Cystathionine γ-lyase mediates cell proliferation, migration, and invasion of nasopharyngeal carcinoma

Qianqian Zhang1,2,7, Yingran Gao1,2,7, Yanxia Zhang1,2, Mirong Jing1,2, Di Wang1,2, Yizhen Wang1,2, Saadullah Khattak2,3, Huwen Qi1,2, Chunbo Cai1,2, Jing Zhang1,2, Ebenezer Erasto Ngowi2,4,5, Nazeer Hussain Khan2,3, Tao Li1,2, Ailing Ji1,2, Qiying Jiang1,2, Xinying Ji1,2,4,5, Yanzhang Li1,2, and Dongdong Wu1,2,6,✉

Nasopharyngeal carcinoma (NPC) is an epithelia-derived malignancy with a distinctive geographic distribution. Cystathionine γ-lyase (CSE) is involved in cancer development and progression. Nevertheless, the role of CSE in the growth of NPC is unknown. In this study, we found that CSE levels in human NPC cells were higher than those in normal nasopharyngeal cells. CSE overexpression enhanced the proliferative, migrative, and invasive abilities of NPC cells and CSE downregulation exerted reverse effects. Overexpression of CSE decreased the expressions of cystochrome C, cleaved caspase (cas)-3, cleaved cas-9, and cleaved poly-ADP-ribose polymerase, whereas CSE knockdown exhibited reverse effects. CSE overexpression decreased reactive oxygen species (ROS) levels and the expressions of phospho (p)-extracellular signal-regulated protein kinase 1/2, p-c-Jun N-terminal kinase, and p-p38, but promoted the expressions of p-phosphatidylinositol 3-kinase (PI3K), p-AKT, and p-mammalian target of rapamycin (mTOR), whereas CSE knockdown showed opposite effects. In addition, CSE overexpression promoted NPC xenograft tumor growth and CSE knockdown decreased tumor growth by modulating proliferation, angiogenesis, cell cycle, and apoptosis. Furthermore, DL-propargylglycine (an inhibitor of CSE) dose-dependently inhibited NPC cell growth via ROS-mediated mitogen-activated protein kinase (MAPK) and PI3K/AKT/mTOR pathways without significant toxicity. In conclusion, CSE could regulate the growth of NPC cells through ROS-mediated MAPK and PI3K/AKT/mTOR cascades. CSE might be a novel tumor marker for the diagnosis and prognosis of NPC. Novel donors/drugs that inhibit the expression/activity of CSE can be developed in the treatment of NPC.

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INTRODUCTION

Hydrogen sulfide (H₂S) is widely regarded as a key gasotransmitter [1–3]. H₂S is generated from L-cysteine catalyzed by cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS) which are pyridoxal-5′-phosphate (PLP)-dependent enzymes. CBS and CSE are mainly found in the cytoplasm [4, 5]. In the presence of α-ketoglutarate, a PLP-independent enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST) can act together with cysteine aminotransferase to generate H₂S. 3-MST can be detected in both cytoplasm and mitochondria [5, 6]. In addition, D-amino acid oxidase could metabolize D-cysteine to 3-mercaptopyruvate, acting as a substrate for 3-MST to generate H₂S in the brain and kidney [7]. H₂S can be scavenged by methaemoglobin and disulfide/metallo-containing molecules that act as sulfane-sulfur and bound-sulfate pools. Oxidation and methylation are another two important pathways for H₂S metabolism [3, 8].

Nasopharyngeal carcinoma (NPC), one of the epithelial malignancies, arises from the most superior portion of the pharynx [9]. NPC has a specific geographic distribution, with the highest incidence in regions of South China, Southeast Asia, and North Africa [10]. A number of factors contribute to the development of NPC, such as host genetics, environmental factors, and Epstein-Barr virus infection [11]. Many patients with NPC at initial diagnosis have locally advanced disease or distant metastasis, leading to a poor clinical prognosis [12, 13]. It is urgent to identify new prognostic marker and molecular target in NPC treatment. It has been proposed that endogenous H₂S can promote cancer cell growth and downregulation of H₂S-producing enzyme can induce cancer cell death [8]. The effects of endogenous H₂S on cancer development and progression have been further demonstrated [14–19]. Nevertheless, the mechanism of action of endogenous H₂S on NPC growth remains unknown.

In this study, we determined the role of CSE in the proliferative, migrative, and invasive activities of NPC cells and detected the effect of CSE on NPC xenograft tumor growth. The function of DL-propargylglycine (PAG, an inhibitor of CSE) on NPC cell growth was further detected.
RESULTS

CSE is upregulated in human NPC cells

We firstly determined the levels of H2S in culture supernatant and human NPC cells. The concentrations of H2S in NPC cells and the supernatant were higher than those in normal nasopharyngeal cells and the supernatant (Fig. 1a). The protein level of CSE was dramatically upregulated in all human NPC cells compared to normal nasopharyngeal cells (Fig. 1b, c). The mRNA level of CSE showed a similar trend (Fig. 1d). To determine the role of CSE in NPC cell growth, overexpression/knockdown of CSE was...
performed. Transfection of CSE cDNA into CNE-1 and C666-1 cells enhanced the protein level of CSE and transfection of sh-CSE reduced the level of CSE (Fig. 1e). Both protein and mRNA levels of CSE showed similar trends (Fig. 1f–h). Furthermore, CSE overexpression increased H2S level in both NPC cells and the supernatant, while CSE knockdown exhibited the reverse effects (Fig. S1). The above data show that CSE overexpression/knockdown experiment has been successfully performed.

CSE mediates the viability and proliferation of human NPC cells

Compared to the Mock group, CSE overexpression promoted the proliferation of CNE-1 and C666-1 cells, whereas CSE knockdown exhibited opposite effects compared to the sh-Scb group (Fig. 2a, b). CSE exerted similar effects on the viability of human NPC cells (Fig. 2c). CSE overexpression enhanced colony formation and CSE knockdown reduced the colony number (Fig. 2d, e). The results of flow cytometric analysis showed that CSE overexpression induced an increased cell population in G2 phase and a decreased population in S phase, whereas CSE knockdown exhibited reversed trends (Fig. 2f, g). It has been shown that a number of cell cycle-related proteins are involved in the regulation of cell cycle progression, such as CDK2/4, cyclin D1/E1, p21, and p27 [20, 21]. As shown in Fig. 2a, CSE overexpression enhanced the expressions of cyclin D1/E1 and CDK2/4, but reduced the expressions of p21 and p27. CSE knockdown exerted reversed effects on the expressions of the proteins. In sum, these data indicate that CSE mediates the viability and proliferation of NPC cells by regulating cell cycle.

