Emerging role of hydrogen sulfide-microRNA crosstalk in cardiovascular diseases

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Am J Physiol Heart Circ Physiol 310: H802–H812, 2016. First published January 20, 2016; doi:10.1152/ajpheart.00660.2015.—Despite an obnoxious smell and toxicity at a high dose, hydrogen sulfide (H₂S) is emerging as a cardioprotective gasotransmitter. H₂S mitigates pathological cardiac remodeling by regulating several cellular processes including fibrosis, hypertrophy, apoptosis, and inflammation. These encouraging findings in rodents led to initiation of a clinical trial using a H₂S donor in heart failure patients. However, the underlying molecular mechanisms by which H₂S mitigates cardiac remodeling are not completely understood. Empirical evidence suggest that H₂S may regulate signaling pathways either by directly influencing a gene in the cascade or interacting with nitric oxide (another cardioprotective gasotransmitter) or both. Recent studies revealed that H₂S may ameliorate cardiac dysfunction by up- or downregulating specific microRNAs. MicroRNAs are noncoding, conserved, regulatory RNAs that modulate gene expression mostly by translational inhibition and are emerging as a therapeutic target for cardiovascular disease (CVD). Few microRNAs also regulate H₂S biosynthesis. The inter-regulation of microRNAs and H₂S opens a new avenue for exploring the cardioprotective role of H₂S. Therefore, H₂S-miRNA crosstalk may have a crucial role in pathophysiology of heart failure. This review embodies regulatory mechanisms that maintain the physiological level of H₂S, exogenous H₂S donors used for increasing the tissue levels of H₂S, H₂S-mediated regulation of CVD, H₂S-microRNAs crosstalk in relation to the pathophysiology of heart disease, clinical trials on H₂S, and future perspectives for H₂S as a therapeutic agent for heart failure.

Keywords: heart failure; inflammation; apoptosis; fibrosis; clinical trial; microRNAs

HYDROGEN SULFIDE (H₂S) was first discovered in 1777 as a colorless gas with a strong “rotten egg” odor. It was thought to be a toxic substance found in sewer gas, swamp gas, and volcanic discharge. Since the discovery that H₂S reacts to oxyhemoglobin similar to nitric oxide (NO) and carbon monoxide (17), a number of studies have been carried out to understand the biological functions of H₂S. The obnoxious odor and toxicity discouraged the attention of researchers until it was revealed that H₂S may have a possible role as an endogenous neuromodulator (1). Subsequently, a plethora of investigations were performed on potential roles of H₂S in cardiovascular disease (CVD), which revealed that physiological levels of H₂S have a pivotal role in maintaining cardiac function, and an exogenous supply of H₂S has the potential to ameliorate heart failure in rodents. Empirical studies elucidated several potential mechanisms of H₂S-mediated cardioprotection in different models of heart failure (21, 117, 123, 128, 150). However, regulation of H₂S functions during heart failure is not completely understood. Recently, it was demonstrated that miRNA regulates endogenous H₂S production (129, 148). MicroRNAs (miRNAs) are noncoding, regulatory RNAs that modulate gene expression mostly by translational repression (10). Interestingly, H₂S also regulates miRNA transcription. However, the crosstalk between H₂S and miRNAs, and its impact on CVD, is poorly understood. Considering differential expression of miRNAs in CVD (27, 133), H₂S-miRNAs crosstalk may have a crucial role in pathophysiology of heart failure. Therefore, H₂S-miRNA crosstalk is important for understanding H₂S-mediated cardioprotection. The goal of this review is to summarize the advancements made in H₂S-mediated cardioprotection, highlight the inter-regulation of miRNAs and H₂S, and emphasize potential future avenues of H₂S-miRNA crosstalk in CVD, which will provide an impetus for developing H₂S-based therapeutics for heart disease.

Regulation of H₂S in vivo. H₂S is toxic at high levels; therefore, the production and degradation of H₂S must be tightly regulated in our body to maintain its physiological level. Multiple synthesis and degradation pathways provide a complex interdependence, which could be crucial for restoring the physiological H₂S level (Figs. 1 and 2). Understanding these pathways is indispensable for developing H₂S-based therapeutics.

