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Hydrogen sulfide mediates the anti-survival effect of sulforaphane on human prostate cancer cells

Yanxi Pei [a,b,1], Bo Wu [a,c,1], Qiuhui Cao [a], Lingyun Wu [c,d], Guangdong Yang [e,*]

[a] Department of Biology, Lakehead University, Thunder Bay, Canada
[b] College of Life Science, Shanxi University, Taiyuan, China
[c] Department of Pathophysiology, Harbin Medical University, Harbin, China
[d] Department of Pharmacology, University of Saskatchewan, Saskatoon, Canada
[e] The School of Kinesiology, Lakehead University, Thunder Bay, Canada

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ABSTRACT

Hydrogen sulfide (H_2S) is a novel gasotransmitter that regulates cell proliferation and other cellular functions. Sulforaphane (SFN) is a sulfur-containing compound that exhibits anticancer properties, and young sprouts of broccoli are particularly rich in SFN. There is consistent epidemiological evidence that the consumption of sulfur-containing vegetables, such as garlic and cruciferous vegetables, may help reduce the occurrence of prostate cancer. Here we found that a large amount of H_2S is released when SFN is added into cell culture medium or mixed with mouse liver homogenates, respectively. Both SFN and NaHS (a H_2S donor) decreased the viability of PC-3 cells (a human prostate cancer cell line) in a dose-dependent manner, and supplement of methemoglobin or oxidized glutathione (two H_2S scavengers) reversed SFN-reduced cell viability. We further found both cystathionine gamma-lyase (CSE) and cystathionine beta-synthase are expressed in PC-3 cells and mouse prostate tissues. H_2S production in prostate tissues from CSE knockout mice was only 20% of that from wild-type mice, suggesting CSE is a major H_2S-producing enzyme in prostate. CSE overexpression enhanced H_2S production and inhibited cell viability in PC-3 cells. In addition, both SFN and NaHS activated p38 mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinase (JNK). Pre-treatment of PC-3 cells with methemoglobin decreased SFN-stimulated MAPK activities. Suppression of both p38 MAPK and JNK reversed H_2S- or SFN-reduced viability of PC-3 cells. Our results demonstrated that H_2S mediates the inhibitory effect of SFN on the proliferation of PC-3 cells, which suggests that H_2S-releasing diet or drug might be beneficial in the treatment of prostate cancer.

Introduction

Prostate cancer is one of the most prevalent malignancies and the second leading cause of cancer deaths in men in North America (Lassi and Dawson, 2011). There is consistent epidemiological evidence that diet and in particular the consumption of cruciferous vegetables have long been associated with a reduced risk in the occurrence of prostate cancer (Zhang et al., 1994). Many of the anticancer effects observed from cruciferous vegetables have been attributed to the sulfide-containing isothiocyanates (ITCs), and sulforaphane (SFN) is postulated to be one of the principle isothiocyanates found in cruciferous vegetables (Keum et al., 2004; Singh et al., 2004; Zhang et al., 2005). The mechanisms of SFN chemoprevention have been well studied and reveal diverse responses depending upon the stage of prostate carcinogenesis, including the induction of apoptosis and cell cycle arrest, inhibition of phase 1 enzymes, induction of phase 2 metabolism enzymes, etc. (Cho et al., 2005; Juge et al., 2007).

Hydrogen sulfide (H_2S) has been traditionally known as a toxic gas with the smell of rotten eggs for centuries. The physiological importance of H_2S surfaced in the mid-1990s. It is clear now that H_2S, joining with other endogenous gasses including nitric oxide and carbon monoxide, is one of gasotransmitters (Wang, 2002, 2011). H_2S at physiologically relevant concentrations hyperpolarizes cell membrane, regulates cell growth, relaxes blood vessels, and modulates neuronal excitability (Calvert et al., 2010; Yang et al., 2008; Yang, 2011; Zhao et al., 2001). Two pyridoxal-5′-phosphate-dependent enzymes, cystathionine beta-synthase (CBS) (EC 4.2.1.22) and cystathionine gamma-lyase (CSE) (EC 4.4.1.1), are responsible for the majority of endogenous production of H_2S in mammalian tissues which use L-cysteine as the main substrate (Wang, 2011; Zhao et al., 2001). The expressions of CBS and CSE have been identified in

