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Potential role of the 3-mercaptopyruvate sulfurtransferase (3-MST)—hydrogen sulfide (H_2S) pathway in cancer cells

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ABSTRACT

Hydrogen sulfide (H_2S), produced by various endogenous enzyme systems, serves various biological regulatory roles in mammalian cells in health and disease. Over recent years, a new concept emerged in the field of H_2S biology, showing that various cancer cells upregulate their endogenous H_2S production, and utilize this mediator in autocrine and paracrine manner to stimulate proliferation, bioenergetics and tumor angiogenesis. Initial work identified cystathionine-beta-synthase (CBS) in many tumor cells as the key source of H_2S. In other cells, cystathionine-gamma-lyase (CSE) has been shown to play a pathogenetic role. However, until recently, less attention has been paid to the third enzymatic source of H_2S, 3-mercaptopyruvate sulfurtransferase (3-MST), even though several of its biological and biochemical features - e.g. its partial mitochondrial localization, its ability to produce polysulfides, which, in turn, can induce functionally relevant posttranslational protein modifications - makes it a potential candidate. Indeed, several lines of recent data indicate the potential role of the 3-MST system in cancer biology. In many cancers (e.g. colon adenocarcinoma, lung adenocarcinoma, urothelial cell carcinoma, various forms of oral carcinomas), 3-MST is upregulated compared to the surrounding normal tissue. According to *in vitro* studies, 3-MST upregulation is especially prominent in cancer cells that recover from oxidative damage and/or develop a multidrug-resistant phenotype. Emerging data with newly discovered pharmacological inhibitors of 3-MST, as well as data using 3-MST silencing approaches suggest that the 3-MST/H_2S system plays a role in maintaining cancer cell proliferation; it may also regulate bioenergetic and cell-signaling functions. Many questions remain open in the field of 3-MST/cancer biology; the last section of current article highlights these open questions and lays out potential experimental strategies to address them.

1. Introduction

Hydrogen sulfide (H_2S) is now commonly accepted as a gaseous biological transmitter in mammalian cells. It belongs to a class of gaseous molecules (gasotransmitters), together with nitric oxide (NO) and carbon monoxide (CO). Several distinct mammalian enzyme systems producing H_2S have been characterize in detail. Most commonly, three 'classical' H_2S-producing enzymes are recognized: cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) (reviewed in 1–3), although there are several additional accepted or putative enzymatic sources of H_2S, including D-amino acid oxidase [4,5] and methanethiol oxidase [6]. Moreover, several non-enzymatic processes can also yield H_2S in biological systems [2].

As a labile, diffusible molecule, H_2S obeys to a different set of rules than most other classes of biological mediators and pharmacological agents. It can rapidly travel from one cell compartment to another, or from one cell to a surrounding cell, and it can initiate signaling through interactions with a wide variety of molecules [1,2]. In these signaling actions, a unique post-translational protein modification process, termed sulfhydration, plays an important role [7,8].

Commonly recognized biological roles of H_2S - at lower concentrations - include actions as a vasodilator, cytoprotectant and anti-inflammatory agent. However, H_2S also has a characteristic bell-shaped or biphasic biological character: it exerts cytotoxic and deleterious effects at higher concentrations [1–3]. The cellular level of H_2S (and consequently, the biological character of H_2S) is heavily influenced not only by its rate of enzymatic production, but also its degradation.

Abbreviations: ATP, adenosine triphosphate; CAT, cysteine aminotransferase; CBS, cystathionine-β-synthase; CO, carbon monoxide; CSE, cystathionine-γ-lyase; DHLA, dihydrolipoic acid; ETHE1, Ethylmalonic Encephalopathy 1 Protein; H_2S, hydrogen sulfide; MCDU, mercaptolactate-cysteine disulfiduria; 3-MST, 3-mercaptopyruvate sulfurtransferase; NO, nitric oxide; ROS, reactive oxygen species; TUM1, tRNA thiouridin modification protein 1

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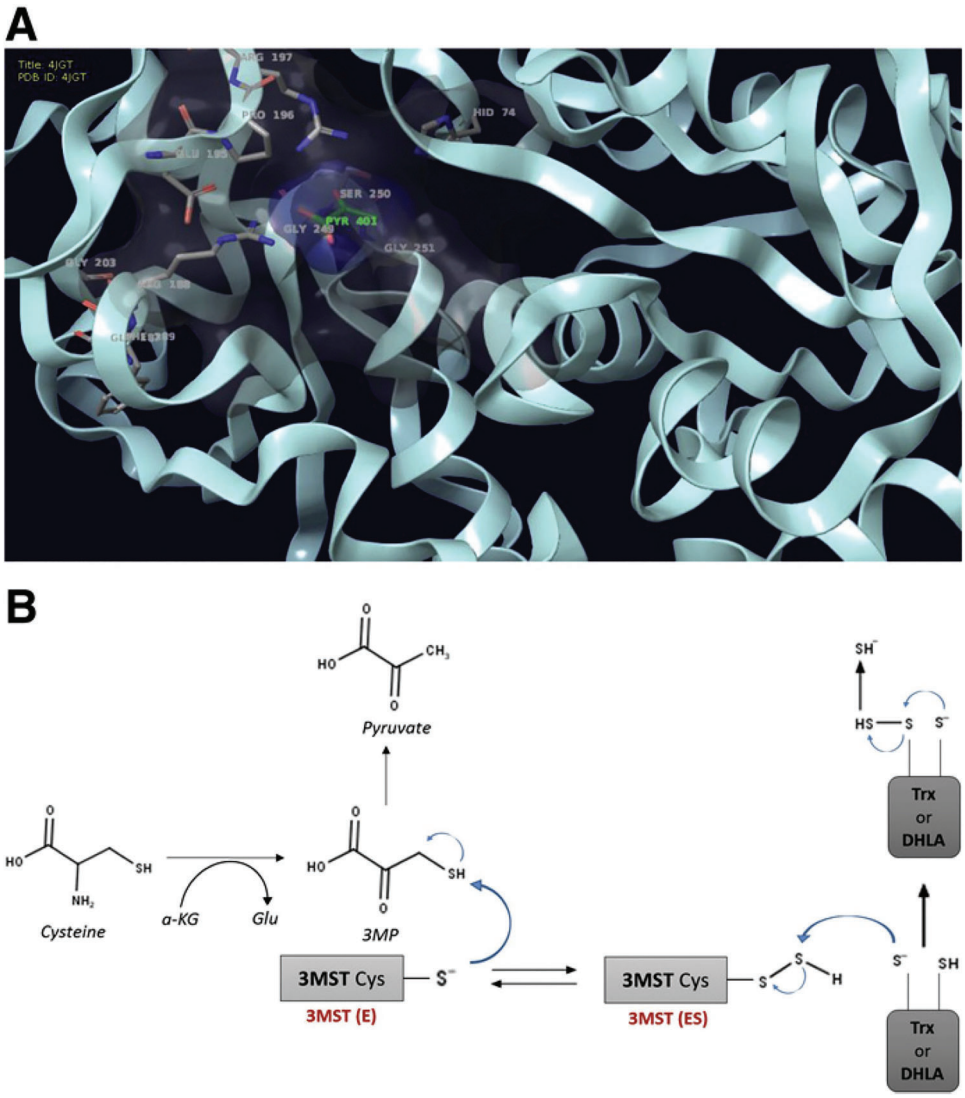