H₂S biosynthesis. H₂S is predominately and primarily produced by three enzymatic pathways, which include cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and the coupling of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfur transferase (3-MST). CBS, CSE, and CAT are pyridoxal 5-phosphate-dependent enzymes. CBS catalyzes the β-replacement of thiosulfides such as homocysteine to cysteine, whereas CSE catalyzes the α- and γ-replacement of

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cysteine to H₂S. CAT deaminates cysteine to mercaptopyruvate, which is followed by transulfuration catalyzed by the zinc-dependent enzyme 3-MST that results in biosynthesis of H₂S. These pathways are elaborated in several excellent review articles (4, 8, 12, 20, 69, 75, 111, 113, 150) and summarized in Fig. 1. Although CBS and CSE are localized in the cytoplasm, 3-MST is localized mainly in mitochondria but has also been reported in the cytoplasm of vascular endothelial cells (106,
H2S is predicted to exist as 14% free H2S gas, 86% HS in members of the gastrointestinal tract by bacterial flora (44, 52, 91, 92). The multiple pathways for H2S synthesis (Fig. 1) suggest an important role of H2S in cell signaling (53, 87, 99, 104).

**Catabolism of H2S.** High levels of H2S are toxic. H2S levels are decreased in our body by several catabolic pathways. H2S is oxidized to thiosulfate and sulfate in the mitochondria of most mammalian tissues; however, the underlying molecular mechanisms and pathways are poorly understood (11, 69). Sulfide quinone oxidoreductase, which is located on the inner mitochondrial membrane, oxidizes H2S to glutathione persulfide using GSH as the sulfide acceptor (55, 90). Persulfide dioxygenase or rhodanese catalyze the oxidation of glutathione persulfide to thiosulfate and sulfate, which is further oxidized to sulfate by sulfite oxidase (90). Conversely, Jackson et al. (63) have shown sulfide quinone oxidoreductase catalyzes the oxidation of H2S directly to thiosulfate using co-enzyme Q as the electron acceptor and sulfite as the acceptor of sulfate sulfur. Although the intermediate pathways involved in the oxidation of H2S are not completely understood, sulfate is the primary by-product of sulfate catabolism, which may be excreted in the urine and feces (63, 69, 90). An additional pathway for excretion of H2S may include expiration by the lungs (61, 142). H2S may be scavenged by disulfide-containing molecules and red blood cells, where H2S binds to methemoglobin forming sulfhemoglobin, which is oxidized to thiosulfate (96). The multiple pathways for reducing H2S levels are presented in Fig. 2.

**Physiological level of H2S.** Physiological levels of H2S range between 15 nM to 300 μM in vivo (62, 84, 85, 96, 110, 140, 153, 154). The wide range of H2S levels results from variable detection methods and the tissues analyzed [see review by Liu et al.(96)]. In vivo conditions (37°C, pH ~7.4), H2S is predicted to exist as 14% free H2S gas, 86% HS^−, and trace levels of S^{2−} (96, 153). Limitations to measuring H2S include 1) free H2S has a short half-life, ranging from 12 to 300 s in various species, and 2) detection methods may release bound sulfur from proteins, resulting in increased H2S concentrations (61, 96, 142, 153).

**Exogenous sulfur donors.** Sodium hydrogen sulfide (NaHS) and sodium sulfide (Na2S) are the two most commonly used sources of H2S. They are water soluble and cost efficient. However, a limitation of NaHS and Na2S is that H2S is a volatile substance resulting in evaporation from drinking water. Furthermore, they give more of a bolus effect of H2S instead of a slow, steady release (19). Diallyl disulfide and diallyl trisulfide (DATS) are organic sulfur compounds found in members of the Allium species (garlic, onions, chives, etc.) that act as H2S donors and have antioxidant, anti-inflammatory, and vasorelaxant properties (14, 94, 116, 132). Slow release sulfide donors such as GYY4137 (88), SG1002 (9, 78, 118), AP39 (138), and S-propargyl-cysteine (58, 149) are potential options for future H2S supplementation. In fact, SG1002 successfully completed Phase I clinical trial and is beginning Phase II clinical trial for increasing plasma H2S levels and mitigating heart failure (clinicaltrials.gov; No. NCT01989208 and No. NCT02278276) (118). Because of the volatile nature and short half-life of H2S, a continuous, low-level H2S release may provide an extended therapeutic potential than a single bolus using high levels as used in many NaHS and Na2S studies (19).

