Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Versatile roles of cysteine persulfides in tumor biology

Klaudia Borbényi-Galambos[1,2], Ágnes Czikora[1], Katalin Erdélyi[1] and Péter Nagy[1,3,4]

Abstract

Rewiring the transsulfuration pathway is recognized as a rapid adaptive metabolic response to environmental conditions in cancer cells to support their increased cysteine demand and to produce Reactive Sulfur Species (RSS) including hydrogen sulfide (H_2S) and cysteine persulfide. This can directly (via RSS) or indirectly (by supplying Cys) trigger chemical or enzyme catalyzed persulfidation on critical protein cysteine residues to protect them from oxidative damage and to orchestrate protein functions, and thereby contribute to cancer cell plasticity. In this review key aspects of persulfide-mediated biological processes are highlighted and critically discussed in relation to cancer cell survival, bioenergetics, proliferation as well as in tumor angiogenesis, adaptation to hypoxia and oxidative stress, and regulation of epithelial to mesenchymal transition.

Addresses

[1] Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, 1122, Hungary
[2] Kálmán Laki Doctoral School, University of Debrecen, Debrecen, Hajdú-Bihar County, 4032, Hungary
[3] Department of Anatomy and Histology, HUN-REN–UVMB Laboratory of Redox Biology Research Group, University of Veterinary Medicine, Budapest, 1078, Hungary
[4] Chemistry Institute, University of Debrecen, Debrecen, Hajdú-Bihar County, 4012, Hungary

Corresponding author: Nagy, Péter (peter.nagy@oncol.hu)

Current Opinion in Chemical Biology 2024, **79:**102440

This review comes from a themed issue on **Chemical Genetics and Epigenetics (2024)**

Edited by **Yimon Aye** and **Christine Winterbourn**

For complete overview of the section, please refer the article collection - Chemical Genetics and Epigenetics (2024)

Available online 28 February 2024

https://doi.org/10.1016/j.cbpa.2024.102440

Keywords

Cancer, Cysteine, Transsulfuration, Persulfidation, Persulfide.

Introduction

Altered regulation of metabolism, a hallmark of cancer [1], enables cancer cells to meet their bioenergetic, biosynthetic and redox demands to support uncontrolled proliferation [2]. Extensive rewiring of metabolic pathways armors them with remarkable flexibility for rapid adaptation to environmental stimuli including immune response and chemo-, radio- or targeted therapy. It is increasingly recognized that reprogramming the transsulfuration pathway is an important element of cancer cell plasticity.

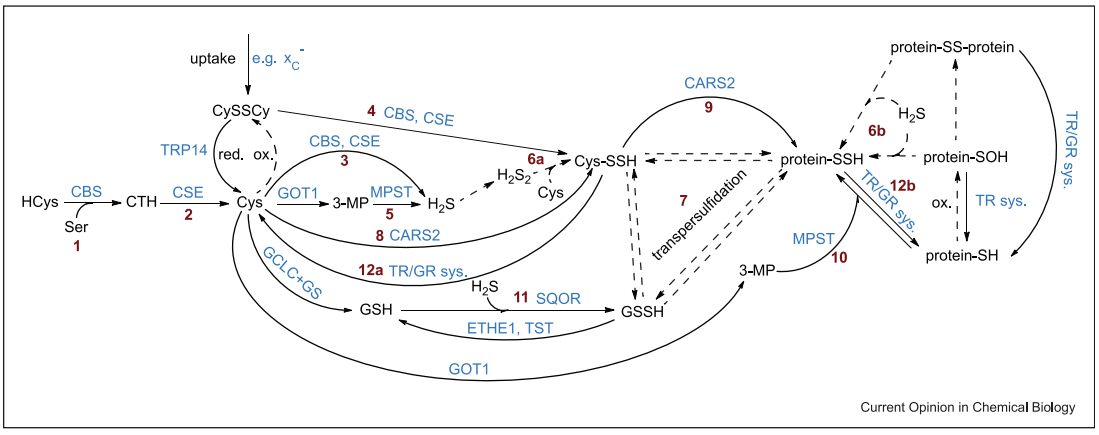
Malignant cells usually have higher levels of reactive oxygen species (ROS) production compared to normal cells, which is induced by oncogenes, hypoxia, disruption of the mitochondrial electron transport chain or by chemo- or radiotherapy [3]. To ameliorate oxidative stress, cancer cells augment antioxidant systems, which require sufficient supply of cysteine (Cys), a biosynthetic precursor for i) redox enzymes that maintain thiol-disulfide homeostasis; ii) essential cofactors including coenzyme A and iron-sulfur clusters; as well as iii) key protective small molecules such as glutathione, taurine, hydrogen sulfide (H_2S) or hydropersulfides [4]. Although representing a new direction in cancer research, a rapidly increasing number of studies report pivotal functions for H_2S and hydropersulfide species in tumor biology. Here we summarize key aspects of this research area and provide our perspective for future studies.

Main text

Transsulfuration pathway

Because in the extracellular milieu Cys is predominantly present in its oxidized dipeptide form, cystine (CySSCy) [5], cellular uptake of Cys occurs mostly in this form, via the x_c^- antiporter system [6]. Since cancer cells rely on Cys availability to support their antioxidant stratagem, upon dietary restriction of CySSCy or inhibition of the x_c^- they undergo an iron-dependent non-apoptotic form of cell death called ferroptosis [7–10]. xCT/SLC7A11, a subunit of the x_c^-, is widely expressed in several human cancers and associated with poor

Figure 1



Metabolism of reactive sulfur species mediated by enzymatic (plain arrow) and non-enzymatic (dashed arrow) reactions. Cys is either taken up from the extracellular space in its oxidized form (CySSCy) via the x_c^- antiporter or it can be synthesized from HCys-derived Cth by the consecutive actions of CBS and CSE (reaction 1&2). CSE and CBS can also catalyze the formation of H_2S from Cys (reaction 3) and Cys-SSH from CySSCy (reaction 4), whereas MPST can generate H_2S indirectly from Cys (reaction 5). RSSH species can be produced by redox reactions involving reduced and oxidized forms of H_2S and thiols (reaction 6a,b), by transpersulfidation reactions (reaction 7), by CARS2 (reaction 8&9), MPST (reaction 10) or SQOR-catalyzed reactions (reaction 11). RSSH can be reduced back to native thiol via the GSH and Trx systems (reaction 12a,b). Red: number of the reaction; blue: proteins.

survival rate [11], corroborating the increased cysteine demand of these tumors [6]. Hence, therapeutic inhibition of the x_c^- is a developing anticancer strategy [11].

