Redox Biology 59 (2023) 102601

Contents lists available at ScienceDirect

Redox Biology

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

Implications of hydrogen sulfide in colorectal cancer: Mechanistic insights and diagnostic and therapeutic strategies

Hanchao Lin [a,b,1], Yixin Yu [c,1], Le Zhu [b], Nannan Lai [a], Luming Zhang [a], Yu Guo [b], Xinxin Lin [a], Dongqin Yang [d,****], Ning Ren [a,e,***], Zhiling Zhu [c,**], Qiongzhu Dong [a,*]

[a] Key Laboratory of Whole-Period Monitoring and Precise Intervention of Digestive Cancer, Shanghai Municipal Health Commission, Minhang Hospital, Fudan University, China
[b] Department of General Surgery, Huashan Hospital & Cancer Metastasis Institute, Fudan University, China
[c] College of Materials Science and Engineering, Qingdao University of Science and Technology, China
[d] Department of Digestive Diseases, Huashan Hospital, Fudan University, China
[e] Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, And Key Laboratory of Carcinogenesis and Cancer Invasion (Ministry of Education), Fudan University, China

ARTICLE INFO

Keywords:
Hydrogen sulfide
Colorectal cancer
Sulfur metabolism
Colorectal carcinogenesis
Diagnosis
Cancer therapy

ABSTRACT

Hydrogen sulfide (H_2S) is an important signaling molecule in colorectal cancer (CRC). It is produced in the colon by the catalytic synthesis of the colonocytes' enzymatic systems and the release of intestinal microbes, and is oxidatively metabolized in the colonocytes' mitochondria. Both endogenous H_2S in colonic epithelial cells and exogenous H_2S in intestinal lumen contribute to the onset and progression of CRC. The up-regulation of endogenous synthetases is thought to be the cause of the elevated H_2S levels in CRC cells. Different diagnostic probes and combination therapies, as well as tumor treatment approaches through H_2S modulation, have been developed in recent years and have become active area of investigation for the diagnosis and treatment of CRC. In this review, we focus on the specific mechanisms of H_2S production and oxidative metabolism as well as the function of H_2S in the occurrence, progression, diagnosis, and treatment of CRC. We also discuss the present challenges and provide insights into the future research of this burgeoning field.

1. Introduction

Hydrogen sulfide (H_2S) is an irritating gas that smells like rotten eggs. As the third identified gasotransmitter after nitric oxide (NO) and carbon monoxide (CO), H_2S has been recognized to have a variety of biological effects on human health and diseases, covering the nervous system, cardiovascular system, immune system, and digestive system [[1–5]]. Moreover, H_2S is widely accepted as a key signaling molecule in cancer biology because of its unique chemical properties, reaction mechanisms, ability to alter proteins, and active participation in many metal redox processes [6].

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers and one of the leading causes of cancer-related deaths worldwide endogenous or exogenous [7]. A number of studies have found that H_2S plays an important role in CRC [[8–13]]. This review summarizes recent studies on H_2S in the field of CRC. We first discuss the production of H_2S in the colon, the mechanism of oxidative metabolism, and the importance of H_2S in the development of CRC. Following that, we review the strategies for using H_2S in the diagnosis and treatment of CRC, and sort and discuss the currently developed probes, donors, therapeutics, and so on according to the diagnosis and treatment strategies.

* Corresponding authors. Key laboratory of whole-period monitoring and precise intervention of digestive cancer, Shanghai Municipal Health Commission (SMHC), Minhang Hospital, Fudan University, 170 Xingsong Road, Shanghai, 201100, China.
** Corresponding author. 53 Zhengzhou Road, Qingdao, Shandong, 266042, China.
*** Corresponding authors. Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai, 200032, China.
**** Corresponding author. 12 Middle Urumuqi Road, Shanghai, 200040, China.
E-mail addresses: kobesakura@fudan.edu.cn (D. Yang), ren.ning@zs-hospital.sh.cn (N. Ren), zlzhu@qust.edu.cn (Z. Zhu), qzhdong@fudan.edu.cn (Q. Dong).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.redox.2023.102601
Received 31 October 2022; Received in revised form 1 January 2023; Accepted 2 January 2023
Available online 7 January 2023

List of abbreviations

ACLY	adenosine triphosphate citrate lyase
ADT	anethole dithione
AIE	aggregation-induced emission
AOAA	aminooxyacetic acid
Apr	adenosine-5′-phosphosulfate reductase
Asr	anaerobic sulfite reductase
AzMC	7-azido-4-methylcoumarin
CAC	colitis-associated cancer
CAT	cysteine aminotransferase
CBS	cystathionine β-synthase
CO	carbon monoxide
CPT	camptothecin
CRC	colorectal cancer
CSE	cystathionine γ-lyase
CT	computed tomography
Cur	curcumin
CyR61	cysteine-rich angiogenic inducer 61
Cys	cysteine
2D	two-dimensional
3D	three-dimensional
DADS	diallyl disulfide
DAO	D-amino acid oxidase
DAS	diallyl sulfide
DATS	diallyl trisulfide
DHLA	dihydrolipoic acid
DHPS	2,3-dihydroxypropane-1-sulfonate
DOX	doxorubicin
Dsr	dissimilatory sulfite reductase
DTTs	dithiothiones
EGCG	epigallocatechin gallate
ETHE1	ethylmalonic encephalopathy protein 1
FR	far-red
5-FU	5-fluorouracil
GRE	glycyl radical enzyme
GSH	glutathione
GSSH	glutathione persulfide
GT	gemcitabine
HAS	human serum albumin
HMPB	hollow mesoporous Prussian blue
H_2O_2	hydrogen peroxide
H_2S	hydrogen sulfide
HSA	human serum albumin
HS-NSAIDs	Hydrogen sulfide-releasing non-steroidal anti-inflammatory drugs
ICG	indocyanine green
IslA	isethionate sulfite-lyase
IslB	isethionate sulfite-lyase's cognate activating enzyme
LA	lauric acid
LP	liposome
MET	mesenchymal-epithelial transition
3-MP	3-mercaptopyruvate
MRI	magnetic resonance imaging
MSOT	multispectral optoacoustic tomography
3-MST	3-mercaptopyruvate sulfurtransferase
NAD(P)H	nicotinamide adenine dinucleotide (phosphate)
NBD	7-nitro-1,2,3-benzoxadiazole
NIR-I/II	Near-infrared-I/II
NO	nitric oxide
NPs	nanoparticles
OH	hydroxyl radical
PA	photoacoustic
PAG	DL-propargylglycine
PAI	photoacoustic imaging
PDA	polydopamine
PDO	persulfide dioxygenase
PDT	photodynamic therapy
PLP	pyridoxal-5′-phosphate
PMSN	polydopamine decorated mesoporous silica nanoparticles
PTT	photothermal therapy
PW	prussian white
Rhd	rhodanese
ROS	reactive oxygen species
SAM	*S*-adenosyl-L-methionine
SarD	sulfoacetaldehyde reductase
Sat	sulfate adenylyltransferase
SATO	*S*-aroylthiooxime
SCM	surface cross-linked micelles
SL	sulfolactate
SOU	sulfide oxidation unit
SPECT	single-photon emission computed tomography
SQ	sulfoquinovose
SQDG	sulfolipid sulfoquinovosyl diacylglycerol
SQR	sulfide: quinone oxidoreductase
SRB	sulfate-reducing bacteria
SUOX	sulfite oxidase
THBH	2,3,4-trihydroxybenzylhydrazine
TME	tumor microenvironment
Toa	taurine:2-oxoglutarate aminotransferase
Trx	thioredoxin
TST	thiosulfate thiotransferase
UCL	upconversion luminescence
UCNPs	upconversion nanophosphors
Xsc	sulfoacetaldehyde acetyltransferase

2. Production and metabolism of H_2S in the colon

2.1. Luminal H_2S produced by intestinal bacteria

The release of luminal H_2S from the intestinal bacterial metabolism is the dominant production mode [13]. Previous studies on microbial sulfidogenesis in the human gut mainly focused on the bacterial inorganic sulfur metabolism. However, increasing evidence suggests that organic sulfur metabolism by intestinal microbiota may be a critical mechanism linking diet and CRC [11]. In this section, we will look at how gut bacteria utilize inorganic sulfur salts or organic substrates (taurine/sulfoquinovose/cysteine/methionine) to produce H_2S.

2.1.1. Metabolism of inorganic sulfur salts

Sulfate-reducing bacteria (SRB) are a very old class of anaerobic bacteria that rely on inorganic sulfates as electron acceptors for energy production [14]. As an important component of the intestinal flora, SRB can colonize the gut of a variety of animals. Among these SRB, *Desulfovibrio*, *Desulfomicrobium*, and *Bilophila* are the main members in the human intestinal tract [15].

SRB produce H_2S in the intestine by reducing sulfur oxidation products like sulfate, sulfite, and thiosulfate, while obtaining energy for their own growth through these biochemical reactions. The main method of producing H_2S from inorganic sulfur salts released by bacteria in the intestinal lumen is dissimilatory sulfate reduction of SRB [16]. This process involves sulfate adenylyltransferase (Sat; an ATP sulfurylase), adenosine phosphosulfate reductase (Apr), dissimilatory sulfite

reductase (Dsr), and anaerobic sulfite reductase (Asr) (Fig. 1A) [[17–19]]. Dsr and Asr catalyze the six-electron reduction of sulfite to H_2S in the final step of the process. Previous studies of SRB concentrate dissimilatory sulfate reduction on bacteria containing Dsr enzyme. Nevertheless, the latest research indicates that the Asr gene is more prevalent in human intestinal bacteria than the Dsr gene, implying that Asr may be a more important contributor to sulfate reduction in the gut than Dsr [11].

2.1.2. Metabolism of taurine

Taurine is the second most abundant free amino acid in colon tissue [20]. It is a substrate for the human gut microbiota that is obtained directly from the diet or from taurine-conjugated bile acids hydrolyzed by bile salt hydrolase [21]. Microbial taurine metabolism has received considerable critical attention after being identified as a potential mechanism by which dietary differences affect the development of colitis and CRC [22,23].

For a long time, *Bilophila wadsworthia* was the only known taurine-reducing bacterium in the human gut. This bacterium is thought to metabolize taurine via a three-step pathway that involves sulfoacetaldehyde acetyltransferase (Xsc) (Fig. 1B) [24]. Surprisingly, recent studies have revealed that *Bilophila wadsworthia* does not have the above pathway, but instead metabolizes taurine via a four-step reduction pathway involving sulfoacetaldehyde reductase (SarD) and glycyl radical enzyme (GRE)-isethionate sulfite-lyase (IslA) and its cognate activating enzyme (IslB) to produce H_2S (Fig. 1C) [11,25].

Given the colon's taurine-rich environment, it is likely that there are other bacteria capable of performing this metabolic process that have yet to be discovered. One study found taurine:2-oxoglutarate aminotransferase (Toa) in *Bifidobacterium kashiwanohense*, which can catalyze the deamination in the first step of taurine reduction [26]. Another recent study looked at the microbiome of sulfur metabolism genes in human gut and identified twelve potential pathways for gut microbes to reduce taurine, as well as new bacterial genera that contain these pathways [11]. Their findings point to new microbial targets for novel enzyme discovery or taurine metabolism research.

2.1.3. Metabolism of sulfoquinovose

Sulfoquinovose (SQ; 6-deoxy-6-sulfoglucose) is the polar head group of sulfolipid sulfoquinovosyl diacylglycerol (SQDG), which is the main organic sulfur reservoir in photosynthetic organisms and a source of carbon and sulfur for various microbial communities [27]. Because vegetarian diets typically contain SQDG and some gut bacteria have the ability to catabolize SQ to H_2S, it is hypothesized that SQ could be a long-neglected source of H_2S in the intestine derived from green diets [28].

In recent years, some advances have been made in the SQ metabolism (described as sulfoglycolysis) of microorganisms. A total of five pathways have been identified, including the sulfo-Embden-Meyerhof-Parnas (sulfo-EMP) pathway [[29–33]], the sulfo-Entner-Doudoroff (sulfo-ED) pathway [34,35], the sulfo-sulfofructose transaldolase (sulfo-SFT; or named sulfo-transaldolase, sulfo-TAL) pathway [36,37], the sulfo-transketolase (sulfo-TK) pathway [33], and the sulfo-sulfoquinovose monooxygenase (sulfo-SMO; or named sulfo-alkanesulfonate monooxygenase, sulfo-ASMO) pathway [33,38]. Among them, the sulfo-SMO pathway allows for complete SQ

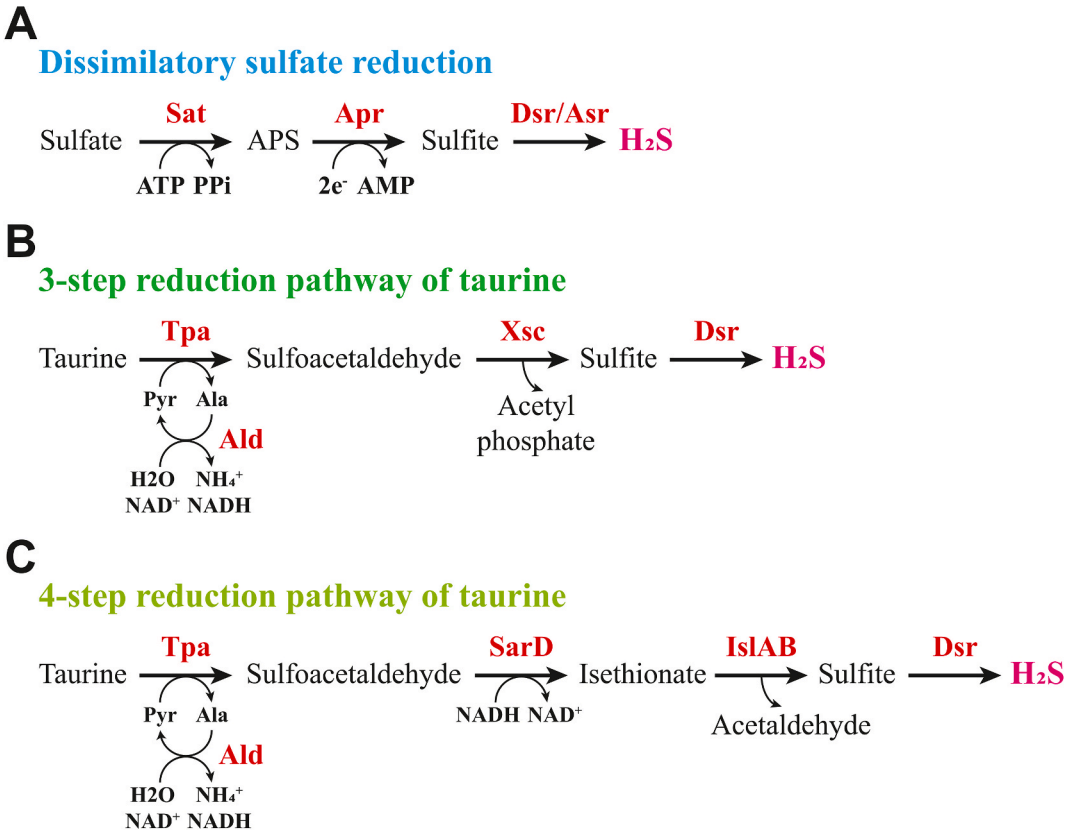


Fig. 1. Metabolic pathways of inorganic sulfur salts and taurine in bacteria. A) Dissimilatory sulfate reduction pathway of SRB. **B)** Previously thought three-step reduction pathway for taurine in *Bilophila wadsworthia*. **C)** Four-step reduction pathway for taurine in *Bilophila wadsworthia*. Ala, alanine; Ald, alanine dehydrogenase; AMP, adenosine monophosphate; Apr, adenosine phosphosulfate reductase; APS, adenosine phosphosulfate; Asr, anaerobic sulfite reductase; ATP, adenosine triphosphate; Dsr, dissimilatory sulfite reductase; IslAB, isethionate sulfite-lyase (IslA) and its cognate activating enzyme (IslB); NAD(P)H, nicotinamide adenine dinucleotide (phosphate); PPi, inorganic pyrophosphate; Pyr, pyruvate; SarD, sulfoacetaldehyde reductase; Sat, sulfate adenylyltransferase; Tpa, taurine: pyruvate aminotransferase; Xsc, sulfoacetaldehyde acetyltransferases.

metabolism and sulfite release, while the other pathways utilize only part of the carbons within the SQ skeleton (Fig. 2). These pathways that incompletely metabolize SQ (with the exception of the sulfo-TK pathway) produce 2,3-dihydroxypropane-1-sulfonate (DHPS) and/or sulfolactate (SL), which are biomineralized to sulfite/sulfate by other members of the microbial community.

SRB can degrade DHPS in the digestive tracts of terrestrial animals, resulting in the conversion of sulfonate sulfur to H_2S. For example, *Desulfovibrio* sp. Strain DF1 can ferment DHPS and SL under anaerobic conditions to produce acetate and H_2S via the DHPS/SL desulfurization pathway (related genes also exist in the SRB of the human gut microbiota) [39]. Furthermore, two oxygen-sensitive GREs in anaerobic bacteria were reported for DHPS degradation through different mechanisms. DHPS sulfolyase (HpsG) in SRB catalyzes *C*–S cleavage to release sulfite, which acts as a terminal electron acceptor in respiration, resulting in production of H_2S [40]. On this basis, a study found that *Eubacterium rectale* (via the sulfo-SFT pathway) and *Bilophila wadsworthia* (via the HpsG pathway) were involved in the interspecies DHPS transfer of human gut microbiota to jointly degrade plant-diet-derived SQ to H_2S. Meanwhile, the impact of SQ on human health and disease was also highlighted as a unique microbial nutrient and an additional source of intestinal H_2S [28].

2.1.4. Metabolism of cysteine/methionine

Some bacteria in the colon can also produce H_2S through cysteine desulfurase-catalyzed degradation of the substrate cysteine. The first L- or D-cysteine desulfurases were discovered in the 1950s in the *Escherichia coli*, which is a well-known resident of the human gut [41,42]. Since then, cysteine desulfurase-mediated desulfurization reactions have been described in a variety of common colonic bacteria, including *Clostridium*, *Enterobacter*, *Klebsiella*, *Streptococcus*, and *Desulfovibrio* of the SRB genus [17].

Notably, a high proportion of bacterial genomes contain mammalian homologous CBS, CSE, and 3-MST-encoding genes that are involved in endogenous H_2S production in cells. Pathogenic strains, such as *Escherichia coli* and *Staphylococcus aureus*, can convert cysteine to H_2S through the activity of one of these enzymes [43]. According to the recent studies, genes involved in microbial cysteine and methionine metabolism are more widely and diversely distributed in the human intestinal microbiome than previously thought [11]. These findings suggest that the sulfur-containing amino acids cysteine and methionine may be understudied and abundant sources of H_2S derived from microbial organosulfur metabolism in the human gut.

2.2. Endogenous production of H_2S

Endogenous H_2S can be produced in mammals via enzymatic reactions as well as non-enzymatic chemical reduction methods. Three enzymes involved in the metabolization of cysteine (Cys) catalyze the production of H_2S: the pyridoxal-5′-phosphate (PLP)-dependent enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), and the PLP-independent enzyme 3-mercaptopyruvate sulfurtransferase (3-MST) (Fig. 3) [1,3,44]. The important substrate cysteine can be derived from dietary absorption or release from endogenous proteins, or it can be synthesized from methionine through the *trans*-sulfuration pathway [45,46].

As the first (and rate-limiting) enzyme in the mammalian *trans*-sulfuration pathway, CBS catalyzes the typical β-replacement reaction for condensation of L-serine and L-homocysteine to form water and L-cystathionine. The latter is the physiological substrate for CSE, which catalyzes its conversion to L-cysteine, α-ketobutyrate and ammonia [47]. CBS can also catalyze multiple H_2S production reactions, such as the reaction of cysteine with L-homocysteine, L-cysteine, and water to generate H_2S. In contrast, CSE is a more promiscuous enzyme than CBS in the *trans*-sulfuration pathway. CSE can generate H_2S from one or 2 mol of homocysteine in addition to common reactions with CBS in H_2S production [1,47,48]. Notably, an intact *trans*-sulfuration pathway can support cell growth in the presence of extracellular cysteine limitation. When the catalytic activity of CBS and CSE is inhibited, cells are unable to synthesize cysteine, which may have deleterious effects on the organism at low cysteine availability [[49–51]].

The 3-MST catalyzed H_2S-producing reactions require the assistance of cysteine aminotransferase (CAT). L-cysteine and α-ketoglutarate are converted to glutamate and 3-mercaptopyruvate (3-MP) through the CAT-based catalysis. The latter is catalyzed by 3-MST to generate pyruvate and MST-bound persulfide, which can produce H_2S in the presence of thioredoxin (Trx) or dihydrolipoic acid (DHLA) [1,52]. Moreover, 3-MP can be synthesized from D-cysteine in the kidneys or the brain by D-amino acid oxidase (DAO) to participate in the subsequent H_2S synthesis [53].

Endogenous H_2S can also be produced by non-enzymatic pathways in addition to enzymatic reactions. Persulfides, thiosulfates, and polysulfides can be converted to H_2S and other metabolites in the presence of reducing equivalents, such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) [45,46,54].

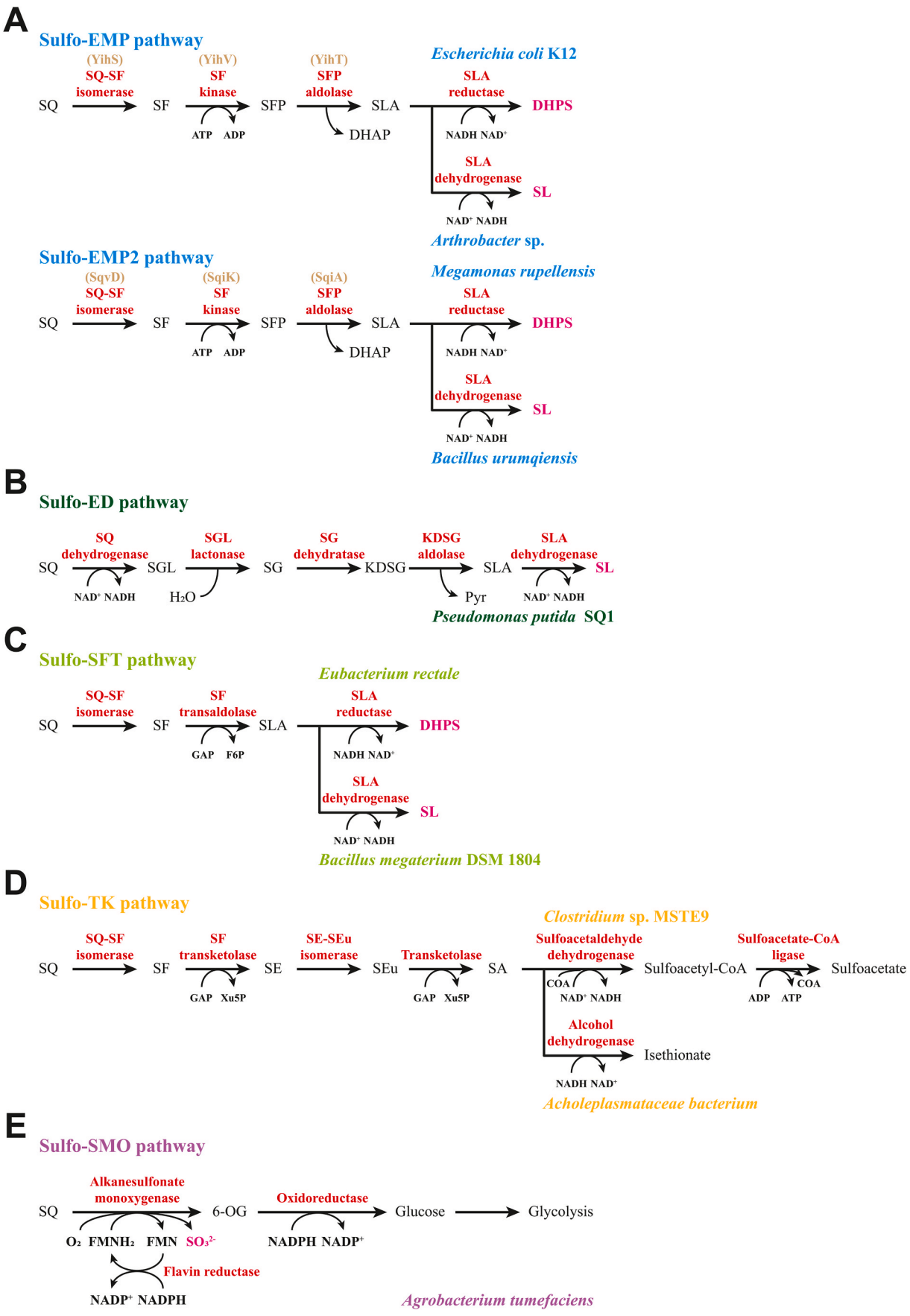
2.3. The catabolism of H_2S

Sulfide can exist in three forms in the intestinal lumen: H_2S gas partially dissolved in the aqueous phase, hydrosulfide anion HS^- and sulfide ion S^{2-} (generally negligible under physiological conditions) [13]. Because of its high membrane permeability, luminal H_2S easily penetrates biofilms and enters colon cells [55,56].

The sulfide oxidation unit (SOU) is a mitochondrial multi-enzyme system that is responsible for the intracellular oxidative metabolism of H_2S in colonic epithelial cells (Fig. 4). This sulfide metabolic process generates electrons that enter the electron transport chain at complex III (from sulfide: quinone oxidoreductase (SQR)) and cytochrome *c*/complex IV (from sulfite oxidase (SUOX)), respectively, driving ATP synthesis [57,58].

