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REVIEW ARTICLE

Homocysteinylation and Sulfhydration in Diseases

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Abstract: Homocysteine (Hcy) is an important intermediate in methionine metabolism and generation of one-carbon units, and its dysfunction is associated with many pathological states. Although Hcy is a non-protein amino acid, many studies have demonstrated protein-related homocysteine metabolism and possible mechanisms underlying homocysteinylation. Homocysteinylated proteins lose their original biological function and have a negative effect on the various disease phenotypes. Hydrogen sulfide (H_2S) has been recognized as an important gaseous signaling molecule with mounting physiological properties. H_2S modifies small molecules and proteins *via* sulfhydration, which is supposed to be essential in the regulation of biological functions and signal transduction in human health and disorders. This review briefly introduces Hcy and H_2S, further discusses pathophysiological consequences of homocysteine modification and sulfhydryl modification, and ultimately makes a prediction that H_2S might exert a protective effect on the toxicity of homocysteinylation of target protein *via* sulfhydration. The highlighted information here yields new insights into the role of protein modification by Hcy and H_2S in diseases.

Keywords: Homocysteine, homocysteinylation, hydrogen sulfide, sulfhydration, protein modification, diseases.

1. INTRODUCTION

Homocysteine (Hcy) is considered to be a common intermediate at the crossing point of two important intracellular metabolic pathways. Hcy participates in the folate cycle with vitamin B_{12}, providing a one-carbon unit for nucleotide and amino acid metabolism. Another pathway is sulfur-containing amino acid metabolism, producing methionine (Met), cysteine (Cys), and even homocysteinylated proteins. Nevertheless, the available source of Hcy in human beings comes from Met, the essential amino acid from diet. In addition, the process of Hcy linking to the target protein means protein homocysteinylation (homocysteine modification). There are two ways of protein homocysteinylation: the isopeptide bond to lysine (Lys) residues (named N-homocysteinylated-protein, N-hcy-protein) [1, 2] or the disulfide bond to Cys residues (named S-homocysteinylated-protein, S-Hcy-protein) [3]. Homocysteine thiolactone (HTL), as the intermediate product in homocysteinylation and only from Hcy, contributes to the protein N-homocysteinylation being the most distinguishing feature for Hcy in comparison to other amino acids. Conversely, the protein S-homocysteinylation is not specific for Hcy because corresponding S-thiolated proteins are produced by the formation of a disulfide bond, with or without Hcy [4]. N-homocysteinylation is a new protein modification, which may have a negative effect on the structure and function of the protein, leading to the onset of human disease [5]. Deficiencies of congenital or acquired factors in Hcy metabolism result in the accumulation of N-Hcy-proteins associated with cardiovascular and neurodegenerative diseases [6].

Hydrogen sulfide (H_2S) is an emerging member of the gasotransmitter family. H_2S was once regarded as a poisonous gas mainly present in the atmosphere, but currently, it is known to be synthesized effectively from Cys in mammals [7]. Based on the fact that nitric oxide (NO) transmits signals *via* covalent modification of Cys in the target protein (nitrosylation), H_2S also modifies Cys through transforming the -SH group of target protein into the -SSH group, called sulfhydration (sulfhydryl modification). While only a small proportion of proteins are nitrosylated, up to 50% of proteins are sulfhydrated in the hepatic tissue [8]. Sulfhydration is a reversible process in the thioredoxin system, like nitrosylation [9]. Sulfhydration is a highly prevalent and abundant protein modification; thus, it participates in a number of pathophysiological processes related to the vascular system and nervous system [7].

Both homocysteine and sulfhydryl modification are relevant to Cys as well as the metabolism of other sulfur-containing amino acids. However, there is a dearth of knowledge regarding the relationship between homocysteinylation and sulfhydration and even the role of these modifications in various disorders. A review of the literature was performed, and relevant publications were identified by searching the following databases: PubMed, Web of Science, and China National Knowledge Infrastructure. Keywords

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included homocysteine, homocysteinylation, hydrogen sulfide, sulfhydration, and protein modification. 136 articles were screened for relevance to the objective of this manuscript, which included one book. This article provides a review of the literature to highlight the importance of homocysteinylation and sulfhydration in different pathological states, for instance, cardiovascular and neurologic diseases.

2. HOMOCYSTEINE

Hcy represents a rather critical part in sulfur-containing amino acid metabolism, together with Cys and Met, which may have a significant impact on the beginning of life on earth, as the origin of coded peptide synthesis [10, 11]. The first sulfur-containing amino acid, disulfide cystine, was originally discovered from a urinary calculus in 1810. Later, Cys was recognized as a major constituent of proteins [12]. The new sulfur-containing amino acid Met was isolated from casein in 1922 [13]. The following study has demonstrated the existence and specific chemical structure of Met [14]. The third sulfur-containing amino acid Hcy, a homologue of Cys, is detected by the chemical decomposition of methionine with sulfuric acid [15]. Subsequent studies have demonstrated the physiological role of Hcy in the Cys and Met metabolic process by the classical transsulfuration and remethylation pathways, respectively [16]. Cys and Met can be used for coding genes, then incorporated into protein by the ribosome to take part in protein transcription and translation processes, whereas Hcy generally is not involved in the genetic code and ribosomal protein biosynthesis, regarded as a non-proteinogenic and auxiliary amino acid [17]. Recently, there has been increasing interest in the role of Hcy as a risk factor for several diseases due to its possible effects on neurons, including augmenting of oxidative stress [18], calcium influx [19], amyloid-β toxicity [20], activation of excitotoxicity [21], and caspase-dependent apoptosis [22]. Especially, it is likely that protein homocysteinylation is the possible mechanism underlying the pathological consequences of elevated Hcy levels.

2.1. Homocysteine Metabolism

Hcy, a result of transmethylation reaction in the cellular metabolism, is produced from Met in the body, whereas Met is from dietary protein, released in the alimentary canal, absorbed by the enterocyte, and transported to different organs *via* blood circulation [6]. Met is metabolized to S-adenosylmethionine (AdoMet or SAM) under the condition of Met S-adenosyltransferase (MAT) and ATP, which can offer activated methyl group for the biological methylated process [23]. The transmethylation reaction of converting AdoMet to S-adenosylhomocysteine (AdoHcy or SAH) leads to the formation of Hcy, which is the primary source of Hcy in the body [24].

Previous studies have clarified that Hcy metabolism is established and maintained stably through several fundamental pathways in mammals (Fig. **1**) [25-27]. Hcy is remethylated to Met under the influence of Met synthase (MS), occurring in each organ of our body. The enzymeization of MS in the 5-methyltetrahydrofolate pathway also requires two cofactors: methyltetrahydrofolate reductase (MTHFR) and vitamin B_{12}, involved in the one-carbon metabolism pathway of Hcy. Another remethylation pathway is that betaine-Hcy methyltransferase (BHMT) transfers the methyl group from betaine to Hcy to form Met [28]. Moreover, Hcy is transsulfurated to Cys by two enzymes, cystathionine β-synthase (CBS) and cystathionine γ–lyase (CSE), with the requirement of vitamin B_6. The transsulfuration by CBS and CSE only occurs in the liver [29], kidneys [30], and brain tissues [31]. Additionally, Hcy metabolizes HTL through methionyl-tRNA synthetase (MetRS), which occurs in each organ of our body [32, 33]. HTL is then synthesized to N-Hcy-protein, a homocysteine modification of the protein. Nonetheless, hydrolysis of HTL by paraoxonase 1 (PON1) into Hcy protects against the accumulation of N-Hcy-protein in human beings. Cytoplasmic bleomycin hydrolase (BLMH) works like HTL [34], as well as mitochondrial biphenol hydrolase-like enzyme (BPHL), but the latter only works *in vitro* [35]. Apart from these, Hcy is converted to AdoHcy *via* the hydrolysis of S-adenosylhomocysteine hydrolase (SAHH). Furthermore, Hcy reacts with the sulfhydryl group of protein to form disulfide, for example, Cys-S-S-Cys protein and Hcy-S-S-Cys protein [36]. The genetic or nutritional deficiencies contribute to the defect of Hcy metabolizing to Cys or Met, resulting in the accumulation of Hcy and its protein-related products and increasing the flux of HTL pathway strongly associated with considerable pathological environments in humans. To our knowledge, the research about Hcy has received amazing development in the past decades due to the desire to understand the effect of Hcy and its related metabolites on cardiovascular and neurologic diseases [37-39].

Moreover, Hcy is also metabolized to excitatory amino acid neurotransmitters, such as homocysteic acid (HCA) and cysteine sulfinic acid [40, 41], which leads to activation of N-methyl-Daspartate (NMDA) glutamate receptor. According to the "glutamatergic hypothesis" of depression, another actor that should be considered in the multifactorial pathogenesis of this disease might be the homocysteic acid (HCA), a neurotoxic compound that derives from Hcy [42]. Increased levels of HCA have been found in the PFC of treatment-resistant depression (TRD) [43]. HCA might contribute to the dysfunction of the glutamatergic system in TRD, and an increase of HCA might be detected in major depressive disorder (MDD) patients with hyperhomocysteinemia (HHcy) [44]. Genetic polymorphisms in MTHFR and CBS genes or a deficiency in essential cofactors in the one-carbon metabolism pathway (vitamin B_6, B_{12}, folate) can lead to HHcy, which is known to decrease the SAM-dependent synthesis of dopamine, noradrenaline [42]. Supplementation with vitamin B_6, B_{12}, and folate is known to reduce HHcy [39]. Almeida *et al.* [45] have found that B vitamins (0.5 mg of vitamin B_{12}, 2 mg of folic acid, and 25 mg of vitamin B_6) enhance and sustain antidepressant response over 1 year.

