

Chapter 8

The Emerging Role of Protein *S*-Nitrosylation in Cancer Metastasis

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Abstract Nitric oxide (NO) has increasingly been recognized as an important cell signaling molecule that controls various steps of cancer development and metastasis. NO regulates a wide range of tumor-associated proteins through *S*-nitrosylation, a reversible coupling of a nitroso moiety to a reactive cysteine thiol (SH) group to form an *S*-nitrosothiol (SNO). In this article, we discuss the various roles of protein *S*-nitrosylation in cancer development with a focus on anoikis resistance, cell invasion and angiogenesis, which are key determinants of cancer metastasis. We specially address the effect of *S*-nitrosylation on protein function and discuss how this post-translational modification affects the aggressive and metastatic behaviors of cancer cells. We propose that dysregulated NO signaling is common in many, if not most, metastatic cancers and that understanding the *S*-nitrosylation process will facilitate the development of novel therapeutic and preventive strategies against cancers.

Keywords Anoikis · Cancer · Invasion · Metastasis · Migration · Nitric Oxide · *S*-nitrosylation

Abbreviations

ABCG2 ATP-binding cassette sub-family G member 2
c-Src cellular Src
DETA diethylenetriamine

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B. Bonavida (ed.), *Nitric Oxide and Cancer: Pathogenesis and Therapy*,
DOI 10.1007/978-3-319-13611-0_8

DISC	death-inducing signaling complex
DPTA	dipropylentriamine
DR	death receptor
DTT	dithiothreitol
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
eNOS (NOS3)	endothelial nitric oxide synthase
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FLIP	FLICE-inhibitory protein
FLIP2CM	FLIP double-cysteine mutant
JNK	Jun N-terminal kinases
IGF	insulin-like growth factor
iNOS (NOS2)	inducible nitric oxide synthase
MKP7	MAP kinase phosphatase 7
MMP	matrix metalloproteinase
NO	nitric oxide
NOS	nitric oxide synthase
nNOS (NOS1)	neuronal NOS
PI3K	phosphoinositide-3-kinase
PTEN	phosphatase and tensin homolog deleted on chromosome ten
PTM	post-translational modification
RNOS	reactive nitrogen-oxygen species
ROS	reactive oxygen species
SDF-1 α	stromal cell-derived factor-1 α
SH	cysteine thiol
SNAP	<i>S</i> -nitroso- <i>N</i> -acetylpenicillamine
SNO	<i>S</i> -nitrosothiol
SNOC	<i>S</i> -nitrosocysteine
VEGF	vascular endothelial growth factor

Introduction

Nitric oxide (NO, formula N=O) is an important signaling molecule that functions as a messenger or effector in various biological processes [1]. NO is synthesized by the metabolism of L-arginine to L-citrulline through a complex reaction catalyzed by NADPH-dependent enzymes called nitric oxide synthases (NOS), which exist in three isoforms, namely, neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3) [2]. Expression of NOS and NO activities are involved in the pathophysiology of cancers, particularly in tumorigenesis and metastasis in various tissues including brain, breast, lung, prostate, and

pancreas [3–6]. With regards to cancers, NO is derived either from tumor cells or neighboring cells, e.g. endothelial cells in the microvasculature and immune and stromal cells in the tumors [6, 7]. NO with its lipophilic nature could diffuse freely across cellular membranes, i.e. of neighboring cells and ultimately exerts its effect on tumor cells. Depending on (i) its activity and cellular sources (tumor or neighboring cells) (ii) localization of NOS (iii) concentration and duration of NO exposure (iv) cellular context and sensitivity to NO and (v) tumor stage, NO appears to exert dichotomous roles (promotion or inhibition) in cancers [8–10].

With its unique chemistry, the reactivity of NO varies under different biological and pathological conditions. The chemical biology of NO is generally classified into direct and indirect effects [11]. Direct effects are defined as those of direct interactions between NO, generally at a low level, and specific molecular targets, e.g. metals, lipids and DNA through free radical reactions. Indirect effects are those mediated by reactive nitrogen-oxygen species (RNOS) derived from the reaction of NO, generally at a high level, with various reactive oxygen species (ROS) leading to nitrosative or oxidative stress. For instance, NO reacts with superoxide anion ($O_2^{\cdot-}$) in the inner-membrane environment that results in the generation of (i) peroxynitrite ($ONOO^-$) in the case of equal concentrations of NO and $O_2^{\cdot-}$ or (ii) dinitrogen trioxide (N_2O_3) in the case of excess NO. The reaction of NO and O_2 (auto-oxidation) yields a nitrogen dioxide (NO_2) intermediate that forms N_2O_3 . N_2O_3 (major species) and $ONOO^-$ are endogenous *S*-nitrosylating agents that lead to *S*-nitrosylation of proteins with reactive sulfhydryl groups.