Fig. 1. Structure and function of 3-MST. (A) 3-MST is a 33-kDa protein where the C-terminal is catalytically active. The catalytic site cysteine, Cys247, is redox-active and is oxidized to form sulfenyl cysteine, which, in turn, gets reduced to the active form by thioredoxin (Trx). Cys247 serves as an intrasubunit redox-sensing switch. **(B)** The production of H_2S by 3-MST in the presence of Trx or dihydrolipoic acid (DHLA). 3-MST reacts with 3 M P to produce H_2S via a persulfide intermediate. Trx or DHLA accepts a sulfur atom from a persulfide intermediate that is attacked by another thiol and releases H_2S. The figure and its legend are reproduced by permission from [2].

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Several H_2S-metabolizing systems have been characterized, including rhodanese and Ethylmalonic Encephalopathy 1 Protein (ETHE1) [9].

The biological roles of H_2S has been intensively studied in health and disease. Specialized, comprehensive review articles focus on the historical aspects of H_2S biology [10], on pharmacological modulation of H_2S biology (such as pharmacological inhibitors of H_2S-producing enzymes, and the various classes of small molecule H_2S donors that can deliver H_2S for biological purposes) [2], as well as on the physiological and pathophysiological roles of H_2S in various fields, such as the cardiovascular system [11,12] or the central and peripheral nervous system [13,14]. The emerging role of H_2S in cancer biology has also been the subject of several recent reviews, predominantly with a focus on the pathogenetic roles of CBS or CSE [15–17].

2. 3-MST, a source of H_2S and polysulfides in mammalian systems

3-MST has been known as a mammalian protein for several decades [18]. It is a 33 kDa, Zn-dependent enzyme, which exists in a monomer-dimer equilibrium, with 3-MST monomer being the active form. Cys154 and Cys263 are involved in intermolecular disulfide formation, which affects the activity of the enzyme [18] (Fig. 1).

3-MST shows both cytosolic and mitochondrial localizations [19]. The intracellular distribution may be, at least in part related to two splice variants of the enzyme. Both of these variants (TUM1-Iso1 and TUM1-Iso2) have similar enzymatic activity, but different localization: TUM1-Iso1 is cytosolic, while TUM1-Iso2 exists both in the cytosol and in mitochondria [20]. 3-MST appears to be present in all mammalian tissues, although its expression levels are tissue-dependent; kidney, liver, brain, testes, large intestine and endocrine organs show especially high expression levels [18].

With respect to 3-MST's biological function, early studies characterized it as an intracellular antioxidant and detoxification enzyme [18]. 3-MST is also involved in the thiolation of cytosolic tRNAs - in this field of research, another nomenclature is used, and 3-MST is termed as is termed TUM1 (tRNA thiouridin modification protein 1) [20].

The fact that 3-MST is a "reactive-sulfur-producing" and/or H_2S-producing enzyme has been accumulating for several decades. The capacity of 3-MST to produce polysulfides has been demonstrated by Hylin and Wood as early as 1959 [21]. Based on studies by Ogasawara and colleagues [22] and Wrobel and colleagues [23], already in 2004, Kamoun was attributing a H_2S-producing capability to 3-MST [24]. Studies by Shibuya and Kimura in 2009 in endothelial cells and in neuronal tissues have further confirmed and extended these findings [25]. Subsequent work by the Kimura group characterized the various sulfur-species produced by 3-MST and identified several species, with putative biological activity such as H_2S_2 and H_2S_3 [26,27].

It is a combined action of 3-MST and a second enzyme (cysteine aminotransferase or CAT; also known as L-cysteine:2-oxoglutarate aminotransferase, aspartate aminotransferase or aspartate/cysteine aminotransferase) is required for the biological synthesis of H_2S. The

molecular mechanism of 3-MST-mediated H_2S production involves the transfer of the sulfur from 3-mercaptopyruvate to a nucleophilic cysteine (Cys247) in its active site; the protein persulfide then yields H_2S through the action of intracellular reductants (e.g. thioredoxin, dihydrolipoic acid) or various intracellular enzymes [18,28,29].

The biochemical properties of 3-MST are such that in the intracellular environment it is more prone to the formation of polysulfides, than the formation of H_2S [26,27]. Polysulfides are now increasingly recognized as biological signaling molecules, with properties that are distinct from those of H_2S. For instance, a solid body of experimental evidence indicates that cysteine sulfhydration (Cys-SH) to Cys-SSH; a functionally important posttranslational modification commonly attributed to H_2S), is the consequence of the action of biologically active polysulfides - and not a direct effect of H_2S [29,30], while H_2S can S-sulfurate Cys-SOH and Cys-SNO to Cys-SSH [31]. The multiple biochemical mechanisms of cysteine-sulfhydration remain to be further studied, especially in the cellular environment, where increased H_2S production and increased polysulfide accumulation tends to go hand in hand.

3-MST contains a critical catalytic site cysteine, Cys247, which is redox sensitive [18]. Accordingly, oxidative stress has been shown to suppress the catalytic activity of 3-MST, thereby suppressing 3-MST-mediated H_2S production [32–34].

It should be mentioned that the substrate of 3-MST, 3-mercaptopyruvate, can also generate H_2S spontaneously (i.e. through non-enzymatic mechanisms) [35,36]. Whether this effect has biological relevance remains to be determined, nevertheless this effect can complicate the interpretation of biological experiments, especially when higher (high micromolar or low millimolar) concentrations of 3-mercaptopyruvate are applied.

