Reactive Nitrogen Posttranslational Modifications of Proteins in Carcinogenesis

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Vasily A. Yakovlev and Ross B. Mikkelsen

Contents

Abstract

Molecular and epidemiological evidence has established important roles for reactive oxygen/reactive nitrogen species (ROS/RNS) in tumor cells, supporting stromal cells and infiltrating leukocytes in tumor initiation and progression. RNS-dependent posttranslational protein modifications (tyrosine nitration and S-nitrosylation) are well-accepted markers of the tissue inflammation, but also modulate the functions of proteins critical in carcinogenesis and tumor growth. Normal tissues utilize predominantly the stable free radical NO• in their

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signaling pathways, whereas tumor tissues appear to thrive under conditions of elevated RNS other than NO•. As a consequence, different downstream pathways can be modulated upon stimulation of nitric oxide synthase (NOS) activity. This switching mechanism may account for the different responses of normal and tumor tissues to an inflammatory environment and explain why NOS activity is cytoprotective for tumor cells.

The hallmarks of cancer comprise biological capabilities acquired during tumor progression and that facilitate tumor evolution and ultimately survival. A recent review by Hanahan and Weinberg updates the list of hallmarks to include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genome instability, inflammation, reprogramming of energy metabolism, and evading immune destruction (Hanahan and Weinberg, 2011). An important question is whether these hallmarks are relatively independent or can influence the activities of each over. This chapter describes how inflammation modulates the other hallmarks by RNS-dependent posttranslational protein modifications.

Keywords

Angiogenesis • Apoptosis • Cancer hallmarks • Carcinogenesis • DNA repair • Genetic instability • Glycolysis • Inflammation • Invasion • Metastasis • Nitric oxide synthase (NOS) • NOS uncoupling • Posttranslational protein modification • Proliferation • Reactive nitrogen species (RNS) • Reactive oxygen species (ROS) • S-nitrosylation • Tyrosine nitration

Introduction

Molecular and epidemiological evidence has established important roles for ROS/RNS in tumor cells, supporting stromal cells and infiltrating leukocytes in tumor initiation and progression (Wink et al. 1998; Mikkelsen and Wardman 2003). For example, a constitutive nitric oxide synthase isoform (eNOS) is essential for oncogenic Ras-driven tumor growth (Lim et al. 2008), and RNS inhibition of protein tyrosine phosphatases (PTP) appears critical for the autocrine activation of receptor tyrosine kinases (Xu et al. 2005; Chen et al. 2008; Lou et al. 2008). Other studies have demonstrated that inhibiting NOS in tumor-bearing animals inhibits tumor growth in part by targeting the tumor vasculature (Jadeski et al. 2003; Sonveaux et al. 2003; Fukumura et al. 2006; Cardnell and Mikkelsen 2011).

NOS catalysis can generate either NO•, as observed in S-nitrosylation of PTP Cys or enhanced cGMP generation, or other RNS such as peroxynitrite. Peroxynitrite generation results in Tyr nitration or oxidation of Cys to sulfenic or sulfinic acid. One mechanism that can explain this dual activity is the coupling state of the NOS catalytic cycle (Alp and Channon 2004; Crabtree et al. 2008, 2009). For all NOS isoforms, there is a transfer of electrons coupled with the oxidation of arginine with the cofactor tetrahydrobiopterin (BH_4) donating electrons to the NOS ferrous dioxygen complex to initiate oxidation. The ratio of BH_4

to its oxidation product dihydrobiopterin (BH_2) is critical since BH_2 binds to NOS with equal affinity but in a catalytically nonproductive way. When BH_4/BH_2 ratio is low, for example, in chronic inflammatory conditions, coupling is less efficient and more superoxide ($O_2^{\bullet -}$) is generated. Under these conditions, NOS is described as a "peroxynitrite synthase" (Mikkelsen and Wardman 2003; Alp and Channon 2004; Crabtree et al. 2008, 2009). NOS uncoupling can also occur under conditions of low arginine, for example, with elevated expression of arginase-2 (Toby et al. 2010; Durante et al. 2007). Regardless of the uncoupling mechanism, the consequences potentially represent a critical switching mechanism for cell growth. When coupled, the primary product of NOS is NO• and downstream signaling is dominated by NO•-dependent pathways including sGC/PKG and S-nitrosylation. Uncoupled NOS on the other hand produces potent oxidants such as peroxynitrite (ONOO−) and •OH initiating different downstream signaling. For example, ONOO− or other nitrating RNS activate NF-κB by nitration of Tyr181 on IκBα (Yakovlev et al. 2007), whereas S-nitrosylation of the p65 subunit, or IKKβ, inhibits NF-κB activity (Marshall and Stamler 2001; Reynaert et al. 2004; Kelleher et al. 2007). Furthermore, prolonged exposure to oxidizing ROS/RNS causes irreversible thiol oxidation and changes in protein function, for example, irreversible inhibition of PTP activity. This has been observed with PTP1B and other PTPs and is associated with constitutive inactivation of PTP activity in tumor cells that express elevated levels of ROS (Xu et al. 2005; Chen et al. 2008; Lou et al. 2008).

