Hydrogen sulfide plays an important protective role by influencing autophagy in diseases

Jun Wang, Dongdong Wu, Honggang Wang*

School of Basic Medical Science, Henan University, Kaifeng, Henan, 475004, China

*Correspongding author Honggang Wang School of Basic Medical Science, Henan University, Kaifeng, Henan, 475004, China E-mail：whg1975316@sina.com

Short title

HYDROGEN SULFIDE INFLUENCES AUTOPHAGY

Summary

Autophagy can regulate cell growth, proliferation, and stability of cell environment. Its dysfunction can be involved in a variety of diseases. Hydrogen sulfide (H_2S) is an important signaling molecule that regulates many physiological and pathological processes. Recent studies indicate that H_2S plays an important protective role in many diseases through influencing autophagy, but its mechanism is not fully understood. This article reviewed the progress about the effect of H_2S on autophagy in diseases in recent years in order to provide theoretical basis for the further research on the interaction of H_2S and autophagy and the mechanisms involved.

KERWORDS: Hydrogen sulfide; Autophagy; Ischemia reperfusion injury; Metabolic diseases

1. Introduction

Autophagy is a process of self-sustaining internal environment stability in eukaryotic cells, in which pathogens, abnormal proteins and organelles are encapsulated by the bilayer membranes to form autophagosomes and then transfered

to lysosome for degredation (Sir *et al*. 2016, Qiu et al. 2014, Murrow *et al.* 2013, Kimura 2014). Autophagy can be classified into macroautophagy, microautophagy, and chaperone-mediated autophagy based on the inducing signals, its timing, types of targets and pathways of delivery of cargo into the lysosome (Gomes *et al.* 2017, Parzych *et al.* 2014). Among them, macroautophagy is the most studied autophagy, in which the content is wrapped by bilayer membrane structure to form autophagosome and then fuses with lysosome for degredation. Microautophagy refers to that the lysosomal membrane directly invaginate and then encapsulate the cell contents. Chaperone-mediated autophagy is selective, in which the cytosolic proteins are transported to the lysosomal chamber after binding to molecular chaperones, and then are digested by lysosomal enzymes (Figure1) (Rubinsztein *et al.* 2012). Under physiological conditions, autophagy is often maintained at the basic level. The internal and external factors such as ischemia, hypoxia, pathogenic infection, hormone therapy, protein misfolding, and nutritional deficiency can induce autophagy (Matsui *et al.* 2007). When the body is in pathological state, the remarkably enhanced autophagy can remove the abnormal protein in the cell, which is beneficial to the survival of the cell. The effect of autophagy on the cell is in a "double-edged sword" mode, since autophagy can cause autophagic death if the autophagy remains at a high level (Garcia-Huerta *et al.* 2016, Liu and Levine 2015). LC3, Beclin1, and other conserved proteins are involved in the process of autophagy, which are called autophagy related proteins (Penaloza *et al.* 2008). Recent studies have shown that autophagy plays important roles in maintaining the balance of synthesis, decomposition, and reutilization of cell components (Wei *et al.* 2016). Abnormal autophagy is involved in the development of the pathological processes such as liver disease, cancer, aging, cardiovascular disease, and kidney disease (Yin and Lu 2017).

Hydrogen sulfide (H_2S) has long been regarded as a flammable, water-soluble, colorless, and toxic gas with the smell of rotten eggs, but since 90s of last century, mounting researches have convincingly proved that H_2S is the third gasotransmitter in various biological systems along with nitric oxide and carbon monoxide (Li *et al.* 2011, Olson 2012, Wang 2012, Kolluru 2013). In mammalian cells, H_2S is produced by three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) (Kimura 2014). The β-replacement reaction of homocysteine with serine is catalyzed by CBS to produce cystathionine(Stipanuk 2004). The α, γ-elimination of cystathionine is catalyzed by CSE to produce cysteine, α-ketobutyrate and NH_3. H_2S is generated from cysteine via β elimination reactions catalyzed by CBS and CSE. 3-mercaptopyruvate is produced by the way in which cysteine aminotransferase transfers the amine group from cysteine to α-ketoglutarate. 3-MST transforms the sulfur of 3-MP into a persulfide to add into the enzyme (3-MST-SSH). The terminal sulfur is released as H_2S by two endogenous reductants thioredoxin or dihydrolipoic acid (Mikami *et al.* 2011, Ishigami *et al.*2009, Shibuya *et al.* 2009). The distribution of H_2S-producing enzymes have tissue specificity. CBS mainly distributes in the liver, kidney, central nerves system, and so on (Distrutti *et al.* 2006, Kimura 2013, Feliers *et al.* 2016). CSE mainly distributes in the cardiovascular system (Polhemus and Lefer 2014). In addition, the gut bacteria are important source of H2S and its derivates (Huc et al. 2016, Tomasov *et al.* 2016). H2S has multiple biological effects depend not on H2S itself but on the formation of new molecules, such as S-nitrosothiols, and its possible mechanisms include reversible protein sulfidation, which alters the function of modified proteins, similar to nitrosation or phosphorylation, direct antioxidant activity and interaction with metalloproteins (Dongó *et al.* 2017). It has been demonstrated

that H_2S plays an important role in many kinds of pathological and physiological processes including development, angiogenesis, carcinogenesis, endoplasmic reticulum stress, and oxidative stress (Huc Paul and Snyder 2012, Kolluru *et al.* 2013, Szabo 2016, Cao and Bian 2016, Xu *et al.* 2017,Feliers *et al.* 2016). Many studies have shown that H_2S might have potential therapeutic effects in the diseases by inhibiting or promoting autophagy in a concentration-dependent manner. So it is necessary to clarify the mechanism that H_2S acts on autophagy.

In this review, we summarize the progress about the effects of H_2S on autophagy in diseases in recent years to provide theoretical basis for the further research on the interaction between H_2S and autophagy and the mechanism involved.