One of the problems that has limited the progress of 3-MST research was the fact that potent or selective pharmacological inhibitors of 3-MST were not available. This situation has recently been, at least in part, rectified, when, through a high-throughput screening of a diverse chemical compound library, Hanaoka and colleagues identified several new small-molecule inhibitors with good potency and fairly good selectivity for 3-MST (over the other H_2S-producing enzymes CBS and CSE) [37] (Fig. 2). (Whether these compounds have secondary pharmacological activity on other enzymes remains to be determined.) Although it would be preferable if several, additional, chemically distinct classes of 3-MST inhibitors were available, the Hanaoka compounds represent a good starting point and initial tools to characterize the biological roles of the 3-MST system through pharmacological experiments.

How the expression and/or activity of 3-MST is regulated by physiological factors, hormones, or various (patho)physiological conditions is largely unknown. An early study by Wrobel and colleagues demonstrated that mitochondrial (but not cytosolic) 3-MST activity is reduced, when animals were treated with thyroxine [38]. In several *in vitro* studies, hypoxia has been demonstrated to increase 3-MST expression [39–41].

Even though 3-MST is ubiquitous, and it appears to be involved in multiple important biological processes, global deletion of 3-MST does not create a robust phenotype [42] - perhaps due to the fact H_2S and polysulfides are also be produced by enzymatic sources other than 3-MST as well. One characteristic feature of the 3-MST-deficient animals is an anxiety-like behavior, which may be associated with the elevated levels of serotonin in the central nervous system of the 3-MST deficient animals [42]. Whether these alterations are the consequences of H_2S or polysulfide signaling processes is currently unknown.

In humans, genetic polymorphisms for 3-MST has been demonstrated, with various intronic polymorphisms and a nonsense mutation; the functional consequence of these polymorphisms is a reduction in 3-MST catalytic activity [43]. There is a very rare human inheritable disease caused by 3-MST deficiency, called mercaptolactate-cysteine disulfiduria (MCDU) [44].

3. Expression of 3-MST in cancer

Since 3-MST is expressed constitutively in all somatic cells, it is probably not surprising that it is also expressed in various cancer cells. One of the early studies focusing on the expression of 3-MST in cancer cells was conducted by Wrobel's group in 2011: significant amounts of 3-MST expression and enzymatic activity were found in all 5 investigated human neoplastic cells lines (the U373 astrocytoma line, the SH-SY5Y neuroblastoma line and two melanoma cell lines, A375 and WM35). Compared to CSE, 3-MST expression and 3-MST specific activity were substantially higher in these cell lines, leading to the conclusion that 3-MST is a more significant source of H_2S than CSE in these cells [45,46]. Additional cancer cell lines where 3-MST expression and/or catalytic activity has been demonstrated include the glioblastoma-astrocytoma cell line U-87 [46], various hepatoma lines (Hepa1c17 and HepG2) [47,48], various colon cancer cell lines (HCT116, LoVo, HT-29) [49–51], various lung adenocarcinoma lines (A549, H522, H1944) [52,53], a renal cell carcinoma line RCC4 [54], various urothelial cancer cell lines [55,56] and in various melanoma cell lines (A375, Sk-Mel-5, Sk-Mel-28 and PES 43) [57]. Please note that many of these cell lines - e.g. A549 cells have abundant thioredoxin-reductase [58]; this would be expected to facilitate the function of the 3-MST system.

Interestingly, 3-MST was found to be upregulated in cancer cell lines that are multidrug-resistant and/or exhibit stem-like properties when they recover from a stressful/cytotoxic stimulus [48,50]. This observation suggests (but certainly does not prove) that 3-MST may confer some cytoprotective or otherwise advantageous (for the tumor cell) roles in advanced/drug-resistant cancers.

As far as primary tumor tissues, 3-MST expression has been demonstrated in human brain gliomas, where its activity tended to decrease with higher grades of the malignancy [59]. In this context, it is also interesting to note that glioblastoma-bearing ipsilateral hemispheres were found to contain greater amounts of H_2Sn than the glioblastoma-free control hemispheres [60]. In contrast, in human melanoma specimens, the expression of 3-MST was detectable with some frequency (25–50% of the sections analyzed) and it was undetectable in the other portion (50–75% of the sections) [57]. In human colon cancer resections [51], in human lung carcinoma resections [52], in human urothelial cell carcinoma of the bladder resections [55,56] in human oral squamous cell carcinoma resections [61] and in oral adenoid cystic carcinoma resections [62] 3-MST expression has been demonstrated by immunohistochemistry and/or by Western blotting; in all of these cancers tumor 3-MST expression was either significantly higher or tended to be higher than 3-MST expression in the surrounding non-cancerous tissues. In contrast, in human papillary thyroid cancer resections 3-MST expression was moderate and was not different from the surrounding tissue [63], while in renal cell carcinoma resections the level of 3-MST expression was highly variable [54].

4. The function of the 3-MST/H_2S system in cancer

What, then, is the functional role of the 3-MST/H_2S system in cancer cells? Because pharmacological inhibitors with suitable potency and selectivity - until recently - were not available, and because silencing of 3-MST has only rarely been used in the published reports, a lot of the published data rely on correlations and inferences, and clearly, additional, definitive studies remain to be conducted to address this question.

Historically, one of the earlies reports that placed the 3-MST system in the context of cancer came from Ernest Kun's group in 1960. They have correlated the metabolism of 3-mercaptopyruvate in tumor cells vs. normal cells and found that tumor cells/tumor tissues appear to have *lower* enzymatic activity than normal tissues [64]. Similarly, Wlodek and colleagues found that the activity of 3-MST (as well as the activity of cysteine aminotransferase) was *lower* in tumor cells (Ehrlich ascites cells) than in normal tissue (mouse liver) [65]. These early