Cysteine is depleted in the tumor microenvironment, which was demonstrated by lower measured levels in tumor interstitial fluids compared to plasma [12]. However, Cys is not an essential amino acid, which means that upon extracellular limitation of CySSCy it can be gained by *de novo* synthesis from serine and methionine-derived homocysteine via the transsulfuration pathway. The consecutive canonical activities of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are responsible for their conversion to Cys via intermediate formation of cystathionine (Cth) (Fig. 1 reactions 1 and 2) [13]. CBS and CSE are constitutively expressed in a subset of cancer cell lines, indicating that extracellular CySSCy availability might not be sufficient to cover cellular demands [10,14] and that the activity of the transsulfuration pathway is critical for tumor growth [4,9,10,15].

Furthermore, apart from their canonical cysteine-producing functions, CBS and CSE are key players in the biogenesis of reactive sulfur species (RSS)[1] including H_2S (Fig. 1 reaction 3) and cysteine persulfide (Cys-SSH) (Fig. 1 reaction 4) [16,17]. The recently revealed vital functions of RSS in human physiology places them in the focus of biomedical and pharmacological research [18,19]. Overexpression of CSE and/or CBS in several cancer cell lines even at sufficient CySSCy availability suggest that their RSS-producing functions are also critical for cancer cell survival [9,20–22].

Overall, the fast adaptive response of the transsulfuration pathway to environmental stimuli including finetuning of the versatile enzyme functions of CBS and CSE is not only vital in healthy cell physiology [23], but also in pathophysiology, including tumor progression [9,10,14,24].

H_2S and cancer

The oncological functions of H_2S, the most widely studied sulfur-containing signaling molecule, were extensively reviewed [20]. Well accepted molecular mechanisms for the versatile biological action of H_2S are: 1) reactions with metalloprotein heme centers (e.g. inhibition of cytochrome c oxidase) [25], 2) regulation of protein functions via persulfidation of cysteine residues [26] and 3) interactions with NO biochemistry [27]. Although the reactions of H_2S with metalloproteins are widely studied, their importance in tumor biology is not well established and the biological relevance of NO–H_2S interactions is just emerging. However, H_2S-mediated persulfidation of key onco-proteins with functional consequences in cancer biology were more extensively investigated.

H_2S was reported to be an important regulator of cancer cell proliferation, cellular bioenergetics and vascularization in a tumor-type specific manner [20]. Although in some cancer types, especially in colorectal cancer, the oncogenic roles of H_2S are widely studied

[1] RSS represents the effector species that can induce persulfidation on thiol residues.

[21,28–30], its pharmacological utilization is still controversial. Some reports promote the therapeutic potential of sulfide donors, others suggest inhibition of endogenous sulfide production. The currently most well accepted view is that due to the delicate balance of different sulfur species in cellular systems, the pharmacological profile of H_2S in oncology represents a bell-shaped curve, where both the inhibition of H_2S producing pathways and the utilization of H_2S donors could have therapeutic potential [20]. We propose that it largely depends on tumor type, disease status and context, therefore, a deeper understanding of sulfide chemical biology in a cancer specific manner is in order.

Furthermore, biochemical studies of H_2S suffer from methodological complications, which makes it currently difficult to link or separate its effects to/from those of hydropersulfides (demonstrated in Refs. [31,32] and reviewed in Refs. [33,34]). Therefore, recent advances and future developments, which allow systematic analyses of sulfur biology via metabolomics, proteomics and fluxomics approaches instead of only measuring steady-state H_2S levels are preferred.

Low molecular weight (LMW) hydropersulfide species

Hydropersulfides (R–SSH) are closely linked biochemically to H_2S via a network of enzyme catalyzed and uncatalyzed reactions [31,33]. Accumulating evidence suggests that R–SSH species play important roles in cellular protection against oxidative stress, modification of key signaling pathways and regulation of cellular metabolism, all three being key to cancer cell survival.

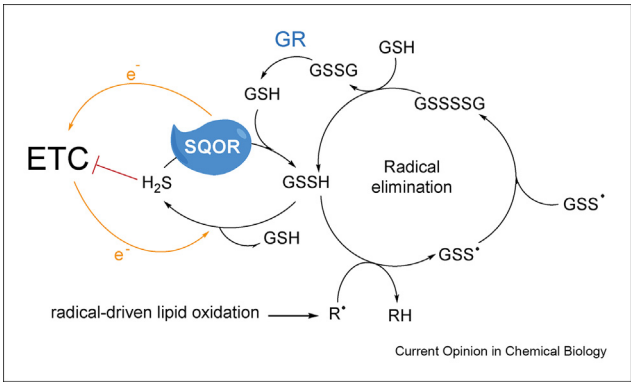
Regarding the chemical properties of R–SSH species, despite being more nucleophilic (mostly due to lower pK_a and the alpha effect), in contrast to thiols (R–SH) they also engage in biochemical reactions as electrophiles [18,35]. Furthermore, R–SSH species are better $1e^-$ reductants than their corresponding thiol derivatives, which is partly due to resonance stabilization of the unpaired electron in perthiyl radicals [36]. This chemical promiscuity calls for further elucidation of molecular targets where this chemistry has functional consequences in cancer biology.

