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The Dual Roles of S-Nitrosylation of Proteins in Cancer: Molecular Mechanisms and Recent Advancements

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ABSTRACT

Protein S-nitrosylation (SNO), emerging as an important posttranslational modification, involves covalent addition of nitric oxide (NO) to the sulfur atom of cysteine in proteins. Accumulated evidence suggests that protein SNO plays crucial roles in pathophysiological mechanisms in cancer, which is attracting great attention. However, there are still controversies about whether S-nitrosylated proteins act as oncogenic proteins or tumor suppressors in cancer. In this review, we provide an overview of the early and latest evidence regarding the underlying mechanism and dual roles of SNO in cancer, in an effort to clarify its contribution in tumor progression. It has been well established that S-nitrosylated proteins restrain tumor progression in several types of cancer, while they have exhibited activities in promoting cell proliferation and inhibiting apoptosis in some other kinds of cancer. Interestingly, emerging evidence also has highlighted both its anti-cancer and pro-tumorigenic roles in several other cancer diseases. Finally, current limitations and future research prospects are presented. The overview of targeting SNO in cancer will provide new opportunities for drug development through in-depth exploration of SNO-mediated signaling pathways.

KEYWORDS

Protein S-nitrosylation; Cancer; Restraining tumor; Inhibiting apoptosis; Signaling pathway

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1. Introduction

Cancer has become one of the most serious diseases which endangers people's life and health. In recent years, the cancer mortality rate has increased annually [1-2]. The main risk factors associated with cancer development including diet, cell dysfunction and the molecular aberrations of chromosomes and proteins [3]. Additionally, environmental, exogenous and endogenous factors, and individual factors including genetic predisposition may also contribute to the progress of cancer [4]. Abnormal accumulation and aberrant activation of proteins may lead to various diseases including human cancers [5,6]. Post-translational modifications of proteins influence protein functions and play crucial roles in approximately all cell biological processes in cancer [7].

It's well known that nitric oxide (NO), a signaling molecule, has contradictory anti-tumor and pro-tumor influence. Anymore, it regulates structure, function, expression, localization, and even their interaction with other protein partners [8]. NO is synthesized from L-arginine and oxygen through a complex reaction catalyzed by nitric oxide synthase (NOS) [9,10]. NOS has three isoforms, including neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and endothelial NOS (eNOS/NOS3) [11]. At low doses, NO regulates homeostatic functions. While at high concentrations, it promotes tissue damage or cytotoxic to cancer cells and induces apoptosis by forming peroxynitrite [12,13]. Further, NO mediates some of the major types of protein posttranslational modifications (PTMs) including S-nitrosylation (SNO), S-glutathionylation, and tyrosine nitration [14]. S-nitrosylated proteins belong to reversible post-translational modifications (PTMs) of cysteine (Cys) residues elicited by NO, contribute significantly to the modulation of physiological functions and many signaling pathways in proteins [15,16]. Abnormal S-nitrosyation level is implicated in many inflammatory disorders including asthma, cancer, hypertension, neurodegeneration, diabetes, and autoimmune disorders [16]. Emerging evidence has indicated that the dysregulation of SNO protein involves in numerous cancer-related pathological events, such as tumor origination, development, metastasis, and treatment resistance [17-20].

S-nitrosylation of proteins could elicit dichotomous effects on cancer development, further complicating matters. In some cases, S-nitrosylation of proteins inhibits tumor progression, whereas, in other cases, it promotes tumor growth [11]. As an activator or inhibitor, protein S-nitrosylation appears to play a dual role in cell signaling for tumor growth and in the induction of cell death in cancer [7]. This review will offer an overview of its dual roles from the points of signaling pathways and recent advancements as well as potential future trends.

2. Protein S-nitrosylation restrains cancer

Numerous studies have demonstrated the elevation of protein SNO levels is a key event in cancer onset that may dramatically increase cancer risk [12,21]. SNO proteins play diverse roles in cancer biology, not only regulating tumor development but also serving as potential targets for cancer therapy [21]. SNO proteins can induce apoptosis of cancer cells through different ways or signaling pathways. Next, we will introduce the specific mechanism by which SNO proteins inhibit various cancer cells.

2.1. S-nitrosylation of peroxiredoxin-2 (Prdx2) promotes apoptosis in lung cancer cells

Peroxiredoxins (Prdxs) constitute a ubiquitous family of thiol proteins that belong to redox-regulated proteins. They can react with hydrogen peroxide and act as antioxidants and sensors in redox-regulated signaling pathways [22]. Prdxs are thiol-specific antioxidant enzymes. In mammals, there are six types of antioxidant enzymes (Prdx1-6). They are typically classified based on the number of cysteine residues directly involved in catalysis, including 2-Cys enzymes (Prdx1-4), atypical 2-Cys enzyme (Prdx5), and 1-Cys enzyme (Prdx6) [23].

Prdx2 is a member of the typical 2-Cys (Prx1) subgroup. The functional unit of Prdx 2 is a non-covalent homodimer containing two active sites, each of which contains a highly reactive Cys residue [24]. It is widely

expressed in various tissues and cells, and regulates cell proliferation, apoptosis, and differentiation [22-24]. S-nitrosoglutathione (GSNO), as an endogenous NO carrier, can transfer its NO moiety to a cysteine thiol, resulting in S-nitrosylation of protein [25]. Interestingly, GSNO could reduce cell viability and cell numbers, as well as inhibit the growth, survival, and colony formation of human lung adenocarcinoma cell lines and squamous-cell carcinoma cell lines [26].

GSNO is also a fundamental component of NO-dependent signaling pathways [27]. GSNO induces cell apoptosis in lung cancer cells through NO production [23]. Excessive NO nitrosylating Prdx2 at Cys51 and Cys172 sites, which was approved by nitrosylation inhibitors, disrupted the formation of Prdx 2 homodimers and successfully inhibited its catalytic cycle, finally leading to the accumulation of hydrogen peroxide [23]. The high concentration of H_2O_2 can increase the phosphorylation levels of 5'-AMP-activated protein kinase (AMPK), which links cell metabolism and cancer [28], in a concentration-dependent manner. The activation of AMPK induces phosphorylation of SIRT1 which is a cellular protective regulator that could be regulated by AMPK, resulting in the loss of SIRT1's deacetylase activity towards p53 [29]. Acetylated p53 increases the expression of caspase-3, further promoting apoptosis of lung cancer cells [23] (Figure 1).