In cancers, *S*-nitrosylation is an important post-translational protein modification (PTM) process that affects virtually all cancer cell phenotypes including cell growth and differentiation, apoptosis, migration and invasion, and angiogenesis [12]. The principal target of protein *S*-nitrosylation is the thiol group of protein's cysteine residues. Not all cysteine residues, however, are susceptible for *S*-nitrosylation and/or responsible for the alteration of protein functions, which depend largely on the degree of hydrophobicity, electrostatic environment, orientation of aromatic residues and proximity of target thiols to redox center, and protein-protein interactions [13–15]. In this article, we will review current findings on NO signaling and its role in cancer with a focus on protein *S*-nitrosylation and its effect on the various steps of cancer progression and metastasis.

Cancer Metastasis

Neoplastic transformation is an early cellular event leading to carcinogenesis. Neoplastic transformation of normal cells is typically a result of chronic or persistent inflammation of tissues in response to stresses or a result of genetic mutations caused by carcinogens, or both [12]. For example, NO has been shown to mediate the neoplastic effect of the carcinogenic metal chromium (VI) on human lung epithelial cells through NO-mediated *S*-nitrosylation of the Bcl-2 protein [16, 17].

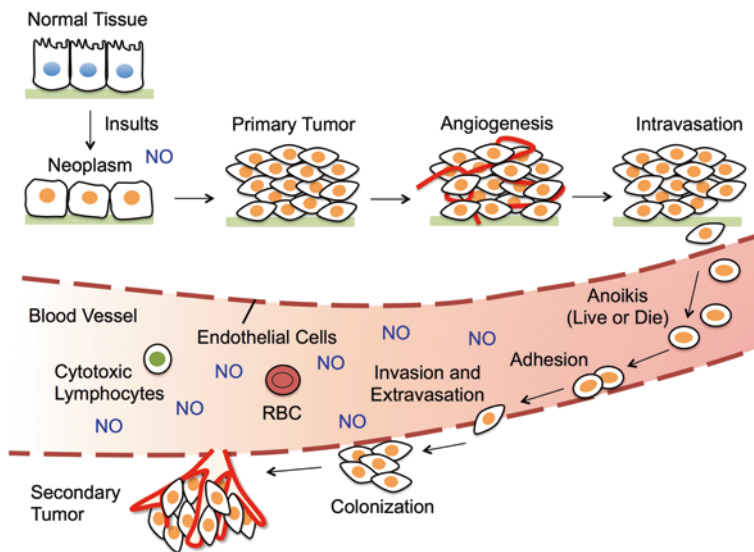


Fig. 8.1 Diagrammatic representation of major steps involved in cancer metastasis. Increased NO production has been associated with many human metastatic tumors. Its effects span from neoplastic transformation to tumor colonization at distant sites

The continuous expansion and progression of a primary tumor frequently leads to metastasis, a process in which a restricted proportion of tumor cells spreads from the primary tumor to form secondary tumors at distant sites. Because metastatic cells are generally resistant to radiation and chemotherapy, they are a major cause of cancer-related death and prime targets for novel cancer therapies [18]. To metastasize, tumor cells must acquire or possess the following properties [19, 20]: (i) unlimited or enhanced proliferative capacity (ii) vascularization within the surrounding host tissues through the synthesis and secretion of angiogenesis factors (iii) local invasion of the host stroma by tumor cells into the blood and/or lymphatic circulation (intravasation) (iv) survival of tumor cells in the circulation (anoikis resistance) (v) adhesion to the capillary wall (vi) invasion and penetration of the cells out of the circulation (extravasation) and (vii) colonization, proliferation, and angiogenesis of tumor cells at distant sites (Fig. 8.1). NO has been shown to participate in all of these steps, which are further discussed below.