CSE mediates the migration and invasion of human NPC cells

We further evaluated the effects of CSE on the migration and invasion of human NPC cells. CSE overexpression increased the migration abilities of CNE-1 and C666-1 cells and CSE knockdown showed opposite effects (Fig. 3a, b). Overexpression of CSE promoted the anchorage-independent growth of CNE-1 and C666-1 cells, whereas the reverse effect was found in sh-CSE group (Fig. 3c, d). The migration/invasion ability of CNE-1 and C666-1 cells was increased in CSE group, whereas the sh-CSE group showed decreased reverse effects (Fig. 3e–h). To confirm the above results, western blot was conducted to detect the expressions of N-cadherin, E-cadherin, Vimentin, MMP-2, and MMP-9, which can play important roles in cellular migration and invasion [22, 23]. The results showed that CSE overexpression increased the expression of E-cadherin, but promoted the expressions of N-cadherin, Vimentin, MMP-2, and MMP-9. CSE knockdown exhibited reverse effects on the expressions of the proteins (Fig. S3). The data together suggest that CSE regulates both migration and invasion of NPC cells.

CSE modulates mitochondrial apoptosis in human NPC cells

The apoptosis was reduced in CSE group compared to Mock group but enhanced in sh-CSE group compared to sh-Scb group (Fig. 4a, b). The Bcl-2 family proteins can act as effector molecules in regulating mitochondria-dependent apoptosis, such as Bax, Bad, Bcl-xl, and Bcl-2 [24]. The enhanced Bax/Bcl-xl and Bax/Bcl-2 ratios are key phenomena in mitochondrial apoptosis [25, 26]. Cyt C can activate the caspase cascade once it is accumulated in cytosol [27]. Caspase-3 and caspase-9 are important members of the caspase family and induce cell apoptosis via the mitochondria-mediated pathway [28]. PARP acts as a cleavage substrate for caspase-3 in the process of apoptosis [29]. CSE overexpression decreased Bad/Bcl-xl and Bax/Bcl-2 ratios and the expressions of Cyt C, cleaved cas-3, cleaved cas-9, and cleaved PARP, while CSE knockdown exhibited reverse effects (Fig. 4c, d). The data reveal that CSE can modulate mitochondria-dependent apoptosis in human NPC cells.

CSE modulates ROS-mediated mitogen-activated protein kinase (MAPK) and PI3K/AKT/mTOR pathways in human NPC cells

ROS are widely regarded as oxygen-containing molecules, such as superoxide anion, hydrogen peroxide, and hydroxyl radical [30, 31]. At low-to-moderate concentrations, ROS can serve as signaling molecules that activate stress-responsive survival pathways and promote cell proliferation and differentiation. Excessive production of ROS induces damages to cellular components, including lipids, DNA, and proteins [30, 32]. The data suggested that CSE overexpression decreased ROS levels and CSE knockdown increased the levels of ROS in CNE-1 and C666-1 cells (Fig. 5a, b), suggesting that CSE can mediate the oxidative stress in human NPC cells. ROS can act as signaling messengers produced during various environmental stresses and play important roles in MAPK and PI3K/AKT/mTOR pathways [21, 33]. These data suggested that CSE overexpression downregulated the phosphorylation levels of ERK, p38, and JNK, but upregulated the expressions of p-PI3K, p-AKT, and p-mTOR. However, CSE knockdown exhibited reversed effects on the expressions of the proteins (Fig. 5c–f). Overall, these data suggest that CSE modulates ROS-mediated MAPK and PI3K/AKT/mTOR cascades in human NPC cells.

CSE regulates human NPC xenograft tumor growth

CNE-1 and C666-1 cells are used to establish subcutaneous xenograft tumors in nude mice [34, 35]. The effects of CSE on NPC xenograft tumor growth were further determined. The tumors were then removed and photographed (Fig. 6a). We observed that CSE overexpression dramatically promoted xenograft tumor growth and CSE knockdown significantly decreased tumor growth (Fig. 6b–h). As shown in Fig. S4a, b, CSE overexpression significantly increased the protein level of CSE and CSE knockdown exhibited reduced CSE level in tumor tissues. In addition, CSE overexpression increased the DT/DC index but decreased TVDT, CSE knockdown showed opposite effects (Fig. S4c, d). However, no obvious difference was observed in body weight (Fig. S4e, f). The proliferation, MVD, and p-AKT of human NPC xenograft tumors were increased in CSE group but decreased in sh-CSE group. Moreover, IHC with p-p38, p21, and cleaved cas-3 antibodies exhibited reverse trends (Fig. S5). In sum, the results show that CSE plays an important role in the regulation of NPC xenograft tumor growth.

CSE inhibitor suppresses human NPC cell growth

PAG, a stereoselective compound, is one of the most commonly used pharmacological inhibitors of CSE [36–38]. The effects of PAG on the growth of human NPC cells were further determined. As

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**Table 1**

| Gene       | Expression Level |
|------------|-----------------|
| CSE        | Increased       |
| CBS        | Decreased       |
| 3-MST      | Increased       |