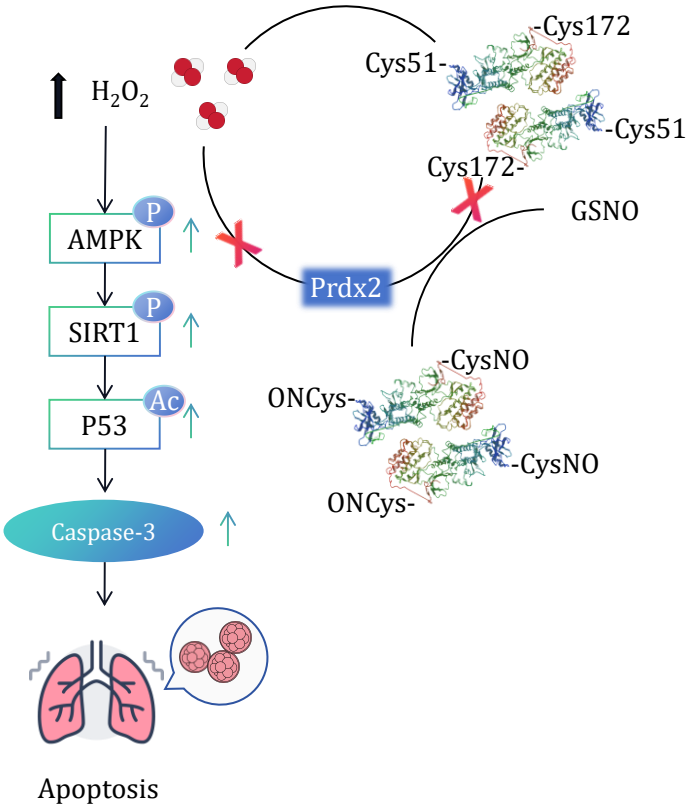

Figure 1. SNO Prdx2 promotes apoptosis in lung cancer cells. GSNO, a nitric oxide donor, it can induce sulfur nitrosylation at Cys51 and Cys171 of Prdx2, disrupting the formation of its homodimers and causing the accumulation of hydrogen peroxide [23]. It synergistically acts with hydrogen peroxide on the AMPK/SIRT1 signaling pathway, upregulates the deacetylation level of P53, and enhances the expression of caspase-3, ultimately inducing apoptosis in lung cancer cells [23].

As reported previously, the catalytic cycle of Prdx2 is associated with the metabolism of hydrogen peroxide [24]. Excessive H_2O_2 caused by metabolic abnormalities or anti-tumor drugs can lead to damage to cancer cells [30]. In agreement with the above reports, S-nitrosylation of Prdx2 synergistically induces apoptosis in lung cancer cells by the high level of H_2O_2 mediated by GSNO and the activation of the AMPK signaling pathway [22].

2.2. S-nitrosylation of colony-stimulating factor 1 receptor (CSF1R) inhibits prostate cancer growth

Prostate cancer is one of the most common cancers in men, which presents clinically similar to other cancers and can progress from a localized indolent disease to a rapidly advancing metastatic disease [31]. There are many studies focusing on the mechanisms of prostate cancer development and potential targets for treatment. Hormone therapy is an option for advanced prostate cancer [32]. However, its success rate is not very high.

Colony-stimulating factors (CSFs) are glycoproteins with a molecular weight ranging from 18 to 70000. They can exert anti-tumor effects in various malignant tumors by regulating the tumor microenvironment [33]. The CSF family consists of macrophage colony-stimulating factor CSF1 (M-CSF), granulocyte-macrophage-stimulating factor CSF2 (GM-CSF), and granulocyte colony-stimulating factor CSF3 (G-CSF) [34]. Colony-stimulating factor 1 (CSF1) is required for the differentiation, survival, proliferation, and renewal of monocytes and macrophages [35].

CSF1R belongs to the type III protein tyrosine kinase receptor family and has the ability to directly influence the differentiation, proliferation, and survival of tissue macrophages [35]. CSF1/CSF1R signaling mediates tumor-associated macrophages recruitment and M2 polarization [36]. CSF1R signaling via CSF1 regulates the production and differentiation of most circulating and tissue resident macrophages, where S-nitrosylation of CSF1R mediated by nitric oxide can induce tumor-suppressive functions [37].

Blocking the interaction between immune receptors and ligands has emerged as an effective paradigm for cancer immunotherapy [38]. CSF1R blockade restrain the progression of some tumours [37]. Uncoupled NOS3 could negatively influence the anti-tumor effectiveness of CSF1R blockade therapy against CRPC [37].

Cells treated with GSNO suggesting that an exogenous increase in NO could inhibit macrophage polarization in-vitro, increased the percentage of M1 macrophages, reduced the percentage of M2 macrophages, rescue increased oxidation, inhibit macrophage polarization and have tumor inhibitory effects against CRPC [37].

Nitric oxide S-nitrosylates CSF1R to induce tumor inhibitory functions. The study proves that GSNO promoted the nitrosylation of the three sites (Cys224, Cys278, and Cys830) on CSF1R, and then it effectively suppressed the tumor of prostatic cancer [36,37]. Increased NO levels augment the action of CSF1R inhibition in the suppression of prostatic cancer (Figure 2).

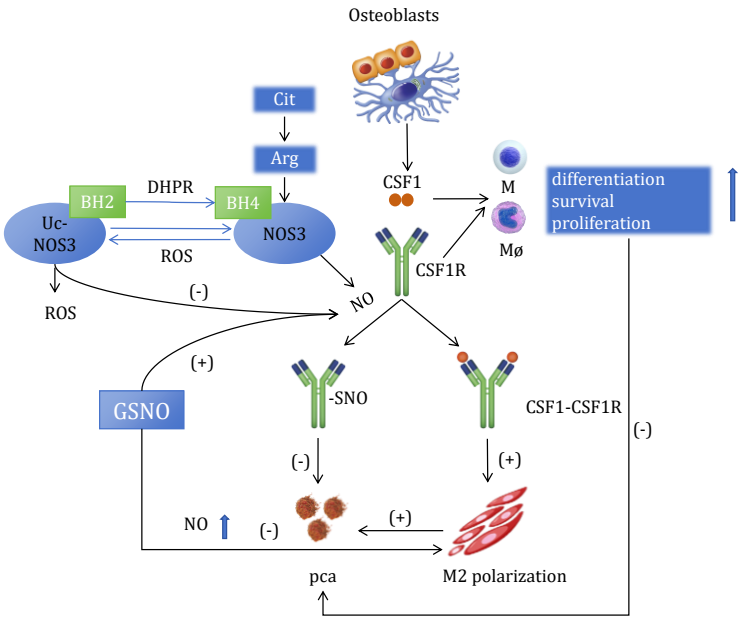

Figure 2. CSF1R blockage against prostate cancer. CSF1/CSF1R signaling mediates tumor-associated macrophages recruitment and M2 polarization [36]. GSNO and coupled NOS3 can increase the concentration of NO, promote sulfur nitrosylation at the Cys224, Cys278, and Cys830 sites of CSF1R [37], exerting an inhibitory effect by blocking CSF1R, which can suppress apoptosis of prostate cancer cells [37]. GSNO and NO can restrain the progression of cancer cells by inhibiting M2 polarization [37].

