Hydrogen Sulfide Oxidation: Adaptive Changes in Mitochondria of SW480 Colorectal Cancer Cells upon Exposure to Hypoxia

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Hydrogen sulfide (H2S), a known inhibitor of cytochrome c oxidase (CcOX), plays a key signaling role in human (patho)physiology. While being able to regulate cell redox homeostasis and other crucial physiological functions at low (nM) concentrations [1–4], at higher (μM) levels, H2S exerts toxicity both inhibiting O2 consumption by cytochrome c oxidase (CcOX) in the mitochondrial electron transport chain [5] and impairing O2 transport/storage through covalent modification of the heme porphyrin ring in globins (reviewed in [6]). It is therefore crucial that cells tightly control H2S bioavailability to prevent toxicity.

1. Introduction

Hydrogen sulfide (H2S) has been increasingly recognized as a key signaling molecule in human (patho)physiology. While being able to regulate cell redox homeostasis and other crucial physiological functions at low (nM) concentrations [1–4], at higher (μM) levels, H2S exerts toxicity both inhibiting O2 consumption by cytochrome c oxidase (CcOX) in the mitochondrial electron transport chain [5] and impairing O2 transport/storage through covalent modification of the heme porphyrin ring in globins (reviewed in [6]). It is therefore crucial that cells tightly control H2S bioavailability to prevent toxicity.

In humans, at least three enzymes are directly involved in H2S synthesis (reviewed in [1, 7, 8]): cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), belonging to the transulfuration pathway, and 3-mercaptoppyruvate sulfurtransferase (MST). Of these, CBS is inhibited with relatively high affinity by nitric oxide (NO) and carbon monoxide (CO), particularly in the presence of the allosteric stimulator S-adenosyl-L-methionine [9–13]. H2S breakdown is instead mostly accomplished by a mitochondrial enzymatic pathway that couples the oxidation of H2S into thiosulfate (S2O32-) and sulfate (SO42-) to ATP synthesis [14]. The first step of sulfide breakdown is catalyzed by the membrane-associated sulfide:quinone oxidoreductase (SQR). This flavoprotein transfers...
electrons from H₂S to coenzyme Q in the mitochondrial electron transfer chain, thus making H₂S the first inorganic substrate that is able to sustain mitochondrial respiration [15]. Concomitantly, SQR transfers the H₂S sulfur atom to an acceptor, leading to the formation of glutathione persulfide (GSSH) [16, 17] or, less likely, S₂O₃²⁻ [18, 19]. Differences in the SQR substrate specificity were recently reported comparing the soluble with the nanodisc-incorporated enzyme [20]. Three additional enzymes, persulfide dioxygenase (ETHE1), thiosulfate sulfurtransferase, and sulfite oxidase, cooperate with SQR in the mitochondrial sulfi de oxidation pathway, to oxidize H₂S into SO₄²⁻ and S₂O₃²⁻. To process 1 H₂S molecule, mitochondria overall consume ~0.75 O₂ molecules (0.25 by CcOX plus 0.5 by ETHER, [21]). Besides being metabolized through the mitochondrial sulfi de-oxidizing pathway, H₂S can be oxidized by several metalloproteins such as globins, heme-based sensors of diatomic gaseous molecules, catalase, and peroxidases (see [8] and references therein) or be catabolized by the cytosolic thiol methyltransferase [22].

In vivo, H₂S can therefore exert a dual effect on cell bioenergetics, at lower concentrations stimulating via SQR mitochondrial respiration and thus ATP synthesis or causing a reversible inhibition of CcOX at higher concentrations (reviewed in [23–26]). Notably, the sulfi de-oxidizing activity varies considerably between different cell types and tissues, spanning from undetectable, as e.g., in neuroblastoma cells, to high, as observed in colonocytes [15, 21, 27]. The high H₂S-detoxicifying ability of colonocytes is perhaps not surprising as these cells are physiologically exposed to the fairly high H₂S levels produced by the gut microbiota (reviewed in [28]). Among other diseases, cancer has been increasingly associated with alterations of H₂S metabolism [29–31]. In particular, CBS has been shown to be overexpressed in cell lines and samples of colorectal cancer [32] and other cancer types [33–36]. In colorectal cancer cell lines, CBS-derived H₂S was proposed to promote cell proliferation and angiogenesis and to sustain cellular bioenergetics by stimulating both oxidative phosphorylation and glycolytic ATP synthesis. The enzyme is therefore currently recognized as a drug target [29, 31, 37]. CSE and CSE-derived H₂S have been recognized as key elements in melanoma progression [38]. All three H₂S-synthesizing enzymes have been posited to contribute to the correlation between increased H₂S production and tumor stage and grade in bladder urothelial cell carcinoma [39]. Moreover, Szczesny et al. [36] observed higher expression levels of all three H₂S-generating enzymes and increased H₂S-producing activity in lung adenocarcinoma samples as compared to the adjacent normal lung tissue. A link between H₂S production and mitochondrial DNA repair was proposed, and the inhibition of CBS and CSE by aminooxyacetic acid or siRNA-mediated depletion of CBS, CSE, or MST in the lung adenocarcinoma A549 cell line resulted in compromised integrity of mitochondrial DNA. Irrespectively of the downstream mechanisms linking increased H₂S levels and cell proliferation and/or tumor progression, it remains to be established how cancer cells circumvent the potentially toxic effects of increased H₂S.