**Figure 1**

The expression levels of CSE, CBS, and 3-MST in human NPC cells were detected and CSE overexpression and knockdown experiments were performed. a The concentrations of H2S in cells and culture supernatant were determined. b Western blot was conducted to detect the protein levels of CSE, CBS, and 3-MST in NP69, CNE-1, CNE-2, HONE-1, and C666-1 cells. GAPDH was used as the loading control. d RT-PCR was performed to detect the mRNA levels of CSE, CBS, and 3-MST in NP69, CNE-1, CNE-2, HONE-1, and C666-1 cells. Data are presented as mean ± SEM of three independent experiments. *P < 0.05, **P < 0.01 compared with human normal nasopharyngeal epithelial cell line NP69. e Fluorescence microscopy of CSE in CNE-1 and C666-1 cells. Original magnification, 400×. g The protein expression of CSE was examined in CNE-1 and C666-1 cells by Western blot. GAPDH was used as the loading control. h RT-PCR was performed to detect the mRNA levels of CSE in CNE-1 and C666-1 cells. Data are presented as mean ± SEM of three independent experiments; **P < 0.01 compared with the Mock group; **P < 0.01 compared with the sh-Scb group.
Fig. 2  Effects of CSE on the proliferation and viability of human NPC cells. a DNA replication activities of CNE-1 and C666-1 cells in each group were examined by EdU assay; original magnification ×100. b The proliferation rate of each group was analyzed. c The percentages of viable cells were determined using MTS and the cell viability of the control group was taken as 100%. d The clonogenic capacity was determined in CNE-1 and C666-1 cells. e The numbers of colonies were calculated. f Flow cytometry assay was used to determine cell cycle distribution. g Cell cycle distribution was analyzed. Data are presented as mean ± SEM of three independent experiments; *P < 0.05, **P < 0.01 compared with the Mock group; #P < 0.05, ##P < 0.01 compared with the sh-Scb group.
Fig. 3  Effects of CSE on the migration and invasion of human NPC cells. a The effect of CSE on cell migration was measured by wound healing assay; original magnification ×100. b The migration rates of CNE-1 and C666-1 cells were calculated. c Soft agar assay was performed to examine the anchorage-independent survival of cells; original magnification ×100. d The number of colonies was calculated. e Transwell assay was performed to assess the migration of CNE-1 and C666-1 cells; original magnification ×200. f The numbers of the migrated cells were calculated. g Transwell assay was performed to assess the invasion of CNE-1 and C666-1 cells; original magnification ×200. h The numbers of the invasive cells were calculated. Data are presented as mean ± SEM of three independent experiments; **P < 0.01 compared with the Mock group; ##P < 0.01 compared with the sh-Scb group.
Fig. 4 Effects of CSE on the apoptosis in human NPC cells. a The apoptotic levels of CNE-1 and C666-1 cells were measured by TUNEL staining; original magnification ×100. b The percentages of TUNEL-positive cells were calculated. c Western blot analysis for the expression levels of Bax, Bcl-2, Bad, Bcl-xl, Cyt C, cleaved cas-3, cleaved cas-9, and cleaved PARP in CNE-1 and C666-1 cells. GAPDH was used as the loading control. d The densitometry analysis of each factor was performed, normalized to the corresponding GAPDH level. The ratios of Bax/Bcl-2 and Bad/Bcl-xl were quantified. Data are presented as mean ± SEM of three independent experiments; *P < 0.05, **P < 0.01 compared with the Mock group; #P < 0.05, ##P < 0.01 compared with the sh-Scb group.
Fig. 5  Effects of CSE on ROS-mediated MAPK and PI3K/AKT/mTOR signaling pathways in human NPC cells. a The intracellular ROS production was detected using the fluorescent probes (shown in red; original magnification, ×100). b The intracellular ROS production was measured. c Western blot analysis for the expression levels of p-ERK, ERK, p-p38, p38, p-JNK, and JNK in CNE-1 and C666-1 cells. GAPDH was used as the loading control. d The densitometry analyses of p-ERK, ERK, p-p38, p38, p-JNK, and JNK were performed, normalized to the corresponding GAPDH level. e Western blot analysis for the expression levels of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, and mTOR in CNE-1 and C666-1 cells. GAPDH was used as the loading control. f The densitometry analyses of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, and mTOR were performed, normalized to the corresponding GAPDH level. Data are presented as mean ± SEM of three independent experiments; *P < 0.05, **P < 0.01 compared with the Mock group; ***P < 0.01 compared with the sh-Scb group.
Fig. 6  Effects of CSE on NPC xenograft tumor growth in nude mice. a Representative xenografts dissected from different groups of nude mice were shown. b The tumor volume was calculated. c, d The tumors were weighed and the inhibition rates of tumor growth were calculated. e Tumor growth of CNE-1 was monitored by bioluminescent imaging at day 0, 7, 14, 21, and 28. f CNE-1 xenograft tumors were removed and imaged. g Tumor growth of C666-1 was monitored by bioluminescent imaging at day 0, 7, 14, 21 and 28. h C666-1 xenograft tumors were removed and imaged. Values are presented as mean ± SEM (n = 6); **P < 0.01 compared with the Mock group; ##P < 0.01 compared with the sh-Scb group.
NPC, an epithelia-derived malignancy, has a very distinctive toxicity. In this study, PAG dose-dependently downregulated the proliferation, MVD, and p-AKT in human NPC xenograft tumors, but showed reverse effects on p-p38 and p21 expression levels and apoptotic index (Fig. 7e–g). However, there was no morphological difference of heart, liver, spleen, lung, kidney, and brain among groups. Moreover, no obvious difference in organ index and body weight was observed among groups (Fig. 8a–d). The data together suggest that CSE inhibitor could suppress human NPC cell growth via ROS-mediated MAPK and PI3K/AKT/mTOR pathways without significant toxicity.

**DISCUSSION**

NPC, an epithelia-derived malignancy, has a very distinctive geographic distribution, including South China, Southeast Asia, and North Africa [9, 10]. In recent years, the developments in application of chemotherapy, radiotherapy technology, and accurate disease staging have dramatically improved the treatment of NPC [39–41]. However, in light of local recurrence and distant metastasis, the prognosis of patients with NPC is unsatisfactory [41, 42]. There is an urgent need for elucidating the underlying mechanism of NPC development for novel therapeutic strategies [43]. CSE is reported to be overexpressed in both PLC/PRF/5 and HepG2 hepatoma cells compared to normal liver cell line HL-7702 [44]. Another study indicates that CSE level in breast cancer tissue from lymph node metastatic patients is higher than that in breast cancer of lymph node non-metastatic samples [17]. Furthermore, it has been revealed that CSE level is upregulated in bone-metastatic PC3 cells [45]. Similar to the previous studies, our data indicated that CSE level was dramatically upregulated in human NPC cells, suggesting that CSE can act as an important biomarker in NPC development.

It has been revealed that CSE is involved in the growth of liver cancer [44], breast cancer [17], and prostate cancer [45]. Nevertheless, the effect of CSE on NPC growth remains unknown. CNE-1 and C666-1 cell lines have been widely used to determine the effect of different agent [34, 35]. Then CNE-1 and C666-1 cells were adopted to detect the role of CSE in NPC cell growth. CSE contributes to the growth of hepatoma cells [44] and gastric cancer cells [46]. In addition, CSE promotes breast cancer metastasis through vascular endothelial growth factor signaling pathway [17], as well as enhances the progression and metastasis of prostate cancer via interleukin-1β/nuclear factor-kappa B (NF-kB) signaling pathways [45]. However, overexpression of CSE could induce spontaneous apoptosis of human melanoma cells via the suppression of NF-kB activity and inhibition of AKT and ERK pathways [47]. Another study indicates that CSE is involved in apoptosis induction in clear cell renal cell carcinoma [48]. These studies together suggest that CSE may exert different effects on tumor development depending on the cell types. Our data showed that CSE overexpression increased the viability, proliferation, migration, and invasion capabilities of NPC cells. Furthermore, overexpression of CSE enhanced the population of cells in G2 phase and reduced the cell percentage in S phase. However, CSE knockdown exhibited completely opposite effects. Collectively, the data indicate that CSE can play important roles in mediating the growth, migration, invasion, and cell cycle of NPC cells.