Previous researches find elevated levels of Hcy in Alzheimer's disease (AD) [46, 47] and cognitive impairment [48]. A preliminary study suggests that plasma HCA may be a useful indicator as an early diagnostic marker for mild cognitive impairment (MCI). HCA is possibly upstream from neurodegeneration in the Alzheimer's disease pathology because it promotes amyloid polymerization and tau phosphorylation in AD [49]. Adequate vitamin B_6, vitamin B_{12}, and folate intakes were significantly associated with better cognitive reserve by affecting methylation levels of specific redox-related genes [50].

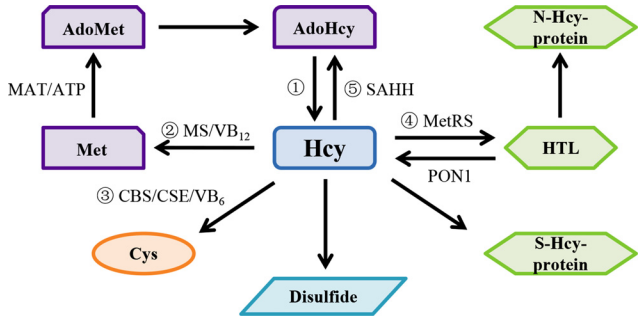

Fig. (1). Pathways of Hcy Metabolism. ① the generative pathway of homocysteine; ② the remethylation pathway; ③ the transsulfuration pathway; ④ the homocysteine-thiolactone pathway; ⑤ the reversible pathway of homocysteine generation. **Abbreviations: Hcy**, homocysteine; **Met**, methionine; **AdoMet**, adenosylmethionine; **AdoHcy**, adenosylhomocysteine; **Cys**, cysteine; **HTL**, homocysteine thiolactone; **N-Hcy-protein**, N-homocysteined protein; **S-Hcy-protein**, S-homocysteined protein; **MS**, Met synthase; **MAT**, Met S-adenosyltransferase; **CBS**, cystathionine β-synthase; **CSE**, cystathionine γ–lyase; **MetRS**, methionyl-tRNA synthetase; **PON1**, paraoxonase 1; **SAHH**, S-adenosylhomocysteine hydrolase; **VB_6**, vitamin B_6; **VB_{12}**, vitamin B_{12}.

2.2. Protein Homocysteinylation

Homocysteinylation is recognized as a chemical non-enzymatic reaction between proteins and Hcy or HTL, including S- or N-homocysteinylation [51]. S-homocysteinylation involves both the addition of reduced Hcy to available cysteinyl residues in proteins and a disulfide exchange reaction with optional disulfide, for example, S-homocysteinylated transthyretin (Fig. **2**) [52-54]. Homocysteine modification affects the function of many proteins, but only a few proteins can be S-homocysteinylated, mainly due to analytical limitations at first. The following study has revealed that in most plasma and tissue, Hcy links to the protein by disulfide bond after treatment with 2-mercaptoethanol [55]. Moreover, exogenous Hcy could link to protein and be detected quantitatively by administration with 2-mercaptoethanol. These investigations have revealed for the first time the phenomenon for protein S-homocysteinylation [56, 57]. S-homocysteinylation alters the original structure of the protein, but the effect of these alterations on health and disease is not well known [5].

N-homocysteinylation is an irreversible process of non-enzymatic acylation between the carbonyl group and Lys residues of proteins, for instance, albumin [58], collagen [59], cytochrome C [60], and fibrinogen [61]; and the level of N-Hcy-protein is dominant directly in plasma 'total Hcy' [62, 63]. N-Hcy-protein was first established in human endothelial cells in the last century [64, 65], and subsequent pieces of research have confirmed the activity of N-Hcy-protein in humans [66] and mice [67]. There are two steps about the mechanism underlying N-Hcy-protein generation (Fig. **2**). The first step is the metabolic conversion of Hcy to HTL catalyzed by MetRS, and the second step is the reaction with HTL and Lys residues of protein to afford N-Hcy-protein. Additionally, homocysteine modification could be induced by two other uncommon ways in humans (Fig. **2**). The one way is the production of S-nitroso-Hcy through nitric oxide-mediated mechanism [68], and another is demethylation of protein Met residues to Hcy catalyzed by cuprum and ferrum [69]. Like other protein modifications, it is well established that protein homocysteinylation leads to alterations in some biological protein structures and functions, for example, protein cross-linking [70], protein aggregation [71], and the generation of autoantibodies directed against homocysteinylated proteins after activation of the immune response [72]. Thus, the role of homocysteinylation in protein damage has gained increasing importance.

N-homocysteinylation results in loss of protein functions due to changes in the protein structure and becomes susceptible to further oxidative damage. It has been observed that the structural and functional consequences resulting from N-homocysteinylation depend on the pI of the proteins. Basic proteins are resistant to structural and functional alterations due to N-homocysteinylation, whereas acidic proteins are sensitive to N-homocysteinylation and cause denaturation of proteins. Therefore, it is a very definite possibility that cells or tissues abundant with acidic proteins might be the major targets for Hcy-induced cytotoxicity [73]. Furthermore, large bodies of evidence support the fact that homocysteinylation results in the accumulation of damaged and dysfunctional products and even diseases [74, 75]. Increased N-homocysteinylation of tau and microtubule-associated protein 1 (MAP1) is a mechanism of brain aging in AD and vascular dementia, which depends on the concentration of Hcy and expression of methionine tRNAsynthetase (MARS) enzyme [76]. It has also been reported that homocysteinylated-proteins affect expressions of vascular endothelial cell-related genes in human beings, associated with cardiovascular and central nervous system diseases [77]. Some blood coagulation genes (*CD9*, *ANXA8*, *SCUBE1*) and lipid metabolism gene (*SULT1E1*) are affected by N- homocysteinylated proteins and Hcy. Most interestingly, epigenetic mechanisms involving chromatin/histone modifications might lead to HTL-induced endothelial dysfunction [78]. Additionally, in human fetal brains, down-regulation in the expressions of neural tube closure-related genes (*Cecr2*, *Smarca4*, and *Dnmt3b*), along with high levels of Hcy and H3K79-Hcy, contributes to the onset of neural tube defects [79]. These pieces of evidence suggest that protein homocysteinylation is an important intermediate process in the pathological consequences of various diseases.

2.3. Hydrogen Sulfide and its Metabolism

Hydrogen sulfide (H_2S) is a kind of colorless, flammable, and rotten egg odor-like gas. It easily crosses the plasma membrane because it is soluble in both water and lipid. In the past, H_2S was known as the environmental toxic gas until first identified as the endogenously generated signaling neuromodulator (Fig. **3**) [80]. Endogenous H_2S is catalyzed synthetization by CBS and CSE, two vitamin B_6-dependent enzymes [81]. Meanwhile, CBS and CSE are associated with the transsulfuration pathway from Hcy to Cys. In particular, the coexistence of Hcy and Cys contributes to H_2S synthesis by CBS [82]. CBS catalyzes Hcy and serine to form cystathionine and H_2O, and serine is replaced by Cys, leading to the formation of H_2S. The CSE-catalyzed reaction to form H_2S is the β-elimination of Cys to ammonia, H_2S, and

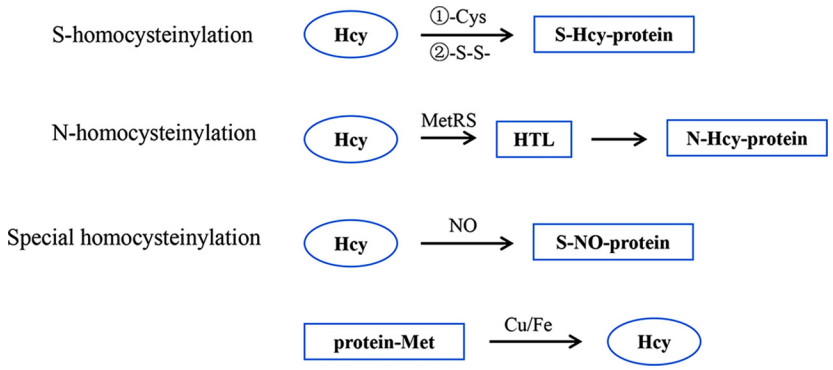

Fig. (2). Pathways of protein homocysteinylation. **Abbreviations: Hcy**, homocysteine; **MetRS**, methionyl-tRNA synthetase; **HTL**, homocysteine thiolactone.

pyruvate, or the γ-elimination of Hcy to α-ketobutyrate [81]. As the Cys level is higher than Hcy in our body, the desulfhydration of Cys is the main mechanism underlying H_2S generation by CSE under normal conditions [83]. The third mechanism of H_2S production is Cys converting to 3-mercaptopytuvate (3-MP) by cysteine aminotransferase (CAT) and 3-MP converting to H_2S by 3-mercaptopyruvate sulfurtransferase (3-MST) [84, 85]. H_2S is also possibly synthesized from Cys by catalysis of D-amino acid oxidase (D-AAO) and 3-MST [86]. H_2S is enzymatically oxidized by sulfide: quinone oxidoreductase (SQR) in the cellular mitochondria at very low concentrations [87], which transfers electrons to ubiquinone (coenzyme Q) to involve in aerobic respiration metabolism [88, 89]. Based on current knowledge, H_2S takes part in many physiological processes, ranging from the regulation of vascular tone to neuroprotective effects [90, 91]. Available pieces of evidence suggest that one of the mechanisms underlying H_2S signaling is probably protein sulfhydration [8].