S-nitrosylation and Anoikis

Apoptosis or programmed cell death is a tightly regulated process characterized by shrinkage of cells, blebbing of plasma membranes, and condensation and fragmentation of chromatin. Acquired apoptosis resistance is a hallmark of most, if not all, types of cancer that is implicated in the neoplastic evolution of pre-malignant cells

and in cancer metastasis [21]. With regards to metastasis, the loss of cell interaction with neighboring cells and the extracellular matrix (ECM) following intravasation into the circulation triggers apoptosis referred to as *anoikis* [22]. Anoikis prevents detached tumor cells from colonizing elsewhere, thereby, it is a critical step in determining cancer metastasis. Surviving anoikis facilitates subsequent reattachment and colonization of tumor cells at distant sites [23]. Clinical evidence has demonstrated a strong correlation between anoikis resistance in advanced stage cancers and poor survival of patients, strengthening the notion that anoikis resistance is a prerequisite for cancer metastasis [23, 24].

Anoikis is regulated by many signaling pathways, notably by the pro-survival signals phosphoinositide-3-kinase (PI3K)/Akt, extracellular signal-regulated kinases (ERK), Jun N-terminal kinases (JNK), and apoptosis-regulatory signals as well as certain membrane microdomains and oncogenes. A number of direct and indirect evidence suggests that increased NO production suppresses anoikis through *S*-nitrosylation of several target proteins described below.

***S*-Nitrosylation and Pro-Survival Signals**

Abnormal regulation of the phosphatases/kinases including PI3K/Akt activates pro-survival signaling and suppresses anoikis [25]. Numajiri et al. demonstrated that PI3K/Akt on-off signaling was regulated through *S*-nitrosylation of phosphatase and tensin homolog deleted on chromosome ten (PTEN) [26]. *S*-nitrosylation of PTEN by a low level ($\leq 10 \mu\text{M}$) of *S*-nitrosocysteine (SNOC) was shown to inhibit its phosphatase activity and subsequently increases Akt phosphorylation, kinase activity, and cell survival. In many cancers such as glioblastoma, prostate, lung and breast carcinoma, loss of PTEN confers resistance to anoikis [27, 28]. Kwak et al. demonstrated that *S*-nitrosylation of PTEN correlated with its ubiquitin-proteasomal degradation [29]. Although this *S*-nitrosylation-based regulation of PTEN was shown in the experimental model of neurons not cancers, it demonstrates a regulatory mechanism that might account for the loss of PTEN in aggressive tumors.

***S*-Nitrosylation and Apoptosis-regulatory Proteins**

As a form of apoptotic cell death, anoikis is regulated through the common death receptor and mitochondrial apoptosis pathways (Fig. 8.2). The extrinsic death receptor pathway is activated through the cell surface death receptors (DRs) upon binding with specific death ligands such as Fas (CD95) ligand, tumor necrosis factor- α (TNF- α), and TNF-related apoptosis-inducing ligand (TRAIL). The death-inducing signaling complex (DISC) then assembles, activates initiator caspases (caspase-8 or FLICE and caspase-10), which subsequently activate effector caspases (caspase-3, caspase-6 and caspase-7) to cleave cellular substrates. FLIP (FLICE-inhibitory protein) has a higher affinity for the DISC than caspase-8, thus inhibiting caspase-8