It is generally accepted that H_2S binds to the SQR enzyme and is oxidized to sulfane sulfur in the first step of the oxidation pathway. However, there are different perspectives on what the physiological receptor of sulfane sulfur is. One view is that the sulfane sulfur generated by the reaction is directly transferred to glutathione (GSH) to form glutathione persulfide (GSSH) [59]. Subsequently, GSSH is oxidized by iron-dependent persulfide dioxygenase (PDO), also known as ethylmalonic encephalopathy protein 1 (ETHE1), regenerating GSH and producing sulfite [60,61], which is oxidized to sulfate by SUOX [62], or reacts with GSSH to generate GSH and thiosulfate under the catalysis of rhodanese (Rhd), or thiosulfate thiotransferase (TST) [59,63]. Another view is that sulfane sulfur uses sulfite as an acceptor to produce thiosulfate, which is then transferred to GSH to form GSSH [64,65]. Nevertheless, this view has been challenged by other studies. First, this indirect pathway was proposed based on in vitro kinetic data, and its research model was inconsistent with physiologically low intracellular sulfite concentrations [66]. Second, the rhodanese-catalyzed sulfur transfer reaction of thiosulfate to GSSH is kinetically unfavorable [59]. Finally, since sulfite itself is an oxidation product of sulfide and is inherently toxic, it is unlikely to be used as a substrate for sulfide oxidation [58]. Overall, the now widely accepted view is that GSH is the primary sulfane sulfur receptor for the SQR reaction, which directly generates GSSH as a product. However, it is worth noting that the importance of sulfite as a receptor in the SQR response may increase under pathological conditions that lead to elevated concentrations [58].

Colonic epithelium express higher levels of SQR compared with other tissues, which may be an adaptation to the colon microenvironment with high sulfide load [67]. The SQR reaction of intestinal epithelial cells prevents H_2S accumulation and produces highly reactive persulfides, which may play an important role in H_2S/polysulfide-mediated protein persulfidation, also known as *S*-sulfuration or



(caption on next page)

Fig. 2. Sulfoglycolytic pathways in bacteria. A) The sulfo-Embden-Meyerhof-Parnas (sulfo-EMP) and sulfo-EMP2 pathways. **B)** The sulfo-Entner-Doudoroff (sulfo-ED) pathway. **C)** The sulfo-sulfofructose transaldolase (sulfo-SFT) pathway. **D)** The sulfo-transketolase (sulfo-TK) pathway. **E)** The sulfo-sulfoquinovose monooxygenase (sulfo-SMO) pathway. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CoA, coenzyme A; DHAP, dihydroxyacetone phosphate; DHPS, 2,3-dihydroxypropanesulfonate; FMN, flavin mononucleotide; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; KDSG, 2-keto-3,6-dideoxy-6-sulfogluconate; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); 6-OG, 6-oxo-glucose; Pyr, pyruvate; SA, sulfoacetaldehyde; SE, 4-deoxy-4-sulfoerythrose; SEu, 4-deoxy-4-sulfoerythrulose; SF, sulfofructose; SFP, sulfofructose-1-phosphate; SG, 6-deoxy-6-sulfogluconate; SGL, 6-deoxy-6-sulfogluconolactone; SL, sulfolactate; SLA, sulfolactaldehyde; SO_3^{2-}, sulfite; SQ, sulfoquinovose; Xu5P; xylulose-5-phosphate.

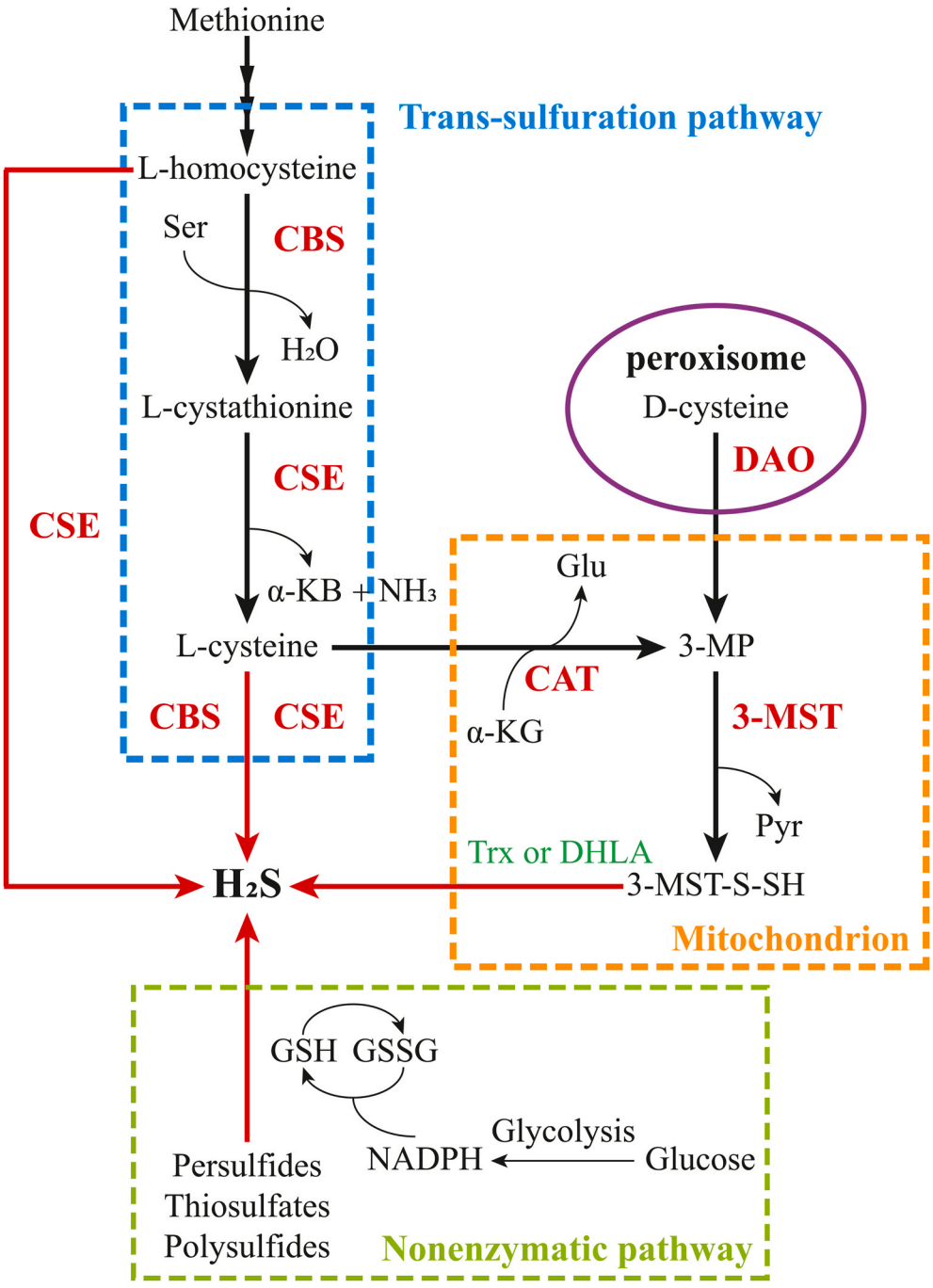


Fig. 3. Enzymatic and non-enzymatic pathways for endogenous H_2S production in mammalian cells. Endogenous H_2S from mammalian cells can be produced by enzymatic reactions with the help of CBS, CSE, 3MST coupled to CAT. As an important substrate in the enzymatic pathway, L-cysteine can be converted from methionine via the *trans*-sulfur pathway (blue dashed rectangle). L-cysteine is catalyzed by CAT to produce 3-MP, which enters the mitochondria for further conversion to H_2S via 3-MST (orange dashed rectangle). In addition, 3-MP can be generated from D-cysteine in brain and kidney, catalyzed by DAO located in the peroxisome (purple circles). A portion of endogenous H_2S can also be obtained by non-enzymatic chemical reduction (green dashed rectangle). The active sulfur species in persulfides, thiosulfates and polysulfides release H_2S, which can be converted from GSSG via NADPH in the presence of thiols such as GSH. α-KB, α-ketobutyrate; α-KG, α-ketoglutarate; CAT, cysteine aminotransferase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; DAO, D-amino acid oxidase; DHLA, dihydrolipoic acid; Glu, glutamate; GSH, glutathione; GSSG, oxidized glutathione; H_2S, hydrogen sulfide; 3-MP, 3-mercaptopyruvate; 3-MST, 3-mercaptopyruvate sulfurtransferase; 3-MST-*S*-SH, 3-MST-bound persulfide; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); NH_3, ammonia; Pyr, pyruvate; Ser, serine; Trx, thioredoxin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

S-sulfhydration [1,58,68–70]. In addition, H_2S can be considered as an inorganic energy substrate for colonocytes [71]. Electrons extracted from H_2S are injected into the mitochondrial respiratory chain via SQR during oxidative metabolism. At excessive concentration, H_2S inhibits the catalytic activity of cytochrome *c* oxidase, leading to inhibition of colonocyte respiration. Whereas micromolar concentration of H_2S is able to increase colonocyte respiration and to stimulate mitochondria allowing these cells to detoxify and to recover energy from luminal sulfide [[71–75]]. Furthermore, the metabolic utilization of H_2S by intestinal epithelial cells may play an important role in the regulation of overall H_2S homeostasis in animals. The researchers found that plasma levels of free H_2S and bound sulfane sulfur were significantly lower in

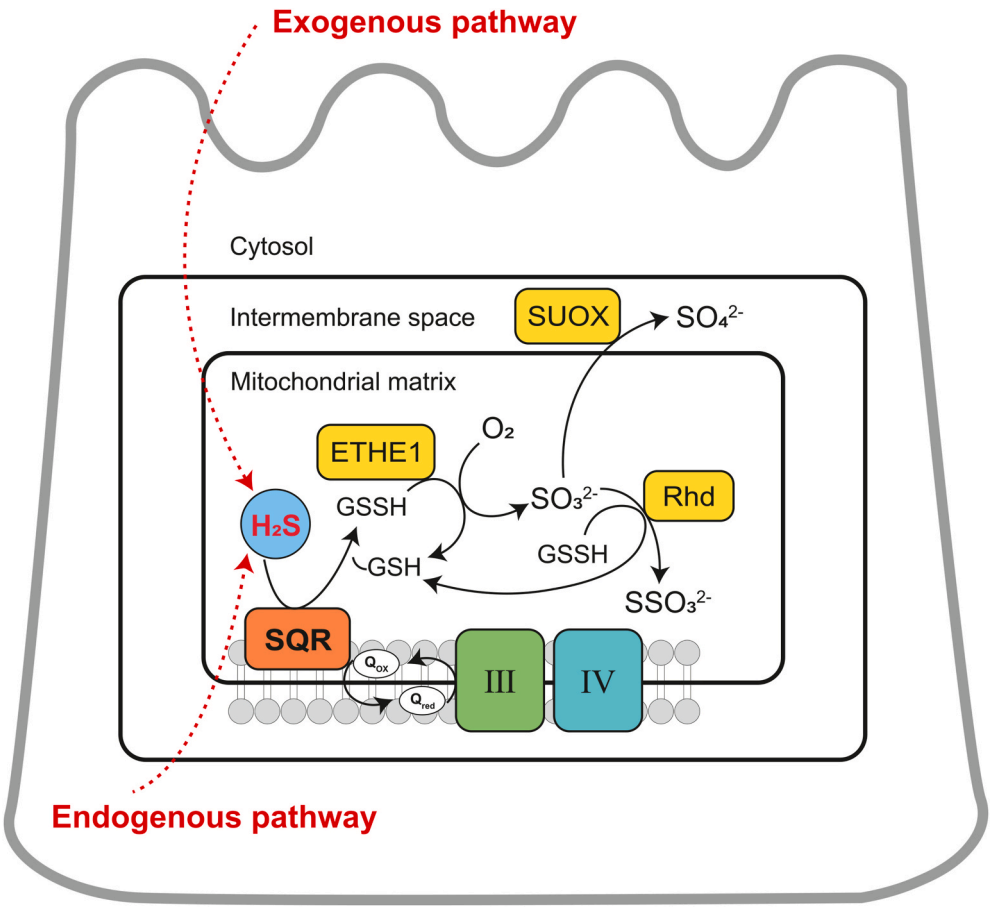


Fig. 4. Oxidation pathway for H_2S in colonic epithelial cells. ETHE1, ethylmalonic encephalopathy protein 1; H_2S, hydrogen sulfide; GSH, glutathione; GSSH, glutathione persulfide; O_2, oxygen; Rhd, rhodanese; SO_3^{2-}, sulfite; SO_4^{2-}, sulfate; SUOX, sulfite oxidase; SSO_3^{2-}, thiosulfate; SQR, sulfide: quinone oxidoreductase; III and IV, complex III and IV.

germ-free mice compared to conventional mice, suggesting that microbial-derived intestinal H_2S is closely related to the total circulating H_2S in animals [76].

Notably, colonic epithelial cells can synthesize H_2S via endogenous synthetic pathways (as described in Section 2.1) in addition to receiving exogenous H_2S from their luminal side. Thus, the intracellular H_2S concentration in colonocytes is determined by the diffusion of luminal H_2S across the cell membrane, cysteine-based intracellular synthesis, and the cellular capacity to metabolize H_2S.

3. The role of H_2S in the pathophysiology of colorectal cancer

The biological effects of H_2S show a typical bell-shaped concentration-response in cancer. Appropriate concentrations of H_2S can promote cancer cell growth, stimulate cellular bioenergetics, enhance angiogenesis, induce dedifferentiation, invasion, and metastasis, and confer chemotherapeutic resistance. When the concentration of H_2S exceeds a certain threshold, it exerts tumor suppressor effects such as reducing cancer cell proliferation, migration and bioenergetics, inducing cancer cells apoptosis, sensitizing cancer cells to chemotherapeutics, and inducing mesenchymal-to-epithelial transition (Fig. 5) [6,68,77].

H_2S-mediated persulfidation (a post-translational modification of proteins) plays a role in regulating tumor growth and metastasis, such as mediating MEK1 persulfidation to regulate ERK1/2 activity and thus affect tumor growth [78,79], and mediating NF-κB persulfidation to induce metastasis-promoting gene expression [80,81]. H_2S acts as a pro-angiogenic factor in vitro and in vivo under different physiological and disease conditions, including cancer. One possibility is H_2S may mediate angiogenesis through persulfidation of Kir 6.1 subunit of KATP channel since inhibition of KATP channel attenuates VEGF mediated endothelial cell migration. The other possibility is the activation of NF-κB/IL-1β signaling through H_2S-mediated NF-κB persulfidation [80,81], since IL-1β is a known pro-angiogenic cytokine through induction of VEGF during cancer progression [82]. Inhibition of mitochondrial complex IV is usually considered as the main mode of cytotoxic action of H_2S, which leads to blocked mitochondrial electron transport and inhibits aerobic ATP production [83]. However, at lower concentrations, H_2S can also act as a metabolic substrate to stimulate the mitochondrial electron transport chain. Oxidation of H_2S by SQR leads to electron transfer to coenzyme Q, which promotes aerobic respiratory ATP synthesis [83,84]. In addition, H_2S increases the catalytic activity of the mitochondrial ATP synthase through persulfidation [85]. In addition to stimulating mitochondrial ATP production, the role of endogenous H_2S in cancer cells includes maintaining mitochondrial organization (preventing mitochondrial fission) and maintaining mitochondrial DNA repair (by stimulating the assembly of mitochondrial DNA repair complexes) [83]. In addition, H_2S regulates the Wnt/β-catenin pathway, which is associated with differentiation/dedifferentiation of tumor cells, by inducing Sp3 transcription factors to activate the promoter of the ATP citrate lyase (ACLY) gene [86,87]. Notably, both endogenous H_2S synthesis in colonic epithelial cells and exogenous H_2S in the lumen are implicated in tumorigenesis in CRC [13,88].

3.1. *Exogenous H_2S and CRC*

Exogenous H_2S in the lumen is primarily derived from the gut

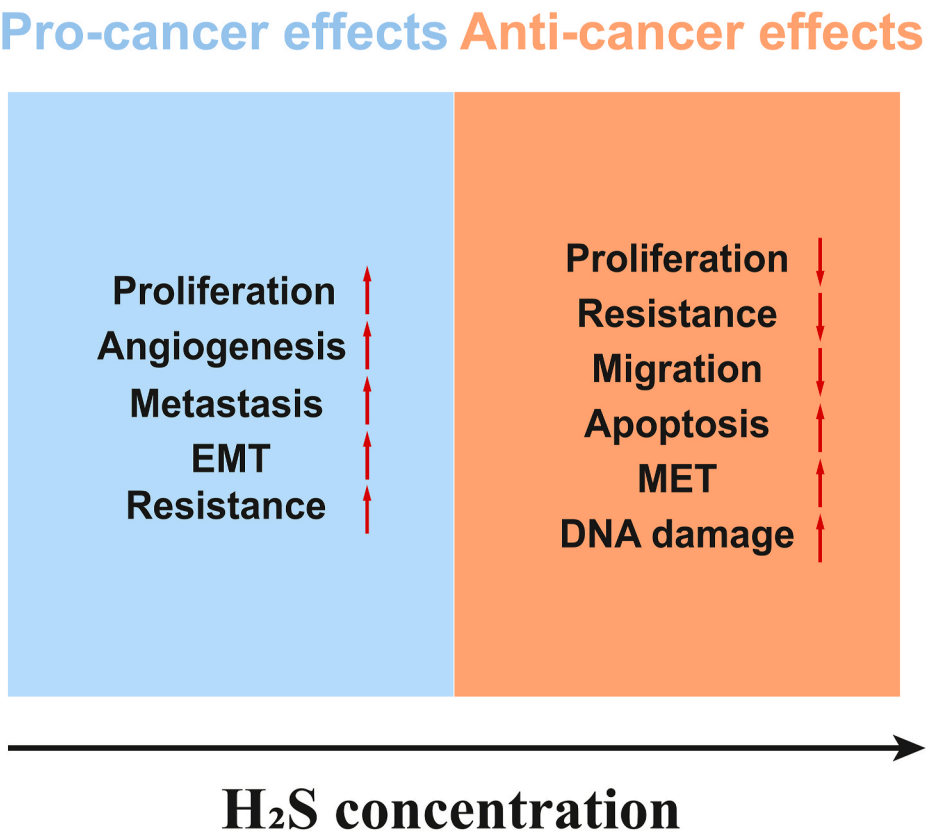


Fig. 5. The schematic diagram of bell-shaped effects of H_2S on cancer. H_2S promotes cancer growth within an appropriate concentration range while acting as a tumor suppressor above the concentration threshold. The models suggest that exogenous delivery of high doses of H_2S by donors and reduction of endogenous H_2S by scavengers or biosynthesis inhibitors represent two strategies to treat cancer.

microbiota, which is associated with the occurrence of CRC [13,89,90]. It has been shown that gastrointestinal exhaust samples recovered from CRC patients contained higher levels of sulfur-containing compounds compared to samples recovered from healthy subjects [91]. Moreover, the colons of CRC patients had a reduced ability to detoxify H_2S [92]. Another study found that fecal H_2S levels were significantly higher in patients with colon tumors and sigmoid surgery compared to the healthy population [93]. However, owing to the good volatility of H_2S, direct measurement of fecal H_2S concentrations does not accurately reflect intestinal H_2S levels. Hale et al. indirectly assessed H_2S production by intestinal bacteria in CRC by quantifying the amino acids produced with H_2S. The experimental results showed that the production of H_2S originated from intestinal microbes was increased in CRC samples, compared to non-cancerous samples [94]. Unfortunately, the combination of only four amino acids (serine, homoserine, lanthionine, and cystathionine) does not fully respond to the intestinal H_2S production. The available evidence suggests that H_2S production by gut microbes is closely related to CRC. Nevertheless, studies directly assessing the role of intestinal bacterial sources of H_2S on CRC are lacking. The precise quantification of the level of H_2S production by intestinal bacteria remains a challenge.

In contrast, quantifying the relative abundance of H_2S-producing bacteria is much less challenging [94]. Multiple studies investigating changes in the gut microbiota of colonic adenomas and/or CRC found that some major groups of sulfur-producing bacteria (eg, *Bilophila*, *Desulfovibrio*, *Bilophila wadsworthia*, *Fusobacterium*) levels increased in those with adenomatous polyps (the most common precursor of CRC) [11,23,95–100]. Among them, *Bilophila*, *Desulfovibrio*, and *Bilophila wadsworthia* are important H_2S-producing bacteria (their mechanisms of H_2S production have been discussed in Section 2.2). *Fusobacterium* is a periodontal disease-related H_2S producer, which has attracted wide attention in recent years due to its close relationship with CRC promotion [[99,101,102]]. These gut bacteria involved in sulfur metabolism use dietary and endogenous organic and inorganic compounds to produce H_2S, which may be the key linking diet, microbiota, and CRC [11,12,97]. In fact, dietary habits, as mediated by intestinal sulfur metabolizing bacteria, have been linked to an increased risk of early-onset adenomas and CRC [12,23]. Long-term adherence to a sulfur microbial diet (characterized by high intakes of low-calorie beverages, red and processed meats, and low intakes of fruits, whole grains, and vegetables, as well as relative abundance of metabolic gut bacteria) is associated with an increased risk of distal CRC and rectal cancer [97]. Another study, which included men and women and used larger, more diverse data as well as an analysis method that accommodated multiple cohorts, found that higher adherence to a sulfuric microbial diet was associated with higher risk of developing distal CRC [103]. Follow-up studies are needed to determine the specific mechanism of action of H_2S as an associated factor in linking dietary pattern-gut microbiota-CRC.

Donors that release H_2S rapidly or slowly can be used to mimic the effect of exogenous H_2S on CRC cell lines. A study reported that treating HCT116 cells with low concentrations (0.3 mM) of GYY4137, a slow-release H_2S donor, enhanced mitochondrial function and glycolysis while also encouraging cell proliferation [104]. Furthermore, H_2S donor stimulated cell proliferation using 0.2 mM NaHS on the same cell line by increasing Akt and ERK activation while inhibiting p21 (Waf1/Cip 1) [105]. Likewise, treatment of colon tumor cells with exogenous H_2S at a concentration of 1 mM using NaHS as a donor inhibited the chemo-preventive PEITC-induced apoptosis [106]. The effects of H_2S are biphasic, concentration- and time-dependent. Exogenous H_2S stimulation at optimum quantities can enhance the proliferation of CRC cells, while high concentration and sustained H_2S therapy can decrease the proliferative vitality of tumor cells. Szabo et al. evaluated the effect

of different concentrations of NaHS (fast-release) or GYY4137 (slow-release) H_2S donors on the proliferation of the human colon cancer cell line HCT116. The results showed that low concentrations (0.03–0.3 mM) of NaHS stimulated tumor cell proliferation, while higher concentrations (1–3 mM) inhibited tumor cell growth. In the case of pretreatment with the CBS inhibitor Aminooxyacetic acid (AOAA), not only the basal proliferation of HCT116 cells was attenuated, but also the toxic effect of higher concentrations of NaHS on the cells was diminished. In addition, without AOAA pretreatment, 0.03–3 mM of GYY4137 stimulated the proliferation of HCT116 cells. In contrast, only 3 mM of GYY4137 stimulated HCT116 cell proliferation beyond the basal growth rate after using AOAA [107]. These studies on the mechanism of action of various H_2S donors in CRC and their therapeutic applications will be described in detail later.

3.2. H_2S endogenous synthesis pathway and CRC

Several experimental and clinical studies have shown that the expression of H_2S endogenous synthase CBS is higher in colon tumor tissues than in normal surrounding tissues [[82,108–110]]. Moreover, CBS levels are closely related to CRC severity/tumor stage as well as patient survival [47,82]. These findings suggest that the CBS/H_2S system is essential in the occurrence and progression of CRC. CBS-derived H_2S has been shown in studies to be involved in maintaining the bioenergetics of CRC cells to support tumor growth and proliferation, as well as promoting angiogenesis and vasodilation to provide blood and nutrients to tumors [82]. In addition, some studies have pointed out that the CBS-H_2S axis may regulate CRC angiogenesis and liver metastasis via positive feedback between the CBS-H_2S axis and VEGF [111]. Considering the bell-shaped effect of H_2S, colon cancer cell proliferation can be suppressed by up-regulating CBS expression or delivering H_2S exogenously in a state where the CBS-H_2S axis is already highly activated. The underlying mechanism could be that H_2S inhibits the functional activity of transcription factor SP-1, thereby negatively regulating CD44 expression [112]. Similar bell-shaped functional responses were observed in HCT116 cells treated with the allosteric activator of CBS, *S*-adenosyl-L-methionine (SAM): lower concentrations/shorter incubation times stimulated HCT116 cell proliferation and bioenergetics, while higher concentrations and longer exposure to SMA inhibited cell viability and exerted cytotoxic effects [113]. Of course, whether the SAM's anti-proliferative mechanism is related to the "overstimulation" of the CBS-H_2S axis is still controversial, and the current consensus is that it is more likely to be an independent mechanism involving CBS. According to a recent study on the oncogenic role of CBS, protein expression of CBS is low in healthy colonic mucosa, but gradually increases with epithelial cell transformation into polyps, hyperplastic polyps, tubular adenomas (dysplasia), and adenocarcinomas (in situ). Furthermore, up-regulation of CBS leads to metabolic reprogramming and the induction of an aggressive tumorigenic phenotype in precancerous colon epithelial cell lines, suggesting that activation of the CBS/H_2S axis may promote colon carcinogenesis [114].

CSE, another key endogenous H_2S synthase, is also expressed in colon tumor cell lines [82,105,115]. The role of CSE/H_2S is currently less known. Activation of the Wnt/β-catenin pathway has been shown to up-regulate CSE expression and stimulate the proliferation of SW480 cells, a CRC cell line, in vitro migration and tumor xenograft growth in vivo [115]. Another study found that stable silencing of CSE via lentiviral transduction (shCSE) or the small molecule inhibitor DL-propargylglycine (PAG) had no effect on H_2S production or cell proliferation in HCT116 cells [82]. Thanki et al. found that loss or inhibition of CSE function promotes excessive inflammation, leading to the development of colitis-associated cancer (CAC). Meanwhile, loss of CSE expression in bone marrow-derived cells alters the balance of mucosal IL-6 and IL-10 expression and accelerates the development and progression of CAC. These results suggest that CAC may result from a failure of normal physiological functions regulating intestinal immune homeostasis and tissue repair via CSE and H_2S [116].