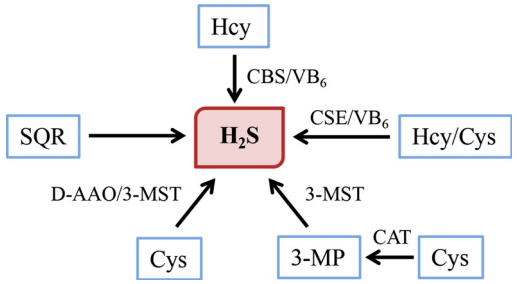

Fig. (3). Pathways of H_2S production. **Abbreviations: Hcy**, homocysteine; **Cys**, cysteine; **SQR**, sulfide: quinone oxidoreductase; **3-MP**, 3-mercaptopytuvate; **CBS**, cystathionine β-synthase; **D-AAO**, D-amino acid oxidase; **3-MST**, 3-mercaptopyruvate sulfurtransferase; **CAT**, cysteine aminotransferase; **CSE**, cystathionine γ–lyase; **VB_6**, vitamin B_6.

2.4. Protein Sulfhydration

Sulfhydration (also named persulfidation or perthiolation) is believed to be a modification of the –SH group of Cys with H_2S to produce persulfide or –SSH group, functioning like nitrosylation [92]. In some sense, persulfidation is more specialized to describe the process in comparison to other terms. H_2S modifies Cys residues after the oxidative forms of Cys, rather than directly reacting. It is noteworthy that the main form of H_2S is its negative ion (HS^-) in soluble liquid, which is the major form of H_2S activity. The reactivity of sulfhydration is mainly dependent on the dissolvent and functional group of Cys residues [93]; for example, Cys of the low acid dissociation constant are more sensitive to sulfhydration [94].

Nevertheless, they are also more susceptible to oxidants (*e.g.*, H_2O_2), resulting in the formation of sulfenic acid (SOH), sulfinic acid (SO_2H), or other sulfonic acid derivatives [95-97]. The formation of SOH is a reversible process, whereas modification of the thiol group of Cys is irreversible. Therefore, protein sulfhydration has an important role in preventing Cys residues of protein against irreversible modification [98]. Sulfhydration occurs by several pathways as mentioned below, which are the reaction of sulfide with oxidized Cys residues, for instance, cysteine sulfenic acids (Cys-SOH) or cysteine disulfides (-S-S-) [99], the reaction between oxidized sulfide species [100], and the reaction of H_2S_2 with cysteine thiol [101]. Previous studies strongly suggest that sulfhydration of target protein contributes to variations in the structure and function of the modified protein [102, 103].

Mechanistically, sulfhydration of the target protein is regarded as a fundamental mechanism underlying H_2S-regulated signaling pathways. Sulfhydration of Cys is an important paradigm of post-translational protein modification in the process of H_2S signaling and a critical redox mechanism to regulate H_2S-mediated neuroprotection and neurogenesis [104]. Several studies have shown that the sulfhydration of Cathepsin S (Cat S) at Cys25 [105] and Akt at Cys77 [106] are required for H_2S-mediated effects and might exert a vital role in the pathological functions of AD. H_2S keeps vascular health *via* the upregulation of vascular endothelial growth factor receptor 2 and neuropilin-1, which mainly results from sulfhydration of a transcription factor specificity protein 1 (SP1) at Cys68 and Cys755 residues [107]. Moreover, SP1, as a potent regulator of CSE, is sequestered and inactivated by mutant huntingtin in early Huntington's disease (HD), leading to cell redox imbalance resulting from depleted Cys biosynthesis [108]. Additionally, sulfhydration of the apoptotic effector protein caspase-3 at Cys163 loses its activity and reduces apoptotic death in primary cortical neurons, suggesting the inhibitory role of protein sulfhydration in enzymatic activities [109]. The sulfhydration of target proteins has a prominent role in the prevention of age-related hippocampal long-term potentiation

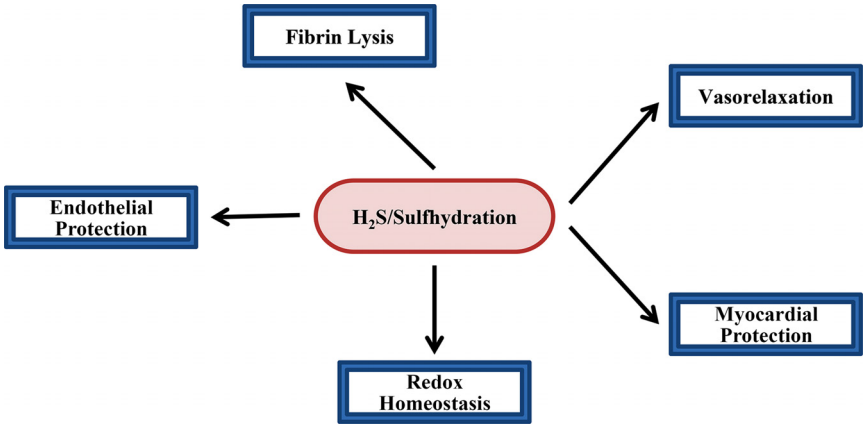

Fig. (4). Effects of H_2S or sulfhydration on diseases. H_2S exerts protective effects on vasorelaxation, myocardial injury, redox homeostasis, endothelial injury, and fibrin lysis.

(LTP) deficit by serine racemase [110] and inhibition of Parkinson's disease-related lesions by Parkin [111] or protein p66Shc (a modulator in mitochondrial redox signaling) [112], suggesting that this modification is also necessary for physiological states and pathological conditions, possibly including cardiovascular and neuronal systems.

2.5. Homocysteinylation and Sulfhydration in Diseases

According to the fact that H_2S protects humans from improper protein modification in contrast to the impairment of Hcy for protein, in a sense, we assume that H_2S may affect the destructive role of homocysteinylated target proteins by sulfhydration in pathological states.

2.5.1. Protein Homocysteinylation and Sulfhydration in Cardiovascular Diseases

Previous research has investigated that vasorelaxation is one of the essential functions of H_2S in human beings (Fig. **4**) [113]. Specifically, H_2S may exert a vasorelaxative effect *via* the reduction of Hcy buildup, ultimately resulting in falling blood pressure [114]. It has been reported that the level of H_2S is significantly reduced in Hcy-treated rats, and exogenous H_2S inhibits Hcy-exposed myocardial injury [115]. High plasma albumin cysteinylation (CysAlb) and Hcy levels are observed in the intestinal ischemia-reperfusion model of rats [116]. Additionally, in the ischemia reperfusion-induced myocardial injury, administration with H_2S obviously decreases infarct size to improve the contractile function of cardiac muscle. Recent findings have shown a high level of sulfhydration during this process (Fig. **4**) [117]. Normal cellular redox homeostasis is an important factor for heart disease. Homocysteinylation of metallothionein (MT) at the Cys residues obstructs zinc linking to the protein and abolishes superoxide dismutase (SOD) activity [118]. However, SOD reacts with H_2S by sulfhydryl modification at Cys111 residue, leading to slower protein unfolding and resistance to oxidation-induced protein aggregation (Fig. **4**) [119, 120]. Sulfhydration of SOD may facilitate endothelial protection and reduce the risk of atherosclerosis (AS). AS is a prevalent and frequent cardiovascular disease among old people that begins with a series of inflammatory reactions induced by HHcy [121], generating HTL to modify Lys residue of the target protein [5]. The subsequent study found that H_2S attenuates aortic atherosclerotic plaque formation, in which the underlying mechanism may be associated with Kelch-like ECH-associated protein 1 (Keap1) sulfhydration at Cys151 to activate nuclear factor-like (Nrf) 2 signaling [122]. Acute myocardial infarction (AMI) is positively related to the increasing Hcy in several case-control and cohort studies [123]. Furthermore, existing investigation evaluated that homocysteinylation of low-density lipoprotein (LDL) is increased in AMI patients [124]. Protective mechanisms of H_2S in AMI involve several physiological and pathological processes, including the direct interaction between H_2S and ATP-sensitive potassium channel (K_{ATP}) channel proteins *via* sulfhydryl modification (Fig. **4**) [125].

2.5.2. Protein Homocysteinylation and Sulfhydration in Neurologic Diseases

Vascular dysfunction induced by HHcy has an important effect on AD [126]. One aspect of vascular dysfunction is the formation of fibrin clots, which are not easy to dissolve and are produced from Hcy-fibrinogen [61]. It has already been shown that protein homocysteinylation promotes the interaction of fibrinogen with Aβ-peptide, which facilitates the development of fibrin clots and difficult fibrinolysis, enhances Aβ deposition in the cerebral vessel, and deteriorates cognitive defect in the AD model [127, 128]. Moreover, the homocysteinylation of Lys residues increases the neurotoxicity of the Aβ peptide by stabilizing soluble oligomeric intermediates [129]. Homocysteinylation of tau at critical amino acids decreases the binding ability of tau to microtubule protein (MTP), leading to the instability of MTP and disability to promote MTP assembly, which could inhibit the function of tau [130]. Nevertheless, H_2S reduces the polymerization of fibrinogen and increases fibrin lysis in the plasma (Fig. **4**) [131]. In AD, owing to diminished protein sulfhydration, exogenous H_2S prevents hyperphosphorylation of tau by sulfhydrating its kinase and glycogen synthase kinase 3β (GSK3β), further ameliorating cognitive deficits [132]. We also find the following pieces of research: the bioactivity of MTHFR is positively associated with its S-sulfhydration level, and H_2S deficiency results in the decrease of MTHFR sulfhydration level and bioactivity in HHcy. However, H_2S donors reverse the decreased bioactivity of MTHFR in HHcy, thus reducing excessive Hcy levels. These studies suggest that H_2S could improve MTHFR bioactivity by sulfhydration, which might provide a candidate therapeutic

strategy for HHcy [133]. In the Hcy-treated mice, supplementation of H_2S donors ameliorates Hcy-induced cerebrovascular pathology, cognitive deficits, and toxicity [134]. Based on these observations, we have speculated that appropriate regulation of sulfhydration by H_2S is essential for the inhibition of homocysteinylated protein, which may merit further investigation.