Apoptosis is responsible for tissue homeostasis and development in multicellular organisms [49]. Two main types of apoptosis exist in mammals: the death receptor and mitochondria-mediated pathways [50]. Cyt C release by the mitochondria is a key feature of mitochondria-mediated apoptosis [51]. Bax protein contributes to the transfer of cytochrome c across the mitochondrial membrane and then activates caspase-3 and caspase-9 [27, 52]. PARP is further proteolytically cleaved by caspase-3 and can result in the occurrence of apoptosis [53]. CSE may play a role in AGS gastric cancer cell proliferation probably via antiapoptotic effects [46]. Furthermore, inhibition of endogenous CSE/HS pathway could significantly enhance mitochondrial disruption and further induce DNA damage and apoptosis [44]. Similarly, our results suggested that CSE overexpression reduced both Bax/Bcl-xl and Bax/Bcl-2 ratios and the expressions of Cyto C, cleaved cas-3, cleaved cas-9, and cleaved PARP, whereas CSE knockdown exhibited reverse effects. Thus, we can conclude that CSE modulates mitochondria-dependent apoptosis in human NPC cells.

Considering different levels of ROS could cause many biological responses, the regulation of ROS levels is essential in cellular homeostasis [30]. Low to moderate levels of ROS contribute to cancer progression either by promoting genomic DNA mutation or acting as signaling molecules. In contrast, high levels of ROS could induce cellular damage and promote cancer cell death [30, 32]. Our data indicated that CSE overexpression decreased the ROS levels and CSE knockdown promoted ROS generation in human NPC cells. Many studies have revealed that MAPK pathway can be regulated by ROS in cancer cells [33, 54]. PI3K/AKT/mTOR pathway is dysregulated in cancer cells and ROS can act as upstream regulators of the pathway [24, 55]. Furthermore, it has been reported that both of these pathways are involved in several types of cancer, such as prostate cancer [56], hepatocellular carcinoma [57], and gastric cancer [58], suggesting that these two pathways may play synergistic effects in the development of cancer. Moreover, H2S can promote autophagy and induce apoptosis in human hepatocellular carcinoma cells and melanoma cells via inhibition of the PI3K/Akt/mTOR pathway [59, 60]. However, H2S exhibits anti-cancer effects by triggering apoptosis though activation of p38 MAPK pathway in rat C6 glioma cells [61]. Another study indicates that H2S dose-dependently decreases the viability of human prostate cancer PC-3 cells via activation of p38 MAPK and JNK [62]. We found that CSE overexpression decreased the expressions of p-ERK, p-JNK, and p-p38, but enhanced the expressions of p-PI3K, p-AKT, and p-mTOR, while CSE knockdown showed reverse effects on the expressions of the proteins. Our data reveal that CSE can modulate ROS-mediated MAPK and PI3K/AKT/mTOR cascades in NPC cells.

Many studies have indicated that CNE-1 and C666-1 cells can be successfully used to establish xenograft tumor models [34, 35]. We then examined the effect of CSE on NPC xenograft tumor growth. CSE overexpression significantly enhanced NPC xenograft tumor growth, while CSE knockdown dramatically decreased tumor growth. Ki67, a cell cycle-related protein, is a key marker in detecting cancer cell proliferation [24, 63–65]. The density of CD31 has been regarded as the tumor MVD [66, 67]. p21 plays an important role in cell cycle arrest in many types of cancer [68, 69]. Cleaved caspase-3 exerts the central effect on the progression of apoptosis [21, 70]. In this study, CSE overexpression increased the proliferation index and MVD, but reduced the ratio of p21 positive cells and the apoptotic index. However, CSE knockdown exhibited completely reverse trends. The data together indicate that CSE can regulate NPC xenograft tumor growth by mediating proliferation, angiogenesis, cell cycle, and apoptosis.

PAG, a selective CSE inhibitor, could suppress the progression of different types of cancer [36]. It has been reported that pretreatment with PAG drastically reduces the migration of tumor-derived endothelial cells [71]. Another study indicates that...
Fig. 7  Effects of PAG on NPC xenograft tumor growth in nude mice. a Representative xenografts dissected from different groups of nude mice were shown. b The tumor volume was calculated. c, d The tumors were weighed and the inhibition rates of tumor growth were calculated. Values are presented as mean ± SEM (n = 6). e Representative photographs of HE, p-AKT, p-p38, Ki67, CD31, p21, and cleaved cas-3 staining in CNE-1 and C666-1 xenograft tumors (original magnification ×400). f, g The p-AKT positive cells, p-p38 positive cells, proliferation index, MVD, p21 positive cells, and apoptotic index were calculated. Data are presented as mean ± SEM of three independent experiments. *P < 0.05, **P < 0.01 compared with the control group.
Fig. 8 Effects of PAG on the toxicity in nude mice. a Representative figures of the heart, liver, spleen, lung, kidney, and brain in nude mice. b The organ index was calculated. c The body weight change curve of each group during the experiment. d The body weight of each group on the first day (day 0) and the last day (day 28). e Current working model of CSE-mediated signaling pathway in NPC development and progression.
PAG concentration dependently suppresses the growth of AGS human gastric cancer cells [46]. In addition, PAG could decrease the proliferation and migration of SW480 human colon cancer cells and reduce tumor xenograft growth [72]. Furthermore, combination of 3,3′-diindolylmethane with PAG synergistically inhibits proliferation and migration but increases apoptosis in human gastric cancer cells [73]. In accordance to previous findings, our data suggested that PAG dose-dependently suppressed the viability, proliferation, migration, and invasion of NPC cells via ROS-mediated MAPK and PI3K/AKT/mTOR pathways. Moreover, no obvious difference was found in body weight, relative organ weight, and morphologies of heart, liver, spleen, lung, kidney, and brain among groups, suggesting no obvious systemic toxicity. Therefore, PAG can be used to inhibit NPC cell growth without significant toxicity.

