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HIGHLIGHT

From *S*-nitrosation targets to drugs: A potential new paradigm in disease treatment



KEY WORDS

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Protein *S*-nitrosation (SNO), an essential posttranslational modification (PTM) elicited by nitric oxide (NO)¹, regulates a broad range of physiological/pathological processes². Recently, a study led by Jonathan Stamler³ published in *Cell* explores the discovery and characterization of an enzyme that selectively *S*-nitrosylates proteins to regulate insulin signaling. This enzyme, termed *S*-nitroso-CoA-assisted nitrosylase (SCAN), catalyzes SNO modification of downstream proteins using *S*-nitroso-CoA (SNO-CoA) as its cofactor. The study provides insights into the mechanistic principles of SCAN and establishes an enzymatic basis for SNO with physiological relevance in insulin signaling. This challenges existing paradigms and sets the stage for further research into the roles of SCAN in metabolic diseases such as diabetes.

The sGC–cGMP pathway-dependent regulation of vascular smooth muscle is one of the most well-known physiological roles of NO. In recent years, the SNO modification of disease-related proteins has garnered increasing attention, as it has been demonstrated to be a crucial mechanism through which NO exerts its diverse physiological effects, including the regulation of protein–biomacromolecule interactions, autophagy, and apoptosis². Traditionally, protein SNO has been considered a non-enzymatic process driven by NO and low molecular weight

S-nitrosothiols such as *S*-nitrosoglutathione, *S*-nitrosocysteine, and SNO-CoA. Zhou et al.³ purified eight SNO-CoA-binding proteins by using SNO-CoA resin, including SCAN. SCAN was identified based on its high affinity for SNO-CoA compared to other SNO-CoA-conjugates. They discovered that SCAN binds to SNO-CoA and target proteins independently, and selectively catalyzes the transfer of NO groups from SNO-CoA to specific target proteins, such as the insulin receptor (INSR) and insulin receptor substrate 1 (IRS1). The discovery of SCAN challenges traditional views by establishing an enzymatic basis for protein SNO, akin to protein phosphorylation. This enzymatic activity reduces insulin signaling by modifying INSR and IRS1, which play crucial roles in insulin signal transduction.

The study observed that SCAN expression correlates with body mass index (BMI) in humans and is associated with increased SNO-INSR in skeletal muscle and adipose tissue. Under physiological conditions, SCAN-mediated SNO of INSR/IRS1 is a regulatory mechanism that modulates insulin signaling. However, in obesity, increased SCAN activity leads to hyper-SNO of INSR/IRS1, contributing to insulin resistance and diabetes (Fig. 1A). This dual role underscores the importance of balanced SCAN activity for maintaining metabolic health and highlights the potential of SCAN as a biomarker and therapeutic target for metabolic disorders. This study not only advances our understanding of NO biology and protein SNO but also offers potential strategies for treating metabolic diseases.

Further research is needed to explore the broader implications of SCAN in other signaling pathways and diseases. Investigating the potential for pharmacological modulation of SCAN activity could pave the way for new treatments for diabetes and other NO-related disorders. Additionally, understanding the structural basis of SCAN's selective binding and activity may facilitate the development of specific inhibitors or activators, offering precise therapeutic strategies. In summary, the identification and characterization of SCAN represent a significant advancement in the field of molecular medicine, providing deeper insights into the

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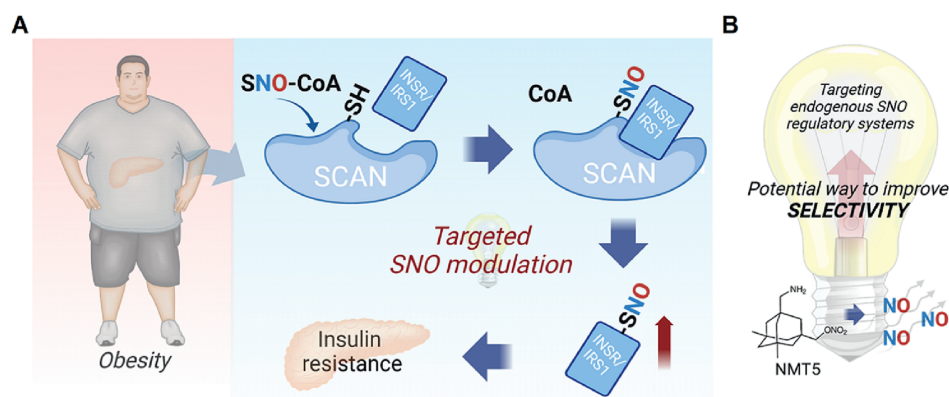


Figure 1 An endogenous SNO regulatory system and its implications for targeted SNO modulation. (A) In individuals with obesity, highly expressed SCAN catalyzes SNO modification of the INSR and IRS1, to inhibit insulin signaling physiologically and lead to insulin resistance. Targeted SNO modulation of proteins within this pathway might provide therapeutic benefits for individuals with obesity. (B) NMT5, a small molecule comprising a classic NO donor and an aminoadamantane moiety, represents an initial effort in targeted SNO modulation. In comparison to conventional nitric oxide synthase inhibitors or NO donors, targeting endogenous SNO regulatory systems through precise chemical strategies may represent a potentially direction to improve target selectivity.

regulation of insulin signaling and the molecular mechanisms underlying diabetes.