3-MST expression and/or catalytic activity has been demonstrated in several CRC cell lines (HCT116, LoVo, HT29, CT26) [6,117]. The recent discovery of the novel pharmacological inhibitor HMPSNE [118] has changed the lack of direct studies on the functional role of 3-MST. Szabo's group discovered that reduction of H_2S biosynthesis by inhibiting the catalytic activity of 3-MST could promote mesenchymal-epithelial transition (MET) in HCT116 cells [87]. The MET regulation mechanism could be related to the involvement of endogenous H_2S in the regulation of ACLY protein expression. ACLY regulates the Wnt/β-catenin pathway, which is an important regulator of EMT/MET balance. In addition, the 3-MST/H_2S system maintains tumor cell proliferation, migration, and cellular bioenergetics in murine CRC cell CT26 [117].

3.3. H_2S oxidation pathway and CRC

In normal colonic epithelium, the sulfide oxidation pathway is mainly located apically at the host-microbiota interface, i.e., the human colonic crypt [13,67]. In contrast, the expression of three enzymes involved in oxidative metabolism (SQR, ETHE1, and TST) was elevated in CRC tissues and showed a diffuse distribution. Moreover, elevated expression of SQR and ETHE1 was observed in all six CRC cell lines (HT29, LoVo, Caco-2, RKO, DLD-1, and HCT116), compared to the non-cancerous colon cell line HCEC [1]. It has been noted that ETHE1 expression is significantly increased in CRC and increases with CRC tumor grade compared to benign colonic epithelium [119]. It has also been found that human colon cancer cells HT-29 Glc (−/+) have an enhanced ability to oxidize sulfide when differentiated spontaneously or induced by butyrate [75]. These results suggest that enhanced SOU function is associated with the development of CRC. However, a different perspective was proposed by Piran et al. They found through meta-analysis that the expression of SQR, ETHE1, and TST genes tended to decrease during the process of colon tumors from normal to primary and then to liver and lung metastases. They speculated that H_2S is a supplementary energy source in the hypoxic environment of colon tumors. Unlike normal colon cells exposed to tubular H_2S, CRC cells may need to avoid excessive consumption of H_2S by reducing sulfide metabolic function during tumorigenesis and metastasis, which helps CRC cells survive from a hypoxic environment [120]. Szabo's group found that in human intestinal epithelial-like organs with harboring early mutations of the Vogelstein sequence, upregulation of H_2S synthase and metabolizing enzymes occurred simultaneously, and live cell imaging did not detect a significant increase in cellular H_2S levels. But, in organoids with late mutations, the expression of H_2S synthase was further increased, while the expression of certain H_2S metabolizing enzymes (e.g., SQR) was reduced and cellular H_2S levels were elevated. This consecutive mutations leads to effects that coincide with the upregulation of cellular bioenergetics (mitochondrial respiration and/or glycolysis) and the upregulation of the Wnt/β-catenin pathway, a key effector of EMT [121]. This study contributes to the proper understanding of the important role of H_2S synthesis and metabolism in the pathogenesis of CRC.

In conclusion, the current study suggests that the oxidative metabolic pathway of H_2S is closely related to the development of CRC. However, direct experiments to validate the role of oxidative pathway-related enzyme systems on CRC, such as observation of the effect of SQR catalytic activity inhibition on CRC by suitable SQR inhibitors, are lacking. Several recent studies have pointed to selective inhibitors of SQR as an attractive target for drug development and have provided some SQR inhibitor candidates, such as STI1, which can bind highly selectively to the coenzyme Q-binding pocket in human SQR [122–124]. Unfortunately, there are no reports of these inhibitors being used in CRC-related biological experiments. It is also important to note that inhibition of the function of these enzymes may to lead to some undesired effects, for example, the restricted function of ETHE1 may lead to the accumulation

of H_2S in the colonic mucosa, liver, muscle, and brain [125,126].

3.4. Persulfidation, persulfides, polysulfides and CRC

Persulfidation (*S*-sulfuration) is a post-translational modification of proteins characterized by the addition of sulfur atoms to specific cysteine residues via H_2S or polysulfides (mainly polysulfides), resulting in peroxisulfide adducts on small molecules and proteins [1,127–129]. This process is considered to be a key step in the biological function of reactive sulfur species [69,70,130,131]. Some of the enzymes involved in H_2S anabolism (mainly 3-MST, SQR), as well as some other proteins (haemoglobin, neuroglobin) and enzymes (catalase, peroxidases, super oxide dismutase, and cysteine tRNA synthetase), have been identified to produce polysulfides, GSSH, and other *S*-sulfurated molecules (*S*-sulfated molecules) [66,132,133].

These generated polysulfides, persulfides, have recently been recognized to play an important role in cancer regulation [68,70,131]. Cysteine-rich angiogenic inducer 61 (CyR61) is a matricellular protein encoded by the CYR61 gene, which promotes colon cancer cell migration, invasion and metastasis, and its high expression is associated with poor CRC prognosis [[134–137]]. Recent studies suggest that H_2S and polysulfides activate the CyR61 promoter in colon cancer cells. Endogenous H_2S/polysulfide biosynthesis of 3-MST in colon cancer cells facilitates the induction of CyR61 mRNA, most likely through the persulfidation of Sp1, and through the activation of S1PR, ATF1 and CREB [137]. It has also been shown that in colon cancer cells, the *trans*-sulfuration pathway –H_2S axis stabilizes xCT (the functional subunit of system Xc-) through the persulfidation of OTUB1, which imports cysteine and converts it to cysteine, providing a substrate for endogenous H_2S biosynthesis. Increasing xCT and the transsulfuration pathway are associated with the metabolic reprogramming of CRC [138]. Furthermore, it was revealed that the stimulatory effect of low concentrations of exogenous H_2S on glycolysis may be due to H_2S/polysulfide-mediated persulfidation of lactate dehydrogenase, which enhances its catalytic activity [104]. *N*-acetylcysteine (NAC) is a commonly used antioxidant in biological experiments as well as in clinical studies [139,140]. NAC treatment was found to promote the expression activity of SW480 cell line 3-MST and SQR, enzymes involved in the production and consumption of H_2S and thiane sulfur. Moreover, NAC can act as a direct thiane sulfur accepting co-substrate for 3-MST and undergo persulfidation. The NAC persulfide formed is hypothesized to promote resistance to oxidative stress and increase drug resistance in colon cancer cells, given the specific novelty of the reaction of the persulfide with electrophilic reagents and the reference to the already reported cysteine persulfide [141]. Thus, further investigations are necessary to validate this hypothesis and whether the effect of NAC on colon cancer cells is mediated through H_2S and related active substances.

Many natural polysulfides have antitumor effects. Studies have pointed out that in CRC, diallyl polysulfides (garlic-derived organosulfur compounds) and their derivatives can exert anticancer activity by inducing cell growth arrest and apoptosis [142–144]. Moreover, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) could promote the expression of multidrug-resistant genes in colo 205 human colon cancer cells [145]. In addition, dibenzyl tetrasulfide, a derivative of diallyl tetrasulfide, was also identified as a mitogenic inhibitor and apoptosis inducer in colon cancer cells and induced cell type-dependent autophagic damage involving p62 [146].

4. H_2S diagnosis and treatment strategies for colon cancer

Existing studies have shown that the moderate supply of exogenous H_2S and the elevated endogenous concentrations of H_2S may be involved in promoting the occurrence of CRC. Increased H_2S production in colon tumor cells is ascribed to increased activity of endogenous H_2S synthases (mainly CBS). These provide new ideas for the diagnosis and treatment of CRC: strategies for H_2S modulation (exogenous delivery of high doses of H_2S or reduced expression of endogenous H_2S) in colon tumor treatment, and using the high level of H_2S in CRC tissue cells to develop diagnostic probes and combination therapies that respond to endogenous H_2S.

4.1. Inhibition and clearance of endogenous H_2S

The reduction of endogenous H_2S is currently receiving wide attention as a method of applying H_2S regulation to tumor therapy [147]. In related studies, a common and well-established approach is to inhibit the endogenous biosynthesis of H_2S. To this end, a series of small molecule inhibitors were screened and synthesized to inhibit three key enzymes involved in endogenous H_2S synthesis, CBS, CSE, and 3-MST. Hence, another more desirable approach is the development of endogenous H_2S scavengers. Targeted H_2S scavenging is achieved by specific, rapid-response, targeted endogenous scavengers. In this section, we summarize and discuss reports on the application of inhibition of H_2S production in the field of CRC, focusing on H_2S-producing enzyme inhibitors and H_2S scavengers (Table 1).

Table 1
Summary of recent reported CRC treatment strategies via H_2S modulation.

Therapeutic agent	Therapeutic strategy	Anticancer mechanism
AOAA	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity; Enhanced sensitivity to oxaliplatin via exaggerating apoptosis induced by ROS.
AOAA derivatives	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity.
Benserazide and its metabolite, THBH	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity; Therapeutic effects on cancer cells with a multidrug-resistant phenotype.
EGCG	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity; CBS specific inhibitor.
PAG	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity.
HMPSNE	Endogenous H_2S biosynthesis inhibitors	HMPSNE exhibited anti-proliferative activity and induced MET through the inhibition of 3-MST, CBS and key enzymes involved in H_2S degradation ETHE1 and TST.
HMPSNE derivatives	Endogenous H_2S biosynthesis inhibitors	Anti-proliferative activity.
CuDDC	Endogenous H_2S biosynthesis inhibitors; H_2S scavenger	Anti-proliferative activity.
VZnO	H_2S scavenger	Anti-proliferative activity.
EA-Fe@BSA	H_2S scavenger	Anti-proliferative activity; CDT/PTT; MRI imaging.
PMSN-Fe-LA-BSA	H_2S scavenger	Anti-proliferative activity; CDT/PTT.
HKUST-1	H_2S scavenger	Anti-proliferative activity; CDT/PTT.
2D Cu-MOF	H_2S scavenger	Anti-proliferative activity; CDT/PTT; PA imaging.
ADT-OH conjugate	H_2S donor	Anti-proliferative activity.
SATO	H_2S donor	Anti-proliferative activity.
SATO-functionalized micelles	H_2S donor	Anti-proliferative activity.
Natural polysulfides	H_2S donor	Anti-proliferative activity.
HS-NSAIDs	H_2S donor	Induced cell proliferation inhibition, apoptosis and G0/G1 phase cell cycle arrest; Phase II metabolic enzyme inducers; Synergistic treatment with NO.
NiNPs	Carrier	H_2S promoted the anticancer efficiency of 5-FU in the presence of NiNPs.

4.1.1. CBS inhibitors

AOAA is one of the best-known CBS inhibitors. In aqueous solution, AOAA is able to react with the aldehyde group of vitamin B6 (pyridoxal form) and generate a stable oxime [148]. As the metabolically active form of vitamin B6, PLP is involved in a series of enzymatic reactions in biological systems and is a cofactor of enzymes such as CBS and CSE. Therefore, in the presence of AOAA, oxime is produced irreversibly, inhibiting the CBS-catalyzed H_2S production. In the field of colon tumor research, Szabo et al. reported that the use of AOAA could inhibit CRC cell proliferation in vitro and reduce tumor growth in vivo [82]. Zhao et al. treated HCT116 cells with AOAA and examined the ability of cancer cells to target H_2S probes through the negative control [149]. Yue et al. found that AOAA sensitizes CRC cells to oxaliplatin via exaggerating apoptosis induced by ROS both in vitro and in vivo [150]. But it is worth noting that AOAA is not a specific inhibitor of CBS, and it inhibits a variety of PLP-dependent enzymes, including CSE. Moreover, AOAA exhibited higher inhibitory potency to CSE than CBS (CSE's IC_{50} is 1 μM in the same report where CBS's IC_{50} is 8 μM) [151]. In addition, AOAA can also indirectly inhibit H_2S formation by the 3-MST system via inhibiting the enzymatic generation of the 3-MST substrate, 3-MP. Furthermore, AOAA can even inhibit some non-enzymatic pathways of H_2S generation [46]. In addition, the poor lipophilicity of AOAA (water/octanol coefficient: 0.0019) makes its action in cells inefficient. In HCT116 cells, up to 100 μM AOAA was required to significantly inhibit H_2S production, which may be attributable to the low cellular uptake [147].

To improve its lipophilicity, Szabo's group designed and synthesized a series of AOAA-based prodrugs that release AOAA after penetrating the cell membrane and hydrolyze the ester bond on the prodrug by cellular esterases [110,152]. Among them, YD0171, a methyl ester derivative of AOAA, possesses stronger lipophilicity and higher inhibition efficiency compared with AOAA. In the treatment of HCT116 cells, CBS-induced inhibition of H_2S production is inhibited with 30 μM YD0171, whereas a similar effect required 100 μM AOAA. The in vivo efficacy is nine times higher than that of AOAA. At the same time, YD0171 administration has low systemic toxicity and can target the metabolism of colon tumors [152]. YD0251, an isopropyl ester derivative of AOAA, inhibits HCT116 tumor growth in vivo 18 times more potently than AOAA and exerts anticancer effects at doses that do not induce severe organ damage. It also inhibited the growth of patient-derived tumor xenografts and exerted anti-proliferative effects on CRC cells with a multidrug-resistant phenotype. Furthemore, they proposed that matching CBS inhibitor therapy to patients with high CBS expression could be a useful application for future CRC therapeutic diagnosis. Likewise, they identified lanthionine, a metabolic intermediate of CBS-mediated H_2S biosynthesis, as a suitable biomarker for identifying CRC target populations with high CBS expression [110].

Some hydrazine derivatives can antagonize the action of vitamin B6. Among them, benserazide was found to inhibit CBS activity and suppress colon cancer cell proliferation and bioenergetics in vitro, as well as tumor growth in vivo [153,154]. It can bind to the active site of the enzyme and inhibit CBS by forming a reversible but kinetically stable Schiff base-like adduct with the formyl portion of pyridoxal, and reacting with PLP cofactors. Moreover, its main metabolite, 2,3,4-trihydroxybenzylhydrazine (THBH), can act as a CBS inhibitor and an anti-proliferative agent for CRC cells. In buffer, the inhibitory effect of benserazide on CBS (IC_{50}: ~30 μM) was much lower than that of AOAA (IC_{50}: ~3 μM). However, it inhibited the proliferation of HCT116 colon cancer cells (IC_{50}: ~20 μM) with greater potency than AOAA (IC_{50}: ~300 μM) in cytological assays, probably owing to the good cellular uptake. Meanwhile, benserazide showed some selectivity for CBS, with the activity inhibition of CSE and 3-MST being 16% and 35%, respectively, after 2 h of 100 μM benserazide treatment, while the activity inhibition of CBS was as high as 66%. It is noted that this conclusion was drawn from the comparison under different substrate concentration conditions, and the inhibition of benserazide is related to the substrate concentration, which remains to be further verified [147]. The efficacy of many anticancer drugs is reduced when cancer cells have a multidrug-resistant phenotype. Szabo's group evaluated the role of AOAA and benserazide in multidrug-resistant phenotypic tumor cells by constructing a 5-FU resistant HCT116 cell line that also exhibited partial resistance to the unrelated chemical oxaliplatin. They found that 5-FU resistance in HCT116 cells was associated with up-regulation of drug-metabolizing enzymes and enhanced endogenous H_2S production. Moreover, 5-FU-resistant cells showed reduced sensitivity to AOAA, but remained sensitive to the anti-proliferative effect of benserazide. This contributes to the development of new strategies for the treatment of advanced CRC [155].

Epigallocatechin gallate (EGCG) is a major bioactive component of green tea with antioxidant, anti-inflammatory, anticancer, and anti-neurodegenerative effects [[156–159]]. Moreover, the anti-proliferative effect of EGCG is known to be more effective than a chemotherapeutic drug, 5-FU, on CRC [160]. Many of the biological effects of EGCG have been attributed to binding to or sterically interfering with certain enzymes or other regulatory proteins, because they can form covalent adducts with protein and non-protein thiols [161]. In the past, many polyphenolic compounds similar to EGCG were selected in the screening of CBS inhibitors, but most of them were not as effective as the most commonly used AOAA for inhibition. In contrast, EGCG inhibits CBS-catalyzed H_2S production at sub-micromolar concentrations (IC_{50}: 0.12 μM) and has been shown to significantly inhibit H_2S production in CRC or Down syndrome cell models in vitro [162]. Remarkably, unlike AOAA and its derivatives or hydrazine CBS inhibitors, the inhibition of CBS by EGCG does not target PLP or response mechanisms, but exploits certain structural or conformational features unique to CBS, which makes it a CBS-specific inhibitor. In addition, it has been proposed that EGCG can oxidize H_2S produced by CBS to form polysulfides [161], indicating that EGCG may act as a scavenger of H_2S. Nonetheless, a study by Szabo's group discovered that EGCG exhibited some H_2S scavenging activity only at higher concentrations and also inhibited non–H_2S–generating HsCBS, indicating that it is a true CBS inhibitor [162]. Although EGCG and other polyphenolic bioflavonoids usually have a variety of pharmacological targets and actions, their therapeutic effects on CRC may not be attributed to CBS inhibition alone. Putting aside the PLP, the studies on EGCG still provide a new idea for the development of CBS inhibitors by targeting specific sites on CBS, resulting in the development of new potent CBS-specific inhibitors.

There are many challenges in translational application of EGCG, including the low systemic bioavailability, less stability in alkaline media, low membrane permeability, high oxidative degradation, and metabolic transformations [156]. A clinical trial of EGCG showed that all the patients treated with EGCG could not respond to it as this unstable EGCG exhibited alterable bioavailability [163]. Therefore, how to improve the stability and bioavailability of EGCG is the focus of further research.

4.1.2. CSE inhibitors

There are few reports on the application of CSE inhibitors in the treatment of CRC, due to the lack of direct evidence to prove that CSE plays a significant role in CRC. DL-propargylglycine (PAG) is an early discovered irreversible CSE inhibitor [164]. Unlike most PLP-dependent enzyme inhibitors, PAG irreversibly changes the CSE active center, thereby inactivating enzyme function. The whole inhibition process is closely related to several key amino acid residues of CSE, including Arg 62, Lys 212 and Tyr114 [147]. Fan et al. reported that treatment of SW480 cells with CSE inhibitors PAG or shCSE effectively reduced tumor cell proliferation, migration, and in vivo tumor xenograft growth [115]. However, this PAG or shCSE-mediated CSE silencing was ineffective in inhibiting H_2S production or cell proliferation in HCT116 cells [82]. The role of CSE inhibitors in the treatment of CRC remains to be explored.

4.1.3. 3-MST inhibitors

High throughput screening has been employed to find suitable endogenous H_2S synthase inhibitors. Hanaoka et al. identified four novel 3-MST inhibitors with IC_{50} values in the micromolar range by high-throughput screening of a library of 174,118 compounds using the H_2S-selective fluorescent probe HSip-1. Among them, (2-[(4-hydroxy-6-methylpyrimidin-2-yl)sulfanyl]-1-(naphthalen-1-yl)ethan-1-one) or HMPSNE is considered to be the most effective and selective [118]. Inhibition of H_2S biosynthesis in CRC cell lines by HMPSNE inhibits tumor cell proliferation, migration, and induces MET [87,117]. Particularly, when treated with 300 μM HMPSNE, expression inhibition of 3-MST, CBS and key enzymes involved in H_2S degradation ETHE1 and TST was observed in HCT116 cells. The effect of HMPSNE on HCT116 cells at this concentration must be explained by the direct inhibition of 3-MST catalytic activity and the combined effect of CBS down-regulation [87]. Bantzi et al. synthesized a library of 63 compounds using the central core of HMPSNE (renamed 1a) to independently modify the pyrimidinone and aryl ketone sides, and then evaluated in vitro the efficacy of these new derivatives as 3-MST inhibitors and their effects on CRC cell proliferation and viability [165]. Six novel 3-MST inhibitors were found to be effective in inhibiting the proliferation of two mouse CRC cells (MC38 and CT26). Although the inhibitory effect of compound (1 b) on recombinant 3-MST was not as good as that of starting compound 1a, it was more effective than 1a in inhibiting tumor growth in tumor-bearing mice in vivo.

4.1.4. H_2S scavengers

The ideal scavenger should have high reactivity and high selectivity to H_2S, and the product after the reaction should have the lowest biological activity [147]. Researchers have developed some small molecule endogenous H_2S scavengers based on sulfonyl azides [166] or NBD [167], because these properties are very similar to those of H_2S sensors. Szabo 's group found that while the clinical drug disulfiram did not directly inhibit H_2S production in CRC cells, its metabolite bis(N, *N*-diethyldithiocarbamate)-copper (II) complex (CuDDC) could be used as an effective inhibitor of CBS and CSE as well as an H_2S scavenger to inhibit CRC cell proliferation [168].

Thanks to the development of modern nanotechnology, researchers have begun to explore the direct elimination of endogenous H_2S by appropriate nanomaterials. Zinc oxide has more prominent desulfurization performance than other metal oxide desulfurizers [169], and has better biocompatibility and low toxicity when made into nanoparticles [170]. Based on these characteristics, Pan et al. developed a ZnO-coated virus-like silica (VZnO) nanoparticle H_2S-responsive nanoplatform for CRC treatment. After entering the cells, VZnO can reduce the level of endogenous H_2S, lead to a significant decrease in intracellular GSH levels, and ultimately lead to ferroptosis in CRC cells. They injected fluorescently labeled VZnO@FITC intravenously into a mouse model of CRC to evaluate the tumor selectivity of this scavenger. The results showed that the tumor tissues had the highest uptake of VZnO@FITC compared with most normal tissues, except the liver. Moreover, the scavenger has no therapeutic effect on the 4T1 breast cancer model (non H_2S rich cancer), suggesting that it may be selective for cells with high H2S levels [171].

Chemodynamic therapy (CDT) is a highly tumor-specific and minimally invasive treatment modality. It uses Fenton or Fenton-like drugs to degrade the highly expressed hydrogen peroxide (H_2O_2) in tumors and generate highly toxic hydroxyl radicals (•OH) to kill cancer cells [[172–174]]. However, its therapeutic efficacy is limited by the low efficiency of •OH production. In particular, in CRC, the high expression of H_2S with strong reducibility leads to the depletion of the generated •OH, which further weakens the efficacy of CDT [175]. Therefore, a therapeutic strategy combining endogenous H_2S scavengers, CDT, and photothermal therapy (PTT) has been proposed for CRC treatment. The key to this effective strategy is that the heating effect of activated PTT can accelerate •OH production while depleting endogenous H_2S, thus improving CDT efficiency and providing better synergistic therapeutic effects.

The classical CDT based on Fe(II)-mediated Fenton reaction is strictly limited by the catalytic efficiency of Fe(II). To this end, Yang 's group assembled EA-Fe@BSA nanoparticles (NPs) using natural polyphenols, Fe(III), and albumin [176]. These NPs have excellent T1-weighted MRI performance as well as enhanced CDT effects through the combined effect of the strong reducing properties of high concentrations of H_2S in CRC, which accelerates Fe(III)/Fe(II) conversion, and the thermal effects of PTT. This holds great potential for effective colon cancer theranostics. In addition, Wang et al. designed an iron-triggered tumor microenvironment (TME)-responsive PMSN-Fe-LA-BSA (PMFLB) nanotherapeutic agent combined with CDT/PTT for colon cancer [177]. It is loaded with Fe^{3+} by polydopamine (PDA) decorated mesoporous silica nanoparticles (PMSN), and uses the phase change ligand lauric acid (LA) to prevent Fe^{3+} leakage. Under NIR laser irradiation, PDA generates a large amount of heat to kill colon tumor cells by hyperthermia, and induces the phase transition of LA to release Fe^{3+}. Fe^{3+} further reacts with endogenous H_2S in TME to form Fe^{2+}, which reacts with H_2O_2 in TME to form •OH and is then converted back to Fe^{3+}. This repeated reaction with endogenous H_2S produces more •OH, thus enhancing CDT.

Cu(II)/Cu(I) pair has lower redox potential (−1.6 eV) and higher catalytic activity than Fe(II)-based Fenton reagents [178,179]. Li et al. developed a non-photoactive copper-based metal-organic framework named HKUST-1, which can be activated by endogenous H_2S in colon tumors to produce photoactive CuS for thermal imaging and PTT [180]. At the same time, HKUST-1 exhibits horseradish peroxidase (HRP) mimetic activity that effectively converts overexpressed H_2O_2 in CRC cells into a more toxic •OH, as well as synergistic therapy of endogenous H_2S scavenging, PTT and CDT. Wang et al. also designed a two-dimensional (2D) Cu-bipyridine [$Cu(bpy)_2(OTf)_2$] metal-organic framework (2D Cu-MOF) nanosheet for combination therapy [175]. This nanotherapeutics can significantly improve the applicability of CDT in CRC treatment by inhibiting the consumption of the generated •OH through rapid depletion of H_2S via 2D Cu-MOF, promoting the Fenton-like reaction by ultra-small CuS produced by in situ vulcanization, and generating CuS with good photothermal properties because of its strong NIR-II absorption.

4.2. H_2S donor therapy

H_2S donor therapy has gained widespread attention in recent years as an important gas chemical transmitter. H_2S donors, i.e., exogenous compounds that can release H_2S, are the key to H_2S gas treatment. Considering the intricate mechanism of the H_2S dose response, its effects are dual in nature and are influenced by many factors such as in vivo concentration and biodistribution [181]. Targeted delivery and controlled release of H_2S is thus critical for efficient and safe precision gas therapy. To this end, scientists have developed a number of small molecules or macromolecular H_2S donors based on biocompatible polymeric materials that can respond to various stimuli, such as pH, thiol-containing compounds, enzymes, and other pathological microenvironments, as well as external stimuli, such as light and ultrasound, resulting in a controlled release of H_2S gas [182]. In this section, we review the application of small and large molecule H_2S donors with different activation mechanisms in the field of CRC research (Table 1) and discuss some new strategies for H_2S donor therapy in CRC.

