.1016/J.REDOX.2019.101096.
59. Bogdándi V, Ida T, Sutton TR, et al. Speciation of reactive sulfur species and their reactions with alkylating agents: do we have any clue about what is present inside the cell? *Br J Pharmacol*. 2019;176:646–670. https://doi.org/10.1111/BPH.14394.
60. Schilling D, Barayeu U, Steimbach RR, et al. Commonly used alkylating agents limit persulfide detection by converting protein persulfides into thioethers. *Angew Chem Int Ed Engl*. 2022;61. https://doi.org/10.1002/ANIE.202203684.
61. Takata T, Jung M, Matsunaga T, et al. Methods in sulfide and persulfide research. *Nitric Oxide*. 2021;116:47–64. https://doi.org/10.1016/J.NIOX.2021.09.002.
62. Kasamatsu S, Ida T, Koga T, et al. High-precision sulfur metabolomics innovated by a new specific probe for trapping reactive sulfur species. *Antioxidants Redox Signal*. 2021;34:1407–1419. https://doi.org/10.1089/ARS.2020.8073.
63. Echizen H, Sasaki E, Hanaoka K. Recent advances in detection, isolation, and imaging techniques for sulfane sulfur-containing biomolecules. *Biomolecules*. 2021;11. https://doi.org/10.3390/BIOM11111553.
64. Chen W, Liu C, Peng B, et al. New fluorescent probes for sulfane sulfurs and the application in bioimaging. *Chem Sci*. 2013;4:2892–2896. https://doi.org/10.1039/C3SC50754H.
65. Takano Y, Hanaoka K, Shimamoto K, et al. Development of a reversible fluorescent probe for reactive sulfur species, sulfane sulfur, and its biological application. *Chem Commun*. 2017;53:1064–1067. https://doi.org/10.1039/C6CC08372B.
66. Umezawa K, Kamiya M, Urano Y. A reversible fluorescent probe for real-time live-cell imaging and quantification of endogenous hydropolysulfides. *Angew Chem Int Ed Engl*. 2018;57:9346–9350. https://doi.org/10.1002/ANIE.201804309.

67. Jortzik E, Wang L, Becker K. Thiol-based posttranslational modifications in parasites. *Antioxidants Redox Signal*. 2012;17:657–673. https://doi.org/10.1089/ARS.2011.4266.
68. Jaffrey SR, Erdjument-Bromage H, Ferris CD, et al. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat Cell Biol*. 2001;3:193–197. https://doi.org/10.1038/35055104.
69. Forrester MT, Foster MW, Benhar M, Stamler JS. Detection of protein S-nitrosylation with the biotin-switch technique. *Free Radic Biol Med*. 2009;46: 119–126. https://doi.org/10.1016/J.FREERADBIOMED.2008.09.034.
70. Paul BD, Snyder SH. H2S: a novel gasotransmitter that signals by sulfhydration. *Trends Biochem Sci*. 2015;40:687–700. https://doi.org/10.1016/J.TIBS.2015.08.007.
71. Dóka É, Pader I, Bíró A, et al. A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems. *Sci Adv*. 2016;2. https://doi.org/10.1126/SCIADV.1500968.
72. Bragazzi NL, Zhong W, Shu J, et al. Burden of heart failure and underlying causes in 195 countries and territories from 1990 to 2017. *Eur J Prev Cardiol*. 2021;28: 1682–1690. https://doi.org/10.1093/EURJPC/ZWAA147.
73. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol*. 2016;13:368–378. https://doi.org/10.1038/NRCARDIO.2016.25.
74. Taylor CJ, Ryan R, Nichols L, et al. Survival following a diagnosis of heart failure in primary care. *Fam Pract*. 2017;34:161–168. https://doi.org/10.1093/FAMPRA/CMW145.
75. Taylor CJ, Ordóñez-Mena JM, Roalfe AK, et al. Trends in survival after a diagnosis of heart failure in the United Kingdom 2000-2017: population based cohort study. *BMJ*. 2019;364. https://doi.org/10.1136/BMJ.L223.
76. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation*. 2013;128:388–400. https://doi.org/10.1161/CIRCULATIONAHA.113.001878.
77. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an International Forum on Cardiac Remodeling. *J Am Coll Cardiol*. 2000;35:569–582. https://doi.org/10.1016/S0735-1097(99)00630-0.
78. Li L, Rose P, Moore PK. Hydrogen sulfide and cell signaling. *Annu Rev Pharmacol Toxicol*. 2011;51:169–187. https://doi.org/10.1146/ANNUREV-PHARMTOX-010510-100505.
79. Szabõ C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov*. 2007;6:917–935. https://doi.org/10.1038/NRD2425.
80. Cadenas S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radic Biol Med*. 2018;117:76–89. https://doi.org/10.1016/J.FREERADBIOMED.2018.01.024.
81. Frank A, Bonney M, Bonney S, et al. Myocardial ischemia reperfusion injury: from basic science to clinical bedside. *Semin CardioThorac Vasc Anesth*. 2012;16: 123–132. https://doi.org/10.1177/1089253211436350.