2.3. S-nitrosylation of cellular inhibitor of apoptosis-1 (clAP1) acts as a tumor suppressor in colon cancer cells

Colon cancer is one of the leading tumours in the world, and along with lung cancer, prostate cancer, it is considered one of the main killers [39]. Cellular inhibitor of apoptosis-1/2 (cIAP1/2) is an anti-apoptotic protein containing-E3-ubiquitine ligase belonging to the inhibitor of apoptosis (IAP) family [40]. The self-ubiquitylation activity of cIAP1 and the subsequent rapid degradation of cIAP1 through the proteasome, leads to cancer cell death [41]. The c-IAP protein is an important regulatory factor of the atypical nuclear factor-kappa B (NF-κB) pathway and ubiquitin ligase. It induces cell death by relying on the tumor necrosis factor (TNF) signaling pathway [42].

TNF-α, primarily originating from monocytes/macrophages, is a type of tumor necrosis factor [43]. Binding of TNF to tumor necrosis factor receptor 1 (TNFR1) can lead to divergent signaling pathways [43]. Upon TNFα stimulation, TNFR1 can mediate signals for either cell survival or cell death through NF-κB pathway and the assemble of caspase [44]. The clAP-mediated lysine 63 (Lys63)-linked ubiquitination of receptor-activating protein 1 (RIP1) is required for TNFα-induced activation of NF-κB [45], which shows the important role of PTM in NF-κB activation [46].

Glyceryl trinitrate (GTN), an agent that releases NO, induces S-nitrosylation of cIAP1 at cysteines 571 and 574 [45], which inhibits the E3 ubiquitin ligase activity of clAP1, resulting in a reduction of Lys-63-linked ubiquitination of RIP1 and further initiation of a death complex assembly [47,48]. S-nitrosylation of cIAP1 affects the signaling pathway induced by TNFα, leading to the activation of the classical cysteine aspartate-specific protease pathway, ultimately resulting in cell apoptosis (Figure 3).

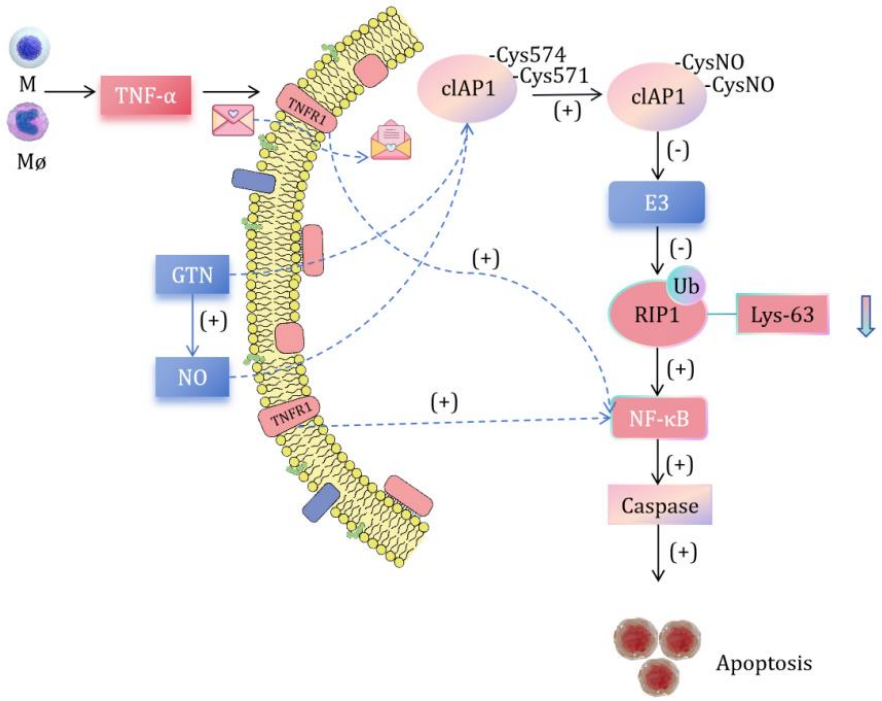

Figure 3. S-nitrosylation of clAP1 promotes apoptosis in colon cancer cells. TNF-α is primarily produced by monocytes/macrophages and belongs to the tumor necrosis factor family [43]. Upon TNFα stimulation, TNFR1 can mediate signals for either cell death through NF-κB pathway and the assemble of caspase [44]. GTN induces S-nitrosylation of cIAP1 at cysteines 571 and 574 [45], and inhibits the E3 ubiquitin ligase activity of cIAP1, resulting in a reduction of Lys-63-linked ubiquitination of RIP1 and initiation of a death complex assembly, inducing apoptosis in cancer cells [47].

2.4 S-nitrosylation of signal transducer and activator of transcription 3 (STAT3) and NF-κB restrain multiple myeloma

Multiple myeloma (MM) is a biologically diverse plasma cell disorder characterized by abnormal proliferation of malignant plasma cells in the bone marrow, which is the second-largest and most common form of hematologic malignancy [49]. However, effective treatment options are currently lacking. STAT3 is a crucial signaling protein that can be activated by numerous cytokines, growth factors, and other stimuli. It can elicit diverse biological outcomes including cellular growth and survival, immunity and apoptosis [50]. STAT3 is a converging point for many signaling pathways of cancers, and it is extensively overactivated in both cancer cells and non-cancer cells within the tumor ecosystem [51]. Numerous reports suggested that aberrant activations of STAT3 and NF-κB promoted survival and proliferation of MM cells [52-54]. Inhibiting the relevant transcription factors can suppress the activities of STAT3 and NF-κB, thereby inhibiting the proliferation and growth of MM cells [55,56].

Interleukin-6 (IL-6) also plays a crucial role in the growth, survival, and malignant progression of MM cells [57]. U266 cells, immortal human B lymphocyte cell lines, can induce STAT3 activation through autocrine IL-6 [58]. The sulfur-nitrosylation inducer S-nitroso-N-acetylcysteine (SNAC) induced SNO of STAT3 and the subunits of NF-κB (p50 and p65) in the IL-6/STAT3 pathway, thereby reducing phosphorylation of STAT3, p50, and p65 [58]. SNAC also reduces the expression of anti-apoptotic members of the Bcl-2 protein family, which are also known to be regulated by STAT3 and NF-κB [59]. The changes in the levels of these apoptotic proteins collectively inhibit multiple myeloma cells [58] (Figure 4).