**CONCLUSIONS**

In conclusion, this study reveals that CSE mediates the proliferation, migration, and invasion of NPC cells via ROS-mediated MAPK and PI3K/AKT/mTOR pathways (Fig. 8c). In light of its role in the progression of human NPC cells, CSE could act as a potential biomarker for the diagnosis and prognosis in NPC patients. In addition, CSE may be an important therapeutic target and novel donors/drugs that inhibit the expression/activity of CSE can be developed in the treatment of NPC.

**MATERIALS AND METHODS**

**Cell culture**

Human NPC cell lines (HONE-1, CNE-1, CNE-2, and C666-1) and normal nasopharyngeal epithelial cell line NP69 were obtained from Fengbio Biosciences (Changsha, Hunan, China). These cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), streptomycin (100 µg/mL), penicillin (100 U/mL) and maintained at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. CNE-1 and C666-1 cells were respectively treated with 5, 10, 20, and 40 µM PAG (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. The control group was treated with phosphate buffer saline (PBS) for 24 h.

**Overexpression/knockdown of CSE**

Human CSE complementary deoxyribonucleic acid (NM_001902) was subcloned between the EcoRI and BamHI sites of GV230 (Shanghai Genechem Ltd, Shanghai, China), identified by gene sequencing and then transfected into human NPC cells using Lipofectamine 3000 reagent (Life Technologies, Carlsbad, CA, USA). The GV230-CSE construct (CSE group) and empty vector (Mock group) were respectively transfected into cancer cells. The short hairpin ribonucleic acid (shRNA) specific for the scramble sequences and CSE were cloned into the HindIII and BamHI sites of GV102 (Genechem), respectively. The scramble shRNA (sh-Scb group) and CSE shRNA (sh-CSE group) were further verified by gene sequencing and transfected into cancer cells. Then G418 (Solarbio, Shanghai, China) was used to screen the stable transfected cell lines. The untransfected cells were used as a negative control group. The localization of CSE was photographed under a Nikon Eclipse Ti microscope (Melville, NY, USA).

**Cell viability and proliferation assays**

The viability was determined with the CellTiter 96 AQueous one solution assay kit (Promega, Madison, WI, USA). 5-Ethynyl-2′-deoxyuridine (EdU) assay was carried out by using the cell-light EdU Apollo 567 in vitro imaging kit (Ribobio, Guangzhou, Guangdong, China) to determine cell proliferation. The cells were observed and counted as the ratio of positive cells to total cells [63].

**Colonies formation assay**

The cells were cultured at 37 °C in 6 well plates (1 × 104/well). After 2 weeks, the colonies were fixed with methanol and stained with crystal violet. Then the plates were scanned and the colony number was counted [24].

**Flow cytometry**

The cells were trypsinized, fixed in 70% ice-cold ethanol, and incubated with propidium iodide and RNase A for 30 min at room temperature. The cell cycle was detected using the FACSVersa flow cytometer (BD, San Jose, CA, USA).

**Scratch assay**

A sterile 200 µL pipette tip was used to scrape the confluent cells. The cells were photographed using a CCKX41 inverted microscope (Olympus, Tokyo, Japan) and calculated using ImageJ software (NIH, Bethesda, MD, USA). The migration rate (MR) was analyzed using formula as below: MR = (A − B)/A × 100%, where A and B is the width at 0 h and 24 h, respectively [64].

**Soft agar assay**

Cells were cultured in the medium with 10% FBS and 0.6% agarose. Then the mixture was overlaid into a basal layer of 1% agarose in 6 well plate (1 × 105/well). After 14 days, the colonies were photographed and counted under an Olympus CCKX41 inverted microscope [65].

**Transwell assay**

Transwell assay was conducted as previously described [65]. The number of stained cells was analyzed by a Zeiss Axioskop 2 microscope (Thornwood, NY, USA).

**Reactive oxygen species (ROS) detection**

The measurement of cellular ROS was performed using the ROS detection assay kit (Beyotime).

**Western blot**

Western blot analysis was conducted to determine the proteins levels. The primary antibodies, such as anti-CSE, anti-CBS, anti-3-MST, anti-cyclin D1, anti-cyclin E1, anti-cyclin-dependent kinase (CDK2, anti-CDK4, anti-p21, anti-p27, anti-antiextracellular signal-regulated protein kinase 1/2 (ERK1/2), anti-phospho (p)-ERK1/2 (Thr202/Tyr204), anti-c-Jun N-terminal kinase (JNK), anti-p-JNK (Thr183/Tyr185), anti-p38, anti-p-p38 (Thr180/Tyr182), anti-phosphorylatedinositol 3-kinase (PI3K), anti-p-pI3K (Tyr199/Tyr548), anti-AKT, anti-p-AKT (Ser473), anti-mammalian target of rapamycin (mTOR), anti-p-mTOR (Ser2448) were purchased from Cell Signaling Technology (CST, Danvers, MA, USA). The primary antibodies, such as anti-vimentin, anti-N-cadherin, anti-E-cadherin, anti-tissue inhibitor of metalloproteinase (TIMP)-1, anti-TIMP-2, anti-matrix metalloproteinase (MMP)-2, anti-MMP-9, anti-B-cell lymphoma-extra-large (Bcl-xl), anti-B-cell lymphoma-2 (Bcl-2), anti-Bcl-x/d/Bcl-2-associated death promoter (Bax), anti-Bcl-2-associated X protein (Bax), anti-cytochrome (Cyt) C, anti-cleaved caspase (casp)-3, anti-cleaved cas-9, anti-cleaved poly-ADP-ribose polymerase (PARP), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and horseradish peroxidase-conjugated secondary antibody were obtained from Proteintech (Chicago, IL, USA). The bands were photographed using a chemiluminescence system (Thermo, Rockford, IL, USA).