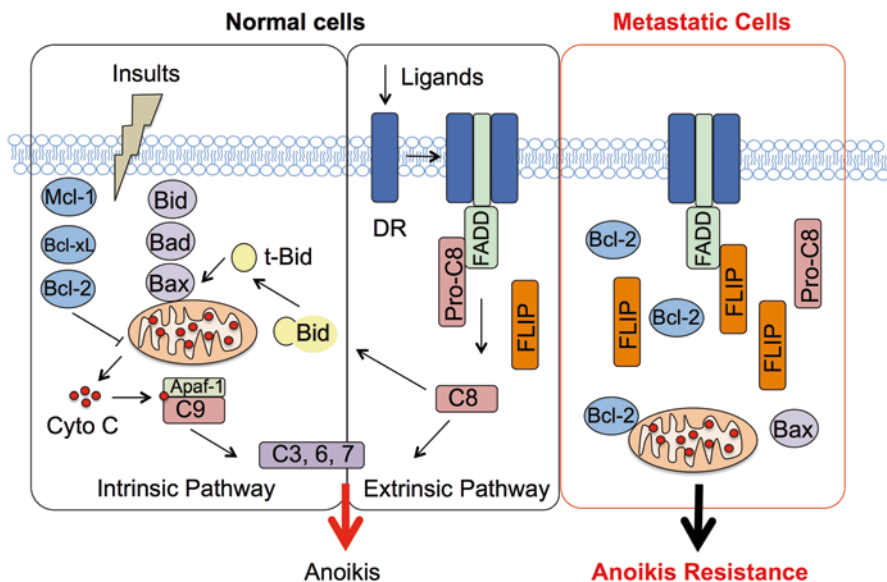


Fig. 8.2 Diagrammatic representation of the intrinsic (mitochondrial) and extrinsic (death receptor) pathway of apoptosis and anoikis subtype. In metastatic cancer cells, increased Bcl-2 and FLIP expression promote anoikis resistance

processing and apoptosis induction [30]. The intrinsic mitochondrial pathway is activated in response to various death signals, e.g. DNA damage, ROS/RNS stress and cytotoxic agents, leading to mitochondrial membrane depolarization, which is controlled by the balance of Bcl-2 family proteins including the anti-apoptotic proteins Bcl-2, Bcl-xL and Mcl-1, and the pro-apoptotic proteins Bax, Bak, Bok, Bim, Bik, Bad and Bid. The subsequent released cytochrome C binds to the caspase adaptor molecule Apaf-1 and recruits the initiator procaspase-9 to form a molecular complex called the apoptosome, which functions to recruit effector caspases to induce apoptosis [31].

Several studies have demonstrated that metastatic malignant cells acquire anoikis resistance through an upregulation of anti-apoptotic proteins such as FLIP [32, 33] and Bcl-2 [34, 35] (Fig. 8.2). NO has been shown to suppress apoptosis induced by various agents including Fas ligand, chemotherapeutic agents, and heavy metals through *S*-nitrosylation of FLIP and Bcl-2 [36–38]. *S*-nitrosylation of these proteins at their cysteine residues prevents their degradation through the ubiquitin-proteasome pathway. We have recently shown that *S*-nitrosylation of FLIP also mediates apoptosis resistance by disrupting its own interaction with an NF- κ B adaptor molecule, receptor-interacting protein 1 (RIP1), which results in NF- κ B activation [39]. Figure 8.3a illustrates that FLIP binds to RIP1 in the absence of the death ligand TNF- α in HEK293 cells, and that this complex is disrupted by TNF- α treatment, which results in the translocation of RIP1 to the cell membrane. Lack of

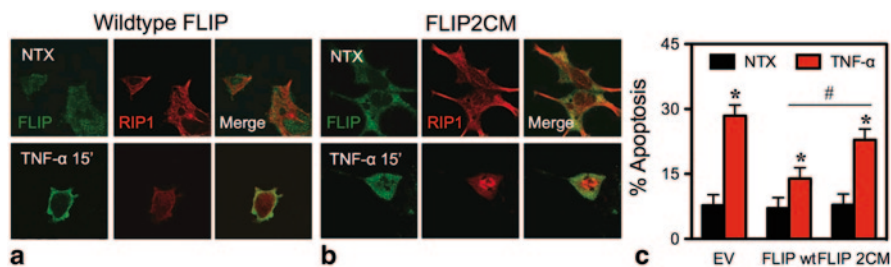


Fig. 3 *S*-nitrosylation of FLIP mediates its interaction with RIP1 and subsequent anti-apoptotic function. **A, B**, HEK-293 cells were transfected with wild-type FLIP **a** or FLIP2CM mutant. **b** Together with RIP1 plasmids. Cells were then treated with TNF- α (50 ng/mL) for 15 min and analyzed for FLIP/RIP1 colocalization by confocal microscopy. **c** Effect of *S*-nitrosylation on anti-apoptotic activity of FLIP. MCF-7 cells were transfected with empty vector (EV), wild-type FLIP or FLIP2CM mutant plasmid, after which they were treated with TNF- α (50 ng/mL) for 16 h. Apoptosis was then determined by flow cytometry using annexin V and propidium iodide assays. Both early and late apoptosis were combined and plotted. * $p < 0.05$ versus non-treated EV control. # $p < 0.05$ versus treated FLIP wild-type cells

FLIP *S*-nitrosylation in a FLIP double-cysteine mutant (FLIP2CM) inhibits the RIP1 translocation (Fig. 8.3b). The FLIP-RIP1 complex is believed to contribute to the reversed anti-apoptotic effect of FLIP in human breast carcinoma MCF-7 cells (Fig. 8.3c). Accordingly, it is postulated that NO might exert its anti-anoikis effect through *S*-nitrosylation of FLIP and Bcl-2.