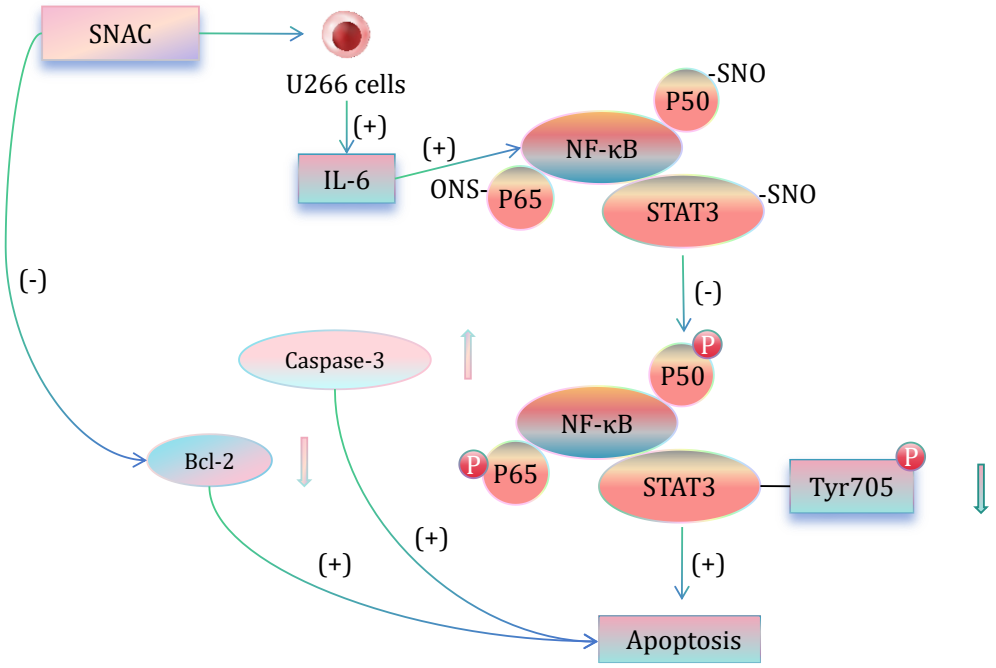

Figure 4. IL-6/STAT3 pathway restrains multiple myeloma. SNAC induces S-nitrosylation of STAT3 and NF-κB (p50 and p65), leading to a reduction in the phosphorylation level, which promotes apoptosis of multiple myeloma cells [50].

3. Aberrant S-nitrosylation of proteins induces cancer

SNO proteins not only have the effect of inhibiting cancer as mentioned above, but also play a carcinogenic role in some others cancers as follows.

3.1. S-nitrosylation of Ezrin acts as an oncogenic protein in non-small cell lung cancer (NSCLC)

NSCLC is a type of lung cancer that accounts for approximately 85% of all the lung cancers [60]. Ezrin is a kind of membrane-cytoskeleton linker proteins, belonging to the ezrin-radixin-moesin (ERM) family, and has been demonstrated to be a driving factor of tumour progression and metastatic spread. Ezrin maintains the cytoskeletal remodelling and cellular signalling pathways, which could regulate cancer-cell survival and metastatic cascade [61]. It has been proved that the up-regulation of SNO ezrin is related to the invasion behavior of NSCLC cells [62].

The experiments indicated that the combination of ezrin and cell membrane depends on Cys117 nitrosylation mediated by iNOS, leading to MF (microfilament) reorganization, ultimately promoting the aggressive phenotype of NSCLC cells. It also revealed the role of ezrin-SNO in modulating the mechanical property of ezrin for promoting the NSCLC cells invasion and metastasis [61].

As a linker protein, ezrin, has no capacity to generate forces. Apparently, the close relationship between MFs and ezrin structure make it easy to comprehend that MF forces are the most important regulator of ezrin tension. However, MT forces also take part in the regulation of ezrin tension. Actually, the mutual interaction between MTs and ERM proteins has been reported [62]. Visually speaking, the MF forces and the MT forces perform as master regulators of cell shape [63,64]. The MF forces are inwards and the MT forces are outward. The joint effect of outward MT and inward MF forces controls the cell shape and plasticity reaching a new balance, in which the cell membrane becomes more flexible and easier to invade and metastasize. Collectively, the SNO of ezrin lead to a number of pathological changes might facilitate cellular mechanical transduction and result in the NSCLC cells invasion and metastasis [61] (Figure 5).

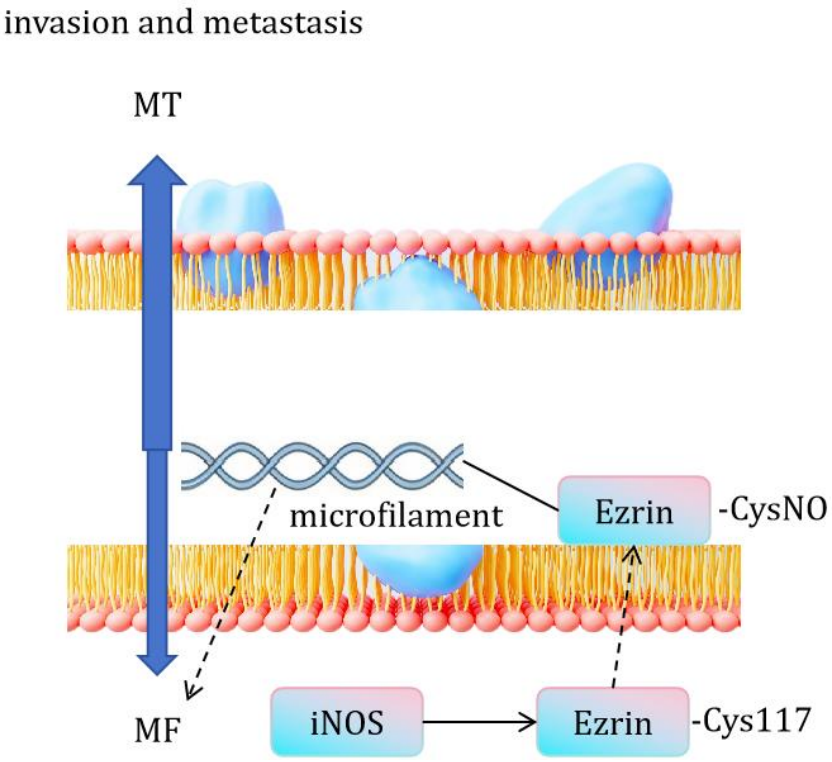

Figure 5. Ezrin is a membrane-cytoskeleton linking protein, and its binding to the cell membrane depends on the iNOS-mediated S-nitrosylation of the Cys117 site [61]. This leads to the reorganization of microfilaments (MF), and the combined action of outward microtubules (MT) and inward MF forces, which controls the shape and plasticity of the cell membrane, making it more flexible and thus more prone to invasion and metastasis [62-64].

3.2. Aberrant S-nitrosylation induced by NO induces lung adenocarcinoma (LUAD)

LUAD is a prevalent subtype of NSCLC, accounting for approximately 40% of cases of NSCLC [65]. Angiogenesis is a crucial step in tumor development and metastasis, and this process is tightly regulated by vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) [66]. Vascular endothelial growth factor (VEGF) is typically secreted by cells near growing blood vessels and acts on receptors on endothelial cells, promoting angiogenesis by maintaining a high local concentration through interaction with the extracellular matrix, thus creating a microenvironment for vascular neogenesis [67].

