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Review Article

Role of hydrogen sulfide in the musculoskeletal system

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ABSTRACT

Hydrogen sulfide (H_2S) has been known as a gasotransmitter, and it contributes to various physiological and pathological processes. Multiple enzymes such as cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-Mercaptopyruvate sulfurtransferase (MST) produce endogenous H_2S, and these are differentially expressed in the various tissue systems including the skeletal system. However, abnormal H_2S production is associated with deregulation of the signaling cascade and imbalanced tissue homeostasis. Several studies have previously provided evidence showing the essential regulatory action of H_2S in skeletal homeostasis. In this review, we have emphasized the novel function of H_2S in both bone and skeletal muscle anabolism, in particular. Additionally, we also reviewed the molecular and epigenetic basis of H_2S signaling in bone development and skeletal muscle function.

1. Introduction

Hydrogen sulfide (H_2S) is a gas with an odor similar to that of a rotten egg and has been considered as a toxic environmental pollutant [1,2]. In the last decades, it has been shown that H_2S exists in the biological system and performs many biological functions, the primary function being maintaining physiological homeostasis. Like nitric oxide and carbon monoxide, it is also considered a gasotransmitter in the tissue system [3]. Physiologically, H_2S is an endogenously released gasotransmitter which is known to be generated in the nervous system, heart, kidneys, vasculature, brain, gastrointestinal tract, skeletal muscle, and bones [1,4,5]. H_2S is mainly produced in mammalian tissue by two pyridoxal-5′-phosphate (PLP)-dependent enzymes, cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), in the transsulfuration pathway of homocysteine metabolism [1,6] and also from 3-Mercaptopyruvate sulfurtransferase (3-MST). However, the synthesis of H_2S and bio-distribution is dependent upon on the tissue-specific action. More detailed information on H_2S biosynthesis is shown in Fig. 1.

CBS is predominantly expressed in bone marrow mesenchymal stem cells (BMMSCs), the central nervous system, ileum, and kidney, as well as in the pancreatic islets. CSE is abundantly expressed in the heart, vascular endothelium, kidney, and vascular smooth muscle [1,7,8]. Abnormal H_2S production is linked to different pathophysiological disorders such as atherosclerosis, diabetes, hypertension, asthma and Alzheimer's disease [1,2,9]. We had previously reported that H_2S maintains the bone anabolism and homeostasis via the epigenetic differentiation of BMMSCs [1,10]. A similar result was obtained from a study also suggesting that H_2S is an important mediator of bone anabolism and the homeostasis of T cells in the immune system [11–14]. It has also been demonstrated that H_2S functions as an antioxidant, and anti-inflammatory molecule, as well as balancing redox homeostasis and inducing antioxidant transcription factor Nrf2 [1,10,15]. Several studies have shown that the abnormal production of H_2S is associated with an array of pathological disturbances [10,11,13]. However, the mechanistic basis of the function of H_2S in the tissue system is not fully understood. Therefore, in this review, we particularly emphasized the recent advancement of research on H_2S as it pertains to skeletal development, with the more precise molecular basis of H_2S signaling in bone formation and skeletal muscle myogenesis.

2. Hydrogen sulfide on BMMSCs function and bone formation

2.1. Hyperhomocysteinemia

H_2S as an endogenous gasotransmitter provides anti-inflammatory, anti-oxidative and anti-apoptotic effects that are closely related to skeletal development. Recent studies on the physiological and pathological role of H_2S have clearly explained its osteo-protective effects in bone disease [1,10,11]. Osteoporosis is a bone disease characterized by an imbalance of bone resorption and bone formation that causes bone fragility and increases the risk of fracture due to high intake of methionine through the typical western diet [16]. A disturbance in the methionine metabolic pathway causes the elevation of serum Hcy, a condition called hyperhomocysteinemia (HHcy) [16]. Both