In addition to the above-mentioned effects of sulfhydration on homocysteinylation, some findings seem to provide completely different perspectives for their relationship. A recent study has demonstrated the inhibitory effect of protein homocysteinylation on enzymatic activities of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) by HTL [135]. However, H_2S causes sulfhydration of GAPDH in neuron dendrites to promote GAPDH binding to ubiquitin-protein ligase E3 (Siah protein). Then, Siah protein links to PSD95, which induces PSD95 degradation and memory dysfunction [136]. The contrary effect of sulfhydration on homocysteinylation may be attributed to the alterations of their substrate and concentration in different pathologic environments.

CONCLUSION

Elevated levels of Hcy and homocysteinylation have been found in many medical diseases. The role of protein homocysteinylation is currently attracting considerable research interest, simply because early intervention to normalize the Hcy-proteins may defer the development of these disorders and protect them from suffering Hcy-proteins-caused cell dysfunction. As discussed in this review, H_2S signaling *via* sulfhydration regulates a wide variety of cellular functions, and the sulfhydration of the target protein is necessary for organismal physiological health and human diseases. Thus, we speculate that to some extent, sulfhydration of H_2S exerts a beneficial effect on protein homocysteinylation under the condition of physiology or pathology. Nevertheless, some findings have deduced inconsistent results. Apparently, further research on these issues is needed with the help of a better understanding of the association of protein modification. Although contradiction exists, this review on homocysteinylation, sulfhydration, and their relationship in various diseases may help toward a better understanding of the current knowledge for further clinical and basic research. There is little doubt that the field of homocysteinylation and sulfhydration will be a topic of significant research in the future.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Jakubowski, H. Homocysteine is a protein amino acid in humans. Implications for homocysteine-linked disease. *J. Biol. Chem.,* **2002**, *277*(34), 30425-30428. http://dx.doi.org/10.1074/jbc.C200267200 PMID: 12093791

[2] Sikora, M.; Marczak, Ł.; Kubalska, J.; Graban, A.; Jakubowski, H. Identification of N-homocysteinylation sites in plasma proteins. *Amino Acids,* **2014**, *46*(1), 235-244. http://dx.doi.org/10.1007/s00726-013-1617-7 PMID: 24292153

[3] Jacovina, A.T.; Deora, A.B.; Ling, Q.; Broekman, M.J.; Almeida, D.; Greenberg, C.B.; Marcus, A.J.; Smith, J.D.; Hajjar, K.A. Homocysteine inhibits neoangiogenesis in mice through blockade of annexin A2-dependent fibrinolysis. *J. Clin. Invest.,* **2009**, *119*(11), 3384-3394. http://dx.doi.org/10.1172/JCI39591 PMID: 19841537

[4] Hortin, G.L.; Seam, N.; Hoehn, G.T. Bound homocysteine, cysteine, and cysteinylglycine distribution between albumin and globulins. *Clin. Chem.,* **2006**, *52*(12), 2258-2264. http://dx.doi.org/10.1373/clinchem.2006.074302 PMID: 17068168

[5] Jakubowski, H. *Homocysteine in protein structure/function and human disease*; Springer-Verlag, **2013**. http://dx.doi.org/10.1007/978-3-7091-1410-0

[6] Jakubowski, H. Homocysteine modification in protein structure/function and human disease. *Physiol. Rev.,* **2019**, *99*(1), 555-604. http://dx.doi.org/10.1152/physrev.00003.2018 PMID: 30427275

[7] Paul, B.D.; Snyder, S.H. H_2S: a novel gasotransmitter that signals by sulfhydration. *Trends Biochem. Sci.,* **2015**, *40*(11), 687-700. http://dx.doi.org/10.1016/j.tibs.2015.08.007 PMID: 26439534

[8] Mustafa, A.K.; Gadalla, M.M.; Sen, N.; Kim, S.; Mu, W.; Gazi, S.K.; Barrow, R.K.; Yang, G.; Wang, R.; Snyder, S.H. H2S signals through protein S-sulfhydration. *Sci. Signal.,* **2009**, *2*(96), ra72. http://dx.doi.org/10.1126/scisignal.2000464 PMID: 19903941

[9] Krishnan, N.; Fu, C.; Pappin, D.J.; Tonks, N.K. H2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Signal.,* **2011**, *4*(203), ra86. http://dx.doi.org/10.1126/scisignal.2002329 PMID: 22169477

[10] Jakubowski, H. Homocysteine editing, thioester chemistry, coenzyme A, and the origin of coded peptide synthesis? *Life (Basel),* **2017**, *7*(1), 6. http://dx.doi.org/10.3390/life7010006 PMID: 28208756

[11] Vallee, Y.; Shalayel, I.; Ly, K-D.; Rao, K.V.R.; De Paëpe, G.; Märker, K.; Milet, A. At the very beginning of life on Earth: the thiol-rich peptide (TRP) world hypothesis. *Int. J. Dev. Biol.,* **2017**, *61*(8-9), 471-478. http://dx.doi.org/10.1387/ijdb.170028yv PMID: 29139533

[12] Johnson, T.B. Sulfur linkages in proteins. *J. Biol. Chem.,* **1911**, *9*, 439-448. http://dx.doi.org/10.1016/S0021-9258(18)91443-2

[13] Howard Mueller, J. A new sulphur-containing amino acid isolated from casein. *Exp. Biol. Med.,* **1922**, *19*(4), 161-163. http://dx.doi.org/10.3181/00379727-19-75

[14] Barger, G.; Coyne, F.P. The amino-acid methionine; constitution and synthesis. *Biochem. J.,* **1928**, *22*(6), 1417-1425. http://dx.doi.org/10.1042/bj0221417 PMID: 16744158

[15] Butz, L.W.; Du Vigneaud, V. The formation of a homologue of cysteine by the decomposition of methionine with sulfuric acid. *J. Biol. Chem.,* **1932**, *99*, 135-142. http://dx.doi.org/10.1016/S0021-9258(18)76074-2

[16] Finkelstein, J.D. Homocysteine: a history in progress. *Nutr. Rev.,* **2000**, *58*(7), 193-204. http://dx.doi.org/10.1111/j.1753-4887.2000.tb01862.x PMID: 10941255

[17] Jakubowski, H. Translational incorporation of S-nitrosohomocysteine into protein. *J. Biol. Chem.,* **2000**, *275*(29), 21813-21816. http://dx.doi.org/10.1074/jbc.C000280200 PMID: 10829011

[18] Herrmann, W.; Obeid, R. Homocysteine: a biomarker in neurodegenerative diseases. *Clin. Chem. Lab. Med.,* **2011**, *49*(3), 435-441. PMID: 21388339

[19] Chan, A.Y.; Alsaraby, A.; Shea, T.B. Folate deprivation increases tau phosphorylation by homocysteine-induced calcium influx and

by inhibition of phosphatase activity: Alleviation by S-adenosyl methionine. *Brain Res.,* **2008**, *1199*, 133-137. http://dx.doi.org/10.1016/j.brainres.2008.01.008 PMID: 18279842

[20] Mendoza-Oliva, A.; Ferrera, P.; Arias, C. Interplay between cholesterol and homocysteine in the exacerbation of amyloid-β toxicity in human neuroblastoma cells. *CNS Neurol. Disord. Drug Targets,* **2013**, *12*(6), 842-848. http://dx.doi.org/10.2174/18715273113129990083 PMID: 23844691

[21] Kuszczyk, M.; Gordon-Krajcer, W.; Lazarewicz, J.W. Homocysteine-induced acute excitotoxicity in cerebellar granule cells *in vitro* is accompanied by PP2A-mediated dephosphorylation of tau. *Neurochem. Int.,* **2009**, *55*(1-3), 174-180. http://dx.doi.org/10.1016/j.neuint.2009.02.010 PMID: 19428823

[22] Kruman, I.I.; Culmsee, C.; Chan, S.L.; Kruman, Y.; Guo, Z.; Penix, L.; Mattson, M.P. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. *J. Neurosci.,* **2000**, *20*(18), 6920-6926. http://dx.doi.org/10.1523/JNEUROSCI.20-18-06920.2000 PMID: 10995836

[23] Finkelstein, J.D.; Martin, J.J. Methionine metabolism in mammals. Adaptation to methionine excess. *J. Biol. Chem.,* **1986**, *261*(4), 1582-1587. http://dx.doi.org/10.1016/S0021-9258(17)35979-3 PMID: 3080429

[24] Zhang, J.; Zheng, Y.G. SAM/SAH analogs as versatile tools for SAM-dependent methyltransferases. *ACS Chem. Biol.,* **2016**, *11*(3), 583-597. http://dx.doi.org/10.1021/acschembio.5b00812 PMID: 26540123

[25] Selhub, J. Homocysteine metabolism. *Annu. Rev. Nutr.,* **1999**, *19*, 217-246. http://dx.doi.org/10.1146/annurev.nutr.19.1.217 PMID: 10448523

[26] Finkelstein, J.D. Pathways and regulation of homocysteine metabolism in mammals. *Semin. Thromb. Hemost.,* **2000**, *26*(3), 219-225. http://dx.doi.org/10.1055/s-2000-8466 PMID: 11011839

[27] Zang, T.; Pottenplackel, L.P.; Handy, D.E.; Loscalzo, J.; Dai, S.; Deth, R.C.; Zhou, Z.S.; Ma, J. Comparison of protein N-homocysteinylation in rat plasma under elevated homocysteine using a specific chemical labeling method. *Molecules,* **2016**, *21*(9), 1195. http://dx.doi.org/10.3390/molecules21091195 PMID: 27617989