2. H_2S influences autophagy in ischemia-reperfusion (I/R) injury

Tissue ischemia, which often affects autophagy, is an important cause of death and disability in the world. After a period of ischemia, the recovery of blood supply further aggravates the injury of tissue and organ, that is, ischemic reperfusion injury. It has been proved that cell damage induced by free oxygen free radical plays a key role in the IR injury. Ischemia causes hypoxia in the tissue and increases the level of lactic acid, hypoxanthine and lipid peroxide, and the restoring of the oxygen supply produces a large number of free radicals, then free radicals reacts with lipid and mitochondria in cells to produce lipid peroxides, which causes cells death and organ damage(Temiz *et al.* 2013, Usul *et al.* 2004). Recent studies have shown that H_2S exerted cellular protection through pro-autophagy or anti-autophagy in the process of tissue I/R injury. However, the exact mechanism is not fully understood.

2.1 The pro-autophagy effect of H_2S

The upregulation of the autophagy level has been found in a variety of spinal

cord injury models, and might play tissue protective roles (Zhang *et al.* 2014, *Hou et al.* 2014). The biological function of miRNA was believed to be widely involved in organic I/R injury (Bijkerk *et al.* 2014). It has been reported that several miRNA were involved in autophagy regulation by regulating the expression of autophagy-related genes (Wang *et al.* 2014). In spinal cord I/R injury, the expression of miR-30c increased, exogenous H_2S upregulated the level of autophagy by inhibiting the expression of miR-30c to protect spinal cord injury (Lei *et al.* 2015,). This indicated that miR-30c might be an important target for reducing spinal cord I/R damage. It has been showed that myocardial I/R promoted autophagosome formation, but not lysosome-autophagosome formation, thus inhibited autophagosome clearance to downregulate autophagy and resulted in myocardial ischemia injury(Ma *et al.* 2012, Zhang *et al*. 2014). H_2S activated adenosine monophosphate-activated protein kinase (AMPK) in several types of cells (Zhou *et al.* 2014, Jia *et al.* 2013). Activated AMPK could induce autophagy through inhibiting mammalian target of rapamycin (mTOR). In myocardial I/R injury, exogenous H_2S upregulated autophagy and activated AMPK to protect cardiomyocytes. In addition, AMPK inhibitors downregulated AMPK activation and abolished the cardioprotective effect of H_2S, suggesting that the activation of AMPK was one pathway for the protection of the heart by H_2S (Xie *et al.* 2015). The mechanism by which H_2S can increase autophagy by activating AMPK needs further study. It probably provide a new therapeutic strategy for the myocardial I/R injury. Hypoxia ischemia (HI) could lead to neuronal loss and severe neurological deficits in premature infants (Hagberg *et al.* 2015). Studies have shown that HI inhibited autophagy clearance at the later stage, resulting in cortical neuron death (Cui *et al.* 2017). LC3 is a marker of autophagy. When autophagy is formed, the cytoplasmic LC3-I will hydrolyze a small fraction of polypeptides and change into (autophagic)

membrane type (LC3-II). The ratio of LC3-II/I can be used to estimate the level of autophagy. Beclin 1, a sign of initiating cell autophagy, is involved in the formation of autophagy by forming a complex with C1assIII PI3K. P62, which can act as a receptor for vesicles to be degraded by autophagy, is integrated with mature autophagosome, so the level of P62 is negatively correlated with autophagy. In neonatal HI mice, HI could lead to elevated levels of LC3-II and P62, followed by a decrease of Beclin 1, which suggested that the accumulation of LC3-II is due to impaired autophagy fluxes. HI did not affect mTOR phosphorylation, but increased LC3-II, which indicated that HI reduced lysosomal degradation, but did not affect autophagy initiation. L-cysteine, a H_2S donor, significantly increased the expression of LC3-II and Beclin 1, but decreased the expression of p62, promoted autophagy, thereby alleviated hypoxic ischemic injury. Moreover, this protective effect was achieved by lowering the phosphorylation level of mTOR (Xin *et al.* 2018).

2.2 The anti-autophagy effect of H_2S

Recent studies have shown that H_2S protected cells against autophagy in the process of tissue I/R injury. Cerebral ischemia is an important cause of death and disability in adults worldwide (Yan *et al.* 2016). Autophagy was over-activated in rat brains subjected to middle cerebral artery occlusion and PC12 cells subjected to oxygen-glucose deprivation/reoxygenation, resulting in autophagic death of a large number of brain cells. NaHS, a H_2S donor, could inhibit the autophagy to greatly alleviate the damage. The inhibition of autophagy by autophagy inhibitor could further reduce injury, while the upregulation of autophagy by autophagy stimulator could aggravate injury, which suggested that exogenous H_2S could attenuate cerebral I/R injury by suppressing overactivated autophagy (Jiang *et al.* 2017). Rat cerebral ischemia could increase the expression of LC3-II, reduce the expression of P62 to

promote the aggregation of autophagosome and maintain the autophagy at a high level. Exogenous H_2S could reverse the above changes and inhibit autophagic death of the brain cells to protect cerebral against I/R injury by reducing autophagosome. However, the reduction of autophagosome did not mean that the level of autophagy was reduced. The above regulatory role of H_2S might be due to reducing the formation of autophagy, or accelerating the degradation of autophagy. In addition, H_2S did not affect the expression of Beclin1. Since the level of Beclin 1 did not accurately reflect the activity of Beclin-1-VPS34-AMBRA1, the role of Beclin-1 complex in regulating cell autophagy by H_2S needed further study (Shui *et al.* 2016, Klionsky e*t al.* 2008, Liu *et al.* 2016). Ischemic heart disease is especially important in the world with high mortality. Reperfusion ,which is the main treatment strategy, can cause damage to the heart again. so it is important to find a way to reduce heart reperfusion injury (Sivaraman *et al.* 2014)[53]. Exogenous H_2S could play protective roles through influencing the level of autophagy by affecting different signaling pathways. It has been known that phosphatidylinositol 3-kinase (PI3K) protected cardiomyocytes by influence apoptosis and autophagy in mammalian cells. SGK1, activated by PI3K, is a downstream target protein of PI3K. GSK3β is an important downstream target of SGK1. During myocardial I/R injury in neonatal rats, exogenous H_2S activated SGK1 through PI3K and then inhibited GSK3β, regulated the PI3K/SGK1/GSK3β signal pathway, and inhibited autophagy to reduce myocardial injury (Park *et al.* 1999, Jiang *et al.* 2016). Previous studies have shown that liver I/R could excessively activate autophagy, which leaded to autophagic death. So blocking the cell death pathways could significantly reduce liver I/R damage (Chen *et al.* 2013, Shen *et al.* 2013). In the course of liver I/R injury, the JNK signal pathway was excessively activated and then inhibited the separation of Bcl-2 and Beclin-1 to promote apoptosis. Exogenous H_2S