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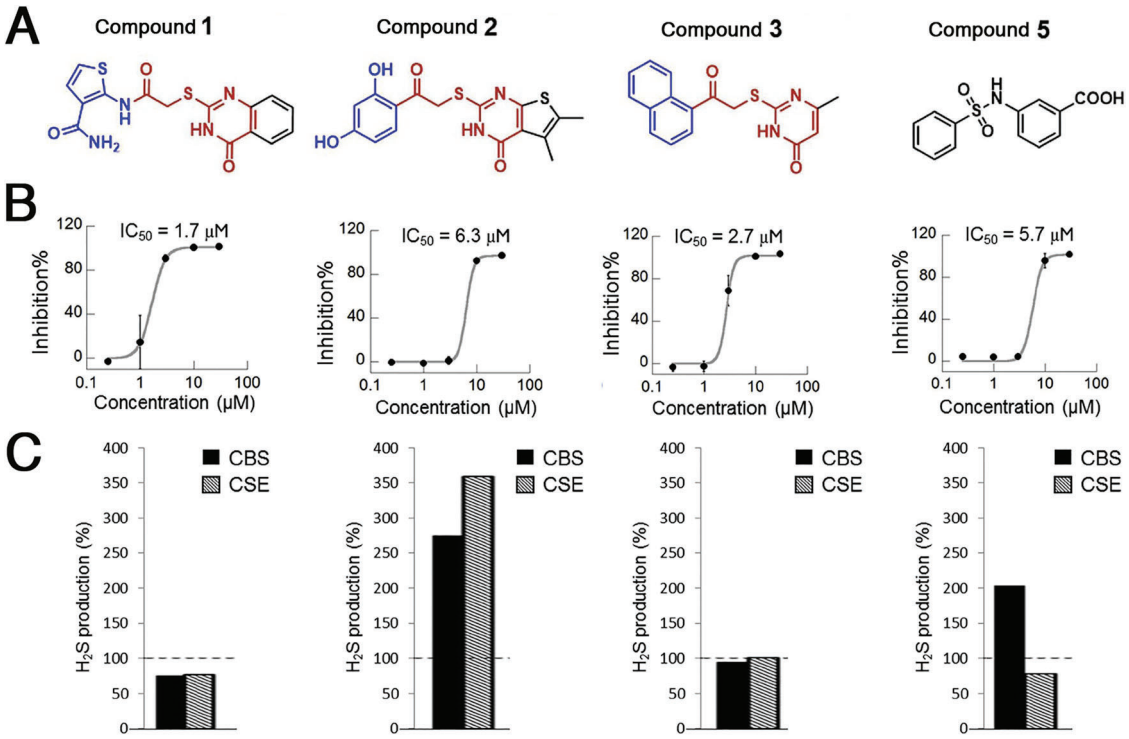

Fig. 2. Recently identified 3-MST inhibitors. Screening of a chemical library of 174,118 compounds identified several 3-MST inhibitors (Compounds 1, 2, 3 and 5). **(A)** The structures of 4 hit compounds are shown, together with **(B)** with dose-response curves of the same compounds against 3-MST activity in a fluorescent H_2S assay. Bar graphs in **(C)** show the inhibitory effect of the same compounds on recombinant CSE and CBS at 100 µM. Note that Compound 3 does not affect CBS or CSE activity and is therefore selective as a 3-MST inhibitor over the two other H_2S-producing enzymes. Reproduced by permission from [35].

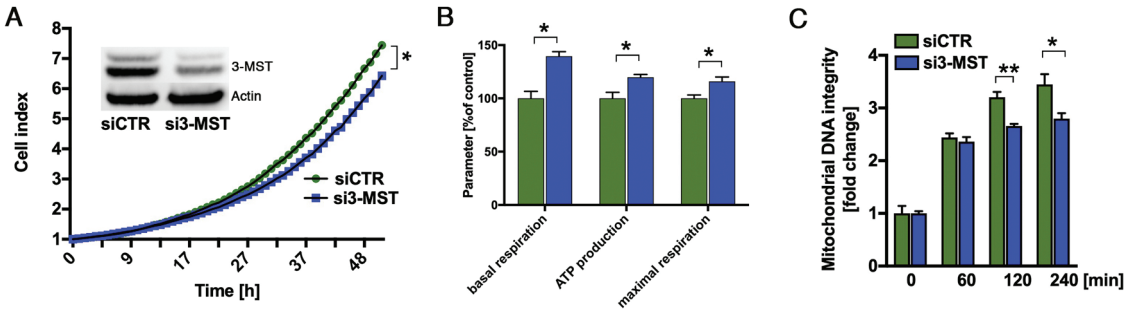

Fig. 3. 3-MST silencing exerts functional effects in A549 lung adenocarcinoma cells. (A) SiRNA-mediated 3-MST silencing (silencing efficiency confirmed by Western blot, inset) attenuates the proliferation of A549 cells (xCELLigence microplate assay) **(B)** 3-MST silencing increases bioenergetic parameters in A549 cells (Seahorse Extracellular Flux analysis). **(C)** 3-MST silencing attenuates the recovery of mitochondrial DNA integrity after oxidative stress (elicited by glucose oxidase) induced mitochondrial DNA damage. n = 5–6, $p < 0.05$. Part C is reproduced by permission from [52].

findings appear to be in contrast to the studies listed in the previous section, showing *high* expression of 3-MST in many tumors. The explanation for this discrepancy is unclear; it is possible that in tumor cells (that typically exhibit increased oxidative stress burden) 3-MST may be in an oxidized (i.e. partially inactivated) state; it is also likely that the *ex vivo* enzymatic assays do not recapitulate the conditions that are present inside living cells. It is important to note that cancer cell 3-MST activity in these early papers was compared to 3-MST activity in liver or kidney homogenates, which have the highest 3-MST expression/activity amongst all parenchymal organs. Thus, cancer cells may have lower 3-MST activity than the activity of these organs, but nevertheless they still have significant (detectable) activity, which can confer significant functional roles.

In 2013 we studied the functional effect of 3-mercaptopyruvate and the effect of 3-MST silencing in the murine hepatoma cell line Hepa1c1c7. The 3-MST substrate induced, at low concentrations, a stimulatory bioenergetic effect, while at higher concentrations, an inhibitory effect was apparent. When 3-MST was silenced, the basal bioenergetic function of the cells was slightly, but significantly suppressed, and the stimulatory effect of 3-mercaptopyruvate was no longer detectable [47]. As expected, the ability of the cells (or mitochondria isolated from them) to produce H_2S in response to 3-mercaptopyruvate was markedly reduced after 3-MST silencing [47]. The 3-mercaptopyruvate-mediated stimulatory bioenergetic effects appeared to be, at least in part, related to direct electron donation (by the H_2S produced from 3-MST) into the mitochondrial electron transport system, because silencing of the enzyme SQR (sulfur-quinone-oxidoreductase, the enzyme that is obligatory for this form of electron donation) attenuated the bioenergetic stimulatory effect of 3-mercaptopyruvate [47].