RNS as signaling molecules have been studied in terms of four primary mechanisms:

(1) binding to metal centers,
(2) nitrosylation of thiol and amine groups,
(3) nitration of Tyr, and
(4) oxidation of thiols.

The most extensively studied is NO• binding to the heme of soluble guanylate cyclase, stimulating cGMP production and activating of protein kinase G. NO• also binds to the heme of cytochrome *c* oxidase with consequential inhibition of cytochrome c oxidase activity. This is one proposed mechanism of mitochondrial electron transport regulation that minimizes mitochondrial ROS generation (Xu et al. 2005). Recent studies on RNS-dependent signal transduction mechanisms were mostly focused on two protein modifications, in particular, S-nitrosylation of thiols and nitration of Tyr residues.

S-nitrosylation fulfills the criteria of a physiologically relevant signal in that it can be specific and reversible, occurs on physiological time scale, and, depending on a target, can result in either activation or inhibition (Janssen-Heininger et al. 2008; Forrester et al. 2009). Protein tyrosine nitration as posttranslational modification in cellular signaling is less understood (Ischiropoulos 2003). Although initially considered to be a marker of oxidative stress, there is a growing body of experimental data suggesting that nitration of tyrosine fulfills the criteria of a signal-transducing mechanism (Ischiropoulos 2003; Gow et al. 2004; Yakovlev and Mikkelsen 2010). For example, tyrosine

nitration has been detected under physiological conditions in most organ systems and in a number of cellular models. Furthermore, accumulating data support a strong link between protein tyrosine nitration and the activation of signaling pathways in a variety of cellular responses and pathological conditions, including the cellular response to irradiation, acute and chronic inflammation, graft rejection, chronic hypoxia, tumor vascularization and the microenvironment, atherosclerosis, myocardial infarction, chronic obstructive pulmonary disease, diabetes, Parkinson disease, and Alzheimer disease (MacMillan-Crow et al. 1996; Giasson et al. 2000; Blantz and Munger 2002; Reynolds et al. 2005; Pacher et al. 2007; Reynolds et al. 2007; Donnini et al. 2008; Naito et al. 2008; Reyes et al. 2008; Upmacis 2008; Jones et al. 2009; Koeck et al. 2009; Pieper et al. 2009; Smith 2009; Brindicci et al. 2010; Kang et al. 2010; Pavlides et al. 2010; Zhang et al. 2010).

The hallmarks of cancer comprise biological capabilities acquired during tumor progression and that facilitate tumor evolution and ultimately survival. A recent review by Hanahan and Weinberg updates the list of hallmarks to include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genome instability, inflammation, reprogramming of energy metabolism, and evading immune destruction (Hanahan and Weinberg 2011). An important question is whether these hallmarks are relatively independent or can influence the activities of each over. This chapter describes how inflammation modulates the other hallmarks (apoptosis, genome instability, invasion and metastasis, etc.) by RNS-dependent posttranslational protein modifications.

Inflammation

Different types of infections and chronic tissue inflammation are recognized risk factors for human cancers at various sites. RNS and ROS produced in infected and inflamed tissues can contribute to the process of carcinogenesis by different mechanisms. RNS-dependent posttranslational proteins modifications (tyrosine nitration and S-nitrosylation) are well-accepted markers of the tissue inflammation, but also modulate the functions of proteins critical in carcinogenesis and tumor growth. RNS-dependent posttranslational modifications can up- or downregulate functions of many proteins. Below, we show examples of such protein modifications and their roles in the stimulation of the different hallmarks of cancer.