could protect the liver by reducing the JNK signal pathway to inhibit apoptosis and autophagy (Cheng *et al.* 2014). Spinal cord I/R injury is a serious complication of thoracoabdominal aortic surgery. About 40% of patients suffer from paraplegia (Zhang *et al.* 2012). Autophagy played an important role in spinal cord injury (Fujita *et al.* 2015). During the reperfusion injury after 1h in spinal cord ischemia, the oxidative stress was enhanced, the autophagy level was upregulated, the autophagic death was increased and the exogenous H_2S could inhibit the oxidative stress level of the cells through influencing the AKT/mTOR signal pathway and reduce the autophagy to mitigate spinal cord I/R injury (Xie *et al.* 2017). In addition, the time windows for the activation of autophagy exerts influence in I/R injury. Early activation of autophagy could alleviate spinal cord I/R injury by inhibiting apoptosis and inflammatory response. However, later excessively elevated autophagy induced autophagic cell death to aggravate I/R damage(Fang *et al.* 2016).

Whether the autophagy plays a beneficial or harmful role after I/R is elusive. Autophagy induced by ischemia can promote survival by degrading lipid and protein in the cells into reusable free fatty acids and amino acids. On the other hand, autophagy also promotes cell death and aggravates I/R damage. Generally, mild injury at the early stage of I/R can activate autophagy to play protective role, and excessive activation of autophagy caused by severe injury at the late stage of I/R can play a destructive role. H_2S not only activates autophagy but also inhibits autophagy, and what role it plays depends on the type of tissue and the basal level of autophagy in the tissue.

3. H_2S influences autophagy in glycometabolic diseases

Hyperglycemia often causes organic lesions by affecting autophagy. It was

reported that the decrease of H_2S caused the deposition and hypertrophy of renal matrix protein in diabetic nephropathy. Exogenous H_2S could improve the above hyperglycemic effect (Lee *et al.* 2012). AMPK is activated through phosphorylation by its upstream liver kinase B1 (LKB1) complex with two other subunits, STE20 related adapters (STAD) and mouse protein 25 (MO25) (Hardie. 2008, Alessi *et al.* 2006). In the lomerular endothelial cells of the high blood glucose mice model, hyperglycemia reduced the expression of CBS, CSE, and H_2S level, thereby inhibiting the activation of LKB1 and AMPK. Inactive AMPK further reduced autophagy and promotes matrix protein synthesis. Exogenous H_2S could reverse the above changes to protect the renal, which suggested that it played protective role in matrix remodeling (Kundu *et al.* 2014). The activation of AMPK inhibits mTOR, a negative regulator of autophagy, and subsequently stimulates autophagy. In diabetic cardiomyopathy, H_2S-induced activation of AMPK inhibited hyperglycemia-induced cardiomyocyte apoptosis and prevented cardiac dysfunction by promoting autophagy via the AMPK/mTOR pathway, which indicated that autophagy was an important target for the treatment of diabetic cardiomyopathy (Yang *et al.* 2017). Further studies are needed to study the relationship between H_2S-induced autophagy and AMPK/mTOR pathway in type I or type II diabetes. In the vascular endothelial cells of type 2 diabetic model rats, exogenous H_2S could promote the transfer of Nrf2 into the nucleus, inhibit high glucose-induced oxidative stress, reduce the phosphorylation level of AMPK, inhibit autophagy and protect the vascular endothelial cells (Liu *et al.* 2016). These above results suggested that the hyperglycemia could affect autophagy and cause tissue damage by changing the phosphorylation level of AMPK, and the use of H_2S could reverse the role of hyperglycemia, which suggested that the activation of AMPK by H_2S could be a new strategy for the treatment of diabetic vascular complications.

Persistent hyperglycemia could induce metabolism, to caue cardiac structural remodeling and myocardial fibrosis (Wang *et al.* 2014). Studies have shown that high glucose could reduce the generation of endogenous H_2S, inhibit PI3K/AKT1 pathway, activate autophagy and induce myocardial fibrosis. Exogenous H_2S could reverse the above changes and protect the myocardium of diabetic myocardium (Xiao *et al.*2016).

4. H_2S influences autophagy in lipid metabolism

Recent studies have shown that autophagy was involved in the regulation of hepatic lipid metabolism (Czaja *et al.* 2010, Settembre and Ballabio 2014, Singh *et al.* 2009). H_2S has been proved to have a protective effect on hypertriglyceridemia (HTG) and nonalcoholic fatty liver disease (NAFLD) (Luo *et al.* 2014, Polyzos *et al.* 2012) . It has been shown that the level of H_2S and the level of autophagy in the hepatocyte of hyper-triglyceride patients were significantly lower than normal. In the hyper-triglyceride hepatocytes, NaHS could reduce the triglyceride in plasma by promoting autophagy, which indicated that the activation of liver autophagy may be a therapeutic target for HTG. In addition, the phosphorylation of AMPK was promoted and the phosphorylation of mTOR was inhibited by H_2S, which suggested that H_2S had exerted the protective role through influencing the AMPK/mTOR pathway (Sun *et al.* 2015). A previous study in our laboratory has shown that in steatosis hepatocytes induced by oleic acid, H_2S could promote the formation and degradation of lipid autophagosomes, improve the expression of autophagy-related protein LC3 II and further promote the degradation of triglyceride by autophagic pathway (Wang *et al.* 2017). Further study is needed to illustrate the mechanism about the effects of H_2S on lowering serum TG and improving NAFLD.