S-Nitrosylation and Caveolin-1

Caveolin-1 is an essential constituent of caveolae, the flask-shaped membrane invaginations that occupy about 20% of the cell membrane [40]. Such invaginations provide a platform for various signaling mechanisms, where caveolin-1 interacts with signaling molecules and controls their subcellular distributions and functions. Caveolin-1 has been shown to play a role in the multidrug resistance of cancer cells partly through its interaction and regulation of multidrug resistance ATP-binding cassette sub-family G member 2 (ABCG2) transporter [41]. In the past decade, the role of caveolin-1 in the regulation of anoikis has gained increasing attention. Caveolin-1 expression has been shown to be associated with poor prognosis and metastasis of several types of cancer, including lung cancer, prostate cancer, renal cell carcinoma, hepatocellular carcinoma, and melanoma [42–44]. Ectopic expression of caveolin-1 was shown to prevent anoikis through various mechanisms, including p53 inactivation, upregulation of insulin-like growth factor (IGF)-I receptor, activation of Akt, and Mcl-1 stabilization in cancer cells [45–48]. A previous study by our group has shown that caveolin-1 expression is downregulated during anoikis through ubiquitin-proteasomal degradation and that NO inhibits this process by inducing *S*-nitrosylation of the protein, thus providing a mechanism by which cancer

cells acquire anoikis resistance [49]. In this study, caveolin-1 was shown to be nitrosylated and resistant to proteasomal degradation upon treatment with NO donors such as sodium nitroprusside (SNP) and diethylenetriamine (DETA) NONOate. Such treatments also inhibited anoikis, the effect that can be reversed by blocking caveolin-1 *S*-nitrosylation, thus supporting the role of *S*-nitrosylation in anoikis resistance of cancer cells.

***S*-Nitrosylation and Cell Migration and Invasion**

Cell migration and invasion are the critical steps of cancer metastasis. To intravasate into the blood or lymphatic circulation and to extravasate out of the circulation, primary tumor cells must migrate and invade through the epithelial and vascular basement membranes and surrounding extracellular matrix [50]. It has been established that only a small fraction of primary tumor cells becomes invasive and eventually metastatic at any given time. NO has been reported to have both promoting and inhibitory effects on tumor cell mobility through the regulation of multiple proteins depending on its concentration. The role of *S*-nitrosylation of specific proteins on cell motility is discussed below.

***S*-Nitrosylation and Caveolin-1**

As mentioned above, caveolin-1 is subjected to *S*-nitrosylation and is associated with metastasis and poor patient survival. In human lung carcinoma cells, we previously reported that NO promoted malignant transformation of the cells through a caveolin-1-dependent mechanism [49]. Caveolin-1 was also shown to promote both cell migration and invasion in human lung cancer and melanoma cells as indicated by their increased motility upon caveolin-1 overexpression and by decreased motility upon caveolin-1 knockdown [51]. A recent study by Sanuphan et al. demonstrated that prolonged exposure of human lung cancer cells, e.g. up to 14 days, to non-cytotoxic concentrations of DPTA NONOate increased cell motility through both caveolin-1-dependent and independent pathways [52]. In the caveolin-1-dependent pathway, caveolin-1 was found to activate focal adhesion kinase (FAK) and its downstream target Akt, whereas in the caveolin-1-independent pathway, Cdc42 and filopodia were activated. It was postulated that *S*-nitrosylation of caveolin-1 might regulate an on-off pattern that controls the FAK-Akt signaling.

S*-Nitrosylation and c-*Src

c-*Src* (cellular *Src*) is a tyrosine kinase that promotes cell invasion and metastasis in many human cancers, including colon, breast, pancreatic and brain cancer [53].