Inorganic sulfide salts, e.g., Na_2S and NaHS, are the simplest and most common class of donors in H_2S-related biological studies. Although commonly categorized as H_2S donors, these sulfide salts are a direct source of H_2S once dissolved in water. They immediately hydrolyze and establish an equilibrium between H_2S, HS^-, and S^{2-}, followed by rapid volatilization of H_2S [183]. These gaseous H_2S substitutes have now been widely used to assess the therapeutic efficacy of exogenous H_2S in a variety of cancer studies, including CRC [184]. However, simple sulfide salts are flawed as chemical tools for studying the biology of H_2S and as

potential therapeutics. Due to their direct and rapid gas release mode, they are difficult to use to mimic the biological effects of endogenous H_2S, and they usually fail to maintain the therapeutic effect and may cause side effects. Furthermore, because sulfide salts lack targeting ability, they are only useful for systemic delivery [185].

Morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate (GYY4137) is a class of Lawson's reagent-derived hydrolysis-activated H_2S donors with slow-release properties that allow for the slow release of H_2S in aqueous solution [186]. It showed in vitro killing effects on several types of cancer cells, including HCT116 [187]. GYY4137 also exhibited anti-proliferative activity against Caco-2 cells, possibly through the production of H_2S-induced G2/M phase cell cycle arrest, apoptosis, and necrosis [188,189]. In addition, NHE1 internalization in colorectal cancer DLD1 cells and uncoupling of the NCX1/NHE1 complex after treatment with GYY4137 resulted in intracellular acidification and apoptosis [190]. Researchers have also proposed a new combination therapy strategy based on the mechanism of action of GYY4137 in CRC. Kajsik et al. reported that GYY4137 could be used to increase the sensitivity of CRC cells to the potent chemotherapeutic agent paclitaxel, which is commonly used in the treatment of a variety of cancers but has low efficacy in CRC [191].

Dithiothiones (DTTs), represented by anethole dithione (ADT), are generally considered as hydrolysis-activated H_2S donors. However, recent studies have shown that H_2S release from ADTs may occur through an enzymatic process in the presence of hepatic microsomes and reduced NADPH [182]. Li et al. designed and synthesized three series of the H_2S-releasing oridonin derivatives using its demethylated analogue (ADT-OH). Among them, compound 12 b showed strong anti-proliferative effect in Caco-2 or HCT116 cells and low toxicity to two human normal cells, making it a potential anticancer candidate [192]. In addition, Hu et al. proposed a new strategy to design H_2S donor-based antitumor drugs by combining a potent natural compound evodiamine with the H_2S donor ADT-OH or α-thioctic acid. They tested the anti-proliferative activity of all synthesized new compounds in five human cancer cell lines and normal cells, and found that the most effective ADT-OH conjugate 12c exhibited significant cytotoxicity against both Caco-2 and HL-60 cell lines with IC_{50} values of 2.02 and 0.58 μM, respectively. And its enhanced anti-proliferative efficacy accompanied by increased selectivity is a class of promising antitumor candidates [193].

Some H_2S donors can be reduced by thiol-containing bioactive compounds lke Cys or GSH to release H_2S, resulting in a smart polymeric H_2S delivery system for therapeutic applications. For example, *S*-aroylthiooxime (SATO) can react with Cys-containing compounds to release H_2S [194] and reduce the proliferation viability of HCT116 cells [195]. In addition, natural polysulfides, such as diallyl trisulfide (DATS), are thiol-activated H_2S donors [196], which are cytotoxic to cancer cell lines like HCT116 [[197–199]].

Hydrogen sulfide-releasing non-steroidal anti-inflammatory drugs (HS-NSAIDs) are a special class of anticancer drugs that covalently link traditional non-steroidal anti-inflammatory drugs with hydrogen sulfide-releasing parts. Kashfi's group reported that H_2S-donating aspirin derivatives (HS-ASA) can act as phase II metabolic enzyme inducers for HT29 cells, with great potential for CRC prevention [200]. They also investigated the effects of four different HS-NSAIDs (HS-aspirin, -sulindac, -iburofen, -naproxen) on the growth characteristics of different human cancer cell lines. The results showed that HS-NSAIDs inhibited cell proliferation, induced apoptosis, and caused G0/G1 cell cycle arrest in a variety of cancer cells [201]. Moreover, it has higher anti-inflammatory activity, tumor growth inhibitory potency, and chemo-preventive effects than conventional counterparts [[201–204]]. Based on NO-releasing NSAIDs (NO-NSAIDs) and HS-NSAIDs, a new strategy for the synergistic cancer treatment with NO and H_2S was proposed. Kashfi's group designed and synthesized a hybrid of NOSH-NSAIDs that can simultaneously release NO and H_2S, including NOSH-aspirin, NOSH-sulindac, and NOSH-naproxen for anti-inflammatory and antitumor studies [205,206]. After a series of in vivo and in vitro studies, these NOSH-NSAID drugs showed excellent performance in inhibiting the growth of CRC cells and are a class of drugs with considerable potential in the prevention and treatment of CRC [[205–212]].

Despite the great progress in the development of small molecule H_2S donors, they usually do not meet the requirements for in vivo applications considering the stability, water solubility, trigger specificity, toxicity, and by-products generated upon H_2S release of the donors themselves. Therefore, macromolecular/supramolecular H_2S donor systems attracted a lot of interest from researchers [213]. For direct release of H_2S from Na_2S and NaHS, polymeric carrier encapsulated delivery is an effective strategy for achieving controlled H_2S delivery. Other sulfide salts, such as MnS, FeS, and ZnS, are less water soluble but slowly hydrolyze at acidic pH to generate H_2S and metal ions, and have been used to develop pH-responsive nanotherapeutic release [182]. Foster et al. prepared an H_2S-releasing micelle from SATO-functionalized polymeric amphiphiles as an in vitro or potential in vivo targeting delivery tool. The micelles were selectively toxic to cancer cells and reduced the survival of CRC cells (HCT116) more significantly than other common H_2S donors including Na_2S, the small molecules SATO and GYY4137 [195]. Furthermore, Housein et al. evaluated the effects of different combinations of nickel nanoparticles (NiNPs) with 5-FU, H_2S, and NO donors on CRC cells. They found that H_2S promoted the anticancer efficiency of 5-FU in the presence of NiNPs, while NO had anti-apoptotic activity on CRC cell lines [214]. This provides a new idea for H_2S donor therapy in combination with chemotherapy. In the future, a new combination therapeutic nanoagent that delivers both H_2S and chemotherapeutics can be developed for colon tumors by loading NiNPs with H_2S donors and 5-FU.

Overall, all donors mentioned in this section failed to achieve targeted delivery and controlled release of H_2S in vivo, even with SATO-functionalized micelles, which were only evaluated in vitro on the selectivity for HCT116 and mouse embryonic fibroblasts NIH/3T3. Indeed, targeted and controlled delivery of H_2S by nanotechnology is feasible. For example, Li et al. provided a strategy combining H_2S donor therapy, PTT, and imaging technology applied to breast cancer treatment to improve the targeting of nanotubes to tumors by responding to GSH enriched in tumor cells [215]. Therefore, an effective strategy to achieve targeted delivery and controlled, on-demand release of H_2S is that combines imaging technology (dynamic monitoring of H_2S levels) and delivery vehicles that respond to tumor microenvironmental factors (e.g., pH, thiols) or external stimuli (e.g., acoustic signals, light signals). In addition, the development of prodrugs through surface modification, or the use of cancer cell-selective or semi-selective enzymes may be an effective way to improve tumor targeting.

4.3. H_2S-mediated colon cancer therapy

Targeted therapy allows for better therapeutic outcomes and reduced side effects compared to conventional CRC treatment. H_2S is a TME factor that can be exploited for targeted therapy in CRC, due to elevated levels of endogenous H_2S in CRC cells [114,127,216]. In this section, we focus on therapeutic agents activated by H_2S designed to target the high levels of endogenous H_2S in CRC, including H_2S-activating chemotherapy, photodynamic therapy, photothermal therapy, chemodynamic therapy, and some combination therapy strategies (Table 2). These promising CRC-targeted therapeutics are currently in development and have not yet been translated into clinical trials.

4.3.1. H_2S in chemotherapy

Chemotherapy is the most commonly used treatment strategy in the clinical practice. Chemotherapeutics that are commonly used include doxorubicin (DOX), camptothecin (CPT), 5-FU, and curcumin (Cur), which are employed in the treatment of many cancers, including CRC [217]. However, chemotherapeutics have a number of limitations in

Table 2
Summary of recent reported H_2S-mediated CRC therapy.

Therapeutic agent	Therapeutic strategy	Imaging method	Stimulus	Colon cancer cells
MSNP-N_3-FA/DOX	Chemotherapy	–	H_2S	HCT116; HT-29
TP-HS	Chemotherapy	Fluorescence imaging	H_2S	HCT116
Ru-NBD	PDT	Fluorescence imaging	H_2S; Light	HCT116
MOF NPs	PDT	Fluorescence imaging	H_2S; Light	HCT116; LoVo
TNP-SO	PDT	NIR imaging	H_2S; Light	HCT116
1^{2+}-PSs-FA	PDT	NIR imaging	H_2S; NIR light	HCT116; HT-29
ZNNPs@FA	PDT	PA imaging	H_2S; Light	HCT116
Nano-PT	PTT	NIR-II imaging	H_2S; Ligh t	HCT116
Cu_2O NPs	PTT	PA imaging	H_2S; NIR light	HCT116
Au@Cu_2O	PTT	PA imaging	H_2S; NIR light	HCT116
Bi:Cu_2O@HA	PTT	CT imaging	H_2S; NIR light	CT26
FeOOH NSs	PTT/ Ferroptosis/ Scavenging endogenous H_2S	MRI imaging	H_2S; NIR light	CT26
PL-Cu	Chemotherapy/ PTT	–	H_2S; NIR light	HCT116
N_3-GT-CPT/ICG	Chemotherapy/ PTT	NIR imaging	H_2S; NIR light	CT26
NPs@BOD/CPT	Chemotherapy/ PTT	NIR-II imaging	H_2S; NIR light	HCT116
CatCry-AgNP-DOX	Chemotherapy/ PTT	NIR-II imaging	H_2S; NIR light	HCT116
Cu-DhaTph COF	PDT/PTT	–	H_2S; NIR light	HCT116
FR-H_2S	PDT/PTT	Fluorescence imaging	H_2S; Far-red light	HCT116
*N*P-Cu	PDT/PTT/CDT	–	H_2S; NIR light;	HCT116
Cu_2O@$CaCO_3$@HA	PDT/PTT/CDT/ Calcium overload	–	H_2S; NIR-II light; pH	CT26
5-Fu/Cur-P@HMPB	Chemotherapy/ CDT/Autophagy	–	H_2S	HCT116

their use, including rapid clearance after administration, non-specific distribution, and non-discriminatory damage to normal tissues [218]. As a result, scientists have been working for a long time to develop a drug carrier that can precisely control the on-demand release of chemotherapeutics in order to increase targeting to tumor tissues and improve therapeutic efficacy.

Recently, considering the high endogenous H_2S levels in some tumor cells (e.g., colon cancer, ovarian cancer [219]), some carriers using H_2S as a specific environmental stimulus to control the release of chemotherapeutics have been developed. Thirumalaivasan et al. designed H_2S-activated folate-modified azide functionalized biocompatible mesoporous silica nanoparticles (MSNPs) capable of targeting tumor cells via folate receptor [220]. In the presence of H_2S, the ester bond in DOX-loaded MSNP-N_3-FA breaks and leads to the release of the drug from the MSNP nanocarriers. At the same time, the therapeutic effect of DOX delivery via MSNP-N_3-FA was verified in HT29 (human colon cancer cells) tumor-bearing mice.

In addition, Bobba et al. developed a therapeutic diagnostic molecular prodrug (TP-HS) in combination with fluorescence imaging technology [221]. It is capable of synergistically releasing rhodol and the active therapeutic component SN-38 under the stimulation of endogenous H_2S, which can be monitored dynamically and quantitatively by fluorescence imaging. Based on the experimental results in colon cancer (HCT116 cells) and lung cancer (A549 cells), it was shown that TP-HS is an effective therapeutic diagnostic delivery system that can be activated in tumor-specific regions and allows the assessment of free drug content by fluorescence modulation.

It is important to note that although these H_2S-activated nanodrug carriers exhibit excellent delivery of chemotherapeutic drugs in cellular or tumor-bearing mouse models, it is difficult to achieve experimental drug delivery in translational applications in organisms due to complex biological barriers.

4.3.2. *H_2S in photodynamic therapy*

Photodynamic therapy (PDT) is primarily based on the accumulation of non-toxic photosensitizers, oxygen and light to produce reactive oxygen species (ROS), particularly singlet oxygen ($^1O_2$), which selectively induces apoptosis and necrosis in cancer cells [222,223]. It is a promising approach for cancer therapy due to its high selectivity, minimal invasiveness, significant efficacy, low impact on the host system, and many other advantages. Monitoring the phototherapy process by detecting changes in H_2S-activated optical signals to guide the on-demand treatment of tumors is another effective strategy that maximizes the effect of PDT. Based on this idea, Yuan et al. developed a multifunctional H_2S probe, Ru-NBD, which can act not only as an effective PDT reagent for H_2S activation, but also for real-time and in situ monitoring of therapeutic effects on tumors through restored luminescence during PDT [224]. Similarly, Ma et al. reported a novel metal-organic framework nanoparticle (MOF NP) for photodynamic therapy of colon cancer via controlled release of H_2S-activated $^1O_2$ in TME [225]. They used zinc-metallized 5,10,15,20-tetra(4-methoxycarbonylphenyl)porphyrin (ZnTCPP) as a photosensitive bridging ligand to construct a new one-component MOF NP PS. Cu^{2+} ions as metal nodes of the network quench ligand-based fluorescence and significantly reduce the release of $^1O_2$. When H_2S appears, Cu^{2+} ions are taken out from the MOF nodes, thereby simultaneously obtaining the luminophore photosensitive ligand. Since Cu^{2+} ions are detached from the MOF network after exposure to H_2S, the fluorescence of the ligand is turned on and the production of $^1O_2$ is enhanced. Through controlled release of photosensitive ligands, this turn-on MOF nanophotosensitizer achieves effective photodynamic therapy for cancer.

To combine NIR imaging with PDT, Wang et al. validated a designed therapeutic diagnostic prodrug, TNP-SO, in human colon cancer cells as well as in tumor-bearing mouse models. It consists of an H_2S -activated imaging probe, NIR-BSO, and a photosensitive drug, 3I-BOD, with both H_2S-activated NIR-emitting light and efficient $^1O_2$ generation [226]. The experimental results showed that the TNP-SO platform accurately guided where light is applied to generate cytotoxic ROS for on-demand cancer treatment through cancer imaging. In addition, Wu et al. doped an electrochromic material based on an organic π-electron structure (bicationic 1,1,4,4-tetraarylbutadiene, 1^{2+}) into semiconductor polymer nanoparticles (SPNs) and constructed tumor-targeted fluorescent probes (1^{2+}-SNP830-FA) by folic acid modification for H_2S-related tumor imaging [227]. On this basis, they further developed a new tumor-targeted photosensitizer (1^{2+}-PSs-FA) by replacing SNP with organic photosensitizer, which generates ROS under 808 nm laser irradiation after H_2S-specific activation. The reagent exhibited a significant

photodynamic therapeutic effect on tumor tissues while showing negligible phototoxicity to normal tissues.

Based on PA imaging technology, Zhang et al. developed a H_2S-responsive and consumable nanoplatform ZNNPs for early diagnosis and treatment of H_2S-related diseases [10]. In the presence of H_2S, ZNNPs exhibited NIR conversion (F_{1070}→F_{720}) and ratiometric PA (PA_{680}/PA_{900}) signals, thereby sensitively and visually detecting endogenous H_2S levels in acute hepatotoxicity, brain hemorrhage models, and colorectal tumors in living mice. In addition, the designed ZNNPs@FA can deplete mitochondrial H_2S in tumors to cause significant ATP reduction and severe mitochondrial damage, and simultaneously activate photodynamic effects for effective inhibition of colorectal tumors in vivo.

Notably, the therapeutic efficacy of PDT is influenced by factors such as penetration depth, photosensitizer distribution and permeability, and especially oxygen supply (the hypoxic environment of most solid tumors results in limited PDT efficacy), which need to be considered when designing PDT drugs.

4.3.3. H_2S in photothermal therapy

PTT is a simple, safe and non-invasive treatment method. It uses the heat generated by photothermal agents under near-infrared light irradiation to achieve local hyperthermia to kill tumor cells [[228–230]]. However, conventional photothermal agents have limitations such as non-specificity and treatment-related side effects. To address these issues, photothermal agents with intelligent responses need to be developed to turn on the photothermal effect at the tumor site through specific stimuli while remaining silent in normal tissues. Furthermore, scientists are interested in combining imaging technology and photothermal therapy to improve photothermal conversion performance and photothermal agent targeting in order to improve the therapeutic effect of PTT on tumors. Taking advantage of the high endogenous H_2S in colon cancer TME, Shi et al. developed an H_2S-activatable nanostructured photothermal agent (Nano-PT) [231]. Through in vivo studies, they found that the Nano-PT probe was specifically activated in H_2S-rich CRC tissues, emitting NIR-II fluorescence and generating high NIR absorption, thereby achieving efficient photothermal conversion under NIR laser irradiation, while remaining inactivated in normal tissues. Based on these characteristics, Nano-PT was able to achieve effective photothermal ablation of CRC tumors using NIR-II imaging. Yang's group designed a switchable diagnostic and therapeutic probe with combined diagnostic and therapeutic functions using the PA signal activated by the in situ reaction between endogenous H_2S and Cu_2O at the colon tumor site and PTT [232]. On this basis, they developed a plasmonic hybrid Au@Cu_2O with intelligent response to H_2S [233]. The photothermal conversion efficiency of Au@Cu_2O in the presence of H_2S is much higher than that of pure Cu_2O due to the localized surface plasmon resonance coupling effect between noble metals and plasmonic semiconductors. Au@Cu_2O can be used for CRC diagnosis and treatment due to its improved PAI and PTT. Bismuth (Bi)-based nanoparticles are widely used as computed tomography (CT) reagents [[234–236]]. Bi doping can improve the NIR absorption of Cu_2O triggered by endogenous H_2S [237]. Based on these features, Cheng et al. developed a smart H_2S-responsive nanoplatform, Bi:Cu_2O@HA NPs, combining tumor-targeted delivery, high-performance CT imaging, and enhanced PTT into one system for colon cancer treatment [238].

Furthermore, MRI can be used to guide photothermal treatment of CRC. Li et al. developed a FeOOH NSs nanotherapeutic drug for MRI, ferroptosis, and H_2S-based cascade-enhanced combination CRC therapy [239]. FeOOH NSs can effectively scavenge endogenous H_2S through reduction reactions and generate H_2S-driven cascades of FeS with photothermal therapeutic ability and Fe^{2+}-mediated ferroptosis. Surprisingly, in vivo experiments revealed that FeOOH NSs do not have curative effects on other cancer types and can be used as a specific therapeutic agent for CRC.

It is important to note that PTT has side effects such as damage to normal tissues around the tumor, inflammatory reaction after treatment, and short-term massive release of tumor cell contents during thermal ablation. For this purpose, mild-temperature photothermal therapy was introduced, which solved some side effects, but some new and other problems emerged, such as decreased therapeutic effect (due to the heat resistance of cancer cells). Combining other therapies, such as chemotherapy and PDT, to synergistically assist the therapeutic effect of PTT is a feasible strategy.

4.3.4. H_2S in combination therapy of chemotherapy and PTT

The combination of nanotechnology-based targeted chemotherapy and PTT can synergistically enhance the efficiency of cancer treatment, thus reducing the systemic toxic effects of the drugs on patients because of their high targeting and lower concentrations of use [240,241]. Taking advantage of the rapid development of nanotechnology, new advancements has been made in the design of a nanotherapeutic platform that integrates PTT and targeted delivery of chemotherapeutics.

Sun et al. reported a copper complex modified drug-loaded liposome nanoplatform (PL-Cu) [242]. The synergistic ablation of colon tumors at mild apparent temperature achieved successfully performed in a mouse model using photothermal inactivation of tumor cells mediated by tumor endogenous H_2S stimulation and drug release induced by thermal effects. To better guide and control drug release, Hou et al. developed a multifunctional therapeutic nanosystem using a small molecule prodrug N_3-GT-CPT designed to link two chemotherapeutic drugs, CPT and gemcitabine (GT), to easily co-precipitate with the photothermal agent indocyanine green (ICG) [243]. The N_3-GT-CPT/ICG nanosystem showed selective H_2S-triggered drug release behavior by a lock GT strategy using a H_2S-responsive azide group. At the same time, a synergistic chemotherapy, PTT and NIR imaging for tumors was achieved by combining N_3-GT-CPT with ICG. In addition, Shi et al. developed a photoresponsive drug delivery system NPs@BOD/CPT combined with NIR-II imaging [244]. NPs@BOD/CPT was developed by co-encapsulating a rationally designed borodipyrrole methylene (InTBOD-Cl) dye (used as an H_2S-activatable NIR photothermal agent) and the clinical drug camptothecin-11 (CPT-11). Under NIR laser irradiation, H_2S induces an increase in local temperature and conformational changes in the thermosensitive matrix, resulting in the instantaneous release of encapsulated CPT to inhibit tumor growth in vivo in a tumor-bearing mouse model of HCT116 (human colon cancer cells).

It is worth noting that the tedious preparation steps, the introduction of toxic reagents, and uncertain long-term toxicity may cause unpredictable limitations to the clinical application of nanocarriers [245]. Zhou et al. introduced catalase nanocrystals (CatCry) as a TME-activated nanoplatform into the CRC treatment and prepared a nanoformulation (CatCry-AgNP-DOX) by a one-step co-crystallization method using catalase, $AgNO_3$, and DOX as substrates [246]. The CatCry-AgNP-DOX nanoformulation reacted in situ to form Ag_2S nanoparticles under the action of large amounts of H_2S in the tumor, which provided NIR-I-activated photothermal effects and NIR-II imaging capabilities while triggering the release of loaded DOX chemotherapeutics. Moreover, CatCry catalyzes the conversion of abundant H_2O_2 in TME to O_2, thereby relieving tumor hypoxia and correspondingly enhancing DOX-induced chemotherapy.

4.3.5. H_2S in the combination therapy of PDT and PTT

At the moment, the combination of PDT and PTT therapy is still the research focus, because it not only improves the treatment efficiency, but also avoids unnecessary damage to normal tissues caused by the high power and prolonged irradiation generated by single-peak phototherapy [247]. However, how to integrate the photosensitizer responsible for ROS production during light irradiation and the photothermal agent responsible for local warming into a single nanosystem to construct a multifunctional therapeutic nanoagent remains a major challenge.

Feng et al. designed a Cu(II)-porphyrin-derived nanoscale covalent

organic framework (Cu-DhaTph COF) [248]. Upon arrival of the Cu-DhaTph COF at the colon tumor site, Cu(II) is captured by endogenous H_2S to form the photothermal agent CuS, allowing the recovery of porphyrin-derived fluorescence. Moreover, the synchronously released photosensitizer DhaTph produces $^1O_2$ in photodynamic mode under light irradiation to achieve in situ activated PDT/PTT combination therapy. In addition, Zhu et al. constructed a far-red (FR) absorbing H_2S -responsive nanosystem (FR- H_2S) based on the prodrug dinitrophenyl BODIPY (DNP-BDP) [249]. DNP-BDP has fluorescence turn-on properties after endogenous H_2S-specific thiolation in tumors, as well as the ability to generate abundant $^1O_2$ and heat for imaging-guided CRC phototherapy.

4.3.6. Other combination therapy

Recently, researchers have provided a three-mode cascade treatment strategy for endogenous H_2S-responsive PDT, PTT, and CDT in CRC treatment. Yang et al. developed an intelligent multifunctional nanoplatform (*N*P–Cu) by clickable assembly of dibenzocyclooctayne (DIBO) functionalized lysine (D-K), the photosensitizer chlorin e6 (Ce6)-responsive prodrug oxyanthraquinone (AQ4N), and cyclen-Cu^{2+} complex [9]. In *N*P-Cu, Cu^{2+} quenched the luminescence of Ce6 in the inactivated state, resulting in PDT in the "turn off" state, and in situ reaction with endogenous H_2S led to the "turn on" state of PDT. In particular, the simultaneous CuS production not only provides additional PTT but also amplifies intracellular hypoxic stress to trigger AQ4N-associated CDT. Chang et al. synthesized a nanoformulation Cu_2O@$CaCO_3$@HA that can respond to colonic TME by in situ mineralization of hollow mesoporous Cu_2O coated with $CaCO_3$ shells and modified with HA [250]. The protective $CaCO_3$ shell decomposes and releases calcium in the acidic colonic TME, allowing the Cu_2O core sulfidated by H_2S to form metabolizable $Cu_{31}S_{16}$ nanocrystals, which exhibit excellent PDT/PTT/CDT effects under 1064 nm laser irradiation. After HA modification, the nanocomposite can achieve synergistic CRC targeting and TME-triggered PDT/PTT/CDT/calcium overload-mediated combination therapy.

In addition, Chen et al. developed a novel 5-Fu/Cur-P@HMPB nanomedicine by co-encapsulating Cur and 5-Fu into a hollow mesoporous Prussian blue (HMPB), combining enhanced chemotherapy and CDT and amplifying autophagy [8]. 5-Fu/Cur-P@HMPB enter CRC's H_2S-rich TME, PB with low Fenton catalytic activity is responsively converted to Prussian white (PW) with high catalytic activity, which in situ produces high levels of •OH to activate CDT while triggering autophagy. The natural anticancer drug Cur can amplify autophagy to induce autophagic cell death, and can also be used as a clinical chemotherapy drug 5-Fu specific chemical sensitizer, with a good synergistic antitumor effect.

It is important to note that combination therapeutic strategies, including sections 4.2.4 and 4.2.5, and the approaches described in this section, require a balance of the characteristics of each system, which increases the difficulty of design development. Moreover, the complexity of the systems may have unknown implications in practical applications and represent a significant challenge for the clinical translation of the drugs.