5. H_2S influencing autophagy in tissue fibrosis diseases

It has been reported that the high level of autophagy leaded to massive loss of myocardial cells, which played an important role in the development of cardiac remodeling and myocardial fibrosis (Ma *et al.* 2015, Jie *et al.* 2015, Zhang *et al.* 2016). In the process of myocardial fibrosis induced by alcohol, H_2S inhibited autophagy to alleviate the myocardial fibrosis caused by alcohol via regulating the expression of miR21, miR211 and the PI3K/AKT1/TGF-β1 signal pathway (Liang *et al.* 2017). Hyperthyroid heart disease is characterized by obvious clinical symptoms and pathological changes, such as myocardial hypertrophy, myocardial fibrosis, arrhythmia, and cardiac dysfunction (Freitas *et al.* 2013, Kaminski *et al.* 2012). Myocardial fibrosis is an important sign of myocardial remodeling in hyperthyroid heart disease, and it is also the main cause of left ventricular dysfunction (Kim *et al.* 2013, Dillmann 2010). Current studies showed that autophagy was involved in the pathogenesis of the myocardial fibrosis and myocardial remodeling. In the model of rat myocardial fibrosis induced by high concentration thyroxine, the protein expression of autophagy related genes was reduced, which suggested that the decreased autophagy was involved in the development and the progression of myocardial fibrosis. H_2S-intervention could significantly reduce collagen deposition, increase autophagy and inhibit myocardial fibrosis. PI3K/AKT is a signal pathway of negative regulation of autophagy. H_2S could reduce the protein expression of PI3K and AKT, which suggested that H_2S promoted autophagy by inhibiting PI3K/AKT signaling pathway (Liu *et al.* 2018). The PI3K/AKT signaling pathway may become a target for the intervention of myocardial fibrosis.

6. H_2S influences autophagy in cancer

H_2S has an important biphasic effect in cancer(Hellmich and Szabo 2015, Wu *et al.* 2015). Autophagy is a double-edged sword, which can both promote and inhibit tumor

growth and development in different experimental environments. In the hepatocellular carcinoma, the treatment of NaHS at a final concentration of 10^{-3} M for 24 h inhibited cell migration, proliferation and cell cycle progression and promoted apoptosis and autophagy. The PI3K/Akt/mTOR signaling pathway played an important role in autophagy. Further studies showed that the use of NaHS could inhibit PI3K/Akt/mTOR signaling pathway. Given that rapamycin induced autophagy by inhibiting the expression of mTOR, rapamycin plus H_2S treatment could enhance autophagy more obviously, which indicated H_2S promote autophagy through the PI3K/Akt/mTOR signaling pathway (Wang *et al.* 2017). These above results suggested a new strategy for the treatment of liver cancer.

7. Conclusion

Autophagy maintains a balance between protein degradation and synthesis, which can protects or destroy cell. The effect of autophagy is determined by specific pathological processes. Nowadays, more and more studies suggest that H_2S related drugs can have potential application in many kinds of diseases by influencing autophagy. However, the mechanism that H_2S affects autophagy has not been thoroughly studied, such as the role of Becline-1 receptor in the regulation of autophagy by H_2S, and whether H_2S can regulate the fusion of autophagosomes and lysosomes. The role of H_2S in influencing autophagy in more diseases needs further study. In addition, the donor of H_2S used in researches of recent years are ususlly NaHS, considering that NaHS can not release H2S for a long time, the new and efficient H_2S-donors need to be further developed and applied to research.

In conclusion, autophagy may be a potential target for H_2S therapy with the in-depth study of the effect of H2S on autophagy.

Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 31300884), the key scientific research project of colleges and universities in Henan, China (No. 16A310001).

References:

ALESSI DR, SAKAMOTO K and BAYASCAS JR: LKB1-dependent signaling pathways. *ANNU REV BIOCHEM* **75**:137-163, 2006.

BIJKERK R, van SOLINGEN C, de BOER HC, van der POL P, KHAIROUN M, de BRUIN RG, van OEVEREN-RIETDIJK AM, LIEVERS E,... van ZONNEVELD AJ: Hematopoietic microRNA-126 protects against renal ischemia/reperfusion injury by promoting vascular integrity. *J AM SOC NEPHROL* **25**:1710-1722, 2014.

CAO X and BIAN JS: The Role of Hydrogen Sulfide in Renal System. *FRONT PHARMACOL* **7**:385, 2016.

CHEN M, Du Y, QUI M, WANG M, CHEN K, HUANG Z, JIANG M, XIONG F,... ZHANG X: Ophiopogonin B-induced autophagy in non-small cell lung cancer cells via inhibition of the PI3K/Akt signaling pathway. *ONCOL REP* **29**:430-436, 2013.

CHENG P, WANG F, CHEN K, SHEN M, DAI W, XU L, ZHANG Y, WANG C,... GUO C: Hydrogen sulfide ameliorates ischemia/reperfusion-induced hepatitis by inhibiting apoptosis and autophagy pathways. *Mediators Inflamm* **2014**:935251, 2014.

CUI D, SUN D, WANG X, YI L, KULIKOWICZ E, REYES M, ZHU J, YANG ZJ,... KOEHLER RC: Impaired autophagosome clearance contributes to neuronal death in a piglet model of neonatal hypoxic-ischemic encephalopathy. *CELL DEATH DIS* **8**:e2919, 2017.