**CVD and H2S.** CVD refers to any disease that leads to dysfunction in the heart and vasculature. Although many therapeutic options are available to treat CVD, the World Health Organization reports CVD remains the leading cause of death globally for both men and women, resulting in 17.5 million deaths in 2012 (155). Further research and better treatment options are necessary to reduce the incidence and burden of CVD. Evidence is mounting that H2S is important in reducing the symptoms associated with CVD. H2S reduces hypertension (2, 3), improves glucose uptake and metabolism (9, 89), protects against ischemia-reperfusion (I/R) injury (70, 81, 141, 161), and decreases cardiac hypertrophy (9, 73, 93, 97). Further evidence shows that free H2S levels are reduced in patients with CVD (64, 79, 118), suggesting H2S supplementation may be a potential therapy for mitigating CVD. However, the underlying molecular mechanisms for H2S-mediated improvement in cardiac function, or amelioration of cardiac dysfunction in CVD, are not completely understood.

**Diabetic cardiomyopathy is mitigated by H2S.** Diabetes is among the top 10 causes of death in the United States (25a). One of the many complications of this debilitating disease is myocardial dysfunction (95, 152). Diabetes increases the risk of heart failure two- to fourfold as compared with age- and sex-matched nondiabetics (25, 100). Diabetes may effect both the heart and the vasculature or it may lead to left ventricular heart failure independent of coronary artery disease, a condition known as diabetic cardiomyopathy (26, 95). Diabetic cardiomyopathy is the end result of chronic exposure to a hyperglycemic environment. The pathophysiology includes cardiac dysfunction due to increased fibrosis and left ventricular hypertrophy (48). High glucose induces cardiovascular dysfunction by activating the PKC/diacylglycerol pathway, calcium dysregulation, inducing endoplasmic reticulum stress, and deregulating autophagy and apoptosis, which have been shown to be mitigated by H2S supplementation (9, 80, 89, 156, 164). Furthermore, plasma H2S levels are decreased in type 2 diabetic patients, high-fat diet–treated mice (type 2 diabetic patients), and streptozotocin-treated rats (9, 64, 66). The decrease in H2S levels in diabetics and the ability of exogenous H2S treatment to ameliorate pathological conditions induced by type 1 and type 2 diabetes suggest that H2S may be a potential novel treatment option for mitigating cardiomyopathy in diabetics.

**H2S mitigates I/R injury.** Ischemia is the restriction of blood to tissues leading to a decrease in oxygen and glucose. Reperfusion injury occurs when blood flow returns to an area following ischemia and can further exacerbate cell damage (23, 146). I/R injury leads to increased cardiomyocyte apoptosis (81). H2S reduces reactive oxygen species (ROS) associated with I/R injury as well as decreases in cardiomyocyte apoptosis during ischemia (36, 56, 70, 81, 135, 141, 161) (Table 1). A major complication following I/R injury is the occurrence of cardiac arrhythmias (37). H2S released by α-lipoic acid treatment activates the potassium ATP-sensitive channels (KATP channels) (37, 67) and decreases post I/R arrhythmias (37). It was further demonstrated that lower plasma H2S levels are associated with greater arrhythmia scores following I/R injury in Wistar rats (137). NaHS treatment inhibited the L-type Ca^{2+} channels.
channels, activated the K_{ATP} channels, and increased the action potential duration in these rats, all of which may result in decreased arrhythmias (137). During myocardial I/R injury, delivery of H_{2}S at the time of reperfusion reduces infarct size and preserves left ventricular function. Moreover, overexpression of cardiac CSE mitigates myocardial I/R injury (39). H_{2}S may protect the heart from I/R injury by increasing NO bioavailability and signaling (76). These findings suggest that H_{2}S treatment may provide protective benefits following I/R injury by decreasing ROS and by mitigating cardiac arrhythmia.

**H_{2}S is hypotensive.** Hypertension (high blood pressure) is defined as having a systolic blood pressure >140 mm mercury (mmHg) or a diastolic pressure >90 mmHg (107a, 109). Hypertension is a major medical concern affecting ~30% of the United States population (109). Chronic high blood pressure creates excessive stress on the vasculature, leading to cardiac hypertrophy over time. An initial response to elevated blood pressure is activation of the renin angiotensin aldosterone system (RAAS) (124, 167). However, chronic activation of the RAAS leads to heart failure (98, 167). H_{2}S inhibits activation of the RAAS in Dahl rats fed with a high-salt diet, mitigating hypertension (59). Furthermore, abrogation of CSE (involved in H_{2}S biosynthesis) decreases the level of H_{2}S and induces hypertension in mice (158). H_{2}S may decrease hypertension by opening voltage-dependent potassium channels, resulting in vasorelaxation and increasing vascular tone through multiple mechanisms (77, 125). H_{2}S also induces vasorelaxation by opening K_{ATP} channels (136, 162) and increasing NO production (38). Contrary to vasorelaxation, Na_{2}S and NaHS increased intracavernoosal pressure by opening potassium channels in penile tissue (68), suggesting that H_{2}S may cause vasoconstriction in other tissues. Therefore, the role of H_{2}S for vasorelaxation or vasoconstriction depends on different conditions and tissues. Nevertheless, H_{2}S has multiple mechanisms for reducing hypertension, suggesting a possible therapeutic role for H_{2}S supplementation in treating hypertension.

