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Regulation of cystathionine gamma-lyase/H_2S system and its pathological implication

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1. ABSTRACT

Hydrogen sulfide (H_2S) is a highly diffusible gasotransmitter, that influence cellular and organ functions by a number of different mechanisms. Cystathionine gamma-lyase (CSE) is one major H_2S-producing enzyme with L-cysteine as the main substrate in mammalian cells. Since the discovery of endogenously-produced H_2S as a biological mediator, there has been an explosion of interest in CSE expression and regulation. CSE expression and activity and ultimately the amount of H_2S synthesis is controlled by a complex integration of transcriptional, post-transcriptional and post-translational mechanisms. Considering the key role that CSE/H_2S system plays in both health and diseases, a better understanding of the regulation of CSE/H_2S system will help us to develop novel and more effective strategies to target CSE and alter H_2S production inside cells. In this review, we summarize the altered expression and activity of CSE and abnormal H_2S production in various pathophysiological conditions. The current knowledge on the signaling and regulatory pathways for CSE expression and H_2S production are also elucidated. As such, our understanding of the pathogenesis of human diseases will be better achieved and the corresponding new therapy can be devised.

2. INTRODUCTION

Physiological importance of hydrogen sulfide (H_2S) surfaced in the mid-1990s. It is clear now that H_2S, joining with other endogenous gases including nitric oxide (NO) and carbon monoxide (CO), is one member of gasotransmitters (1-6). Since the discovery of endogenously-produced H_2S in various tissues, there has been an explosion of interest in H_2S as a biological mediator. The reactivity of H_2S in biological system is complex and permits H_2S to exert a wide range of actions (1,7,8). H_2S is involved in the regulation of vascular tone, myocardial contractility, neurotransmission, insulin secretion, energy generation, inflammation, and nociception, etc (3,8-18). In the past 5 years, redox modification of cysteine residues by H_2S through *S*-sulfhydration garnered considerable attention as a mechanism of intracellular signaling (7,8,15,19-21). *S*-sulfhydration is increasingly recognized for its ability to influence protein function in a manner analogous to *S*-nitrosylation and phosphorylation, and the dysregulation of protein *S*-sulfhydation may be involved in a wide spectrum of human diseases (22-26).

It is clear now that cystathionine gamma-lyase (CSE, CTH or CGL, EC 4.4.1.1.) acts as a major H_2S-producing enzyme in cardiovascular system, liver, kidney,

Table 1. Altered CSE expression/activty in some pathological cases

Diseases	Species/ Tissues	Alteration	mRNA/ protein	REFERENCES
Myocardial ischemia	Rat/Heart	Decrease	mRNA	50,51
Hypoxia pulmonary hypertension	Rat/Lung	Decrease	mRNA	121
Hypertension	Rat/Aorta	Decrease	mRNA	3,40
Atherosclerosis	Mouse/Aorta	Decrease	Protein	46
Chronic kidney disease	Rat, Human/Kidney	Decrease	mRNA	68
Diabetes	Rat/Pancreas and Liver	Increase	Both	28,128,129
Diabetes	Mouse/Kidney	Decrease	Protein	69
Ischaemia/Reperfusion Injury	Mouse and Rat/Liver and Kidney	Increase	Proteine	129
Allergic rhinitis	Human/Nasal mucosa	Increase	Both	130
Radioadaptive response	Human/Liver cells	Increase	Protein	131
Drug resistance	Human/Cancer cell line	Increase	Both	1
Liver cirrhosis	Rat/Serum	Decrease	Protein	132
Cecal ligation and puncture-induced sepsis	Mouse/Liver	Increase	mRNA	70
Acute pancreatitis.	Mouse/Pancreas	Increase	N/A	133
Transplant tolerance	Rat/Kidney	Decrease	mRNA	56
Severe Burn Injury–Induced Inflammation	Mouse/Liver and Lung	Increase	mRNA	134
Acute inflammation	Mouse and Rat/Whole body	Decrease	N/A	90
Inflammatory bowel disease	Mouse/Bowel	Increase	mRNA	91
Asthma	Human, Rat/Lung	Decrease	Protein and mRNA	135,136
Hyperhomocysteinemic	Mouse/Brain	Increase	mRNA	137
Myocarditis	Mouse/Heart	Increase	mRNA and protein	138
Colitis	Mouse/ Gastrointestinal tract	Increase	mRNA	139
Partial Ileal Obstruction	Mouse/Ileal smooth muscle tissues	Decrease	Protein	140
Balloon injury	Rat Carotid/ arteries	Decrease	mRNA	44
Aging	Rat/Lense	Decrease	mRNA and protein	79
Preeclampsia	Human/Placenta	Decrease	mRNA	87
Werner syndrome	Human/fibroblast cells	Decrease	mRNA and protein	89