[28] Teng, Y-W.; Mehedint, M.G.; Garrow, T.A.; Zeisel, S.H. Deletion of betaine-homocysteine S-methyltransferase in mice perturbs choline and 1-carbon metabolism, resulting in fatty liver and hepatocellular carcinomas. *J. Biol. Chem.,* **2011**, *286*(42), 36258-36267. http://dx.doi.org/10.1074/jbc.M111.265348 PMID: 21878621

[29] Zhao, Y.; Wu, S.; Gao, X.; Zhang, Z.; Gong, J.; Zhan, R.; Wang, X.; Wang, W.; Qian, L. Inhibition of cystathionine β-synthase is associated with glucocorticoids over-secretion in psychological stress-induced hyperhomocystinemia rat liver. *Cell Stress Chaperones,* **2013**, *18*(5), 631-641. http://dx.doi.org/10.1007/s12192-013-0416-0 PMID: 23512717

[30] James, D. House, Margaret E. Brosnan, and John T. Brosnan, Characterization of homocysteine metabolism in the rat kidney. Biochem. *J.,* **1997**, *328*(1), 287-292. http://dx.doi.org/10.1042/bj3280287

[31] Hensley, K.; Denton, T.T. Alternative functions of the brain transsulfuration pathway represent an underappreciated aspect of brain redox biochemistry with significant potential for therapeutic engagement. *Free Radic. Biol. Med.,* **2015**, *78*, 123-134. http://dx.doi.org/10.1016/j.freeradbiomed.2014.10.581 PMID: 25463282

[32] Jakubowski, H. Quality control in tRNA charging -- editing of homocysteine. *Acta Biochim. Pol.,* **2011**, *58*(2), 149-163. http://dx.doi.org/10.18388/abp.2011_2259 PMID: 21643559

[33] Jakubowski, H. Quality control in tRNA charging. *Wiley Interdiscip. Rev. RNA,* **2012**, *3*(3), 295-310. http://dx.doi.org/10.1002/wrna.122 PMID: 22095844

[34] Zimny, J.; Sikora, M.; Guranowski, A.; Jakubowski, H. Protective mechanisms against homocysteine toxicity: the role of bleomycin hydrolase. *J. Biol. Chem.,* **2006**, *281*(32), 22485-22492. http://dx.doi.org/10.1074/jbc.M603656200 PMID: 16769724

[35] Marsillach, J.; Suzuki, S.M.; Richter, R.J.; McDonald, M.G.; Rademacher, P.M.; MacCoss, M.J.; Hsieh, E.J.; Rettie, A.E.; Furlong, C.E. Human valacyclovir hydrolase/biphenyl hydrolase-like protein is a highly efficient homocysteine thiolactonase. *PLoS One,* **2014**, *9*(10), e110054. http://dx.doi.org/10.1371/journal.pone.0110054 PMID: 25333274

[36] Sengupta, S.; Chen, H.; Togawa, T.; DiBello, P.M.; Majors, A.K.; Büdy, B.; Ketterer, M.E.; Jacobsen, D.W. Albumin thiolate anion is an intermediate in the formation of albumin-S-S-homocysteine. *J. Biol. Chem.,* **2001**, *276*(32), 30111-30117. http://dx.doi.org/10.1074/jbc.M104324200 PMID: 11371573

[37] Maron, B.A.; Loscalzo, J. The treatment of hyperhomocysteinemia. *Annu. Rev. Med.,* **2009**, *60*(1), 39-54. http://dx.doi.org/10.1146/annurev.med.60.041807.123308 PMID: 18729731

[38] Refsum, H.; Ueland, P.M.; Nygård, O.; Vollset, S.E. Homocysteine and cardiovascular disease. *Annu. Rev. Med.,* **1998**, *49*(1), 31-62. http://dx.doi.org/10.1146/annurev.med.49.1.31 PMID: 9509248

[39] Smith, A.D.; Refsum, H. Homocysteine, B vitamins, and cognitive impairment. *Annu. Rev. Nutr.,* **2016**, *36*(1), 211-239. http://dx.doi.org/10.1146/annurev-nutr-071715-050947 PMID: 27431367

[40] Bleich, S.; Degner, D.; Wiltfang, J.; Maler, J.M.; Niedmann, P.; Cohrs, S.; Mangholz, A.; Porzig, J.; Sprung, R.; Rüther, E.; Kornhuber, J. Elevated homocysteine levels in alcohol withdrawal. *Alcohol Alcohol.,* **2000**, *35*(4), 351-354. http://dx.doi.org/10.1093/alcalc/35.4.351 PMID: 10905999

[41] Obeid, R.; Herrmann, W. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. *FEBS Lett.,* **2006**, *580*(13), 2994-3005. http://dx.doi.org/10.1016/j.febslet.2006.04.088 PMID: 16697371

[42] Bhatia, P.; Singh, N. Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. *Fundam. Clin. Pharmacol.,* **2015**, *29*(6), 522-528. http://dx.doi.org/10.1111/fcp.12145 PMID: 26376956

[43] Francis, P.T.; Poynton, A.; Lowe, S.L.; Najlerahim, A.; Bridges, P.K.; Bartlett, J.R.; Procter, A.W.; Bruton, C.J.; Bowen, D.M. Brain amino acid concentrations and Ca^{2+}-dependent release in intractable depression assessed antemortem. *Brain Res.,* **1989**, *494*(2), 315-324. http://dx.doi.org/10.1016/0006-8993(89)90600-8 PMID: 2570624

[44] Folstein, M.; Liu, T.; Peter, I.; Buell, J.; Arsenault, L.; Scott, T.; Qiu, W.W.; Qiu, W.W. The homocysteine hypothesis of depression. *Am. J. Psychiatry,* **2007**, *164*(6), 861-867. http://dx.doi.org/10.1176/ajp.2007.164.6.861 PMID: 17541043

[45] Almeida, O.P.; Ford, A.H.; Hirani, V.; Singh, V.; vanBockxmeer, F.M.; McCaul, K.; Flicker, L. B vitamins to enhance treatment response to antidepressants in middle-aged and older adults: results from the B-VITAGE randomised, double-blind, placebo-controlled trial. *Br. J. Psychiatry,* **2014**, *205*(6), 450-457. http://dx.doi.org/10.1192/bjp.bp.114.145177 PMID: 25257064

[46] Robert, C.A.; David, S.; Kim, A. Jobst, H.R.; Leslsy, S.; Per, M.U. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer Disease. *JAMA Neurol.,* **1998**, *55*(11), 1449-1455.

[47] Seshadri, S.; Beiser, A.; Selhub, J.; Jacques, P.F.; Rosenberg, I.H.; D'Agostino, R.B.; Wilson, P.W.F.; Wolf, P.A. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N. Engl. J. Med.,* **2002**, *346*(7), 476-483. http://dx.doi.org/10.1056/NEJMoa011613 PMID: 11844848

[48] Wright, C.B.; Lee, H.S.; Paik, M.C.; Stabler, S.P.; Allen, R.H.; Sacco, R.L. Total homocysteine and cognition in a tri-ethnic cohort: the Northern Manhattan Study. *Neurology,* **2004**, *63*(2), 254-260. http://dx.doi.org/10.1212/01.WNL.0000129986.19019.5D PMID: 15277617

[49] Hasegawa, T.; Kosoku, Y.; Sano, Y.; Yoshida, H.; Kudoh, C.; Tabira, T. Homocysteic acid in blood can detect mild cognitive impairment: a preliminary study. *J. Alzheimers Dis.,* **2020**, *77*(2), 773-780. http://dx.doi.org/10.3233/JAD-200234 PMID: 32741817

[50] An, Y.; Feng, L.; Zhang, X.; Wang, Y.; Wang, Y.; Tao, L.; Qin, Z.; Xiao, R. Dietary intakes and biomarker patterns of folate, vitamin B_6, and vitamin B_{12} can be associated with cognitive impairment by hypermethylation of redox-related genes NUDT15 and TXNRD1. *Clin. Epigenetics,* **2019**, *11*(1), 139. http://dx.doi.org/10.1186/s13148-019-0741-y PMID: 31601260

[51] Jakubowski, H. Molecular basis of homocysteine toxicity in humans. *Cell. Mol. Life Sci.,* **2004**, *61*(4), 470-487. http://dx.doi.org/10.1007/s00018-003-3204-7 PMID: 14999406

[52] Blom, H.J. Consequences of homocysteine export and oxidation in the vascular system. *Semin. Thromb. Hemost.,* **2000**, *26*(3), 227-232. http://dx.doi.org/10.1055/s-2000-8467 PMID: 11011840

[53] Sass, J.O.; Nakanishi, T.; Sato, T.; Sperl, W.; Shimizu, A. S-homocysteinylation of transthyretin is detected in plasma and serum of humans with different types of hyperhomocysteinemia. *Biochem. Biophys. Res. Commun.,* **2003**, *310*(1), 242-246. http://dx.doi.org/10.1016/j.bbrc.2003.08.089 PMID: 14511677

[54] Lim, A.; Sengupta, S.; McComb, M.E.; Théberge, R.; Wilson, W.G.; Costello, C.E.; Jacobsen, D.W. *In vitro* and *in vivo* interactions of homocysteine with human plasma transthyretin. *J. Biol. Chem.,* **2003**, *278*(50), 49707-49713. http://dx.doi.org/10.1074/jbc.M306748200 PMID: 14507924

[55] Kang, S.S.; Wong, P.W.; Becker, N. Protein-bound homocyst(e)ine in normal subjects and in patients with homocystinuria. *Pediatr. Res.,* **1979**, *13*(10), 1141-1143. http://dx.doi.org/10.1203/00006450-197910000-00012 PMID: 503641