R–SSH can be formed non-enzymatically via redox reactions between thiol and H_2S derivatives, where one of the reaction partners is in its oxidized state. For example, i) a disulfide or a sulfenic acid form of R–SH can react with H_2S or ii) thiols are oxidized by inorganic polysulfides to give R–SSH species [37] (Fig. 1 reaction 6a,b). Moreover, R–SSH can react with R′-SH in transpersulfidation reactions which can yield R′-SSH species [16] (Fig. 1 reaction 7).

LMW R–SSH are also metabolized via versatile enzyme catalyzed reactions. For example, cysteinyl-tRNA synthetase 2 (CARS2) can generate Cys-SSH from Cys (Fig. 1 reaction 8) [38]. In addition, using CySSCy not Cys as a substrate, direct formation of Cys-SSH is catalyzed by CBS and CSE (Fig. 1 reaction 4). Although intracellular concentrations of CySSCy compared to Cys are low under normal conditions and hence Cys was proposed to outcompete CySSCy as a substrate for CSE or CBS [39], cancer cells are characterized by increased oxidative stress and therefore could have elevated CySSCy:Cys ratios [3]. This together with the fact that under equimolar substrate conditions the catalytic reactions of CSE and CBS with CySSCy are orders of magnitude faster than with Cys [16,40], may provide reaction 4 as an alternate route for persulfide production in cancer cells. Beside the non-enzymatic reactions, glutathione persulfide (GSSH), an abundant form of LMW persulfide species can be generated by mitochondrial oxidative catabolism of H_2S through sulfide:quinone oxidoreductase (SQOR) (Fig. 1 reaction 11) [41]. Reduction of persulfide species to thiols and H_2S are catalyzed by the thioredoxin and glutathione reductase systems [42,43].

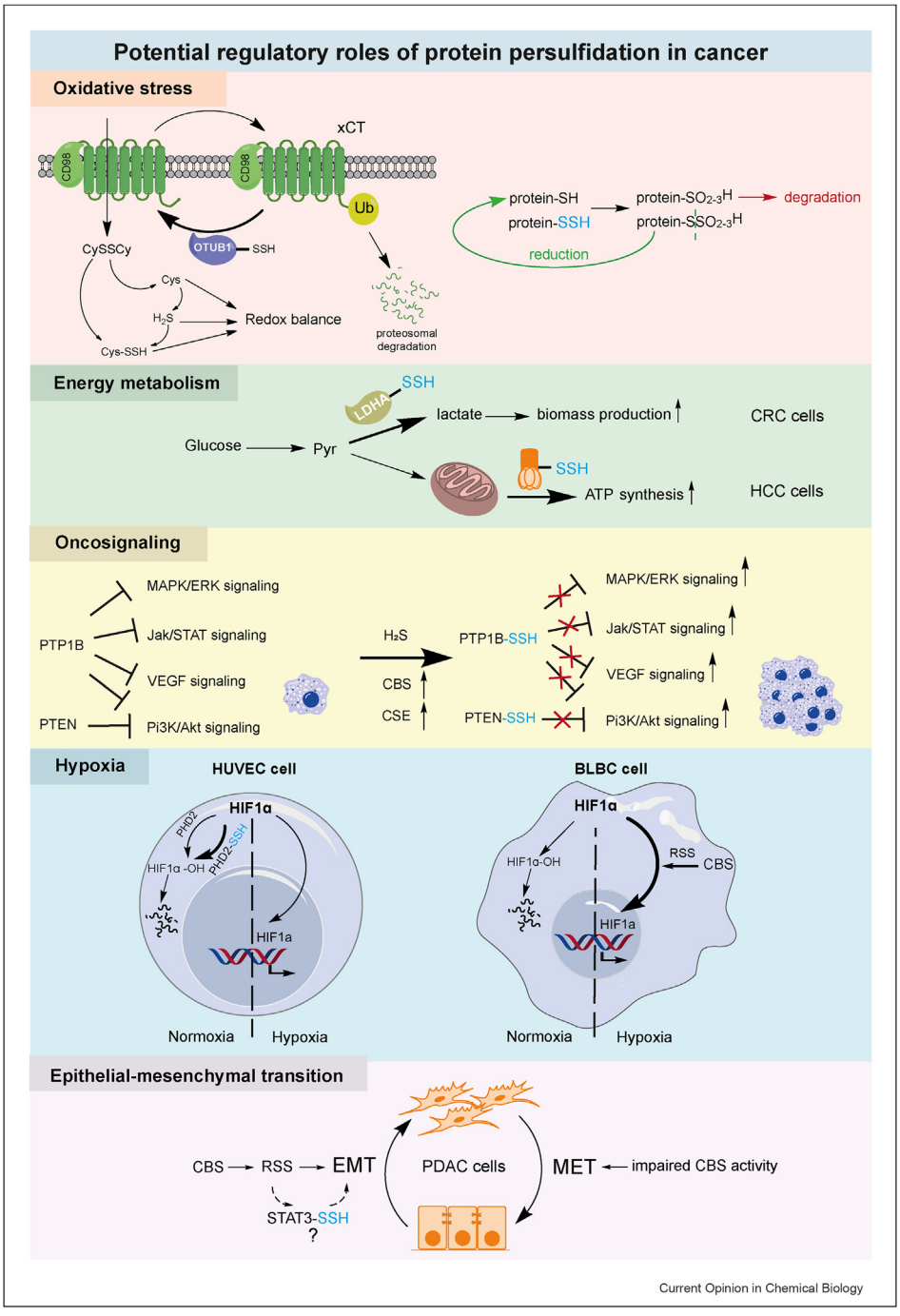
The physiological relevance of these LMW R–SSH in cell biology are increasingly recognized. They were recently shown to be potent inhibitors of lipid-peroxidation-driven ferroptosis [44,45] (Fig. 2), which is recognized as a protecting property of cancer cells [9]. Moreover, persulfides were shown to directly or indirectly accept electrons from the electron transport chain [38,46], potentiating them as mediators of mitochondrial respiration (Fig. 2). Careful dissection of these