pancreas and prostate (3,7,10,27-29). CSE, also named as cystathionase, is an enzyme in the transsulfuration pathway to catalyze cystathionine to cysteine, ammonia, and α-ketobutyrate in most eukaryotes and actinomycetes species of prokaryotes (30). CSE has broad substrate specificity. CSE can breakdown cysteine into pyruvate, ammonia and thiocysteine (31,32). Thiocysteine could be further catalyzed by CSE to produce H_2S (33). CSE can also utilize homocysteine as substrate to generate H_2S (34). Deficiency of CSE is associated with cystathioninuria, hyperhomocysteinemia, and lower plasma cysteine (35). Either knockout or cell-specific overexpression of CSE gene has enabled a unique appreciation of the ability of H_2S to modulate cellular functions and various diseases (3,9). The expression and activity of CSE is under tight regulation to ensure its proper function. Together with CSE, there are other two enzymes involved in H_2S production in mammals, cystathionine beta-synthase (CBS, EC 4.2.1.2.2.) and 3-mercaptosulfurtransferase (3-MPST, 2.8.1.2.) (36). All these three enzymes can generate H_2S with L-cysteine as the main substrate. It is updated that 3-MPST could catalyze D-cysteine to produce H_2S in the cerebellum and kidney recently, which supplied a novel pathway for H_2S synthesis in mammals (37). CBS is reported to be the predominant H_2S-generating enzyme in the brain and nervous system, while 3-MPST could contributes to H_2S production in vascular system, brain and kidney (1).

The gasotransmitter's roles of H_2S in mammalian have been reviewed everywhere (1,2,4,6,23,38). This review focuses on the altered expression of CSE and abnormal H_2S production in various physiological and/or pathophysiological conditions. The current knowledge on the signaling and regulatory pathways for these abnormal expression and/or function of CSE/H_2S system is also elucidated. As such, our understanding of the pathogenesis of human diseases will be better achieved and the corresponding new therapy can be devised.

3. ABNORMAL CSE EXPRESSION AND H_2S PRODUCTION IN BOTH HEALTH AND DISEASES

Down-regulation of CSE/H_2S system was observed in various animal models of arterial and pulmonary hypertension, Alzheimer's disease, gastric mucosal injury, and liver cirrhosis. Over-activation of H_2S was found in diabetes, sepsis, shock, and pancreatitis. In Table 1, we summarized the alterations of CSE expression and activity in some diseases which were reported in the past decade.

3.1. Cardiovascular diseases

H_2S is capable of inducing vasorelaxation and lowering blood pressure in rats, due to mostly a direct action of H_2S on smooth muscle cells via activation of K_{ATP} channels and partially an endothelium-dependent mechanism (8,10,39). Reduced H_2S production as a result of CSE inhibition in rats increased blood pressure and administration of H_2S attenuated blood pressure elevation (40). The deficiency of CSE in mice leads to decreased endogenous H_2S level, age-dependent increase in blood pressure, and impaired endothelium-dependent vasorelaxation (3).

Vascular neointimal formation is a common consequence following pathological lesion, and arterial smooth muscle cell (SMC) phenotypic switching is a key element in the development of neointimal formation. Accumulating evidence has demonstrated that CSE/H_2S system plays a vital role in regulating SMC differentiation, migration, and proliferation (41-43). Serum deprivation

induced SMC differentiation and increased CSE expression and H_2S production in cultured human aorta SMCs (43). Carotid artery ligation in mice resulted in down-regulation of CSE expression and enhanced neointima formation (42). Earlier study also showed that CSE expression and H_2S production were reduced during the development of balloon injury-induced neointimal hyperplasia in rats, and treatment with H_2S significantly reduced neointimal lesion formation by inhibiting SMC proliferation (44). Although H_2S mediated estrogen-inhibited proliferation of SMCs via selective activation of ERα/cyclin D1 pathways, estrogen had little effect on CSE expression (45). We recently proved that knockdown of CSE decreases endogenous H_2S production and predispose the mice to vascular remodeling and early development of atherosclerosis, pointing to the CSE/H_2S pathway as an important therapeutic target for protection against atherosclerosis (46). Low blood levels of H_2S have been found in haemodialysis patients partially through transcriptional deregulation of CSE gene, which would provide some hints for the mechanisms of hyperhomocysteinaemia in uraemia as well as hypertension and premature atherosclerosis (47).

In vascular SMCs, CSE is localized in the cytosol under normal condition, however high level of calcium induced CSE expression and stimulated CSE translocation to mitochondria (11). Tom20 is involved in the process of CSE mitochondrial translocation, because Tom20 siRNA significantly inhibited mitochondrial translocation of CSE and mitochondrial H_2S production. Increased CSE expression and CSE mitochondrial translocation on specific stress stimulations is probably a unique mechanism to promote H_2S production inside mitochondria to maintain sufficient mitochondrial ATP production (11).

Osteoclast differentiation has been linked to advanced atherosclerotic plaques, and CSE is reported to be involved in early stages of osteoclastogenesis (48). Both CSE protein and mRNA expression is up-regulated in osteoclast-like cells differentiated from RAW264.7. cells in response to receptor activator of nuclear factor κ-B ligand induction, a common *in vitro* model for osteogenesis (48). Knockdown of CSE by short interfering RNA (siRNA) and blockage of CSE activity by DL-propargylglycine (PPG) attenuated receptor activator of nuclear factor κ-B ligand-induced tartrate-resistant acid phosphatase type 5 activity and pit formation. Similarly mechanical force promoted the mRNA expression of CSE in the receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL) in human periodontal ligament cells (hPDLCs), suggesting H_2S could promote osteogenic differentiation (49).