Abbreviations: CBS, cystathionine beta-synthase; CSE, cystathionine gamma-lyase; ERK, extracellular signal-regulated kinase; GSH, glutathione; GSSG, oxidized glutathoine; H_2S, hydrogen sulfide; ITCs, isothiocyanates; JNK, c-Jun N-terminal kinase; KO, knockout; MAPK, mitogen-activated protein kinase; NaHS, sodium hydrogen sulfide; SFN, sulforaphane; WT, wild type.

* Corresponding author at: The School of Kinesiology, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, Canada P7B 5E1. Fax: +1 807 346 7873.
E-mail address: gyang@lakeheadu.ca (G. Yang).

[1] These authors made equal contributions to this work.

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many human and other mammalian cells, including those from liver, kidney, brain, smooth muscle, pancreas, and lymphocytes (Wang, 2002; Yang et al., 2007; Zhao et al., 2001). Endogenously produced or exogenously applied H_2S regulates cell growth or death in a multitude of settings, and unbalanced cell proliferation and apoptosis due to the altered metabolism and functions of H_2S under different pathological conditions have been documented (Gobbi et al., 2009; Papapetropoulos et al., 2009; Yang, 2011; Yang et al., 2004b).

Recently, H_2S was discovered to mediate the major beneficial effects of garlic on cardiac functions, which suggest that sulfur compounds contained within plants may be transformed chemically or enzymatically in the human body with subsequent formation of H_2S (Benavides et al., 2007). Given cruciferous vegetables such as broccoli tend to release strong smell of H_2S when cooked, stored for a long time, or when rotten, ITCs may release H_2S which would mediate the beneficial effect of ITCs on prostate cancer (Chiao et al., 2002; Giovannucci et al., 2003). It has been shown that intake of SFN enriched food ameliorates both hypertension and atherosclerotic changes in spontaneously hypertensive stroke-prone rats, and H_2S administration generated the same beneficial effects as SFN on these cardiovascular disorders (Wu et al., 2004). It is essential to decipher the signaling and regulatory effects of H_2S and also its mediation on ITCs chemoprevention in prostate cancer.

Here we first investigated the release of H_2S from SFN and the mediation of H_2S on anti-survival effect of SFN on human prostate cancer cells (PC-3). The expressions of CSE and CBS as well as the endogenous production of H_2S in mouse prostate tissues and PC-3 cells were determined. We further explored the signal transduction pathway activated by both SFN and H_2S on stimulating PC-3 cell death.

Methods

Cell culture. Human prostate cancer PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA) and were cultured with F-12K Nutrient Mixture medium (Sigma, Oakville, ON) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. The experiments were performed when the cells reached 70–80% confluence between passages 2 and 6. In all studies, cells were first incubated in the serum-free medium overnight and maintained at a quiescent state (G0 phase), and then 10% serum was added together with different agents.

Cellular viability assays. Cell viabilities were assessed based on conversion of trazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals by living cells (Cao et al., 2010; Yang et al., 2004a). Briefly, cells of equal number were plated onto each well of 96-well plates for 24 h. After different treatments, 20 μl (5 mg/ml) MTT was added to each well. The cells were then cultured at 37 °C for 4 h, and absorbance of formazan products at 570 nm was measured in a FLUOstar OPTIMA microplate spectrophotometer (BMG LABTEch, Germany). The cells incubated with control medium were considered 100% viable.