CZAJA MJ: Autophagy in health and disease. 2. Regulation of lipid metabolism and storage by autophagy: pathophysiological implications. *Am J Physiol Cell Physiol* **298**:C973-C978, 2010.

DILLMANN W: Cardiac hypertrophy and thyroid hormone signaling. *HEART FAIL REV* **15**:125-132, 2010.

DONGO E, BELICZAI-MAROSI G, DYBVIG AS and KISS L: The mechanism of action and role of hydrogen sulfide in the control of vascular tone. *Nitric Oxide*, 2017.

FANG B, LI XQ, BAO NR, TAN WF, CHEN FS, PI XL, ZHANG Y,... MA H: Role of autophagy in the bimodal stage after spinal cord ischemia reperfusion injury in rats. *NEUROSCIENCE* **328**:107-116, 2016.

FELIERS D, LEE HJ and KASINATH BS: Hydrogen Sulfide in Renal Physiology and Disease. *Antioxid Redox Signal* **25**:720-731, 2016.

FREITAS F, ESTATO V, CARVALHO VF, TORRES RC, LESSA MA and TIBIRICA E: Cardiac microvascular rarefaction in hyperthyroidism-induced left ventricle dysfunction. *MICROCIRCULATION* **20**:590-598, 2013.

FUJITA S, SAKURAI M, BABA H, ABE K and TOMINAGA R: Autophagy-mediated stress response in motor neurons after hypothermic spinal cord ischemia in rabbits. *J VASC SURG* **62**:1312-1319, 2015.

GARCIA-HUERTA P, TRONCOSO-ESCUDERO P, JEREZ C, HETZ C and VIDAL RL: The intersection between growth factors, autophagy and ER stress: A new target to treat neurodegenerative diseases? *BRAIN RES* **1649**:173-180, 2016.

GOMES LR, MENCK C and CUERVO AM: Chaperone-mediated autophagy prevents cellular transformation by regulating MYC proteasomal degradation. *AUTOPHAGY* **13**:928-940, 2017.

HAGBERG H, MALLARD C, FERRIERO DM, VANNUCCI SJ, LEVISON SW, VEXLER ZS and GRESSENS P: The role of inflammation in perinatal brain injury. *NAT REV NEUROL* **11**:192-208, 2015.

HARDIE DG: AMPK: a key regulator of energy balance in the single cell and the whole organism. *Int J Obes (Lond)* **32 Suppl 4**:S7-S12, 2008.

HELLMICH MR and SZABO C: Hydrogen Sulfide and Cancer. *Handb Exp Pharmacol* **230**:233-241, 2015.

HOU H, ZHANG L, ZHANG L, LIU D, MAO Z, DU H and TANG P: Acute spinal cord injury in rats induces autophagy activation. *TURK NEUROSURG* **24**:369-373, 2014.

HUC T, JURKOWSKA H, WROBEL M, JAWORSKA K, ONYSZKIEWICZ M and UFNAL M: Colonic hydrogen sulfide produces portal hypertension and systemic hypotension in rats. *Exp Biol Med*

(Maywood) **243**:96-106, 2018.

ISHIGAMI M, HIRAKI K, UMEMURA K, OGASAWARA Y, ISHII K and KIMURA H: A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal* **11**:205-214, 2009.

JIA J, XIAO Y, WANG W, QING L, XU Y, SONG H, ZHEN X, AO G,... CHENG J: Differential mechanisms underlying neuroprotection of hydrogen sulfide donors against oxidative stress. *NEUROCHEM INT* **62**:1072-1078, 2013.

JIANG H, XIAO J, KANG B, ZHU X, XIN N and WANG Z: PI3K/SGK1/GSK3beta signaling pathway is involved in inhibition of autophagy in neonatal rat cardiomyocytes exposed to hypoxia/reoxygenation by hydrogen sulfide. *EXP CELL RES* **345**:134-140, 2016.

JIANG WW, HUANG BS, HAN Y, DENG LH and WU LX: Sodium hydrosulfide attenuates cerebral ischemia/reperfusion injury by suppressing overactivated autophagy in rats. *FEBS OPEN BIO* **7**:1686-1695, 2017.

KAMINSKI G, MAKOWSKI K, MICHALKIEWICZ D, KOWAL J, RUCHALA M, SZCZEPANEK E and GIELERAK G: The influence of subclinical hyperthyroidism on blood pressure, heart rate variability, and prevalence of arrhythmias. *THYROID* **22**:454-460, 2012.

KIM BH, CHO KI, KIM SM, KIM N, HAN J, KIM JY and KIM IJ: Heart rate reduction with ivabradine prevents thyroid hormone-induced cardiac remodeling in rat. *HEART VESSELS* **28**:524-535, 2013.

KIMURA H: Physiological role of hydrogen sulfide and polysulfide in the central nervous system. *NEUROCHEM INT* **63**:492-497, 2013.

KIMURA H: The physiological role of hydrogen sulfide and beyond. *Nitric Oxide* **41**:4-10, 2014.

KLIONSKY DJ, ABDALLA FC, ABELIOVICH H, ABRAHAM RT, ACEVEDO-AROZENA A, ADELI K, AGHOLME L, AGNELLO M,... ZUCKERBRAUN B: Guidelines for the use and interpretation of assays for monitoring autophagy. *AUTOPHAGY* **8**:445-544, 2012.

KOLLURU GK, SHEN X, BIR SC and KEVIL CG: Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric Oxide* **35**:5-20, 2013.

KUNDU S, PUSHPAKUMAR S, KHUNDMIRI SJ and SEN U: Hydrogen sulfide mitigates hyperglycemic remodeling via liver kinase B1-adenosine monophosphate-activated protein kinase signaling. *Biochim Biophys Acta* **1843**:2816-2826, 2014.

