

Nitric oxide-mediated S-Nitrosylation contributes to signaling transduction in human physiological and pathological status (Review)

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Received April 16, 2025; Accepted July 10, 2025

DOI: 10.3892/ijmm.2025.5593

Abstract. In the complex development of various diseases, nitric oxide-mediated S-nitrosylation is increasingly recognized for its distinct regulatory function. Recent research has advanced our knowledge of how this nitric oxide-dependent modification is dynamically controlled under both physiological and pathological conditions. S-nitrosylation plays a key role in regulating mitochondrial function, gene transcription, cellular homeostasis and metabolism and it is also involved in the pathogenesis of cardiovascular disorders, neurological conditions and cancer. The present review outlined the signaling pathways driven by nitric oxide and describes the formation, specificity and factors that influence S-nitrosylation levels. It also compared the strengths and limitations of different detection methods for S-nitrosylation reactions. The

present review discussed the cellular regulatory mechanisms affected by S-nitrosylation to clarify how certain major diseases are connected to specific S-nitrosylated proteins. These insights may guide the development of targeted repair strategies for malfunctioning proteins by focusing on defined S-nitrosylation sites, offering theoretical support for disease intervention and treatment.

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Abbreviations: AD, Alzheimer's disease; AGT, angiotensinogen; ANTI, adenine nucleotide translocator 1; ATP, adenosine triphosphate; cGMP, cyclic guanosine monophosphate; CTSB, recombinant cathepsin B; DD, diastolic dysfunction; Drp1, dynamin-related protein 1; ED, endothelial dysfunction; GSNOR, S-Nitrosoglutathione Reductase; IR, insulin resistance; NF- κ B, nuclear factor kappa-B; NO, nitric oxide; NOS, nitric oxide synthase; NSCLC, non-small cell carcinoma; PD, Parkinson's disease; PDAC, pancreatic ductal adenocarcinoma; PTM, post-translational modification; SCAN, S-nitro-coenzyme A-assisted nitrotransferase; SNO, S-nitrosothiol; TRX, thioredoxin reductase

Key words: post-translational modification, nitric oxide, S-nitrosylation, S-nitrosothiols, biotin switch assay

1. Introduction

Protein post-translational modifications (PTMs) increase the functional diversity of the proteome by controlling protein activity, localization, expression and interactions with other cellular components through the covalent addition or removal of chemical groups at amino acid residues. These changes ultimately influence cellular phenotype and biogenesis (1). At present, nearly all proteins are subject to some form of PTM, which markedly expands the range of proteome functions (2).

Protein S-nitrosylation refers to the oxidative modification of cysteine residues by nitric oxide (NO), leading to the formation of protein S-nitrosothiols (SNOs). This modification mediates redox-dependent signal transduction and reflects how NO regulates various cellular processes (3). As a widespread PTM, S-nitrosylation transmits the effects of NO throughout

the cell and is involved in multiple biological activities. Under physiological conditions, S-nitrosylation governs protein structure, stability, subcellular distribution and protein-protein interactions, allowing NO to function beyond the classical cyclic guanosine monophosphate (cGMP)-dependent signaling pathway (4). Under pathological conditions, S-nitrosylation targets diverse substrates, including transcription factors, enzymes, receptors and signaling molecules, in an effort to restore disrupted cellular balance. An increasing body of evidence suggests that abnormal NO levels can interfere with the regulation of S-nitrosylation, ultimately affecting the physiological and pathological processes central to life (2).

The first author conducted a thorough literature search in databases such as PubMed, Web of Science and ScienceDirect using the terms ‘post-translational modification’, ‘nitric oxide’, ‘S-nitrosylation’, ‘S-nitrosothiols’ and ‘biotin switch assay’ to explore the association between S-nitrosylation and disease. Articles were initially screened by reviewing titles and abstracts and 119 relevant publications were selected for detailed examination.

2. NO generation and signaling

NO synthase. NO is generated through the oxidation of L-arginine by NO synthase (NOS) and is involved in key physiological functions, including vasodilatation, platelet aggregation, immune defense, cell proliferation and mitochondrial respiration (5-7). As NO is a gas with a short half-life in the body, its activity is concentration-dependent, often confining its action to local target sites (7). Identifying NOS-specific inhibitors can contribute to understanding NO-driven signaling pathways and may provide a basis for developing therapies for conditions linked to abnormal NO levels.

In mammals, NOS exists in three major isoforms: inducible (iNOS/NOS2), neural (nNOS/NOS1) and endothelial (eNOS/NOS3), each showing distinct expression patterns and biological functions across various tissues and cell types (8,9). Under resting conditions, iNOS is generally not expressed. Its expression is triggered by stimuli such as inflammatory mediators, endotoxins and hypoxic environments (9). Pro-inflammatory cytokines including tumor necrosis factor- α , interleukin-1, interferon- γ and lipopolysaccharide bind to membrane receptors, activating intracellular kinases that phosphorylate target proteins and initiate specific transcription factors. These transcription factors then translocate into the nucleus, bind promoter sequences and induce iNOS gene expression, leading to sustained, high-output NO production (10,11).

By contrast, nNOS and eNOS are constitutively expressed and their enzymatic activities are fine-tuned by phosphorylation, S-nitrosylation, protein-protein interactions and calcium signaling (9). eNOS is primarily located in endothelial cells and is the predominant isoform involved in regulating vascular tone and function (12). NO derived from endothelial cells inhibits platelet aggregation, prevents thrombus formation and regulates the expression of genes associated with atherosclerosis (AS), thereby blocking leukocyte adhesion and migration to the endothelium, which helps prevent AS progression (13,14). nNOS is mainly found in neurons and is regulated by Ca^{2+}

and calmodulin, playing essential roles in learning, memory and neurogenesis (15). Under physiological conditions, nNOS rapidly produces small amounts of NO to maintain internal stability and contribute to neuroprotection (16).

NO-mediated signaling. NO signaling functions through both classical and non-classical mechanisms to regulate diverse cellular activities (17). In the classical pathway, NO binds to the heme moiety of soluble guanylate cyclase, facilitating the enzymatic conversion of guanosine triphosphate into cGMP (18). Acting as a second messenger, cGMP activates cGMP-dependent protein kinase G, which in turn regulates downstream targets, including cGMP-regulated kinases, phosphodiesterases and ion channels, thereby regulating mitochondrial dynamics and biogenesis.

By contrast, the non-classical signaling pathway involves the covalent post-translational modification of biomolecules by NO and its reactive derivatives. This mode of action, primarily through S-nitrosylation, plays a regulatory role across various tissues and organ systems (19,20). S-nitrosylation has emerged as a critical form of redox-based signaling that enables cells to manage oxidative stress and limit reactive oxygen species levels (21,22). Importantly, S-nitrosylation is a selective and reversible modification encompassing nitrosylation, transnitrosylation and denitrosylation processes (23). Two principal classes of denitrosylating enzymes have been identified: The Trx system and low-molecular-weight SNO-cleaving enzymes, which include S-nitrosoglutathione reductase (GSNOR) and S-nitroso-coenzyme A reductase (24). Enzymes such as GSNOR and thioredoxin reductase (TrxR) catalyze the denitrosylation of SNOs, restoring free sulfhydryl groups. In parallel, SNOs can donate NO groups to the cysteine thiols of target proteins, a process facilitated by transnitrosylases (Fig. 1). Recent findings indicate that NO-driven S-nitrosylation markedly affects essential cellular processes including transcription, DNA repair, cell growth, differentiation and apoptosis (17,25).