Measurement of H_2S production and concentration. H_2S production rate was measured as described previously (Zhao et al., 2001). Briefly, the cells or tissues were collected and homogenized in 50 mM ice-cold potassium phosphate buffer (pH 6.8). The flasks

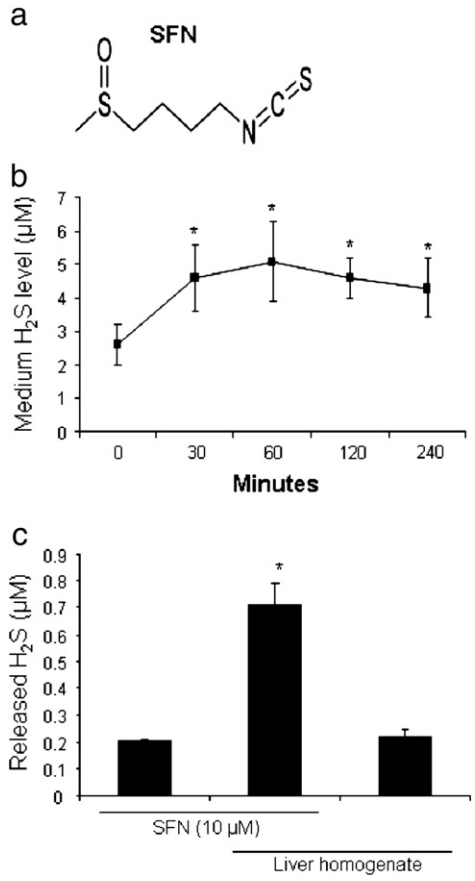

Fig. 1. SFN functions as a H_2S donor. a, Chemical structure of SFN constituting of parent moiety (glucosinolate) and ITCs group (−N=C=S). b, Release of H_2S from SFN when administered into cell culture medium. SFN (10 μM) was added into cell culture medium in the presence of PC-3 cells, and H_2S concentration in the medium was measured at the indicated time point. n=4. * $p<0.05$. c, More H_2S was released from SFN in the presence of liver homogenate. SFN (10 μM) was mixed with mouse liver homogenate at 37 °C for 90 min, and released H_2S was trapped and measured by using methylene blue method. n=3. * $p<0.05$.

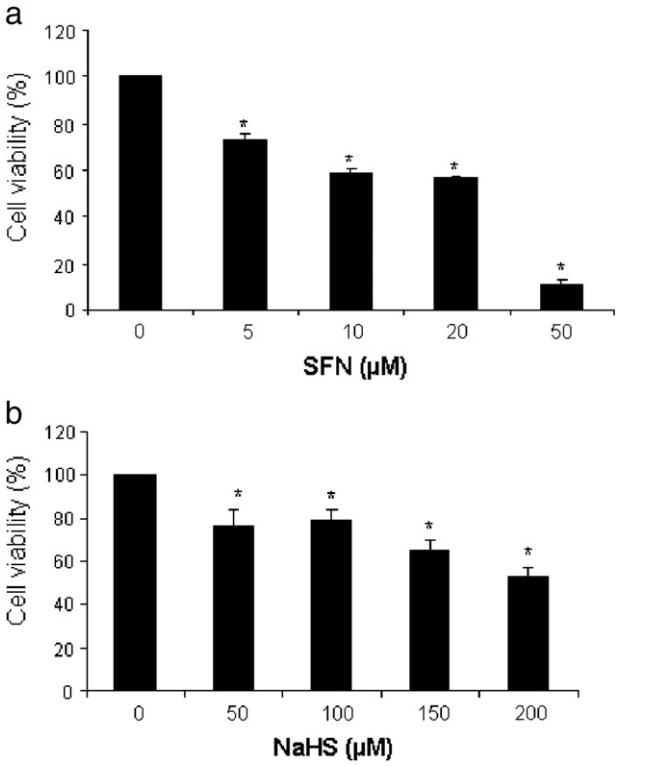

Fig. 2. Both SFN and H_2S reduced PC-3 cell viability. a, SFN decreased PC-3 cell viability. After the cells were incubated with different concentrations of SFN (5–50 μM) for 24 h, cell viability was analyzed by MTT assay. n=4. * $p<0.05$. b, NaHS decreased PC-3 cell viability. After the cells were incubated with different concentrations of NaHS (50–200 μM) for 24 h, cell viability was analyzed by MTT assay. n=4. * $p<0.05$.