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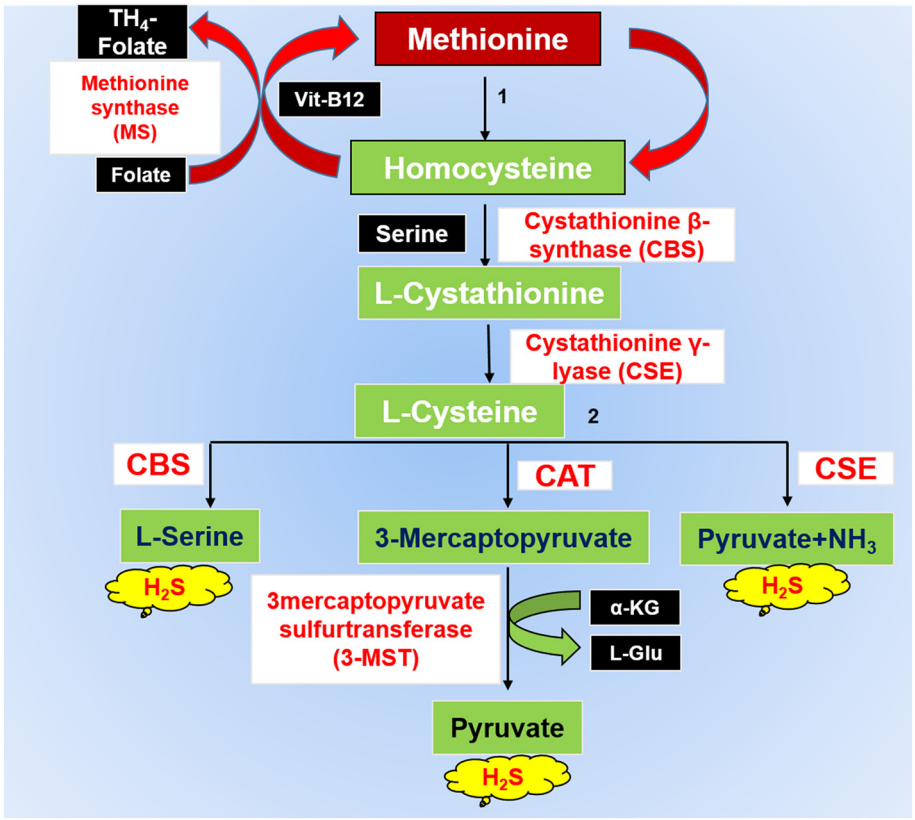

Fig. 1. Endogenous H_2S production, metabolism during methionine cycle. First, Methionine is sequentially converted to L-cysteine through homocysteine intermediate. Methionine can also be re-synthesized from homocysteine by methionine synthase (MS) activity. Second, H_2S biosynthesis from L-cysteine, via the trans-sulfuration pathway by CBS, CSE, and CAT/3MST enzymes. H_2S; hydrogen sulfide, CBS; cystathionine-beta-synthase, CSE; cystathionine-gamma-lyase, CAT; cysteine amino-transferase 3MST; 3-Mercaptopyruvate sulfur-transferase, DAO; D-amino acid oxidase.