3. Protein S-nitrosylation

The formation of protein S-nitrosylation. In 1992, Stamler *et al* (26) first identified SNO, a product formed when NO reacts with bovine serum albumin. Protein S-nitrosylation, a covalent modification involving the attachment of NO to reactive cysteine residues, has since been recognized as a key mechanism by which NO mediates its biological functions (27). As NO itself is not an oxidant and does not efficiently interact with protein thiols directly (17), most S-nitrosylation events proceed through an intermediate oxidation process. Initially, NO reacts with O_2 to form NO_2 , which then combines with another NO molecule to produce N_2O_3 , a more reactive intermediate that reacts with protein thiols to yield nitrosothiols (28). Additionally, NO_2 can oxidize thiols to generate thiol radicals, which subsequently react with NO to form nitrosothiols (17). Other pathways also exist. During the auto-nitrosylation of hemoglobin or in the formation of GSNO by cytochrome *c*, NO is oxidized by a transition metal such as Fe^{3+} or Cu^{2+} within a metalloenzyme, forming a nitrosonium ion (18). This positively charged intermediate then reacts with a nearby thiol group close to the catalytic center, producing a stable nitrosothiol (18) (Fig. 2).

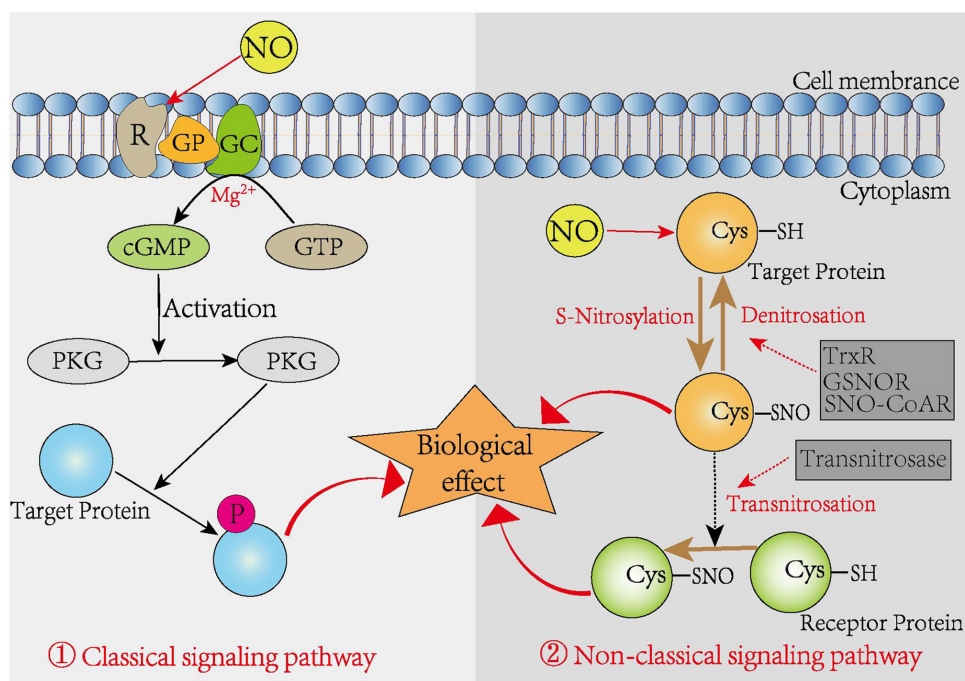


Figure 1. NO-mediated 'classical' and 'non-classical' signaling pathways. (1) Classical signaling pathway: NO binds to a specific R on the cell membrane and activates the GC system. In the presence of Mg^{2+} , GC converts GTP to cGMP. cGMP activates inactive PKG, which influences effector proteins to catalyze the phosphorylation of various intracellular proteins, enabling the target cell to exert biological effects. (2) Non-classical signaling pathway: NO interacts with specific cysteine sulfhydryl groups on target proteins to form SNOs, which exert biological functions. SNOs can be denitrosylated and reduced to free sulfhydryl groups under TrxR and GSNOR. Additionally, SNOs can transfer S-nitroso to cysteine sulfhydryl groups of receptor proteins through Transnitrosase to exert biological effects. NO, nitric oxide; R, receptor; GC, guanylate cyclase; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; SNOs, S-nitrosothiols; TrxR, thioredoxin reductase; GSNOR, S-nitrosogluthathione reductase.

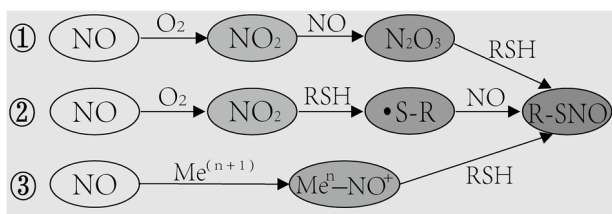


Figure 2. Three S-nitrosylation reactions. (1) NO reacts with O_2 to form a series of nitrogen oxides. Subsequently, N_2O_3 reacts with a protein thiol to produce nitrite and a nitrosothiol. (2) NO reacts with O_2 to form NO_2 , which reacts with a thiol to produce a thiol radical and nitrite. Then, NO reacts with a thiol radical to form a nitrosothiol. (3) NO is oxidized by the transition metal (such as Fe^{3+} or Cu^{2+}) to yield nitrosonium. Nitrosonium then reacts with a thiol near the catalytic center to form a nitrosothiol. NO, nitric oxide; RSH, a protein thiol; R-SNO, a nitrosothiol.

Specificity of the S-nitrosylation reaction. S-nitrosylation does not occur randomly across all cysteine residues; it is selective and occurs only on specific thiols within proteins (29). The target cysteine typically needs to be spatially close to the NO source for direct recognition by NOS. It is often located in a hydrophobic environment shaped by the tertiary protein structure or membranes, which avoids steric hindrance and increases reaction probability (29,30). Moreover, certain motifs, such as I/L-X-C-X2-D/E, are preferentially targeted by NOS. These motifs often lie within α -helices that present a broad surface area, making them more accessible for S-nitrosylation (31). Proteomic analyses have revealed that the likelihood of S-nitrosylation is determined not only by the surrounding amino acid sequence but also by the pKa

of the cysteine thiol, the local pH, the electrophilic nature of nitrosothiols, NOS enzymatic activity and the concentration of external NO (22,27,32-34).