In a follow-up study we have started to test the functional effect of 3-MST silencing in A549 cells (a human lung adenocarcinoma line). Silencing of 3-MST resulted in a reduced proliferation rate of the cells, and attenuated their mitochondrial DNA repair rate after mitochondrial

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DNA damage (Fig. 3) [52]. However, in these cells, 3-MST silencing elevated (rather than decreased) bioenergetic function (Fig. 3). Since H_2S has bell-shaped effect on cellular bioenergetics, it is possible that the degree of 3-MST silencing affects the direction in which the bioenergetic alterations change after 3-MST silencing; cell-type-dependent differences are also possible. In RCC4 cells (renal cell carcinoma), the degree of cellular apoptosis - elicited by AIK (Apoptosis Inducer Kit, Abcam) - was unaffected by 3-MST silencing [54], once again, suggesting that the functional role/importance of the 3-MST system may be cell-type and/or stimulus and context-dependent.

The recent availability of pharmacological 3-MST inhibitors enabled a new wave of pharmacological studies exploring the various roles of the 3-MST system in health and disease. As far as the effect of 3-MST inhibitors on cancer cells, the only published paper, so far, evaluates the effect of "Compound 3″ - (or HMPSNE: 2-[(4-hydroxy-6-methylpyrimidin-2-yl)sulfanyl]-1-(naphthalen-1-yl)ethan-1-one) originally described by Hanaoka and colleagues (Fig. 2) [37] - in HCT116 cells and demonstrates that the inhibitor exerts a concentration-dependent suppression of cellular proliferation rate, as well as - at higher concentrations, above 300 μM - an inhibition of cell viability (assessed by the MTT assay) [51].

Cancer cells benefit from a well-organized and multifunctional microenvironment, which consists of many cell types, including endothelial cells and other vascular cells, immune cells, fibroblasts and others [66,67]. Whether the 3-MST system plays a role in the organization or function of this tumor cell microenvironment is largely unknown. There are a limited number of studies indicating that the 3-MST system (i.e. 3-MST-derived H_2S produced by the endothelial cells themselves) plays a role in vascular relaxation, endothelial cell proliferation, migration and angiogenesis, especially under hypoxic conditions [35,41,68,69]. Part of these responses appear to be dependent on the regulation of endothelial cell bioenergetics and metabolomics by the 3-MST system [69]. Whether these findings have any significance for the function of the tumor microenvironment remains to be tested in future experiments.

So far, there are no published in vivo studies with 3-MST inhibitors or 3-MST deficient systems in cancer. Therefore, future work is needed to assess whether 3-MST - similar to CBS and CSE, which, in many forms of cancer, support tumor-promoting functions, and inhibition of which can suppress tumor growth [16] - can affect tumor growth, angiogenesis or other functional parameters in tumor-bearing animals in vivo.

When interpreting the existing body of literature (or future publications) we always have to keep in mind that the 3-MST system's roles go beyond H_2S production. Thus, the functional effects of 3-MST inhibitors and 3-MST silencing are likely to be a combination of H_2S-related effects, as well as effects on various redox processes that 3-MST regulates. In addition, with 3-MST silencing, 3-MST-mediated protein-protein interactions would also become suppressed, with potential functional consequences. Such protein-protein interactions, in fact, have been reported; for example, 3-MST physically associates with the L-cysteine desulfurase NFS1 and the rhodanese-like protein MOCS3 [20].

5. Unanswered questions, future research directions

Within the tumor cells, H_2S elicits a bell-shaped concentration-response curve, where an optimal (i.e. optimal for the cancer cell, not for the tumor-bearing host) level of H_2S (produced by some combination of the three main enzymes, CBS, CSE and 3-MST) contributes to the formation of a pro-energetic and pro-proliferative as well as cytoprotective environment [16]. How exactly the 3-MST system fits into this scheme - in a presumably tumor cell-type- and perhaps tumor-stage-dependent fashion - remains to be further defined. In the previous section we have presented some data, in hepatoma cells, that suggests that part of the role of the 3-MST system may be to support mitochondrial function (electron transport, mitochondrial DNA repair) [47,52]. A potential extension of this work would be to conduct a full metabolommic analysis, to map out all the intracellular metabolic processes that are affected by 3-MST.

Whether the 3-MST/H_2S system also "drives" proliferative or cytoprotective signaling (and if so, through what pathway) remains to be evaluated in future studies. In general, endogenously produced H_2S can stimulate these pathways through a number of pathways, including the PI3K pathway, the guanylate cyclase pathway, the Nrf2 pathway and many others [2]. Since the 3-MST system may be more of a source of polysulfides than 'free' H_2S, and since polysulfides are important molecules to elicit sulfhydration (a functionally relevant posttranslational protein modification), it is conceivable that the presence or absence (or functional activity) of 3-MST has wide-ranging roles in the modulation of the activity of various (mitochondrial and cytoplasmic) proteins. A full 'sulfhydrome' analysis in cancer cells with/without 3-MST inhibition or 3-MST silencing could address this question.

Another, almost completely open question is how H_2S (3-MST-derived, or otherwise) interacts the various constituents of the cellular microenvironment. As mentioned in the previous section, the 3-MST system plays a role in the stimulation of angiogenesis within the endothelial cells. It is also conceivable that tumor-derived H_2S (since H_2S is a diffusible intercellular paracrine mediator) also enters the space in the tumor microenvironment and modulates the function of various non-tumor-cell elements (vascular, immune, scaffold/matrix-related, etc.) This area remains to be explored, in general (with respect to H_2S, regardless of its enzymatic source) and in particular (in the context of 3-MST-derived H_2S and polysulfides).

As mentioned in the previous section, it is intriguing that as cancer cells recover from damage (e.g. as they develop stem-cell-like and multidrug-resistant phenotypes), 3-MST expression increases [48,50]. The functional role of this phenomenon remains to be explored.