The “Big Players”

The “Big Players” are the proteins involved in modulation of multiple signaling pathways and are able to regulate a number of cancer hallmarks simultaneously. It makes them very attractive targets for posttranslational modification or/and mutations in the process of tumor development.

p53: Apoptosis, Initiation of the DNA Repair, and Glycolysis

p53 transactivates genes encoding proteins responsible for DNA repair, cell-cycle arrest, and/or the induction of apoptosis in cells harboring non-repairable damaged DNA. High doses of exogenous RNS donors induce DNA damage and promote p53 transcriptional activity by a classical ATM/ATR-dependent mechanism (Hofseth et al. 2003; Wang et al. 2003). These early studies report that lower RNS donor concentrations also stimulate unequivocal nuclear retention of p53 but by mechanism(s) not requiring ATM/ATR-dependent p53 Ser15 phosphorylation. It was further explored showing that low RNS donor concentrations stimulate nitration of Y327 in the p53 tetramerization domain promoting oligomerization, nuclear accumulation, and increased DNA-binding activity without Ser15 phosphorylation (Yakovlev et al. 2010). A difference in the pattern of p53 target gene expression at low and high doses of RNS is also observed. Regulation of Bax, Cyclin B, GADD45, MDM2, and MSH2 expression levels by high doses of the NO• donor mimics the ionizing radiation-dependent regulation pattern and is significantly different quantitatively and qualitatively from that following treatment at lower doses of the donor. Other studies have demonstrated that depending on concentration, RNS can alternatively decrease or enhance p53 transcriptional activity (Calmels et al. 1997). There are at least two possible mechanisms for this. Because eight of the nine tyrosine residues present in p53 are located in the critical DNA-binding region, additional nitration of some of these residues may downregulate the p53/DNA-binding activity. Secondly ONOO- is reactive to the Zn^{2+}-thiolate center of many zinc finger transcription factors and thus may modify the zinc-bound Cys residues in the core domain of p53 and compromise the protein's activity (Hainaut and Mann 2001). p53 downregulation results not only in the inhibition of apoptosis, senescence, and DNA repair but also in the stimulation of aerobic glycolysis (Warburg effect), via TP53-dependent glycolysis and apoptosis regulator (TIGAR)-dependent pathway (Bensaad et al. 2006).

Hence, different types of RNS at different doses produce variable patterns of p53 posttranslational modifications and as a consequence variable changes in p53 functions. These types of the structural/functional protein modifications are not unique to p53. As discussed below, numerous proteins exhibit a variety of posttranslational modifications depending on the amount and type of RNS (Bayden et al. 2011). This in large part explains the variable and in many cases conflicting physiological responses to RNS exposure reported in the literature.

NF-κB: Proliferation, Invasion, Angiogenesis, and Metastasis

Constitutive activation of the transcription factor NF-κB is an emerging hallmark of various types of tumors. Many of the signaling pathways implicated in cancer are networked to the activation of NF-κB. Its activation controls the expression of

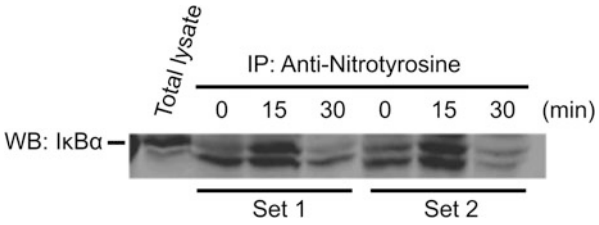

Fig. 127.1 *Tyrosine nitration of IκBα.* Two sets of mice with MDA-231 breast cancer xenografts were irradiated. Tumor cell lysates were prepared at indicated times and immunopurified nitrated proteins were analyzed by Western blot for IκBα. Each lane represents a single animal. Reasons for the doublet in the lysate and IPs are not known (Original unpublished data from R. Mikkelsen)

genes that mediate transformation, proliferation, invasion, angiogenesis, and metastasis (Grivennikov et al. 2010; Prasad et al. 2010). NF-κB can induce several of these cellular alterations by producing inflammation and has been shown to be associated with development of cancer. Suppression of NF-κB inhibits the growth of tumor cells, leading to the concept of "NF-κB addiction" in cancer cells (Chaturvedi et al. 2011).