Tons of studies reported decreased CSE expression and H_2S levels in myocardium with ischemia reperfusion (I/R) injury, and supply of exogenous H_2S provides a cardiac protective effect (50,51). CSE/H_2S is also involved in the attenuation of diabetic myocardial injury (52). High glucose reduced CSE expression in the primary neonatal rat cardiomyocytes, and treatment with NaHS significantly reversed the diabetic rat hearts function (53). Besides these, many other factors were shown to protect heart via regulating CSE/H_2S system. Estrogen decreased oxidative stress and inflammatory status in the myocardium of ovariectomized rats by increasing CSE expression and H_2S production (54). Sulfur dioxide preconditioning could significantly reduce I/R-induced myocardial injury by upregulating CSE/H_2S pathway (55). Silly R *et al*. demonstrated that CSE is down-modulated in transplant tolerance of heart and plays a critical role in regulating IL-12 in monocytes and dendritic cells, possibly involving in the maintenance of the tolerant state (56).

3.2. Diabetes

CSE is a major H_2S-producing enzyme in pancreatic beta cells and regulates beta cell function. Altered H_2S production is involved in the development of diabetes (28,57,58). Overexpression of CSE inhibited insulin release from cultured insulin-secreting beta cells (INS-1E), but lowering endogenous H_2S production by PPG or knockdown of CSE by siRNA had the opposite effect (58). H_2S formation was significantly higher in the pancreas of Zuker diabetic fatty rats compared with nondiabetic animals (57). Cysteine level was also elevated in diabetic patients with diabetic nephropathy renal complications (59). Streptozotocin (STZ) injection induced pancreatic CSE activity and H_2S production as well as hyperglycemia and hypoinsulinemia in mice (28). The application of PPG to inhibit CSE activity protected mice from STZ-induced diabetes. STZ also significantly stimulated H_2S production in INS-1E cells (28). The reduced pancreatic CSE expression and activity in diabetes may be due to high level of glucose. High glucose inhibited H_2S production in INS-1E cells and freshly isolated rat pancreatic islets (12). CSE mRNA expression, CSE activity and protein abundance were also profoundly reduced by high glucose. The accurate regulation of CSE/H_2S system by glucose may be involved in the fine control of glucose-induced insulin secretion (12).

In liver cells, insulin at the physiological range inhibited CSE expression, but CSE expression was increased in insulin-resistant state induced by exposing the cells to high levels of insulin and glucose (60). Adenovirus-mediated CSE overexpression or exogenously applied H_2S decreased insulin-stimulated phosphorylation of Akt and lowered glycogen content in liver cells, suggesting that the interaction of H_2S and insulin in liver plays a pivotal role in regulating insulin sensitivity and glucose metabolism (60). Another studies showed that enhanced CSE activation and H_2S generation contributed to vitamin D-induced glucose uptake and glucose utilization in adipocytes (61). Vitamin D supplement also induced CSE expression and H_2S formation in glucose-treated U937 monocytes, indicating that CSE/H_2S system contributes to vitamin D-modulated glucose metabolism (62).

Quite differently, CSE expression and H_2S production was reduced in insulin-insensitive kidney tissue from hyperglycemic Akita mice, and H_2S would generate therapeutic potential to prevent adverse diabetic renal remodeling (63). In addition, in a rat model of type I diabetes induced by STZ, CSE mRNA expression and endogenous H_2S production was significantly increased in cerebral arteries with reduced cerebral artery endothelial

function (64). Thus, upregulation of endogenous H_2S in diabetes may play a vasoprotective role.

3.3. Liver and kidney diseases

Liver produces a large amount of H_2S compared all other tissues in our body. Enhanced formation of H_2S may contribute to the liver injury (65,66). Lipopolysaccharide (LPS) induced endotoxemia in liver following enhanced H_2S concentration and CSE expression. Pretreatment with NaHS strengthened the LPS-induced liver damage; however, blockage of CSE activity by PPG reversed the increase of liver H_2S concentration and reduced the liver damage. Inhibition of H_2S synthesis may provide a useful therapeutic strategy against the liver injury associated with endotoxemia (65). In another case, the mRNA and protein levels of CSE in the liver were significantly elevated in mice fed a high fat diet, and upregulation of CSE/H_2S system might play an adaptive role against oxidative stress by maintaining total glutathione levels in the liver (67).

In a mouse model of unilateral ureteral obstruction (UO)-induced kidney fibrosis, CSE expression in the kidney was decreased (68). Treatment with sodium hydrogen sulfide (NaHS, a H_2S producer) during UO-induced oxidative stress with preservations of catalase, copper-zinc superoxide dismutase, and manganese superoxide dismutase expression, and glutathione level. NaHS treatment attenuated UO-induced increases in levels of TGF-β1, activated Smad3, and activated NF-κB, suggesting that H_2S and its transsulfuration pathway may be a potential target for development of therapeutics for fibrosis-related diseases (68). Methionine sulfoxide reductase A protects the kidney against I/R injury. Interestingly, I/R reduced the expression and activities of CSE in the kidney, and the reductions were more profound in the methionine sulfoxide reductase A knockout mice (69).

CSE expression was also shown to be up-regulated or down-regulated in many other cases. In a mouse model of partial obstruction-induced dysfunction of the interstitial cells of Cajal (ICC), CSE expression was lower in the tunica muscularis of the obstructed intestine (70). H_2S reduced but PPG elevated the expression of TNF-α mRNA in the tunica muscularis of the ileum, pointing to the protective role of H_2S in partial intestinal obstruction-induced loss of ICC. In contrast, in a bile duct ligation induced-acute acalculous cholecystitis model in guinea pigs, immunohistochemistry analysis showed that CSE expression is progressively increased in the gallbladder epithelium, suggesting CSE may be involved in the development of acalculous cholecystitis (71).

