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Review

Cancer Research

Reactive Oxygen and Nitrogen Species–Induced Protein Modifications: Implication in Carcinogenesis and Anticancer Therapy

Nurbubu T. Moldogazieva[1], Sergey V. Lutsenko[1], and Alexander A. Terentiev[2]

Abstract

Cancer is a complex disorder extremely dependent on its microenvironment and highly regulated by multiple intracellular and extracellular stimuli. Studies show that reactive oxygen and nitrogen species (RONS) play key roles in cancer initiation and progression. Accumulation of RONS caused by imbalance between RONS generation and activity of antioxidant system (AOS) has been observed in many cancer types. This leads to alterations in gene expression levels, signal transduction pathways, and protein quality control machinery, that is, processes that regulate cancer cell proliferation, migration, invasion, and apoptosis. This review focuses on the latest advancements evidencing that RONS-induced modifications of key redox-sensitive residues in regulatory proteins, that is, cysteine oxidation/S-sulfenylation/S-glutathionylation/S-nitrosylation and tyrosine nitration, represent important molecular mechanisms underlying carcinogenesis. The oxidative/nitrosative modifications cause alterations in activities of intracellular effectors of MAPK- and PI3K/Akt-mediated signaling pathways, transcription factors (Nrf2, AP-1, NFκB, STAT3, and p53), components of ubiquitin/proteasomal and autophagy/lysosomal protein degradation systems, molecular chaperones, and cytoskeletal proteins. Redox-sensitive proteins, RONS-generating enzymes, and AOS components can serve as targets for relevant anticancer drugs. Chemotherapeutic agents exert their action via RONS generation and induction of cancer cell apoptosis, while drug resistance associates with RONS-induced cancer cell survival; this is exploited in selective anticancer therapy strategies. *Cancer Res; 78(21); 1–8.* ©2018 AACR.

Introduction

Cancer cells grow in low oxygen concentration environment denoted as hypoxia and adapt their metabolism to meet elevated requirements in energy and nutrients for proliferation and survival. Metabolic reprogramming is a hallmark of cancer cell phenotype that is characterized by increased anaerobic glycolysis, deficiency in oxidative phosphorylation and ATP generation, and the overall mitochondrial dysfunction (1).

In response to hypoxia, an elevated production of reactive oxygen and nitrogen species (RONS) has been observed in various cancer cells (2). Mitochondria and Nox family NADPH oxidases are major internal sources of primary endogenous reactive oxygen species (ROS), superoxide anion radical ($O_2^{\bullet -}$). The latter spontaneously or enzymatically, by superoxide dismutases (SOD), gives rise to hydrogen peroxide (H_2O_2) that, in turn, in the presence of Fe^{2+} produces hydroxyl radical (HO•; refs. 3, 4). Nitric oxide synthases (NOS) use L-arginine to produce primary RNS type, nitric oxide radical, •NO, that interacts with $O_2^{\bullet -}$ yielding another RNS, peroxynitrite ($ONOO^-$; ref. 5).

High reactivity of RONS and toxicity of their elevated concentrations for cells dictate the necessity of scavenging mechanisms. Under physiologic conditions, cellular redox homeostasis is maintained by balance between RONS generation and activity of endogenous antioxidant system (AOS; ref. 6). Both enzymatic and nonenzymatic AOS components terminate RONS propagation to result in low intracellular RONS concentrations that cause reversible oxidative/nitrosative modifications of redox-sensitive residues in regulatory proteins. This underlies redox switches in activities of intracellular effectors of cell signaling pathways and transcription factors. This phenomenon denoted as ROS/RNS signaling plays a key role in regulation of cell proliferation, differentiation, migration, and survival (7).

Oppositely, disbalance between RONS generation and AOS activity causes RONS accumulation and oxidative/nitrosative stress, which contribute to pathogenesis of various diseases including cancer (8). Studies evidence that oxidative/nitrosative stress causes alterations in signal transduction pathways, activity of transcription factors, ubiquitin/proteasomal and lysosomal/autophagy-mediated protein degradation, and cytoskeletal organization to contribute to disturbances in cellular homeostasis and higher level of proteotoxic stress in cancer cells as compared with normal cells (9). This review focuses on recent advancements in studying RONS-induced protein modifications as mechanisms underlying the abovementioned alterations to cause cancer initiation and progression.