**Molecular mechanisms of H_{2}S-mediated cardioprotection.** The cardioprotective effects of H_{2}S are related to several important signaling and regulatory pathways including antioxidant, anti-apoptotic, antifibrotic, and anti-inflammatory regulation (Fig. 3).

**Antioxidant effects of H_{2}S.** ROS refer to small reactive molecules such as superoxide (O_{2}^{−}), hydrogen peroxide (H_{2}O_{2}), hydroxyl (OH^{−}), and hypochlorite (OCl^{−}), which react readily with other cellular molecules (29, 51). ROS are primarily generated as by-products of oxidative phosphorylation within the mitochondrial electron transport chain (29). Healthy mitochondria are vital for the overall health of the heart, because mitochondrial ATP generation via oxidative phosphorylation is essential for energy supply and for survival of cardiomyocytes. The generation and removal of ROS are tightly controlled during normal homeostasis but ROS generation increases during pathological conditions (18, 46). Superoxide radicals activate JNK, which is a stress-activated protein kinase. Induction of JNK leads to the activation of NF-κB, an important transcription factor of many cytokines, resulting in increased expression of NF-κB (80). High glucose (33 mM) treatment increases expression of JNK, NF-κB, and the pro-apoptotic marker caspase-3, which results in increased apoptosis in cardiomyocytes (80).

In humans, red blood cells convert organic polysulfide (derived from garlic) into H_{2}S, and it is facilitated by allyl substituents (14). DATS inhibits apoptosis by preventing caspase-3 activation, blunting phosphorylation of JNK and c-JUN, and inhibiting nuclear translocation of NF-κB (80). H_{2}S mitigates upregulation of ROS and prevents cell death associated with several disease states including hyperglycemia, hyper-homocysteinemia, smoke exposure, and I/R injury (49, 54, 144, 145, 156, 165).

Nuclear factor erythroid 2 related factor (Nrf2) is a primary regulator for the transcription of antioxidant response elements (72). NaHS (8 μM·kg^{-1}·day^{-1}) increases Nrf2 levels in cardiomyocytes, resulting in decreased ROS levels in smoke-exposed rats (165). Furthermore, Nrf2 activation by DATS (10 μM) reduces ROS in high glucose–treated cardiomyocytes (143). In both cases, Nrf2 activation is the result of increased PI3K/Akt signaling induced by H_{2}S supplementation (143, 165). H_{2}S administration before myocardial ischemia increases

Table 1. Disease models and signaling mechanisms showing the cardioprotective role of H_{2}S in cardiovascular diseases