If, indeed, the 3-MST/H_2S system plays a functional role in cancer cell energetics, signaling and survival (and perhaps in tumor angiogenesis as well) (Fig. 4), then one would expect that these effects translate into functional effects detectable in *in vivo* models. With the availability of 3-MST pharmacological inhibitors (as well as cell lines with stable 3-MST silencing), tumor-bearing mice studies can be initiated. In these experiments one will have to keep in mind that the role of the H_2S system is probably tumor-cell-type dependent, and even in those tumor types where H_2S plays important functional roles, the 'driver' enzyme may be CBS, or CSE, or 3-MST, or some combination of these proteins. If these experiments identify a functional role of the 3-MST system in the promotion of tumor growth or anticancer drug

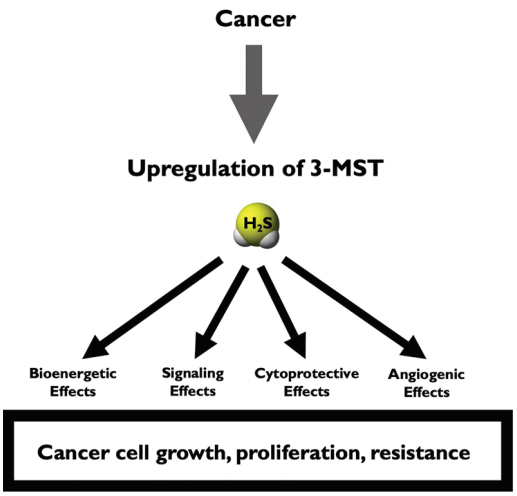

Fig. 4. Potential mechanisms of action and functional roles of the 3-MST system in cancer. 3-MST-mediated H_2S production may support cancer cell functions through several different, but potentially interrelated mechanisms.

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resistance *in vivo*, one can start to think about potentially translating these findings for ultimate clinical therapeutic benefit. The fact that the 3-MST deficient animals do not show a severe adverse phenotype suggests that systemic 3-MST inhibition may be well tolerated in tumor-bearing organisms. However, before start to speculate about efficacy and selectivity and human translation, a number of key preclinical experiments remain to be conducted.

We can conclude that there are bad news and good news. The bad news are (a) that the cancer cell 3-MST/H_2S system appears to be a highly complex one, and may well be highly cell-type and context-dependent and (b) that several key experiments remain to be conducted, before we can reach definitive conclusions regarding the pathogenetic role of this system. The good news is that the molecular and pharmacological tools are readily available to define the relative functional role of these systems in the pathogenesis of cancer over the coming years.

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References

[1] R. Wang, Physiological implications of hydrogen sulfide: a whiff exploration that blossomed, Physiol. Rev. 92 (2012) 791–896.

[2] C. Szabo, A. Papapetropoulos, International union of basic and clinical pharmacology. CII: pharmacological modulation of H_2S levels: H_2S donors and H_2S biosynthesis inhibitors, Pharmacol. Rev. 69 (2017) 497–564.

[3] P. Rose, P.K. Moore, Y.Z. Zhu, H_2S biosynthesis and catabolism: new insights from molecular studies, Cell. Mol. Life Sci. 74 (2017) 1391–1412.

[4] N. Shibuya, S. Koike, M. Tanaka, M. Ishigami-Yuasa, Y. Kimura, Y. Ogasawara, K. Fukui, N. Nagahara, H. Kimura, A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells, Nat. Commun. 4 (2013) 1366.

[5] L.K. Souza, T.S. Araújo, N.A. Sousa, F.B. Sousa, K.M. Nogueira, L.A. Nicolau, J.V. Medeiros, Evidence that d-cysteine protects mice from gastric damage via hydrogen sulfide produced by d-amino acid oxidase, Nitric Oxide 64 (2017) 1–6.

[6] A. Pol, G.H. Renkema, A. Tangerman, E.G. Winkel, U.F. Engelke, A.P.M. de Brouwer, K.C. Lloyd, R.S. Araiza, L. van den Heuvel, H. Omran, H. Olbrich, M. Oude Elberink, C. Gilissen, R.J. Rodenburg, J.O. Sass, K.O. Schwab, H. Schäfer, H. Venselaar, J.S. Sequeira, H.J.M. Op den Camp, R.A. Wevers, Mutations in SELENBP1, encoding a novel human methanethiol oxidase, cause extraoral halitosis, Nat. Genet. 50 (2018) 120–129.

[7] G. Meng, S. Zhao, L. Xie, Y. Han, Y. Ji, Protein S-sulfhydration by hydrogen sulfide in cardiovascular system, Br. J. Pharmacol. 175 (2018) 1146–1156.

[8] B.D. Paul, S.H. Snyder, Gasotransmitter hydrogen sulfide signaling in neuronal health and disease, Biochem. Pharmacol. 149 (2018) 101–109.

[9] K.R. Olson, H_2S and polysulfide metabolism: Conventional and unconventional pathways, Biochem. Pharmacol. 149 (2018) 77–90.

[10] C. Szabo, A timeline of hydrogen sulfide (H_2S) research: from environmental toxin to biological mediator, Biochem. Pharmacol. 149 (2018) 5–19.

[11] E. Donnarumma, R.K. Trivedi, D.J. Lefer, Protective actions of H_2S in acute myocardial infarction and heart failure, Compr. Physiol. 7 (2017) 583–602.

[12] N.L. Kanagy, C. Szabo, A. Papapetropoulos, Vascular biology of hydrogen sulfide, Am. J. Physiol. Cell Physiol. 312 (2017) C537–549.

[13] K. Kida, F. Ichinose, Hydrogen sulfide and neuroinflammation, Handb. Exp. Pharmacol. 230 (2015) 181–189.

[14] U. Shefa, M.S. Kim, N.Y. Jeong, J. Jung, Antioxidant and cell-signaling functions of hydrogen sulfide in the central nervous system, Oxid. Med. Cell. Longev. 2018 (2018) 1873962.

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

[16] C. Szabo, Gasotransmitters in cancer: from pathophysiology to experimental therapy, Nat. Rev. Drug Discov. 15 (2016) 185–203.

[17] X. Cao, L. Ding, Z.Z. Xie, Y. Yang, M. Whiteman, P.K. Moore, J.S. Bian, A review of hydrogen sulfide synthesis, metabolism, and measurement: is modulation of hydrogen sulfide a novel therapeutic for cancer? Antioxid. Redox. Signal (2018) In press.

[18] N. Nagahara, Multiple role of 3-mercaptopyruvate sulfurtransferase: antioxidative function, H_2S and polysulfide production and possible SOx production, Br. J. Pharmacol. 175 (2018) 577–589.

[19] N. Nagahara, T. Ito, H. Kitamura, T. Nishino, Tissue and subcellular distribution of mercaptopyruvate sulfurtransferase in the rat: confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis, Histochem. Cell Biol. 110 (1998) 243–250.

[20] B. Fräsdorf, C. Radon, S. Leimkühler, Characterization and interaction studies of two isoforms of the dual localized 3-mercaptopyruvate sulfurtransferase TUM1 from humans, J. Biol. Chem. 289 (2014) 34543–34556.

[21] J.W. Hylin, J.L. Wood, Enzymatic formation of polysulfides from mercaptopyruvate, J. Biol. Chem. 234 (1959) 2141–2144.