Elevated RONS Generation in Cancer

Oxidative/nitrosative stress may both cause and modify tumor growth through multiple cellular and molecular events such as DNA damage and genome instability, shaping tumor

[1]Department of Biotechnology, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia. [2]Department of Biochemistry and Molecular Biology, N.I. Pirogov Russian National Research Medical University, Moscow, Russia.

Corresponding Author: Nurbubu T. Moldogazieva, I.M. Sechenov First Moscow State Medical University (Sechenov University), 8 Trubetskaya street, Moscow 119991, Russia. Phone/Fax: 7-499-2480181; E-mail: nmoldogazieva@mail.ru

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microenvironment, and alteration in cell signaling, which transforms normal cells into malignant and neoplastic cells. Various human cancer types including ovarian, prostate, hepatic, bladder, breast, gastric, oral, and pharyngeal cancers can produce *in vitro* much greater amount of RONS and protein oxidation adducts and have decreased AOS activity as compared with nontransformed cells (10–13). Indeed, in HT29 colon carcinoma cell line, c-Src tyrosine kinase induces ROS generation by Nox1 through Rac-dependent mechanism (14). Hepatocellular carcinoma (HCC) cells overexpressed Nox1, DUOX1, and DUOX2, while normal cells failed to express all the three NADPH oxidases (15). Both DUOX1 and DOUX2 were highly expressed in MHCC-97H and MHCC-97L, but not in Bel-7402 cells. High Nox1/2/5 levels in patients with HCC correlated with expression of genes associated with cell survival and metastasis and poor prognosis (16).

Growth factors and cytokines can stimulate RONS production (Fig. 1). Indeed, TGFβ1 has been shown to activate Rac1 and stimulates ROS generation by NADPH oxidase accompanied by NFκB and MMP-2 activation, IL6 release, and enhanced SW1990 invasiveness (17). In addition, EGF stimulates NADPH oxidase and heme oxygenase-1 to activate NFκB via c-Src and PI3K/PKB/Akt-mediated signaling and to enhance HT-29 colon cancer cells' proliferation (18). Prolonged •NO production by iNOS promoted angiogenesis and oral squamous cell carcinoma progression (19). In addition, •NO generation by tumor-associated macrophages prevented tumor cells apoptosis and caused chemoresistance to cisplatin (20). However, in breast, colorectal, epidermoid, head, and neck tumors decrease in tetrahydrobiopterin level causes NOS uncoupling and production of O_2•$^-$ and NOO^- instead of NO• to promote tumorigenesis (21). Reconstruction of coupled NOS activity shifted downstream signaling toward increased cGMP-dependent PKG activation, reduced β-catenin expression, and decreased NFκB activity.

However, in some solid and hematologic malignancies, oxidative stress can induce apoptosis, and this may be exploited in selective anticancer therapy strategies (22). Ionizing radiation and chemotherapeutic agents work through either direct or indirect RONS generation followed by accumulation of oxidized proteins, DNA damage, and cell-cycle arrest. Indeed, tamoxifen, paclitaxel, and As_2O_3 cytotoxicity associates with O_2•$^-$, H_2O_2, and NO• accumulation, while cancer cell chemoresistance to these agents is proportional to activity of antioxidant genes (23, 24). Furthermore, docosahexaenoic acid (DHA) sensitizes As_2O_3-resistant HL-60 cells to ROS-inducing anticancer agents and enhances apoptosis through increased proapoptotic Bax protein expression, caspase-3 activation, and mitochondrial inner membrane disruption (25). DHA-induced cytotoxicity was prevented by intracellular GSH and glutathione peroxidase (GPx) elevation, which enhances cancer cell motility and metastatic capacity (26). Thus, both RONS-generating enzymes and AOS components can be targets for anticancer therapy.

Relevance of RONS-Induced Protein Modifications to Cancer Cell Signaling

RONS-MAPK signaling

Studies show that in cancer tissues, RONS activates all three members of MAPK family stress-responsible protein kinases including ERK1/2, JNK, and p38 (Fig. 1). Stimulation of RONS-MAPK signaling is involved in proliferation, migration, and invasion of human breast, liver, prostate, lung, skin, and pancreatic cancer cells. Indeed, dose-dependent O_2•$^-$ and H_2O_2-induced migration of MDA-MB-231 breast cancer cells via activation of lysophosphatidic acid–stimulated PI3K/PAK1/ERK signaling has been reported (27). ROS caused ERK1/2 and p38 activation, and IL1α-enhanced IL8 secretion in human pancreatic cancer cells (28), while HGF-induced H_2O_2 generation and JNK phosphorylation were observed in HCC cells (29).