Vascular endothelial growth factor D (VEGFD) induces angiogenesis through binding with vascular endothelial growth factor receptor 2 (VEGFR2) [68]. SNO promotes tumor metastasis by regulating epithelial-to-mesenchymal metastasis, migration, and invasion, enabling tumors to absorb intravascular nutrients and achieve rapid development [69].

In LUAD, both mRNA and protein levels of VEGFD significantly decrease [70]. Low protein expression of VEGFD is closely associated with tumor occurrence in LUAD, which is a consequence of excessive SNO at the Cys277 site of VEGFD induced by NO [71]. Additionally, sustained inhibition of S-nitrosoglutathione reductase leads to a reduction in denitrosylation of VEGFD at the Cys277 site, thereby promoting the progression of LUAD [71] (Figure 6).

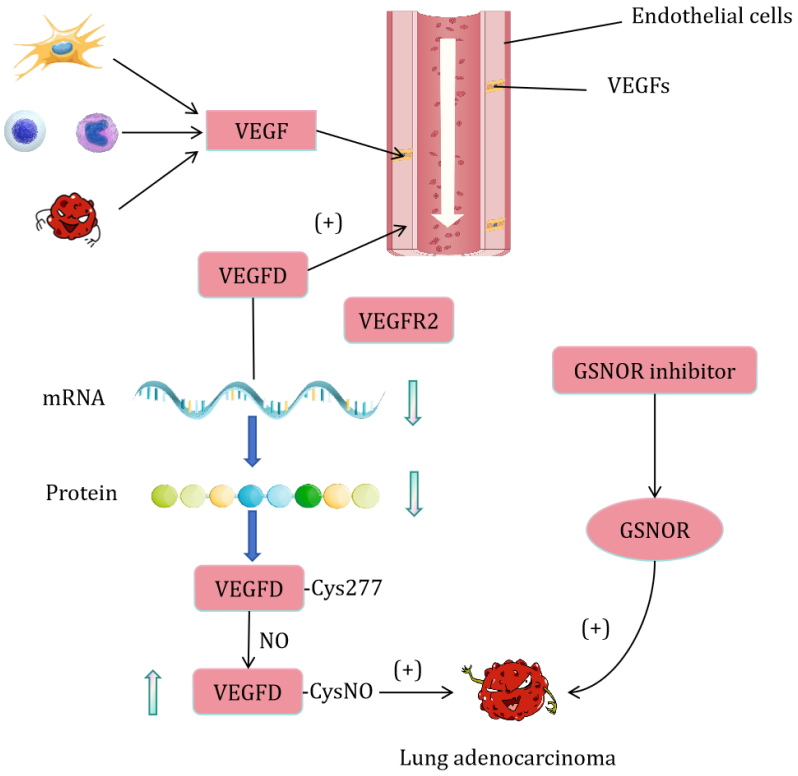

Figure 6. The regulation of VEGFs and VEGFRs is an important mechanism for controlling angiogenesis and tumor formation and metastasis [66]. NO induced the S-nitrosylation of VEGFD at the Cys277 site. GSNOR inhibitors inhibited the activity of GSNOR, reduced the denitrosylation function [69-71]. The aforementioned mechanisms collectively promoted the progression of LUAD.

3.3. Role of S-nitrosylation level of STAT3 in pancreatic ductal adenocarcinoma (PDAC)

PDAC is an aggressive solid malignant cancer which originated from exocrine cells in pancreas, accounts for approximately 85-90% of all malignant tumors in the pancreas [72], is the most common pancreatic cancer subtype [73]. The characteristic of PDAC is poor prognosis, with a 5-year survival rate of less than 8% [74]. NOS is a key

signaling molecule in PDAC with the ability to regulate cell proliferation and apoptosis [75]. NO participates in tumorigenesis by regulating other proteins through SNO [76,77]. The levels of nitric oxide synthase (NOS) and total S-nitrosylation proteins in the tissues of PDAC patients is significantly higher than non-cancerous tissues that corresponding adjacent. This suggests that NO-mediated protein modifications may play a central role in the pathogenesis of PDAC [78]. Particularly, SNO of wild-type Ras protein is required for the initiation and maintenance of PDAC tumor growth [79].

PANC-1 is a human pancreatic cancer cell line that can be used as a model cell for experimental purposes [80]. In order to confirm the SNO of Raf-1 and STAT3 proteins in the pancreatic cancer pathway, Tan et al. used biotin switching method to detect the SNO level of proteins [78,81]. The results revealed that both Raf-1 and STAT 3 proteins had high SNO levels in PANC-1 cells and PDAC tissues. NOS inhibitors led to a decrease in S-nitrosylation level of STAT3, an increase in phosphorylation level of STAT3, and enhanced pancreatic cancer cell viability [78] (Figure 7). The elevated levels of S-nitrosylation proteins, such as wild-type Ras, Raf-1, and STAT3, ultimately promote the development of PDAC.

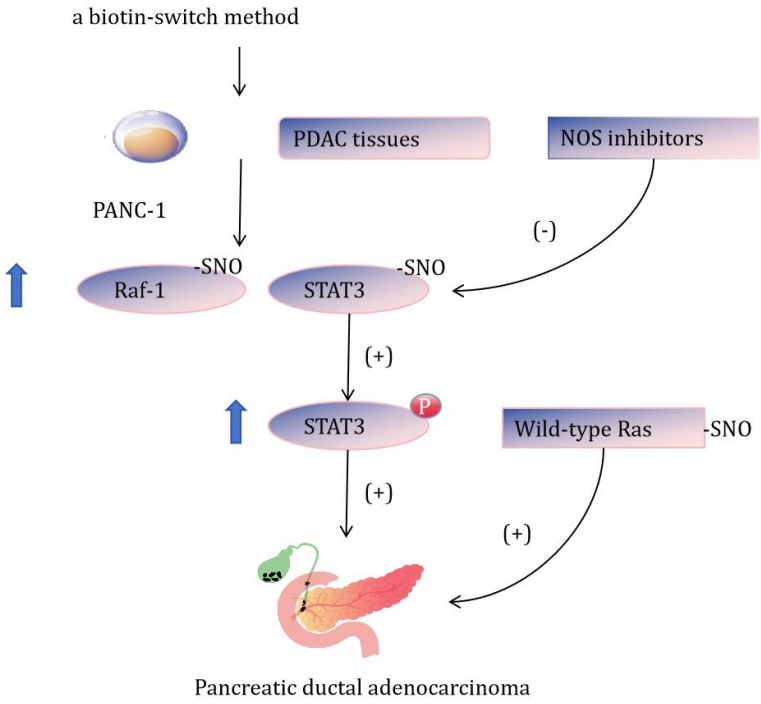

Figure 7. The levels of NOS and total SNO protein are significantly higher in pancreatic ductal adenocarcinoma (PDAC) tissues compared to corresponding non-cancerous tissues [72-74]. Among them, the S-nitrosylation levels of Raf-1 and STAT3 proteins are elevated, and S-nitrosylation of wild-type Ras protein can promote PDAC tumor growth [78-81]. Inhibition of NOS leads to a decrease in STAT3 S-nitrosylation levels, an increase in STAT3 phosphorylation levels, and enhanced viability of pancreatic cancer cells [78].