Clinically relevant doses of ionizing radiation (IR) activate NF-κB predominantly by a mechanism requiring constitutive NOS activity, nitration of Y181 of the inhibitor protein IκBα and its release from the active transcription factor complex, p50/p65 (Yakovlev et al. 2007). Nitration is oscillatory with a 15 min frequency similar to IκBα phosphorylation after treatment of cells with TNF (Hoffmann et al. 2002). This mechanism differs from treatment with TNF or high IR doses (>10 Gy) is not involving IKKβ-dependent phosphorylation and proteolysis of IκBα. IKKβ-dependent phosphorylation of the p65 subunit may, however, still be a requirement for nuclear import of the p50/p65 transcription factor. This nitro-oxidative mechanism is also relevant to basal NF-κB activity of tumor cells since 30–50 % of basal NF-κB activity is sensitive to either NOS inhibitors or dominant negative NOS mutants (Yakovlev et al. 2007). IR-induced Tyr nitration of IκBα with identical transient kinetics is also observed in the breast cancer cell line MDA-MB-231 grown as a tumor xenograft (Fig. 127.1).

As in case of p53, NF-κB also demonstrates RNS-dependent downregulation. Interesting, for both nuclear factors (p53 and NF-κB) mechanisms of activation depend on Tyr nitration, whereas inhibition depends on S-nitrosylation. RNS repress NF-κB activity by direct S-nitrosylation of p65 as well as p50 (Marshall and Stamler 2001; Kelleher et al. 2007), or by S-nitrosylation and inhibition of IKKβ (Reynaert et al. 2004). As in the case of p53, S-nitrosylation of p65 or p50 decreases DNA-binding activity of p65/p50 heterodimer and block activation of NF-κB-dependent genes. S-nitrosylation of IKKβ, more proximal target, inhibits activity of this kinase and downregulates IKK-dependent signals of NF-κB activation.

Akt: Proliferation, Apoptosis, Angiogenesis, and Glycolysis

Phosphoinositide 3-kinase (PI3K) pathway exerts its effects through Akt, its downstream target molecule, and thereby regulates various cell functions including cell proliferation, cell transformation, apoptosis, tumor metabolism, and angiogenesis. PTEN, the phosphatase and tensin homologue deleted on chromosome 10, is one of the most frequently mutated tumor suppressors in human cancers. The lipid phosphatase activity of PTEN dephosphorylates PIP3 to generate PIP2 and thus antagonizes the PI3K activity in the activation of Akt. It was recently shown that PTEN is exclusively S-nitrosylated by low concentrations of RNS at Cys83. This posttranslational modification inhibits PTEN activity and consequently stimulates downstream Akt signaling (Numajiri et al. 2011). Activated Akt plays a key role in mediating signals for cell growth, cell survival (anti-apoptotic), and cell-cycle progression (Brazil et al. 2004). Activation of Akt also plays a crucial role in the stimulation of vascular endothelial growth factor (VEGF) and other factors of tumor angiogenesis (Chen et al. 2005; Ma et al. 2009). PI3K/Akt/mTOR pathway regulates glycolysis through the HIF1α/c-Myc–hnRNPs/PKM2 network (Sun et al. 2011). Activation of PI3K/Akt/mTOR switches on PKM2 production through upregulation of HIF1α-mediated transcriptional activation, and the c-Myc–hnRNPs regulated alternative splicing. It was recently shown that mTOR is a major positive regulator of the aerobic glycolysis (Warburg effect), not only in cancer cells but also in benign tumor cells and even in premature senescent primary cells (Sun et al. 2011).

Apoptosis

Inhibition of spontaneous and metabolically induced apoptosis is one mechanism underlying carcinogenesis, facilitating tumor progression and limiting the effectiveness of cancer treatment. RNS has been shown to possess both pro-apoptotic and anti-apoptotic functions, depending on the cell type, cellular redox state, and most importantly on the concentration and flux of RNS (Kolb 2000; Davis et al. 2001). Induction of apoptosis by *exogenous* RNS has been attributed to its ability to induce oxidative stress and caspase activation (Klein and Ackerman 2003). In contrast, *endogenous* RNS production or the exposure to RNS levels characteristic of chronic inflammation in various in vivo and in vitro experimental models leads to apoptosis inhibition, e.g. (Liu et al. 1998; Chung et al. 2001).