Figure 2



Biological roles of LMW persulfides with potential relevance in cancer. By oxidizing H_2S, SQOR donates electrons to the electron transport chain (ETC) to generate GSSH, which can act as a potent electron acceptor to feedback H_2S, thus promoting mitochondrial function (orange arrows). However, in increased doses, H_2S is a potent inhibitor of the ETC by inhibiting cytochrome c oxidase (red inhibitory arrow). GSSH can also act as a radical scavenger to counteract lipid peroxidation-induced ferroptosis.

Figure 3



Examples for the versatile roles of protein persulfidation in cancer cell survival and metastasis. Persulfidation of deubiquitinase OTUB1 could enhance x_c^- stabilization and thereby contribute to CySSCy uptake and redox balance. Persulfidation of Cys residues protect proteins from irreversible overoxidation (orange panel). Persulfidation of LDHA in CRC cells and ATP synthase in HCC may enhance enzyme activity leading to altered energy metabolism (green panel). Persulfidation of regulatory Cys of key tumor suppressors PTP1B and PTEN inhibit their function potentially leading to increased oncosignaling (yellow panel). Persulfidation of PHD2 could increase its function in HUVEC cells leading to increased HIF1α degradation under normoxic conditions. In contrast, in BLBC cells CBS-derived RSS promote HIF1α function and loss of CBS activity results in impaired HIF1α activation and therefore impaired angiogenesis (blue panel). CBS-derived RSS promote EMT in PDAC cells potentially via persulfidation of STAT3 (pink panel).

LMW functions in a cancer cell specific manner holds premises for tumor biology.

Protein persulfidation in cancer

Protein persulfidation is a reversible posttranslational modification of Cys residues, which can be reduced back to thiols by the thioredoxin (TRP14/Trx/TrxR) and glutathione (GSH/GSH reductase and glutaredoxin) systems [42,43,47] (Fig. 1 reaction 12b). Although the exact mediators of protein persulfidation in the intracellular milieu are still debated, recent reports suggested that CARS2 (Fig. 1 reaction 9) [38] and mercaptopyruvate sulfur transferase (MPST) [48] may be key players (Fig. 1 reaction 10). However, by producing H_2S and LMW Cys-SSH, CBS and CSE (Fig. 1 reaction 3&4) could also contribute indirectly to protein persulfidation via redox- and transpersulfidation-pathways [37].

Persulfidation and oxidative stress

Increased transsulfuration activity is critical under limiting Cys availability in neuroblastoma, glioblastoma, melanoma and prostate cancer to counteract oxidative stress by allowing sufficient GSH production utilizing the transsulfuration pathway derived Cys [10]. Apart from regulation of protein activity, persulfidation also protects protein Cys residues from oxidative damage. Irreversible oxidation of Cys residues impairs enzyme functions and leads to protein replacement. In contrast, oxidized persulfide (Cys-$SSO_{1\text{-}3}H$) derivatives can be reduced at the SS-bond back to native thiols (Cys-SH) by the Trx or GSH systems and thereby preserve protein function [47,49]. For example, we reported earlier that overexpression of CBS is involved in BLBC tumor progression. Our data highlight that, among other effects, CBS- and, in its absence, CSE-produced H_2S/R−SSH species and protein persulfidation protect BLBC cells from oxidative stress-induced cell death [9], but the contributions of persulfidation reactions at individual protein Cys residues remain to be elucidated.

Persulfidation and cancer cell metabolism

Regulation of metabolic pathways via protein Cys persulfidation is increasingly recognized. For example, in pancreatic β cells, metabolic plasticity was conferred by Gao et al. based on the profiles of the measured 827 proteins that they found to be persulfidated upon endoplasmic reticulum stress [50]. The degree of persulfidation was associated with the induction of genes encoding CSE and SLC7A11/xCT, which are mediated by the stress response activating transcription factor 4 (ATF4). In line with these observations, persulfidation of the OTU domain-containing ubiquitin aldehyde-binding ubiquitin protein 1 (OTUB1) Cys-91 was shown to increase the stability of xCT in colorectal cancer [51] (Fig. 3 first panel), revealing a reciprocal regulation between cystine uptake and the transsulfuration pathway. H_2S-induced persulfidation of lactate dehydrogenase A (LDHA), another key enzyme in cancer cell metabolism, was also shown to be prominent in colon cancer cells [52]. Moreover, the enzyme activity of ATP synthase was proposed to be activated by persulfidation of its α subunit to support mitochondrial bioenergetics [53] (Fig. 3 second panel).

Persulfidation and oncosignaling

Persulfidation of catalytic or regulatory cysteines can alter protein function and therefore contribute to the regulation of key signaling events that are relevant in cancer research. In the past decade, the activity of several transcription factors and other proteins involved in signal transduction were reported to be regulated by persulfidation.