Inspired by this study, we propose a novel perspective for disease treatment: developing SNO modulators based on disease-associated SNO targets. For diseases characterized by abnormally elevated SNO levels, such as the increased SCAN-SNO levels reported in obese individuals, developing SCAN-SNO-targeted scavengers could benefit patients with insulin resistance. For the opposite scenario, a classic example is SNO-mediated pyruvate kinase M2 (PKM2) inhibition. The SNO-PKM2 shunted metabolites through serine and pentose phosphate pathway, which promotes the repair of acute kidney injury, suggesting that PKM2-targeted SNO agents could represent a new therapeutic approach for acute kidney injury⁴.

In fact, to our knowledge, Oh et al.⁵ have already initiated efforts in targeted SNO modulation. They designed a small molecule compound, NMT5, which can *S*-nitrosylate angiotensin-converting enzyme 2 (ACE2). All-atom molecular dynamics simulations suggest that the addition of *S*-nitrosylation at the side chain of Cys498, located near Gln175, may be sufficient to induce rearrangement in the packing of the secondary structural elements in this region. This rearrangement could, in turn, disrupt the only point of contact between the two peptidase domains of ACE2. The loss of this contact may potentially lead to further destabilization at the dimeric interface between the neck domains, thereby preventing the interaction between ACE2 and the SARS-CoV-2 spike protein⁵. In this manner, NMT5 protects against SARS-CoV-2 infection *in vivo* through mechanisms distinct from classic targets such as RNA-dependent RNA polymerase (RdRp), 3C-like protease (3CLpro), and papain-like protease (PLpro)⁶. These findings demonstrate that SNO-based therapeutic strategies have broad potential and could offer new treatment options for various diseases. The aminoadamantane moiety of NMT5 can guide a therapeutic warhead to target ACE2. However, it should be noted that the nitrate group in the NMT5 structure is a classic NO donor. Generally, enzymes such as aldehyde dehydrogenase 2 and xanthine oxidase, as well as low molecular weight reducing molecules like cysteine and ascorbic acid, participate in the release of nitric oxide from organic nitrate¹. The released NO from nitrate may freely diffuse, reducing the selectivity of SNO

modifications and causing unpredictable physiological effects (Fig. 1B). Therefore, improving the selectivity of SNO modulators could lead to more desirable therapeutic benefits.

Endogenous SNO regulatory systems provide insights for improving the selectivity of SNO modulators. Apart from the *S*-nitrosylase using SNO-CoA reported by Jonathan Stamler's lab, there are other targeted SNO systems in organisms, such as the inducible nitric oxide synthase (iNOS)-based heterotrimeric *S*-nitrosylase complex⁷, neuronal nitric oxide synthase (nNOS)-target protein complex^{8,9}, and noncanonical transnitrosylation network¹⁰. Based on these novel discoveries, we propose several strategies for highly selective SNO modulation, which may represent potential new directions for disease treatment. On the one hand, proteolysis targeting chimera (PROTAC) for components of endogenous targeted SNO systems or protein-protein interaction inhibitors for these components could regulate the aberrant activation of the endogenous system. On the other hand, molecular glues could be used to promote the assembly or enhance the stability of endogenous targeted SNO systems, thereby upregulating the SNO levels of downstream targets. These strategies may potentially offer higher target selectivity compared to traditional NOS inhibitors or NO donors.

A substantial body of physiological and pathological research has revealed the association between SNO and diseases. There is an urgent need for medicinal chemists and chemical biologists to leverage SNO targets for drug development. We believe that SNO-based target discovery and drug development would significantly accelerate the discovery of new drug targets and the generation of new drugs.

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Author contributions

Hui Ye: Writing – original draft, Investigation. Jianbing Wu: Writing – original draft, Investigation, Funding acquisition. Chen Zhang: Writing – review & editing, Investigation. Duorui Ji: Writing – review & editing, Investigation. Yihua Zhang: Writing – review & editing, Funding acquisition. Zhangjian Huang: Writing – review & editing, Supervision, Project administration, Funding acquisition.

Conflicts of interest

The authors have no conflicts of interest to declare.

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