Caspase activation occurs through two general mechanisms: death receptors and/or mitochondrial cytochrome *c* release (Mannick et al. 1997; Ghafourifar et al. 1999). The death receptor pathway is initiated by caspase-8, whereas the mitochondrial pathway is dependent on caspase-9. Both death receptor- and mitochondria-initiated apoptotic cascades involve the downstream of caspase-3, caspase-6, and caspase-7 (Thornberry and Lazebnik 1998). RNS inhibit apoptosis

downstream of cytochrome *c* release by blocking caspase-9 activation through nitrosylation of the procaspase (Torok et al. 2002). Others have shown S-nitrosylation of active site Cys and as a consequence inhibition of caspase-3 and caspase-8 (Kim et al. 1997, 2000; Rossig et al. 1999).

Protein kinase Bα (PKBα, or Akt1) has a crucial role in programmed cell death. Akt1 is activated by phosphorylation at multiple sites and subsequently phosphorylates and activates eNOS. The high levels of RNS that are produced under stress conditions result in *S*-nitrosylation of PKBα/Akt1 on Cys296, blocking disulfide bond formation between Cys296 and Cys310 and suppressing the biological effects of this protein (Lu et al. 2005).

S-nitrosylation can prevent apoptosis by the stabilization of anti-apoptotic proteins, such as c-FLIP and Bcl-2 (Azad et al. 2006; Iyer et al. 2008). Bcl-2 is a key apoptosis regulatory protein of the mitochondrial death pathway. The oncogenic potential of Bcl-2 is well established, with its overexpression reported in various cancers. Generation of RNS has no effect on Bcl-2 phosphorylation but induces S-nitrosylation of the protein, inhibiting its ubiquitination and subsequent proteasomal degradation (Azad et al. 2006; Chanvorachote et al. 2006). S-nitrosylation of Bcl-2 also has a crucial role in apoptosis resistance and malignant transformation of human lung epithelial cells in response to Hexavalent chromium Cr (VI) exposure (Azad et al. 2010b). Interestingly, RNS-mediated *S*-nitrosylation and stabilization of Bcl-2 protein is the primary mechanism involved in the malignant transformation of non-tumorigenic lung epithelial cells in response to long-term carcinogen exposure (Azad et al. 2010a). FLICE-inhibitory protein (FLIP) is a key apoptosis regulatory protein of the death receptor-mediated pathway. FLIP inhibits apoptotic signaling by interfering with the binding of caspase-8 to FADD at the death-inducing signaling complex (Irmler et al. 1997; Tschopp et al. 1998). FLIP is involved in rendering cells resistant to death receptor-mediated apoptosis (Irmler et al. 1997; Tschopp et al. 1998; Abedini et al. 2004), and elevated expression of FLIP is associated with tumor cells that can escape from immune surveillance in vivo (Medema et al. 1999). Furthermore, downregulation of FLIP by cytotoxic agents has been shown to sensitize cells to Fas-mediated apoptosis (Kinoshita et al. 2000). Recent findings demonstrate an important role of RNS in FLIP regulation and its anti-apoptotic function in Fas death signaling (Chanvorachote et al. 2005). The mechanism by which RNS regulate FLIP involves *S*-nitrosylation and its inhibition of ubiquitin-proteasomal degradation.

Apoptosis signal-regulating kinase 1 (ASK1) is a serine/threonine protein kinase that functions as a MAPK kinase in the JNK and p38 MAPK signaling pathways (Ichijo et al. 1997). ASK1 is activated by exposure of cells to various stimuli, including tumor necrosis factor-α, Fas ligand, hydrogen peroxide, genotoxic agents, microtubule-interfering drugs, and osmotic stress (Ichijo et al. 1997; Ichijo 1999). Activation of ASK1 is associated with apoptotic cell death under various conditions (Ichijo et al. 1997; Ichijo 1999; Hatai et al. 2000). For example, RNS mediate the interferon-γ-induced inhibition of ASK1 in L929 cells by direct S-nitrosylation of ASK1 (Park et al. 2004). Based on co-immunoprecipitation data, RNS inhibit binding of wild type of ASK1, but not mutant ASK1(C869S), to its substrates – mitogen-activated protein kinase kinase 3 (MKK3), also known as MEK3, and MKK6.