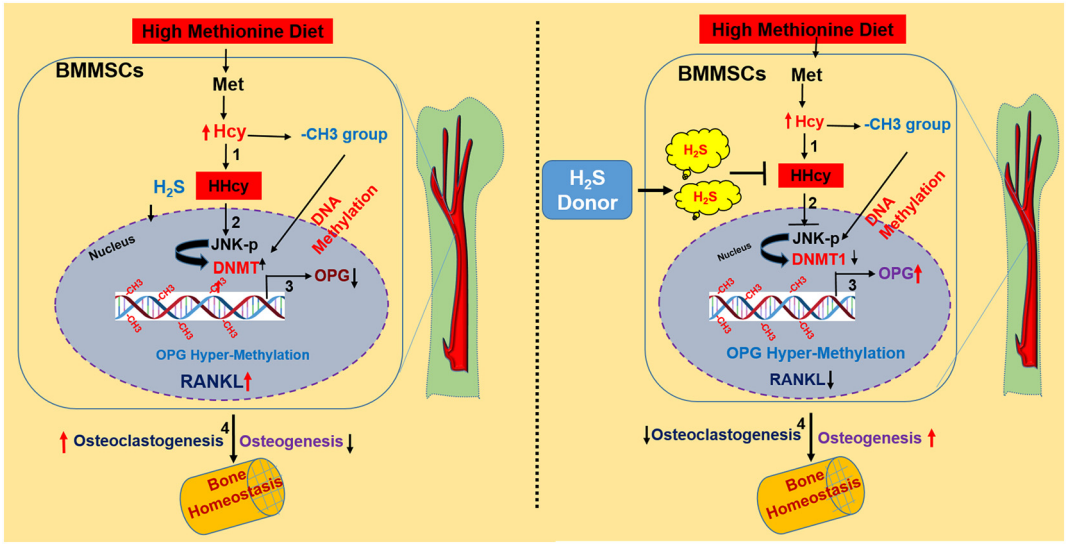

Fig. 2. H_2S epigenetically accelerates bone formation in HHcy mice model. (A): A high methionine diet (HMD) induces the HHcy condition in mice by decreasing endogenous H_2S production (1). HHcy condition activates C-Jun/JNK-p signaling and further transcriptionally regulates DNMT1 expression (2). Increased DNMT1 causes OPG promoter hypermethylation, leading to BMMSCs-derived osteoblast dysfunction (3). The upregulation of RANKL during HHcy increases osteoclastogenesis and bone loss (4). (B): Proposed mechanism of H_2S signaling that reverses the HHcy phenotype (1). Exogenous H_2S administration inhibits JNK-p-DNMT1 signaling (2). Decreased DNMT1 balances OPG-RANKL production in BMMSCs (3). The upregulation of OPG upon H_2S administration increases osteogenesis and bone homeostasis (4).

epidemiological and clinical studies have suggested that a patient having severe HHcy due to decreased expression of CBS could have a detrimental risk factor for the onset of bone loss and fracture [11]. Considering this, we have previously investigated the potential role of H_2S in reversing HHcy-induced bone loss using a high methionine diet (HMD) enhanced HHcy model in mice, which evaluated the potential role of H_2S in reversing HHcy-induced bone loss [1]. Results from our lab have shown that BMMSCs express the CBS protein and enhance H_2S levels that maintain osteogenesis and bone formation in BMMSCs. In this study, the high methionine diet (HMD) fed mice developed HHcy phenotypes, leading to oxidative stress and further epigenetic changes in the CpG islands of the RANKL/OPG promoter through c-Jun/JNK signaling [1]. Administration of an H_2S donor (sodium hydrosulfide; NaHS) prevent the HHcy-induced oxidative damage and bone loss, thus

displaying an osteo-protective property. The detailed H_2S mediated preventive action on bone formation during HHcy is depicted in Fig. 2. Indeed, the study of Xu et al. reported that H_2S protects against oxidative stress via inhibition of mitogen-activated protein kinase (MAPK) signaling in cultured osteoblastic MC3T3-E1 cells [17]. Another study demonstrated that HHcy is associated with the bone loss by decreasing osteoblast activity in a rat model [18]. This study demonstrated that Hcy induces phosphorylation of the protein phosphatase 2 A (PP2A) to inhibit FOXO1/P38 signaling and OPG synthesis. However, administration of *N*-acetyl cysteine reverses the HHcy mediated bone loss and reduction of bone quality [18]. Also, Yang et al. confirmed that H_2S prevented dexamethasone (Dex)-induced apoptosis in MC3T3-E1 cells via AMP-activated protein kinase (AMPK) signaling and inhibited ROS production [19].

2.2. CBS deficiency causes bone loss

H_2S is important for BMMSCs function in that it maintains cell proliferation and differentiation [10,11]. H_2S deficiency in BMMSCs attenuates osteogenesis and proliferation. Interestingly, CBS deficient ($CBS^{+/-}$) mice have decreased serum and intracellular levels of H_2S, causing a severe osteoporotic phenotype [10,11]. However, administration of H_2S via an H_2S donor (NaHS or GYY4137) can restore normal bone homeostasis. The biochemical data suggest that CBS deficient mice have increased levels of Hcy in the plasma, and this leads to oxidative damage and dysfunction of the BMMSCs [10]. The mechanistic study revealed that H_2S deficiency causes decreased intracellular Ca^{2+} influx due to reduced protein sulfhydration of cysteine residues on multiple Ca^{2+} transient receptor potential (TRP) channels [11]. The reduced intracellular level of Ca^{2+} flux further downregulates PKC dependent Wnt/β-catenin signaling, leading to ablation of osteogenic differentiation of BMMSCs [11] (Fig. 3B). Indeed, we have also provided evidence of the epigenetic role of H_2S in CBS deficiency-induced bone loss [10]. Our study strongly suggested that H_2S deficiency caused inhibition of HDAC3 activity and subsequent inflammation by enhancing oxidative damage [10]. Mechanistically, inflammatory cytokines (IL-6, TNF-α) are transcriptionally activated by an acetylated lysine residue in histone (H3K27ac) of chromatin by binding to its promoter. Further, we demonstrate that IL-6 secreted by BMMSCs induces osteoclast differentiation and bone resorption [10]. However, H_2S administration in $CBS^{+/-}$ mice attenuated histone acetylation-dependent inflammatory signaling by restoring HDAC3 activity in BMMSCs and promoted bone formation via RUNX2 in a sulfhydration dependent manner [10]. Collectively, restoration of H_2S may provide a novel anti-osteoporotic property for CBS-deficiency induced metabolic osteoporosis (Fig. 3A).