LEE HJ, MARIAPPAN MM, FELIERS D, CAVAGLIERI RC, SATARANATARAJAN K, ABBOUD HE, CHOUDHURY GG,... KASINATH BS: Hydrogen sulfide inhibits high glucose-induced matrix protein synthesis by activating AMP-activated protein kinase in renal epithelial cells. *J BIOL CHEM* **287**:4451-4461, 2012.

LI L, JIANG HK, LI YP and GUO YP: Hydrogen sulfide protects spinal cord and induces autophagy via miR-30c in a rat model of spinal cord ischemia-reperfusion injury. *J BIOMED SCI* **22**:50, 2015.

LI L, ROSE P and MOORE PK: Hydrogen sulfide and cell signaling. *Annu Rev Pharmacol Toxicol* **51**:169-187, 2011.

LIANG B, XIAO T, LONG J, LIU M, LI Z, LIU S and YANG J: Hydrogen sulfide alleviates myocardial fibrosis in mice with alcoholic cardiomyopathy by downregulating autophagy. *INT J MOL MED* **40**:1781-1791, 2017.

LIU J, WU J, SUN A, SUN Y, YU X, LIU N, DONG S, YANG F,... ZHANG W: Hydrogen sulfide decreases high glucose/palmitate-induced autophagy in endothelial cells by the Nrf2-ROS-AMPK signaling pathway. *CELL BIOSCI* **6**:33, 2016.

LIU M, LI Z, LIANG B, LI L, LIU S, TAN W, LONG J, TANG F,... YANG J: Hydrogen sulfide ameliorates rat myocardial fibrosis induced by thyroxine through PI3K/AKT signaling pathway. *ENDOCR J* **65**:769-781, 2018.

LIU Y and LEVINE B: Autosis and autophagic cell death: the dark side of autophagy. *CELL DEATH DIFFER* **22**:367-376, 2015.

LUO ZL, TANG LJ, WANG T, DAI RW, REN JD, CHENG L, XIANG K,... TIAN FZ: Effects of treatment with hydrogen sulfide on methionine-choline deficient diet-induced non-alcoholic steatohepatitis in rats. *J Gastroenterol Hepatol* **29**:215-222, 2014.

MA S, WANG Y, CHEN Y and CAO F: The role of the autophagy in myocardial ischemia/reperfusion injury. *Biochim Biophys Acta* **1852**:271-276, 2015.

MA X, LIU H, FOYIL SR, GODAR RJ, WEINHEIMER CJ, HILL JA and DIWAN A: Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury. *CIRCULATION* **125**:3170-3181, 2012.

MATSUI Y, TAKAGI H, QU X, ABDELLATIF M, SAKODA H, ASANO T, LEVINE B,... SADOSHIMA J: Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *CIRC RES* **100**:914-922, 2007.

MIKAMI Y, SHIBUYA N, KIMURA Y, NAGAHARA N, OGASAWARA Y and KIMURA H: Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. *BIOCHEM J* **439**:479-485, 2011.

MURROW L and DEBNATH J: Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. *Annu Rev Pathol* **8**:105-137, 2013.

OLSON KR: A practical look at the chemistry and biology of hydrogen sulfide. *Antioxid Redox Signal* **17**:32-44, 2012.

PARK J, LEONG ML, BUSE P, MAIYAR AC, FIRESTONE GL and HEMMINGS BA: Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J* **18**:3024-3033, 1999.

PARZYCH KR and KLIONSKY DJ: An overview of autophagy: morphology, mechanism, and

regulation. *Antioxid Redox Signal* **20**:460-473, 2014.

PENALOZA C, ORLANSKI S, YE Y, ENTEZARI-ZAHER T, JAVDAN M and ZAKERI Z: Cell death in mammalian development. *Curr Pharm Des* **14**:184-196, 2008.

POLHEMUS DJ and LEFER DJ: Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. *CIRC RES* **114**:730-737, 2014.

POLYZOS SA, KOUNTOURAS J, PATSIAOURA K, KATSIKI E, ZAFEIRIADOU E, DERETZI G, ZAVOS C, GAVALAS E,... SLAVAKIS A: Serum homocysteine levels in patients with nonalcoholic fatty liver disease. *ANN HEPATOL* **11**:68-76, 2012.

QIU DM, WANG GL, CHEN L, XU YY, HE S, CAO XL, QIN J, ZHOU JM,... E Q: The expression of beclin-1, an autophagic gene, in hepatocellular carcinoma associated with clinical pathological and prognostic significance. *BMC CANCER* **14**:327, 2014.

RUBINSZTEIN DC, CODOGNO P and LEVINE B: Autophagy modulation as a potential therapeutic target for diverse diseases. *NAT REV DRUG DISCOV* **11**:709-730, 2012.

SEO JY, KIM YH, KIM JW, KIM SI and HA KY: Effects of Therapeutic Hypothermia on Apoptosis and Autophagy After Spinal Cord Injury in Rats. *Spine (Phila Pa 1976)* **40**:883-890, 2015.

SETTEMBRE C and BALLABIO A: Lysosome: regulator of lipid degradation pathways. *TRENDS CELL BIOL* **24**:743-750, 2014.

SHEN M, LU J, DAI W, WANG F, XU L, CHEN K, HE L, CHENG P,... GUO C: Ethyl pyruvate ameliorates hepatic ischemia-reperfusion injury by inhibiting intrinsic pathway of apoptosis and autophagy. *Mediators Inflamm* **2013**:461536, 2013.

SHIBUYA N, TANAKA M, YOSHIDA M, OGASAWARA Y, TOGAWA T, ISHII K and KIMURA H: 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* **11**:703-714, 2009.

SHUI M, LIU X, ZHU Y and WANG Y: Exogenous hydrogen sulfide attenuates cerebral ischemia-reperfusion injury by inhibiting autophagy in mice. *Can J Physiol Pharmacol* **94**:1187-1192, 2016.

SINGH R, KAUSHIK S, WANG Y, XIANG Y, NOVAK I, KOMATSU M, TANAKA K, CUERVO AM,... CZAJA MJ: Autophagy regulates lipid metabolism. *NATURE* **458**:1131-1135, 2009.