A previous study by Rahman et al. demonstrated that *S*-nitrosylation of c-Src at cysteine 498 in breast cancer MCF-7 cells is critical for its activation and cell invasion induced by SNAP and β -estradiol [54]. In breast cancer cells that have estrogen receptors with minimal invasive property such as MCF-7 cells, the promoting effect of β -estradiol is dependent on NO through eNOS induction. Further, FAK was found to be a substrate of c-Src since c-Src activation led to tyrosine phosphorylation of FAK. The authors suggested that FAK might be subjected to *S*-nitrosylation since it has the cysteine residue that corresponds to cysteine 498 of c-Src.

***S*-Nitrosylation and EGFR**

Epidermal growth factor receptor (EGFR) contributes to the aggressive nature of basal-like subtype of breast cancer and colon cancers [55]. Previous studies have shown that iNOS expression is associated with EGFR phosphorylation and poor disease outcome of estrogen receptor negative (ER⁻) breast cancer patients [56]. Likewise, NO, at physiological concentrations, promoted ER⁻ cell migration, thus suggesting that iNOS/NO signaling is involved in the cell aggressiveness [56]. *S*-nitrosylation of EGFR (and c-Src) in ER⁻ breast cancer MDA-MB-468 cells induced by DETA/NO resulted in the activation of EGFR/c-Src kinases, which led to the induction of oncogenic c-Myc, Akt, STAT3, and β -catenin signaling pathways as well as the inhibition of tumor suppressor PPA2 [57]. One of the clinically relevant phenotypes of basal-like breast cancer is the CD44⁻/CD24⁺ cancer stem cell subpopulation, which requires STAT3 signaling for its proliferation. NO signaling via *S*-nitrosylation of EGFR was shown to upregulate CD44 expression concomitantly with STAT3 phosphorylation, suggesting its role in cancer stem cell regulation [57].

***S*-Nitrosylation and Ras**

The Ras superfamily of small GTPase consists of many subfamilies, including Ras, Rho, and Rab. Among these, H-Ras, N-Ras, and K-Ras are the clinically most notable members because of their implication in cancers and their role as proto-oncogenes [58]. Mutations and activation of Ras proto-oncogenes have been found in about 30% of all human cancers. Ectopic expression of human or rodent H-Ras in noncancerous cells leads to increased invasiveness and acquisition of the metastatic phenotype [59]. Interestingly, these Ras proteins contain redox active residues that are sensitive to NO modifications [60]. Lim et al. reported that *S*-nitrosylation of H-Ras is required for its tumor promoter function [61]. Knockdown of wild-type H-Ras in the oncogenic K-Ras-driven pancreatic tumor CFPac1 cells reduced tumor xenograft growth in immunocompromised mice, the effect that can be reversed by re-expression of the wild-type H-Ras but not the H-Ras mutant lacking *S*-nitrosylation at cysteine 118. However, Raines et al. reported the suppressive role of *S*-nitrosylation on the tumorigenic effect of H-Ras in N293 cells (HEK-293

ectopically transfected with nNOS) [62]. It was suggested that the difference in NO sources, e.g. nNOS or iNOS, may attribute to the differential tumorigenic response.

K-Ras was reported to regulate colon cancer cell migration through a caveolin-1-dependent mechanism [63]. Ectopic expression of K-Ras in colon cancer HCT116 cells upregulated the expression of caveolin-1 through the Akt pathway, and caveolin-1 was, in turn, required for K-Ras signaling in promoting the HCT116 cell migration. Although the role of NO in the K-Ras/caveolin-1 regulatory axis has not been firmly established, it is likely that NO regulates this axis as both K-Ras and caveolin-1 are known targets for *S*-nitrosylation.

***S*-Nitrosylation and FLIP**

FLIP is a key anti-apoptotic protein involved in the regulation of cell death. It also plays a role in cancer cell motility [64]. Downregulation of FLIP in human cervical cancer HeLa cells by siRNA impaired cell motility by enhancing Akt activity. As described earlier, *S*-nitrosylation of FLIP inhibits its ubiquitination and subsequent proteasomal degradation, thereby, stabilizing the protein and sustaining its anti-apoptotic activity. Although there is no direct evidence for the role of *S*-nitrosylation in HeLa cell motility, indirect evidence suggests its involvement. For example, inhibition of iNOS and NO production was reported to suppress HeLa cell migration and invasion as well as xenograft tumor growth [65, 66].