2.3. Ovariectomy (OVX)

Postmenopausal osteoporosis is a common skeletal disease associated with the declining level of estrogen, leading to bone loss and increased risk of fracture [12,20–22]. Due to the lack of estrogen, the bone resorption process is primarily increased by osteoclast maturation [23]. This led to both trabecular and cortical bone changes after estrogen deficiency [23,24]. Other have shown that genetic factors may potentially modulate bone loss subsequent to estrogen deficiency using different inbred strains of mice [24]. However, future research is still needed to delineate the genetic factors that govern the skeletal changes to estrogen deficiency. For laboratory practice in a small animal such as rats or mice, the acute effect of menopause is modeled by ovariectomy (OVX), which intensifies the bone resorption by increasing osteoclast formation [23,25]. However, the mechanism of preventing osteoclast-mediated bone loss by restoring bone formation needs to be addressed. The work of Grassi et al. investigated whether estrogen deficiency impairs the H_2S level and the role of H_2S in OVX induced bone loss [12]. Grassi et al. showed that administration of the GYY4137 (H_2Sdonor) increases bone formation and completely prevents both trabecular and cortical bone loss caused by ovariectomy via restoring the level of H_2S and increasing BMMSC osteogenic differentiation [12]. Mechanistic studies showed that GYY4137 increases osteoblastogenesis through the activation of the Wnt signaling cascade by increased production of the Wnt ligands Wnt16, Wnt2b, Wnt6, and Wnt10b in the bone marrow [12]. Further, in vitro treatment with 17b-estradiol in human BM stromal cells (hSCs), upregulates the expression of CBS and CSE and produces normal H_2S synthesis. Therefore, restoration of H_2S levels could be a potential osteoprotective approach for postmenopausal osteoporosis [12].

2.4. Tissue regeneration and bone fracture healing

In the past decades, the advancement of research in mesenchymal stem cell (MSCs) transplantation has brought milestones in regenerative medicine, as it has been found that MSCs have a high potential for tissue regeneration. Additionally, H_2S has recently been proposed as a modulator or inhibitor of cell viability/apoptosis in various organ systems. Recent studies demonstrate that administration of H_2S could potentiate MSCs proliferation and survival by preventing multiple forms of stress (low oxygen, oxidative damage, or serum deprivation) induced apoptosis [26–30]. The work of Fox et al. reported that H_2S might represent a novel mechanism of cytoprotection in inflammatory joint pain and rheumatoid arthritis [26]. H_2S is also known to regulate MSC function through upregulating the expression of the anti-apoptosis gene *Bcl-2* to attenuate the hypoxia-mediated effect [30]. Recent studies reported that H_2S (GYY4137) promotes bone fracture healing in the rabbit model of distraction osteogenesis [31]. However, the