In malignant lung epithelial cells, stimulation of caspase-9 and p38/MAPK associates with increased •NO and antiapoptotic Bcl-2 levels, suggesting that Bcl-2 S-nitrosylation is critical for tumorigenesis (30). Furthermore, TPA-dependent ROS generation induces PKCα activation, sustained ERK1/2 phosphorylation, and integrin-α5, -α6, -β1 expression followed by HepG2 cell migration (31). This involves FAK, Src, and paxillin phosphorylation to enhance tumor progression.

In addition, chemotherapeutic agents trigger cancer cell apoptosis and autophagy through dose-dependent stimulation of ROS-MAPK signaling. Indeed, ROS-ERK1/2 signaling was involved in HCT116 human colon cancer cell autophagy caused by short-term treatment with MS-275, histone deacetylase inhibitor (32). Long-term treatment with MS-275 led to ROS-p38/MAPK-mediated autophagy-to-apoptosis switch. Besides, salinomycin induces autophagy-to-apoptosis switch in chemoresistant PC-3 prostate cancer cells via both ROS-ERK/p38 and ROS-PI3K/Akt/mTOR signaling (33). Furthermore, AICAR-stimulated ROS-mediated AMPK, energy sensor, activation induces DU-145 prostate cancer cell apoptosis through JNK phosphorylation, caspase-3 activation, and mTOR inhibition (34).

Mechanisms underlying RONS-induced MAPK activation involve oxidative modification and inhibition of various PTPs that dephosphorylate MAPKs. Specific oxidation of redox-sensitive Cys215 in PTPB1 followed by its inhibition was observed in HepG2 and A341 cancer cells (35). At 0.1 mmol/L H_2O_2, Cys215 was oxidized both reversibly to sulfenic acid, and irreversibly to sulfinic/sulfonic acids, while at 10 mmol/L H_2O_2 only irreversible Cys215 oxidation occurs (Table 1). In addition, H_2O_2-induced PTEN catalytic domain oxidation/inactivation causes both ERK1/2 and Akt phosphorylation/activation to enhance DU145 cell migration and invasion (36).

Direct MAPK oxidation also contributes to cancer cell proliferative phenotype. Indeed, selective H_2O_2-induced oxidation/S-sulfenylation of ERK1/2, JNK, and p38/MAPK increases their binding to cognate upstream MAPKKs/MEKs and sustained LP07 murine tumor cell proliferation (37). Thus, in addition to intricate ways of MAPK spatiotemporal localization and phosphorylation dynamics, endogenous reversible oxidation/S-sulfenylation of redox-sensitive Cys in MAPKs and PTPs serve as fine-tuning mechanisms underlying regulation of MAPK-mediated signaling.

RONS-PI3K/Akt signaling

ROS-induced PI3K/Akt signaling plays a key role in acquisition of malignant phenotype by normal cells and survival of cancer cells through Akt activation or its negative regulator, PTEN, inactivation (Fig. 1). ROS trigger PI3K activation and PKB/Akt-mediated upregulation of cell-cycle genes (*cdc2*, *PRC1*, and *PCNA*) contributing to E2- and 4-OH-E2–induced malignant transformation of MCF-10A human mammary epithelial cells (38). In addition, exogenous LPA-induced ERK and Akt phosphorylation/stimulation, and NFκB activation promote SKOV3 ovarian cancer cell proliferation through O_2•$^-$ and H_2O_2