Hypoxia is a common factor in the microenvironment of solid tumors that has been recognized to be associated to drug resistance and promotion of cancer progression, metas- tasis, and angiogenesis (see [40] for a review). The effect of hypoxia on cancer metabolism has been extensively investigated (reviewed in [41–43]). Among other changes, hypoxic cells undergo a reduction in mitochondrial mass, resulting from reduced biogenesis of this organelle and enhanced mitophagy [44–46]. Because mitochondria are the main site of sulfide oxidation, in the absence of compensatory mechanisms, hypoxic cells are expected to display a reduced ability to detoxify sulfi de. The intricate interplay between H₂S and O₂ has been extensively investigated (reviewed in [47, 48]). As O₂ facilitates both the chemical and enzymatic oxidative decomposition of H₂S into persulfides and polysulfides, at low O₂ tension a higher stability of H₂S is expected. Furthermore, hypoxic/ischemic conditions have been reported to enhance H₂S synthesis, through upregulation or stimulation of the sulfi de-synthesizing enzymes [49, 50], accumulation of CBS in mitochondria, likely augmenting the H₂S mitochondrial levels [51], and release of CO-mediated inhibition of CBS and CSE [52, 53]. Hypoxia is thus expected to increase H₂S bioavailability, a condition that can have opposite physiological consequences. Indeed, while H₂S has been shown to be protective against ischemic injuries [54, 55], the enhanced biosynthesis and chemical stability of H₂S, combined with the reduced content in mitochondria (the main sites of sulfide disposal), may increase the risk of H₂S toxicity in hypoxic cells.

This information prompted us to investigate in the present study the effect of hypoxia on the mitochondrial sulfi de-oxidizing activity and SQR expression in colorectal cancer cells.

2. Materials and Methods

2.1. Materials. The human colon cancer cell line SW480 was purchased from the American Type Culture Collection (ATCC no. CCL228™). Sodium sulfide nonahydrate (Na₂S·9H₂O, 431648), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acetyl coenzyme A, oxaloacetate, CellUtlytic™ MT cell lysis reagent, protease inhibitor cocktail (P8340), and rabbit polyclonal antibody against human SQR (HPA017079) were purchased from Sigma. The bicinchoninic acid assay (BCA) kit was from Thermo Fisher Scientific. Cell culture media and antibiotics were from Sigma, EuroClone, or Gibco. Mini-PROTEAN TGX Stain-Free Precast Gels, the Clarity Western ECL Substrate, and the Laemmli protein sample buffer were purchased from Bio-Rad. Bovine serum albumin was from AppliChem.

2.2. Preparation of Sulfi de Stock Solutions. Stock solutions of Na₂S were prepared by quickly washing the surface of a crystal of sodium sulfide nonahydrate with degassed ultrapure (Milli-Q®) water and then dissolving it in degassed Milli-Q water under N₂ atmosphere, as reported in [56]. The concentration of Na₂S in solution was measured spectrophotometrically using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) according to Nashef et al. [57] in a
Cary 60 UV-VIS spectrophotometer. The concentration of \( \text{Na}_2\text{S} \) was then adjusted to 3-5 mM by dilution with degassed ultrapure (Milli-Q\textsuperscript{®}) water in a gas-tight glass syringe.