Genome Instability

Accumulative DNA damage can result from the following: (a) DNA damage in excess of a cell's repair capacity, (b) DNA damage that is incapable of being repaired, or (c) inactivation of DNA repair mechanisms. Although RNS and ROS can directly damage DNA, mammalian cells have complex and powerful mechanisms to repair the genome including base excision repair (BER), nucleotide excision repair, transcription-coupled repair, double strand break repair, and mismatch repair. Several key DNA repair enzymes are inhibited by RNS-mediated S-nitrosylation of active site Cys. DNA repair enzymes with vulnerable active site Cys residues include 6-*O*-alkyl DNA transferase, formamidopyrimidine glycosylase, xeroderma pigmentosum A protein, and DNA ligases (see review (Jaiswal et al. 2001)).

Although DNA oxidative lesions, the predominant mechanism of DNA damage in inflammation, can be excised from DNA by multiple pathways, BER is quantitatively the most important. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), the most abundant oxidative DNA lesion, is excised in humans by 8-oxodeoxyguanosine DNA glycosylase 1 (hOgg1) (Rosenquist et al. 1997). HOgg1 contains critical cysteines in a Zn^{2+} finger at its active sites (Tani et al. 1998). hOgg1 activity can be inhibited by NO• and ONOO− by *S*-nitrosylation of the Zn^{2+} finger Cys residues and ejection/loss of Zn^{2+} (Jaiswal et al. 2000).

O(6)-Alkylguanine-DNA alkyltransferase (AGT) plays a critical role in protection from the carcinogenic effects of simple alkylating agents by repairing O(6)-alkylguanine adducts via a direct transfer reaction. hAGT is readily but reversibly inactivated by the formation of S-nitrosylcysteine at Cys145, which is the alkyl acceptor site (Liu et al. 2002). The facile reaction of this Cys residue with RNS is attributable to its interaction with other residues in AGT including His146 and Glu172 that activate the sulfhydryl group of Cys145 facilitating its nucleophilic attack on DNA adducts. Although the S-nitrosylcysteine adduct in hAGT is readily reversible by reaction with other cellular thiols, the formation of S-nitrosocysteine at Cys145 leads to the rapid degradation of the hAGT protein in vivo. This degradation is brought about by the ubiquitin/proteasomal system. The formation of an S-nitrosylcysteine at Cys145 in hAGT in response to RNS stimulates the ubiquitination of the protein.

Abundance and activity of *S*-nitrosoglutathione reductase (GSNOR), a protein critical for control of protein *S*-nitrosylation, are significantly decreased in about 50 % of hepatocellular carcinoma (HCC) patients. It was shown that one of the proteins highly susceptible to *S*-nitrosylation by iNOS is AGT and that protection of AGT from *S*-nitrosylation requires GSNOR. In vitro recombinant human AGT is susceptible to *S*-nitrosylation at the enzyme active site Cys145 (Liu et al. 2002). These data, taken together, establish a critical role for GSNOR in protection of AGT from hyper-*S*-nitrosylation and proteasomal degradation. In a few human HCC samples that are deficient in GSNOR activity, AGT protein amount is decreased, suggesting possible protection of AGT by GSNOR in human tumors (Wei et al. 2010).

The breast cancer type 1 susceptibility protein (BRCA1) contributes to cell viability in multiple ways, including DNA repair, cell-cycle checkpoint control, transcription, and regulation of chromosome segregation. BRCA1 expression is negatively regulated at the transcriptional level by the repressive complex of retinoblastoma-like protein 2 (RBL2) and E2F4. Formation of the repression RBL2/E2F4 complex can be accelerated by, for example, RBL2 dephosphorylation. Recently, protein phosphatase 2A (PP2A), an enzyme responsible for RBL2 dephosphorylation, was shown to be activated by nitration on Tyr284 (Ohama and Brautigan 2010). Inflammatory levels of RNS/ROS, which don't induce significant DNA damage and maintains the ATM/ATR-dependent pathways intact, stimulate substantial dephosphorylation of RBL2. RBL2 dephosphorylation promotes a repressive RBL2/E2F4 complex formation with subsequent block of BRCA1 expression (Yakovlev 2013). As result, BRCA1-dependent mechanisms of genetic stability are significantly compromised. Interestingly, the same mechanism of BRCA1 downregulation takes place in the different types of cells under hypoxic condition (Bindra et al. 2005). NOS activity and RNS generations are stimulated under certain hypoxic conditions (Feelisch et al. 2008; Mikula et al. 2009; Strijdom et al. 2009), suggests that some pro-carcinogenic effects of hypoxic microenvironment are also RNS dependent.