Regulation of S-nitrosylation levels. Intracellular SNO levels are shaped not only by the cellular redox environment but also by the action of specific nitrosylases (35,36). Among the most abundant endogenous SNOs, GSNO acts as a key NO donor for protein modification *in vivo* (37). GSNO is metabolized by GSNOR, a member of the alcohol dehydrogenase family, which catalyzes its breakdown into oxidized glutathione and ammonia (38). In addition to GSNOR, the Trx enzyme family, primarily located in the cytoplasm, forms a central part of the denitrosylation system (39). Unlike the GSNOR system, which specifically targets GSNO, TrxR enables denitrosylation across a broader range of nitrosylated proteins. Changes in the expression or function of reductases such as GSNOR and TrxR can disturb S-nitrosylation dynamics and contribute to disease development (4,40). S-nitrosylation is also regulated through transnitrosylation, a process in which the NO group is transferred from low molecular weight nitrosothiols such as GSNO to cysteine thiols on target proteins (41). GSNO can transfer its S-nitroso group to various substrates, affecting cellular physiology or contributing to disease progression through protein S-nitrosylation (17,41). Together, nitrosylation, denitrosylation and transnitrosylation work in concert to maintain SNO homeostasis *in vivo*, playing essential roles in normal physiology. Disruptions in these processes can lead to imbalances in SNO levels, resulting in functional disturbances.

4. S-nitrosylation detection method

As interest in protein S-nitrosylation continues to grow, understanding its physiological functions has become a major research focus. Although significant advances have been made in exploring the role of S-nitrosylation in health and disease, detecting protein's SNO levels within complex biological systems remains difficult due to their low abundance and instability under physiological conditions. The present review outlined the main techniques currently available for identifying and analyzing protein S-nitrosylation (Table I). Common approaches include iodide/copper-based reduction methods and immunohistochemistry (42,43). However, the S-NO bond in nitrosylated proteins is inherently unstable and prone to breakdown under reducing agents or UV exposure, complicating direct detection efforts (43).

Liu *et al* (44) proposed a label-free detection strategy using resonance Raman spectroscopy of ferrous cytochrome *c*. In this method, light releases NO from S-nitrosylated proteins, which then reacts with the heme center of ferrous cytochrome *c*. Shifts in the resonance Raman spectra allow for quantitative measurement of S-nitrosylated protein levels.

Currently, the biotinylation-based method is the most widely applied technique for detecting protein S-nitrosylation. In 2001, Jaffrey *et al* (45) introduced a protocol in which biotin is covalently linked to the sulfhydryl groups of nitrosylated proteins, stabilizing otherwise labile nitroso groups. This method permits reliable analysis of S-nitrosylated protein expression via relative quantification of biotin-labeled proteins. It also allows detection of S-nitrosylation at specific thiol sites within protein mixtures. The procedure involves three main steps: Alkylation to block free thiols, selective reduction of nitrosothiols to free sulfhydryls and labeling of these with biotin. Biotinylated proteins are then purified and analyzed to reflect the S-nitrosylation status of target cysteine residues. Later, Forrester *et al* (46) showed that the biotin switch assay had become a widely used and foundational tool for identifying S-nitrosylated proteins in complex samples. They also addressed potential technical limitations of the method and proposed updated versions, highlighting directions for further development in the expanding field of S-nitrosylation research.

With the development of molecular and cellular biology techniques, refined protocols such as the idoTMT transformation method (47), the Cys-Boost technique (48) and surface plasmon resonance (49) have improved both the sensitivity and precision of protein nitrosylation detection. Direct identification of protein S-nitrosylation through mass spectrometry has made high-throughput analysis possible, allowing for the detection of previously unknown post-translational modifications, pinpointing modification sites and quantifying expression or modification levels at the proteome scale (48). Qin *et al* (50) introduced a fluorine affinity tag-switch chemical proteomics approach to enrich and detect mercapto-nitrosylated peptide fragments. This technique enabled accurate quantitative analysis of mercapto-nitrosylated groups in plants and revealed their key involvement in regulating endoplasmic reticulum stress. However, the instability of the S-NO bond on cysteine poses challenges for mass spectrometry. Accurate identification of S-nitrosylation sites requires careful experimental planning and targeted analysis to distinguish these

modifications from other thiol-based changes, minimizing the risk of both false positives and false negatives (51). Standardized operating procedures, along with strict quality control systems, are necessary to maintain consistency and reliability across different sample batches.

5. Regulatory functions of S-nitrosylation in cellular life activities

S-nitrosylation plays an essential role in both physiological and pathological conditions (9). S-nitrosylated proteins are found throughout the cell, including in membrane domains, the cytosol, mitochondria, nucleus and extracellular matrix, where they affect numerous signaling pathways. These include synaptic transmission, transcription factor regulation, mitochondrial dynamics and receptor or ion channel activity, all of which help maintain tissue homeostasis (42). Moreover, nitroso groups can be regulated through transnitrosylation or secretion, influencing broader tissue-level processes. The sections below outline the regulatory roles of S-nitrosylation in three fundamental cellular activities (Fig. 3).

Regulating mitochondrial function. Mitochondria, central to cell growth and cycle regulation, function not only as the primary source of cellular ATP but also participate in signal transduction, differentiation and apoptosis. Murray *et al* (43) identified several proteins modified by S-nitrosylation in rat heart tissue following co-incubation with the NO donor GSNO. These proteins were mainly involved in mitochondrial quality control, energy metabolism and mitochondria-mediated apoptosis. This suggests that S-nitrosylation-mediated control of mitochondrial biogenesis and dynamics may offer a potential strategy for addressing diseases linked to mitochondrial dysfunction (52,53). In plant mitochondria, protein S-nitrosylation has been shown to participate in multiple metabolic pathways, including photorespiration (54), the tricarboxylic acid cycle (55-67), oxidative phosphorylation (54,58), protein processing and turnover (59) and other core metabolic processes (60). Beyond metabolic regulation, S-nitrosylation also influences mitochondrial autophagy. Parkin, a ubiquitin E3 ligase of the RING-between-RING (RBR) family, is a key player in mitochondrial quality control. Ozawa *et al* (61) reported that S-nitrosylation levels of Parkin increased markedly in GSNO-treated HeLa cells, which increased Parkin's E3 ligase activity and promoted mitochondrial degradation. In addition to its role in regulating mitochondrial pathways, Tang *et al* (38) demonstrated that mitochondrial GSNOR is critical for maintaining mitochondrial homeostasis. Through the denitrosylation of ANT1, GSNOR contributes to preserving mitochondrial integrity and improving mitochondrial performance.

Regulating transcription factors. S-nitrosylation not only affects mitochondrial function but also modifies key transcription factors and their associated regulatory proteins, including NF- κ B, p53, hypoxia-inducible factor (HIF) and nuclear respiratory factor 2 (52,62-64). NF- κ B is a major transcriptional regulator involved in immune and inflammatory responses, development, cell proliferation and apoptosis. It activates transcription by binding specific DNA sequences within

Table 1. Different methods for detection and characterization of protein S-nitrosylation have respective advantages and drawbacks.