***S*-Nitrosylation and MMP-9**

There has been a strong correlation between matrix metalloproteinases (MMPs) and ECM degradation and cancer cell invasion [67]. MMP-9 is a key proteinase that efficiently degrades native collagen type IV and V, fibronectin, entactin, and elastin. Its expression is elevated in various solid malignancies, including breast, bladder, prostate and ovarian cancer [68]. *S*-nitrosylation of MMP-9 was facilitated by its colocalization with iNOS at the leading edge of migrating trophoblasts, where NO production occurred. *S*-nitrosylation of MMP-9 resulted in its activation and increased trophoblast migration and invasion [69]. It is conceivable that the promoting effect of NO on tumor cell motility may be mediated, in part, through *S*-nitrosylation of MMP-9.

***S*-Nitrosylation and Angiogenesis**

Angiogenesis, the physical process of new blood vessel formation, is an essential step for tumor growth and metastasis. Angiogenesis involves endothelial cell migration and vascular permeability which are subjected to NO regulation [70, 71]. In this

process, NO is synthesized through eNOS upon stimulation with angiogenic factors such as the vascular endothelial growth factor (VEGF) [72]. While *S*-nitrosylation of eNOS itself suppresses its enzymatic activity and, thus, NO production, *S*-nitrosylation of many other target proteins in the close proximity of eNOS and in the microenvironment enriched with NO enhance angiogenesis as further discussed below.

***SDF-1 α* and *S*-Nitrosylation of MKP7**

Stromal cell-derived factor-1 α (SDF-1 α), also called CXCL12, is one of the most potent pro-angiogenic CXC chemokines that plays a role in angiogenesis. In aortic endothelial cells, SDF-1 α stimulated cell migration through eNOS activation [73]. Increased NO production by eNOS led to *S*-nitrosylation of MAP kinase phosphatase 7 (MKP7) and subsequent suppression of its activity. Under a basal condition, JNK3 is inactivated by MKP7. *S*-nitrosylation of MKP7 causes sustained JNK3 activation and ultimately endothelial cell migration and angiogenesis.

***S*-nitrosylation and β -catenin**

An increase in vascular permeability is one of the early events during angiogenesis and a key characteristic of the newly formed vasculature in tumors. The vascular permeability is controlled by the adherens junction complex consisting of β -catenin and VE-cadherin. In aortic endothelial cells, such complex is regulated by *S*-nitrosylation of β -catenin, which facilitates its dissociation from VE-cadherin and reorganization of the adherens junction [74]. Together with its tyrosine phosphorylation by Src, *S*-nitrosylation of β -catenin promotes the disruption of adherens junction and increases endothelial permeability.

Conclusion

The effect of NO on tumor biology is broad, spanning from tumor initiation of cellular transformation to tumor progression of the metastatic cascade. Protein *S*-nitrosylation is a PTM process that has gained increasing prominence rivaling other known PTMs such as phosphorylation and ubiquitination. *S*-nitrosylation controls the function and activity of many cancer-associated proteins, thus, its dysregulation could lead to carcinogenesis and metastasis. Currently, numerous efforts have been made to develop novel anticancer therapeutics based on *S*-nitrosylation [75]. In this article, we review the role of protein *S*-nitrosylation in anoikis resistance, cell migration and invasion, and angiogenesis, which are key determinants of cancer metastasis. While most studies have indicated the positive regulatory role of

protein *S*-nitrosylation in cancer progression and metastasis, the suppressive role of this post-translational process has also been reported similarly to the observed dichotomous effects of NO. As we move forward, it will be essential to identify the key determining factors of these effects and answer some unresolved questions such as what tumor-associated proteins are involved, what are their mechanisms of action, how localization of NOS contributes to the protein *S*-nitrosylation, and how differential amounts of NO regulate the *S*-nitrosylation process. The past decade has provided exciting new discoveries on the diverse role of protein *S*-nitrosylation in cancer biology, but obviously we have only just started.

Acknowledgements This work was supported by the National Institutes of Health Grants R01-HL095579 and R01-ES022968.

Conflicts of Interest No potential conflicts of interest were disclosed.

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