Evading Growth Suppressors

Two extensively investigated critical suppressors of proliferation are p53, which was discussed above, and retinoblastoma protein (Rb). Practically all tumors have either inactivated p53 or the Rb pathway. Inactivation of Rb by phosphorylation is crucial for the initiation of G1/S transition during the cell-cycle progression. It was shown that high levels of RNS induce mitosis in human breast cancer cells by stimulation of Rb hyper-phosphorylation and inactivation (Radisavljevic 2004). The same RNS-dependent effect was revealed in vivo for mouse models with colitis. The mechanism of RNS-dependent Rb hyper-phosphorylation and inactivation is not completely understood, but preliminary results indicate the sGC/cGMP/PKG and MEK/ERK1/2 pathways (Ying et al. 2007).

Invasion and Metastasis

c-Src is a tyrosine kinase that promotes cancer cell invasion and metastasis. RNS S-nitrosylates c-Src at Cys498 stimulating its kinase activity (Rahman et al. 2010). Cys498 is conserved among Src family kinases. For example, Cys506 of c-Yes (another member of SRC family) corresponding to Cys498 is also important for the RNS-mediated activation of c-Yes. This suggests possible mechanisms for how

estrogens interact synergistically with RNS to induce the proliferation and migration of breast cancer cells. For example, beta-estradiol induces the expression of eNOS with subsequent increase of RNS production in MCF7 cells and mediates activation of c-Src by the S-nitrosylation of Cys498. Furthermore, RNS-dependent activation of c-Src is involved in beta-estradiol stimulation of E-cadherin junctions and enhancement of cell invasion (Rahman et al. 2010).

Anoikis, a detachment-induced apoptosis, is an important mechanism for inhibiting tumor cell metastasis. Tumor cells that acquire anoikis resistance are frequently observed in metastatic disease. In the human lung carcinoma H460 cells, RNS donors sodium nitroprusside and diethylenetriamine NONOate inhibit detachment-induced apoptosis, whereas the NOS inhibitors aminoguanidine and NO• scavenger, 2-(4-carboxyphenyl) tetramethylimidazoline-1-oxyl-3-oxide, promote anoikis (Chanvorachote et al. 2009). Resistance to anoikis in H460 cells is mediated by Cav-1, which is significantly downregulated after cell detachment through a non-transcriptional mechanism involving ubiquitin-proteasomal degradation. RNS inhibit this downregulation by interfering with Cav-1 ubiquitination through a process that involves protein *S*-nitrosylation preventing Cav-1 proteasomal degradation and induction of anoikis.

hTIMP-4 is a member of a group of metalloproteinase inhibitors of matrix metalloproteinase-2 (MMP-2). Activation of hTIMP-4 reduces basal or growth factor-induced invasiveness of both endothelial and fibrosarcoma tumor cells. hTIMP-4 treatment with ONOO− promotes its nitration on tyrosine residues (Y114, Y195, Y188, and Y190) and oligomerization. ONOO−-treated hTIMP-4 loses its inhibitory effect toward MMP-2 activity, consequently increasing endothelial and tumor cell invasiveness (Donnini et al. 2008).

Avoiding Immune Destruction

Antigen-specific CD8+ T cell tolerance, induced by myeloid-derived suppressor cells (MDSCs), is one of the main mechanisms of tumor escape from immune surveillance. MDSCs in vivo directly disrupt the binding of specific peptide major histocompatibility complex (pMHC) dimers to CD8-expressing T cells through nitration of tyrosines in a T cell receptor (TCR)-CD8 complex. This process makes CD8-expressing T cells unable to bind pMHC and to respond to the specific peptide, although they retain their ability to respond to nonspecific stimulation. Nitration of TCR-CD8 is induced by MDSCs through hyperproduction of reactive oxygen species and ONOO− during direct cell-cell contact. Molecular modeling suggests specific sites of nitration that might affect the conformational flexibility of TCR-CD8 and its interaction with pMHC (Nagaraj et al. 2007). This mechanism may explain the well-established fact that tumor-bearing hosts do not have profound systemic immune deficiency and T cells retain their ability to respond to other stimuli, including viruses, lectins, and interleukin 2 (IL-2), among others.