First author, year	Detection method	Key points	Advantages	Drawbacks	(Refs)
Marley <i>et al</i> , 2000	Iodide/copper reduction.	NO release, indirect detection by chemiluminescence.	Simple operation and high sensitivity	High inter-individual variability and poor reproducibility.	(107)
Gow <i>et al</i> , 2002	Immunohistochemistry.	Antibody specifically recognizing-SNO.	Evidence for active coupling of protein S-nitrosylation to each major form of NOS.	The antibody dependence is strong and the quantitative ability is weak.	(108)
Liu <i>et al</i> , 2020	Resonance Raman spectroscopy.	Light induces the release of NO, which undergoes oxidation with heme in ferrous cytosolic heme c.	Rapid and sensitive quantitative detection.	The substrate stability is poor and the applicability of actual samples is low.	(44)
Jaffrey <i>et al</i> , 2001	Biotin switch assay.	Labeling of biotin on sulfhydryl groups of proteins undergoing nitrosylation.	Conversion of unstable nitroso to stable biotinylation for improved sensitivity.	Poor stability, insufficient specificity and cumbersome steps.	(45)
Qu <i>et al</i> , 2014	IdoTMT transformation method.	Irreversible labeling of SNO-Cys using idoTMTsixplex reagent for immunoaffinity detection and enrichment of iodine TMT-labeled peptides by anti-TMT resin.	Quantification of S-nitrosylation in complex samples; identification of potential therapeutic targets for disease.	Enrichment based on anti-TMT antibodies had low specificity and sensitivity.	(47)
Ruzanna <i>et al</i> , 2019	Cys-Boost technology.	Efficient LC-MS-based quantitative analysis of Cys PTM based on bioorthogonal cleavable junction enrichment and conversion technology combined with TMT labeling.	High sensitivity, specificity and precision.	Off-target effect, high cost and complex process.	(48)
Zhang <i>et al</i> , 2018	Surface Plasmon Resonance Technology.	Obtaining specific signals of biomolecular interactions through dynamic changes in SPR angles during biological reactions.	Real-time analysis, simple and fast, dynamic monitoring of the whole process of biomolecular interactions in the interaction.	Poor sensitivity, resolution and stability.	(49)
Qin <i>et al</i> , 2023	Fluorine affinity labeling replacement method.	Fluoro-affinity tag-switching chemical proteomics approach for the enrichment.	High sensitivity and peptide identification efficiency.	Lack of commercial antibodies recognizing the F-tag.	(50)
Forrester <i>et al</i> , 2009	Resin assisted-capture of SNO-proteins.	Substitutes thiopropyl sepharose for biotin-avidin.	Fewer steps, more efficient detection.	Detection sensitivity and efficiency dependent on mass spectrometry technology.	(109)
Seneviratne <i>et al</i> , 2016	SNO trapping by triaryl phosphine.	Using phosphine-based chemical probes.	Direct tagging.	Limited ionization suppression.	(110)

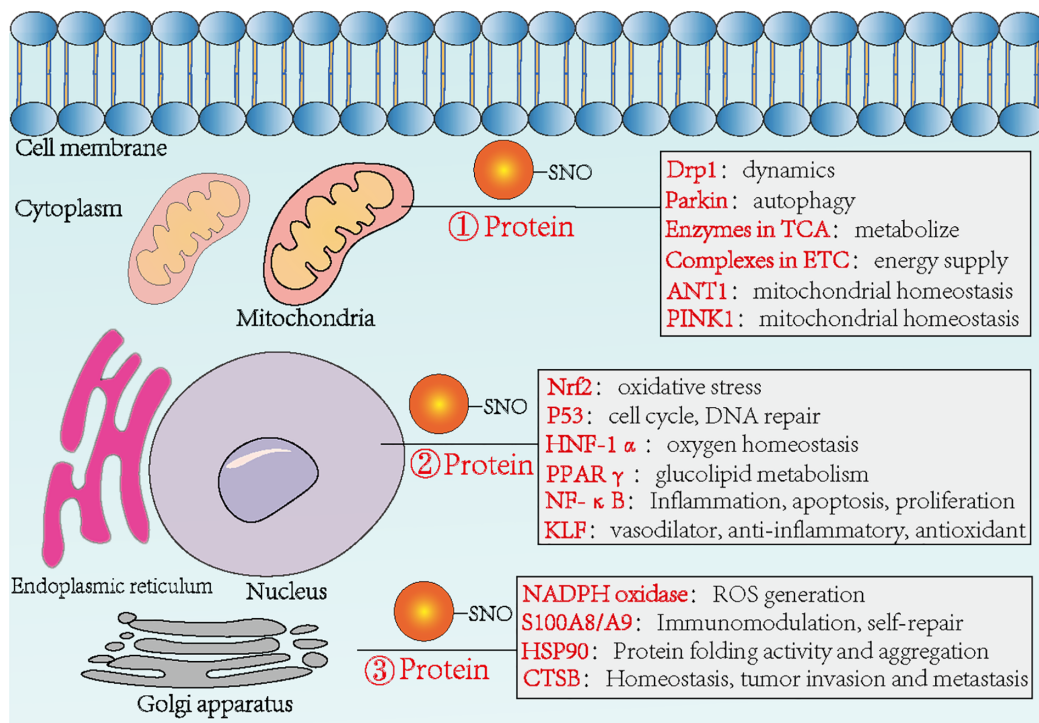


Figure 3. Regulation of nitrosylated proteins in cellular activities. (1) Proteins regulating mitochondrial function: Drp1, Parkin, enzymes in the tricarboxylic acid cycle, complexes I, II, III and IV in the electron transport chain, ANT1, PINK1. (2) Proteins regulating gene transcription: Nrf1, P53, HNF-1 α , PPAR γ , NF- κ B, KLF. (3) Proteins regulating homeostasis: NADPH oxidase, S100A8/A9, HSP90, CTSB. Drp1, Dynamin-related protein 1; ANT1, adenine nucleotide translocator 1; PINK1, PTEN-induced putative kinase 1; Nrf1, Nuclear respiratory factor 1; HNF-1 α , Hepatic Nuclear factor 1 α ; PPAR γ , Peroxisome proliferator-activated receptor γ ; NF- κ B, Nuclear factor kappa-B; KLF, Kruppel-like factor; NADPH, Nicotinamide adenine dinucleotide phosphate; HSP90, Heat shock protein 90; CTSB, Cathepsin B.

promoter regions. S-nitrosylation of the NF- κ B p50 subunit at Cys62 and the p65 subunit at Cys38 impairs this DNA-binding ability, thereby reducing transcriptional activity (65,66). Matthews *et al* (62) showed that in A549 human lung cancer cells, treatment with the NO donor GSNO led to S-nitrosylation of NF- κ B, which suppressed its transcriptional activity and increased the cells' sensitivity to tumor necrosis factor- α -induced apoptosis. Additionally, under excitotoxic conditions triggered by glutamate, eNOS caused S-nitrosylation of the NF- κ B p65 subunit. This modification decreased NF- κ B activity and offered neuroprotection, improving neuronal survival (67).

S-nitrosylation also influences p53. Specifically, modification at Cys124 within its DNA-binding domain increases the regulation of downstream targets such as superoxide dismutase and catalytic subunits, helping reduce oxidative stress (50). Beyond NF- κ B and p53, S-nitrosylation of HIF-1 α stabilizes the protein, increasing its protective function in hypoxic environments (68).