A well-studied example is protein tyrosine phosphatase 1B (PTP1B), which is a regulator of several key signaling pathways. Inactivation of PTP1B phosphatase activity by persulfidation of its active site cysteine (Cys-215) was published more than a decade ago [54]. In squamous adenocarcinoma cells, we demonstrated that epidermal growth factor (EGF) mediated tyrosine phosphorylation is markedly increased by inhibiting PTP1B via polysulfide-treatment [47]. We also showed that silencing CBS in basal-like breast cancer (BLBC) cells results in decreased levels of tyrosine phosphorylation upon EGF treatment under cystine deprivation. The underlying contribution of PTP1B was demonstrated by i) significantly lower phosphorylation at Tyr-1068 of EGFR, which is known to be dephosphorylated by PTP1B and by ii) phosphorylation of the downstream MEK1/2 upon EGF treatment [9] (Fig. 3 third panel).

Redox regulation of another key tumor suppressor, phosphatase and tensin homolog (PTEN), which acts as a major antagonist of the phosphoinositide 3-kinase (PI3K)-Akt pathway, is also well established. Similarly to PTP1B, the activity of PTEN is reversibly inhibited by persulfidation of its active site cysteine (Cys-124) [55] (Fig. 3 third panel).

Persulfidation and hypoxia

Adaptation to hypoxic conditions of the tumor microenvironment is key to cancer cell survival [1]. It was demonstrated in non-cancerous cell systems that under normoxic conditions, persulfidation augments prolyl hydroxylase 2 (PHD2) function, which leads to proteasomal degradation of hypoxia inducible factor-1α (HIF1α). By depletion of endogenous H_2S, impaired persulfidation of PHD2 was proposed to reduce its activity leading to HIF1α stabilization and hence enhanced hypoxia signaling [56]. In contrast with these observations, in BLBC we found that disruption of CBS inhibited hypoxic response and tumor angiogenesis in mouse xenografts. Moreover, our *in vitro* studies revealed that CBS silencing resulted in impaired HIF1α activation [9] (Fig. 3 fourth

panel). These contradicting observations might be caused by the used different cellular systems including disease versus normal conditions. Future studies should investigate the roles of RSS in regulation of the tumor hypoxic response in a tumor specific manner.

Persulfidation and EMT

Epithelial-to-mesenchymal transition (EMT) of cancer cells is a major contributing factor to tumor invasiveness and metastasis. The role of CBS in EMT was extensively studied in pancreatic ductal adenocarcinoma (PDAC) [57]. Using an orthotopic mouse model, we showed that by silencing CBS, PDAC tumors gave lower number of liver metastases compared to controls. CBS silencing decreased WNT5a and SNAI1 gene expression as well as increased E-cadherin and lowered Vimentin signals in mouse tumors. This together with cell migration and colony formation experiments indicated a primary role for CBS in mediating the EMT of cancer cells in PDAC tumor progression (Fig. 3 fifth panel). Furthermore, our data suggested that CBS-induced RSS may protect signal transducer and activator of transcription 3 (STAT3), a key downstream regulator of the Wnt pathway, from oxidative inactivation [57].

Conclusions

Although genomic and genetic studies still dominate the cancer research continuum, it is becoming increasingly recognized that protein−protein interactions as well as altered metabolic regulation of protein functions contribute to tumor progression. Recent advances demonstrated that reprogramming of the transsulfuration pathway produce RSS, which are active metabolites in cancer cell plasticity. This review article is dedicated to provide an overview of recent findings on how cancer cells utilize RSS for promoting proliferation and survival. Initially, studies were focused on the small signaling metabolite H_2S, but it became clear that it cannot at present be easily discriminated from the actions of Cys persulfide species. Here we highlighted that both LMW and protein persulfides have pivotal functions in tumor biology. Realigned transsulfuration can induce dynamic Cys-, GSH- and protein Cys persulfidation to mediate key oncogenic processes including cancer cell proliferation, angiogenesis, survival and metastasis via regulating transcription, cellular signal transduction, hypoxic response, EMT and antioxidant protection. Importantly, more detailed studies recently demonstrated that these mechanisms are utilized for tumor progression in a cancer specific manner. It is also important to point out that protein persulfide detection methodologies suffer from serious caveats (e.g. Refs. [33,34]). In particular, the specificity of the modified biotin switch assay, which was used in a number of tumor biology studies, was criticized [58]. Therefore, further corroboration of these observations and more detailed mechanistic studies are in order.

Nevertheless, H_2S and Cys-persulfide species appear to be key in the rapid adaptation of cancer cells to different environmental stimuli and therefore better understanding of RSS-mediated tumor progression via utilizing available state-of-the-art methods as well as novel technological advances is vital to design more effective targeted therapeutic interventions.

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

The article only contains published data and no original research data.

Acknowledgments

The project was implemented with the support from the National Research, Development and Innovation Fund of the Ministry of Culture and Innovation under the National Laboratories Program (National Tumor Biology Laboratory (2022-2.1.1-NL-2022-00010)) and the Hungarian Thematic Excellence Program (under project TKP2021-EGA-44) Grant Agreements with the National Research, Development and Innovation Office (NRDIO). This project has received funding from the HUN-REN Hungarian Research Network (grant 1500207). K.B.G. acknowledges financial support from the Richter Gedeon Talentum Foundation founded by Richter Gedeon Plc.