Sir D, TIAN Y, CHEN WL, ANN DK, YEN TS and OU JH: The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc Natl Acad Sci U S A* **107**:4383-4388, 2010.

SIVARAMAN V and YELLON DM: Pharmacologic therapy that simulates conditioning for cardiac ischemic/reperfusion injury. *J Cardiovasc Pharmacol Ther* **19**:83-96, 2014.

STIPANUK MH: Sulfur amino acid metabolism: pathways for production and removal of homocysteine

and cysteine. *ANNU REV NUTR* **24**:539-577, 2004.

SUN L, ZHANG S, YU C, PAN Z, LIU Y, ZHAO J, WANG X, YUN F,... LI Y: Hydrogen sulfide reduces serum triglyceride by activating liver autophagy via the AMPK-mTOR pathway. *Am J Physiol Endocrinol Metab* **309**:E925-E935, 2015.

SZABO C: Gasotransmitters in cancer: from pathophysiology to experimental therapy. *NAT REV DRUG DISCOV* **15**:185-203, 2016.

TEMIZ C, SOLMAZ I, TEHLI O, KAYA S, ONGURU O, ARSLAN E and IZCI Y: The effects of splenectomy on lipid peroxidation and neuronal loss in experimental spinal cord ischemia/reperfusion injury. *TURK NEUROSURG* **23**:67-74, 2013.

TOMASOVA L, KONOPELSKI P and UFNAL M: Gut Bacteria and Hydrogen Sulfide: The New Old Players in Circulatory System Homeostasis. *MOLECULES* **21**, 2016.

USUL H, CAKIR E, COBANOGLU U, ALVER A, PEKSOYLU B, TOPBAS M and BAYKAL S: The effects of tyrphostine Ag 556 on experimental spinal cord ischemia reperfusion injury. *Surg Neurol* **61**:45-54, 54, 2004.

WANG H, ZHANG Y, HUANGFU C, WANG J, WU D, ZHONG P, WANG G,... LI Y: Effect of GYY4137 on cytosolic decomposition in Mouse Primary Hepatocytes. p 1520-1523, 2017.

WANG L, YUAN T, Du G, ZHAO Q, MA L and ZHU J: The impact of 1,25-dihydroxyvitamin D3 on the expression of connective tissue growth factor and transforming growth factor-beta1 in the myocardium of rats with diabetes. *Diabetes Res Clin Pract* **104**:226-233, 2014.

WANG P, LIANG J, LI Y, LI J, YANG X, ZHANG X, HAN S, LI S,... LI J: Down-regulation of miRNA-30a alleviates cerebral ischemic injury through enhancing beclin 1-mediated autophagy. *NEUROCHEM RES* **39**:1279-1291, 2014.

WANG R: Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *PHYSIOL REV* **92**:791-896, 2012.

WANG SS, CHEN YH, CHEN N, WANG LJ, CHEN DX, WENG HL, DOOLEY S,... DING HG: Hydrogen sulfide promotes autophagy of hepatocellular carcinoma cells through the PI3K/Akt/mTOR signaling pathway. *CELL DEATH DIS* **8**:e2688, 2017.

WEI C, GAO J, LI M, LI H, WANG Y, LI H and XU C: Dopamine D2 receptors contribute to cardioprotection of ischemic post-conditioning via activating autophagy in isolated rat hearts. *INT J CARDIOL* **203**:837-839, 2016.

WU D, SI W, WANG M, LV S, JI A and LI Y: Hydrogen sulfide in cancer: Friend or foe? *Nitric Oxide* **50**:38-45, 2015.

XIAO T, LUO J, WU Z, LI F, ZENG O and YANG J: Effects of hydrogen sulfide on myocardial fibrosis and PI3K/AKT1-regulated autophagy in diabetic rats. *MOL MED REP* **13**:1765-1773, 2016.

XIE H, XU Q, JIA J, AO G, SUN Y, HU L, ALKAYED NJ, WANG C,... CHENG J: Hydrogen sulfide protects against myocardial ischemia and reperfusion injury by activating AMP-activated protein kinase to restore autophagic flux. *Biochem Biophys Res Commun* **458**:632-638, 2015.

XIE L, YU S, YANG K, LI C and LIANG Y: Hydrogen Sulfide Inhibits Autophagic Neuronal Cell Death by Reducing Oxidative Stress in Spinal Cord Ischemia Reperfusion Injury. *OXID MED CELL LONGEV* **2017**:8640284, 2017.

XIN D, CHU X, BAI X, MA W, YUAN H, QIU J, LIU C, LI T,... WANG Z: l-Cysteine suppresses hypoxia-ischemia injury in neonatal mice by reducing glial activation, promoting autophagic flux and mediating synaptic modification via H2S formation. *BRAIN BEHAV IMMUN* **73**:222-234, 2018.

XU D, JIN H, WEN J, CHEN J, CHEN D, CAI N, WANG Y, WANG J,... WANG X: Hydrogen sulfide protects against endoplasmic reticulum stress and mitochondrial injury in nucleus pulposus cells and ameliorates intervertebral disc degeneration. *PHARMACOL RES* **117**:357-369, 2017.

XU J, QIN X, CAI X, YANG L, XING Y, LI J, ZHANG L, TANG Y,... GAO F: Mitochondrial JNK activation triggers autophagy and apoptosis and aggravates myocardial injury following ischemia/reperfusion. *Biochim Biophys Acta* **1852**:262-270, 2015.

YAN A, ZHANG T, YANG X, SHAO J, FU N, SHEN F, FU Y,... XIA W: Thromboxane A2 receptor antagonist SQ29548 reduces ischemic stroke-induced microglia/macrophages activation and enrichment, and ameliorates brain injury. *Sci Rep* **6**:35885, 2016.