**Reverse transcription-polymerase chain reaction (RT-PCR)**

Total RNA was extracted from cells using TRIzol reagent, treated with DNase I, and purified using the RNA clean-up kit (CwbioTech, Beijing, China). Total RNA (1 µg) was used for reverse transcription with a cDNA kit (CwbioTech). Primers were used as previously described: CSE, forward 5′-GACCTCTACATGGCGAGATCCG-3′ and reverse 5′-AACCTGACATCAGGAAGTG-3′; CBS, forward 5′-GGCGGATCACTGAGATTTG-3′ and reverse 5′-AGACGTCGCCAGTGTCG-3′ [74]; 3-MST, forward 5′-GACCCCGGCTTCTG-3′ and reverse 5′-GATGGTACATGACAA-3′; GAPDH, forward 5′-TATGACACAGAAGTTGCTACAG-3′ and reverse 5′-GATTGTACATGACAGAGTGC-3′ [64]. The reaction was performed in the volume of 20 µL according to these thermal cycling parameters: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min. The result was normalized to the expression level of GAPDH.
Animal study

Animal experiments were approved by the Committee of Medical Ethics and Welfare for Experimental Animals of Henan University School of Medicine (HUSOM-2017-191). Animal studies were carried out according to a previously reported method with slight modifications [63]. BALB/c nude mice (n = 6/group, 4-week-old, male) were obtained from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). 2 x 10^5 CNE-1 and C666-1 cells with overexpression/knockdown of CSE were inoculated subcutaneously into the right flanks of nude mice. For the CSE inhibitor experiment, PAG (5, 10, 20, and 40 mg/kg/day) was administered subcutaneously for 4 weeks. The control group was treated with PBS for 4 weeks. The tumor volume and body weight of nude mice were monitored daily. The tumor volume was measured: volume (V) = 1/2 x L x W^2, where L is the longest dimension parallel to the skin surface and W is the dimension perpendicular to L [24]. The parameter of DT/DC (%) was measured, where DT = T – Do and DC = C – C/10 (C/10 represents the volume of the treated/untreated tumor; Do represents the initial average tumor volume) [65]. The tumor volume doubling time (TVDT) was measured as TVDT = (T – T0) x log2/log(V2/V1), where (T – T0) is the time interval and V2/V1 respectively represents the tumor volume at two measurement time [64]. Tumor growth was weekly monitored by bioluminescent imaging (IVIS® Lumina III In Vivo Imaging System, PerkinElmer, Hopkinton, MA, USA). Then the tumors were removed, weighted, and imaged. The inhibition rate (IR) = ([A – B]/A) x 100%, where A/B is the average tumor weight of the control/treatment group [24].

Measurements of H2S concentrations

H2S levels in culture supernatant and cells were determined by enzyme-linked immunosorbent assay (ELISA) kits (Lanpaibio, Shanghai, China). Briefly, 50 µL of streptavidin-horseradish peroxidase (HRP) and 50 µL of standard solution were added to the wells in the antibody pre-coated microplates. Then 40 µL of samples to be tested, 50 µL of streptavidin-HRP and 10 µL of H2S-antibody were added to each well. The plates were covered and incubated at 37 °C for 40 min. After incubation, the wells were washed 3 times with the 20 x wash solution. Then the wells were incubated with 50 µL chromogen A and 50 µL chromogen B at 37 °C for 15 min in dark. To stop the reaction, 50 µL of the stop solution was added to each well which immediately turned the color of the solution into yellow. For the blank wells, only chromogen A and chromogen B and the step solution were added. The optical density (OD) of each well was determined spectrophotometrically at 450 nm using a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The value for the blank was subtracted from the sample OD. A calibration curve was plotted relating the concentration of each standard solution on the x axis to the OD value on the y axis. The standard curve linear regression equation was created and the H2S concentration was calculated from the standard curve.

Hematoxylin and eosin (HE) staining

Tumor tissues were routinely formalin-fixed, paraffin-embedded, 4 µM sectioned, and stained with HE. The results were photographed using a Zeiss Axioskop 2 microscope.

Immunohistochemistry (IHC)

Cluster of differentiation 31 (CD31) is a key biomarker for vascular endothelial cells. Its density has been regarded as microvesSEL density (MVD) [66]. Tumor tissues were respectively stained with anti-CD31 (CST), anti-Ki67 (CST), anti-p-AKT, anti-p-p38, anti-p21, and anti-cleaved cas-3 antibody. The results were photographed using a Zeiss Axioskop 2 microscope. Then the MVD was measured and the proliferation index, apoptotic index, p-AKT positive cells, p-p38 positive cells, and p21 positive cells were determined by the ratios of positive cells to total cells.

Statistical analysis

All data are expressed as mean and standard error of the mean (SEM). The differences among groups were analyzed using one-way analysis of variance using SPSS statistical software followed by Tukey’s test. P < 0.05 was regarded as statistically significant.

DATA AVAILABILITY

The data of the study are available from the corresponding author on reasonable request.