2.3. Cell Culture. The human colon cancer cell line SW480 was maintained in Dulbecco’s Modified Eagle Medium (DMEM) containing 4.5 g.L\(^{-1}\) glucose, supplemented with 2 mM l-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U.mL\(^{-1}\) penicillin, and 100 \( \mu \)g.mL\(^{-1}\) streptomycin. Cells at 37°C and 5% CO\(_2\) in 25 cm\(^2\) or 75 cm\(^2\) flasks were grown under normoxic conditions (air O\(_2\)) or incubated for 24 h under hypoxic conditions (1% O\(_2\)) in a Galaxy 14 S incubator (Eppendorf) designed to maintain cell cultures at controlled O\(_2\) tension. After trypsinization, the cells were washed in the culture medium, counted using the trypan blue dye exclusion test, centrifuged at 1000 \( \times \) g for 5 min, and resuspended in fresh medium at a final density of 8 \( \times \) 10\(^6\) cells.mL\(^{-1}\). Trypan blue-positive cells were always less than 5%. Cells grown under air conditions or exposed to hypoxia are, respectively, referred to as “normoxic” and “hypoxia-treated” cells.

2.4. Measurements of the Mitochondrial Sulfide-Oxidizing Activity. The mitochondrial sulfide-oxidizing activity of tested cells was evaluated as described in [25], by measuring the stimulatory effect of sulfide on cellular O\(_2\) consumption. Measurements were carried out at 37°C, using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria), equipped with two 1.5 mL chambers and a micropump (TIP-2k) allowing for steady injections of relatively small amounts of sulfide into the chambers. According to Abou-Hamdan et al. [25], in these assays, sulfide is injected into a cell suspension at increasing flux (determined by the pump rate) and the mitochondrial sulfide-detoxifying activity is evaluated from the observed stimulation of cellular O\(_2\) consumption. Indeed, upon increasing the rate of sulfide injection, the concentration of sulfide in solution and, in turn, the sulfide-sustained cellular O\(_2\) consumption increase until the concentration of injected sulfide becomes inhibitory for CoXO. In colorectal cancer cells, SQR-mediated sulfide detoxification was shown to promote both forward electron transfer to O\(_2\) via quinol-cytochrome c reductase (complex III)/cytochrome c/CoXO and reverse electron transfer through complex I [21]. Therefore, measurements were herein carried out in the presence of rotenone, a known inhibitor of complex I, to prevent electrons derived from SQR-mediated sulfide oxidation to be partially diverted from O\(_2\) reduction with consequent underestimation of the mitochondrial sulfide-oxidizing activity. Herein, the assays were typically conducted in FBS-supplemented cell medium under stirring as follows. A suspension of four million cells was added into the respirometer chamber, and the basal respiration was measured for 10 min. Afterwards, following the addition of 5 \( \mu \)M rotenone resulting in O\(_2\) consumption inhibition, a solution of 3-5 mM sulfide was injected for time intervals of 180 s at increasing rates (10 nL.s\(^{-1}\), 20 nL.s\(^{-1}\), 40 nL.s\(^{-1}\), 80 nL.s\(^{-1}\), and 160 nL.s\(^{-1}\)) and the effect on O\(_2\) consumption was measured. Control experiments were carried out in the presence of both rotenone (5 \( \mu \)M) and antimycin A (5 \( \mu \)M), an inhibitor of complex III. The latter assays allowed us to evaluate the effect of sulfide on extramitochondrial and nonenzymatic O\(_2\) consumption and thus obtain by subtraction (from the experiments performed in the absence of antimycin A) the genuine mitochondrial O\(_2\) consumption activity due to sulfide oxidation and from it an estimate of the H\(_2\)S-oxidizing activity, considering that \(~\)1.33 molecules of H\(_2\)S per O\(_2\) molecule are reportedly consumed by the mitochondrial sulfide-oxidizing pathway [21].

2.5. Evaluation of Mitochondrial Content by the Citrate Synthase Assay. Cells were harvested and lysed using the CellLytic™ MT cell lysis reagent and protease inhibitor cocktail from Sigma according to the manufacturer’s instructions. Cell extracts were assayed spectrophotometrically for citrate synthase in 100 mM Tris-HCl, 0.3 mM acetyl-CoA, 0.1 mM DTNB and 0.1 mM oxaloacetate, as described in [58].