Regulating protein homeostasis. S-nitrosylation, as part of redox-based signaling, influences protein function by affecting enzyme activity, subcellular localization and protein-protein interactions. Under conditions of proliferative stress, hematopoietic stem cells show increased levels of NO and SNO, which are associated with the accumulation of misfolded or aggregated proteins. Yi *et al* (69) identified a mechanistic connection between GSNOR-regulated protein S-nitrosylation and the CHOP-mediated unfolded protein response, which plays a role in maintaining hematopoietic stem cell activity.

Their findings suggest that adjusting S-nitrosylation levels could support the expansion of functional hematopoietic stem cells without compromising viability. Lin *et al* (70) reported that S-nitrosylated recombinant cathepsin B (CTSB) regulates its mRNA reprogramming through the ADD1/MATR3/ADAR1 signaling pathway, affecting the steady-state expression of CTSB. S-nitrosylation may either promote or suppress protein activity depending on the cellular context, timing and location (71). This highlights the need to evaluate how SNO-modified proteins influence downstream signaling processes under specific conditions.

6. Regulatory functions of S-nitrosylation in human diseases

S-nitrosylation has emerged as a central mechanism for transmitting NO signals within cells and tissues, functioning similarly to phosphorylation (4). Unlike irreversible post-translational modifications that permanently alter protein regulation, S-nitrosylation of cysteine residues is reversible, enabling dynamic control over numerous biological processes in both healthy and diseased states (72). Under normal physiological conditions, protein S-nitrosylation is tightly controlled to preserve redox balance (73,74). However, disrupted regulation of S-nitrosylation in pathological settings can affect key proteins, contributing to the development of diseases such as AS, Parkinson's disease (PD) and cancer. These changes may represent promising targets for therapeutic intervention (Fig. 4).

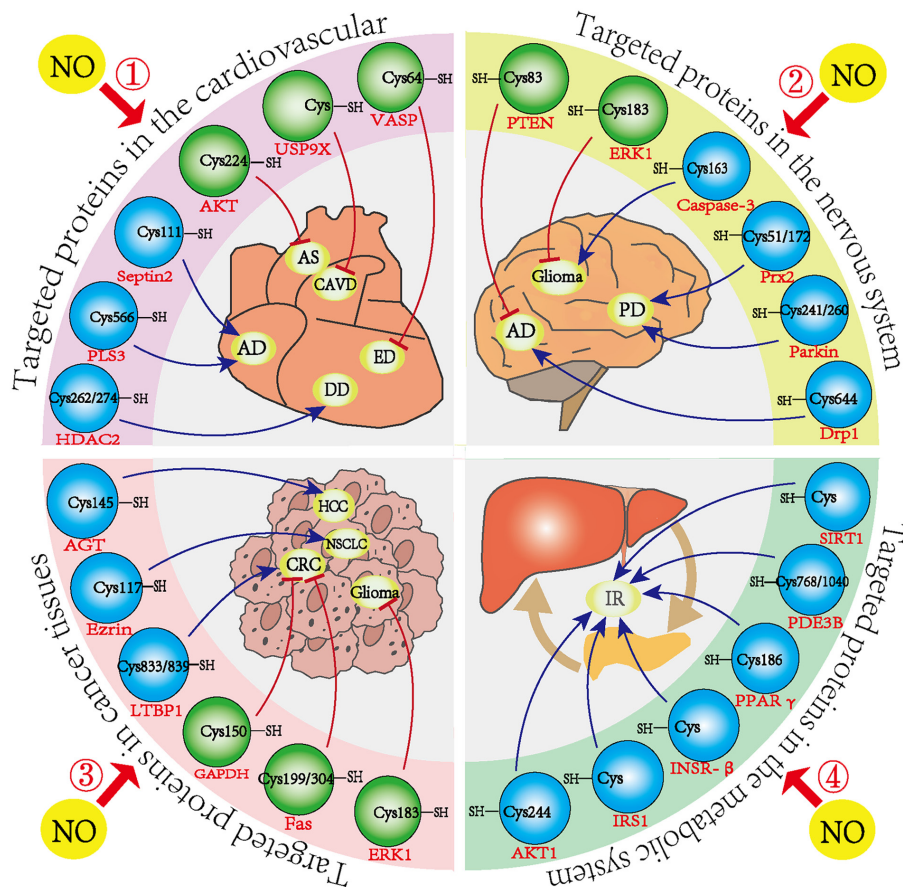


Figure 4. Regulatory functions of S-nitrosylation in human diseases. (1) In the cardiovascular system, NO affects specific cysteine sites of Plastin 3, Septin2 and PLS3 target proteins, leading to the development of AD and DD. Conversely, the action of NO on cysteine sites of USP9X, AKT and VASP target proteins can mitigate cardiovascular disorders such as AS and ED. (2) In the nervous system, the interaction of NO with cysteine sites of Caspase-3, Parkin, Prx2 and Drp1 target proteins leads to the onset of glioma, PD and AD. However, the action of NO on cysteine sites of ERK1 and PTEN helps alleviate neurological disorders associated with glioma and PD. (3) In tumor tissues, the interaction of NO with specific cysteine sites of LTBP1, RAB3GAP1, AGT and Ezrin target proteins induces CRC, PDAC, HCC and NSCLC, while the effect of NO on cysteine sites of Fas, GAPDH and ERK1 promotes apoptosis of cancer cells. (4) In metabolic diseases, S-nitrosylation of INSR- β , IRS1 and AKT1 disrupts insulin signaling; S-nitrosylation of PPAR γ and PDE3B impairs adipocyte function, leading to IR in adipocytes; and S-nitrosylation of SIRT1 sustains an inflammatory state, enhancing IR. PLS3, Plastin 3; AD, Alzheimer's disease; DD, diastolic dysfunction; USP9X, ubiquitin specific peptidase 9; AKT, protein kinase B; VASP, vasodilator-stimulated phosphoprotein; AS, atherosclerosis; ED, endothelial dysfunction; Prx2, peroxiredoxin 2; Drp1, Dynamin-related protein 1; PD, Parkinson's disease; LTBP1, latent transforming growth factor beta binding protein 1; RAB3GAP1, rab3 gtpase activating protein catalytic subunit 1; AGT, angiotensinogen; CRC, colon cancer; PDAC, pancreatic ductal adenocarcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell carcinoma; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ERK1, extracellular signal-regulated kinase 1; INSR- β , Insulin Receptor- β ; IRS1, insulin receptor substrate 1; AKT1, RAC-alpha serine/threonine-protein kinase; PPAR γ , peroxisome proliferator-activated receptor γ ; PDE3B, phosphodiesterase 3B; SIRT1, silent information regulator 1; IR, insulin resistance.

Cardiovascular system. In the complex development of cardiovascular diseases, protein S-nitrosylation is gaining recognition for its distinct role in regulating inflammation, apoptosis, angiogenesis, myocardial contractility and electrophysiological signaling transduction (Table II). Yoon *et al* (75) showed that increased nNOS activity in hearts with diastolic dysfunction (DD) raised NO levels *in vivo*, leading to S-nitrosylation of histone deacetylase 2 and worsening clinical symptoms in patients with DD. This study identified protein S-nitrosylation as a contributing factor in DD pathogenesis and proposed it as a potential therapeutic target for treatment-resistant heart failure.