<table>
<thead>
<tr>
<th>Disease and Role of H_{2}S</th>
<th>Pathways Regulated by H_{2}S</th>
<th>Reference (No.)</th>
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<tbody>
<tr>
<td>Ischemia-reperfusion</td>
<td>Anti-apoptosis</td>
<td>Kang et al. (70)</td>
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<td></td>
<td>Anti-inflammatory and anti-apoptosis</td>
<td>Zhang et al. (161)</td>
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<td></td>
<td>Anti-inflammatory</td>
<td>Toldo et al. (141)</td>
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<td></td>
<td>Anti-apoptosis</td>
<td>Lambert et al. (81)</td>
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<td></td>
<td>Anti-apoptosis</td>
<td>Hu et al. (56)</td>
</tr>
<tr>
<td>Smoke-induced cardiomyopathy</td>
<td>Anti-inflammatory, anti-apoptosis</td>
<td>Zhou et al. (163)</td>
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<tr>
<td></td>
<td>Antioxidant</td>
<td>Zhou et al. (165)</td>
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<tr>
<td>Hypertension</td>
<td>Glucose utilization</td>
<td>Liang et al. (89)</td>
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<td></td>
<td>Antioxidant</td>
<td>Xu et al. (156)</td>
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<td></td>
<td>Anti-apoptosis Antioxidant</td>
<td>Wei et al. (151)</td>
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<td></td>
<td>Antioxidant, anti-apoptosis, antifibrosis</td>
<td>Kuo et al. (80)</td>
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<td></td>
<td>Antioxidant, anti-apoptosis</td>
<td>Barr et al. (9)</td>
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<td>Tsai et al. (143)</td>
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<tr>
<td>Vasorelaxation</td>
<td>Vaso dilatation</td>
<td>Sun et al. (136)</td>
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<td>Zhao et al. (162)</td>
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<td>Eberhardt et al. (38)</td>
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GLUT, glucose transporter; H_{2}S, hydrogen sulfide; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species.
Fig. 3. Cardioproteective effects of H$_2$S. The pathways involved in H$_2$S cardioprotection and the signaling mechanisms that have been shown to be induced (↑) or downregulated/blocked (↓) by H$_2$S are indicated. The signaling molecules involved in each pathway and their references are included. CTGF, connective tissue growth factor; NOX, NADPH oxidase; SMA, smooth muscle actin; NO, nitric oxide; ROS, reactive oxygen species; ER, endoplasmic reticulum.

H$_2$S promotes cell survival. Apoptosis or “programmed cell death” can be a protective mechanism at physiological levels for removing damaged cells. However, in pathological conditions, apoptosis is deregulated, which causes increased cell death and tissue damage. As previously mentioned, ROS induces apoptosis and ROS can be mitigated by the antioxidant properties of H$_2$S (see H$_2$S mitigates I/R injury). In addition, H$_2$S inhibits apoptosis by suppressing autophagy (65, 163). Autophagy is lysosome-mediated degradation and recycling of damaged and/or unnecessary cellular components (84). NaHS treatment suppresses cigarette smoke-induced upregulation of the autophagy inducers: Beclin-1, LC3II, and AMPK (163). H$_2$S not only downregulates autophagy but it also induces PI3K and GSK3β. PI3K and GSK3β upregulate Nrf2 for cardioprotection (65, 143, 165). In an in vivo I/R model, NaHS (100 μM) increased phosphorylated mTOR complex 2, resulting in inactivation of cell death initiator Bim (Bcl-2 interacting mediator of cell death) for cell survival (166). Conversely, GYY4137 (100 μM) increases phosphorylated AMPK and decreases mTOR, which prevented high glucose-induced cardiac hypertrophy (151).

Members of the MAPK family, p38-MAPK and ERK1/2, are upregulated in response to cellular stress, leading to cardiomyocyte apoptosis (50, 156). p38-MAPK is induced in several disease conditions including hyperglycemia, I/R injury, and hypoxia (16, 49, 50, 134, 156). NaHS (400 μM) inhibits the activation of p38-MAPK in response to the antitumor drug doxorubicin, resulting in decreased apoptosis (50). Furthermore, pretreatment of H9c2 cardiomyocytes with NaHS (400 μM) reduces the cytotoxicity associated with hyperglycemia by inhibiting p38-MAPK and ERK1/2 (156). These findings demonstrate that H$_2$S provides cardioprotection by promoting cell survival via suppressing autophagy and inhibiting multiple proteins involved in apoptosis signaling (Fig. 3).

H$_2$S mitigates pathological cardiac remodeling by inhibiting fibrosis and hypertrophy. Cardiac fibrosis results from the proliferation of myofibroblasts, leading to increased extracellular matrix deposition in the muscle (34, 47). Excessive fibrosis decreases contractility and cardiac output and is often associated with cardiomyopathy. NaHS (100 μM) reduces fibroblast proliferation in human atrial fibroblast cells (130). Furthermore, it decreases NADPH oxidase 4, a stimulator of fibrogenic markers α-smooth muscle actin and connective tissue growth factor (114). Oral supplementation of SG1002 (20 mg·kg$^{-1}$·day$^{-1}$) reduces fibrosis in a transverse aortic constriction model as assessed by Masson Trichrome and Picrosirius Red staining (78). These findings suggest that H$_2$S inhibits fibrosis (Fig. 3), and H$_2$S supplementation has a therapeutic potential for decreasing cardiac fibrosis.