2.6. Immunoblotting Assays. Cells were harvested and lysed as described in the previous section, and after total protein content determination by the bicinchoninic acid method, proteins (20 \( \mu \)g per lane) were separated by SDS-PAGE using Mini-PROTEAN TGX Stain-Free Precast Gels (Bio-Rad). The formulation of these gels includes trihalo compounds which lead to UV fluorescence emission upon reaction with proteins [59], allowing estimation of the total protein load in a gel lane, using a ChemiDoc MP imaging system (Bio-Rad) without resorting to staining procedures or housekeeping proteins for normalization purposes. Proteins commonly used as housekeepers, such as glyceraldehyde 3-phosphate dehydrogenase and β-actin, indeed are known to change their expression levels under hypoxia [60, 61]. Afterwards, the proteins separated by SDS-PAGE were transferred onto a polyvinylidene difluoride membrane using a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (from Bio-Rad) at 180 mA for 30 min. The membrane was blocked with PBS-T (phosphate-buffered saline with 0.1% Tween 20 (v/v)) containing 3% bovine serum albumin (BSA, w/v) and then incubated overnight at 4°C with the antibody against human SQR (1:150, in PBS-T with 3% BSA (w/v)). After three washing steps with PBS-T (15 min), the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (1:5000, in PBS-T with 3% BSA (w/v)), followed by three washing steps with PBS-T (15 min) and detection by enhanced chemiluminescence (Clarity Western ECL Substrate, Bio-Rad). Finally, the blotted membrane was subjected to densitometric analysis using the Image Lab software (Bio-Rad), followed by the normalization of the target protein band intensity to the total protein load determined as described above.

2.7. Data Analysis. Oxygen consumption rates (OCR) were calculated using the software DatLab4 (Oroboros Instruments, Austria). Data are reported as mean ± standard error of the mean (SEM). Statistical significance (P) was estimated using Student’s t-test in Microsoft Excel. *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 were considered significant.
3. Results

Colorectal cancer SW480 cells were either grown under normoxic (air O_2) conditions or exposed for 24 h to hypoxia (1% O_2), and their sulfide-oxidizing activity was assayed by high-resolution respirometry, according to Abou-Hamdan et al. [25], as described in Materials and Methods. A representative oxygraphic trace acquired with untreated ("normoxic") cells is shown in Figure 1(a). The trace shows that ~80% of oxygen consumption was blocked by the addition of the complex I inhibitor rotenone, added to prevent sulfide oxidation through reversal of complex I activity, as described in [21, 62]. Sulfide was then injected five times at increasing rates into the oxygraphic chamber via a micro-pump. The first four injections led to the stimulation of O_2 consumption, pointing to a fully operative mitochondrial sulfide-oxidizing pathway in the tested cells (Figures 1(a) and 1(b)). The stimulation persisted for the entire duration (3 minutes) of sulfide injection, after which the O_2 consumption rate (OCR) declined back to the value measured in the absence of sulfide. The decline took a few minutes, as if some sulfide persisted in solution, sustaining cell respiration even after the injection was stopped. The extent of O_2 consumption stimulation by sulfide increased with the rate of sulfide injection (up to 80 nL·s^{-1}, Figures 1(a) and 1(c)). However, upon further increasing the injection rate (to 160 nL·s^{-1}), a decline...
in OCR was observed already before sulfide injection was stopped, likely due to CcOX inhibition by sulfide, as suggested previously [25].

For comparison, the measurements described above were carried out on the same cells after 24 h exposure to hypoxic conditions. A representative oxygraphic trace is shown in Figure 1(b). Hypoxia-treated cells displayed a lower basal respiratory activity compared to untreated cells (6.3 ± 0.5 nM O₂·s⁻¹ vs. 17.1 ± 1.1 nM O₂·s⁻¹ per million cells). Yet, as observed for normoxic cells, after rotenone addition a progressive stimulation of cell respiration was observed upon injecting sulfide at an increasing rate (Figures 1(b) and 1(d)), until the amount of injected sulfide exceeded the detoxifying activity of the cells, and CcOX inhibition occurred, leading to impairment of cell respiration (see last sulfide injection in Figure 1(b), top).