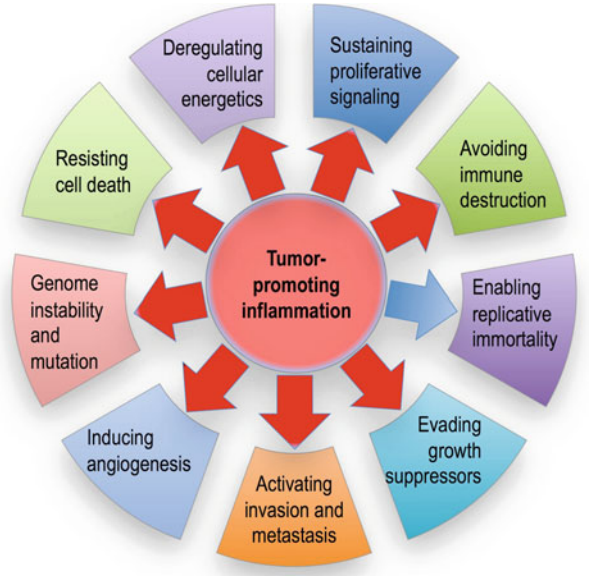

Fig. 127.2 RNS generated under inflammatory conditions modulate all hallmarks of carcinogenesis. *Red arrows* – modulation of hallmarks with revealed examples of RNS-dependent posttranslational modification of the target proteins; *Blue arrow* – modulation of the hallmark is RNS-dependent; however, RNS-specific protein modifications responsible for these modulations are not revealed yet

Enabling Replicative Immortality

Presently there is no direct evidence linking specific RNS-dependent posttranslational modification of target proteins involved in replicative immortality. However, treatment with RNS prevents age-related downregulation of telomerase activity and delays senescence of endothelial cells (Vasa et al. 2000). One of the possible mechanisms of telomerase activation – through the modulation of the PTEN/PI3K/Akt/VEGF pathway – was discussed above. RNS-dependent VEGF activation is capable of inducing the telomerase reverse transcriptase (TERT) expression and telomerase activity (Zaccagnini et al. 2005).

Deregulating Cellular Energetics

The best-characterized metabolic phenotype observed in tumor cells is the Warburg effect, which is a shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations (see review (Cairns et al. 2011)). According to the present knowledge, this effect is regulated mostly by the PI3K/Akt, hypoxia-inducible factor 1(HIF-1), p53, and MYC. HIF-1 is a master transcriptional regulator activated under low oxygen level. HIF-1 targets a large number of genes involved in regulation of glucose metabolism, as well as angiogenesis, apoptosis, and invasion/metastasis. HIF-1 activity is usually suppressed due to the rapid, oxygen-dependent degradation of one of its two subunits, HIF-1a. Recently it was shown that S-nitrosylation of HIF-1α on Cys533

prevents its oxygen-dependent degradation (Li et al. 2007). This mechanism appears to be independent of the prolyl hydroxylase-based pathway that is involved in oxygen-dependent regulation of HIF-1α.

Conclusion

RNS-dependent modifications of the key regulatory proteins in cell signaling pathways are a relatively new area of the research. Nonetheless, a substantial body of evidence demonstrates RNS-dependent modulation of practically all the hallmarks of carcinogenesis (Fig. 127.2). Inflammation, a designated hallmark of carcinogenesis, upregulates the RNS tissue level modulating all other hallmarks of carcinogenesis simultaneously. Normal tissues utilize predominantly the stable free radical NO• in their signaling pathways, whereas tumor tissues appear to thrive under conditions of elevated RNS other than NO•. As a consequence, different downstream pathways can be activated with stimulation of NOS activity. This switching mechanism may account for the different responses of normal and tumor tissues to an inflammatory environment and explain why NOS activity is cytoprotective for tumor cells. Because chronic inflammatory conditions are associated with uncoupled NOS activity, it may also provide a mechanism for the increased incidence of cancer and mortality in patients with chronic inflammatory diseases (such as type 2 diabetes) (Alp and Channon 2004).

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Cross-References

- ▶ Caveolin-1 Regulation of Endothelial Nitric Oxide Synthase Function and Oxidative Stress in the Endothelium
- ▶ Environmental Reactive Oxygen Species and Cancer
- ▶ Pathophysiological Roles of Reactive Oxygen and Nitrogen Species
- ▶ Peroxynitrite Biology
- ▶ Reactive Oxygen Species and Reactive Nitrogen Species in Epigenetic Modifications

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