YANG F, ZHANG L, GAO Z, SUN X, YU M, DONG S, WU J, ZHAO Y,... LU F: Exogenous H2S Protects Against Diabetic Cardiomyopathy by Activating Autophagy via the AMPK/mTOR Pathway. *CELL PHYSIOL BIOCHEM* **43**:1168-1187, 2017.

YIN F and LU LM: [Advances in mechanism of autophagy in renal tubular injury]. *Sheng Li Xue Bao* **69**:723-729, 2017.

ZHANG L, DING WY, WANG ZH, TANG MX, WANG F, LI Y, ZHONG M, ZHANG Y,... ZHANG W: Early administration of trimetazidine attenuates diabetic cardiomyopathy in rats by alleviating fibrosis, reducing apoptosis and enhancing autophagy. *J TRANSL MED* **14**:109, 2016.

ZHANG Q, HUANG C, MENG B, TANG T, SHI Q and YANG H: Acute effect of Ghrelin on ischemia/reperfusion injury in the rat spinal cord. *INT J MOL SCI* **13**:9864-9876, 2012.

ZHANG Q, HUANG C, MENG B, TANG TS and YANG HL: Changes in autophagy proteins in a rat model of spinal cord injury. *Chin J Traumatol* **17**:193-197, 2014.

ZHANG YL, YAO YT, FANG NX, ZHOU CH, GONG JS and LI LH: Restoration of autophagic flux in myocardial tissues is required for cardioprotection of sevoflurane postconditioning in rats. *ACTA PHARMACOL SIN* **35**:758-769, 2014.

ZHOU X, CAO Y, AO G, HU L, LIU H, WU J, WANG X, JIN M,... CHENG J: CaMKKbeta-dependent activation of AMP-activated protein kinase is critical to suppressive effects of hydrogen sulfide on

neuroinflammation. *Antioxid Redox Signal* **21**:1741-1758, 2014.

Table 1. H_2S influences autophagy in ischemia-reperfusion injury

Experimental models	Effects	Proposed mechanisms	References
Spinal cord ischemia-reperfusion injury(rat)	Intraperitoneally injection with NaSH (1.68mg/L/kg), dissolved in saline) 30 minutes improved spinal cord injury and motor function in rat model of I/R injury	Induction autophagy via miR-30c	Lei *et a*l. 2015
Spinal cord ischemia-reperfusion injury(rat)	Intraperitoneally administation of ADT,a H2S donor, at 50mg/kg immediately at the beginning of reperfusion protected against myocardial I/R injury	Induction autophagy by activating AMP	Xie *et al.* 2015
Brain hypoxia-ischemic injury (neonatal mice)	L-Cysteine, an H2S donor, alleviated hypoxic ischemic injury.	promoting autophage via H2S formation	Xin *et al.* 2018
Cerebral ischemia/ reperfusion injury(rat)	NaHS (5.6mg/kg/day) supplementation attenuates cerebral ischemia/reperfusion	Overactivated autophagy was suppressed	Jiang *et al.* 2017

	injury		
Cerebral ischemia-reperfusion injury(mice)	NaHS(2mg/kg) intraperitoneally injection at the onset of ischemia attenuates cerebral ischemia/reperfusion injury	Inhibiting autophagy by decreasing autophagosome accumulation	Shui *et al*. 2016
Cardiomyocytes exposed to hypoxia/reoxygenation (rat)	Treatment with NaHS (1.68mg/L) 0.5 h before hypoxia/reoxygenation exert cardioprotection	Inhibition of autophagy by regulatingPI3K/SGK1/GSK3β signaling pathway	Park *et al*. 1999
Liver ischemia/reperfusion injury(mice)	Intraperitoneal injection of 1 mL of NaHS solution (1.56mg/L/kg) 30 min before I/Rameliorates ischemia/reperfusion-induced hepatitis	Inhibition of autophagy by reducing the JNK signal pathway	Cheng et al. 2014
Spinal cord ischemia reperfusion/ injury(rat)	Intraperitoneally injection with NaHS · H2O (5.6 mg/kg) 1 h before the onset of spinal cord I/R ameliorates Spinal Cord Ischemia Reperfusion Injury	Inhibition autophagy by reducing the oxidative stress level	Xie et al. 2017

Table 2. H_2S influences autophagy in metabolic diseases

Experimental models	Effects	Proposed mechanisms	References
Diabetic cardiomyopathy (Rat)	Intraitoneal injection of NaHS (5.6mg/ L) for 4 or 8 weeks protect myocardium against hyperglycaemia injury	Activation of autophagy via the AMPK/mTOR pathway	Yang *et al.* 2017
Diabetic arterial endothelial	NaHS (5.6mg/ L/kg) given by	Decreases high	Liu *et al.* 2016

cells(mice)	intraperitoneal injection every 2 days protects arterial endothelial cells	glucose/palmitate-induced autophagy by inhibiting activation of AMPK signaling pathway	
Diabetic cardiomyopathy (Rat)	Intraperitoneally administeration with NaHS at a dose of 5.6mg/ L/kg for 8 weeks ameliorated myocardial fibrosis	Attenuation of autophagy via the upregulation of the PI3K/AKT1 signaling pathway	Xiao *et al*.2016
Hypertriglyceride rat	NaHS(3.1mg/ L/·kg/·day) administrated by intraperitoneal injection for 8 weeks reduce the triglyceride in plasma	Activation autophagy via the AMPK-mTOR pathway	Sun *et al*.2015

Figure1. The general process of macroautophagy, microautophagy, and chaperone-mediated autophagy.

In the process of macroautophagy, the content is wrapped by bilayer membrane structure to form autophagosome and then fuses with lysosome for degredation. Microautophagy refers to that the lysosomal membrane directly invaginate and then encapsulate the cell contents. In the process of chaperone-mediated autophagy, the cytosolic proteins are transported to the lysosomal chamber after binding to molecular chaperones, and then are digested by lysosomal enzymes.