4.4. H_2S-activated probes

Abnormal H_2S levels in organisms have been associated with the development of many diseases, including tumors, Alzheimer's disease, and cardiovascular disease [127]. Therefore, highly sensitive probes targeting H_2S concentrations in animals are very important. They can help us to understand the effects of H_2S on various physiological and pathological processes and serve as potential diagnostic tools for relevant diseases. Optical imaging techniques are widely used for the development of H_2S probes because of their high sensitivity, short response time, non-invasiveness, and real-time monitoring capability. For example, the H_2S fluorescent probe SF7-AM based on visible light imaging allows for direct, real-time visualization of endogenous H_2S produced in live human umbilical vein endothelial cells stimulated by VEGF [251]. Nanoprobe 1-PEI-DCNPs based on near-infrared imaging can monitor endogenous H_2S in lipopolysaccharide-induced liver inflammation in animal models [[252]]. Some other imaging modalities, such as photoacoustic imaging (PAI), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and combined imaging, have also been explored for potential use in H_2S detection and diagnosis. Recently, due to the increased levels of H_2S in CRC, many probes and H_2S-based in vivo diagnosis have been developed to detect intracellular levels of H_2S in CRC cells [[181,253,254]]. In this section, we summarize the H_2S-activatable probes relevant in the field of CRC research (Table 3) and described in detail below.

4.4.1. Visible light imaging probes

In living systems, selective detection and differentiation of endogenous H_2S are challenging considering the interference of other active substances such as intracellular GSH [255]. For H_2S fluorescent probes, the selectivity of the sensing reaction should be considered as one of the most important properties. Probes based on H_2S-mediated azide reduction are a widely adopted design strategy for H_2S fluorescent probes [[256–258]]. Upon reduction by H_2S, the electron-withdrawing azido group will be converted into an electron-donating amino group and turn on the fluorescent signal of the fluorophore. 7-Azido-4-methylcoumarin (AzMC) is a commercial fluorescent probe which has been applied in CRC-related biological studies, including screening potential CRC therapeutic drugs (CBS inhibitors) and evaluating the role of 3-MST in CRC, because AzMC is easy to synthesize and can selectively and sensitively detect H_2S levels in colon tumor cells [[154] [117,153,168]]. Compared with H_2S-mediated azide reduction, H_2S-mediated thiolysis of 7-nitro-1,2,3-benzoxadiazole (NBD) amines possess better selectivity for H_2S even in the presence of millimolar GSH [[259–261]]. Taking advantage of NBD amines, Ye et al. developed a cell-capable probe (AM-BODIPY-NBD) for highly selective and ultrasensitive imaging of intracellular H_2S to reveal a positive correlation between endogenous H_2S and the metastatic potential of CRC and breast cancer cells [259].

Most of the traditional fluorescent probes still suffered from the aggregation-caused quenching effect which adversely impacts the performance of the probes. In contrast, molecules with aggregation-induced emission (AIE) characteristics usually exhibit weak emission when dissolved in solvents but intense fluorescence in the aggregated state [262,263]. Based on these characteristics, Xu et al. developed a fluorescent probe based on a triphenylamine benzopyridine platform (TPANF) with AIE characteristics to identify changes in H_2S concentration in living cells and to image endogenous H_2S in HCT116 (human colon cancer cells) xenograft tumor tissue [264]. This probe has the advantages of high stability to photobleaching and low background fluorescence interference compared with traditional fluorescent probes. Notably, AzMC and AM-BODIPY-NBD are usually only used to detect H_2S levels in cells, while TPANF with high photostability can be applied to in vivo animal imaging, but it is limited by the low tissue penetration and poor spatial resolution of visible light imaging mode.

4.4.2. Near-infrared-I/II (NIR-I/II) imaging probes

Because of the numerous problems that arise during the transition from solution, cells, and tissues to whole organisms, NIR fluorescent probes with good penetration and low phototoxicity are preferable to fluorescent probes emitting in the visible region for H_2S monitoring and imaging in living animal tissues [265]. Zhang and co-workers developed a cyanine-based NIR-I probe (Probe 1) for rapid and highly selective NIR-I imaging of endogenous H_2S in live cells, tissues and mouse models via H_2S-mediated thiolysis of NBD amines [266]. Compared to conventional visible light (450–750 nm) and NIR-I (750–900 nm) imaging, fluorescence imaging in the second near-infrared window (NIR-II, 1000–1700 nm) has higher in vivo spatial resolution compared to

Table 3
Summary of recent reported H_2S-activated probes in CRC.

H_2S probe	Imaging method	Signal detection	Detection limit	In vivo Experimental subject
AzMC	Visible light	450 nm	200 nM	–
AM-BODIPY-NBD	Visible light	520 nm	15.7 nM	–
TPANF	Visible light	468 nm	170 nM	HCT116 tumor mice
Probe 1	NIR-I	796 nm	39.6 nM	HCT116, HT29 tumor mice
Ag-CEW	NIR-II	1090 nm	35 nM	HCT116 tumor mice
NanoBOD-SCM	NIR-I	710/610 nm	198 nM	–
NIR-II@Si	NIR-II	700/900 nm	37 nM	HCT116 tumor mice
DCNP@HSA-Ag^+	NIR-II	1050/1550 nm	210 μM	MC38-luc tumor mice
$F1^{2+}$-ANP	Afterglow luminescent	790 nm	100 nM	–
TPAMC-UCNPs@PEG	UCL	540/800 nm	220 nM	HCT116 tumor mice
Si@BODPA	PA	780 nm	53 nM	HCT116 tumor mice
AzHD-LP	PA	680 nm	500 nM	HCT116 tumor mice
NR-NO_2	PA	710 nm	40 nM	HCT116 tumor mice
[^{99m}Tc]Tc-Gluconate	SPECT	gamma-ray	–	–
Fe_3O_4@Cu_2O-lipid-mPEG	PA; MRI	770–1100 nm; MRI signals	110/4.10 μM; 214/2.15 μM (wide/narrow concentration ranges)	HCT116 tumor mice
Amphiphilic probes 1 and 2	PA; NIR-I	718 nm	60 nM	HCT116 tumor mice
SiO_2@Ag	PA; NIR-II	1000–1400 nm	0.92 nM	HCT116 tumor mice

conventional visible (450–750 nm) and NIR-I (750–900 nm) imaging [267]. On this basis, Deng et al. developed an in situ H_2S-activatable NIR–II–emitting nanoprobe for illumination of colorectal cancer in vivo [268]. This probe is based on an Ag-chicken egg white (Ag-CEW) complex, which forms Ag_2S quantum dots via endogenous H_2S-induced in situ chemistry with effective NIR-II emission at 1000–1400 nm, enabling specific visualization of colon cancer guided by NIR-II imaging as well as precise localization.

Experiment results for most single-window response fluorescent probes tend to fluctuate with changes in experimental conditions [269]. In contrast, ratiometric fluorescent probes with two or more emissions construct a self-calibrating system that minimizes the interference caused by experimental conditions [270]. Liu and co-workers prepared nanoprobes (NanoBOD-SCM) by trapping a small molecule probe BODVA-Cl inside the hydrophobic interior of organic nanoparticles made from surface cross-linked micelles (SCM) [271]. This probe provided H_2S-specific ratiometric fluorescence patterns (I_{710}/I_{610}) and was successfully used to image H_2S-rich colon cancer cells in a dual-color imaging modality. To visualize colorectal cancer in vivo, Xu et al. designed an activatable NIR-II nanoprobe (NIR-II@Si) consisting of two dyes, boron-dipyrromethene (ZX-NIR) and aza-BODIPY (aza-BOD) [267]. This targeted probe exhibits high selectivity and proportional fluorescence response to H_2S, allowing deep tissue imaging and identification of colon tumors in animal models. In addition, Wang and co-workers bound human serum albumin (HSA) to Ag^+ on the surface of DCNP to form the DCNP@HSA-Ag^+ nanoprobe, which generate H_2S concentration-dependent ratiometric $F_{1050Em,\ 808Ex}/F_{1550Em,\ 980Ex}$ signals based on H_2S-induced in situ reduction reaction to precisely localize CRC in vivo [255].

In conclusion, H_2S-activated NIR imaging probes have the following features: NIR-II has higher spatial resolution compared to NIR-I imaging probes; ratiometric probes reduce interference caused by experimental conditions; and probes based on H_2S-mediated in situ reactions (Ag-CEW and DCNP@HSA-Ag^+) allow precise localization of CRC in vivo.

4.4.3. Afterglow luminescent probes

Afterglow luminescence probes, also known as persistent luminescence probes, which capture excitation energy in defects and slowly release photons after the cessation of photoexcitation, have recently emerged as promising tools to overcome the limitations of fluorescent probes in biosensing and molecular imaging [[272–274]]. Wu et al. designed and developed H_2S-activatable NIR afterglow luminescence probes ($F1^{2+}$-ANP) by doping EM $F1^{2+}$ (an organic electrochromic material) and NIR775 (a NIR photosensitizer) into MEH-PPV-based organic afterglow nanoparticles [275]. This probe exhibited a fast response rate ($1563 \pm 141\ M^{-1}\ s^{-1}$) and large afterglow turn-on ratio (~122-fold) toward H_2S, which can rapidly quantify H_2S concentrations in blood samples from healthy individuals and patients diagnosed with CRC or hepatocellular carcinoma. The afterglow signal can persist for more than 40 min after the stop of the pre-irradiated laser with a half-life of ~6.6 min, and can be charged by multiple irradiations with the 808 nm laser for long-term imaging (validated with 15 cycles over 3 days).

It is noted that there are no afterglow imaging probes for the in vivo detection of colonic tumors. It might be possible to obtain these probes through surface modifications. For example, Wu et al. prepared $F1^{2+}$-ANP-Gal by introducing β-Gal (targeting the β-Gal receptor on HepG2 cells) to the F1 surface of the abovementioned probe for detecting subcutaneous and in situ tumors in living mice, as well as for depicting tumor lesions in clinical liver resection specimens [275].

4.4.4. Upconversion luminescence (UCL) probes

Upconversion nanophosphors (UCNPs) can absorb NIR light and convert it to high-energy light in the wavelength range from UV to NIR [[276–278]]. Based on the UCL system, Li et al. developed a lysosomal-assisted mitochondrial targeting probe (TPAMC-UCNPs@PEG) consisting of a pH-sensitive PEG shell and a sensing core of merocyanine triphenylamine-merocyanine (TPAMC)-modified UCNPs [270]. TPAMC, which acts as an H_2S -responsive site, is initially hidden under the PEG shell and upon entering the cell via endocytosis the mitochondria-targeting site is exposed due to the acidic environment in the lysosome enabling further attachment to the mitochondria. The strong Förster resonance energy transfer effect between UCNPs and TPAMC allows sensitive detection of H_2S by using ratiometric UCL signals. The group revealed the acid-activated mitochondrial targeting processes via lysosomal delivery of this probe, and demonstrated that this probe can be used to monitor mitochondrial H_2S levels in living cells and a mouse model of CRC by ratiometric UCL imaging.

Compared to conventional two-photon materials, they have higher conversion efficiency due to the presence of stable intermediate states, which also allow continuous-wave excitation at lower power densities [259,260,279,280]. Considering that mitochondria are the main metabolic site of H_2S in living organisms, UCL nanoprobes are expected to be an effective and promising tool for studying the biological and pathological roles of mitochondrial H_2S.

4.4.5. Photoacoustic (PA) imaging probes

Researchers have developed a probe with a new imaging modality, PA imaging, which relies on the translation of excitation light into ultrasonic waves based on the PA effect. This imaging method combines the advantages of high resolution of optical imaging and high penetration depth of ultrasound imaging for deep tissues and whole animals imaging [254,281].

Efforts have been made in recent years to develop PA probes for the detection of H_2S in vivo. Shi et al. developed an H_2S-activated Si@BODPA PA probe by encapsulating a hemicyanine-BODIPY hybrid dye (BODPA) inside a silica nanocomposite [282]. The probe produces a strong PA signal output in the NIR region in the presence of H_2S, converting BODPA within nanoparticles to BOD-HS via aromatic nucleophilic substitution. The probe shows an extremely fast response and can detect transient changes in H_2S, which allows direct PA tracking of endogenous H_2S production in a CRC cell-bearing mouse model. To reduce the interference of experimental results by factors such as instrumentation, probe concentration and external environment, Ma et al. developed an AzHD-LP ratiometric PA nanoprobe consisting of a liposome (LP) encapsulating an H_2S-responsive NIR dye (AzHD) for in vivo detection of H_2S [283]. Upon H_2S-mediated reduction of the probe's azide group to amine, the absorption of AzHD-LP at 600 nm decreased while the absorption at 700 nm increased, producing a proportional PA signal. By combining AzHD-LP with the tumor-targeting peptide c (RGDyK), this probe enabled the detection of intra-tumor H2S production in a mouse model of CRC with ratiometric PA signal.

Multispectral optoacoustic tomography (MSOT) is an emerging technology combining optoacoustic tomography and multiwavelength illumination, which allows each light absorber to be visualized individually in the target tissue and allows the generation of three-dimensional (3D) MSOT images [[284–286]]. Sun et al. proposed a new strategy for the effective assessment of drug side effects by tracking their metabolism-related products through H_2S turn-on optoacoustic probes (NR-NO_2) [287]. This probe was first developed for monitoring liver injury induced by metformin, which induces excessive H_2S expression, through detecting hepatic H_2S. It can successfully identify and precisely localize liver injury with the help of MSOT imaging method, while visualizing the volume of liver injury. It has also achieved desirable results in the evaluation of the effects of AOAA (a CBS inhibitor) used in a mouse model of colon tumors [287].

In general, compared with conventional optical imaging, PA imaging overcomes the challenges of tissue penetration and in vivo spatial resolution, and the reconstruction of 3D photoacoustic images by MSOT has promising applications in visual cancer diagnosis, drug efficacy assessment, and other fields. It is to be noted that the spectral coloring effect and the spectral crosstalk are the two main challenges of multispectral PA imaging, and further research is needed to deal with them.

4.4.6. SPECT imaging

SPECT is an in vivo imaging modality that uses the SPECT camera to measures gamma rays emitted by radiotracers injected into the body [[288–290]]. Technetium-99 m (^{99m}Tc) is one of the most commonly used isotopes for diagnostic purposes, which has a short half-life of 6 h and is cheap and can be easily purchased from hospitals or obtained from ^{99}Mo/^{99m}Tc generators [291,292]. Jeong's group labeled various α-hydroxy acids with ^{99m}Tc and developed ^{99m}Tc-labeled reagents for in vitro and in vivo H_2S quantification [293]. These ^{99m}Tc-labeled reagents, particularly [^{99m}Tc] Tc-gluconate, can form insoluble complexes in the presence of H_2S, thus enabling imaging of endogenously produced H_2S. In addition, after evaluating a mouse CRC cell model under hypoxic conditions and a mouse model of acute hindlimb ischemia-reperfusion, they found a significant increase in the uptake of [^{99m}Tc] Tc-gluconate under hypoxic conditions, implying that this imaging agent could be used to detect endogenous H_2S produced in hypoxic tissues [294]. Considering that SPECT is a commonly utilized imaging technique in clinical practice [295,296], assays based on SPECT imaging principles, such as [^{99m}Tc]Tc-Gluconate, have great promise for translational applications.

4.4.7. Dual-mode imaging probe

The limitations of single-modal probes in practical applications, such as poor tissue penetration and photostability of conventional optical imaging probes, are exacerbated in complex physiological settings. As a result, it is critical to develop novel dual-modality probes that are sensitive to H_2S. Moreover, single-modal imaging rarely provides enough information to make an accurate cancer diagnosis. Hence, it is important to develop novel dual-mode probes that are sensitive to H_2S [297].

Magnetic probes are not affected by fluorescence quenching or problems associated with tissue penetration depth and have been widely used for ultrasensitive detection of bacteria, proteins, viruses, and nucleic acids [[298–301]]. However, the resolution of magnetic probes is insufficient to detect H_2S in vivo. In contrast, PA probes have recently been used to detect H_2S in vivo because of their high resolution and the absence of ambiguous photoluminescence signal caused by tissue-induced optical extinction and sporadic autofluorescence in the visible range when using conventional optical imaging techniques. Thus, combining the two modes into a single system can provide complementary benefits and is expected to greatly improve the accuracy of H_2S detection in vivo. Yan and co-workers constructed core-shell Fe_3O_4@Cu_2O nanoparticles as a magnetic-optoacoustic dual-mode probe for CRC diagnosis based on the in situ response of Cu_2O to endogenous H_2S in colon tumors [297]. The obtained Cu_2O with weak NIR absorption was sulfated in the presence of H_2S to generate Cu_9S_8 with strong NIR absorption and activated PA signal. Meanwhile, the in situ response of Cu_2O formed a layer of cavities between the core and the initial coating, leading to enhanced r1-and T1-weighted MRI.

In addition, other researchers have combined NIR-I/II probes with PA probes to form complementary multimodal probes that provide ultra-spatial resolution and sensitivity for tumor imaging and monitoring. Wang et al. designed and synthesized two small molecule probes using hydrophilic *N*-methylpyridinium to act as an electron-withdrawing group and a hydrophobic monochlorinated BODIPY core to act as an H_2S-responsive unit [149]. The designed amphiphilic probes 1 and 2 are able to spontaneously self-assemble into nanoprobes with well-defined nanostructures and show an unprecedented aggregation-enhanced responsiveness to H_2S. This probe in the aggregated state rather than the molecularly soluble state emits NIR fluorescence as well as PA signals upon specific activation by H_2S, thus allowing in vivo visualization and differentiation of CRC based on differences in H_2S content. In addition, Bi and co-workers developed a NIR-II/PA dual-mode nanoprobe (SiO_2@Ag) based on the H_2S-mediated in situ sulfation reaction for specific monitoring of colorectal tumors [302]. Upon activation by endogenous H_2S, this nanoprobe present turned-on NIR-II fluorescence from 1000 to 1400 nm as well as an excellent PA signal, showing high sensitivity and specificity in the diagnosis of colon cancer in vivo.

The development of H_2S-activated dual-mode imaging probes and even multimode imaging probes is currently an emerging field. It remains a challenge to rationally integrate multimodal imaging modalities into a single probe while responding to endogenous H_2S.

5. Conclusion and outlook

H_2S has been identified as an important regulator of CRC biology among numerous compounds released by the colon and colonic microbiota. CBS, CSE, and 3-MST are the three major H_2S-generating enzymes that catalyze the production of endogenous H_2S in colon cells to varying degrees. In addition, H_2S produced by gut microbes using inorganic or organic substrates can easily penetrate the cell membrane and enter colon cells. These intracellular H_2S molecules are oxidatively metabolized in mitochondria to generate energy or used in other biological reactions. The occurrence and progression of CRC are influenced by both endogenous synthesis of colonic epithelial cells and exogenous delivery

of H_2S from the luminal side. The effect of H_2S on CRC showed duality, promoting energy metabolism, proliferation, and migration of tumor cells within an appropriate concentration range while acting as a tumor suppressor above the concentration threshold. As a result, determining H_2S release kinetics, concentration-dependent effect curves, and optimal therapeutic doses is critical when using it in therapy. Furthermore, considering the close relationship between intestinal H_2S, microbes, and diet, H_2S is regarded as an important factor linking dietary patterns, gut microbiota, and CRC. Modifying dietary patterns to reduce *S*-containing substrates for H_2S production and/or limiting the number of H_2S-producing gut microbes may be a promising approach to CRC prevention.

In terms of H_2S synthase inhibitors, the current focus needs to be on finding effective, selective, and non-toxic inhibitors that can be translated. Traditional small molecule inhibitors such as AOAA and EGCG were once of great interest, however, they were not finally used in clinical treatment because of their respective shortcomings. Unlike AOAA, the inhibition of CBS by EGCG does not target PLP, but exploits certain structural or conformational features unique to CBS, which makes it a CBS-specific inhibitor. According to this idea, the development of new potent specific inhibitors targeting specific sites on the synthase is a feasible strategy. In addition, the search for suitable inhibitors from compound libraries by high-throughput screening techniques also deserves researchers' attention. In terms of scavengers, the H_2S scavenger options available in CRC therapy are still restricted. Moreover, H_2S scavengers require higher doses to deal with the continuous generation of H_2S and its byproducts [147], which is still under investigation. It is worth mentioning that H_2S enzymes are prevalent in the body and perform important biological functions, and that endogenous H_2S is an important signaling molecule in physiological or pathological states. Inhibitors or scavengers are likely to have undesired consequences during practical use. For example, CBS and CSE are key enzymes in the cellular synthesis of cysteine and H_2S plays an important role in the maintenance of vascular tone and angiogenesis. Inhibition of these effects inevitably causes damage to the body. Therefore, a comprehensive assessment must be undertaken during drug development and translation to target and eliminate or attenuate possible side effects.

Although conventional small molecule H_2S donors offer numerous advantages over sulfide salts in H_2S donor therapy, they are intrinsically unstable molecules. To achieve improved therapeutic effects, stimulus-responsive delivery as well as gradual and prolonged release must be established. One of the tactics that has received a lot of attention and is effective in managing the transport and release of H_2S gas is the use of macromolecular/supramolecular polymer carriers in the development of delivery vehicles. Delivery systems that combine carriers and detection imaging technologies that respond to the TME as well as to external stimuli are one way to improve the therapeutic targeting of H_2S donors and to achieve controlled and on-demand gas release.

The development of tumor-targeted intelligent diagnostic and therapeutic drugs based on TME characteristics is currently a popular cancer treatment strategy [218,303–306]. The high level of H_2S in CRC cells makes it a promising endogenous microenvironment molecule for the design and synthesis of H_2S-responsive diagnostic therapeutics targeting CRC. Currently, H_2S-activated therapeutics developed in combination with nanotechnology are being investigated in CRC mainly in the fields of chemotherapy, PDT, PTT, and CDT. Combining these therapeutic strategies may lead to better therapeutic outcomes, such as the combination of CDT and PTT (thermal effects can accelerate •OH production), the combination of chemotherapy and PTT (controlled drug delivery through thermal effects), and the combination of PDT and PTT (many photosensitizers have photothermal effects themselves). The combination of H_2S-activated therapeutic agents with the combination of other emerging therapeutic modalities, such as immunotherapy, sonodynamic therapy, and electrotherapy, remains to be further explored.

It is to be noted that these nanotherapeutics, including nanocarriers introduced for controlled gas delivery and H_2S-activated targeted nanodrugs, can be used in a tumor-bearing mouse model to achieve good tumor suppression by direct injection of the therapeutic agent at the tumor site, but the actual application in the organism often does not yield as effective therapeutic results as in vitro experiments. A complex set of biological barriers limit the bioavailability of drugs at specific sites and prevent good therapeutic outcomes. These barriers include mononuclear phagocyte systems, nonspecific distribution, blood rheology/vascular flow restrictions, pressure gradients, cellular internalization, escape from endosomal and lysosomal compartments, and drug efflux pumps [216,307]. In addition, nanomedicines, prior to clinical translation, must undergo long-term pharmacodynamic and toxicological studies to fully assess the effects on the human body, due to the frequently complex and inconsistent biological profiles of nanomaterials. In the future, these barriers should be addressed in a targeted manner while rationally incorporating innovative designs in the development of new generations of CRC tumor-targeting nanotherapeutics to achieve optimal therapeutic outcomes.

In the last twenty years, H_2S detection methods have evolved from initial methods that require handling and/or destruction of tissues or cells, including colorimetric method, electrochemical method, gas chromatography and sulfide precipitation method, to visible light imaging fluorescent probes that can be used on living cells, to optical imaging modalities, e.g., NIR-I/II imaging, UCL imaging and afterglow imaging, and other non-optical imaging modalities, e.g., PA imaging, SPECT imaging, MRI imaging. In response to H_2S in the tumor environment, NBD amines have a higher selectivity for H_2S compared to azides. The probes developed based on the H_2S-mediated in situ response are able to illuminate tumor sites in situ when applied in vivo. Conventional visible light imaging modalities have many limitations such as low tissue penetration, poor spatial resolution of deep tissue, interference from autofluorescence, and poor photostability when applied in vivo to animals. In contrast, novel optical imaging modalities such as NIR-I/II imaging has higher spatial resolution, UCL imaging has high tissue penetration and strong photostability, afterglow imaging does not require real-time external light excitation, is not subject to autofluorescence interference, and has the potential for long-term imaging. Compared to optical imaging modalities, PA imaging combines the features of optical imaging and ultrasound imaging and allows the reconstruction of 3D photoacoustic images by MSOT. Remarkably, PA imaging has been clinically translated in the field of gynecology with high-resolution imaging (down to capillary size resolution) of the cervical microvascular system by transvaginal fast-scan photoacoustic endoscopy [308]. Likewise, there have been several studies on handheld photoacoustic imaging devices and platforms that have greatly increased the potential of this modality for commercial as well as clinical applications [309]. Similar to the vagina, researchers can develop high-performance PA imaging probes through CRC tumor microenvironmental factors, e.g., H_2S, or other specific targets to detect PA signals transintestinally or directly in vitro for accurate diagnosis of CRC. Other non-optical imaging modalities, such as SPECT imaging and MRI imaging, are already widely used imaging techniques in clinical practice, and the development of corresponding CRC diagnostic probes based on this technology may facilitate clinical translation.

Overall, H_2S-related researches are fruitful in the areas of mechanistic studies of colon cancer development, diagnosis, and treatment. The current focus is on how to translate the existing researches into clinical applications. In addition, researchers need to further explore new H_2S-mediated therapeutic modalities, such as H_2S-mediated anticancer immunotherapy or H_2S-mediated antibacterial therapy in CRC, whose mechanisms or therapeutic effects are still not fully known.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by the National Key Research and Development Program of China [2021YFC2500403]; the Shanghai International Science and Technology Collaboration Program [214907137000]; and the Program of Shanghai Academic Research Leader [20XD1400900].