4. Dual roles of S-nitrosylation in cancers

4.1. Role of S-nitrosylation in liver cancer

The liver cancer is the sixth most common site of primary cancer in humans, with notable features that late onset and low survival rate [82]. The etiology of hepatocellular carcinoma (HCC) is most likely attributed to chronic hepatitis caused by hepatitis B or C viral infections [83]. Nevertheless, the molecular mechanisms by which risk

factors promote the occurrence of liver cancer are still largely unknown [84]. Recently, many scholars have been studying SNO-related metabolic biomarkers in HCC and utilizing them for treatment [85].

SNO from S-nitrosoglutathione reductase (GSNOR) deficiency promotes Hepatocarcinogenesis. GSNOR, a highly evolutionarily conserved enzyme of the denitrosylating enzymatic system, its regulation of protein SNO is implicated in the development of many diseases [86,87]. The dysregulation of GSNOR can significantly alter cellular homeostasis and plays a important regulatory role in processes such as smooth muscle relaxation, immune function, inflammation, neuronal development, and cancer progression [87]. There is a significant decrease in both the abundance and activity of GSNOR in the half of HCC patients pproximately. It suggests that GSNOR may be a key protein involved in controlling protein S-nitrosylation [86]. The deficiency of GSNOR induces the S-nitrosylation of certain proteins, further promoting tumor development [88]. In addition, protein S-nitrosylation is a potential modulator of cellular processes important for tumorigenesis, including key mechanisms of signaling pathways involved in critical cellular processes such as transcription regulation, DNA repair, and apoptosis [89].

In human liver cancer tissues, GSNOR activity is significantly reduced. Immunoblot analysis indicates that the decreased GSNOR activity is associated with a reduction in the amount of GSNOR protein. Thus, the reduced protein levels may be the main cause of the decreased enzyme activity [90].

Angiotensinogen (AGT) is the sole precursor of all angiotensin peptides that repair mutagenic lesions in DNA and is an important target for cancer prevention and chemotherapy [91,92]. It catalyzes the unique, single-step, direct DNA damage reversal repair of O6-alkylguanines by selectively transferring the O6-alkyl adduct to an internal cysteine residue [92]. The SNO of AGT and proteasomal degradation contribute to the development of hepatocellular carcinoma [93].

Research on GSNOR-deficient mice indicates that the lack of GSNOR in the inflammatory response following intraperitoneal injection of diethylnitrosamine (DEN) and lipopolysaccharide (LPS) results in SNO, proteasomal degradation, and depletion of AGT in the liver [90]. As a result, the function of O6-alkylguanine in the liver is impaired, leading to an increased risk of liver cancer [93].

Sorafenib is the first generation of targeted therapeutic drugs for the treatment of liver cancer, and Keap1-Nrf2/ARE/Trx-1 is the main antioxidant signaling pathway of sorafenib, which plays an important role in oxidative damage, liver apoptosis and mitochondrial dysfunction in related diseases [94]. Kelch-like ECH-associated protein 1 (Keap1), is a master negative regulator 2 of Nrf2 [95]. Activation of the Keap1-Nrf2 signaling pathway can trigger antioxidant responses. Conformational changes in Keap1 lead to the dissociation of Nrf2, which subsequently translocates into the nucleus, binds to antioxidant response elements (AREs), and initiate the expression of diverse antioxidant and metabolic genes, such as thioredoxin (Trx) [96].

Thioredoxin-1 (Trx-1) is a small protein with a characteristic of reduction-oxidation (redox) that widely exists in humans [97]. Trx-1 induced activation of NOS3 and S-nitrosyation of CD95 leading to an increase of caspase-8 activity and caspase-3 activity [98] (Figure 8). This mechanism ultimately leads to reduced liver damage and promotes apoptosis of liver cancer cells [99].

4.2. Role of S-nitrosylation in breast cancer

The development of the mammary gland is influenced by many complex factors [100]. Imbalances in certain signaling pathways or endogenous substances levels in the body can lead to the occurrence of breast cancer [101]. The elevation of cellular NO levels leads to an increase in the level of SNO in breast cancer, which is an important mechanism promoting the breast cancer [102].

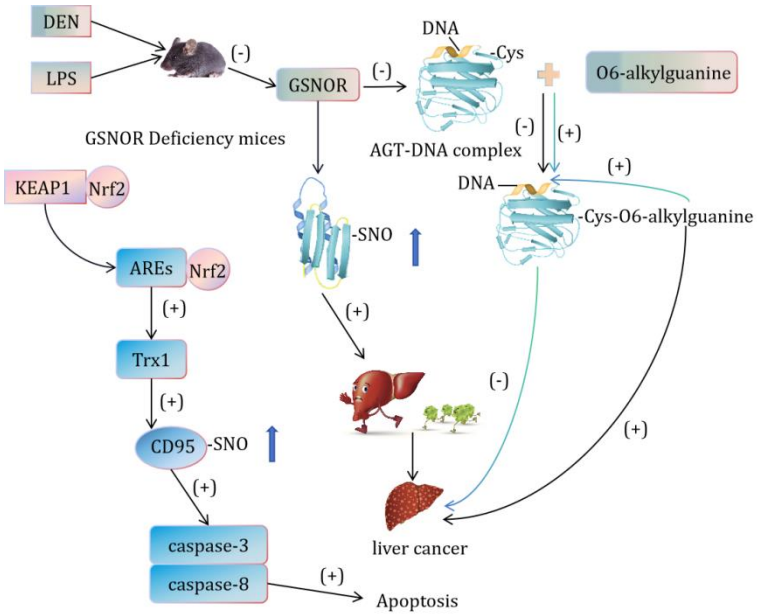

Figure 8. S-nitrosylation proteins have a dual role in cancer progression. GSNOR may be a key protein involved in controlling protein S-nitrosylation [85]. GSNOR-deficient leads to an increase in S-nitrosylation protein levels and the consumption of AGT, which is impaired in the liver, which increases the risk of liver cancer [92]. Keap1-Nrf2/ARE-Trx-1 plays a crucial role in liver apoptosis [94]. Conformational changes in Keap1 lead to the dissociation of Nrf2, which subsequently translocates into the nucleus, binds to antioxidant response elements (AREs), and initiate the expression of Trx1 [95,96]. Trx1 increase the S-nitrosylation level of CD95, caspase-8 activity and caspase-3 activity [100]. This mechanism ultimately leads to reduced liver damage and promotes apoptosis of liver cancer cells.