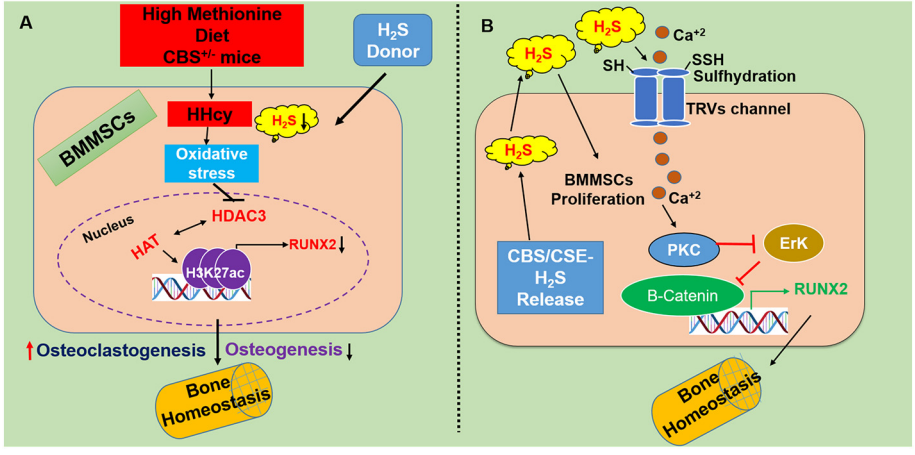

Fig. 3. H_2S deficiency accelerates bone loss in CBS-deficient mice. A. Proposed mechanism for H_2S mediated bone homeostasis in $CBS^{+/-}$ mice. CBS-deficiency in mice causes HHcy condition and decreased H_2S production. This leads to inhibition of histone deacetylase activity through an oxidative stress mechanism. Inhibition of HDAC3 further epigenetically regulates histone acetylation (H3K27ac) and further decreases RunX2 sulfhydration and osteogenesis. In other words, H_2S deficiency enhances osteoclastogenesis and bone loss. B. Proposed mechanism of H_2S signaling in mesenchymal stem cell (MSC) function via regulating Ca^{+2} channel. The endogenous or exogenous H_2S administration affects sulfhydration of calcium channels and calcium influx. This leads to β-catenin-mediated Runx2 dependent osteogenesis and bone homeostasis.

mechanistic basis of H_2S mediated bone healing is still unclear. The work of Zheng et al. [32] reported that Cystathionine gamma-lyase enzyme (CSE) is the major expressed enzyme generating H_2S in osteoblasts. Mechanistically, the CSE-H_2S system promotes increased osteogenesis activity via RUNX2 sulfhydration as a novel transactivation regulator, thereby promoting bone healing. These findings suggest that modulation of H_2S metabolism or H_2S donors might serve as a therapeutic approach for treating osteoporosis or other bone diseases. However, the molecular mechanism needs to be investigated.

CSE-H_2S induces bone fracture healing using a fixed bone fracture model in the rat [32]. Zheng et al., also showed that using micro-computed tomography scanning and 3D reconstruction, those bone fracture lesions were well repaired with increasing trabecular numbers and reducing the trabecular spacing [32]. Furthermore, H_2S is able to prevent inflammatory cell filtration and helps in the deposition of more collagen and osteocytes. Mechanistically, the CSE-H_2S system promotes increased osteogenesis activity via RUNX2 sulfhydration as a novel transactivation regulator, thereby promoting bone healing. These findings suggest that modulation of H_2S metabolism or H_2S donors might serve as a therapeutic approach for treating osteoporosis or other bone diseases, including HHcy induced bone injury or bone fracture in individuals. However, more research is needed to understand further the role of H_2S on transplanted MSCs and direct administration in clinical practice.

2.5. Periodontal disease and orthodontics

Mesenchymal stem cells (MSCs) have been identified from the specialized craniofacial tissue such as exfoliated deciduous teeth, apical papillae, and gingiva [2,33–35]. Dental pulp stem cells (DPSCs), also enriched in tooth pulp, exhibit self-renewal, and multilineage differentiation potential, as observed in BMMSCs [2]. However, DPSCs more specifically undergo odontogenic lineage, and play an important role in tooth development [2,36]. Several studies have reported the novel function of H_2S in these dental stem cells [36]. The work of Cen et al. demonstrates that an optimal concentration of endogenous H_2S is required for periodontal ligament stem cell (PDLSC) osteogenesis via Wnt/β-catenin signaling [37]. Other studies also showed that H_2S is indispensable for PDLSCs and involved in osteogenic and adipogenic differentiation. Interestingly, CBS enzyme is the main source of endogenous H_2S in PDLSC [38]. This study indicates that H_2S is required for periodontal tissue homeostasis. Periodontal inflammation and alveolar bone remodeling are involved in tooth movement [39]. In this study, CSE-H_2S system contributes to osteoclastogenesis during bone remodeling induced by mechanical loading [39]. The data demonstrate that CSE-H_2S was produced endogenously during osteoclast formation and orthodontic tooth movement (OTM) and played a pro-inflammatory role. Furthermore, using $CSE^{+/-}$ mice, they confirmed that CSE-H_2S is essential for bone remodeling induced by mechanical loading [39]. In addition to H_2S mediated periodontal tissue remodeling, the work of Pu et al. investigated the effect of H_2S on the alveolar bone remodeling that is associated with tooth movement [40]. The data provided evidence that H_2S was caused to increase in the rate of tooth movement in vivo by promoting osteogenesis and osteoclastogenesis in alveolar bone [40]. This finding provides a novel understanding of how to increase tooth movement and shorten the treatment time, demonstrating the potential therapeutic value of H_2S as orthodontic treatment.