82. Elrod JW, Calvert JW, Morrison J, et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A*. 2007;104:15560–15565. https://doi.org/10.1073/PNAS.0705891104.
83. Ji Y, Pang Q feng, Xu G, et al. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur J Pharmacol*. 2008;587:1–7. https://doi.org/10.1016/J.EJPHAR.2008.03.044.
84. Osipov RM, Robich MP, Feng J, et al. Effect of hydrogen sulfide in a porcine model of myocardial ischemia-reperfusion: comparison of different administration regimens and characterization of the cellular mechanisms of protection. *J Cardiovasc Pharmacol*. 2009;54:287–297. https://doi.org/10.1097/FJC.0B013E3181B2B72B.
85. Luan HF, Zhao Z Bin, Zhao QH, et al. Hydrogen sulfide postconditioning protects isolated rat hearts against ischemia and reperfusion injury mediated by the JAK2/STAT3 survival pathway. *Braz J Med Biol Res*. 2012;45:898–905. https://doi.org/10.1590/S0100-879X2012007500090.
86. King AL, Polhemus DJ, Bhushan S, et al. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. *Proc Natl Acad Sci U S A*. 2014;111:3182–3187. https://doi.org/10.1073/PNAS.1321871111.
87. Módis K, Coletta C, Erdélyi K, et al. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *Faseb J*. 2013;27:601–611. https://doi.org/10.1096/FJ.12-216507.
88. Szabo C, Ransy C, Módis K, et al. Regulation of mitochondrial bioenergetic function by hydrogen sulfide. Part I. Biochemical and physiological mechanisms. *Br J Pharmacol*. 2014;171:2099–2122. https://doi.org/10.1111/BPH.12369.
89. Fryer RM, Hsu AK, Eells JT, et al. Opioid-induced second window of cardioprotection: potential role of mitochondrial KATP channels. *Circ Res*. 1999; 84:846–851. https://doi.org/10.1161/01.RES.84.7.846.
90. Nozawa Y, Miura T, Miki T, et al. Mitochondrial KATP channel-dependent and -independent phases of ischemic preconditioning against myocardial infarction in the rat. *Basic Res Cardiol*. 2003;98:50–58. https://doi.org/10.1007/S00395-003-0378-Y.
91. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J*. 2001;20:6008–6016. https://doi.org/10.1093/EMBOJ/20.21.6008.
92. Abramochkin DV, Moiseenko LS, Kuzmin VS. The effect of hydrogen sulfide on electrical activity of rat atrial myocardium. *Bull Exp Biol Med*. 2009;147:683–686. https://doi.org/10.1007/S10517-009-0607-Y.
93. Kang M, Hashimoto A, Gade A, Akbarali HI. Interaction between hydrogen sulfide-induced sulfhydration and tyrosine nitration in the KATP channel complex. *Am J Physiol Gastrointest Liver Physiol*. 2015;308:G532–G539. https://doi.org/10.1152/AJPGI.00281.2014.
94. Pharoah BM, Khodade VS, Eremiev A, et al. Hydropersulfides (RSSH) outperform post-conditioning and other reactive sulfur species in limiting ischemia-reperfusion injury in the isolated mouse heart. *Antioxidants*. 2022;11. https://doi.org/10.3390/ANTIOX11051010.
95. Shao M, Zhuo C, Jiang R, et al. Protective effect of hydrogen sulphide against myocardial hypertrophy in mice. *Oncotarget*. 2017;8:22344–22352. https://doi.org/10.18632/ONCOTARGET.15765.
96. Kondo K, Bhushan S, King AL, et al. H_2S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. *Circulation*. 2013;127:1116–1127. https://doi.org/10.1161/CIRCULATIONAHA.112.000855.
97. Snijder PM, Frenay AR, De Boer RA, et al. Exogenous administration of thiosulfate, a donor of hydrogen sulfide, attenuates angiotensin II-induced hypertensive heart disease in rats. *Br J Pharmacol*. 2015;172:1494–1504. https://doi.org/10.1111/BPH.12825.
98. Meng G, Xiao Y, Ma Y, et al. Hydrogen sulfide regulates krüppel-like factor 5 transcription activity via specificity protein 1 S-sulfhydration at Cys664 to prevent myocardial hypertrophy. *J Am Heart Assoc*. 2016;5. https://doi.org/10.1161/JAHA.116.004160.
99. Wu D, Hu Q, Tan B, et al. Amelioration of mitochondrial dysfunction in heart failure through S-sulfhydration of Ca2+/calmodulin-dependent protein kinase II. *Redox Biol*. 2018;19:250–262. https://doi.org/10.1016/J.REDOX.2018.08.008.
100. Wu D, Tan B, Sun Y, Hu Q. Cystathionine γ lyase S-sulfhydrates Drp1 to ameliorate heart dysfunction. *Redox Biol*. 2022;58. https://doi.org/10.1016/J.REDOX.2022.102519.