3.4. Cancer

Hydrogen sulfide (H_2S) has been shown to regulate cancer cell growth and tumor progression. We recently observed that H_2S mediates the anti-cancer effect of sulforaphane in an androgen-independent prostate cancer cell line (PC-3) (27). GYY413 (a slow-releasing H_2S donor) and S-propargyl-cysteine (a CSE activator) have also been shown to generate anti-cancer effects (72,73). Butyrate inhibits terminal differentiation of a variety of human colon cancer cell lines and induces the incidence of colon cancer (74). Cao *et al*. demonstrated that butyrate increased cell production of H_2S and upregulated CSE expressions in colon cancer cells (75). However, blockade of CBS, but not CSE, decreased butyrate-stimulated H_2S production and reversed butyrate-inhibited cell viability. Another report also showed that silencing of CSE does not affect colon tumor growth or bioenergetics (76). In human neuroblastoma tissue specimens, no CSE activity was detectable. A variety of differentiating agents, including butyric acid, dimethyl sulfoxide, serum-free medium, or sodium citrate, were hard to induce CSE activity and neuroblastoma cell differentiation, suggesting that neuroblastomas have a biochemical block in the transsulfuration enzymes at the level of CSE (77). In addition, treatment of neuroblastoma mouse xenografts with a CSE construct resulted in near complete cessation of tumor growth (78).

3.5. Aging

It is well known that the CSE/H_2S system is involved in development as well as the ageing process. The mRNA and protein levels of CSE are lower in the lenses from old rats, and inhibition of CSE activity leads to cataractogenesis *in vitro* (79). CSE activity as well as the level of cysteine is lower in the livers of older mice (80). We provided evidence recently showing that CSE protein expression is significantly reduced in mouse embryonic fibroblasts at older passage, which display increased oxidative stress and accelerated cellular senescence (15). H_2S incubation significantly reduced oxidative stress and rescued the cells from cellular senescence. It also has been reported that H_2S improves the function of senescent human umbilical vein endothelial cells, potentially through modulation of SIRT1 activity (81). In caenorhabditis elegans, although H_2S reduced detrimental age-dependent changes, genetic deficiency of CSE did not affect their lifespan, suggesting other H_2S-producing enzyme but not CSE contributes to the lifespan-prolonging and health-promoting effects of H_2S (14).

3.6. Asthma

CSE has been shown to be one of the major enzymes H_2S in lungs, participating in the regulation of respiratory functions and playing a protective role in the development of asthma. By using ovalbumin (OVA)-induced acute asthma model in mice, Zhang *et al*. demonstrated that OVA challenge decreases lung CSE expression and H_2S production following aggravated airway hyperresponsiveness and inflammation in bronchoalveolar lavage fluid (82). More importantly, NaHS supplement rescued the mice from the aggravated pathological process of asthma. This study paves a way for developing a new therapeutic potential for asthma via targeting CSE/H_2S metabolism (82,83). In another rat model of asthma, plasma H_2S levels and CSE expression in the lung was also decreased, and budesonide alleviated airway inflammation in asthmatic rats possibly by reversing the expression of CSE and endogenous production of H_2S (84). In human patients with asthma, the serum H_2S level was also significantly lower when compared with normal

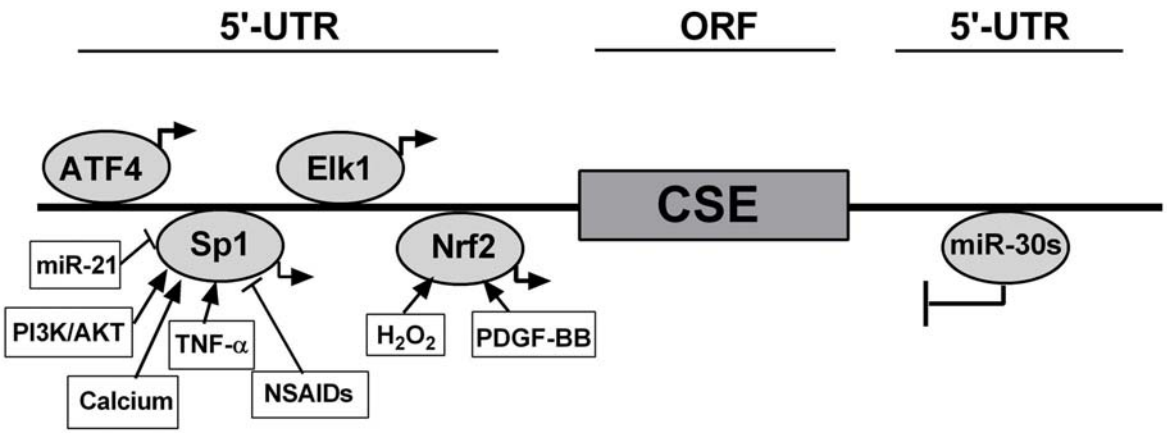

Figure 1. The regulation of CSE at the transcriptional and translational levels. A number of transcript factors, including Sp1, Nrf2, ATF4, and Elk1, regulate CSE transcription through direct or indirect binding with CSE promoter. MiR-21 and NSAIDs are reported to suppress but PI3K and TNF-α stimulate CSE transcription by targeting Sp1 gene. H_2O_2 and PDGF-BB induce CSE transcription by targeting at Nrf2. MicroRNAs, including miR-30s, bind at 3'-UTR of CSE gene for translational repression. UTR, untranslated region; ORF, open reading frame.

controls, implicating endogenous H_2S may be involved in the pathogenesis of asthma and can be used as a marker for asthma diagnosis (85). In a mouse model of tobacco smoke-induced chronic obstructive pulmonary disease, CSE expression was reduced in the lung, and NaHS supplement could protect against tobacco smoke-induced oxidative stress, airway inflammation, and remodeling and ameliorate the development of emphysema and pulmonary hypertension (86).