To evaluate the contribution of mitochondria to the observed sulfide-oxidizing activity, we used antimycin A, a known inhibitor of complex III that blocks quinol oxidation in the respiratory chain and thus prevents sulfide oxidation by mitochondria [25]. As shown in Figures 1(a) and 1(b) (bottom traces), in the presence of rotenone, antimycin A considerably prevented O₂ consumption stimulation by sulfide in both normoxic and hypoxia-treated cells, proving that under the tested conditions sulfide oxidation occurs mostly at the mitochondrial level. The effect of sulfide on mitochondrial O₂ consumption was quantitatively evaluated by subtracting the OCR values measured during sulfide injection.
in the presence of both rotenone and antimycin A from those measured at identical sulfide injection rates in the presence of rotenone only (see legend of Figure 1 for more details). According to this analysis, at the highest non-inhibitory (for CcOX) injection rate sulfide sustained a mitochondrial O$_2$ consumption of 9.7 ± 1.2 nM O$_2$·s$^{-1}$ and 7.3 ± 0.8 nM O$_2$·s$^{-1}$ per million cells, in normoxic and hypoxia-treated cells, respectively. Considering that the mitochondrial sulfide-oxidizing pathway overall was reported to consume ~1.33 molecules of H$_2$S per O$_2$ molecule [21], a mitochondrial sulfide-oxidizing activity of 12.8 ± 1.5 nM H$_2$S·s$^{-1}$ per million cells was estimated for normoxic and hypoxia-treated cells, respectively (Figure 2(a)). To evaluate the mitochondrial content in the tested cells, we carried out citrate synthase activity assays, a validated surrogate biomarker of mitochondrial content ([63] and references therein). Normoxic and hypoxia-treated cells displayed, respectively, a citrate synthase activity of 1.1 ± 0.1 μmol·min$^{-1}$·10$^6$·cells$^{-1}$ and 0.6 ± 0.1 μmol·min$^{-1}$·10$^6$·cells$^{-1}$ (Figure 2(b)), consistent with a reduction in the mitochondrial content upon exposure to hypoxia [44–46]. The measured citrate synthase activity was used to normalize the calculated mitochondrial sulfide-oxidizing activity, which proved to be in hypoxia-treated cells ~1.4-fold higher than in normoxic cells (Figure 2(c)). Finally, we have assayed by immunoblotting combined with “stain-free” imaging technology the SQR expression level in the tested cells (Figure 3(a)) and found that hypoxia-treated cells display 1.4-fold higher SQR protein levels than normoxic cells (Figure 3(b)). Considering that hypoxia-treated cells

![Figure 3: Effect of hypoxia on SQR expression. Representative Western blot analyzing SQR expression in normoxic and hypoxia-exposed SW480 cells (a), with the corresponding total protein load quantitation by stain-free imaging technology (see Materials and Methods). SQR levels in normoxic (n = 4 in triplicate, blue bars) and hypoxia-treated cells (n = 4 in triplicate, red bars), as normalized to total protein (b) or citrate synthase activity (c). ***P ≤ 0.001.](image-url)
have a lower mitochondrial content (based on citrate synthase activity assays, Figure 2(b)), we estimate that the mitochondria of hypoxia-treated cells contain 2.6-fold more SQR than those of normoxic cells (Figure 3(c)).

4. Discussion

O₂ and H₂S are key molecules in living systems, able to control each other’s availability, and regulate numerous processes in human (patho)physiology. As reviewed in [47], the interplay between H₂S and O₂ is intricate and based on several mechanisms: (i) direct reaction between the two, (ii) O₂-dependent H₂S breakdown through the mitochondrial sulfide-oxidizing pathway, (iii) H₂S-mediated stimulation or inhibition of mitochondrial O₂ consumption, (iv) O₂-dependent regulation of expression and cellular relocalization of the H₂S-synthesizing enzymes, and (v) O₂-dependent control of CO-mediated inhibition of H₂S production by CBS. H₂S has indeed been recognized as an O₂ sensor [64]. Despite this, to our knowledge no studies have been conducted yet to explore the effect of prolonged exposure to hypoxia on the cell ability to dispose of H₂S, which represented the main objective of the present study.

Under hypoxic conditions, H₂S plays a key protective role against ischemia/reperfusion damages [54, 55] through only partly understood molecular mechanisms including induction of antioxidant and vasorelaxation effects on microcirculation. Moreover, H₂S appears to mediate the repair of damaged mitochondrial DNA [36], occurring in ischemia/reperfusion, and to protect from hypoxia-induced proteostasis disruption, as demonstrated in Caenorhabditis elegans [65]. In knockdown experiments with Hepa1-6 cells, H₂S-mediated protection during O₂ deprivation was found to require SQR [66], pointing to a key role of H₂S catabolism in the cellular protective responses to hypoxia. Consistently, under hypoxic conditions, thiosulfate, a major product of H₂S oxidation, has been shown to exert protective effects against ischemia/reperfusion damage [66–68] and also to generate H₂S [69]. In this context, it is noteworthy that H₂S is able to mimic hypoxia-induced responses such as vasodilation [70], neoangiogenesis [71], and expression of the hypoxia-inducible factor (HIF-1α, [72]), a master gene regulator promoting cell survival under hypoxic conditions shown to stimulate CBS expression in hypoxia [49]. The occurrence of H₂S under hypoxic conditions is therefore likely part of a more general adaptive response adopted by...
the cells to ensure survival and protection from damages resulting from $O_2$ deprivation (and possible reoxygenation).

