Emerging role of hydrogen sulfide-microRNA crosstalk in cardiovascular diseases

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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, highlight the inter-regulation of H2S and microRNAs, and emphasize potential future avenues of investigation in relation to the pathophysiology of heart disease, clinical trials on H2S, and future perspectives for H2S as a therapeutic agent for heart failure.

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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-mercapto-pyruvate 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 cystathionine by cysteine.
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, 151).
H2S is predicted to exist as 14% free H2S gas, 86% HS. Options for future H2S supplementation. In fact, SG1002 successfully completed Phase I clinical trial and is beginning 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/or sulfide, 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 sulfane sulfur. Although the intermediate pathways involved in the oxidation of H2S are not completely understood, sulfate is the primary by-product of sulfide 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 (47). 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 traces of S2~ (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). Dialyl 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 GYY-4137 (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 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 diabetes 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 Ca2+...
Table 1. Disease models and signaling mechanisms showing the cardioprotective role of H2S in cardiovascular diseases

<table>
<thead>
<tr>
<th>Disease and Role of H2S</th>
<th>Pathways Regulated by H2S</th>
<th>Reference (No.)</th>
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<tbody>
<tr>
<td>Ischemia-reperfusion</td>
<td>Anti-apoptosis</td>
<td>miR-1, Bcl-2</td>
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<td>Anti-inflammatory and anti-apoptosis</td>
<td>CD11b<em>Gr-1</em> myeloid cells, Bcl-2</td>
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<td>Anti-inflammatory</td>
<td>miR-21, Inflammasome induction</td>
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<td>Anti-apoptosis</td>
<td>Erk1/2 signaling BCl-xL, Bad, GSK3β</td>
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<td>Anti-apoptosis</td>
<td>Na+/H+ exchanger-1, pH</td>
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<td>Smoke-induced cardiomyopathy</td>
<td>Anti-inflammatory, anti-apoptosis</td>
<td>p38, JNK, caspase 3, Bcl-2</td>
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<td></td>
<td>Antioxidant</td>
<td>PI3K/AKT, Nrf2</td>
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<tr>
<td>Hypertension</td>
<td>Glucose utilization</td>
<td>GLUT1, GLUT4</td>
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<td></td>
<td>Antioxidant</td>
<td>p38-MAPK, ERK1/2</td>
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<td>Anti-apoptosis</td>
<td>p-AMPK, Mammalian target of rapamycin</td>
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<td></td>
<td>Angiotensin Antioxidant</td>
<td>p-JNK, p-cJun, NF-κB, ROS</td>
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<td>Antioxidant, anti-apoptosis, antifibrosis</td>
<td>Estrogen receptor ROS, caspase-12, p-JNK, Picrosirius red, GLUT4</td>
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<td>Antioxidant, anti-apoptosis</td>
<td>ROS, caspase-3, PI3K/Akt, Nrf2</td>
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<td>Hypertension</td>
<td>Vasorelaxation</td>
<td>KATP channels, Endothelin-1</td>
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<td></td>
<td>Vasodilation</td>
<td>Nitric oxide</td>
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GLUT, glucose transporter; H2S, hydrogen sulfide; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species.

channels, activated the KATP 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 H2S at the time of reperfusion reduces infarct size and preserves left ventricular function. Moreover, overexpression of cardiac CSE mitigates myocardial I/R injury (39). H2S may protect the heart from I/R injury by increasing NO bioavailability and signaling (76). These findings suggest that H2S treatment may provide protective benefits following I/R injury by decreasing ROS and by mitigating cardiac arrhythmia.

**H2S 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). H2S inhibits activation of the RAAS in Dahl rats fed with a high-salt diet, mitigating hypertension (59). Furthermore, abrogation of CSE (involved in H2S biosynthesis) decreases the level of H2S and induces hypertension in mice (158). H2S may decrease hypertension by opening voltage-dependent potassium channels, resulting in vasorelaxation and increasing vascular tone through multiple mechanisms (77, 125). H2S also induces vasorelaxation by opening KATP channels (136, 162) and increasing NO production (38). Contrary to vasorelaxation, Na2S and NaHS increased intracavernosal pressure by opening potassium channels in penile tissue (68), suggesting that H2S may cause vasoconstriction in other tissues. Therefore, the role of H2S for vasorelaxation or vasoconstriction depends on different conditions and tissues. Nevertheless, H2S has multiple mechanisms for reducing hypertension, suggesting a possible therapeutic role for H2S supplementation in treating hypertension.

**Molecular mechanisms of H2S-mediated cardioprotection.** The cardioprotective effects of H2S are related to several important signaling and regulatory pathways including antioxidant, anti-apoptotic, antifibrotic, and anti-inflammatory regulation (Fig. 3).

**Antioxidant effects of H2S.** ROS refer to small reactive molecules such as superoxide (O2·−), hydrogen peroxide (H2O2), 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 cardiac cell death (80). High glucose (33 mM) treatment increases expression of JNK, NF-κB, and the pro-apoptotic marker caspase-3, which resulted in increased apoptosis in cardiomyocytes (80).

In humans, red blood cells convert organic polysulfide (derived from garlic) into H2S, 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). H2S 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, 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 H2S supplementation (143, 165). H2S administration before myocardial ischemia increases...
the nuclear localization of Nrf2 and protects the ischemic heart from injury (21). These studies suggest that H2S regulates several signaling molecules to suppress oxidants in the heart (Fig. 3).

H2S 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 H2S (see H2S mitigates I/R injury). In addition, H2S 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). H2S not only downregulates autophagy but it also induces PI3K and GSK3β. PI3K and GSK3β upregulate Nrf2 for cardioprotection (65, 143, 156). In an in vivo I/R model, NaHS (100 μM) increases 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 prevents 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 H2S provides cardioprotection by promoting cell survival via suppressing autophagy and inhibiting multiple proteins involved in apoptosis signaling (Fig. 3).

H2S 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 fibroblast proliferation in human atrial fibroblast cells (130). 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 H2S inhibits fibrosis (Fig. 3), and H2S 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 H2S 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 H2S treatment mitigates cardiac hypertrophy by upregulating miR-133a (73), an antihypertrophy (24, 41) and antiﬁbrosis (28, 101) miRNA. To increase the levels of miR-133a, H2S activates myosin enhancer factor-2c (MEF2C), a transcription factor of the MAPK pathway and their references are included. CTGF, connective tissue growth factor; NO, nitric oxide; ROS, reactive oxygen species; ER, endoplasmic reticulum.

**Fig. 3. Cardioprotective effects of H2S.** The pathways involved in H2S cardioprotection and the signaling mechanisms that have been shown to be induced (↑) or downregulated/ blocked (↓) by H2S 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.
μM/Kg body wt) suppress leukocyte adhesion by activating $K_{ATP}$ channels in rats injected with aspirin, an inductor 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 inflammation in response to I/R injury (subheading 4.2), suggest that H$_2$S plays a pivotal role in mitigating inflammation (Fig. 3).

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 shock protein 70 (148). Ovariectomized rats have decreased 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.

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 (antihyper trophy and antifibrotic 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.
miR-21 in primary cardiomyocytes and heart tissue. However, levels translate into significant benefits in treating or preventing normal pregnancy, suggesting that low H2S level may induce placental tissue from high risk pregnancy as compared with increased miR-21 levels and decreased CSE levels are found in reported. This provides an avenue to explore the effect of H2S on n ure is required to elucidate H2S-miRNA crosstalk in CVD. Therefore, more research using different models of heart failure (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). Transcriptome 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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