3.7. Others

Placental CSE expression and plasma levels of H_2S were significantly lower in preeclampsia women. Blockage of CSE activity by PPG reduced placenta growth factor production in human placental explants and decreased fetal growth in mice. In addition, a slow releasing H_2S-generating compound, GYY4137 restored fetal growth (87). Another group also showed that CSE immunoreactivity was reduced in placentas from pregnancies with severe early-onset growth-restriction, and exposure of villus explants to hypoxia-reoxygenation significantly reduced CSE protein and mRNA (88). These suggest that CSE/H_2S system is required for healthy placental vasculature and a decrease in CSE/H_2S activity may contribute to the pathogenesis of preeclampsia.

Werner syndrome protein is involved in DNA repair and its mutation causes Werner syndrome, an autosomal recessive genetic disorder with a premature aging phenotype (89). CSE expression was shown to be lower in Werner syndrome fibroblasts with increasing levels of oxidative stress and excess activation of the mTOR (mammalian target of rapamycin) pathway (89). The authors further provided evidence showing that NaHS treatment blocks mTOR activity, abrogates protein aggregation and normalizes the phenotype of Werner syndrome fibroblasts, suggesting CSE/H_2S system would be a target for preventing Werner syndrome (89).

In a rat model of nonsteroidal anti-inflammatory drug (NSAID) gastropathy, NSAIDs reduced H_2S formation and CSE expression (mRNA and protein) leading to increased inflammation and gastric injury (90). The authors identified CSE as a novel target for NSAIDs and suggested a physiologic role for H_2S in regulating the gastric microcirculation. Altered CSE expression or H_2S production was shown in various inflammation conditions. In some inflammatory diseases, CSE expression and H_2S formation were increased, while decreased in some other inflammatory status (91). These results suggest that the roles of H_2S in inflammatory diseases are dependent on the inflammatory types.

4. THE REGULATORY MECHANISMS UNDERLYING ALTERED CSE EXPRESSION AND H_2S PRODUCTION

The CSE gene has been characterized in many organisms including human, mouse, rat, amphibian, and plants, showing high sequence identity between phylogenetically distant organisms, which indicates the evolutionary conservation of this enzyme (1). The first mammal cDNA of CSE was cloned by screening cDNA library of rat in 1990 (92). Two years later, human CSE cDNA was cloned as well and two isoforms of CSE mRNA were detected based on the sequence analysis (93). These two forms of human CSE mRNA have high similarity except 132 bp internal missing in the shorter one. Overexpression of the short form of CSE did not contribute to activity increase while longer form did (93). The three-dimensional structure of human CSE has been explored via X-ray crystallography (94). CSE protein consists of four identical monomers with a covalently bound pyridoxal 5'-phosphate (PLP) cofactor in each monomer (95). CSE is an inducible gene in many types of cells and tissues, contributing to the development of various disorders. The signaling and regulatory pathways for abnormal expression and/or function regulation of CSE/H_2S system in these pathological conditions are dynamic and complicated. Many factors have been discovered to regulate CSE expression and activity at multiple levels, including