In hypoxic cells, H$_2$S bioavailability therefore needs to be finely regulated for this gaseous molecule to occur at physiologically protective yet non-poisonous levels. In this regard, it seems relevant to gain insight into the regulation of H$_2$S production and breakdown at low $O_2$ tensions. Previous studies focused on the H$_2$S-synthesizing enzymes have shown that, under hypoxic conditions, H$_2$S synthesis is enhanced [47] through multiple mechanisms [49–53] (see Introduction). In addition, H$_2$S breakdown via both chemical and enzymatic reaction pathways is negatively affected by low $O_2$ tensions. Evidence for a lower mitochondrial sulfide-oxidizing activity at lower $O_2$ concentrations was initially provided in [73] working on immortalized cells derived from alveolar macrophages and, then, corroborated by Abou-Hamdan et al. in a more recent investigation on CHO cells [74].

In the present study, using SW480 colorectal cancer cells as a model, we tested the effect of prolonged (24 h) exposure to 1% $O_2$ on the cellular ability to dispose of sulfide at the mitochondrial level. Exposure to hypoxia leads to a notable (2.7-fold) reduction in basal respiration and to a marked (1.8-fold) decrease in the mitochondrial content (Figure 2(b)), as previously documented and suggested to result from enhanced mitophagic activity and reduced organelle biogenesis [44–46]. Hypoxia-treated cells also display a lower ability to dispose of H$_2$S as compared to normoxic cells (Figure 2(a)). However, considering the above-mentioned decrease in mitochondrial content, the sulfide-detoxyfying capacity of hypoxia-treated cells normalized to their minor mitochondrial content actually turned out to be 1.4-fold higher than that of untreated cells, pointing to an enhanced sulfide disposal capacity of mitochondria in hypoxia-treated cells. To gain further insight, we analyzed the SQR expression by immunoblotting, employing “stain-free” imaging technology for total protein quantitation and normalization purposes. Using this approach, we made the somewhat puzzling observation that hypoxia-treated cells, though displaying slightly reduced overall sulfide-oxidizing activity, have modestly (∼1.4-fold) increased SQR levels. Interestingly, normalizing the SQR expression to the mitochondrial content revealed that, in line with their enhanced sulfide-oxidizing capacity, mitochondria of hypoxia-treated SW480 cells have ∼2.6-fold higher levels of SQR than those of normoxic cells. Altogether, these results are intriguing in that they suggest that mitochondria in hypoxia-treated cells display lower mass but are enriched in SQR. The increased SQR levels could have a protective role in hypoxic cells preventing mitochondria to be poisoned by enhanced production of sulfide (Figure 4).

5. Conclusions

This is to our knowledge the first study in which the effect of prolonged cell exposure to hypoxia on the mitochondrial sulfide-oxidizing activity has been evaluated. The evidence collected here on SW480 colorectal cancer cells shows that hypoxia-treated cells metabolize sulfide with overall reduced maximal efficacy and have reduced mitochondrial content, but mitochondria are better equipped to dispose of H$_2$S. Physiologically, this may represent a regulatory mechanism to ensure higher protective H$_2$S levels, while protecting mitochondria from H$_2$S toxicity.

Abbreviations

H$_2$S: Hydrogen sulfide  
SQR: Sulfidequinone oxidoreductase  
NO: Nitric oxide  
CO: Carbon monoxide  
CcOX: Cytochrome c oxidase  
CBS: Cystathionine $\beta$-synthase  
CSE: Cystathionine $\gamma$-lyase  
MST: 3-Mercaptoppyruvate sulfurltransferase  
$SO_4^{2-}$: Sulfate  
$S_2O_3^{2-}$: Thiosulfate  
DTNB: 5,5′-Dithiobis-(2-nitrobenzoic acid)  
PBS-T: Phosphate-buffered saline with 0.1% Tween 20 (v/v)  
OCR: Oxygen consumption rate.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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