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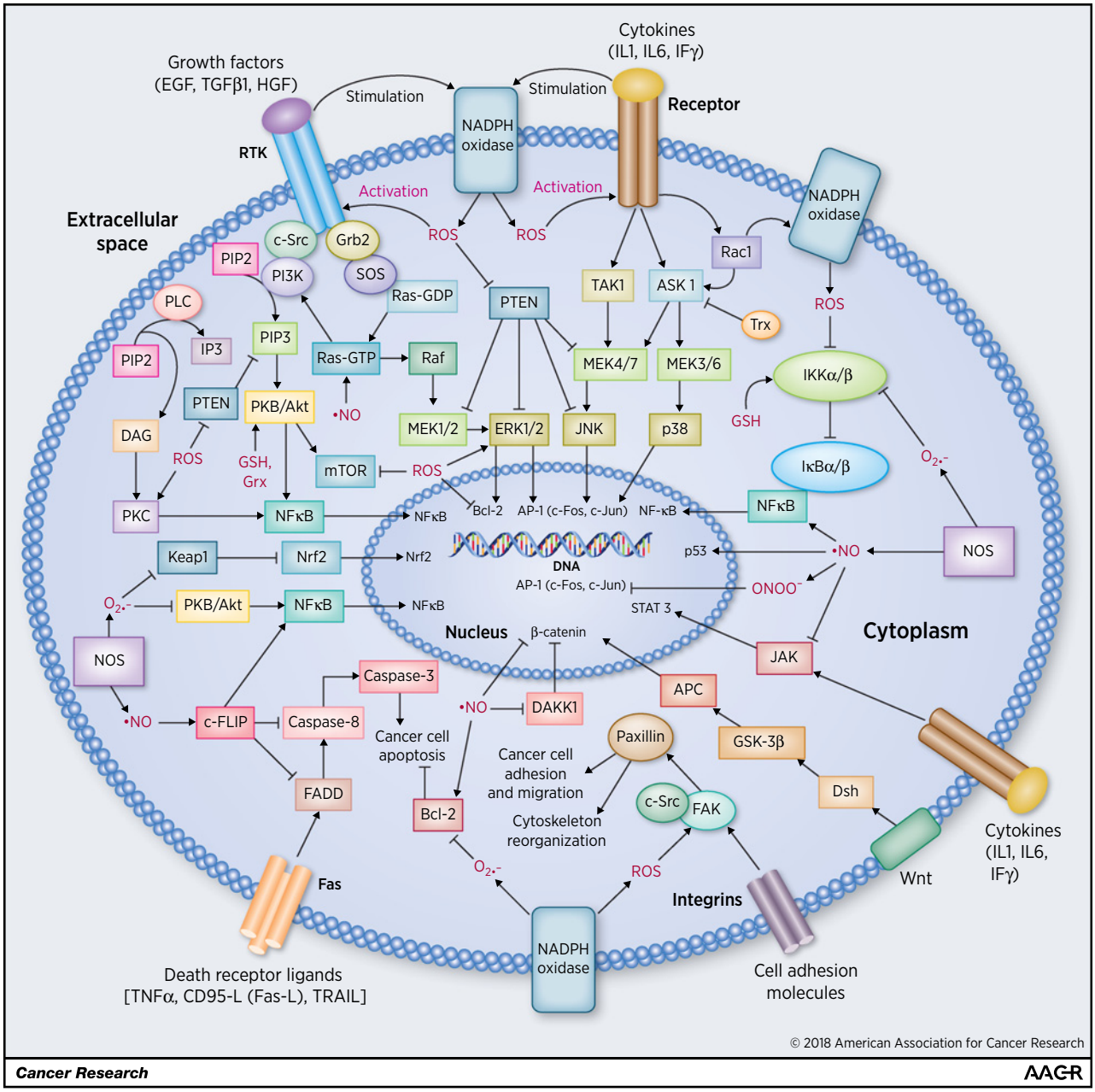

Figure 1.

RONS-regulated cell signaling pathways and transcription factors involved in cancer initiation and progression. Reversible oxidative/nitrosative modifications of redox-sensitive residues including Cys oxidation/S-sulfenylation/S-glutathionylation/S-nitrosylation and Tyr oxidation/nitration in enzymes, intracellular effectors of signal transduction pathways, and transcription factors enable redox switches in their activities. This provides regulation of cancer cell proliferation, differentiation, migration and apoptosis, and cytoskeleton reorganization.

production (39). Mechanism of LPA-mediated cancer cell signaling involves Akt2 Cys124 oxidation/S-sulfenylation followed by loss of its kinase activity, G_1–S cell-cycle transition and stimulated cell migration (40).