References

[1] G. Cirino, C. Szabo, A. Papapetropoulos, Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs, Physiol. Rev. 103 (1) (2023) 31–276.
[2] R. Gupta, M. Sahu, R. Tripathi, R.K. Ambasta, P. Kumar, Protein S-sulfhydration: unraveling the prospective of hydrogen sulfide in the brain, vasculature and neurological manifestations, Ageing Res. Rev. 76 (2022), 101579.
[3] G.K. Kolluru, R.E. Shackelford, X. Shen, P. Dominic, C.G. Kevil, Sulfide regulation of cardiovascular function in health and disease, Nat. Rev. Cardiol. (2022) 1–17.
[4] N. Dilek, A. Papapetropoulos, T. Toliver-Kinsky, C. Szabo, Hydrogen sulfide: an endogenous regulator of the immune system, Pharmacol. Res. 161 (2020), 105119.
[5] U. Głowacka, T. Brzozowski, M. Magierowski, Synergisms, discrepancies and interactions between hydrogen sulfide and carbon monoxide in the gastrointestinal and digestive system physiology, pathophysiology and pharmacology, Biomolecules 10 (3) (2020) 445.
[6] S. Khattak, M.A. Rauf, N.H. Khan, Q.Q. Zhang, H.J. Chen, P. Muhammad, et al., Hydrogen sulfide biology and its role in cancer, Molecules 27 (11) (2022) 3389.
[7] E. Dekker, D.K. Rex, Advances in CRC prevention: screening and surveillance, Gastroenterology 154 (7) (2018) 1970–1984.
[8] J. Chen, F. Xue, W. Du, H. Yu, Z. Yang, Q. Du, et al., An endogenous H_2S-activated nanoplatform for triple synergistic therapy of colorectal cancer, Nano Lett. 22 (15) (2022) 6156–6165.
[9] L. Yang, Y. Zhu, L. Liang, C. Wang, X. Ning, X. Feng, Self-assembly of intelligent nanoplatform for endogenous H_2S-triggered multimodal cascade therapy of colon cancer, Nano Lett. 22 (10) (2022) 4207–4214.
[10] Y. Zhang, J. Fang, S. Ye, Y. Zhao, A. Wang, Q. Mao, et al., A hydrogen sulphide-responsive and depleting nanoplatform for cancer photodynamic therapy, Nat. Commun. 13 (1) (2022) 1685.
[11] P.G. Wolf, E.S. Cowley, A. Breister, S. Matatov, L. Lucio, P. Polak, et al., Diversity and distribution of sulfur metabolic genes in the human gut microbiome and their association with colorectal cancer, Microbiome 10 (1) (2022) 64.
[12] L.H. Nguyen, Y. Cao, J. Hur, R.S. Mehta, D.R. Sikavi, Y. Wang, et al., The sulfur microbial diet is associated with increased risk of early-onset colorectal cancer precursors, Gastroenterology 161 (5) (2021) 1423–1432, e4.
[13] F. Blachier, M. Andriamihaja, P. Larraufie, E. Ahn, A. Lan, E. Kim, Production of hydrogen sulfide by the intestinal microbiota and epithelial cells and consequences for the colonic and rectal mucosa, Am. J. Physiol. Gastrointest. Liver Physiol. 320 (2) (2021). G125-g35.
[14] R. Wang, Physiological implications of hydrogen sulfide: a whiff exploration that blossomed, Physiol. Rev. 92 (2) (2012) 791–896.
[15] I. Kushkevych, D. Dordević, M. Vítězová, Possible synergy effect of hydrogen sulfide and acetate produced by sulfate-reducing bacteria on inflammatory bowel disease development, J. Adv. Res. 27 (2021) 71–78.
[16] I. Kushkevych, J. Cejnar, J. Treml, D. Dordević, P. Kollar, M. Vítězová, Recent advances in metabolic pathways of sulfate reduction in intestinal bacteria, Cells 9 (3) (2020) 698.
[17] F. Carbonero, A.C. Benefiel, A.H. Alizadeh-Ghamsari, H.R. Gaskins, Microbial pathways in colonic sulfur metabolism and links with health and disease, Front. Physiol. 3 (2012) 448.
[18] A.L. Müller, K.U. Kjeldsen, T. Rattei, M. Pester, A. Loy, Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases, ISME J. 9 (5) (2015) 1152–1165.
[19] K. Anantharaman, B. Hausmann, S.P. Jungbluth, R.S. Kantor, A. Lavy, L. A. Warren, et al., Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle, ISME J. 12 (7) (2018) 1715–1728.
[20] B. Ahlman, C.E. Leijonmarck, C. Lind, E. Vinnars, J. Wernerman, Free amino acids in biopsy specimens from the human colonic mucosa, J. Surg. Res. 55 (6) (1993) 647–653.
[21] J.M. Ridlon, P.G. Wolf, H.R. Gaskins, Taurocholic acid metabolism by gut microbes and colon cancer, Gut Microb. 7 (3) (2016) 201–215.
[22] S. Devkota, Y. Wang, M.W. Musch, V. Leone, H. Fehlner-Peach, A. Nadimpalli, et al., Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in $Il10^{-/-}$ mice, Nature 487 (7405) (2012) 104–108.
[23] C. Yazici, P.G. Wolf, H. Kim, T.L. Cross, K. Vermillion, T. Carroll, et al., Race-dependent association of sulfidogenic bacteria with colorectal cancer, Gut 66 (11) (2017) 1983–1994.
[24] Y. Wei, Y. Zhang, Glycyl radical enzymes and sulfonate metabolism in the microbiome, Annu. Rev. Biochem. 90 (2021) 817–846.
[25] S.C. Peck, K. Denger, A. Burrichter, S.M. Irwin, E.P. Balskus, D. Schleheck, A glycyl radical enzyme enables hydrogen sulfide production by the human intestinal bacterium Bilophila wadsworthia, Proc. Natl. Acad. Sci. U. S. A. 116 (8) (2019) 3171–3176.
[26] M. Li, Y. Wei, J. Yin, L. Lin, Y. Zhou, G. Hua, et al., Biochemical and structural investigation of taurine:2-oxoglutarate aminotransferase from Bifidobacterium kashiwanohense, Biochem. J. 476 (11) (2019) 1605–1619.
[27] A.J.D. Snow, L. Burchill, M. Sharma, G.J. Davies, S.J. Williams, Sulfoglycolysis: catabolic pathways for metabolism of sulfoquinovose, Chem. Soc. Rev. 50 (24) (2021) 13628–13645.
[28] B.T. Hanson, K. Dimitri Kits, J. Löffler, A.G. Burrichter, A. Fiedler, K. Denger, et al., Sulfoquinovose is a select nutrient of prominent bacteria and a source of hydrogen sulfide in the human gut, ISME J. 15 (9) (2021) 2779–2791.
[29] K. Denger, M. Weiss, A.K. Felux, A. Schneider, C. Mayer, D. Spiteller, et al., Sulphoglycolysis in Escherichia coli K-12 closes a gap in the biogeochemical sulphur cycle, Nature 507 (7490) (2014) 114–117.
[30] M. Sharma, P. Abayakoon, R. Epa, Y. Jin, J.P. Lingford, T. Shimada, et al., Molecular basis of sulfosugar selectivity in sulfoglycolysis, ACS Cent. Sci. 7 (3) (2021) 476–487.
[31] M. Sharma, P. Abayakoon, J. Lingford, R. Epa, A. John, Y. Jin, et al., Dynamic structural changes accompany the production of dihydroxypropanesulfonate by sulfolactaldehyde reductase, ACS Catal. 10 (4) (2020) 2826–2836.
[32] A. Kaur, P.L. van der Peet, J.W. Mui, M. Herisse, S. Pidot, S.J. Williams, Genome sequences of Arthrobacter spp. that use a modified sulfoglycolytic Embden-Meyerhof-Parnas pathway, Arch. Microbiol. 204 (3) (2022) 193.
[33] J. Liu, Y. Wei, K. Ma, J. An, X. Liu, Y. Liu, et al., Mechanistically diverse pathways for sulfoquinovose degradation in bacteria, ACS Catal. 11 (24) (2021) 14740–14750.
[34] A.K. Felux, D. Spiteller, J. Klebensberger, D. Schleheck, Entner-Doudoroff pathway for sulfoquinovose degradation in Pseudomonas putida SQ1, Proc. Natl. Acad. Sci. U. S. A. 112 (31) (2015) E4298–E4305.
[35] J. Li, R. Epa, N.E. Scott, D. Skoneczny, M. Sharma, A.J.D. Snow, et al., A sulfoglycolytic entner-doudoroff pathway in rhizobium leguminosarum bv. trifolii SRDI565, Appl. Environ. Microbiol. 86 (15) (2020) e00750-20.
[36] B. Frommeyer, A.W. Fiedler, S.R. Oehler, B.T. Hanson, A. Loy, P. Franchini, et al., Environmental and intestinal phylum firmicutes bacteria metabolize the plant sugar sulfoquinovose via a 6-Deoxy-6-sulfofructose transaldolase pathway, iScience 23 (9) (2020), 101510.
[37] Y. Liu, Y. Wei, Y. Zhou, E.L. Ang, H. Zhao, Y. Zhang, A transaldolase-dependent sulfoglycolysis pathway in Bacillus megaterium DSM 1804, Biochem. Biophys. Res. Commun. 533 (4) (2020) 1109–1114.
[38] M. Sharma, J.P. Lingford, M. Petricevic, A.J.D. Snow, Y. Zhang, M.A. Järvå, et al., Oxidative desulfurization pathway for complete catabolism of sulfoquinovose by bacteria, Proc. Natl. Acad. Sci. U. S. A. 119 (4) (2022), e2116022119.
[39] A. Burrichter, K. Denger, P. Franchini, T. Huhn, N. Müller, D. Spiteller, et al., Anaerobic degradation of the plant sugar sulfoquinovose concomitant with H_2S production: Escherichia coli K-12 and Desulfovibrio sp. strain DF1 as Co-culture model, Front. Microbiol. 9 (2018) 2792.
[40] J. Liu, Y. Wei, L. Lin, L. Teng, J. Yin, Q. Lu, et al., Two radical-dependent mechanisms for anaerobic degradation of the globally abundant organosulfur compound dihydroxypropanesulfonate, Proc. Natl. Acad. Sci. U. S. A. 117 (27) (2020) 15599–15608.
[41] M.A. Metaxas, E.A. Delwiche, The L-cysteine desulfhydrase of Escherichia coli, J. Bacteriol. 70 (6) (1955) 735–737.
[42] A. Saz, L.W. Brownell, D-Cysteine desulfhydrase in Escherichia coli, Arch. Biochem. Biophys. 52 (1) (1954) 291–293.
[43] K. Shatalin, E. Shatalina, A. Mironov, E. Nudler, H_2S: a universal defense against antibiotics in bacteria, Science 334 (6058) (2011) 986–990.
[44] H.J. Sun, Z.Y. Wu, X.W. Nie, X.Y. Wang, J.S. Bian, Implications of hydrogen sulfide in liver pathophysiology: mechanistic insights and therapeutic potential, J. Adv. Res. 27 (2021) 127–135.
[45] C. Szabó, Hydrogen sulphide and its therapeutic potential, Nat. Rev. Drug Discov. 6 (11) (2007) 917–935.
[46] K. Zuhra, F. Augsburger, T. Majtan, C. Szabo, Cystathionine-β-Synthase: molecular regulation and pharmacological inhibition, Biomolecules 10 (5) (2020) 697.
[47] K. Ascenção, C. Szabo, Emerging roles of cystathionine β-synthase in various forms of cancer, Redox Biol. 53 (2022), 102331.
[48] R. Banerjee, Catalytic promiscuity and heme-dependent redox regulation of H_2S synthesis, Curr. Opin. Chem. Biol. 37 (2017) 115–121.
[49] J. Zhu, M. Berisa, S. Schwörer, W. Qin, J.R. Cross, C.B. Thompson, Transsulfuration activity can support cell growth upon extracellular cysteine limitation, Cell Metabol. 30 (5) (2019) 865–876, e5.
[50] H.F. Zhang, R.I. Klein Geltink, S.J. Parker, P.H. Sorensen, Transsulfuration, minor player or crucial for cysteine homeostasis in cancer, Trends Cell Biol. 32 (9) (2022) 800–814.
[51] J.I. Sbodio, S.H. Snyder, B.D. Paul, Regulators of the transsulfuration pathway, Br. J. Pharmacol. 176 (4) (2019) 583–593.
[52] F. Augsburger, C. Szabo, Potential role of the 3-mercaptopyruvate sulfurtransferase (3-MST)-hydrogen sulfide (H_2S) pathway in cancer cells, Pharmacol. Res. 154 (2020), 104083.
[53] N. Shibuya, S. Koike, M. Tanaka, M. Ishigami-Yuasa, Y. Kimura, Y. Ogasawara, et al., A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells, Nat. Commun. 4 (2013) 1366.

[54] K.R. Olson, E.R. Deleon, Y. Gao, K. Hurley, V. Sadauskas, C. Batz, et al., Thiosulfate: a readily accessible source of hydrogen sulfide in oxygen sensing, Am. J. Physiol. Regul. Integr. Comp. Physiol. 305 (6) (2013) R592–R603.

[55] J.C. Mathai, A. Missner, P. Kügler, S.M. Saparov, M.L. Zeidel, J.K. Lee, et al., No facilitator required for membrane transport of hydrogen sulfide, Proc. Natl. Acad. Sci. U. S. A. 106 (39) (2009) 16633–16638.

[56] S.D. Hemelrijk, M.C. Dirkes, M.H.N. van Velzen, R. Bezemer, T.M. van Gulik, M. Heger, Exogenous hydrogen sulfide gas does not induce hypothermia in normoxic mice, Sci. Rep. 8 (1) (2018) 3855.

[57] K.R. Olson, K.D. Straub, The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling, Physiology 31 (1) (2016) 60–72.

[58] A.P. Landry, D.P. Ballou, R. Banerjee, Hydrogen sulfide oxidation by sulfide quinone oxidoreductase, Chembiochem 22 (6) (2021) 949–960.

[59] M. Libiad, P.K. Yadav, V. Vitvitsky, M. Martinov, R. Banerjee, Organization of the human mitochondrial hydrogen sulfide oxidation pathway, J. Biol. Chem. 289 (45) (2014) 30901–30910.

[60] V. Tiranti, C. Viscomi, T. Hildebrandt, I. Di Meo, R. Mineri, C. Tiveron, et al., Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy, Nat Med 15 (2) (2009) 200–205.

[61] O. Kabil, R. Banerjee, Characterization of patient mutations in human persulfide dioxygenase (ETHE1) involved in H_2S catabolism, J. Biol. Chem. 287 (53) (2012) 44561–44567.

[62] B.K. Maiti, Cross-talk between (Hydrogen)Sulfite and metalloproteins: impact on human health, Chemistry 28 (23) (2022), e202104342.

[63] M. Libiad, A. Sriraman, R. Banerjee, Polymorphic variants of human rhodanese exhibit differences in thermal stability and sulfur transfer kinetics, J. Biol. Chem. 290 (39) (2015) 23579–23588.

[64] M.R. Jackson, S.L. Melideo, M.S. Jorns, Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite, Biochemistry 51 (34) (2012) 6804–6815.

[65] S.L. Melideo, M.R. Jackson, M.S. Jorns, Biosynthesis of a central intermediate in hydrogen sulfide metabolism by a novel human sulfurtransferase and its yeast ortholog, Biochemistry 53 (28) (2014) 4739–4753.

[66] A.P. Landry, D.P. Ballou, R. Banerjee, H_2S oxidation by nanodisc-embedded human sulfide quinone oxidoreductase, J. Biol. Chem. 292 (28) (2017) 11641–11649.

[67] M. Libiad, V. Vitvitsky, T. Bostelaar, D.W. Bak, H.J. Lee, N. Sakamoto, et al., Hydrogen sulfide perturbs mitochondrial bioenergetics and triggers metabolic reprogramming in colon cells, J. Biol. Chem. 294 (32) (2019) 12077–12090.

[68] R.H. Wang, Y.H. Chu, K.T. Lin, The hidden role of hydrogen sulfide metabolism in cancer, Int. J. Mol. Sci. 22 (12) (2021).

[69] M. Iciek, A. Bilska-Wilkosz, M. Kozdrowicki, M. Górny, Reactive sulfur species and their significance in health and disease, Biosci. Rep. 42 (9) (2022), BSR20221006.

[70] K. Zuhra, C.S. Tomé, E. Forte, J.B. Vicente, A. Giuffrè, The multifaceted roles of sulfane sulfur species in cancer-associated processes, Biochim. Biophys. Acta Bioenerg. 1862 (2) (2021), 148338.

[71] M. Goubern, M. Andriamihaja, T. Nübel, F. Blachier, F. Bouillaud, Sulfide, the first inorganic substrate for human cells, Faseb. J. 21 (8) (2007) 1699–1706.

[72] F. Blachier, A.M. Davila, S. Mimoun, P.H. Benetti, C. Atanasiu, M. Andriamihaja, et al., Luminal sulfide and large intestine mucosa: friend or foe? Amino Acids 39 (2) (2010) 335–347.

[73] A. Abou-Hamdan, H. Guedouari-Bounihi, V. Lenoir, M. Andriamihaja, F. Blachier, F. Bouillaud, Oxidation of H_2S in mammalian cells and mitochondria, Methods Enzymol. 554 (2015) 201–228.

[74] M. Beaumont, M. Andriamihaja, A. Lan, N. Khodorova, M. Audebert, J.M. Blouin, et al., Detrimental effects for colonocytes of an increased exposure to luminal hydrogen sulfide: the adaptive response, Free Radic. Biol. Med. 93 (2016) 155–164.

[75] S. Mimoun, M. Andriamihaja, C. Chaumontet, C. Atanasiu, R. Benamouzig, J. M. Blouin, et al., Detoxification of H_2S by differentiated colonic epithelial cells: implication of the sulfide oxidizing unit and of the cell respiratory capacity, Antioxidants Redox Signal. 17 (1) (2012) 1–10.

[76] X. Shen, M. Carlström, S. Borniquel, C. Jädert, C.G. Kevil, J.O. Lundberg, Microbial regulation of host hydrogen sulfide bioavailability and metabolism, Free Radic. Biol. Med. 60 (2013) 195–200.

[77] R.E. Shackelford, I.Z. Mohammad, A.T. Meram, D. Kim, F. Alotaibi, S. Patel, et al., Molecular functions of hydrogen sulfide in cancer, Pathophysiology 28 (3) (2021) 437–456.

[78] K. Zhao, Y. Ju, S. Li, Z. Altaany, R. Wang, G. Yang, S-sulfhydration of MEK1 leads to PARP-1 activation and DNA damage repair, EMBO Rep. 15 (7) (2014) 792–800.

[79] Z. Lu, S. Xu, ERK1/2 MAP kinases in cell survival and apoptosis, IUBMB Life 58 (11) (2006) 621–631.

[80] Y.H. Wang, J.T. Huang, W.L. Chen, R.H. Wang, M.C. Kao, Y.R. Pan, et al., Dysregulation of cystathionine γ-lyase promotes prostate cancer progression and metastasis, EMBO Rep. 20 (10) (2019), e45986.

[81] B. Hoesel, J.A. Schmid, The complexity of NF-κB signaling in inflammation and cancer, Mol. Cancer 12 (1) (2013) 86.

[82] C. Szabo, C. Coletta, C. Chao, K. Módis, B. Szczesny, A. Papapetropoulos, et al., Tumor-derived hydrogen sulfide, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer, Proc. Natl. Acad. Sci. U. S. A. 110 (30) (2013) 12474–12479.

[83] C. Szabo, Hydrogen sulfide, an endogenous stimulator of mitochondrial function in cancer cells, Cells 10 (2) (2021) 220.

[84] B.D. Paul, S.H. Snyder, K. Kashfi, Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics, Redox Biol. 38 (2021), 101772.

[85] K. Módis, Y. Ju, A. Ahmad, A.A. Untereiner, Z. Altaany, L. Wu, et al., S-Sulfhydration of ATP synthase by hydrogen sulfide stimulates mitochondrial bioenergetics, Pharmacol. Res. 113 (2016) 116–124.

[86] J. Wen, X. Min, M. Shen, Q. Hua, Y. Han, L. Zhao, et al., ACLY facilitates colon cancer cell metastasis by CTNNB1, J. Exp. Clin. Cancer Res. 38 (1) (2019) 401.

[87] K. Ascenção, N. Dilek, F. Augsburger, T. Panagaki, K. Zuhra, C. Szabo, Pharmacological induction of mesenchymal-epithelial transition via inhibition of H_2S biosynthesis and consequent suppression of ACLY activity in colon cancer cells, Pharmacol. Res. 165 (2021), 105393.

[88] F.F. Guo, T.C. Yu, J. Hong, J.Y. Fang, Emerging roles of hydrogen sulfide in inflammatory and neoplastic colonic diseases, Front. Physiol. 7 (2016) 156.

[89] Y. Fang, C. Yan, Q. Zhao, J. Xu, Z. Liu, J. Gao, et al., The roles of microbial products in the development of colorectal cancer: a review, Bioengineered 12 (1) (2021) 720–735.

[90] N. Dalal, R. Jalandra, N. Bayal, A.K. Yadav, Harshulika, M. Sharma, et al., Gut microbiota-derived metabolites in CRC progression and causation, J. Cancer Res. Clin. Oncol. 147 (11) (2021) 3141–3155.

[91] K. Yamagishi, K. Onuma, Y. Chiba, S. Yagi, S. Aoki, T. Sato, et al., Generation of gaseous sulfur-containing compounds in tumour tissue and suppression of gas diffusion as an antitumour treatment, Gut 61 (4) (2012) 554–561.

[92] S. Ramasamy, S. Singh, P. Taniere, M.J.S. Langman, M.C. Eggo, Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2) (2006) G288–G296.

[93] K. Kanazawa, F. Konishi, T. Mitsuoka, A. Terada, K. Itoh, S. Narushima, et al., Factors influencing the development of sigmoid colon cancer: bacteriologic and biochemical studies, Cancer 77 (8) (1996) 1701–1706.

[94] D.R. Linden, M.D. Levitt, G. Farrugia, et al., Endogenous production of H_2S in the gastrointestinal tract: still in search of a physiologic function, Antioxidants Redox Signal. 12 (9) (2010) 1135–1146.

[95] Q. Feng, S. Liang, H. Jia, A. Stadlmayr, L. Tang, Z. Lan, et al., Gut microbiome development along the colorectal adenoma-carcinoma sequence, Nat. Commun. 6 (2015) 6528.

[96] V.L. Hale, J. Chen, S. Johnson, S.C. Harrington, T.C. Yab, T.C. Smyrk, et al., Shifts in the fecal microbiota associated with adenomatous polyps, Cancer Epidemiol. Biomarkers Prev. 26 (1) (2017) 85–94.

[97] L.H. Nguyen, W. Ma, D.D. Wang, Y. Cao, H. Mallick, T.K. Gerbaba, et al., Association between sulfur-metabolizing bacterial communities in stool and risk of distal colorectal cancer in men, Gastroenterology 158 (5) (2020) 1313–1325.

[98] V.L. Hale, P. Jeraldo, M. Mundy, J. Yao, G. Keeney, N. Scott, et al., Synthesis of multi-omic data and community metabolic models reveals insights into the role of hydrogen sulfide in colon cancer, Methods 149 (2018) 59–68.

[99] S. Yachida, S. Mizutani, H. Shiroma, S. Shiba, T. Nakajima, T. Sakamoto, et al., Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer, Nat Med 25 (6) (2019) 968–976.

[100] V.A. Romanov, I.A. Karasev, N.S. Klimenko, S.I. Koshechkin, A.V. Tyakht, O. A. Malikhova, Luminal and tumor-associated gut microbiome features linked to precancerous lesions malignancy risk: a compositional approach, Cancers 14 (21) (2022) 5207.

[101] N. Wang, J.Y. Fang, Fusobacterium nucleatum, a key pathogenic factor and microbial biomarker for colorectal cancer, Trends Microbiol. 14 (23) (2022) 5813.

[102] S. Bullman, C.S. Pedamallu, E. Sicinska, T.E. Clancy, X. Zhang, D. Cai, et al., Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer, Science 358 (6369) (2017) 1443–1448.

[103] Y. Wang, L.H. Nguyen, R.S. Mehta, M. Song, C. Huttenhower, A.T. Chan, Association between the sulfur microbial diet and risk of colorectal cancer, JAMA Netw. Open 4 (11) (2021), e2134308.

[104] A.A. Untereiner, G. Oláh, K. Módis, M.R. Hellmich, C. Szabo, H_2S-induced S-sulfhydration of lactate dehydrogenase a (LDHA) stimulates cellular bioenergetics in HCT116 colon cancer cells, Biochem. Pharmacol. 136 (2017) 86–98.

[105] W.J. Cai, M.J. Wang, L.H. Ju, C. Wang, Y.C. Zhu, Hydrogen sulfide induces human colon cancer cell proliferation: role of Akt, ERK and p21, Cell Biol. Int. 34 (6) (2010) 565–572.

[106] P. Rose, P.K. Moore, S.H. Ming, O.C. Nam, J.S. Armstrong, M. Whiteman, Hydrogen sulfide protects colon cancer cells from chemopreventative agent beta-phenylethyl isothiocyanate induced apoptosis, World J. Gastroenterol. 11 (26) (2005) 3990–3997.

[107] M.R. Hellmich, C. Coletta, C. Chao, et al., The therapeutic potential of cystathionine β-synthetase/hydrogen sulfide inhibition in cancer, Antioxidants Redox Signal. 22 (5) (2015) 424–448.

[108] G. Oláh, K. Módis, G. Törö, M.R. Hellmich, B. Szczesny, C. Szabo, Role of endogenous and exogenous nitric oxide, carbon monoxide and hydrogen sulfide in HCT116 colon cancer cell proliferation, Biochem. Pharmacol. 149 (2018) 186–204.

[109] S. Chen, T. Yue, Z. Huang, J. Zhu, D. Bu, X. Wang, et al., Inhibition of hydrogen sulfide synthesis reverses acquired resistance to 5-FU through miR-215-5p-EREG/TYMS axis in colon cancer cells, Cancer Lett. 466 (2019) 49–60.

[110] M.R. Hellmich, C. Chao, K. Módis, Y. Ding, J.R. Zatarain, K. Thanki, et al., Efficacy of novel aminooxyacetic acid prodrugs in colon cancer models: towards clinical translation of the cystathionine β-synthase inhibition concept, Biomolecules 11 (8) (2021).

[111] S. Guo, J. Li, Z. Huang, T. Yue, J. Zhu, X. Wang, et al., The CBS-H_2S axis promotes liver metastasis of colon cancer by upregulating VEGF through AP-1 activation, Br. J. Cancer 126 (7) (2022) 1055–1066.