[56] Glushchenko, A.V.; Jacobsen, D.W. Molecular targeting of proteins by L-homocysteine: mechanistic implications for vascular disease. *Antioxid. Redox Signal.,* **2007**, *9*(11), 1883-1898. http://dx.doi.org/10.1089/ars.2007.1809 PMID: 17760510

[57] Perła-Kaján, J.; Twardowski, T.; Jakubowski, H. Mechanisms of homocysteine toxicity in humans. *Amino Acids,* **2007**, *32*(4), 561-572. http://dx.doi.org/10.1007/s00726-006-0432-9 PMID: 17285228

[58] Sikora, M.; Marczak, L.; Twardowski, T.; Stobiecki, M.; Jakubowski, H. Direct monitoring of albumin lysine-525 N-homocysteinylation in human serum by liquid chromatography/mass spectrometry. *Anal. Biochem.,* **2010**, *405*(1), 132-134. http://dx.doi.org/10.1016/j.ab.2010.04.034 PMID: 20659604

[59] Perła-Kajan, J.; Utyro, O.; Rusek, M.; Malinowska, A.; Sitkiewicz, E.; Jakubowski, H. N-Homocysteinylation impairs collagen cross-linking in cystathionine β-synthase-deficient mice: a novel mechanism of connective tissue abnormalities. *FASEB J.,* **2016**, *30*(11), 3810-3821. http://dx.doi.org/10.1096/fj.201600539 PMID: 27530978

[60] Perła-Kaján, J.; Marczak, Ł.; Kaján, L.; Skowronek, P.; Twardowski, T.; Jakubowski, H. Modification by homocysteine thiolactone affects redox status of cytochrome C. *Biochemistry,* **2007**, *46*(21), 6225-6231. http://dx.doi.org/10.1021/bi602463m PMID: 17474717

[61] Sauls, D.L.; Lockhart, E.; Warren, M.E.; Lenkowski, A.; Wilhelm, S.E.; Hoffman, M. Modification of fibrinogen by homocysteine thiolactone increases resistance to fibrinolysis: a potential mechanism of the thrombotic tendency in hyperhomocysteinemia. *Biochemistry,* **2006**, *45*(8), 2480-2487. http://dx.doi.org/10.1021/bi052076j PMID: 16489740

[62] Jakubowski, H. Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. *FASEB J.,* **1999**, *13*(15), 2277-2283. http://dx.doi.org/10.1096/fasebj.13.15.2277 PMID: 10593875

[63] Jakubowski, H. Aminoacyl-tRNA synthetases and the evolution of coded peptide synthesis: the Thioester World. *FEBS Lett.,* **2016**, *590*(4), 469-481. http://dx.doi.org/10.1002/1873-3468.12085 PMID: 26831912

[64] Jakubowski, H. Metabolism of homocysteine thiolactone in human cell cultures. Possible mechanism for pathological consequences of elevated homocysteine levels. *J. Biol. Chem.,* **1997**, *272*(3), 1935-1942. http://dx.doi.org/10.1016/S0021-9258(19)67504-6 PMID: 8999883

[65] Jakubowski, H.; Zhang, L.; Bardeguez, A.; Aviv, A. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. *Circ. Res.,* **2000**, *87*(1), 45-51. http://dx.doi.org/10.1161/01.RES.87.1.45 PMID: 10884371

[66] Jakubowski, H.; Boers, G.H.J.; Strauss, K.A. Mutations in cystathionine beta-synthase or methylenetetrahydrofolate reductase gene increase N-homocysteinylated protein levels in humans. *FASEB J.,* **2008**, *22*(12), 4071-4076. http://dx.doi.org/10.1096/fj.08-112086 PMID: 18708589

[67] Jakubowski, Hieronim; Perla-Kaján, Joanna; Finnell, Richard H.; Cabrera, Robert M.; Wang, Hong; Gupta, Sapna; Kruger, Warren D.; Kraus, Jan P.; Shih, Diana M. Genetic or nutritional disorders in homocysteine or folate metabolism increase protein N-homocysteinylation in mice. *FASEB J,* **2009**, *23*(6), 1721-1727. http://dx.doi.org/10.1096/fj.08-127548 PMID: 19204075

[68] Jakubowski, H. Translational accuracy of aminoacyl-tRNA synthetases: implications for atherosclerosis. *J. Nutr.,* **2001**, *131*(11), 2983S-2987S. http://dx.doi.org/10.1093/jn/131.11.2983S PMID: 11694633

[69] Borowczyk, K.; Suliburska, J.; Jakubowski, H. Demethylation of methionine and keratin damage in human hair. *Amino Acids,* **2018**, *50*(5), 537-546. http://dx.doi.org/10.1007/s00726-018-2545-3 PMID: 29480334

[70] Liu, M.; Zhang, Z.; Zang, T.; Spahr, C.; Cheetham, J.; Ren, D.; Zhou, Z.S. Discovery of undefined protein cross-linking chemistry: a comprehensive methodology utilizing 18O-labeling and mass spectrometry. *Anal. Chem.,* **2013**, *85*(12), 5900-5908. http://dx.doi.org/10.1021/ac400666p PMID: 23634697

[71] Sauls, D.L.; Warren, M.; Hoffman, M. Homocysteinylated fibrinogen forms disulfide-linked complexes with albumin. *Thromb. Res.,* **2011**, *127*(6), 576-581. http://dx.doi.org/10.1016/j.thromres.2011.01.009 PMID: 21316742

[72] Undas, A.; Perła, J.; Lacinski, M.; Trzeciak, W.; Kaźmierski, R.; Jakubowski, H. Autoantibodies against N-homocysteinylated proteins in humans: implications for atherosclerosis. *Stroke,* **2004**, *35*(6), 1299-1304. http://dx.doi.org/10.1161/01.STR.0000128412.59768.6e PMID: 15131313

[73] Sharma, G.S.; Kumar, T.; Singh, L.R. N-homocysteinylation induces different structural and functional consequences on acidic and basic proteins. *PLoS One,* **2014**, *9*(12), e116386. http://dx.doi.org/10.1371/journal.pone.0116386 PMID: 25551634

[74] Berlett, B.S.; Stadtman, E.R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.,* **1997**, *272*(33), 20313-20316. http://dx.doi.org/10.1074/jbc.272.33.20313 PMID: 9252331

[75] Vlassara, H. Recent progress on the biologic and clinical significance of advanced glycosylation end products. *J. Lab. Clin. Med.,* **1994**, *124*(1), 19-30. PMID: 8035098

[76] Bossenmeyer-Pourié, C.; Smith, A.D.; Lehmann, S.; Deramecourt, V.; Sablonnière, B.; Camadro, J.M.; Pourié, G.; Kerek, R.; Helle, D.; Umoret, R.; Guéant-Rodriguez, R.M.; Rigau, V.; Gabelle, A.; Sequeira, J.M.; Quadros, E.V.; Daval, J.L.; Guéant, J.L. N-homocysteinylation of tau and MAP1 is increased in autopsy specimens of Alzheimer's disease and vascular dementia. *J. Pathol.,* **2019**, *248*(3), 291-303. http://dx.doi.org/10.1002/path.5254 PMID: 30734924

[77] Jakubowski, H. Quantification of urinary S- and N-homocysteinylated protein and homocysteine-thiolactone in mice. *Anal. Biochem.,* **2016**, *508*, 118-123. http://dx.doi.org/10.1016/j.ab.2016.06.002 PMID: 27293214

[78] Gurda, D.; Handschuh, L.; Kotkowiak, W.; Jakubowski, H. Homocysteine thiolactone and N-homocysteinylated protein induce pro-atherogenic changes in gene expression in human vascular endothelial cells. *Amino Acids,* **2015**, *47*(7), 1319-1339. http://dx.doi.org/10.1007/s00726-015-1956-7 PMID: 25802182

[79] Zhang, Q.; Bai, B.; Mei, X.; Wan, C.; Cao, H.; Dan Li, ; Wang, S.; Zhang, M.; Wang, Z.; Wu, J.; Wang, H.; Huo, J.; Ding, G.; Zhao, J.; Xie, Q.; Wang, L.; Qiu, Z.; Zhao, S.; Zhang, T. Elevated H3K79 homocysteinylation causes abnormal gene expression during neural development and subsequent neural tube defects. *Nat. Commun.,* **2018**, *9*(1), 3436. http://dx.doi.org/10.1038/s41467-018-05451-7 PMID: 30143612

[80] Abe, K.; Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.,* **1996**, *16*(3), 1066-1071. http://dx.doi.org/10.1523/JNEUROSCI.16-03-01066.1996 PMID: 8558235

[81] Kabil, O.; Banerjee, R. Enzymology of H2S biogenesis, decay and signaling. *Antioxid. Redox Signal.,* **2014**, *20*(5), 770-782. http://dx.doi.org/10.1089/ars.2013.5339 PMID: 23600844

[82] Singh, S.; Padovani, D.; Leslie, R.A.; Chiku, T.; Banerjee, R. Relative contributions of cystathionine β-synthase and γ-cystathionase

to H2S biogenesis *via* alternative trans-sulfuration reactions. *J. Biol. Chem.,* **2009**, *284*(33), 22457-22466. http://dx.doi.org/10.1074/jbc.M109.010868 PMID: 19531479

[83] Chiku, T.; Padovani, D.; Zhu, W.; Singh, S.; Vitvitsky, V.; Banerjee, R. H2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. *J. Biol. Chem.,* **2009**, *284*(17), 11601-11612. http://dx.doi.org/10.1074/jbc.M808026200 PMID: 19261609

[84] Shibuya, N.; Tanaka, M.; Yoshida, M.; Ogasawara, Y.; Togawa, T.; Ishii, K.; Kimura, H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid. Redox Signal.,* **2009**, *11*(4), 703-714. http://dx.doi.org/10.1089/ars.2008.2253 PMID: 18855522