Ets-1 is an oncogenic transcription factor involved in the progression of breast cancer [103]. It has an elevated expression level in primary breast cancer [104]. NO activated the transcriptional activity of Ets-1 through the Ras/MAPK/MEK/ERK signaling pathway, and its mechanism involves the S-nitrosylation of Ras. S-nitrosylation of Ras leads to activation of Ets-1, which is caused by MAPK-dependent phosphorylation and results in an aggressive breast cancer phenotype [105,106].

The NO signal also activated EGFR and Src kinases through SNO, leading to oncogenic signaling pathways such as Akt, STAT3, and β-catenin and inhibiting the tumor suppressor PP2A, ultimately inducing a basal-like breast cancer phenotype in human cell lines [107].

Hypoxia-inducible factor 1α (HIF-1α) is a key transcription factor involved in cancer progression and targeted cancer therapy [108]. S-NO of HIF-1α at the Cys533 site can lead to an increase in the stability of the protein and the expression of vascular endothelial growth factor, promoting angiogenesis. This is a key process in breast tumor growth and metastasis [108,109].

Some SNO proteins can promote the occurrence of breast cancer, while others may also play an inhibitory role. Human monocarboxylate transporter 1 (hMCT1) is overexpressed in many cancers and is associated with the prognosis of certain cancers. Exposure to S-nitrosocysteine (CysNO), the thiols on human monocarboxylate transporter 1 (hMCT1) undergo S-nitrosylation, leading to inhibition of substrate uptake and suppression of breast cancer cell growth [110,111] (Figure 9).

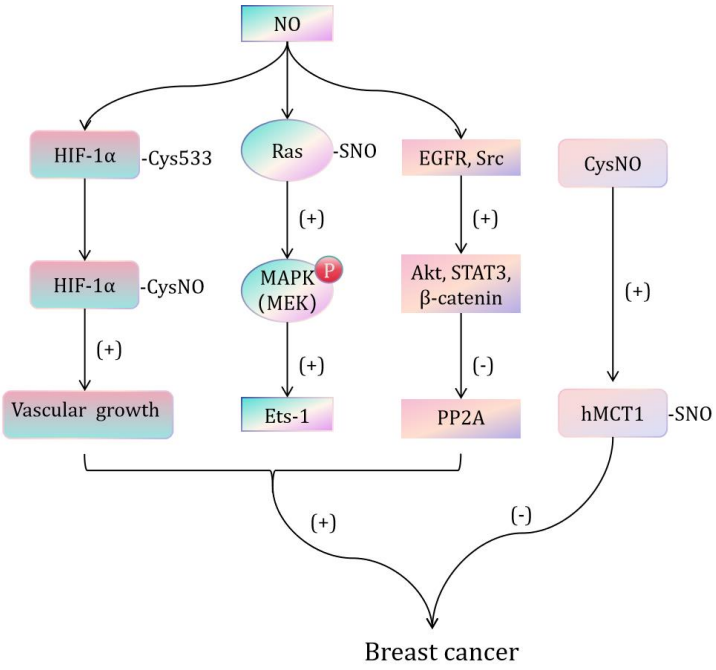

Figure 9. Ras/MAPK/MEK/ERK signaling pathway is closely associated with the occurrence of multiple types of cancer [105]. NO activates the transcriptional activity of Ets-1 through the Ras/MAPK/MEK/ERK signaling pathway, and the s-nitrosylation of Ras leads to the activation of Ets-1 and consequently the invasive breast cancer phenotype. Nitric oxide induced the S-nitrosylation of HIF-1α at Cys 533 site, promoting angiogenesis and facilitating the development of breast cancer. It activated EGFR and Src kinases inhibits the tumor suppressor factor PP2A [107], promoting the development of breast cancer. There also had some S-nitrosylation proteins inhibiting the development of breast cancer, such as hMCT1 [110,111].

4.3. Role of S-nitrosylation in ovarian cancer

Ovarian cancer is one of the invasive gynecological cancers [112]. The change of energy metabolism is the main biochemical features of cancer cells [113]. Glucose metabolism in cancer cells is primarily characterized by two major biochemical events including increased glucose intake and aerobic glycolysis [113]. Phosphofructokinase1 (PFK1) as a member of the phosphofructokinase (PFKM) family, it is one of the most important regulatory enzymes of glycolysis [114]. PFKM binds to NOS1 through the PDZ domain [115].

NOS1 induces the S-nitrosylation of Cys351 on PFKM. The S-nitrosylation of Cys351 further promotes the formation of PFKM tetramers, enhances NOS1-mediated PFKM activity, and regulates the increased glucose metabolism in ovarian cancer cells, thereby promoting ovarian cancer cell proliferation and metastasis [116]. NOS2 and the accompanied release of NO are associated with the initiation and progression of cancer [117]. NOS2 promotes angiogenesis and tumor proliferation, inducing the development of invasive type II ovarian tumors [118].

As a nitrosylating agent, GSNO can inhibit the proliferation and invasion of cancer cells [119]. It can mediate the S-nitrosylation of various proteins in ovarian cancer cells, including Akt, the p65 of NF-kB, and STAT3 [120]. It reduces the levels of phosphorylated Akt and STAT3, inhibiting the activation of STAT3 and Akt [121]. This, in turn, suppresses the invasion and migration of breast cancer cells. Blocking signaling pathways such as Akt and STAT3 can also inhibit cell proliferation by inducing apoptosis and cell cycle arrest [121,122]. The levels of SNO proteins in ovarian cancer may have both promotive and inhibitory effects through different pathways, as shown in Figure 10.