2.6. Hydrogen Sulfide on skeletal muscle development

The emerging evidence suggests that H_2S has a multifaceted biological role and acts as an anti-inflammatory, anti-oxidative and anti-apoptotic molecule [17,41,42]. However, the exact biological role of these effects on skeletal muscle function in the pathological setting remained to be studied. Recent evidence demonstrates that H_2S may protect from the mitochondrial damage associated with skeletal muscle dysfunction, as mitochondria provide the energy sources for the skeletal muscle function [43]. Therefore, future study to be warranted to understand the detailed mechanistic role of H_2S in reversing skeletal muscle myopathy and dysfunction.

Species-specific expression of H_2S producing enzymes (CBS, CSE) is well documented in skeletal muscles [44]. For example, human skeletal muscle expresses ample amounts of these enzymes, whereas mouse skeletal muscle expresses these enzymes in much smaller amounts [44,45], but these enzymes were present at a detectable level in rat skeletal muscles [46]. However, the species-specific contribution of H_2S production in the skeletal muscle system is not clear. To ameliorate the paucity of knowledge about the role of H_2S in muscle function, several pieces of evidence have been put forth to understand the physiological function of H_2S in skeletal muscle wasting and homeostasis. It was suggested from one study that diabetic patients have a lower level of H_2S in the plasma, potentially leading to skeletal muscle myopathy [47]. Studies by Parsanathan et al. using high glucose-induced C_2C_{12} myoblasts, demonstrated that H_2S donors significantly upregulate the CSE expression and restoration of normal H_2S levels [47]. Further, using the CSE knockdown approach, the data demonstrate that expression of the glucose transporter type 4 (GLUT4) transporter and the key transcription factors (VDR, PGC1α, PPARα, and PPARγ) were decreased in C_2C_{12} myotubes [47]. Another study also explored that H_2S has a protective role in the diaphragmatic muscle function of type 1 diabetic rats [48]. The data demonstrate found that H_2S could improve the diaphragm contractility and ultrastructural damage of diaphragmatic muscle [48]. Furthermore, the mechanistic study showed that H_2S administration increases the activity of superoxide dismutase (SOD) and the ratio of Bcl2/Bax mRNA levels, indicating that H_2S administration in diabetic rats promotes an anti-apoptotic mechanism [48].

In another recent study from our lab, we demonstrated that CBS-deficient mice having hyperhomocysteinemia (HHcy) that can cause skeletal muscle dysfunction [49]. We found CBS-deficiency inhibits H_2S production and induces HHcy, causing redox imbalance and endoplasmic reticulum (ER) stress in the skeletal muscle in vivo. Furthermore, the data revealed that HHcy was detrimental to skeletal muscle, particularly the gastrocnemius and quadriceps muscle weights and muscle atrophy, via JNK/Atrogen 1 signaling. Administration of an H_2S donor, such as NaHS, is beneficial in mitigating HHcy-mediated skeletal injury incited by oxidative/ER-stress responses in $CBS^{+/-}$ mouse models [49] (Fig. 4A). Others have reported that children born with CBS homozygous mutation ($CBS^{-/-}$) die after the age of 15–16 years, however children can survive with the heterozygous mutation ($CBS^{+/-}$), and the single functional allele is able to produce sufficient CBS enzyme to produce at least some of the H_2S required for proper physiological function [49].