[85] Shibuya, N.; Mikami, Y.; Kimura, Y.; Nagahara, N.; Kimura, H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J. Biochem.,* **2009**, *146*(5), 623-626. http://dx.doi.org/10.1093/jb/mvp111 PMID: 19605461

[86] Shibuya, N.; Koike, S.; Tanaka, M.; Ishigami-Yuasa, M.; Kimura, Y.; Ogasawara, Y.; Fukui, K.; Nagahara, N.; Kimura, H. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat. Commun.,* **2013**, *4*(1), 1366. http://dx.doi.org/10.1038/ncomms2371 PMID: 23340406

[87] Hildebrandt, T.M.; Grieshaber, M.K. Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *FEBS J.,* **2008**, *275*(13), 3352-3361. http://dx.doi.org/10.1111/j.1742-4658.2008.06482.x PMID: 18494801

[88] Bouillaud, F.; Blachier, F. Mitochondria and sulfide: a very old story of poisoning, feeding, and signaling? *Antioxid. Redox Signal.,* **2011**, *15*(2), 379-391. http://dx.doi.org/10.1089/ars.2010.3678 PMID: 21028947

[89] Jackson, M.R.; Melideo, S.L.; Jorns, M.S. Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry,* **2012**, *51*(34), 6804-6815. http://dx.doi.org/10.1021/bi300778t PMID: 22852582

[90] Yang, G.; Wu, L.; Jiang, B.; Yang, W.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A.K.; Mu, W.; Zhang, S.; Snyder, S.H.; Wang, R. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science,* **2008**, *322*(5901), 587-590. http://dx.doi.org/10.1126/science.1162667 PMID: 18948540

[91] Zhang, Q.; Yuan, L.; Liu, D.; Wang, J.; Wang, S.; Zhang, Q.; Gong, Y.; Liu, H.; Hao, A.; Wang, Z. Hydrogen sulfide attenuates hypoxia-induced neurotoxicity through inhibiting microglial activation. *Pharmacol. Res.,* **2014**, *84*, 32-44. http://dx.doi.org/10.1016/j.phrs.2014.04.009 PMID: 24788079

[92] Stamler, J.S.; Simon, D.I.; Osborne, J.A.; Mullins, M.E.; Jaraki, O.; Michel, T.; Singel, D.J.; Loscalzo, J. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc. Natl. Acad. Sci. USA,* **1992**, *89*(1), 444-448. http://dx.doi.org/10.1073/pnas.89.1.444 PMID: 1346070

[93] Marino, S.M.; Gladyshev, V.N. Redox biology: computational approaches to the investigation of functional cysteine residues. *Antioxid. Redox Signal.,* **2011**, *15*(1), 135-146. http://dx.doi.org/10.1089/ars.2010.3561 PMID: 20812876

[94] Greiner, R.; Pálinkás, Z.; Bäsell, K.; Becher, D.; Antelmann, H.; Nagy, P.; Dick, T.P. Polysulfides link H2S to protein thiol oxidation. *Antioxid. Redox Signal.,* **2013**, *19*(15), 1749-1765. http://dx.doi.org/10.1089/ars.2012.5041 PMID: 23646934

[95] Poole, L.B.; Nelson, K.J. Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr. Opin. Chem. Biol.,* **2008**, *12*(1), 18-24. http://dx.doi.org/10.1016/j.cbpa.2008.01.021 PMID: 18282483

[96] Klomsiri, C.; Karplus, P.A.; Poole, L.B. Cysteine-based redox switches in enzymes. *Antioxid. Redox Signal.,* **2011**, *14*(6), 1065-1077. http://dx.doi.org/10.1089/ars.2010.3376 PMID: 20799881

[97] Finkel, T. From sulfenylation to sulfhydration: what a thiolate needs to tolerate. *Sci. Signal.,* **2012**, *5*(215), 10. http://dx.doi.org/10.1126/scisignal.2002943 PMID: 22416275

[98] Paul, B.D.; Snyder, S.H. H_2S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.,* **2012**, *13*(8), 499-507. http://dx.doi.org/10.1038/nrm3391 PMID: 22781905

[99] Francoleon, N.E.; Carrington, S.J.; Fukuto, J.M. The reaction of H(2)S with oxidized thiols: generation of persulfides and implications to H(2)S biology. *Arch. Biochem. Biophys.,* **2011**, *516*(2), 146-153. http://dx.doi.org/10.1016/j.abb.2011.09.015 PMID: 22001739

[100] Zhang, D.; Macinkovic, I.; Devarie-Baez, N.O.; Pan, J.; Park, C-M.; Carroll, K.S.; Filipovic, M.R.; Xian, M. Detection of protein S-sulfhydration by a tag-switch technique. *Angew. Chem. Int. Ed. Engl.,* **2014**, *53*(2), 575-581. http://dx.doi.org/10.1002/anie.201305876 PMID: 24288186

[101] Nagy, P.; Winterbourn, C.C. Rapid reaction of hydrogen sulfide with the neutrophil oxidant hypochlorous acid to generate polysulfides. *Chem. Res. Toxicol.,* **2010**, *23*(10), 1541-1543. http://dx.doi.org/10.1021/tx100266a PMID: 20845929

[102] Ju, Y.; Fu, M.; Stokes, E.; Wu, L.; Yang, G. H2S-mediated protein S-sulfhydration: a prediction for its formation and regulation. *Molecules,* **2017**, *22*(8), 1334. http://dx.doi.org/10.3390/molecules22081334 PMID: 28800080

[103] Zhang, D.; Du, J.; Tang, C.; Huang, Y.; Jin, H. H2S-induced sulfhydration: biological function and detection methodology. *Front. Pharmacol.,* **2017**, *8*, 608. http://dx.doi.org/10.3389/fphar.2017.00608 PMID: 28932194

[104] Sun, H-J.; Wu, Z-Y.; Nie, X-W.; Bian, J-S. Role of hydrogen sulfide and polysulfides in neurological diseases: focus on protein S-Persulfidation. *Curr. Neuropharmacol.,* **2021**, *19*(6), 868-884. http://dx.doi.org/10.2174/1570159X18666200905143550 PMID: 32888271

[105] Cao, L.; Cao, X.; Zhou, Y.; Nagpure, B.V.; Wu, Z-Y.; Hu, L.F.; Yang, Y.; Sethi, G.; Moore, P.K.; Bian, J-S. Hydrogen sulfide inhibits ATP-induced neuroinflammation and $A\beta_{1-42}$ synthesis by suppressing the activation of STAT3 and cathepsin S. *Brain Behav. Immun.,* **2018**, *73*, 603-614. http://dx.doi.org/10.1016/j.bbi.2018.07.005 PMID: 29981830

[106] Sen, T.; Saha, P.; Jiang, T.; Sen, N. Sulfhydration of AKT triggers Tau-phosphorylation by activating glycogen synthase kinase 3β in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(8), 4418-4427. http://dx.doi.org/10.1073/pnas.1916895117 PMID: 32051249

[107] Saha, S.; Chakraborty, P.K.; Xiong, X.; Dwivedi, S.K.; Mustafi, S.B.; Leigh, N.R.; Ramchandran, R.; Mukherjee, P.; Bhattacharya, R. Cystathionine β-synthase regulates endothelial function *via* protein S-sulfhydration. *FASEB J.,* **2016**, *30*(1), 441-456. http://dx.doi.org/10.1096/fj.15-278648 PMID: 26405298

[108] Dunah, A.W.; Jeong, H.; Griffin, A.; Kim, Y-M.; Standaert, D.G.; Hersch, S.M.; Mouradian, M.M.; Young, A.B.; Tanese, N.; Krainc, D. Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science,* **2002**, *296*(5576), 2238-2243. http://dx.doi.org/10.1126/science.1072613 PMID: 11988536

[109] Marutani, E.; Yamada, M.; Ida, T.; Tokuda, K.; Ikeda, K.; Kai, S.; Shirozu, K.; Hayashida, K.; Kosugi, S.; Hanaoka, K.; Kaneki, M.; Akaike, T.; Ichinose, F. Thiosulfate mediates cytoprotective effects of hydrogen sulfide against neuronal ischemia. *J. Am. Heart Assoc.,* **2015**, *4*(11), e002125. http://dx.doi.org/10.1161/JAHA.115.002125 PMID: 26546573

[110] Li, Y-L.; Wu, P-F.; Chen, J-G.; Wang, S.; Han, Q-Q.; Li, D.; Wang, W.; Guan, X-L.; Li, D.; Long, L.H.; Huang, J.G.; Wang, F. Activity-dependent sulfhydration signal controls N-methyl-D-aspartate subtype glutamate receptor-dependent synaptic plasticity *via* increasing D-serine availability. *Antioxid. Redox Signal.,* **2017**, *27*(7), 398-414. http://dx.doi.org/10.1089/ars.2016.6936 PMID: 28051338

[111] Vandiver, M.S.; Paul, B.D.; Xu, R.; Karuppagounder, S.; Rao, F.; Snowman, A.M.; Ko, H.S.; Lee, Y.I.; Dawson, V.L.; Dawson, T.M.; Sen, N.; Snyder, S.H. Sulfhydration mediates neuroprotective actions of parkin. *Nat. Commun.,* **2013**, *4*, 1626. http://dx.doi.org/10.1038/ncomms2623 PMID: 23535647

[112] Xie, Z-Z.; Shi, M-M.; Xie, L.; Wu, Z-Y.; Li, G.; Hua, F.; Bian, J-S. Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. *Antioxid. Redox Signal.,* **2014**, *21*(18), 2531-2542. http://dx.doi.org/10.1089/ars.2013.5604 PMID: 24766279