[112] Y. Zhang, S. Chen, J. Zhu, S. Guo, T. Yue, H. Xu, et al., Overexpression of CBS/H_2S inhibits proliferation and metastasis of colon cancer cells through downregulation of CD44, Cancer Cell Int. 22 (1) (2022) 85.

[113] K. Módis, C. Coletta, A. Asimakopoulou, B. Szczesny, C. Chao, A. Papapetropoulos, et al., Effect of S-adenosyl-L-methionine (SAM), an allosteric activator of cystathionine-β-synthase (CBS) on colorectal cancer cell proliferation and bioenergetics in vitro, Nitric Oxide 41 (2014) 146–156.

[114] C.M. Phillips, J.R. Zatarain, M.E. Nicholls, C. Porter, S.G. Widen, K. Thanki, et al., Upregulation of cystathionine-β-synthase in colonic epithelia reprograms metabolism and promotes carcinogenesis, Cancer Res. 77 (21) (2017) 5741–5754.

[115] K. Fan, N. Li, J. Qi, P. Yin, C. Zhao, L. Wang, et al., Wnt/β-catenin signaling induces the transcription of cystathionine-γ-lyase, a stimulator of tumor in colon cancer, Cell. Signal. 26 (12) (2014) 2801–2808.

[116] K.K. Thanki, P. Johnson, E.J. Higgins, M. Maskey, C. Phillips, S. Dash, et al., Deletion of cystathionine-γ-lyase in bone marrow-derived cells promotes colitis-associated carcinogenesis, Redox Biol. 55 (2022), 102417.

[117] F. Augsburger, E.B. Randi, M. Jendly, K. Ascencao, N. Dilek, C. Szabo, Role of 3-mercaptopyruvate sulfurtransferase in the regulation of proliferation, migration, and bioenergetics in murine colon cancer cells, Biomolecules 10 (3) (2020) 447.

[118] K. Hanaoka, K. Sasakura, Y. Suwanai, S. Toma-Fukai, K. Shimamoto, Y. Takano, et al., Discovery and mechanistic characterization of selective inhibitors of H_2S-producing enzyme: 3-mercaptopyruvate sulfurtransferase (3MST) targeting active-site cysteine persulfide, Sci. Rep. 7 (2017), 40227.

[119] E. Ozluk, D. Coppola, I.Z. Mohammad, T. Islam, G. Ghali, C.G. Kevil, et al., Ethylmalonic encephalopathy 1 protein is increased in colorectal adenocarcinoma, Anticancer Res. 41 (10) (2021) 4719–4723.

[120] M. Piran, N. Sepahi, A. Moattari, A. Rahimi, A. Ghanbariasad, Systems biomedicine of primary and metastatic colorectal cancer reveals potential therapeutic targets, Front. Oncol. 11 (2021), 597536.

[121] K. Ascenção, N. Dilek, K. Zuhra, K. Módis, T. Sato, C. Szabo, Sequential accumulation of 'driver' pathway mutations induces the upregulation of hydrogen-sulfide-producing enzymes in human colonic epithelial cell organoids, Antioxidants 11 (9) (2022) 1823.

[122] M.R. Jackson, K.D. Cox, S.D.P. Baugh, L. Wakeen, A.A. Rashad, P.Y.S. Lam, et al., Discovery of a first-in-class inhibitor of sulfide:quinone oxidoreductase that protects against adverse cardiac remodelling and heart failure, Cardiovasc. Res. 118 (7) (2021) 1771–1784.

[123] S.D.P. Baugh, M.R. Jackson, A.A. Rashad, A.B. Reitz, P.Y.S. Lam, M.S. Jorns, Synthesis and evaluation of potent novel inhibitors of human sulfide:quinone oxidoreductase, Bioorg. Med. Chem. Lett 54 (2021), 128443.

[124] D. Linden, J. Furne, G. Stoltz, M. Abdel-Rehim, M. Levitt, J. Szurszewski, Sulphide quinone reductase contributes to hydrogen sulphide metabolism in murine peripheral tissues but not in the CNS, Br. J. Pharmacol. 165 (7) (2012) 2178–2190.

[125] I. Di Meo, C. Lamperti, V. Tiranti, Mitochondrial diseases caused by toxic compound accumulation: from etiopathology to therapeutic approaches, EMBO Mol. Med. 7 (10) (2015) 1257–1266.

[126] M. Grings, M. Wajner, G. Leipnitz, Mitochondrial dysfunction and redox homeostasis impairment as pathomechanisms of brain damage in ethylmalonic encephalopathy: insights from animal and human studies, Cell. Mol. Neurobiol. 42 (3) (2022) 565–575.

[127] M.R. Filipovic, J. Zivanovic, B. Alvarez, R. Banerjee, Chemical biology of H_2S signaling through persulfidation, Chem. Rev. 118 (3) (2018) 1253–1337.

[128] A.K. Mustafa, M.M. Gadalla, N. Sen, S. Kim, W. Mu, S.K. Gazi, et al., H_2S signals through protein S-sulfhydration, Sci. Signal. 2 (96) (2009) ra72.

[129] H.J. Sun, Z.Y. Wu, X.W. Nie, J.S. Bian, Role of hydrogen sulfide and polysulfides in neurological diseases: focus on protein S-persulfidation, Curr. Neuropharmacol. 19 (6) (2021) 868–884.

[130] H. Kimura, Signalling by hydrogen sulfide and polysulfides via protein S-sulfuration, Br. J. Pharmacol. 177 (4) (2020) 720–733.

[131] C.T. Yang, N.O. Devarie-Baez, A. Hamsath, X.D. Fu, M. Xian, S-Persulfidation, Chemistry, chemical biology, and significance in health and disease, Antioxidants Redox Signal. 33 (15) (2020) 1092–1114.

[132] Y. Kimura, S. Koike, N. Shibuya, D. Lefer, Y. Ogasawara, H. Kimura, 3-Mercaptopyruvate sulfurtransferase produces potential redox regulators cysteine- and glutathione-persulfide (Cys-SSH and GSSH) together with signaling molecules H_2S_2, H_2S_3 and H_2S, Sci. Rep. 7 (1) (2017), 10459.

[133] N. Shibuya, M. Tanaka, M. Yoshida, Y. Ogasawara, T. Togawa, K. Ishii, et al., 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain, Antioxidants Redox Signal. 11 (4) (2009) 703–714.

[134] X. Huang, L. Xiang, Y. Li, Y. Zhao, H. Zhu, Y. Xiao, et al., Snail/FOXK1/Cyr61 signaling Axis regulates the epithelial-mesenchymal transition and metastasis in colorectal cancer, Cell. Physiol. Biochem. 47 (2) (2018) 590–603.

[135] Y. Monnier, P. Farmer, G. Bieler, N. Imaizumi, T. Sengstag, G.C. Alghisi, et al., CYR61 and alphaVbeta5 integrin cooperate to promote invasion and metastasis of tumors growing in preirradiated stroma, Cancer Res. 68 (18) (2008) 7323–7331.

[136] M. Baek, S. Bae, D. Jeong, Relationship of pro-angiogenic factor Cyr61 to colorectal cancer development and prognosis, J. Clin. Oncol. 29 (4_suppl) (2011) 446.

[137] K. Ascenção, B. Lheimeur, C. Szabo, Regulation of CyR61 expression and release by 3-mercaptopyruvate sulfurtransferase in colon cancer cells, Redox Biol. 56 (2022), 102466.

[138] S. Chen, D. Bu, J. Zhu, T. Yue, S. Guo, X. Wang, et al., Endogenous hydrogen sulfide regulates xCT stability through persulfidation of OTUB1 at cysteine 91 in colon cancer cells, Neoplasia 23 (5) (2021) 461–472.

[139] B. Pedre, U. Barayeu, D. Ezeriņa, T.P. Dick, The mechanism of action of N-acetylcysteine (NAC): the emerging role of H_2S and sulfane sulfur species, Pharmacol. Ther. 228 (2021), 107916.

[140] B. Kalyanaraman, NAC, NAC, Knockin' on Heaven's door: interpreting the mechanism of action of N-acetylcysteine in tumor and immune cells, Redox Biol. 57 (2022), 102497.

[141] K. Zuhra, C.S. Tomé, L. Masi, G. Giardina, G. Paulini, F. Malagrinò, et al., N-acetylcysteine serves as substrate of 3-mercaptopyruvate sulfurtransferase and stimulates sulfide metabolism in colon cancer cells, Cells 8 (8) (2019) 828.

[142] C. Busch, C. Jacob, A. Anwar, T. Burkholz, L. Aicha Ba, C. Cerella, et al., Diallylpolysulfides induce growth arrest and apoptosis, Int. J. Oncol. 36 (3) (2010) 743–749.

[143] N.E.B. Saidu, S. Valente, E. Bana, G. Kirsch, D. Bagrel, M. Montenarh, Coumarin polysulfides inhibit cell growth and induce apoptosis in HCT116 colon cancer cells, Bioorg. Med. Chem. 20 (4) (2012) 1584–1593.

[144] C.P. Siegers, B. Steffen, A. Röbke, R. Pentz, The effects of garlic preparations against human tumor cell proliferation, Phytomedicine 6 (1) (1999) 7–11.

[145] K.C. Lai, C.L. Kuo, H.C. Ho, J.S. Yang, C.Y. Ma, H.F. Lu, et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells in vitro and in vivo, Phytomedicine 19 (7) (2012) 625–630.

[146] E. Yagdi Efe, A. Mazumder, J.Y. Lee, A. Gaigneaux, F. Radogna, M.J. Nasim, et al., Tubulin-binding anticancer polysulfides induce cell death via mitotic arrest and autophagic interference in colorectal cancer, Cancer Lett. 410 (2017) 139–157.

[147] Y. Wang, X. Ni, R. Chadha, C. McCartney, Y. Lam, B. Brummett, et al., Methods for suppressing hydrogen sulfide in biological systems, Antioxidants Redox Signal. 36 (4–6) (2022) 294–308.

[148] D.B. McCormick, E.E. Snell, Pyridoxal phosphokinases. II. Effects of inhibitors, J. Biol. Chem. 236 (1961) 2085–2088.

[149] R. Wang, X. Gu, Q. Li, J. Gao, B. Shi, G. Xu, et al., Aggregation enhanced responsiveness of rationally designed probes to hydrogen sulfide for targeted cancer imaging, J. Am. Chem. Soc. 142 (35) (2020) 15084–15090.

[150] T. Yue, S. Zuo, D. Bu, J. Zhu, S. Chen, Y. Ma, et al., Aminooxyacetic acid (AOAA) sensitizes colon cancer cells to oxaliplatin via exaggerating apoptosis induced by ROS, J. Cancer 11 (7) (2020) 1828–1838.

[151] A. Asimakopoulou, P. Panopoulos, C.T. Chasapis, C. Coletta, Z. Zhou, G. Cirino, et al., Selectivity of commonly used pharmacological inhibitors for cystathionine β synthase (CBS) and cystathionine γ lyase (CSE), Br. J. Pharmacol. 169 (4) (2013) 922–932.

[152] C. Chao, J.R. Zatarain, Y. Ding, C. Coletta, A.A. Mrazek, N. Druzhyna, et al., Cystathionine-beta-synthase inhibition for colon cancer: enhancement of the efficacy of aminooxyacetic acid via the prodrug approach, Mol. Med. 22 (2016) 361–379.

[153] N. Druzhyna, B. Szczesny, G. Olah, K. Módis, A. Asimakopoulou, A. Pavlidou, et al., Screening of a composite library of clinically used drugs and well-characterized pharmacological compounds for cystathionine β-synthase inhibition identifies benserazide as a drug potentially suitable for repurposing for the experimental therapy of colon cancer, Pharmacol. Res. 113 (Pt A) (2016) 18–37.

[154] M.K. Thorson, T. Majtan, J.P. Kraus, A.M. Barrios, Identification of cystathionine β-synthase inhibitors using a hydrogen sulfide selective probe, Angew Chem. Int. Ed. Engl. 52 (17) (2013) 4641–4644.

[155] A.A. Untereiner, A. Pavlidou, N. Druzhyna, A. Papapetropoulos, M.R. Hellmich, C. Szabo, Drug resistance induces the upregulation of H_2S-producing enzymes in HCT116 colon cancer cells, Biochem. Pharmacol. 149 (2018) 174–185.

[156] S. Mehmood, M. Maqsood, N. Mahtab, M.I. Khan, A. Sahar, S. Zaib, et al., Epigallocatechin gallate: phytochemistry, bioavailability, utilization challenges, and strategies, J. Food Biochem. 46 (8) (2022), e14189.

[157] J.M. Kim, H.J. Heo, The roles of catechins in regulation of systemic inflammation, Food Sci. Biotechnol. 31 (8) (2022) 957–970.

[158] M. Alam, S. Ali, G.M. Ashraf, A.L. Bilgrami, D.K. Yadav, M.I. Hassan, Epigallocatechin 3-gallate: from green tea to cancer therapeutics, Food Chem. 379 (2022), 132135.

[159] A. Payne, S. Nahashon, E. Taka, G.M. Adinew, K.F.A. Soliman, Epigallocatechin-3-Gallate (EGCG): new therapeutic perspectives for neuroprotection, aging, and neuroinflammation for the modern age, Biomolecules 12 (3) (2022) 371.

[160] G.J. Du, Z. Zhang, X.D. Wen, C. Yu, T. Calway, C.S. Yuan, et al., Epigallocatechin Gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea, Nutrients 4 (11) (2012) 1679–1691.

[161] K.R. Olson, A. Briggs, M. Devireddy, N.A. Iovino, N.C. Skora, J. Whelan, et al., Green tea polyphenolic antioxidants oxidize hydrogen sulfide to thiosulfate and polysulfides: a possible new mechanism underpinning their biological action, Redox Biol. 37 (2020), 101731.

[162] K. Zuhra, M. Petrosino, B. Gupta, T. Panagaki, M. Cecconi, V. Myrianthopoulos, et al., Epigallocatechin gallate is a potent inhibitor of cystathionine beta-synthase: structure-activity relationship and mechanism of action, Nitric Oxide 128 (2022) 12–24.

[163] D. Mereles, W. Hunstein, Epigallocatechin-3-gallate (EGCG) for clinical trials: more pitfalls than promises? Int. J. Mol. Sci. 12 (9) (2011) 5592–5603.

[164] R.H. Abeles, C.T. Walsh, Acetylenic enzyme inactivators. Inactivation of gamma-cystathionase, in vitro and in vivo, by propargylglycine, J. Am. Chem. Soc. 95 (18) (1973) 6124–6125.

[165] M. Bantzi, F. Augsburger, J. Loup, Y. Berset, S. Vasilakaki, V. Myrianthopoulos, et al., Novel aryl-substituted pyrimidones as inhibitors of 3-mercaptopyruvate sulfurtransferase with antiproliferative efficacy in colon cancer, J. Med. Chem. 64 (9) (2021) 6221–6240.
[166] C.T. Yang, Y. Wang, E. Marutani, T. Ida, X. Ni, S. Xu, et al., Data-driven identification of hydrogen sulfide scavengers, Angew Chem. Int. Ed. Engl. 58 (32) (2019) 10898–10902.
[167] I. Ismail, Z. Chen, L. Sun, X. Ji, H. Ye, X. Kang, et al., Highly efficient H_2S scavengers via thiolysis of positively-charged NBD amines, Chem. Sci. 11 (30) (2020) 7823–7828.
[168] K. Zuhra, T. Panagaki, E.B. Randi, F. Augsburger, M. Blondel, G. Friocourt, et al., Mechanism of cystathionine-β-synthase inhibition by disulfiram: the role of bis(N, N-diethyldithiocarbamate)-copper(II), Biochem. Pharmacol. 182 (2020), 114267.
[169] D. Liu, B. Li, J. Wu, Y. Liu, Sorbents for hydrogen sulfide capture from biogas at low temperature: a review, Environ. Chem. Lett. 18 (1) (2020) 113–128.
[170] B. Aksoy, N. Atakan, H.M. Aksoy, G.G. Tezel, N. Renda, H.A. Ozkara, et al., Effectiveness of topical zinc oxide application on hypertrophic scar development in rabbits, Burns 36 (7) (2010) 1027–1035.
[171] X. Pan, Y. Qi, Z. Du, J. He, S. Yao, W. Lu, et al., Zinc oxide nanosphere for hydrogen sulfide scavenging and ferroptosis of colorectal cancer, J. Nanobiotechnol. 19 (1) (2021) 392.
[172] C. Jia, Y. Guo, F.G. Wu, Chemodynamic therapy via Fenton and fenton-like nanomaterials: strategies and recent advances, Small 18 (6) (2022), e2103868.
[173] Y. Zhou, S. Fan, L. Feng, X. Huang, X. Chen, Manipulating intratumoral Fenton chemistry for enhanced chemodynamic and chemodynamic-synergized multimodal therapy, Adv. Mater. 33 (48) (2021), e2104223.
[174] Z. Tang, Y. Liu, M. He, W. Bu, Chemodynamic therapy: tumour microenvironment-mediated Fenton and fenton-like reactions, Angew Chem. Int. Ed. Engl. 58 (4) (2019) 946–956.
[175] C. Wang, F. Xue, M. Wang, L. An, D. Wu, Q. Tian, 2D Cu-bipyridine MOF nanosheet as an agent for colon cancer therapy: a three-in-one approach for enhancing chemodynamic therapy, ACS Appl. Mater. Interfaces 14 (34) (2022) 38604–38616.
[176] Q. Tian, L. An, Q. Tian, J. Lin, S. Yang, Ellagic acid-Fe@BSA nanoparticles for endogenous H_2S accelerated Fe(III)/Fe(II) conversion and photothermal synergistically enhanced chemodynamic therapy, Theranostics 10 (9) (2020) 4101–4115.
[177] S. Wang, Y. Yang, H. Wu, J. Li, P. Xie, F. Xu, et al., Thermosensitive and tum or microenvironment activated nanotheranostics for the chemodynamic/ photothermal therapy of colorectal tumor, J. Colloid Interface Sci. 612 (2022) 223–234.
[178] B. Ma, S. Wang, F. Liu, S. Zhang, J. Duan, Z. Li, et al., Self-assembled copper-amino acid nanoparticles for in situ glutathione "AND" H_2O_2 sequentially triggered chemodynamic therapy, J. Am. Chem. Soc. 141 (2) (2019) 849–857.
[179] W. Wu, L. Yu, Q. Jiang, M. Huo, H. Lin, L. Wang, et al., Enhanced tumor-specific disulfiram chemotherapy by in situ Cu^{2+} chelation-initiated nontoxicity-to-toxicity transition, J. Am. Chem. Soc. 141 (29) (2019) 11531–11539.
[180] Y. Li, J. Zhou, L. Wang, Z. Xie, Endogenous hydrogen sulfide-triggered MOF-based nanoenzyme for synergic cancer therapy, ACS Appl. Mater. Interfaces 12 (27) (2020) 30213–30220.
[181] H. Ding, J. Chang, F. He, S. Gai, P. Yang, Hydrogen sulfide: an emerging precision strategy for gas therapy, Adv Healthc Mater 11 (4) (2022), e2101984.
[182] F. Rong, T. Wang, Q. Zhou, H. Peng, J. Yang, Q. Fan, et al., Intelligent polymeric hydrogen sulfide delivery systems for therapeutic applications, Bioact. Mater. 19 (2023) 198–216.
[183] D. Wu, Q. Hu, Y. Zhu, Therapeutic application of hydrogen sulfide donors: the potential and challenges, Front. Med. 10 (1) (2016) 18–27.
[184] E.E. Ngowi, A. Afzal, M. Sarfraz, S. Khattak, S.U. Zaman, N.H. Khan, et al., Role of hydrogen sulfide donors in cancer development and progression, Int. J. Biol. Sci. 17 (1) (2021) 73–88.
[185] C.R. Powell, K.M. Dillon, J.B. Matson, A review of hydrogen sulfide (H_2S) donors: chemistry and potential therapeutic applications, Biochem. Pharmacol. 149 (2018) 110–123.
[186] L. Li, M. Whiteman, Y.Y. Guan, K.L. Neo, Y. Cheng, S.W. Lee, et al., Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide, Circulation 117 (18) (2008) 2351–2360.
[187] Z.W. Lee, J. Zhou, C.S. Chen, Y. Zhao, C.H. Tan, L. Li, et al., The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects in vitro and in vivo, PLoS One 6 (6) (2011), e21077.
[188] S. Sakuma, S. Minamino, M. Takase, Y. Ishiyama, H. Hosokura, T. Kohda, et al., Hydrogen sulfide donor GYY4137 suppresses proliferation of human colorectal cancer Caco-2 cells by inducing both cell cycle arrest and cell death, Heliyon 5 (8) (2019), e02244.
[189] W. Xu, K. Watanabe, Y. Mizukami, Y. Yamamoto, T. Suzuki, Hydrogen sulfide suppresses the proliferation of intestinal epithelial cells through cell cycle arrest, Arch. Biochem. Biophys. 712 (2021), 109044.
[190] I. Szadvari, S. Hudecova, B. Chovancova, M. Matuskova, D. Cholujova, L. Lencesova, et al., Sodium/calcium exchanger is involved in apoptosis induced by H_2S in tumor cells through decreased levels of intracellular pH, Nitric Oxide 87 (2019) 1–9.
[191] M. Kajsik, B. Chovancova, V. Liskova, P. Babula, O. Krizanova, Slow sulfide donor GYY4137 potentiates effect of paclitaxel on colorectal carcinoma cells, Eur. J. Pharmacol. 922 (2022), 174875.
[192] H. Li, J. Mu, J. Sun, S. Xu, W. Liu, F. Xu, et al., Hydrogen sulfide releasing oridonin derivatives induce apoptosis through extrinsic and intrinsic pathways, Eur. J. Med. Chem. 187 (2020), 111978.
[193] X. Hu, R. Jiao, H. Li, X. Wang, H. Lyu, X. Gao, et al., Antiproliferative hydrogen sulfide releasing evodiamine derivatives and their apoptosis inducing properties, Eur. J. Med. Chem. 151 (2018) 376–388.
[194] J.C. Foster, C.R. Powell, S.C. Radzinski, J.B. Matson, S-aroylthiooximes, A facile route to hydrogen sulfide releasing compounds with structure-dependent release kinetics, Org. Lett. 16 (6) (2014) 1558–1561.
[195] J.C. Foster, S.C. Radzinski, X. Zou, C.V. Finkielstein, J.B. Matson, H_2S-Releasing polymer micelles for studying selective cell toxicity, Mol. Pharm. 14 (4) (2017) 1300–1306.
[196] E. Piragine, L. Flori, L. Di Cesare Mannelli, C. Ghelardini, E. Pagnotta, R. Matteo, et al., Eruca sativa Mill. seed extract promotes anti-obesity and hypoglycemic effects in mice fed with a high-fat diet, Phytother Res. 35 (4) (2021) 1983–1990.
[197] D. Bhattacherjee, C. Basu, Q. Bhardwaj, S. Mal, S. Sahu, R. Sur, et al., Design, synthesis and anti-cancer activities of benzyl analogues of garlic-derived diallyl disulfide (DADS) and the corresponding diselenides, ChemistrySelect 2 (24) (2017) 7399–7406.
[198] G. Gojon, G.A. Morales, SG1002 and catenated divalent organic sulfur compounds as promising hydrogen sulfide prodrugs, Antioxidants Redox Signal. 33 (14) (2020) 1010–1045.
[199] E. Yagdi, C. Cerella, M. Dicato, M. Diederich, Garlic-derived natural polysulfanes as hydrogen sulfide donors: friend or foe? Food Chem. Toxicol. 95 (2016) 219–233.
[200] M. Chattopadhyay, R. Kodela, N. Nath, C.R. Street, C.A. Velázquez-Martínez, D. Boring, et al., Hydrogen sulfide-releasing aspirin modulates xenobiotic metabolizing enzymes in vitro and in vivo, Biochem. Pharmacol. 83 (6) (2012) 733–740.
[201] M. Chattopadhyay, R. Kodela, N. Nath, Y.M. Dastagirzada, C.A. Velázquez-Martínez, D. Boring, et al., Hydrogen sulfide-releasing NSAIDs inhibit the growth of human cancer cells: a general property and evidence of a tissue type-independent effect, Biochem. Pharmacol. 83 (6) (2012) 715–722.
[202] W. Elsheikh, R.W. Blackler, K.L. Flannigan, J.L. Wallace, Enhanced chemopreventive effects of a hydrogen sulfide-releasing anti-inflammatory drug (ATB-346) in experimental colorectal cancer, Nitric Oxide 41 (2014) 131–137.
[203] R. Kodela, N. Nath, M. Chattopadhyay, D.E. Nesbitt, C.A. Velázquez-Martínez, K. Kashfi, Hydrogen sulfide-releasing naproxen suppresses colon cancer cell growth and inhibits NF-κB signaling, Drug Des. Dev. Ther. 9 (2015) 4873–4882.
[204] M. Paul-Clark, W. Elsheikh, N. Kirkby, M. Chan, P. Devchand, T.A. Agbor, et al., Profound chemopreventative effects of a hydrogen sulfide-releasing NSAID in the $APC^{Min/+}$ mouse model of intestinal tumorigenesis, PLoS One 11 (2) (2016), e0147289.
[205] R. Kodela, M. Chattopadhyay, K. Kashfi, NOSH-aspirin: a novel nitric oxide-hydrogen sulfide-releasing hybrid: a new class of anti-inflammatory pharmaceuticals, ACS Med. Chem. Lett. 3 (3) (2012) 257–262.
[206] R. Kodela, M. Chattopadhyay, K. Kashfi, Synthesis and biological activity of NOSH-naproxen (AVT-219) and NOSH-sulindac (AVT-18A) as potent anti-inflammatory agents with chemotherapeutic potential, Medchemcomm 4 (11) (2013) 1472–1481.
[207] M. Chattopadhyay, R. Kodela, K.R. Olson, K. Kashfi, NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid is a potent inhibitor of colon cancer cell growth in vitro and in a xenograft mouse model, Biochem. Biophys. Res. Commun. 419 (3) (2012) 523–528.
[208] F. Vannini, R. Kodela, M. Chattopadhyay, K. Kashfi, NOSH-aspirin inhibits colon cancer cell growth: effects of positional isomerism, Redox Biol. 5 (2015) 421.
[209] F. Vannini, A.C. MacKessack-Leitch, E.K. Eschbach, M. Chattopadhyay, R. Kodela, K. Kashfi, Synthesis and anti-cancer potential of the positional isomers of NOSH-aspirin (NBS-1120) a dual nitric oxide and hydrogen sulfide releasing hybrid, Bioorg. Med. Chem. Lett 25 (20) (2015) 4677–4682.
[210] F. Vannini, M. Chattopadhyay, R. Kodela, P.P.N. Rao, K. Kashfi, Positional isomerism markedly affects the growth inhibition of colon cancer cells by NOSH-aspirin: COX inhibition and modeling, Redox Biol. 6 (2015) 318–325.
[211] M. Chattopadhyay, R. Kodela, P.L. Duvalsaint, K. Kashfi, Gastrointestinal safety, chemotherapeutic potential, and classic pharmacological profile of NOSH-naproxen (AVT-219) a dual NO- and H_2S-releasing hybrid, Pharmacol Res Perspect 4 (2) (2016), e00224.
[212] K. Kashfi, M. Chattopadhyay, R. Kodela, NOSH-sulindac (AVT-18A) is a novel nitric oxide- and hydrogen sulfide-releasing hybrid that is gastrointestinal safe and has potent anti-inflammatory, analgesic, antipyretic, anti-platelet, and anti-cancer properties, Redox Biol. 6 (2015) 287–296.
[213] K. Kaur, R.J. Carrazzone, J.B. Matson, The benefits of macromolecular/ supramolecular approaches in hydrogen sulfide delivery: a review of polymeric and self-assembled hydrogen sulfide donors, Antioxidants Redox Signal. 32 (2) (2020) 79–95.
[214] Z. Housein, T.S. Kareem, A. Salihi, In vitro anticancer activity of hydrogen sulfide and nitric oxide alongside nickel nanoparticle and novel mutations in their genes in CRC patients, Sci. Rep. 11 (1) (2021) 2536.
[215] J. Li, L. Xie, B. Li, C. Yin, G. Wang, W. Sang, et al., Engineering a hydrogen-sulfide-based nanomodulator to normalize hyperactive photothermal immunogenicity for combination cancer therapy, Adv. Mater. 33 (22) (2021), e2008481.
[216] S. Peng, F. Xiao, M. Chen, H. Gao, Tumor-microenvironment-responsive nanomedicine for enhanced cancer immunotherapy, Adv. Sci. 9 (1) (2022), e2103836.