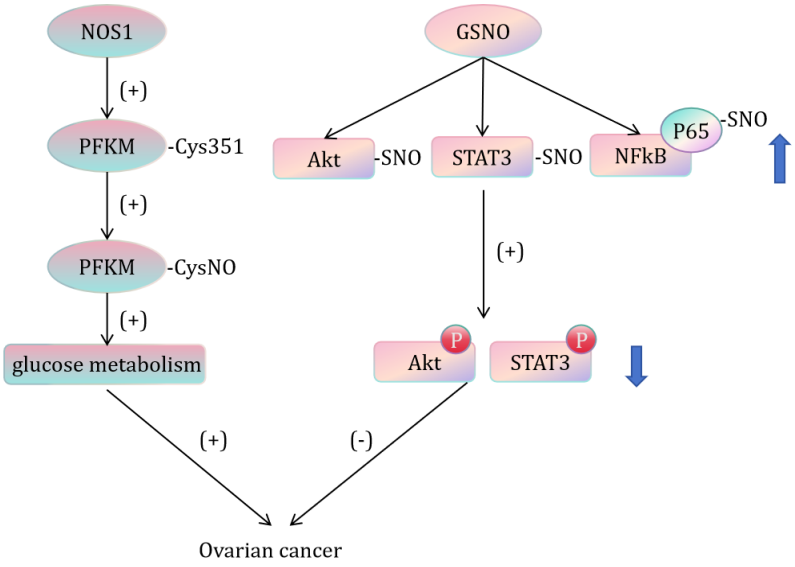

Figure 10. NOS1 induces S-nitrosylation of Cys351 on PFKM, regulating increased glucose metabolism in ovarian cancer cells, thereby promoting the proliferation and metastasis of ovarian cancer cells [114,115]. GSNO increased the level of S-nitrosylation of multiple proteins in ovarian cancer cells, including Akt, the p65 of NF-κB, and STAT3. It reduced the phosphorylation of Akt and STAT3, inhibited the activation of STAT3 and Akt, and thereby suppressed the invasion and migration of ovarian cancer cells [119-122].

The aforementioned studies indicated that SNO proteins had dual roles in cancer. Through different signaling pathways and mechanisms, they can promote the occurrence or inhibit the progression in a certain cancer.

5. Challenges and limitations

Abnormal expression of proteins may not only cause abnormal cell proliferation, but may also cause abnormal differentiation of cells, which promotes tumor formation and development [18]. At present, many studies have found the deregulation of S-nitrosylation in various cancer tissues, suggesting that S-nitrosylation is closely related to the development of cancer, and it can play a pro-cancer or inhibited role in different cancer microenvironments [11].

The two most widely accepted method to evidence the S-nitrosylation of proteins is the biotin switch technique, it relys on decomposition of the S-NO bond and detection of generated products in the form of free thiol, another is the chemiluminescence for NO [123]. With the development of instrument analysis technology, mass spectrometry has become an indispensable analytical tool in all types of drug discovery applications [124]. It is also an important means of studying proteomics. S-nitrosocysteine enrichment by anti-S-nitrosylated cysteine antibodies followed by proteomic analysis by LC-MS/MS can provide site-specific identification of modified cysteine residues and effective systematic screening of related biomarkers, such as upstream and downstream proteins [125]. These methods and techniques are very critical and important for studying the role of S-nitrosylation proteins in cancer.

However, SNO and targeting SNO as cancer treatment strategies still have some limitations. First, because the process of S-nitrosylation is reversible and only S-nitrosylation of specific cysteine residues [15]. Since proteins may contain many cysteines, as well as the destabilizing nature of SNO, S-nitrosylated cysteines may be difficult to detect and difficult to distinguish from non-S-nitrosylated amino acids. All of these factors are potential challenges that need to be addressed urgently. SNO protein signaling function is also involved in the pathogenesis of many diseases, which has also become a key point for studying its targeted drug development. Its related upstream and downstream proteins may be relevant targets for cancer treatment.

Besides the detection of S-nitrosylated proteins, change in any physiological indicator is one of the key treatments. Changing the level of NO can affect other biochemical indicators to achieve the purpose of treating the disease. At present, the development of NO donor drugs has become a mature therapeutic method in clinical practice. Many NO donor drugs, such as nitroglycerin, sodium nitroprusside, nicorandil, Bidil, etc., have been widely used in the clinic to treat angina, heart failure, and other cardiovascular diseases [14]. The accumulated findings show that S-nitrosylation of cathepsins under the influence of NO-donors can prevent the invasion of cancer and cause cancer cell death by blocking the activity of cathepsins as well as the major denitrosylase systems using a multi-way approach [126]. In summary, regulating the levels of the proteins of S-nitrosylation by changing the levels of NO may treat some related diseases. It also provides ideas for future research on targeted therapy of SNO proteins.

Due to the complexity of proteomics, these still need a lot of scientific research to further study the mechanism of targeting SNO for cancer treatment in the future, that can provide strong evidence for the potential of targeting SNO in drug development. As shown in Table 1, in order to be more concise, we summarize the key proteins and biomarkers and their effects mentioned in the paper.

Table 1. The overview of S-nitrosylation proteins in cancer.

Types of cancer	Key proteins	Biomarkers	effect
Lung cancer	Prdx-2	AMPK, SIRT1, p53, caspase-3	+
Prostate cancer	CSF1R	GSNO, NO, CSF1	-
Colon cancer	clAP1	TNFα, TNFR1, NF-κB, RIP1	-
Multiple myeloma	STAT3, NF-κB	IL-6, Bcl-2	-
non-small cell lung cancer	Ezrin	iNOS	+
Lung adenocarcinoma	VEGFD	VEGFD and the mRNA	+
Pancreatic ductal adenocarcinoma	wild-type Ras, Raf-1	NOS, STAT 3	+
Liver cancer	AGT, CD95	GSNOR, NOS3, caspase-8, caspase-3	±
Breast cancer	Ras, HIF-1α	Ets-1, MAPK, Akt, STAT3 and β-catenin	±
Ovarian cancer	PFKM	NOS1, NOS2, Akt, NF-κB, and STAT3	±

Notes："+": Promotes the progression of cancer, "-": inhibits cancer progression, "±": represents dual effects.

6. Conclusions and prospect

In summary, various evidences have showed that SNO of proteins are widely involved in the occurrence and development of cancer, and different proteins will undergo modifications of function gain or loss after SNO, which can inhibit tumor progression or promote cell proliferation.

In this review, we summarized the mechanisms of SNO proteins in promoting apoptosis of cells in lung cancer, prostate cancer, colon cancer, and multiple myeloma by regulating AMPK, CSF1R, and NF-κB signaling pathways. They can also play roles in promoting the proliferation of cells in non-small cell lung cancer, lung adenocarcinoma, and pancreatic ductal adenocarcinoma by modulating Ezrin, VEGF, and STAT3 signaling pathways. In addition, SNO proteins have the dual effect of inhibiting and promoting cell proliferation in a certain cancer, which may be due to differences in the target of action.

Understanding in the roles and regulatory mechanisms of SNO proteins had great significance for treatment of cancers. Thus, our investigation provides a strong reference basis for the further development of targeted therapeutic drugs for cancer. Until now, there are not many relevant studies. Therefore, it is urgent to further explore the roles of SNO proteins in cancers. With further research, it is expected to open up new avenues for clinical treatment of cancer.

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Conflict of interest

All the authors claim that the manuscript is completely original. The authors also declare no conflict of interest.

Author contributions

Conceptualization: Hongmei Yang; Investigation: Yi Wu, Yanqi Li, Tong Wu; Methodology: Hongmei Yang, Yi Wu; Writing–original draft: Yi Wu, Yanqi Li; Writing–review & editing: Hongmei Yang, Tong Wu.

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