Abbreviations

BLBC basal-like breast cancer
CARS2 cysteine-tRNA ligase, mitochondrial
CBS cystathionine-β-synthase
CRC colorectal cancer
CSE cystathionine γ-lyase
Cth cystathionine
Cys cysteine
Cys-SSH cysteine persulfide
CySSCy cystine
EGF epidermal growth factor
EGFR epidermal growth factor receptor
EMT epithelial−mesenchymal transition
ETHE1 persulfide dioxygenase
GCLC glutamate-cysteine ligase catalytic subunit
GS glutathione synthetase
GSH glutathione
GSSG oxidized glutathione
GSSH glutathione persulfide
HCC hepatocellular carcinoma
HCys homocysteine
HIF1α hypoxia inducible factor 1α
HMW high molecular weight
HUVEC human umbilical vein endothelial cells
LDHA lactate dehydrogenase A
LMW low molecular weight
MET mesenchymal−epithelial transition
MPST mercaptopyruvate sulfurtransferase
OTU ovarian tumor

OTUB1	OTU deubiquitinase, ubiquitin aldehyde binding 1
PDAC	pancreatic ductal adenocarcinoma
PHD2	prolyl hydroxylase domain-containing protein 2
PTEN	phosphatase and tensin homolog
PTP1B	protein tyrosine phosphatase 1B
Pyr	pyruvate
RSS	reactive sulfur species
Ser	serine
STAT3	signal transducer and activator of transcription 3
SUOX	sulfite oxidase
SQOR	sulfide:quinone oxidoreductase
TRP14	thioredoxin domain-containing protein 17
Trx	thioredoxin
TrxR1	thioredoxin reductase 1
TST	thiosulfate sulfurtransferase
Ub	ubiquitin
x_c^-	cystine/glutamate antiporter system

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation**. *Cell* 2011, **144**:646–674.

2. Pavlova NN, Zhu J, Thompson CB: **The hallmarks of cancer metabolism: still emerging**. *Cell Metab* 2022, **34**:355–377.

3. Hayes JD, Dinkova-Kostova AT, Tew KD: **Oxidative stress in cancer**. *Cancer Cell* 2020, **38**:167–197.

4. Bonifacio VDB, Pereira SA, Serpa J, Vicente JB: **Cysteine metabolic circuitries: druggable targets in cancer**. *Br J Cancer* 2021, **124**:862–879.

5. Jones DP, Mody Jr VC, Carlson JL, Lynn MJ, Sternberg Jr P: **Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses**. *Free Radic Biol Med* 2002, **33**:1290–1300.

6. Combs JA, DeNicola GM: **The non-essential amino acid cysteine becomes essential for tumor proliferation and survival**. *Cancers* 2019, **11**.

7. Cramer SL, Saha A, Liu J, Tadi S, Tiziani S, Yan W, Triplett K, Lamb C, Alters SE, Rowlinson S, Zhang YJ, *et al.*: **Systemic depletion of l-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth**. *Nat Med* 2017, **23**:120–127.

8. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison 3rd B, *et al.*: **Ferroptosis: an iron-dependent form of nonapoptotic cell death**. *Cell* 2012, **149**:1060–1072.

9. ** Erdelyi K, Ditroi T, Johansson HJ, Czikora A, Balog N, Silwal-Pandit L, Ida T, Olasz J, Hajdu D, Matrai Z, Csuka O, *et al.*: **Reprogrammed transsulfuration promotes basal-like breast tumor progression via realigning cellular cysteine persulfidation**. *Proc Natl Acad Sci U S A* 2021, **118**.

This is a comprehensive study, which emerged from proteomic analyses of patient samples showing that the persulfide producing function of cystathionine-β-synthase is vital for progression of basal-like breast cancer, the most aggressive breast cancer subtype.

10. ** Zhu J, Berisa M, Schworer S, Qin W, Cross JR, Thompson CB: **Transsulfuration activity can support cell growth upon extracellular cysteine limitation**. *Cell Metab* 2019, **30**:865–876 e865.

This study demonstrated that the canonical cysteine producing functions of the transsulfuration pathway is utilized in neuroblastoma and glioblastoma cells to provide adequate glutathione supply to counteract intrecellular oxidative stress under conditions when extracellular cysteine is limited.

11. Lin W, Wang C, Liu G, Bi C, Wang X, Zhou Q, Jin H: **Slc7a11/xCT in cancer: biological functions and therapeutic implications**. *Am J Cancer Res* 2020, **10**:3106–3126.

12. Sullivan MR, Danai LV, Lewis CA, Chan SH, Gui DY, Kunchok T, Dennstedt EA, Vander Heiden MG, Muir A: **Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability**. *eLife* 2019:8.

13. Stipanuk MH: **Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine**. *Annu Rev Nutr* 2004, **24**:539–577.

14. Zhang HF, Klein Geltink RI, Parker SJ, Sorensen PH: **Transsulfuration, minor player or crucial for cysteine homeostasis in cancer**. *Trends Cell Biol* 2022, **32**:800–814.

15. Min JY, Chun KS, Kim DH: **The versatile utility of cysteine as a target for cancer treatment**. *Front Oncol* 2022, **12**:997919.

16. Ida T, Sawa T, Ihara H, Tsuchiya Y, Watanabe Y, Kumagai Y, Suematsu M, Motohashi H, Fujii S, Matsunaga T, Yamamoto M, *et al.*: **Reactive cysteine persulfides and s-polythiolation regulate oxidative stress and redox signaling**. *Proc Natl Acad Sci U S A* 2014, **111**:7606–7611.

17. Kumar R, Banerjee R: **Regulation of the redox metabolome and thiol proteome by hydrogen sulfide**. *Crit Rev Biochem Mol Biol* 2021, **56**:221–235.

18. Cortese-Krott MM, Koning A, Kuhnle GGC, Nagy P, Bianco CL, Pasch A, Wink DA, Fukuto JM, Jackson AA, van Goor H, Olson KR, *et al.*: **The reactive species interactome: evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine**. *Antioxid Redox Signal* 2017, **27**:684–712.

19. Wallace JL, Wang R: **Hydrogen sulfide-based therapeutics: exploiting a unique but ubiquitous gasotransmitter**. *Nat Rev Drug Discov* 2015, **14**:329–345.

20. Szabo C: **Gasotransmitters in cancer: from pathophysiology to experimental therapy**. *Nat Rev Drug Discov* 2016, **15**:185–203.