[217] B. Li, H. Shao, L. Gao, H. Li, H. Sheng, L. Zhu, Nano-drug co-delivery system of natural active ingredients and chemotherapy drugs for cancer treatment: a review, Drug Deliv. 29 (1) (2022) 2130–2161.
[218] N. Singh, J. Kim, J. Kim, K. Lee, Z. Zunbul, I. Lee, et al., Covalent organic framework nanomedicines: biocompatibility for advanced nanocarriers and cancer theranostics applications, Bioact. Mater. 21 (2023) 358–380.
[219] S.C. Nunes, C. Ramos, F. Lopes-Coelho, C.O. Sequeira, F. Silva, S. Gouveia-Fernandes, et al., Cysteine allows ovarian cancer cells to adapt to hypoxia and to escape from carboplatin cytotoxicity, Sci. Rep. 8 (1) (2018) 9513.
[220] N. Thirumalaivasan, P. Venkatesan, P.S. Lai, S.P. Wu, In vitro and in vivo approach of hydrogen-sulfide-responsive drug release driven by azide-functionalized mesoporous silica nanoparticles, ACS Appl. Bio Mater. 2 (9) (2019) 3886–3896.
[221] K.N. Bobba, G. Saranya, P.T. Sujai, M.M. Joseph, N. Velusamy, A. Podder, et al., Endogenous H_2S-assisted cancer-cell-specific activation of theranostics with emission readout, ACS Appl. Bio Mater. 2 (3) (2019) 1322–1330.
[222] J. Choi, I.C. Sun, H. Sook Hwang, H. Yeol Yoon, K. Kim, Light-triggered photodynamic nanomedicines for overcoming localized therapeutic efficacy in cancer treatment, Adv. Drug Deliv. Rev. 186 (2022), 114344.
[223] N.M. Carigga Gutierrez, N. Pujol-Solé, Q. Arifi, J.L. Coll, T. le Clainche, M. Broekgaarden, Increasing cancer permeability by photodynamic priming: from microenvironment to mechanotransduction signaling, Cancer Metastasis Rev. (2022) 1–36.
[224] J. Yuan, T.B. Ren, S. Xu, C.J. Wang, X.B. Zhang, L. Yuan, A unique multifunctional luminescent probe for self-monitoring photodynamic therapy by detecting H_2S in cancer cells, ACS Appl. Bio Mater. 4 (8) (2021) 6016–6022.
[225] Y. Ma, X. Li, A. Li, P. Yang, C. Zhang, B. Tang, H_2S-Activable MOF nanoparticle photosensitizer for effective photodynamic therapy against cancer with controllable singlet-oxygen release, Angew Chem. Int. Ed. Engl. 56 (44) (2017) 13752–13756.
[226] R. Wang, K. Dong, G. Xu, B. Shi, T. Zhu, P. Shi, et al., Activatable near-infrared emission-guided on-demand administration of photodynamic anticancer therapy with a theranostic nanoprobe, Chem. Sci. 10 (9) (2019) 2785–2790.
[227] L. Wu, Y. Sun, K. Sugimoto, Z. Luo, Y. Ishigaki, K. Pu, et al., Engineering of electrochromic materials as activatable probes for molecular imaging and photodynamic therapy, J. Am. Chem. Soc. 140 (47) (2018) 16340–16352.
[228] M. Chang, Z. Hou, M. Wang, C. Li, J. Lin, Recent advances in hyperthermia therapy-based synergistic immunotherapy, Adv. Mater. 33 (4) (2021), e2004788.
[229] C. Xu, K. Pu, Second near-infrared photothermal materials for combinational nanotheranostics, Chem. Soc. Rev. 50 (2) (2021) 1111–1137.
[230] S. Liu, X. Pan, H. Liu, Two-dimensional nanomaterials for photothermal therapy, Angew Chem. Int. Ed. Engl. 59 (15) (2020) 5890–5900.
[231] B. Shi, Q. Yan, J. Tang, K. Xin, J. Zhang, Y. Zhu, et al., Hydrogen sulfide-activatable second near-infrared fluorescent nanoassemblies for targeted photothermal cancer therapy, Nano Lett. 18 (10) (2018) 6411–6416.
[232] L. An, X. Wang, X. Rui, J. Lin, H. Yang, Q. Tian, et al., The in situ sulfidation of Cu_2O by endogenous H_2S for colon cancer theranostics, Angew Chem. Int. Ed. Engl. 57 (48) (2018) 15782–15786.
[233] C. Tao, L. An, J. Lin, Q. Tian, S. Yang, Surface plasmon resonance–enhanced photoacoustic imaging and photothermal therapy of endogenous H_2S-triggered Au@Cu_2O, Small 15 (44) (2019), 1903473.
[234] O. Rabin, J. Manuel Perez, J. Grimm, G. Wojtkiewicz, R. Weissleder, An X-ray computed tomography imaging agent based on long-circulating bismuth sulphide nanoparticles, Nat. Mater. 5 (2) (2006) 118–122.
[235] K. Ai, Y. Liu, J. Liu, Q. Yuan, Y. He, L. Lu, Large-scale synthesis of Bi_2S_3 nanodots as a contrast agent for in vivo X-ray computed tomography imaging, Adv. Mater. 23 (42) (2011) 4886–4891.
[236] M.H. Yao, M. Ma, Y. Chen, X.Q. Jia, G. Xu, H.X. Xu, et al., Multifunctional Bi_2S_3/PLGA nanocapsule for combined HIFU/radiation therapy, Biomaterials 35 (28) (2014) 8197–8205.
[237] R. Zhang, Y. Li, W. Zhang, Y. Sheng, M. Wang, J. Liu, et al., Fabrication of Cu_2O/Bi_2S_3 heterojunction photocatalysts with enhanced visible light photocatalytic mechanism and degradation pathways of tetracycline, J. Mol. Struct. 1229 (2021), 129581.
[238] Y. Cheng, H. Bo, R. Qin, F. Chen, F. Xue, L. An, et al., Hyaluronic acid-coated Bi: Cu_2O: an H_2S-responsive agent for colon cancer with targeted delivery and enhanced photothermal performance, J. Nanobiotechnol. 20 (1) (2022) 346.
[239] Y. Li, W. Chen, Y. Qi, S. Wang, L. Li, W. Li, et al., H_2S-Scavenged and activated iron oxide-hydroxide nanospindles for MRI-guided photothermal therapy and ferroptosis in colon cancer, Small 16 (37) (2020), e2001356.
[240] X.Q. Pu, X.J. Ju, L. Zhang, Q.W. Cai, Y.Q. Liu, H.Y. Peng, et al., Novel multifunctional stimuli-responsive nanoparticles for synergetic chemo-photothermal therapy of tumors, ACS Appl. Mater. Interfaces 13 (24) (2021) 28802–28817.
[241] B. Liu, W. Wang, J. Fan, Y. Long, F. Xiao, M. Daniyal, et al., RBC membrane camouflaged prussian blue nanoparticles for gamabutolin loading and combined chemo/photothermal therapy of breast cancer, Biomaterials 217 (2019), 119301.
[242] J. Sun, Y. Liu, X. Zhu, X. Liao, L. Wang, J. Yuan, et al., Endogenous H_2S-activable liposomal nanoplatform for synergistic colorectal tumor ablation at mild apparent temperature, ACS Appl. Bio Mater. 3 (10) (2020) 6680–6687.
[243] M. Hou, M. Ye, L. Liu, M. Xu, H. Liu, H. Zhang, et al., Azide-locked prodrug Co-assembly into nanoparticles with indocyanine green for chemophotothermal therapy, Mol. Pharm. 19 (9) (2022) 3279–3287.
[244] B. Shi, N. Ren, L. Gu, G. Xu, R. Wang, T. Zhu, et al., Theranostic nanoplatform with hydrogen sulfide activatable NIR responsiveness for imaging-guided on-demand drug release, Angew Chem. Int. Ed. Engl. 58 (47) (2019) 16826–16830.
[245] S. Bhanushali, V. Tanna, Y. Nimbalkar, P. Ravikumar, S.P. Sawarkar, Safety of nanobiomaterials for cancer nanotheranostics, Cancer Nanotheranostics (2021) 333–368.
[246] R. Zhou, T.Y. Ohulchanskyy, Y. Xu, R. Ziniuk, H. Xu, L. Liu, et al., Tumor-microenvironment-activated NIR-II nanotheranostic platform for precise diagnosis and treatment of colon cancer, ACS Appl. Mater. Interfaces 14 (20) (2022) 23206–23218.
[247] L. Cheng, C. Wang, L. Feng, K. Yang, Z. Liu, Functional nanomaterials for phototherapies of cancer, Chem. Rev. 114 (21) (2014) 10869–10939.
[248] J. Feng, W.X. Ren, F. Kong, Y.B. Dong, A covalent organic framework-based nanoagent for H_2S-activable phototherapy against colon cancer, Chem. Commun. 57 (59) (2021) 7240–7243.
[249] Y. Zhu, C. Chen, Q. Wu, G. Yang, Z. Liu, E. Hao, et al., Single-wavelength phototheranostics for colon cancer via the thiolytic reaction, Nanoscale 12 (22) (2020) 12165–12171.
[250] M. Chang, Z. Hou, D. Jin, J. Zhou, M. Wang, M. Wang, et al., Colorectal tumor microenvironment-activated bio-decomposable and metabolizable Cu_2O@$CaCO_3$ nanocomposites for synergistic oncotherapy, Adv. Mater. 32 (43) (2020), e2004647.
[251] V.S. Lin, A.R. Lippert, C.J. Chang, Cell-trappable fluorescent probes for endogenous hydrogen sulfide signaling and imaging H_2O_2-dependent H_2S production, Proc. Natl. Acad. Sci. U. S. A. 110 (18) (2013) 7131–7135.
[252] Q. Liu, Y. Zhong, Y. Su, L. Zhao, J. Peng, Real-time imaging of hepatic inflammation using hydrogen sulfide-activatable second near-infrared luminescent nanoprobes, Nano Lett. 21 (11) (2021) 4606–4614.
[253] X. Wang, L. An, Q. Tian, K. Cui, Recent progress in H_2S activated diagnosis and treatment agents, RSC Adv. 9 (58) (2019) 33578–33588.
[254] L. Kong, W. Lu, X. Cao, Y. Wei, J. Sun, Y. Wang, The design strategies and biological applications of probes for the gaseous signaling molecule hydrogen sulfide, J. Mater. Chem. B 10 (39) (2022) 7924–7954.
[255] C. Wang, M. Niu, W. Wang, L. Su, H. Feng, H. Lin, et al., In situ activatable ratiometric NIR-II fluorescence nanoprobe for quantitative detection of H_2S in colon cancer, Anal. Chem. 93 (27) (2021) 9356–9363.
[256] L. Wu, A.C. Sedgwick, X. Sun, S.D. Bull, X.P. He, T.D. James, Reaction-based fluorescent probes for the detection and imaging of reactive oxygen, nitrogen, and sulfur species, Acc. Chem. Res. 52 (9) (2019) 2582–2597.
[257] F. Yu, X. Han, L. Chen, Fluorescent probes for hydrogen sulfide detection and bioimaging, Chem. Commun. 50 (82) (2014) 12234–12249.
[258] X. Kang, X. Cai, L. Yi, Z. Xi, Multifluorinated aryl azides for the development of improved H_2S probes, and fast strain-promoted azide-alkyne cycloaddition and staudinger reactions, Chem. Asian J. 15 (9) (2020) 1420–1429.
[259] H. Ye, L. Sun, Z. Pang, X. Ji, Y. Jiao, X. Tu, et al., Cell-Trappable BODIPY-NBD dyad for imaging of basal and stress-induced H_2S in live biosystems, Anal. Chem. 94 (3) (2022) 1733–1741.
[260] Z. Pang, H. Ye, D. Ma, X. Tu, L. Yi, Z. Xi, A H_2S-specific ultrasensitive fluorogenic probe reveals TMV-induced H_2S production to limit virus replication, Chembiochem 22 (13) (2021) 2292–2299.
[261] H.A. Henthorn, M.D. Pluth, Mechanistic insights into the H_2S-mediated reduction of aryl azides commonly used in H_2S detection, J. Am. Chem. Soc. 137 (48) (2015) 15330–15336.
[262] H. Wang, E. Zhao, J.W. Lam, B.Z. Tang, AIE luminogens: emission brightened by aggregation, Mater. Today 18 (7) (2015) 365–377.
[263] Y. Wu, J. Chen, L. Sun, F. Zeng, S. Wu, A nanoprobe for diagnosing and mapping lymphatic metastasis of tumor using 3D multispectral optoacoustic tomography owing to aggregation/deaggregation induced spectral change, Adv. Funct. Mater. 29 (8) (2019), 1807960.
[264] L. Xu, L. Ni, L. Sun, F. Zeng, S. Wu, A fluorescent probe based on aggregation-induced emission for hydrogen sulfide-specific assaying in food and biological systems, Analyst 144 (22) (2019) 6570–6577.
[265] H.D. Li, Q.C. Yao, J.L. Fan, N. Jiang, J.Y. Wang, J. Xia, et al., A fluorescent probe for H_2S in vivo with fast response and high sensitivity, Chem. Commun. 51 (90) (2015) 16225–16228.
[266] K. Zhang, J. Zhang, Z. Xi, L.Y. Li, X. Gu, Q.Z. Zhang, et al., A new H_2S-specific near-infrared fluorescence-enhanced probe that can visualize the H_2S level in colorectal cancer cells in mice, Chem. Sci. 8 (4) (2017) 2776–2781.
[267] G. Xu, Q. Yan, X. Lv, Y. Zhu, K. Xin, B. Shi, et al., Imaging of colorectal cancers using activatable nanoprobes with second near-infrared window emission, Angew Chem. Int. Ed. Engl. 57 (14) (2018) 3626–3630.
[268] Z. Deng, M. Jiang, Y. Li, H. Liu, S. Zeng, J. Hao, Endogenous H_2S-triggered in situ synthesis of NIR-II-emitting nanoprobe for in vivo intelligently lighting up colorectal cancer, iScience 17 (2019) 217–224.
[269] Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, et al., A ratiometric fluorescent probe for rapid detection of hydrogen sulfide in mitochondria, Angew Chem. Int. Ed. Engl. 52 (6) (2013) 1688–1691.
[270] X. Li, H. Zhao, Y. Ji, C. Yin, J. Li, Z. Yang, et al., Lysosome-assisted mitochondrial targeting nanoprobe based on dye-modified upconversion nanophosphors for ratiometric imaging of mitochondrial hydrogen sulfide, ACS Appl. Mater. Interfaces 10 (46) (2018) 39544–39556.
[271] H. Liu, G. Xu, T. Zhu, R. Wang, J. Tan, C. Zhao, et al., Rational design of water-dispersible and biocompatible nanoprobes with H_2S-triggered NIR emission for cancer cell imaging, J. Mater. Chem. B 8 (28) (2020) 6013–6016.
[272] T. Maldiney, A. Lecointre, B. Viana, A. Bessière, M. Bessodes, D. Gourier, et al., Controlling electron trap depth to enhance optical properties of persistent luminescence nanoparticles for in vivo imaging, J. Am. Chem. Soc. 133 (30) (2011) 11810–11815.

[273] A. Abdukayum, J.T. Chen, Q. Zhao, X.P. Yan, Functional near infrared-emitting Cr^{3+}/Pr^{3+} co-doped zinc gallogermanate persistent luminescent nanoparticles with superlong afterglow for in vivo targeted bioimaging, J. Am. Chem. Soc. 135 (38) (2013) 14125–14133.
[274] Y. Jiang, J. Huang, X. Zhen, Z. Zeng, J. Li, C. Xie, et al., A generic approach towards afterglow luminescent nanoparticles for ultrasensitive in vivo imaging, Nat. Commun. 10 (1) (2019) 2064.
[275] L. Wu, Y. Ishigaki, Y. Hu, K. Sugimoto, W. Zeng, T. Harimoto, et al., H_2S-activatable near-infrared afterglow luminescent probes for sensitive molecular imaging in vivo, Nat. Commun. 11 (1) (2020) 446.
[276] F. Auzel, Upconversion and anti-Stokes processes with f and d ions in solids, Chem. Rev. 104 (1) (2004) 139–173.
[277] W. Feng, C. Han, F. Li, Upconversion-nanophosphor-based functional nanocomposites, Adv. Mater. 25 (37) (2013) 5287–5303.
[278] J. Zhou, Z. Liu, F. Li, Upconversion nanophosphors for small-animal imaging, Chem. Soc. Rev. 41 (3) (2012) 1323–1349.
[279] C. Ma, T. Bian, S. Yang, C. Liu, T. Zhang, J. Yang, et al., Fabrication of versatile cyclodextrin-functionalized upconversion luminescence nanoplatform for biomedical imaging, Anal. Chem. 86 (13) (2014) 6508–6515.
[280] Y.I. Park, K.T. Lee, Y.D. Suh, T. Hyeon, Upconverting nanoparticles: a versatile platform for wide-field two-photon microscopy and multi-modal in vivo imaging, Chem. Soc. Rev. 44 (6) (2015) 1302–1317.
[281] K. Pu, A.J. Shuhendler, J.V. Jokerst, J. Mei, S.S. Gambhir, Z. Bao, et al., Semiconducting polymer nanoparticles as photoacoustic molecular imaging probes in living mice, Nat. Nanotechnol. 9 (3) (2014) 233–239.
[282] B. Shi, X. Gu, Q. Fei, C. Zhao, Photoacoustic probes for real-time tracking of endogenous H_2S in living mice, Chem. Sci. 8 (3) (2017) 2150–2155.
[283] T. Ma, J. Zheng, T. Zhang, D. Xing, Ratiometric photoacoustic nanoprobes for monitoring and imaging of hydrogen sulfide in vivo, Nanoscale 10 (28) (2018) 13462–13470.
[284] V. Ntziachristos, D. Razansky, Molecular imaging by means of multispectral optoacoustic tomography (MSOT), Chem. Rev. 110 (5) (2010) 2783–2794.
[285] A. Taruttis, V. Ntziachristos, Advances in real-time multispectral optoacoustic imaging and its applications, Nat. Photonics 9 (4) (2015) 219–227.
[286] V. Ntziachristos, Going deeper than microscopy: the optical imaging frontier in biology, Nat. Methods 7 (8) (2010) 603–614.
[287] L. Sun, Y. Wu, J. Chen, J. Zhong, F. Zeng, S. Wu, A turn-on optoacoustic probe for imaging metformin-induced upregulation of hepatic hydrogen sulfide and subsequent liver injury, Theranostics 9 (1) (2019) 77–89.
[288] B. Broens, J.W. Duitman, G.J.C. Zwezerijnen, E.J. Nossent, C.J. van der Laken, A. E. Voskuyl, Novel tracers for molecular imaging of interstitial lung disease: a state of the art review, Autoimmun. Rev. 21 (12) (2022), 103202.
[289] M.H. Younis, Z. Tang, W. Cai, Multimodality imaging of nanoparticle-based vaccines: shedding light on immunology, Wiley Interdiscip Rev Nanomed Nanobiotechnol 14 (5) (2022), e1807.
[290] C.A. Boswell, M.W. Brechbiel, Development of radioimmunotherapeutic and diagnostic antibodies: an inside-out view, Nucl. Med. Biol. 34 (7) (2007) 757–778.
[291] N. Urbano, M. Scimeca, V. Tancredi, E. Bonanno, O. Schillaci, (99)mTC-sestamibi breast imaging: current status, new ideas and future perspectives, Semin. Cancer Biol. 84 (2022) 302–309.
[292] E. Roussel, U. Capitanio, A. Kutikov, E. Oosterwijk, I. Pedrosa, S.P. Rowe, et al., Novel imaging methods for renal mass characterization: a collaborative review, Eur. Urol. 81 (5) (2022) 476–488.
[293] J.Y. Park, Y.J. Kim, J.Y. Lee, Y.S. Lee, J.M. Jeong, Imaging of the third gasotransmitter hydrogen sulfide using (99m)Tc-labeled alpha-hydroxy acids, Nucl. Med. Biol. 76–77 (2019) 28–35.
[294] Y. Kweon, J.Y. Park, Y.J. Kim, Y.S. Lee, J.M. Jeong, Imaging hydrogen sulfide in hypoxic tissue with [(99m)Tc]Tc-gluconate, Molecules 26 (1) (2020) 96.
[295] X. Qin, H. Jiang, Y. Liu, H. Zhang, M. Tian, Radionuclide imaging of apoptosis for clinical application, Eur. J. Nucl. Med. Mol. Imag. (2021) 1–15.
[296] J. Pellico, P.J. Gawne, R.T. de Rosales, Radiolabelling of nanomaterials for medical imaging and therapy, Chem. Soc. Rev. 50 (5) (2021) 3355–3423.
[297] C. Yan, D. Liu, L. An, Y. Wang, Q. Tian, J. Lin, et al., Magnetic-photoacoustic dual-mode probe for the visualization of H_2S in colorectal cancer, Anal. Chem. 92 (12) (2020) 8254–8261.
[298] D. Ni, W. Bu, E.B. Ehlerding, W. Cai, J. Shi, Engineering of inorganic nanoparticles as magnetic resonance imaging contrast agents, Chem. Soc. Rev. 46 (23) (2017) 7438–7468.
[299] H. Li, T.J. Meade, Molecular magnetic resonance imaging with Gd(III)-Based contrast agents: challenges and key advances, J. Am. Chem. Soc. 141 (43) (2019) 17025–17041.
[300] G. Angelovski, Heading toward macromolecular and nanosized bioresponsive MRI probes for successful functional imaging, Acc. Chem. Res. 50 (9) (2017) 2215–2224.
[301] G. Angelovski, What we can really do with bioresponsive MRI contrast agents, Angew Chem. Int. Ed. Engl. 55 (25) (2016) 7038–7046.
[302] S. Bi, Z. Deng, Q. Jiang, M. Jiang, S. Zeng, A H_2S-triggered dual-modal second near-infrared/photoacoustic intelligent nanoprobe for highly specific imaging of colorectal cancer, Anal. Chem. 93 (39) (2021) 13212–13218.
[303] X.J. Wang, J. Cheng, L.Y. Zhang, J.G. Zhang, Self-assembling peptides-based nano-cargos for targeted chemotherapy and immunotherapy of tumors: recent developments, challenges, and future perspectives, Drug Deliv. 29 (1) (2022) 1184–1200.
[304] V. Yagublu, A. Karimova, J. Hajibabazadeh, C. Reissfelder, M. Muradov, S. Bellucci, et al., Overview of physicochemical properties of nanoparticles as drug carriers for targeted cancer therapy, J. Funct. Biomater. 13 (4) (2022) 196.
[305] C.G.A. Das, V.G. Kumar, T.S. Dhas, V. Karthick, C.M.V. Kumar, Nanomaterials in anticancer applications and their mechanism of action - a review, Nanomedicine 47 (2022), 102613.
[306] X. Zhang, Y. Chen, X. He, Y. Zhang, M. Zhou, C. Peng, et al., Smart nanogatekeepers for tumor theranostics, Small 17 (47) (2021), e2103712.
[307] E. Blanco, H. Shen, M. Ferrari, Principles of nanoparticle design for overcoming biological barriers to drug delivery, Nat. Biotechnol. 33 (9) (2015) 941–951.
[308] Y. Qu, C. Li, J. Shi, R. Chen, S. Xu, H. Rafsanjani, et al., Transvaginal fast-scanning optical-resolution photoacoustic endoscopy, J. Biomed. Opt. 23 (12) (2018) 1–4.
[309] M.W. Schellenberg, H.K. Hunt, Hand-held optoacoustic imaging: a review, Photoacoustics 11 (2018) 14–27.