REFERENCES

1. Szabo C. Gasotransmitters in cancer: from pathophysiology to experimental therapy. Nat Rev Drug Discov. 2016;15:185–203.
2. Giovannazzo D, Bursac B, Sbodio J, Nalluru S, Vignane T, Snowman AM, et al. Hydrogen sulfide is neuroprotective in Alzheimer’s disease by sulfhydrating GSXK3B and inhibiting Tau hyperphosphorylation. Proc Natl Acad Sci USA. 2021;118:e201725118.
3. Wallace JL, Wang R. Hydrogen sulfide-based therapeutics: exploiting a unique but ubiquitous gasotransmitter. Nat Rev Drug Discov. 2015;14:329–45.
4. Filipovic MR, Zivanovic J, Alvarez B, Banerjee R. Chemical biology of H2S signaling through persulfidation. Chem Rev. 2018;118:1253–337.
5. Harte MD, Pluth MD. A practical guide to working with H2S at the interface of chemistry and biology. Chem Soc Rev. 2016;45:108–17.
6. Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev. 2012;92:791–896.
7. Shibuya N, Koike S, Tanaka M, Ishigami-Yuasa M, Kimura Y, Ogasawara Y, et al. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. Nat Commun. 2013;4:1366.
8. Wu DD, Wang DY, Li HM, Guo JC, Duan SF, Ji XY. Hydrogen sulfide as a novel regulatory factor in liver health and disease. Oxid Med Cell Longev. 2019;2019:3831713.
9. Bruce JP, Yip K, Bratman SV, Ito E, Liu FF. Nasopharyngeal cancer: molecular landscape. J Clin Oncol. 2015;33:3346–55.
10. Zhang Y, Chen L, Hu GQ, Zhang N, Zhu XD, Yang KJ, et al. Gemcitabine and cisplatin induction chemotherapy in nasopharyngeal carcinoma. N Engl J Med. 2019;381:1124–35.
11. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet. 2019;394:64–80.
12. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, Chan SL, et al. Analysis of plasma Epstein-Barr virus DNA to screen for nasopharyngeal cancer. N Engl J Med. 2017;377:513–22.
13. Ke L, Zhou H, Wang C, Xiong G, Xiang Y, Ling Y, et al. Nasopharyngeal carcinoma super-enhancer-driven ETV6 correlates with prognosis. Proc Natl Acad Sci USA. 2017;114:9683–8.
14. Ascencio K, Dilek N, Augsburger F, Panagaki T, Zuhra K, Szabo C. Pharmacological induction of mesenchymal-epithelial transition via inhibition of H2S bio-synthesis and consequent suppression of ACLY activity in colon cancer cells. Pharmacol Res. 2021;165:105393.
15. Younessi RA, Gad AZ, Sanber K, Ahn YJ, Lee GH, Khalaff E, et al. Targeting hydrogen sulfide signaling in breast cancer. J Adv Res. 2020;27:177–90.
16. Augsburger F, Randi EB, Jendly M, Ascencio K, Dilek N, Szabo C. Role of 3-mercaptopyrurate sulfotransferase in the regulation of proliferation, migration, and bioenergetics in murine colon cancer cells. Biomolecules 2020;10:447.
17. Wang L, Shi H, Liu Y, Zhang W, Duan X, Li M, et al. Cystathionine-γ-lyase promotes the metastasis of breast cancer via the VEGF signaling pathway. Int J Oncol. 2019;55:473–87.
18. Chakraborty PK, Murphy B, Mustafi SB, Dey A, Xiong X, Rao G, et al. Cystathionine β-synthase regulates mitochondrial morphogenesis in ovarian cancer. FASEB J. 2018;32:4145–57.
19. Phillips CM, Zatarain JR, Nicholls ME, Porter C, Widen SG, Thanki K, et al. Upregulation of cystathionine-β-synthase in colonic epithelia reprograms metabolism and promotes carcinogenesis. Cancer Res. 2017;77:5741–54.
20. Shen Q, Eun JW, Lee K, Kim HS, Yang HD, Kim SY, et al. Barrier to autointegration factor 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3, and splicing factor 3b subunit 4 as early-stage cancer decision markers and drivers of hepatocellular carcinoma. Hepatology. 2018;67:1360–71.
21. Su D, Zhong P, Wang Y, Zhang Q, Li J, Liu Z, et al. Hydrogen sulfide attenuates high-fat diet-induced non-alcoholic fatty liver disease by inhibiting apoptosis and promoting autophagy through reactive oxygen species/phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin signaling pathway. Front Pharmacol. 2020;11:585860.
22. Zhao Y, Tang H, Zeng X, Ye D, Liu J. Resveratrol inhibits proliferation, migration and invasion via Akt and ERK1/2 signaling pathways in renal cell carcinoma cells. Biomed Pharmacother. 2018;98:36
23. Shan B, Man H, Liu J, Wang L, Zhu T, Ma M, et al. TIM-3 promotes the metastasis of esophageal squamous cell carcinoma by targeting epithelial-mesenchymal transition via the Akt/GSK3β/Snail signaling pathway. Oncol Rep. 2016;36:1551–61.
24. Dong Q, Yang B, Han JG, Zhang MM, Liu W, Zhang X, et al. A novel hydrogen sulfide-releasing donor, HA-ADT, suppresses the growth of human breast cancer cells through inhibiting the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK signaling pathways. Cancer Lett. 2019;455:60–72.
25. Chen M, Wang X, Zha D, Cai F, Zhang W, He Y, et al. Apigenin potentiates TRAIL therapy of non-small cell lung cancer via upregulating DR4/DR5 expression in a p33-dependent manner. Sci Rep. 2016;6:35468.
26. Pitchakam P, Suzuki S, Ogawa K, Pompimon W, Takahashi S, Asamoto M, et al. Induction of G1 arrest and apoptosis in androgen-dependent human prostate cancer by Kugucin A, a triterpenoid from Momordica charantia leaf. Cancer Lett. 2011;306:142–50.

27. Sur S, Steele R, Shi X, Ray RB. miRNA-29b inhibits prostate tumor growth and induces apoptosis by increasing Bim expression. Cells 2019;8:1455.

28. Hu Y, Huang L, Liu M, Liu Y, Gu W, Wu Y, et al. Pioglitazone protects compression-mediated apoptosis in nucleus pulposus mesenchymal stem cells by suppressing oxidative stress. Oxid Med Cell Longev. 2019;10:4764071.

29. Beneke S, Diefenbach J, Bürkle A. Poly(ADP-ribose)lation inhibitors: promising drug candidates for a wide variety of pathophysiological conditions. Int J Cancer. 2004;111:813–8.

30. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov. 2013;12:931–47.

31. D’Auteleurs B, Toledoano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol. 2007;8:813–24.

32. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov. 2009;8:579–91.

33. Li W, Ma F, Zhang L, Huang Y, Li X, Zhang A, et al. S-Propargyl-cysteine exerts a role of the gaseous mediator hydrogen sulphide (H2S) in inhibition of human nasopharyngeal carcinoma cells. Cell Death Dis. 2019;10:588.

34. Fu D, Wu D, Cheng W, Gao J, Zhang Z, Ge J, et al. Costunolide induces autophagy and apoptosis by activating ROS/MAPK signaling pathways in renal cell carcinoma. Front Oncol. 2020;10:247237.

35. Martinez-Cutillas M, Gil V, Mané N, Clavé P, Gallego D, Martin MT, et al. Potential targets of the NKIRAS2/NF-kB pathway. Cancer Lett. 2019;447:33–40.

36. Wang J, Kong J, Nie Z, Chen D, Qiang J, Gao W, et al. Circular RNA Hsa_circ_0066755 as an Oncogene via sponging miR-651 and as a promising diagnostic biomarker for nasopharyngeal carcinoma. Int J Med Sci. 2020;17:1499–507.

37. Zeng Q, Liu YM, Liu J, Han J, Guo XJ, Lu S, et al. Inhibition of ZIPA reverses epithelial-to-mesenchymal transition and enhances the radiosensitivity in human nasopharyngeal carcinoma cells. Cell Death Dis. 2019;10:558.

38. Szabo C, Papapetropoulos A. International union of basic and clinical pharmacology. Cl: pharmacological modulation of H2 S Levels: H2S donors and H2S biosynthesis inhibitors. Pharmacol Rev. 2017;69:497–564.