[113] Hosoki, R.; Matsuki, N.; Kimura, H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with

nitric oxide. *Biochem. Biophys. Res. Commun.,* **1997**, *237*(3), 527-531.
http://dx.doi.org/10.1006/bbrc.1997.6878 PMID: 9299397

[114] Huang, S.; Li, H.; Ge, J. A cardioprotective insight of the cystathionine γ -lyase/hydrogen sulfide pathway. *Int. J. Cardiol. Heart Vasc.,* **2015**, *7*, 51-57.
http://dx.doi.org/10.1016/j.ijcha.2015.01.010 PMID: 28785645

[115] Chang, L.; Geng, B.; Yu, F.; Zhao, J.; Jiang, H.; Du, J.; Tang, C. Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. *Amino Acids,* **2008**, *34*(4), 573-585.
http://dx.doi.org/10.1007/s00726-007-0011-8 PMID: 18071843

[116] Bar-Or, D.; Curtis, C.G.; Sullivan, A.; Rael, L.T.; Thomas, G.W.; Craun, M.; Bar-Or, R.; Maclean, K.N.; Kraus, J.P. Plasma albumin cysteinylation is regulated by cystathionine β -synthase. *Biochem. Biophys. Res. Commun.,* **2004**, *325*(4), 1449-1453.
http://dx.doi.org/10.1016/j.bbrc.2004.10.191 PMID: 15555590

[117] Sun, J.; Aponte, A.M.; Menazza, S.; Gucek, M.; Steenbergen, C.; Murphy, E. Additive cardioprotection by pharmacological postconditioning with hydrogen sulfide and nitric oxide donors in mouse heart: S-sulfhydration *vs*. S-nitrosylation. *Cardiovasc. Res.,* **2016**, *110*(1), 96-106.
http://dx.doi.org/10.1093/cvr/cvw037 PMID: 26907390

[118] Barbato, J.C.; Catanescu, O.; Murray, K.; DiBello, P.M.; Jacobsen, D.W. Targeting of metallothionein by L-homocysteine: a novel mechanism for disruption of zinc and redox homeostasis. *Arterioscler. Thromb. Vasc. Biol.,* **2007**, *27*(1), 49-54.
http://dx.doi.org/10.1161/01.ATV.0000251536.49581.8a PMID: 17082481

[119] Briggs, R.G.; Fee, J.A. Sulfhydryl reactivity of human erythrocyte superoxide dismutase. On the origin of the unusual spectral properties of the protein when prepared by a procedure utilizing chloroform and ethanol for the precipitation of hemoglobin. *Biochim. Biophys. Acta,* **1978**, *537*(1), 100-109.
http://dx.doi.org/10.1016/0005-2795(78)90606-2 PMID: 718975

[120] de Beus, M.D.; Chung, J.; Colón, W. Modification of cysteine 111 in Cu/Zn superoxide dismutase results in altered spectroscopic and biophysical properties. *Protein Sci.,* **2004**, *13*(5), 1347-1355.
http://dx.doi.org/10.1110/ps.03576904 PMID: 15096637

[121] Libby, P. Inflammation and cardiovascular disease mechanisms. *Am. J. Clin. Nutr.,* **2006**, *83*(2), 456S-460S.
http://dx.doi.org/10.1093/ajcn/83.2.456S PMID: 16470012

[122] Xie, L.; Gu, Y.; Wen, M.; Zhao, S.; Wang, W.; Ma, Y.; Meng, G.; Han, Y.; Wang, Y.; Liu, G.; Moore, P.K.; Wang, X.; Wang, H.; Zhang, Z.; Yu, Y.; Ferro, A.; Huang, Z.; Ji, Y. Hydrogen sulfide induces keap1 S-sulfhydration and suppresses diabetes-accelerated atherosclerosis *via* Nrf2 activation. *Diabetes,* **2016**, *65*(10), 3171-3184.
http://dx.doi.org/10.2337/db16-0020 PMID: 27335232

[123] Gomes, E.; Duarte, R.; Reis, R.P.; Cândido, A.; Cardim, N.; Correia, M.J.; Castela, S.; Cordeiro, R.; Ramos, A.; Lobo, J.L.; Correia, J.F.M. Homocysteine increase after acute myocardial infarction--can it explain the differences between case-control and cohort studies? *Rev. Port. Cardiol.,* **2002**, *21*(5), 575-581.
PMID: 12174520

[124] Zinellu, A.; Sotgia, S.; Scanu, B.; Deiana, L.; Talanas, G.; Terrosu, P.; Carru, C. Low density lipoprotein S-homocysteinylation is increased in acute myocardial infarction patients. *Clin. Biochem.,* **2012**, *45*(4-5), 359-362.
http://dx.doi.org/10.1016/j.clinbiochem.2011.12.017 PMID: 22240067

[125] Donnarumma, E.; Trivedi, R.K.; Lefer, D.J. Protective actions of H2S in acute myocardial infarction and heart failure. *Compr Physiol.,* **2017**, *7*(2), 583-602.
http://dx.doi.org/10.1002/cphy.c160023 PMID: 28333381

[126] Cortes-Canteli, M.; Paul, J.; Norris, E.H.; Bronstein, R.; Ahn, H.J.; Zamolodchikov, D.; Bhuvanendran, S.; Fenz, K.M.; Strickland, S. Fibrinogen and beta-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. *Neuron,* **2010**, *66*(5), 695-709.
http://dx.doi.org/10.1016/j.neuron.2010.05.014 PMID: 20547128

[127] Ahn, H.J.; Zamolodchikov, D.; Cortes-Canteli, M.; Norris, E.H.; Glickman, J.F.; Strickland, S. Alzheimer's disease peptide beta-amyloid interacts with fibrinogen and induces its oligomerization. *Proc. Natl. Acad. Sci. USA,* **2010**, *107*(50), 21812-21817.
http://dx.doi.org/10.1073/pnas.1010373107 PMID: 21098282

[128] Chung, Y.C.; Kruyer, A.; Yao, Y.; Feierman, E.; Richards, A.; Strickland, S.; Norris, E.H. Hyperhomocysteinemia exacerbates Alzheimer's disease pathology by way of the β-amyloid fibrinogen interaction. *J. Thromb. Haemost.,* **2016**, *14*(7), 1442-1452.
http://dx.doi.org/10.1111/jth.13340 PMID: 27090576

[129] Khodadadi, S.; Riazi, G.H.; Ahmadian, S.; Hoveizi, E.; Karima, O.; Aryapour, H. Effect of N-homocysteinylation on physicochemical and cytotoxic properties of amyloid β -peptide. *FEBS Lett.,* **2012**, *586*(2), 127-131.
http://dx.doi.org/10.1016/j.febslet.2011.12.018 PMID: 22200570

[130] Karima, O.; Riazi, G.; Khodadadi, S.; Aryapour, H.; Khalili, M.A.; Yousefi, L.; Moosavi-Movahedi, A.A. Altered tubulin assembly dynamics with N-homocysteinylated human 4R/1N tau *in vitro*. *FEBS Lett.,* **2012**, *586*(21), 3914-3919.
http://dx.doi.org/10.1016/j.febslet.2012.09.024 PMID: 23041345

[131] Olas, B.; Kontek, B. The possible role of hydrogen sulfide as a modulator of hemostatic parameters of plasma. *Chem. Biol. Interact.,* **2014**, *220*, 20-24.
http://dx.doi.org/10.1016/j.cbi.2014.06.001 PMID: 24929049

[132] Giovinazzo, D.; Bursac, B.; Sbodio, J.I.; Nalluru, S.; Vignane, T.; Snowman, A.M.; Albacarys, L.M.; Sedlak, T.W.; Torregrossa, R.; Whiteman, M.; Filipovic, M.R.; Snyder, S.H.; Paul, B.D. Hydrogen sulfide is neuroprotective in Alzheimer's disease by sulfhydrating GSK3β and inhibiting Tau hyperphosphorylation. *Proc. Natl. Acad. Sci. USA,* **2021**, *118*(4), e2017225118.
http://dx.doi.org/10.1073/pnas.2017225118 PMID: 33431651

[133] Ji, D.; Luo, C.; Liu, J.; Cao, Y.; Wu, J.; Yan, W.; Xue, K.; Chai, J.; Zhu, X.; Wu, Y.; Liu, H.; Wang, W. Insufficient S-sulfhydration of methylenetetrahydrofolate reductase contribute to the progress of hyperhomocysteinemia. *Antioxid Redox Signal,* **2021**, *36*(1-3), 1-14.
http://dx.doi.org/10.1089/ars.2021.0029 PMID: 34409847

[134] Kamat, P.K.; Kyles, P.; Kalani, A.; Tyagi, N. Hydrogen sulfide ameliorates homocysteine-induced Alzheimer's disease-like pathology, blood–brain barrier disruption, and synaptic disorder. *Mol. Neurobiol.,* **2016**, *53*(4), 2451-2467.
http://dx.doi.org/10.1007/s12035-015-9212-4 PMID: 26019015

[135] Stroylova, Y.Y.; Semenyuk, P.I.; Asriyantz, R.A.; Gaillard, C.; Haertlé, T.; Muronetz, V.I. Creation of catalytically active particles from enzymes crosslinked with a natural bifunctional agent--homocysteine thiolactone. *Biopolymers,* **2014**, *101*(9), 975-984.
http://dx.doi.org/10.1002/bip.22514 PMID: 24912753

[136] Mir, S.; Sen, T.; Sen, N. Cytokine-induced GAPDH sulfhydration affects PSD95 degradation and memory. *Mol. Cell,* **2014**, *56*(6), 786-795.
http://dx.doi.org/10.1016/j.molcel.2014.10.019 PMID: 25435139