101. Ladino-Orjuela G, Gomes E, da Silva R, et al. Metabolic pathways for degradation of aromatic hydrocarbons by bacteria. *Rev Environ Contam Toxicol*. 2016;237: 105–121. https://doi.org/10.1007/978-3-319-23573-8_5.
102. Spinelli JB, Rosen PC, Sprenger HG, et al. Fumarate is a terminal electron acceptor in the mammalian electron transport chain. *Science*. 2021;374:1227–1237. https://doi.org/10.1126/SCIENCE.ABI7495.
103. Kim HK, Vasileva EA, Mishchenko NP, et al. Multifaceted clinical effects of echinochrome. *Mar Drugs*. 2021;19. https://doi.org/10.3390/MD19080412.
104. Marutani E, Morita M, Hirai S, et al. Sulfide catabolism ameliorates hypoxic brain injury. *Nat Commun*. 2021;12. https://doi.org/10.1038/S41467-021-23363-X.
105. Griffiths K, Ida T, Morita M, et al. Cysteine hydropersulfide reduces lipid peroxidation and protects against myocardial ischaemia-reperfusion injury - are endogenous persulfides mediators of ischaemic preconditioning? *Redox Biol*. 2023; 60. https://doi.org/10.1016/J.REDOX.2023.102605.
106. Polhemus DJ, Kondo K, Bhushan S, et al. Hydrogen sulfide attenuates cardiac dysfunction after heart failure via induction of angiogenesis. *Circ Heart Fail*. 2013; 6:1077–1086. https://doi.org/10.1161/CIRCHEARTFAILURE.113.000299.
107. Nishimura A, Shimauchi T, Tanaka T, et al. Hypoxia-induced interaction of filamin with Drp1 causes mitochondrial hyperfission-associated myocardial senescence. *Sci Signal*. 2018;11. https://doi.org/10.1126/SCISIGNAL.AAT5185.
108. Song M, Dorn GW. Mitoconfusion: noncanonical functioning of dynamism factors in static mitochondria of the heart. *Cell Metabol*. 2015;21:195–205. https://doi.org/10.1016/J.CMET.2014.12.019.
109. Ashrafian H, Docherty L, Leo V, et al. A mutation in the mitochondrial fission gene Dnm1l leads to cardiomyopathy. *PLoS Genet*. 2010;6:1–18. https://doi.org/10.1371/JOURNAL.PGEN.1001000.
110. Cho DH, Nakamura T, Fang J, et al. S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science*. 2009;324:102–105. https://doi.org/10.1126/SCIENCE.1171091.
111. Kim YM, Youn SW, Sudhahar V, et al. Redox regulation of mitochondrial fission protein Drp1 by protein disulfide isomerase limits endothelial senescence. *Cell Rep*. 2018;23:3565–3578. https://doi.org/10.1016/J.CELREP.2018.05.054.
112. Mishra N, Kar R, Singha PK, et al. Inhibition of mitochondrial division through covalent modification of Drp1 protein by 15 deoxy-Delta(12,14)-prostaglandin J2. *Biochem Biophys Res Commun*. 2010;395:17–24. https://doi.org/10.1016/J.BBRC.2010.03.093.
113. Fujita M, Iwashima K. Estimation of organic and total mercury in seawater around the Japanese archipelago. *Environ Sci Technol*. 1981;15:929–933. https://doi.org/10.1021/ES00090A008.
114. Guallar E, Sanz-Gallardo MI, Veer P van't, et al. Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med*. 2002;347:1747–1754. https://doi.org/10.1056/NEJMOA020157.
115. Salonen JT, Seppänen K, Nyyssönen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. *Circulation*. 1995;91:645–655. https://doi.org/10.1161/01.CIR.91.3.645.
116. Galougahi KK, Antoniades C, Nicholls SJ, et al. Redox biomarkers in cardiovascular medicine. *Eur Heart J*. 2015;36:1576–1582. https://doi.org/10.1093/EURHEARTJ/EHV126.
117. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. *Am J Physiol Heart Circ Physiol*. 2011;301:2181–2190. https://doi.org/10.1152/AJPHEART.00554.2011.
118. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2016;18:891–975. https://doi.org/10.1002/EJHF.592.
119. Wolfram R, Oguogho A, Palumbo B, Sinzinger H. Enhanced oxidative stress in coronary heart disease and chronic heart failure as indicated by an increased 8-epi-

PGF(2alpha). *Eur J Heart Fail*. 2005;7:167–172. https://doi.org/10.1016/J.EJHEART.2004.05.007.
120. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. *Eur J Heart Fail*. 2019;21:425–435. https://doi.org/10.1002/EJHF.1320.
121. Goszcz K, Deakin SJ, Duthie GG, et al. Antioxidants in cardiovascular therapy: panacea or false hope? *Front Cardiovasc Med*. 2015;2. https://doi.org/10.3389/FCVM.2015.00029.
122. Pavlidis G. Oxidative stress and antioxidant therapy in cardiovascular diseases-clinical challenge. *J Clin Med*. 2022;11. https://doi.org/10.3390/JCM11133784.