[22] Y. Ogasawara, S. Isoda, S. Tanabe, Reconstitution of an iron–sulfur cluster with bound sulfur: a possible source of acid-labile sulfur in biological systems, Biol. Pharm. Bull. 18 (1995) 1045–1048.

[23] M. Wróbel, T. Ubuka, W.B. Yao, T. Abe, L-cysteine metabolism in guinea pig and rat tissues, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 116 (1997) 223–226.

[24] P. Kamoun, Endogenous production of hydrogen sulfide in mammals, Amino Acids 26 (2004) 243–254.

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

[26] Y. Kimura, Y. Toyofuku, S. Koike, N. Shibuya, N. Nagahara, D. Lefer, Y. Ogasawara, H. Kimura, Identification of H_2S_3 and H_2S produced by 3-mercaptopyruvate sulfurtransferase in the brain, Sci. Rep. 5 (2015) 14774.

[27] 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 (2017) 10459.

[28] Y. Mikami, N. Shibuya, Y. Kimura, N. Nagahara, Y. Ogasawara, H. Kimura, Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide, Biochem. J. 439 (2011) 479–485.

[29] H. Kimura, Physiological roles of hydrogen sulfide and polysulfides, Handb. Exp. Pharmacol. 230 (2015) 61–81.

[30] N. Lau, M.D. Pluth, Reactive sulfur species (RSS): persulfides, polysulfides, potential, and problems, Curr. Opin. Chem. Biol. 49 (2018) 1–8.

[31] O. Kabil, N. Motl, R. Banerjee, H_2S and its role in redox signaling, Biochim. Biophys. Acta 1844 (2014) 1355–1366.

[32] N. Nagahara, A. Katayama, Post-translational regulation of mercaptopyruvate sulfurtransferase via a low redox potential cysteine-sulfenate in the maintenance of redox homeostasis, J. Biol. Chem. 280 (2005) 34569–34576.

[33] M. Wróbel, H. Jurkowska, Menadione effect on l-cysteine desulfuration in U373 cells, Acta Biochim. Pol. 54 (2007) 407–411.

[34] K. Módis, A. Asimakopoulou, C. Coletta, A. Papapetropoulos, C. Szabo, Oxidative stress suppresses the cellular bioenergetic effect of the 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway, Biochem. Biophys. Res. Commun. 433 (2013) 401–407.

[35] C. Coletta, K. Módis, B. Szczesny, A. Brunyánszki, G. Oláh, E.C. Rios, K. Yanagi, A. Ahmad, A. Papapetropoulos, C. Szabo, Regulation of vascular tone, angiogenesis and cellular bioenergetics by the 3-mercaptopyruvate sulfurtransferase/H_2S pathway: functional impairment by hyperglycemia and restoration by DL-α-lipoic acid, Mol. Med. 21 (2015) 1–14.

[36] E. Mitidieri, T. Tramontano, D. Gurgone, V. Citi, V. Calderone, V. Brancaleone, A. Katsouda, N. Nagahara, A. Papapetropoulos, G. Cirino, R. d'Emmanuele di Villa Bianca, R. Sorrentino, Mercaptopyruvate acts as endogenous vasodilator independently of 3-mercaptopyruvate sulfurtransferase activity, Nitric Oxide 75 (2018) 53–59.

[37] K. Hanaoka, K. Sasakura, Y. Suwanai, S. Toma-Fukai, K. Shimamoto, Y. Takano, N. Shibuya, T. Terai, T. Komatsu, T. Ueno, Y. Ogasawara, Y. Tsuchiya, Y. Watanabe, H. Kimura, C. Wang, M. Uchiyama, H. Kojima, T. Okabe, Y. Urano, T. Shimizu, T. Nagano, 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.

[38] M. Wróbel, T. Ubuka, W.B. Yao, T. Abe, Effects of thyroxine on L-cysteine desulfuration in mouse liver, Acta Med. Okayama 54 (2000) 9–14.

[39] J. Sun, L. Sun, N. Zhang, X. Lu, H. Zhang, A20 is up-regulated in primary mouse hepatocytes subjected to hypoxia and reperfusion, Cell Biochem. Funct. 30 (2012) 683–686.

[40] M. Li, L. Nie, Y. Hu, X. Yan, L. Xue, L. Chen, H. Zhou, Y. Zheng, Chronic intermittent hypoxia promotes expression of 3-mercaptopyruvate sulfurtransferase in adult rat medulla oblongata, Auton. Neurosci. 179 (2013) 84–89.

[41] B. Tao, R. Wang, C. Sun, Y. Zhu, 3-Mercaptopyruvate sulfurtransferase, not cystathionine β-synthase nor cystathionine γ-lyase, mediates hypoxia-induced migration of vascular endothelial cells, Front. Pharmacol. 8 (2017) 657.

[42] N. Nagahara, M. Nagano, T. Ito, K. Shimamura, T. Akimoto, H. Suzuki, Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase knockout mice exhibit increased anxiety-like behaviors: a model for human mercaptolactate–cysteine disulfiduria, Sci. Rep. 3 (2013) 1986.

[43] I. Billaut-Laden, E. Rat, D. Allorge, A. Crunelle-Thibaut, C. Cauffiez, D. Chevalier, J.M. Lo-Guidice, F. Broly, Evidence for a functional genetic polymorphism of the human mercaptopyruvate sulfurtransferase (MPST), a cyanide detoxification enzyme, Toxicol. Lett. 165 (2006) 101–111.

[44] N. Nagahara, N. Sawada, The mercaptopyruvate pathway in cysteine catabolism: a physiologic role and related disease of the multifunctional 3-mercaptopyruvate sulfurtransferase, Curr. Med. Chem. 13 (2006) 1219–1230.

[45] H. Jurkowska, W. Placha, N. Nagahara, M. Wróbel, The expression and activity of cystathionine-γ-lyase and 3-mercaptopyruvate sulfurtransferase in human neoplastic cell lines, Amino Acids 41 (2011) 151–158.

[46] P. Bronowicka-Adamska, A. Bentke, M. Wróbel, Hydrogen sulfide generation from l-cysteine in the human glioblastoma-astrocytoma U-87 MG and neuroblastoma SHSY5Y cell lines, Acta Biochim. Pol. 64 (2017) 171–176.