Studies by Veeranki et al. demonstrated the mechanistic basis of HHcy induced skeletal muscle weakness and fatigability through mitochondrial dysfunction and epigenetic alternation using $CBS^{+/-}$ mice [50]. $CBS^{+/-}$ can cause a reduction in the number of large muscle fibers, and it reduced mitochondrial ATP production with a decrease in mitochondrial transcription factor A (mtTFA) expression, and, consequently, the reduction of muscular dystrophin level in skeletal muscle [50]. The molecular alteration observed in $CBS^{+/-}$ in mice was reversed after physical treadmill exercise. These results suggest that exercise plays a causal role in reversing the HHcy mediated effect in skeletal muscles in $CBS^{+/-}$ mice [50]. Another study has reported that treadmill exercise regulates endogenous H_2S generation and expression of CSE enzyme, thereby attenuating inflammation in the skeletal muscles of obese rats [51], indicating that treadmill exercise could enhance the H_2S synthesis in skeletal muscle to combat H_2S deficiency associated skeletal muscle dysfunction and weakness. Studies by Du et.al also revealed that H_2S could be endogenously generated by rats' skeletal muscles (H_2S: (2.06 ± 0.43) nmol/mg) and its level was, indeed,

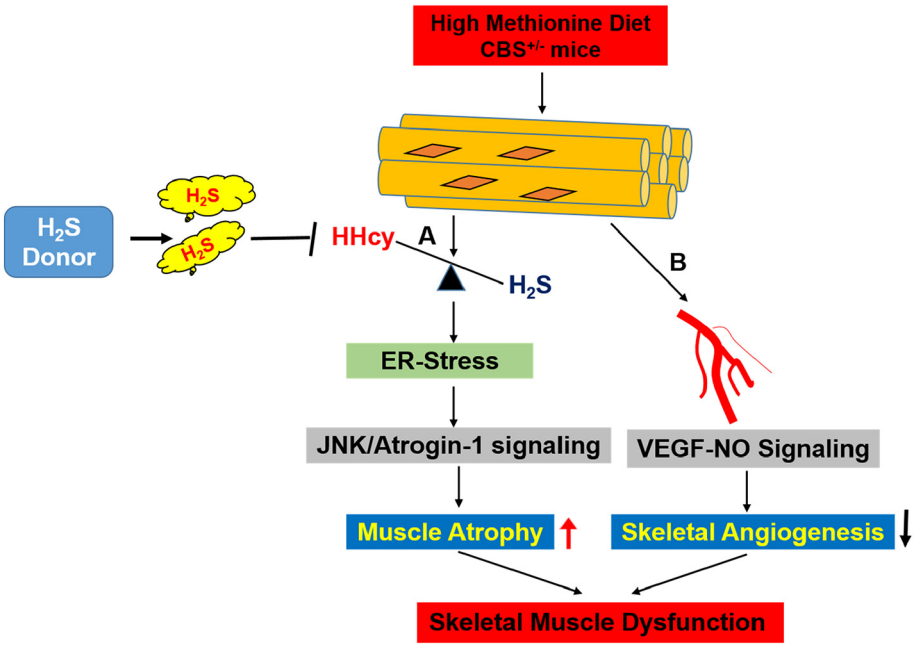

Fig. 4. H_2S promotes skeletal muscle development in CBS-deficient mice. A. The proposed mechanism of H_2S mediated recovery of skeletal muscle dysfunction via ER stress-dependent muscle atrophy. HHcy causes an ER stress response via the oxidative stress-dependent mechanism. This leads to activation of JNK-Atrogen 1 signaling and subsequently causes muscle atrophy. B. The proposed model of HHcy mediated ablation of skeletal muscle angiogenesis. HHcy causes decreased endothelial NO production via VEGF-eNOS signaling. This leads to disruption of endothelium in skeletal muscle. H_2S administration reverses the above effect in HHcy mice model.

down-regulated, mediated through increased oxidative damage in the skeletal muscle of rats with ischemic reperfusion (I-R) skeletal muscle injury [52]. However, H_2S treatment significantly protected rat skeletal muscle against I-R injury [52].