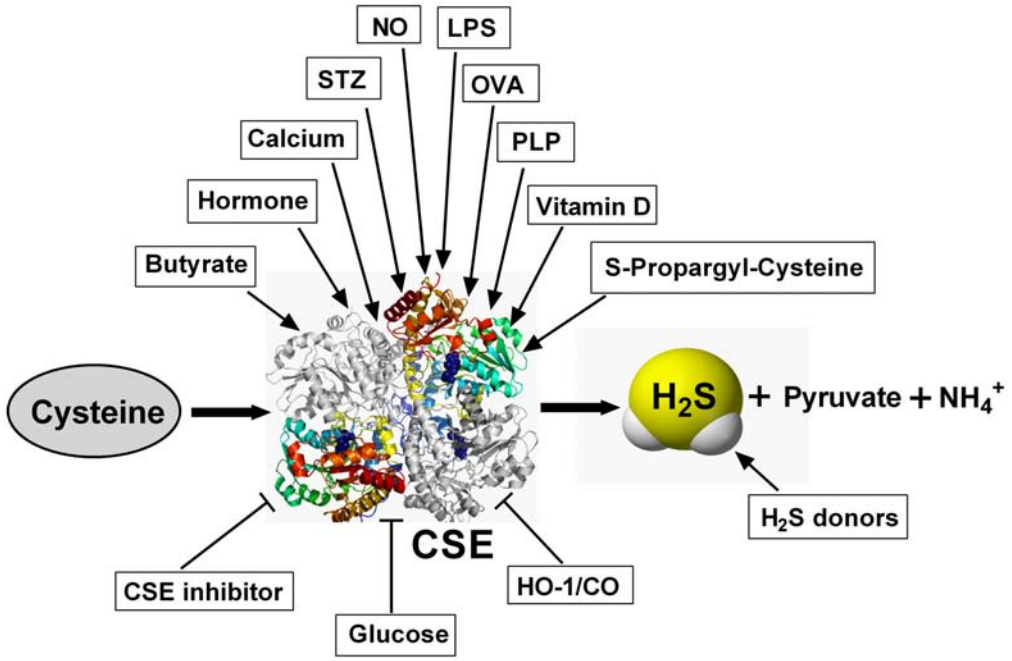

Figure 2. A numbers of factors are involved in the modification of CSE activity and H_2S production. CSE activity and H_2S production can be activated or burst by tons of compounds, including butyrate, specific hormone, calcium, streptozotocin (STZ), nitric oxide (NO), lipopolysaccharide (LPS), ovalbumin (OVA), pyridoxal-5'-phosphate (PLP), vitamin D, S-propargyl-cysteine, and various H_2S donors, etc. In contrast, glucose, HO-1/CO system, and various CSE inhibitors are shown to suppress CSE activity and H_2S production.

transcriptional, post-transcriptional and post-translational levels, as shown in Figure 1 and 2.

4.1. Transcription factor regulation of CSE transcription

Specificity protein-1 (Sp1) is a zinc finger transcription factor that binds to GC-rich motifs of many promoters (96). It was first reported that the promoter region -157 to +18 of mouse CSE gene displayed strongest activity with Sp1 binding in HEK-293 cells (97). In human aorta SMCs, there were two Sp1 consensus binding sites present in the core promoter region of human CSE gene (43). Incubation of human aorta SMCs with Sp1 binding inhibitor mithramycin inhibited CSE mRNA expression, while overexpression of Sp1 increased the activity of human CSE core promoter and CSE expression (43). The direct binding of Sp1 to human CSE promoter was demonstrated by chromatin immunoprecipitation (CHIP) assay. Moreover, PI3K/AKT regulated CSE transcription and promoter activity by activating on Sp1 in human hepatocellular carcinoma cell lines. CSE expression was reduced by the PI3K inhibitor or Akt deletion, while enhanced with the Akt activator (98). CHIP assay demonstrated that PI3K/AKP stimulated the binding of Sp1 with CSE core promoter. Exposure to NSAIDs inhibited Sp1 phosphorylation, which leads to reduced binding of Sp1 to CSE promoter and lower CSE expression in HEK-293 cells transfected with a vector containing the core CSE promoter (13). Another report demonstrated that TNF-α treatment triples H_2S generation by stimulating binding of Sp1 to the CSE promoter in liver cells, and H_2S generated by CSE stimulates DNA binding and gene activation of NF-κB and maintains its anti-apoptotic properties (21). Similarly in pancreatic beta cells, Calcium-dependent CSE expression is mediated via direct binding of Sp1 with CSE promoter as demonstrated by reporter assay (99).

CSE gene is tightly modulated by oxidative stress. It has been reported that H_2O_2 induced CSE protein and mRNA expression in human lung adenocarcinoma cells (A549 cells) or human liver cancer cells (SMMC-7721 cells), possibly through enhanced CSE transcription, because H_2O_2 stimulated CSE promoter activity (100). Moderate oxidative stress also up-regulates hepatic CSE expression in the fetal to neonatal transition (101). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) as a transcription factor is involved extensively in antioxidant responses (102). In response to oxidative stress, Nrf2 binds to antioxidant response element (ARE) and promotes ARE-mediated transcription of the genes, including CSE gene (103). A recent reported showed that platelet-derived growth factor PDGF-BB induced CSE expression in rat renal mesangial cells in a dose- and time-dependent manner, and this process was possibly through Nrf2 activation (104). The evidence from electrophoretic mobility shift assay demonstrated that PDGF-BB induced binding of Nrf2 to a corresponding consensus antioxidant element in CSE promoter a redox-dependent manner (104).

Elk1, an ETS domain-containing protein, has been shown to regulate CSE transcription. Knockdown of Elk-1 blunted CSE expression in pancreatic beta cells;

however, the reporter assay showed that Elk1 does not directly bind with CSE promoter, suggesting Elk-1 indirectly regulates CSE expression via other pathways (99). In mouse embryonic fibroblasts, deficiency of activating transcription factor 4 (ATF4) caused ablation of CSE expression (105). After searched promoter region of murine CSE, the authors did not find ATF4 binding sites according to its consensus in the promoter region, suggesting ATF4 may bind with the DNA sequence which is not strictly consistent to the consensus, but with several variant bases (105). CSE transcript can also be regulated by its product H2S through feedback response. Luciferase assay demonstrated that exogenous H2S at 120 μM increases the transcription and expression of CSE, while at a concentration of over 160 μM, the transcription and expression of CSE are completely inhibited, suggesting higher level of H2S may become toxic (106).

CSE gene from Neurospora crassa was cloned and characterized, which contains no introns and encodes a protein of 417 amino acids with conserved PLP binding site (107). Gel mobility shift analysis demonstrated that the presence of four CYS3 transcriptional activator binding sites on Neurospora crassa CSE promoter, and mutation of these binding sites caused inactivation of promoter activity (107).