[47] K. Módis, C. Coletta, K. Erdélyi, A. Papapetropoulos, C. Szabo, Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains

http://doc.rero.ch

mitochondrial electron flow and supports cellular bioenergetics, FASEB J. 27 (2013) 601–611.
[48] E.A. Ostrakhovitch, S. Akakura, R. Sanokawa-Akakura, S. Goodwin, S. Tabibzadeh, Dedifferentiation of cancer cells following recovery from a potentially lethal damage is mediated by H_2S-Nampt, Exp. Cell Res. 330 (2015) 135–150.
[49] C. Szabo, C. Coletta, C. Chao, K. Módis, B. Szczesny, A. Papapetropoulos, M.R. Hellmich, 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 (2013) 12474–12479.
[50] 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.
[51] 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.
[52] B. Szczesny, M. Marcatti, J.R. Zatarain, N. Druzhyna, J.E. Wiktorowicz, P. Nagy, M.R. Hellmich, C. Szabo, Inhibition of hydrogen sulfide biosynthesis sensitizes lung adenocarcinoma to chemotherapeutic drugs by inhibiting mitochondrial DNA repair and suppressing cellular bioenergetics, Sci. Rep. 6 (2016) 36125.
[53] Y.W. Bai, M.J. Ye, D.L. Yang, M.P. Yu, C.F. Zhou, T. Shen, Hydrogen sulfide attenuates paraquat-induced epithelial-mesenchymal transition of human alveolar epithelial cells through regulating transforming growth factor-β1/Smad2/3 signaling pathway, J. Appl. Toxicol. (2018) In press.
[54] J. Breza Jr, A. Soltysova, S. Hudecova, A. Penesova, I. Szadvari, P. Babula, B. Chovancova, L. Lencesova, O. Pos, J. Breza, K. Ondrias, O. Krizanova, Endogenous H_2S producing enzymes are involved in apoptosis induction in clear cell renal cell carcinoma, BMC Cancer 18 (2018) 591.
[55] J.W. Gai, W. Qin, M. Liu, H.F. Wang, M. Zhang, M. Li, W.H. Zhou, Q.T. Ma, G.M. Liu, W.H. Song, J. Jin, H.S. Ma, Expression profile of hydrogen sulfide and its synthases correlates with tumor stage and grade in urothelial cell carcinoma of bladder, Urol. Oncol. 34 (166) (2016) e15–20.
[56] W. Wahafu, J. Gai, L. Song, H. Ping, M. Wang, F. Yang, Y. Niu, N. Xing, Increased H_2S and its synthases in urothelial cell carcinoma of the bladder, and enhanced cisplatin-induced apoptosis following H_2S inhibition in EJ cells, Oncol. Lett. 15 (2018) 8484–8490.
[57] E. Panza, P. De Cicco, C. Armogida, G. Scognamiglio, V. Gigantino, G. Botti, D. Germano, M. Napolitano, A. Papapetropoulos, M. Bucci, G. Cirino, A. Ianaro, Role of the cystathionine γ lyase/hydrogen sulfide pathway in human melanoma progression, Pigment Cell Melanoma Res. 28 (2015) 61–72.
[58] N. Fujiwara, T. Fujii, J. Fujii, N. Taniguchi, Functional expression of rat thioredoxin reductase: selenocysteine insertion sequence element is essential for the active enzyme, Biochem. J. 340 (1999) 439–444.
[59] M. Wróbel, J. Czubak, P. Bronowicka-Adamska, H. Jurkowska, D. Adamek, B. Papla, Is development of high-grade gliomas sulfur-dependent? Molecules 19 (2014) 21350–21362.
[60] M. Shiota, M. Naya, T. Yamamoto, T. Hishiki, T. Tani, H. Takahashi, A. Kubo, D. Koike, M. Itoh, M. Ohmura, Y. Kabe, Y. Sugiura, N. Hiraoka, T. Morikawa, K. Takubo, K. Suina, H. Nagashima, O. Sampetrean, O. Nagano, H. Saya, S. Yamazoe, H. Watanabe, M. Suematsu, Gold-nanofève surface-enhanced Raman spectroscopy visualizes hypotaurine as a robust anti-oxidant consumed in cancer survival, Nat. Commun. 9 (2018) 1561.
[61] A.T. Meram, J. Chen, S. Patel, D.D. Kim, B. Shirley, P. Covello, D. Coppola, E.X. Wei, G. Ghali, C.G. Kevil, R.E. Shackelford, Hydrogen sulfide is increased in oral squamous cell carcinoma compared to adjacent benign oral mucosae, Anticancer Res. 38 (2018) 3843–3852.
[62] K. Dongsoo, J. Chen, E. Wei, J. Ansari, A. Meram, S. Patel, G. Ghali, C. Kevil, R.E. Shackelford, Hydrogen sulfide and hydrogen sulfide-synthesizing enzymes are altered in a case of oral adenoid cystic carcinoma, Case Rep. Oncol. 11 (2018) 585–590.
[63] Y. Xu, N. Ma, P. Wei, Z. Zeng, J. Meng, Expression of hydrogen sulfide synthases and Hh signaling pathway components correlate with the clinicopathological characteristics of papillary thyroid cancer patients, Int. J. Clin. Exp. Pathol. 11 (2018) 1818–1824.
[64] E. Kun, C. Klausner, D.W. Fanshier, The rate of cleavage of beta-mercaptopyruvate by rapidly dividing cells, Experientia 16 (1960) 55–56.
[65] L. Włodek, M. Wróbel, J. Czubak, Transamination and transsulphuration of L-cysteine in Ehrlich ascites tumor cells and mouse liver. The nonenzymatic reaction of L-cysteine with pyruvate, Int. J. Biochem. 25 (1993) 107–112.
[66] M.R. Junttila, F.J. de Sauvage, Influence of tumour micro-environment heterogeneity on therapeutic response, Nature 501 (2013) 346–354.
[67] D. Hanahan, L.M. Coussens, Accessories to the crime: functions of cells recruited to the tumor microenvironment, Cancer Cell 21 (2012) 309–322.
[68] N. Shibuya, Y. Mikami, Y. Kimura, N. Nagahara, H. Kimura, Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide, J. Biochem. 146 (2009) 623–626.
[69] A.A. Govar, G. Törő, P. Szaniszlo, A. Pavlidou, S.I. Bibli, K. Thanki, V.A. Resto, C. Chao, M.R. Hellmich, C. Szabo, A. Papapetropoulos, K. Módis, 3-mercaptopyruvate sulfurtransferase supports endothelial cell angiogenesis and bioenergetics, Br. J. Pharmacol. (2018) in press.

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