39. Martinez-Cutillas M, Gil V, Mané N, Clavé P, Gallego D, Martin MT, et al. Potential role of the gaseous mediator hydrogen sulphide (H2S) in inhibition of human colorectal cancer. Pharmacol Res. 2015;93:52–63.

40. Li W, Ma F, Zhang L, Huang Y, Li X, Zhang A, et al. S-Propargyl-cysteine exerts a novel protective effect on methionine and choline deficient diet-induced fatty liver via Akt/NF2/HD-1 pathway. Oxid Med Cell Longev. 2016;2016:4690857.

41. Luo M, Wu C, Guo E, Peng S, Zhang L, Sun W, et al. FOXO3α knockdown promotes radioresistance in nasopharyngeal carcinoma by inducing epithelial-mesenchymal transition and the Wnt/β-catenin signaling pathway. Cancer Lett. 2019;455:26–35.

42. Lee AW, Ma BB, Ng WT, Chan AT. Management of nasopharyngeal carcinoma: current practice and future perspective. J Clin Oncol. 2015;33:3356–64.

43. Han JM, Sohng JK, Lee WH, Oh TJ, Jung HJ. Identification of Cyclin A as a potential anticancer target of novel nargenicin A1 analog in AGS gastric cancer cells. Int J Mol Sci. 2021;22:2473.

44. Wang SS, Chen YH, Chen N, Wang LJ, Chen DK, Wang H, et al. Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway. Cell Death Dis. 2017;8:e2688.

45. Xiao Q, Ying J, Qiao Z, Yang Y, Dai X, Xu Z, et al. Exogenous hydrogen sulfide inhibits human melanoma cell development via suppression of the PI3K/AKT/mTOR pathway. J Dermatol Sci. 2020:98:26–34.

46. Zhao L, Wang Y, Yan Q, Lu W, Zhang Y, He S. Exogenous hydrogen sulfide exhibits anti-cancer effects though p38 MAPK signaling pathway in C6 glioma cells. Biol Chem. 2013;396:1247–53.

47. Pei Y, Wu B, Cao Q, Wu L, Yang H. Hydrogen sulfide mediates the anti-sulfurane effect on human prostate cancer cells. Toxicol Appl Pharmacol. 2011;257:420–8.

48. Wu DD, Liu SY, Gao YR, Lu D, Hong Y, Chen YG, et al. Tumour necrosis factor-α-induced protein 8-like 2 is a novel regulator of proliferation, migration, and invasion in human rectal adenocarcinoma cells. J Cell Mol Med. 2019;23:1698–713.

49. Wu DD, Gao YR, Li T, Wang DY, Lu D, Liu SY, et al. PEST-containing nuclear protein mediates the proliferation, migration, and invasion of human neuroblastoma cells through MAPK and PI3K/AKT/mTOR signaling pathways. BMC Cancer. 2018;18:499.

50. Wu D, Tian W, Li J, Zhang Q, Wang H, Zhang L, et al. Peptide P11 suppresses the growth of human thyroid carcinoma by inhibiting the PI3K/AKT/mTOR signaling pathway. Mol Biol Rep. 2019;46:2665–78.

51. Wu D, Liu Z, Li J, Zhang Q, Zhong P, Teng T, et al. Epigallocatechin3-gallate inhibits the growth and increases the apoptosis of human thyroid carcinoma cells through suppression of EGFR/RAS/RAF/MEK/ERK signaling pathway. Cancer Cell Int. 2019;19:43.

52. Tolaney SM, Boucher Y, Duda DG, Martin JD, Seano G, Ancukiewicz M, et al. Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients. Proc Natl Acad Sci USA. 2015;112:14325–30.

53. Zhu L, Ding R, Zhang J, Zhang J, Lin Z. Cyclin-dependent kinase 5 acts as a promising biomarker in clear cell renal carcinoma cells. BMC Cancer. 2019;19:1698.

54. Xie Y, Li S, Sun L, Liu S, Wang F, Wen B, et al. Fungal immunomodulatory protein from nectria haematococca suppresses growth of human lung adenocarcinoma by inhibiting the PI3K/Akt pathway. Int J Mol Sci. 2018;19:3429.

55. Hsu HY, Lin TY, Hu CH, Shu DTF, Lu MK. Fucoidan upregulates TRLA4/CHOP-mediated caspase-3 and PARP activation to enhance cisplatin-induced cytotoxicity in human lung cancer cells. Cancer Lett. 2018;432:112–20.

56. Pupo E, Pla AF, Avanzato D, Moccia F, Cruz JE, Tanzi F, et al. Hydrogen sulfide promotes calcium signals and migration in tumor-derived endothelial cells. Free Radic Biol Med. 2011;51:1765–73.

57. Fan K, Li N, Qin J, Yin P, Zhao C, Wang L, et al. Wnt/β-catenin signaling induces the transcription of cystathionine-γ-lyase, a stimulator of tumor in colon cancer. Cell Signal. 2014;26:2801–8.

58. Ye F, Li X, Sun K, Xu W, Shi H, Bian J, et al. Inhibition of endogenous hydrogen sulfide biosynthesis enhances the anti-cancer effect of 3,3'-diindolylmethane in human gastric cancer cells. Life Sci. 2020;261:113848.

59. Chun-Mei J, Wu C, Guo-Liang M, Yue G, Ning C, Ji Y. Production of endogenous hydrogen sulfide in human gingival tissue. Arch Oral Biol. 2017;74:1108–13.

60. Jurkowska H, Placha W, Nagahara N, Wrobel M. The expression and activity of cystathionine-γ-lyase and 3-mercaptopyruvate sulfurtransferase in human neo-
cell renal cancer. Amino Acids. 2011;41:151–8.
AUTHOR CONTRIBUTIONS
DDW, YZL and XYJ conceived the study and drafted the manuscript. DDW, QQZ, YRG, YXZ, MRU, DW, YZW, SK, HWQ, CBC, JZ, EEN, NHK, TL, ALJ and QYJ designed and performed the experiments. DDW, QQZ and YRG analyzed the data and prepared the figures. All authors read and approved the final manuscript. The data of the study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS
The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Animal experiments were approved by the Committee of Medical Ethics and Welfare for Experimental Animals of Henan University School of Medicine (HUSOM-2017-191) in compliance with the Experimental Animal Regulations formulated by the National Science and Technology Commission, China.

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Correspondence and requests for materials should be addressed to Xinying Ji, Yanzhang Li or Dongdong Wu.

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