4.2. MicroRNA regulation of CSE protein translation

Recent discovery of microRNAs (miRs) has revolutionized our understanding of the mechanisms of gene expression regulation (108,109). MiRs are endogenous, small, non-coding RNAs that control gene expression by targeting at the 3' untranslational regions (UTRs) of mRNAs for degradation and/or translational repression. The human genome encodes more than 1000 miRs, and these molecules are proposed to regulate up to one-third of all human genes (109). We first demonstrated that miR-21 repressed CSE mRNA and protein expression by directly targeting at transcript factor Sp1 in human aorta SMCs (110). Furthermore, we provided experimental evidence that miR-21 overexpression reduces H_2S production, stimulates SMC proliferation, and represses expression of SMC differentiation marker. One year later, this discovery was confirmed by Cindrova-Davies T *et al*., who found that miR-21 is increased in placentas with abnormal Doppler waveforms, and exposure of villus explants to hypoxia-reoxygenation significantly increases miR-21 expression but reduces CSE protein and mRNA expression (88). It also has been shown that miR-30 family members regulate endogenous H_2S production through interaction with CSE mRNA in primary cardiac myocytes, and inhibition of miR-30s increased CSE expression (111,112). Several bioinformatics software predicts that there are more than 20 types of miRs which could target at human CSE mRNA. However, most of these CSE-targeted miRs have not been experimentally validated, and identification of these miRs may provide new insight into how miRs control expression of CSE genes in various pathological conditions.

4.3. PLP regulation of CSE activity

PLP is an active phosphorylated derivative of vitamin B6 (pyridoxine), which acts as a prosthetic group of certain enzymes, including CSE (113). This co-factor can stabilize carbanionic intermediates in both substitution and elimination reactions involving aminated compounds (114). The supplement of exogenous PLP is crucial for maximal CSE-catalyzed enzymatic activity. Huang *et al*. proved that crystallization of human CSE apoenzyme is attained only when L-cysteine is added to the crystallization conditions, providing the direct evidence that the binding of PLP to CSE during the generation of H_2S. Binding of the substrate to PLP in the first step of the reaction is common for all PLP-dependent enzymes. It is predicted that the presence of PLP binds to human CSE via the active site Lys212 residue (115). Upon the addition of L-cysteine, the internal aldimine is most likely cleaved off following binding of L-cysteine to PLP (35,114,115). Zhu *et al*. confirmed that two pathogenic T67I and Q240E missense mutations in human CSE gene weak the affinity of PLP to CSE, and the PLP content of the T67I and Q240E mutants are about 4-fold and 80-fold lower than that of wild-type enzyme, respectively (95). Preincubation of these mutants with PLP restored activity to wild-type levels, and pyridoxine therapy would be a better choice for cystathionuric patients with these mutations (95).

4.4. Key residues involved in the catalysis of H_2S

With the aid of structure-based site-directed mutagenesis of human CSE, Huang *et al*. explored the critical residues involved in the catalysis of H_2S (115). Mutation of glutation 339 residue in human CSE to a more hydrophobic residue such as alanine or tyrosine leads to an approximately 6-fold enhancement in H_2S production (115). These observations suggest that the extent of hydrophobicity determines the rate of H_2S production by CSE and the affinity of these mutations for the cysteine substrate may be much lower than the wild type (115). The residues of Ser209, Thr211, and Glu349 are most probably not catalytically involved in the production of H_2S, because mutation of these residues did not change H_2S production rate. In contrast, mutation of other residues in human CSE, including Tyr60, Arg62, Tyr114, Asp187, Thr189, Lys212, and Arg375, displayed a complete loss of H_2S production activity (115). These amino acids are either highly conserved across different CSE homologs or in close proximity to the PLP cofactor or substrate binding site of the enzyme.

4.5. Hormone regulation of CSE activity

Testosterone has a high androgenic and anabolic potency. CSE activity has been shown to be unregulated by testosterone in vascular tissues, which lead to a concentration-dependent vasodilatation of rat aortic rings *in vitro* (116). Further evidence showed that testosterone did not change CSE protein expression in aorta tissues, suggesting that testosterone possibly modulates CSE activity at post-translational level. 17β-estradiol has a higher estrogenic effect, and injection of ovariectomized Sprague-Dawley rats with 17β-estradiol for 12 weeks increased CSE expression and H_2S generation in the myocardium (54). 17β-estradiol also decreased oxidative stress and inflammatory status, suggesting that estrogens might exert cardioprotective effects through up-regulation of CSE expression and H_2S generation (54). In contrast, in

SMCs, 17β-estradiol had no effect on CSE expression and H_2S production (45). Dexamethasone as an anti-inflammatory and immunosuppressant glucocorticoid hormone, was reported to suppress LPS-induced CSE expression and H_2S production rate in macrophage, suggesting the involvement of CSE/H_2S system in coordinating the balance between pro- and anti-inflammatiory mediators (117). Another similar report also showed that inhibition of CSE expression in neutrophils contributes to the anti-inflammatory effect of dexamethasone in rat endotoxic shock (118). Some other stress hormones, such as phenylephrine or glucagon were reported to increase CSE activity in fetal hepatocytes, pointing to the critical role of CSE in regulating oxidative stress during the fetal-to-neonatal transition (119). We recently proved that CSE expression was increased in insulin-resistant state induced by exposing hepatocytes to high levels of insulin (500 nM) and glucose (33 mM), suggest that the interaction of H_2S and insulin in liver plays a pivotal role in regulating insulin sensitivity and glucose metabolism (60).