2.7. Hydrogen sulfide on skeletal muscle angiogenesis

Angiogenesis is the process of new capillary growth from the pre-existing vasculature, improving blood flow under ischemic conditions and accelerating wound healing. Therefore, therapeutic angiogenesis might be suggested as an alternative approach in the treatment of ischemia. The proangiogenic function of H_2S and its capacity for the improvement of regional blood flow in ischemic organs is still unknown, though the work of Wang et al. reports that H_2S is a new gasotransmitter promoting angiogenesis in a rat model of hindlimb ischemia [53]. Wang et al. found that H_2S donor (NaHS) administration significantly increased collateral vessel growth, capillary density, and blood flow in ischemic hindlimb skeletal muscles compared with the controls, and there was a subsequent increase in vascular endothelial growth factor (VEGF) expression and vascular endothelial growth factor receptor 2 (VEGFR2) phosphorylation. Mechanistically, the proangiogenic effect of NaHS resulted in VEGF dependent VEGFR2-Protein kinase B (Akt) signaling in skeletal muscle cells, and improved the regional blood flow [53].

The earlier report suggests that CBS is an important Hcy metabolizing enzyme, actively participating in the transsulfuration pathway of methionine-Hcy metabolism [54]. However, mice with heterozygous CBS deficiency ($CBS^{+/-}$) develop the mild to severe HHcy phenotype [55]. Taking this into account, Majumder et al. used an HHcy mouse model ($CBS^{+/-}$) to investigate the effect of H_2S on neoangiogenesis in ischemic skeletal muscle [56]. The data suggested that H_2S donor (GYY4137) administration significantly improved collateral vessel density and blood flow in hindlimb femoral artery ligation (FAL) or ischemic hindlimb skeletal muscles of $CBS^{+/-}$ mice compared with WT mice. The mechanistic study revealed that the GYY4137 treatment augmented VEGF-eNOS-NO signaling in skeletal muscle cells via an HHcy antagonizing effect, and GYY4137 could serve as a potential neoangiogenic modulator to treat the angiogenic defect in hindlimb ischemia of the skeletal muscle in $CBS^{+/-}$ mice [56] (Fig. 4B). In another study, it was reported that restoration or administration of H_2S improves bone marrow (BM) cell function and subsequent preservation of skeletal muscle architecture in a diabetic type-2 FAL mice model (db/db + FAL) [57]. In vitro data showed that treatment of H_2S donor diallyl trisulfide (DATS) or overexpression of CSE restored H_2S synthesis and BMC angiogenic activity in high glucose (HG)-treated BMCs. In vivo administration of DATS or CSE-overexpressing BMCs greatly improved blood perfusion, capillary/arteriole density, and skeletal muscle architecture in ischemic hind limbs of db/db mice. Mechanistically, DATS administration in BMC upregulates NO signaling mediated angiogenesis and restores skeletal muscle function [57].

2.8. Future challenges and conclusive remarks

H_2S, a colorless irritant gas and considered as a toxic gas and environment hazard [58]. It exhibits different effects in a dose-dependent manner. At low doses, it is beneficial and is highly toxic in high doses. Till date, there is no antidote available to combat or treat the H_2S toxicity in pathophysiological settings [59]. In particular, a knowledge gap exists about the physiological and pathological role of H_2S in the past decades. However, H_2S based therapy remained to be a great challenge for the development of suitable H_2S donors with good tissue-specific action [59,60]. To understand the mechanistic role of H_2S as well as safe use of H_2S, a new method must be developed with a low limit of detection. This might help in measuring the H_2S concentration in diseased tissue and organs at the clinics. Further, the use of the cost-effective animal model that mimics the human condition following acute H_2S inhalation might be needed to understand the mechanistic response to candidate H_2S donors. Accumulated evidence suggests that H_2S gas plays a wide variety of roles in both the physiological and pathological processes of the skeletal system. The data found that H_2S is known to regulate BMMSCs and skeletal muscle function, ensuring bone and skeletal muscle homeostasis. H_2S also plays a crucial role in cell proliferation and differentiation of BMMSCs in the HHcy mouse model. Another study also demonstrates that H_2S regulates BMMSCs function in both OVX and bone fracture mouse models. During skeletal muscle homeostasis, H_2S is known to regulate the skeletal muscle function by regulating muscle angiogenesis. Mechanistic insight suggests that H_2S governs key cellular signaling pathways, protein sulfhydration, and epigenetic remodeling of chromatin landscapes in the skeletal tissue. Therefore, future research is warranted to make a thorough evaluation of the physiological and pathophysiological roles of H_2S in the skeletal tissue and further novel H_2S releasing drugs to be discovered for use as

a therapeutic module in clinical settings.

Conflicts of interest

None.

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