Cardiomyocyte hypertrophy occurs in response to chronic increases in pressure or stress. NaHS (100 μM) prevents phenylephrine- and isoproterenol-induced hypertrophy in cultured cardiomyocytes and in rat hearts. Furthermore, it downregulates mitochondrial ROS production, apoptosis, and improves glucose uptake through upregulation of the glucose transporters Glut-4 and Glut-1 (89, 97). The H$_2$S donor SG1002 prevents cardiac hypertrophy and cardiac dysfunction by reducing endoplasmic reticulum stress in high-fat diet–fed mice (9). Our laboratory has demonstrated that H$_2$S treatment mitigates cardiac hypertrophy by upregulating miR-133a (73), an antihypertrophy (24, 41) and antifibrosis (28, 101) microRNA. To increase the levels of miR-133a, H$_2$S activates myosin enhancer factor-2c (MEF2C), a transcription factor of miR-133a, by releasing MEF2C from MEF2C-HDAC1 complex (inactivated state) (73). These studies suggest that H$_2$S plays a crucial role in inhibiting cardiac hypertrophy (Fig. 3).

Anti-inflammatory role of H$_2$S. H$_2$S treatments reduce inflammation by multiple mechanisms. NaHS and Na$_2$S (100 μM) inhibits IL-1β (156) and IL-6 (156), leukocyte adhesion (160), and NO (78).
μM/Kg body wt) suppress leukocyte adhesion by activating K_{ATP} channels in rats injected with aspirin, an inducer of leukocyte adhesion (160). Furthermore, inhibition of CSE reduces H$_2$S biosynthesis and promotes leukocyte adhesion (160). H$_2$S also regulates NF-κB, a transcription factor that is induced in stress, apoptosis, and inflammation signaling. NaHS (400 μM) suppresses high glucose-induced increases in NF-κB and the pro-inflammatory cytokine IL-1β in H9c2 cells (156). These studies along with the previously mentioned decrease in therapeutic targets for CVD (22, 103, 108, 122, 126). H$_2$S translational inhibition (10). MiRNAs are rapidly emerging as mRNA and protein expression through mRNA degradation or croRNAs are small (22 nt), noncoding RNAs that regulate /H$_1$11 expression through estrogen receptor (anti-apoptosis) and Akt (anti-oxidant) either directly or via regulating miR-1 and miR-21, respectively. H$_2$S may have synergistic effect on these pathways by

H$_2$S regulates microRNAs to ameliorate heart failure. MicroRNAs are small (~22 nt), noncoding RNAs that regulate mRNA and protein expression through mRNA degradation or translational inhibition (10). MiRNAs are rapidly emerging as therapeutic targets for CVD (22, 103, 108, 122, 126). H$_2$S regulates mRNA expression (70, 73, 93); however, the interaction between H$_2$S and miRNAs in CVD is poorly understood. Only a few publications document that miRNAs may regulate enzymes that control H$_2$S biosynthesis and that H$_2$S regulates miRNAs. H$_2$S and miRNA inter-regulations are summarized in Fig. 4.

MiRNAs regulate H$_2$S biosynthesis. Three miRNAs, miR-21, miR-22, and miR-30, have been shown to control H$_2$S biosynthesis by regulating CSE gene expression. MiR-30 family members are upregulated concomitant with downregulation of CSE in the infarct and border zones following myocardial infarction (MI) in rat hearts (129). Luciferase reporter assay and in vivo silencing of miR-30 have confirmed that miR-30 targets CSE and inhibition of miR-30 can protect the MI heart by upregulating CSE (129). Estrogen (E$_2$) also induces CSE expression through estrogen receptor α, which upregulates transcription of specificity protein-1 (SP1). SP1 directly binds to the promoter region of CSE to upregulate CSE transcription and H$_2$S biosynthesis (148). Ovariectomized rats have increased levels of myocardial miR-22, which are normalized by E$_2$ therapy. Furthermore, addition of miR-22 mimic inhibits estrogen receptor α and SP1 and downregulated CSE transcription (148). This suggests that miR-22 modulates H$_2$S production in females. Therefore, E$_2$ status and its regulation by miR-22 could be crucial in females with CVD. However, the role of anti-miR-22 treatment on cardiac function in males is unclear. It would be an interesting avenue to assess impact of anti-miR-22 treatment on cardiac CSE and H$_2$S levels in males. These studies demonstrate that miRNAs play a crucial role in regulating H$_2$S biosynthesis (Fig. 4).