21. * Szabo C, Coletta C, Chao C, Modis K, Szczesny B, Papapetropoulos A, Hellmich MR: **Tumor-derived hydrogen sulfide, produced by cystathionine-beta-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer**. *Proc Natl Acad Sci U S A* 2013, **110**:12474–12479.

The first study showing that cystathionine-β-synthase produced hydrogen sulfide is involved in colon cancer progression.

22. Szczesny B, Marcatti M, Zatarain JR, Druzhyna N, Wiktorowicz JE, Nagy P, Hellmich MR, Szabo C: **Inhibition of hydrogen sulfide biosynthesis sensitizes lung adenocarcinoma to chemotherapeutic drugs by inhibiting mitochondrial DNA repair and suppressing cellular bioenergetics**. *Sci Rep* 2016, **6**:36125.

23. Sbodio JI, Snyder SH, Paul BD: **Regulators of the transsulfuration pathway**. *Br J Pharmacol* 2019, **176**:583–593.

24. Ascencao K, Szabo C: **Emerging roles of cystathionine beta-synthase in various forms of cancer**. *Redox Biol* 2022, **53**, 102331.

25. Doman A, Doka E, Garai D, Bogdandi V, Balla G, Balla J, Nagy P: **Interactions of reactive sulfur species with metalloproteins**. *Redox Biol* 2023, **60**:102617.

26. * Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH: **H2s signals through protein s-sulfhydration**. *Sci Signal* 2009, **2**:ra72.

The first study which reported that hydrogen sulfide may signal through inducing persulfide formation on protein cysteine residues.

27. Kevil C, Cortese-Krott MM, Nagy P, Papapetropoulos A, Feelisch M, Szabo C: *Cooperative interactions between no and H2S: chemistry, biology, physiology, pathophysiology. . Nitric oxide: biology and pathobiology*. 3rd ed. 2017:57–83.

28. * Libiad M, Vitvitsky V, Bostelaar T, Bak DW, Lee HJ, Sakamoto N, Fearon E, Lyssiotis CA, Weerapana E, Banerjee R: **Hydrogen**

sulfide perturbs mitochondrial bioenergetics and triggers metabolic reprogramming in colon cells. *J Biol Chem* 2019, **294**:12077–12090.
This work provided mechanistic insights how hydrogen sulfide can feed the mitochondrial electron transport chain and induce metabolic reprograming in colon cancer cells.

29. Vitvitsky V, Kumar R, Libiad M, Maebius A, Landry AP, Banerjee R: **The mitochondrial NADH pool is involved in hydrogen sulfide signaling and stimulation of aerobic glycolysis**. *J Biol Chem* 2021, **296**:100736.

30. Yue T, Li J, Zhu J, Zuo S, Wang X, Liu Y, Liu J, Liu X, Wang P, Chen S: **Hydrogen sulfide creates a favorable immune microenvironment for colon cancer**. *Cancer Res* 2023, **83**: 595–612.

31. Bogdandi V, Ida T, Sutton TR, Bianco C, Ditroi T, Koster G, Henthorn HA, Minnion M, Toscano JP, van der Vliet A, Pluth MD, *et al.*: **Speciation of reactive sulfur species and their reactions with alkylating agents: do we have any clue about what is present inside the cell?** *Br J Pharmacol* 2019, **176**:646–670.

32. Schilling D, Barayeu U, Steimbach RR, Talwar D, Miller AK, Dick TP: **Commonly used alkylating agents limit persulfide detection by converting protein persulfides into thioethers**. *Angew Chem Int Ed Engl* 2022, **61**, e202203684.

33. Nagy P, Doka E, Ida T, Akaike T: **Measuring reactive sulfur species and thiol oxidation states: challenges and cautions in relation to alkylation-based protocols**. *Antioxid Redox Signal* 2020, **33**:1174–1189.

34. Schilling D, Ditroi T, Barayeu U, Juranyi EP, Nagy P, Dick TP: **The influence of alkylating agents on sulfur-sulfur bonds in per- and polysulfides**. *Curr Opin Chem Biol* 2023, **76**:102368.

35. Switzer CH: **How super is supersulfide?: reconsidering persulfide reactivity in cellular biology**. *Redox Biol* 2023, **67**: 102899.

36. Bianco CL, Chavez TA, Sosa V, Saund SS, Nguyen QNN, Tantillo DJ, Ichimura AS, Toscano JP, Fukuto JM: **The chemical biology of the persulfide (RSSH)/perthiyl (RSS.) redox couple and possible role in biological redox signaling**. *Free Radic Biol Med* 2016, **101**:20–31.

37. Nagy P: **Mechanistic chemical perspective of hydrogen sulfide signaling**. *Methods Enzymol* 2015, **554**:3–29.

38. * Akaike T, Ida T, Wei FY, Nishida M, Kumagai Y, Alam MM, Ihara H, Sawa T, Matsunaga T, Kasamatsu S, Nishimura A, *et al.*: **Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics**. *Nat Commun* 2017, **8**:1177.
This publication revealed the moonlighting cysteine-persulfide producing function of cysteinyl-tRNA synthetase 2 (CARS2) and how it may trigger cysteine-persulfide incorporation into nascent polypeptides inside the ribosome.

39. Yadav PK, Martinov M, Vitvitsky V, Seravalli J, Wedmann R, Filipovic MR, Banerjee R: **Biosynthesis and reactivity of cysteine persulfides in signaling**. *J Am Chem Soc* 2016, **138**: 289–299.

40. Jeitner TM, Azcona JA, Ables GP, Cooke D, Horowitz MC, Singh P, Kelly JM, Cooper AJL: **Cystine rather than cysteine is the preferred substrate for beta-elimination by cystathionine gamma-lyase: implications for dietary methionine restriction**. *Geroscience* 2023, https://doi.org/10.1007/s11357-023-00788-4.