4.6. Calcium regulation of CSE activity

CSE is physiologically activated by calcium-calmodulin. In endothelial cells, H_2S formation was markedly augmented by the calcium ionophore A23187, but blocked by the calcium chelator BAPTA and calmodulin antagonist W7 (3). Catalytic activity of pure CSE was increased more than two-fold by calcium and calmodulin but not by either substance alone (3). Using recombinant CSE protein, it was shown that CSE directly binds with calmodulin, which was abolished by the calcium chelator EGTA and W7 (3). In SMCs, both A23187 and thapsigargin induced the increased of intracellular calcium following increased CSE expression and H_2S production, all of which sustains mitochondrial ATP production under hypoxic conditions (11). Similarly, blockage of calcium/calmodulin-dependent protein kinase (CaMK) II reversed glucose- and thapsigargin-induced CSE expression in pancreatic beta cells (99). Mikami Y *et al.* also showed that CSE efficiently produces H_2S at the steady-state low calcium concentrations but is suppressed at high calcium concentrations in the presence of PLP. In contrast, in the absence of PLP, H_2S production maintains the suppressed levels at high calcium concentrations and decreased further at low calcium concentrations, suggesting that calcium may interact or compete with PLP to regulate CSE activity (120).

4.7. NO and CO regulation of CSE activity

Similar to H_2S, NO and CO are another two members in gasotransmitter family. Zhao *et al*. first discovered that incubation of rat aortic tissue homogenate with a NO donor significantly increased CSE activity (10). L-arginine, the substrates to produce NO, also has been shown to augment the expression of CSE mRNA as well as H_2S production in rat lung tissue and significantly attenuated pulmonary artery pressure (121). In contrast, another report showed that L-arginine decreased CSE expression in myocardium and improved myocardial function (122). In line with this, administration of nitroflurbiprofen, a NO donor, resulted in a dose-dependent inhibition of LPS-mediated increase in liver CSE expression and H_2S synthesis, contributing to the anti-inflammatory activity of this compound and highlighting the existence of 'crosstalk' between NO and H_2S in inflammation status (123).

Interaction of CO and H_2S has also been shown to be involved in multiple conditions. The H_2S content in the medium and CSE expression was markedly increased by ZnPP (a known inhibitor of heme oxygenase-1 (HO-1)) compared with the control group in SMCs, suggesting that endogenous CO/HO pathway inhibits CSE/H_2S system under physiological conditions (124). Similar to this finding, another study demonstrated that the concentration of H_2S was negatively correlated with CO in nasal mucosa from guinea pigs with allergic rhinitis (125).

4.8. CSE inhibitors

Several chemicals have been reported to inhibit CSE activity, including β,β,β-trifluoroalanine (F_3Ala), PPG, aminoethoxyvinylglycine (AVG), β-cyanoalanine (BCA), aminooxyacetic acid (AOAA), and hydroxylamine (HA), etc. Steegborn *et al.* investigated the characteristics of F_3Ala, PPG and AVG with purified human CSE protein and concluded that PPG and F_3Ala could compete with PLP to bind CSE and formed an irrevisible enzyme-inhibitor (EI) complex while AVG is a slow-binding inhibitor (126). Asimakopoulou *et al.* compared the binding ability of inhibitors to CSE and got the conclusion that AOAA is the most potent inhibitor of CSE with inhibitor constant (IC) 50 of 1.1. + 0.1. μM (127). This challenged our common knowledge that AOAA is a specific inhibitor of CBS, another H_2S producing enzyme. Besides with AOAA, HA and F_3Ala have also been reported to act as CBS inhibitors (1,126,127).

5. PERSPECTIVE

The molecular, biological, biochemical and experimental evidence supports an important role of CSE/H_2S system in both health and diseases. Corruption of CSE/H_2S signaling is considered an early and common mechanism underlying numerous pathologies. Although knowledge about the gasotransmitter's role of H_2S is rapidly increasing, we are still at the beginning of understanding as to when and how mammalian cells generate H_2S. This review summarizes the available progress on how CSE/H_2S system is involved in various pathological disorders. A comprehensive, multi-tiered regulation network contributes to the expression change of CSE as well as H_2S production. There is a great need of significant trails that further explore the importance of CSE/H_2S system in clinical stage. Modulating CSE through changing the bioavailability of its substrate and cofactors, altering its transcription, and interfering with other modulators of CSE/H_2S system, has high potential for effective treatment of various diseases. We also need to carefully evaluate the perspectives of pharmacological enhancement or inhibition of CSE activity as a strategy for new drug design. More studies in the future are needed to develop CSE specific inhibitors/activators that can be used safely and effectively for disease treatment in real clinic stage.

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Abbreviations: 3-MPST, 3-mercaptosulfurtransferase; ATF4, activating transcription factor 4; CBS, cystathionine beta-synthase; CHIP, chromatin immunoprecipitation; CSE, cystathionine gamma-lyase; H_2S, hydrogen sulfide;

HO-1, heme oxygenase-1; I/R, ischemia-reperfusion; LPS, lipopolysaccharide; miRs; microRNAs; NO, nitric oxide; OVA, ovalbumin; PLP, pyridoxal 5'-phosphate; PPG, $_{DL}$-propargylglycine; siRNA, short interfering RNA; SMCs, smooth muscle cells; Sp1, specificity protein-1; STZ, streptozotocin

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