H$_2$S regulates miRNAs. Not only do miRNAs regulate the biosynthesis of H$_2$S, but H$_2$S has also been demonstrated to regulate miRNAs. NaHS (100 μM) treatment upregulates cardioprotective miR-133a in primary cultures of neonatal rat cardiomyocytes to inhibit phenylepinephrine-induced cardiomyocyte hypertrophy (93). Furthermore, Na$_2$S (30 μM) treatment increased miR-133a level to suppress hyperhomocysteinemia-induced cardiomyocyte hypertrophy in HL1 cardiomyocytes (73). These studies suggest that H$_2$S may have an important role in miR-133a-mediated alleviation of cardiac dysfunction. Because diabetic human heart failure patients have reduced levels of miR-133a (107), the role of H$_2$S in these hearts will be an interesting area for investigation. Measuring the cardiac levels of H$_2$S and designing experiments with late-stage heart failure with H$_2$S supplementation will provide insight on H$_2$S-mediated cardioprotection in the failing heart.

MiR-1 is a bicistronic transcript with miR-133a and is upregulated in response to I/R stress (70). I/R-induced apoptosis is mediated through upregulation of miR-1 (70). MiR-1 is pro-apoptotic and directly suppresses anti-apoptotic proteins including Bcl-2 (70, 139), heat shock protein 60 (127), and heat

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Fig. 4. Interactions between H$_2$S and microRNAs (miRNAs). MiR-21 and miR-22 inhibit specificity protein 1 [SP1, a transcription factor for cystathionine gamma lyase (CSE)], whereas miR-30 directly inhibits CSE, an enzyme responsible for H$_2$S production, resulting in decreased H$_2$S levels. Estrogen (E) activates estrogen receptor α (ERα), which binds to SP1 increasing CSE production and H$_2$S. ERα also inhibits miR-22, which is an inhibitor of SP1. MiR-22 may also inhibit ERα, providing a secondary pathway for reducing CSE expression. H$_2$S inhibits miRNAs including miR-221 (anti-neovasculogenic miRNA) and induces miR-133a (anti hypertrophy and anti-fibrotic miRNA). H$_2$S may inhibit and/or induce miR-21, which is a prohypertrophic miRNA but protects the heart during ischemia-reperfusion injury. H$_2$S and miR-21 have crosstalk as miR-21 regulates H$_2$S biosynthesis by inhibiting SP1 and CSE. H$_2$S regulates Bcl-2 (anti-apoptosis) and Akt (anti-oxidant) either directly or via regulating miR-1 and miR-21, respectively. H$_2$S may have synergistic effect on these pathways by direct regulation and indirect via miRNA regulation. PTEN, phosphatase and tensin homolog.
I/R injury (141). These findings suggest that H2S-miRNA reduces infarct size and attenuates inflammation in response to miR-21 in primary cardiomyocytes and heart tissue. However, levels translate into significant benefits in treating or preventing placental tissue from high risk pregnancy as compared with DATS releases H2S (120) and downregulates anti-neovascularure is required to elucidate H2S-miRNA crosstalk in CVD. An example of H2S-miRNA crosstalk is that H2S downregulates miR-21 to mitigate phenylephrine-induced cardiomyocyte hypertrophy (93), and miR-21 targets SP1 to decrease CSE transcription and H2S production (157). Furthermore, increased miR-21 levels and decreased CSE levels are found in placental tissue from high risk pregnancy as compared with normal pregnancy, suggesting that low H2S level may induce miR-21 and contribute to increased pregnancy complications (32). Perfusion of placental extracts with NaHS improves vasodilation; however, it is unclear whether NaHS perfusion alters miR-21 levels. Although these studies demonstrate that miR-21 may have a negative impact as it reduces H2S levels, several studies show the beneficial effects of miR-21 in cardiac cells, including inhibiting apoptosis (30, 121), protecting cardiomyocytes from H2O2 (30) and I/R damage by suppressing phosphatase and tensin homolog (158). Na2S (10 μM) induces miR-21 in primary cardiomyocytes and heart tissue. However, it had no effect on miR-21 knockout mice even though it reduces infarct size and attenuates inflammation in response to I/R injury (141). These findings suggest that H2S-miRNA crosstalk may vary in a context-dependent manner and may have different roles in different disease conditions (Fig. 4). Therefore, more research using different models of heart failure is required to elucidate H2S-miRNA crosstalk in CVD.