As mentioned earlier, mitochondrial GSNOR is critical for maintaining mitochondrial homeostasis via denitrosylation of ANT1. A decline in mitochondrial GSNOR expression during heart failure increases S-nitrosylation of ANT1 at Cys160. In line with this, overexpression of either mitochondrial GSNOR or a non-nitrosylated mutant, ANT1

Cys160A, markedly improved mitochondrial function, offering a new potential target for heart failure therapy (38). Aortic aneurysm and coarctation, life-threatening conditions that can lead to aortic dissection and sudden death, have also been associated with increased SNO levels. Aortic tissues from patients with coarctation showed elevated S-nitrosylation compared to healthy controls (76). Pan *et al* (77) identified Plastin 3, modified at Cys566, as a key protein affected by S-nitrosylation in this disease. Additionally, Zhang *et al* (78) found that the S-nitrosylation level of Septin2 was markedly increased in diseased vascular tissues and peripheral blood mononuclear cells of patients with aortic coarctation. This modification altered the TIAM1/RAC1/NF- κ B signaling cascade, contributing to macrophage-driven vascular inflammation and aortic pathology. Two compounds, NSC23766 and R-ketoglutarate, targeting this pathway, were found effective in preventing and treating the condition.

Table II. S-nitrosylation targeted proteins and SNO sites for common diseases in cardiovascular system.

First author, year	Target protein	Expanded name	SNO site	Type of disease	(Refs)
Liu, 2017	ANT1	Adenine nucleotide translocator 1	Cys160	Myocardial hypertrophy	(111)
Zamorano <i>et al</i> , 2017	VASP	Vasodilator-stimulated phosphoprotein	Cys64	ED	(112)
Li <i>et al</i> , 2018	AKT	Protein Kinase B	Cys224	Atherosclerosis	(113)
Pan <i>et al</i> , 2020	PLS3	Plastin 3	Cys566	Aortic dissection	(77)
Majumdar <i>et al</i> , 2021	USP9X	Ubiquitin specific peptidase 9 X-linked	Not mentioned	Calcified aortic valve	(114)
Yoon <i>et al</i> , 2021	HDAC2	Histone deacetylase 2	Cys262/Cys274	Diastolic dysfunction	(75)
Chao <i>et al</i> , 2021	GNAI2	G protein subunit alpha I2	Cys66	Atherosclerosis	(115)
Tang <i>et al</i> , 2023	ANT1	Adenine nucleotide translocator 1	Cys160	Heart failure	(38)
Zhang <i>et al</i> , 2024	Septin2	-	Cys111	Aortic aneurysm/clamping	(78)

SNO, S-nitrosothiol; ED, endothelial dysfunction.

In conclusion, the levels of a number of protein SNOs are upregulated in various cardiovascular diseases. De-nitrosylation can play a role in protecting the heart and alleviating vascular damage. The research on SNO modification in cardiovascular physiology and pathology has made certain progress, suggesting that S-nitrosylation of proteins plays an important role in regulating cardiovascular diseases. These findings suggest that detailed exploration of the molecular role of S-nitrosylation in cardiovascular physiology and disease may guide new drug development and help identify novel therapeutic strategies. In addition, continuously promoting the research on the targets and mechanisms between SNO and cardiovascular diseases and integrating them with clinical treatment, will bring good news to more patients with cardiovascular diseases.

Nervous system. Studies have shown that NO is involved in a wide range of neural processes, including development, neurotransmitter release and synaptic plasticity (7). However, abnormal S-nitrosylation driven by excessive NO can contribute to neuronal damage in various neurodegenerative diseases (79) (Table III). Nakamura *et al* (29) reported that under normal conditions, nitrosylated NMDA receptors remain inactive, helping maintain physiological NO levels and supporting normal neuronal function. By contrast, under pathological conditions, overactivation of the extrasynaptic NMDA-nNOS pathway leads to excess NO production, driving the formation of abnormal nitrosylated proteins and accelerating disease progression. In Alzheimer's disease (AD), NO targets the Cys644 residue of guanosine triphosphate-bound Drp1, forming SNO-Drp1, which overactivates the protein and triggers mitochondrial fragmentation. This results in insufficient energy for neurotransmission and contributes to synaptic failure and cognitive decline (80). S-nitrosylation of Drp1 also promotes mitochondrial overactivation and fission (81,82). Wang *et al* (82) found that S-nitrosylation of Drp1 was present in all cases of sporadic AD. Inhibiting this modification prevented A β -induced mitochondrial fragmentation, synaptic dysfunction and neuronal death, suggesting that SNO-Drp1 could serve

as a potential therapeutic target for preserving neuronal and synaptic integrity in AD. Furthermore, Nakamura *et al* (83) demonstrated, using *in vitro* and *in vivo* AD models and postmortem brain samples from AD patients, that enzymes from various biochemical pathways can undergo a cascade of S-nitrosylation events, ultimately leading to synapse loss.

In PD, both animal models and human tissue studies have shown that S-nitrosylation of the parkin protein disrupts its neuroprotective function by impairing substrate ubiquitination and suppressing its ubiquitin ligase activity (84). Tsang *et al* (85) further confirmed that nitrosative stress contributes to PD progression by impairing prosurvival proteins such as parkin and X-linked inhibitor of apoptosis protein via distinct pathways, indicating that abnormal S-nitrosylation is a key factor in neurodegeneration. In glioma cells, S-nitrosylation of extracellular signal-regulated kinase 1/2 (ERK1/2) within the MAPK pathway suppresses its phosphorylation, promoting apoptosis (86). On the other hand, S-nitrosylation of caspase-3 in glioma cells inhibits apoptosis and facilitates tumor progression (87). Altogether, aberrant S-nitrosylation plays a critical role in the pathogenesis of AD, PD and gliomas.

In the clinic, neurodegenerative diseases can be prevented or treated by controlling NO production through drug delivery or the modulation of NOS activity (88). In addition, by inhibiting the excessive release of α -synuclein, which oligomerizes, aggregates and deposits in the cytoplasm, Parkin SNO can be reduced and cells can be protected from extracellular α -synuclein oligomer-induced toxicity, thus treating diseases (89). Therefore, gaining a deeper understanding of how dysregulated S-nitrosylation pathways contribute to neurological disorders will improve insight into their molecular basis and support the search for therapeutic targets.

Tumors. S-nitrosylation plays dual roles in cancer, acting as both a tumor suppressor and promoter and is involved across multiple stages of tumor progression. Abnormal S-nitrosylation patterns have been identified in various cancers, including pancreatic, lung, liver and prostate, where they influence

Table III. S-nitrosylation targeted proteins and SNO sites for common diseases in nervous system.