41. Libiad M, Yadav PK, Vitvitsky V, Martinov M, Banerjee R: **Organization of the human mitochondrial hydrogen sulfide oxidation pathway**. *J Biol Chem* 2014, **289**:30901–30910.

42. Doka E, Pader I, Biro A, Johansson K, Cheng Q, Ballago K, Prigge JR, Pastor-Flores D, Dick TP, Schmidt EE, Arner ES, *et al.*: **A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems**. *Sci Adv* 2016, **2**, e1500968.

43. Wedmann R, Onderka C, Wei S, Szijarto IA, Miljkovic JL, Mitrovic A, Lange M, Savitsky S, Yadav PK, Torregrossa R, Harrer EG, *et al.*: **Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation**. *Chem Sci* 2016, **7**: 3414–3426.

44. ** Barayeu U, Schilling D, Eid M, Xavier da Silva TN, Schlicker L, Mitreska N, Zapp C, Grater F, Miller AK, Kappl R, Schulze A, *et al.*: **Hydropersulfides inhibit lipid peroxidation and ferroptosis by scavenging radicals**. *Nat Chem Biol* 2022.

45. * Wu Z, Khodade VS, Chauvin JR, Rodriguez D, Toscano JP, Pratt DA: **Hydropersulfides inhibit lipid peroxidation and protect cells from ferroptosis**. *J Am Chem Soc* 2022, **144**: 15825–15837.
These two studies (55 and 56) simultaneously provided mechanistic insights, how the radical scavenging activity of glutathione persulfide protects against lipid peroxidation induced ferroptosis in cells.

46. Alam MM, Kishino A, Sung E, Sekine H, Abe T, Murakami S, Akaike T, Motohashi H: **Contribution of NRF2 to sulfur metabolism and mitochondrial activity**. *Redox Biol* 2023, **60**:102624.

47. * Doka E, Ida T, Dagnell M, Abiko Y, Luong NC, Balog N, Takata T, Espinosa B, Nishimura A, Cheng Q, Funato Y, *et al.*: **Control of protein function through oxidation and reduction of persulfidated states**. *Sci Adv* 2020, **6**, eaax8358.
This study elucidated the mechanism how persulfidation of functional or regulatory protein cysteine residues protect them from intracellular oxidative damage.

48. Pedre B, Talwar D, Barayeu U, Schilling D, Luzarowski M, Sokolowski M, Glatt S, Dick TP: **3-Mercaptopyruvate sulfur transferase is a protein persulfidase**. *Nat Chem Biol* 2023.

49. * Zivanovic J, Kouroussis E, Kohl JB, Adhikari B, Bursac B, Schott-Roux S, Petrovic D, Miljkovic JL, Thomas-Lopez D, Jung Y, Miler M, *et al.*: **Selective persulfide detection reveals evolutionarily conserved antiaging effects of s-sulfhydration**. *Cell Metab* 2019, **30**:1152–1170 e1113.
This study demonstrated the cysteine protective function of protein persulfidation in aging.

50. Gao XH, Krokowski D, Guan BJ, Bederman I, Majumder M, Parisien M, Diatchenko L, Kabil O, Willard B, Banerjee R, Wang B, *et al.*: **Quantitative h2s-mediated protein sulfhydration reveals metabolic reprogramming during the integrated stress response**. *eLife* 2015, **4**, e10067.

51. Chen S, Bu D, Zhu J, Yue T, Guo S, Wang X, Pan Y, Liu Y, Wang P: **Endogenous hydrogen sulfide regulates xCT stability through persulfidation of otub1 at cysteine 91 in colon cancer cells**. *Neoplasia* 2021, **23**:461–472.

52. Untereiner AA, Olah G, Modis K, Hellmich MR, Szabo C: **H(2)s-induced s-sulfhydration of lactate dehydrogenase a (LDHA) stimulates cellular bioenergetics in HCT116 colon cancer cells**. *Biochem Pharmacol* 2017, **136**:86–98.

53. Modis K, Ju Y, Ahmad A, Untereiner AA, Altaany Z, Wu L, Szabo C, Wang R: **S-Sulfhydration of ATP synthase by hydrogen sulfide stimulates mitochondrial bioenergetics**. *Pharmacol Res* 2016, **113**(Pt A):116–124.

54. Krishnan N, Fu C, Pappin DJ, Tonks NK: **H2S-induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response**. *Sci Signal* 2011, **4**:ra86.

55. Greiner R, Palinkas Z, Basell K, Becher D, Antelmann H, Nagy P, Dick TP: **Polysulfides link H2S to protein thiol oxidation**. *Antioxid Redox Signal* 2013, **19**:1749–1765.

56. Dey A, Prabhudesai S, Zhang Y, Rao G, Thirugnanam K, Hossen MN, Dwivedi SKD, Ramchandran R, Mukherjee P, Bhattacharya R: **Cystathione beta-synthase regulates HIF-1alpha stability through persulfidation of PHD2**. *Sci Adv* 2020, **6**.

57. ** Czikora A, Erdelyi K, Ditroi T, Szanto N, Juranyi EP, Szanyi S, Tovari J, Strausz T, Nagy P: **Cystathionine beta-synthase overexpression drives metastatic dissemination in pancreatic ductal adenocarcinoma via inducing epithelial-to-mesenchymal transformation of cancer cells**. *Redox Biol* 2022, **57**:102505.
This study revealed that cystathionine-β-synthase overexpression in metastatic pancreatic ductal adenocarcinoma is a driver of epithelial–mesenchymal transition of cancer cells by regulating the Wnt signaling pathway through protecting Signal transducer and activation of tranription 3 (STAT3) activity from oxidative inactivation.

58. Pan J, Carroll KS: **Persulfide reactivity in the detection of protein s-sulfhydration**. *ACS Chem Biol* 2013, **8**:1110–1116.