Potential new areas for investigation on H2S-miRNA crosstalk. H2S and miRNAs are relatively new research areas in CVD, and there is a lot of potential inter-regulation between H2S and miRNAs. Although miR-21, miR-22, and miR-30 are demonstrated to modulate CSE gene expression (Fig. 4), no miRNAs are reported that control CBS or 3-MST expression, suggesting a gap in knowledge. There are ample opportunities for investigations on potential miRNAs regulating genes involved in H2S biosynthesis. Furthermore, many miRNAs have been shown to regulate CVD, yet the effect of H2S on only a few miRNAs is reported. This provides an avenue to explore the effect of H2S on crucial miRNAs involved in the regulation of heart failure. For example, miR-24 is upregulated following MI, and it directly inhibits endothelial NO synthase (102). H2S provides cardioprotective effects through upregulating endothelial NO synthase (76, 78), yet it is unclear whether H2S suppresses miR-24. Interestingly, H2S may regulate anti-apoptotic Bcl-2 by either directly upregulating it or by suppressing miR-1, an inhibitor of Bcl-2. Similarly, H2S may regulate Akt directly or by regulating miR-21 (Fig. 4). Future studies in these areas will elucidate the complex regulatory network of H2S-miRNA crosstalk in CVD.

H2S involved in clinical trials. Clinical trials with SG1002 will be exciting to follow and to see if the increase in H2S levels translate into significant benefits in treating or preventing heart failure. Results from the initial clinical trial with SG1002 have recently been reported, demonstrating that SG1002 increases H2S and nitrite levels with few, mild adverse events (118). Furthermore, sulfur donors are being attached to current drug treatments, such as naproxen, improving their anti-inflammatory effectiveness and decreasing gastrointestinal and cardiovascular side effects (13, 19, 35, 42, 105). H2S has beneficial effects in other organs including neurons, kidney, pancreas, and gastrointestinal and in arthritis (15, 43). This suggests organ-specific delivery systems for H2S may need to be developed to localize therapeutic effects. As H2S levels are decreased in diabetics and MI patients, H2S may be a useful biomarker for CVD. A clinical trial has been recently completed where H2S was measured as a biomarker for peripheral artery disease (clinicaltrials.gov; No. NCT01407172). Another clinical trial is recruiting to measure H2S levels in women with CVD (clinicaltrials.gov; No. NCT02180074).

Future directions. Many mechanisms of H2S signaling have been elucidated; however, there are still a lot of unknowns on how H2S levels influence cardiovascular health. As detection methods improve for more accurate measurements of H2S and for measuring real-time H2S generation, we will get a better understanding of how cells maintain H2S balance. H2S mitigates many pathologies associated with CVD, and the therapeutic benefits of H2S are currently being explored in other organ systems. For example, H2S levels are decreased in the neurodegenerative disorders Alzheimer’s and Parkinson’s diseases (40, 57), and H2S treatments have mitigated renal dysfunction by many of the same pathways as involved in cardiomyopathy (115).

The inter-regulation of H2S and miRNAs suggests H2S may have a greater role in maintaining cellular homeostasis than previously thought. For example, H2S may directly regulate Bcl-2 and Akt, or it may control these genes by regulating miR-1 and miR-21, respectively. Besides, H2S may have a synergistic effect using direct regulation and indirect regulation via miRNAs (Fig. 4). More mechanistic studies are required to elucidate the growing role of H2S on miRNA regulation and its potential to regulate epigenetic modifications. MiRNAs regulate pathological remodeling in the heart and may be important treatment options for mitigating CVD (103). Two miRNAs or anti-miRNA therapies are undergoing clinical trial for non-CVD including anti-miR-122 (Miravirsen) for treatment of Hepatitis C (clinicaltrials.gov; No. NCT01200420) (112) and a miR-34 mimic for the treatment of primary liver cancer and other solid tumors (clinicaltrials.gov; No. NCT01829971). The role of these miRNAs on H2S or role of H2S in the regulation of miRNAs is awaiting.

Long noncoding RNAs (lncRNAs), RNAs with >200 nucleotides, are emerging as regulators of genes, miRNAs, and proteins involved in CVD (5, 60, 71, 119). Transcriptional analyses show distinct patterns of lncRNA profiles in heart failure conditions (82, 86), yet lncRNA-mediated regulation of H2S or any effect of H2S on lncRNAs is an open area for investigation. Future studies exploring the crosstalk among lncRNAs, miRNAs, and H2S may elucidate novel regulatory mechanisms for CVD and provide new strategies for treatment of heart failure.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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