First author, year	Target protein	Expanded-name	SNO site	Type of disease	(Refs)
Chung <i>et al</i> , 2004	Parkin	-	Cys241/Cys260	Parkinson's	(84)
Martínez-Ruiz <i>et al</i> , 2005	Hsp90	Heat shock protein 90	Cys597	Alzheimer's	(116)
Hara <i>et al</i> , 2006	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Cys150	Parkinson's	(117)
Fang <i>et al</i> , 2007	Prx2	Peroxiredoxin 2	Cys51/Cys172	Parkinson's	(103)
Cho <i>et al</i> , 2009	Drp1	Dynamin-related protein 1	Cys644	Alzheimer's	(81)
Kwak <i>et al</i> , 2010	PTEN	Mutated in multiple advanced cancers 1	Cys83	Alzheimer's	(118)
Nakamura <i>et al</i> , 2010	XIAP	X-linked inhibitor of apoptosis protein	Cys450	Parkinson's	(119)
Abrams <i>et al</i> , 2011	ApoE3	Apolipoprotein E 3	Cys112	Alzheimer's	(120)
Qu <i>et al</i> , 2011	Cdk5	Cyclin dependent kinase 5	Cys83/Cys157	Alzheimer's	(121)
Shen <i>et al</i> , 2016	Caspase-3	CysteinyI aspartate specific proteinase	Cys163	Glioma	(87)
Jin <i>et al</i> , 2018	ERK1	Extracellular signal-regulated kinase 1	Cys183	Glioma	(86)

SNO, S-nitrosothiol.

tumor behavior in both positive and negative directions (Table IV). In pancreatic ductal adenocarcinoma (PDAC), dysregulated NO production by iNOS and eNOS contributes to tumor development and correlates with poor prognosis, although the detailed mechanisms remain unclear (90,91). Tan *et al* (92) used site-specific proteomics to identify 585 S-nitrosylation sites on 434 proteins in PDAC tissues and PANC-1 cell lines, reporting markedly more S-nitrosylated proteins in tumor samples than in adjacent normal tissue. Deng *et al* (93) demonstrated that tumor necrosis factor-related apoptosis-inducing ligand triggered apoptosis in human thyroid carcinoma FRO cells by increasing NO levels, which led to S-nitrosylation of GAPDH and its accumulation in the nucleus. In another study, increased S-nitrosylation of the parkin protein, driven by iNOS and GSNOR deficiency, was found to contribute to hepatocellular carcinoma formation in mice (94). Similarly, Wei *et al* (95) identified S-nitrosylation of O6-alkylguanine-DNA alkyltransferase (AGT) at Cys145 in both *in vivo* and *in vitro* models. This modification promoted AGT degradation through the proteasome and led to the accumulation of mutagenic alkylamines, accelerating liver tumor progression.

In the context of non-small cell carcinoma (NSCLC), Zhang *et al* (96) found that inflammatory conditions caused S-nitrosylation of the cytoskeletal regulator ezrin, promoting mechanical signaling between the cytoskeleton and membrane and increasing cell invasiveness and metastatic capacity. This provides a new perspective on NSCLC progression and potential therapeutic approaches. S-nitrosylation has also been shown to influence the function of epigenetic regulators. DNA methyltransferases (DNMTs), which control DNA methylation patterns in mammals, are key epigenetic enzymes. Okuda *et al* (97) discovered that S-nitrosylation of DNMT suppressed its enzymatic activity and promoted tumor formation. They also identified DBIC, a compound

that blocks S-nitrosylation of DNMT3B at low concentrations ($IC_{50} \leq 100$ nM), without interfering with its catalytic function. DBIC markedly reduced tumor cell proliferation and malignant transformation. Taken together, these findings show that disruptions in S-nitrosylation contribute to cancer development.

Although SNO modification accounts for a relatively low proportion of overall protein PTMs, it still plays an important role in tumor development and inhibition (98). Increasing the level of SNO modification in certain proteins by developing NO donors, inhibiting GSNOR and denitrication and enhancing the targeting of SNO modification and protein active sites with nanomaterials may provide a new approach for tumor cell therapy. Conversely, reducing the SNO levels of certain cancer-promoting proteins can promote their ubiquitination and degradation and reverse inhibit tumor growth. This can be achieved by using NO scavengers or NOS inhibitors to reduce the activity of certain pro-cancer proteins and using exogenous GSNOR to enhance cell denitrication and inhibit tumor growth. Investigating the role of S-nitrosylation in tumorigenesis can help identify potential targets for early intervention. For patients already diagnosed with cancer, targeting S-nitrosylation-modified proteins may provide new therapeutic options and help delay the onset of drug resistance.

Metabolic diseases. Diabetes mellitus is closely linked to disrupted S-nitrosylation, which affects all stages of insulin activity, from its synthesis and secretion to signaling and degradation in target tissues (99). This regulation plays an important role in insulin-related metabolic disorders. In type 2 diabetes, insulin resistance (IR) is influenced by S-nitrosylation through three key mechanisms: i) S-nitrosylation of insulin receptor- β , insulin receptor substrate 1 and AKT1 either suppresses their activity or accelerates their degradation, thereby impairing

Table IV. S-nitrosylation targeted proteins and SNO sites for multiple tumors.

First author, year	Target protein	Expanded-name	SNO site	Type of disease	(Refs)
Kornberg <i>et al</i> , 2010	SIRT1	Silent information regulator 1	Cys387/Cys390	Gastric cancer	(122)
Leon-Bollotte <i>et al</i> , 2011	Fas	Tumor necrosis factor receptor superfamily member 6	Cys199/Cys304	Colorectal cancer	(123)
Wei <i>et al</i> , 2011	AGT	Angiotensinogen	Cys145	Liver cancer	(95)
Tang <i>et al</i> , 2013	Parkin	-	Not mentioned	Liver cancer	(94)
Jin <i>et al</i> , 2018	ERK1	Extracellular signal-regulated kinase 1	Cys183	Glioma	(86)
Zhang <i>et al</i> , 2019	Ezrin	-	Cys117	Non-small cell lung cancer	(96)
Tan <i>et al</i> , 2019	RAB3GAP1	RAB3 GTPase activating protein catalytic subunit 1	Cys-858	Pancreatic ductal adenocarcinoma	(92)
Zhao <i>et al</i> , 2020	LTBP1	Latent transforming growth factor β binding protein 1	Cys833/Cys839	Colorectal cancer	(124)
Li <i>et al</i> , 2020	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Cys150	Colorectal cancer	(104)
Okuda <i>et al</i> , 2023	DNMT3B	DNA methyltransferase 3 β	Cys651	Cancer	(97)

SNO, S-nitrosothiol.

insulin signaling. ii) S-nitrosylation of PPAR γ and PDE3B reduces their activity, weakening adipocyte function and contributing to adipocyte IR. iii) S-nitrosylation of SIRT1 increases acetylation of NF- κ B p65, boosting NF- κ B's transcriptional activity, which triggers pro-inflammatory gene expression and worsens IR.

More recently, attention has turned to the role of S-nitrosylation in cellular metabolism, particularly in relation to type 2 diabetes and insulin resistance. Zhou *et al* (100) demonstrated that insulin signaling is directly tied to protein S-nitrosylation, where NO covalently binds to cysteine residues to form SNOs, serving as functional effectors of insulin action. This finding highlights the relevance of S-nitrosylation in metabolic regulation and supports its growing importance in diabetes research.

Zhou *et al* (99) identified a new enzyme, S-nitro-coenzyme A-assisted nitrotransferase (SCAN), which uses SNO-CoA as a cofactor to S-nitrosylate various target proteins. SCAN has been shown to regulate insulin signaling under normal conditions, while its dysregulation may contribute to the development of diabetes. This discovery advances the understanding of islet signaling pathways and suggests new possibilities for treating diseases such as obesity and diabetes. In addition, a number of individuals with diabetes develop neuropathy. Li *et al* (101) found that in the hippocampus of diabetic rats, defective autophagy caused by S-nitrosylation of the ATG4B protein plays a significant role in CNS neuropathy. This finding reveals a novel mechanism involved in diabetes-related CNS complications.

Carvalho-filho *et al* (102) indicate that aspirin treatment not only reduces iNOS protein levels but also S-nitrosylation of IR β , IRS-1 and Akt. These changes are associated with improved insulin resistance and signaling, suggesting a novel mechanism of insulin sensitization evoked by aspirin

treatment. These findings also suggest that inhibition of iNOS might be a major mediator of the insulin-sensitizing effects of IKK β inhibition. In conclusion, targeting S-nitrosylation presents an opportunity for novel or refined drug development that could provide a novel and more precise approach for the management of metabolic diseases.

Fig. 4 systematically summarized the aforementioned 24 studies related to 'S-nitrosylation modification and disease occurrence and development'. Among them, 16 studies have shown that NO-mediated S-nitrosylation modification of target proteins has a promoting effect on the occurrence of diseases. For example, in a study where the protein S-nitrosylation of HDAC2 plays a key role in the development of DD, NO reduction and protein denitrosylation markedly improved the mitral early wave/mitral annulus movement ratio and the effect on movement time in DD mice, but it did not markedly affect the ejection fraction (75). The results indicated that the denitrosylation of HDAC2 induced by antioxidants could alleviate DD. Furthermore, in another study to determine whether the level of SNO-Prx2 in the brains of Patients with PD has pathophysiological significance, the researchers found that the ratio of SNO-Prx2 to total Prx2 was comparable to the ratio of cell death in PD cell-based Models (103). The results indicated that there were pathophysiological-related amounts of SNO-Prx2 in the human brain of patients with PD and the production of SNO-Prx2, interfering with the normal antioxidant system, might lead to neuronal cell death. Conversely, eight studies have shown that S-nitrosylation modification of target proteins has an inhibitory effect on the occurrence of diseases. For instance, Microcystins-LR induces apoptosis of human colon cancer cells (SW480) through the SNO-GAPDH-Siah1 signaling pathway (with significant differences between the treatment group and the control group) (104). This study provides further understanding of the *in vitro* molecular

mechanism of Microcystins-LR-induced colorectal toxicity. Finally, it is worth noting that in metabolic system diseases, all six studies revealed that S-nitrosylation modification of target proteins can aggravate insulin resistance. Therefore, targeted inhibition of S-nitrosylated proteins may be an effective strategy for treating metabolic system diseases.

7. Conclusions and future perspectives

Protein S-nitrosylation, a covalent modification involving the addition of NO to reactive cysteine thiols, plays a critical role in redox-based signaling and contributes to altered signaling under pathological conditions. This modification changes protein structure and function, influencing bioactivity, stability, subcellular distribution and interactions with other proteins, thereby affecting a broad range of physiological and pathological processes (25). The cellular level of S-nitrosylation is controlled by the dynamic balance between S-nitrosylation and denitrosylation in both space and time. Disruption of this balance is linked to the development of multiple diseases.

At present, there is an ongoing debate over the dual roles of S-nitrosylation in disease, where it may act as either a protective or pathogenic factor depending on context. For instance, the same protein may be beneficial in one disease and harmful in another when modified by S-nitrosylation (105). Moreover, technical limitations in current detection methods raise concerns. The biotin switch assay, for example, may generate false positives due to the nonspecific reduction of disulfide bonds by ascorbic acid (46). There are also significant gaps in understanding, such as the inability to distinguish the functional impact of SNOs at different cysteine residues within the same protein (75). Most studies capture only static snapshots of nitrosylation, failing to reflect real-time fluctuations. The inherent instability of SNOs and the absence of reliable biomarkers further complicate clinical translation. Furthermore, the molecular reaction mechanism of denitrosylation, its role and its relationship with nitrosylation remain unclear. Meanwhile, the level of nitrosylation *in vivo* and different tissues determines the different physiological roles it plays. Improving our understanding of the protein nitrosylation network requires a comprehensive and systematic analysis using an interdisciplinary approach that integrates insights from biochemistry, molecular biology and pharmacology, aiming to provide new solutions and directions for the clinical control and treatment of various diseases caused by the imbalance of protein nitrosylation levels.

To date, no theranostic strategies have been reported for addressing dysregulated SNO in disease models. Given the challenges associated with current detection technologies, limited sensitivity, poor stability, interference issues and cumbersome protocols, future research should aim to develop a highly specific, real-time detection platform. Integrating nanomaterials, microfluidics and AI-driven analysis may provide tools capable of meeting the demands of environmental monitoring, drug safety testing and clinical diagnostics. Ye *et al* (106) developed a theranostic SNO probe, P-EHC, capable of detecting SNO level changes in cells under oxygen-glucose deprivation/reperfusion (OGD/R) conditions while simultaneously enhancing cell viability. To the best of the authors' knowledge, this is the first report of such a

concept. P-EHC serves as a powerful tool for analyzing SNO level dynamics in OGD/R cells and offers new perspectives for the treatment of ischemia-reperfusion diseases.

Due to the limitations of animal models (such as species differences and inability to simulate the dynamic changes of the microenvironment) as well as the bottlenecks in clinical sample analysis (such as poor sample stability and lack of standardized detection procedures), there are obstacles between disease models and clinical translation. Further work should also address the dynamic nature of nitrosylation, the mismatch between site-specificity and disease complexity and how these influence downstream biochemical responses. Only through cross-disciplinary and multi-scale collaboration can we uncover how S-nitrosylation initiates, drives and worsens disease processes, ultimately enabling the development of targeted, effective therapeutic interventions.

Acknowledgements

Not applicable.

Funding statement

The present study was supported by the National Natural Science Foundation of China, (grant no. 32060232); the Natural Science Foundation of Jiangxi Province, (grant no. 20212BAB206075); the Foundation of Jiangxi Provincial Key Laboratory of Tissue Engineering (grant no. 2024SSY06291); the Foundation of Technology Innovation Team of First Affiliated Hospital of Gannan Medical University (grant no. 2021CXTD-08); the Science and Technology Project of Ganzhou (grant no. 202101034530), First Affiliated Hospital of Gannan Medical University, Doctor Start-up Fund (grant no. QD076) and the Stem Cell Clinical Research Bi-Filing Project of First Affiliated Hospital of Gannan Medical University (grant no. SC-BiFR-001).

Availability of data and materials

Not applicable.

Authors' contributions

YX performed the literature search and drafted the manuscript. XW, XZ, LP, JY, YZ and NW retrieved the relevant literature. JY were major contributors to revision of the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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