Indirect stimulation of ROS-Akt signaling occurs through oxidative modification/inactivation of tumor suppressors, PTEN, and CDC25A phosphatases. In E2-dependent MCF-7 cells, this causes NRF-1 activation, p27 inhibition, and stimulated cell proliferation (41). PTEN transient inactivation results from H_2O_2-induced Cys124 oxidation/S-sulfenylation and Cys71-Cys124 disulfide bonding (42). Oxidative regulation of PI3K/Akt signaling involves GSH, peroxiredoxins (Prx), and glutaredoxins (Grx) that control ROS and oxidized protein levels.

Stimulation of PI3K/Akt signaling and cancer cell proliferation can be achieved through eNOS and •NO-induced Cys118 oxidation/S-nitrosylation in wild-type Ras small GTPases (43). It is

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Table 1. Types of protein oxidative/nitrosative modifications responsible for redox switches in protein activities and relevant to cancer growth

RONS types	Type of a protein modification	Modified residues	Modified protein	Cellular response	References
H_2O_2 (dose-dependent)	Oxidation/S-sulfenylation/ sulfinylation/sulfonylation	Cys215	PTPB1 phosphatase	ROS-MAPK signaling	35
$O_2^{\bullet -}$- and H_2O_2	Oxidation/S-sulfenylation	Cys124	Akt2	Cell migration, G_1–S cell-cycle transition	40
H_2O_2	Oxidation/S-sulfenylation, Cys71-Cys124 disulfide bonding	Cys124	PTEN	Stimulation of ROS-PI3K/Akt signaling and cell proliferation	36, 42
•NO	Oxidation/S-nitrosylation	Cys118	Wild-type Ras	Activation of PI3K/PKB/Akt pathway	43
H_2O_2	Oxidation/S-glutathionylation	Cys179	IKKβ	Prevention of TNFα-induced NFκB activation	46
•NO	Oxidation/nitration	Tyr181	IκBα	IKK-independent activation of NFκB	47
•NO	Oxidation/S-nitrosylation	Cys254	c-FLIP	IKK-independent activation of NFκB	50
•NO	Oxidation/nitration	Tyr1007/Tyr1008	JAK2	Progression of JAK/STAT/Akt signaling	51
•NO, $ONOO^-$	Oxidation/nitration	Tyr327	Wild-type p53	p53 DNA-binding and nuclear accumulation	54
H_2O_2 and HOCl	Oxidation/Cys226-Cys613 disulfide bond formation	Cys151, Cys226, Cys273, Cys288, Cys613	Keap1	Prevention of Nrf2 UPS-mediated degradation	57
H_2O_2	Oxidation/S-sulfenylation	Cys293	MKP-3	MKP-3 UPS-mediated degradation and stimulation of ROS-ERK1/2 signaling	62
•NO	Oxidation/S-nitrosylation	Cys128, Cys226	Bcl-2	Prevention of Bcl-2 UPS-mediated degradation and Bcl-2 stabilization	30, 66
H_2O_2, $O_2^{\bullet -}$	Oxidation/S-sulfenylation	Cys128, Cys226	Bcl-2	Bcl-2 UPS-mediated degradation	66
H_2O_2	Oxidation/S-glutathionylation	Cys442	HSP60	ERK1/2 phosphorylation and activation	69
H_2O_2	Oxidation/S-glutathionylation	Cys18	Protein disulfide isomerase	ERK1/2 phosphorylation and activation	69
H_2O_2	Oxidation/S-glutathionylation	Cys374, Met44, Met47	Actin and vimentin	Cytoskeleton remodeling	75, 76

known that mutations in *ras* genes lead to permanently activated Ras proteins, and mutated human H-Ras, K-Ras, and N-Ras remain in GTP-bound oncogenic state. Induction of ROS production by Nox1 in K-Ras–dependent manner is considered as a key step in cellular malignant transformation and tumorigenesis (44).

Activation of transcription factors

Coordinated ROS-mediated activation of NFκB, p53, AP-1, HIF-1α, and STAT3 transcription factors is involved in expression of many genes controlling cancer cell metabolism and survival (Fig. 1). Indeed, EGF- and TNFα-induced ROS generation and MAPK-mediated signaling cause coordinated activation of NFκB and AP-1 during skin carcinogenesis (45). ROS increase DNA binding capacity of both NFκB and AP-1 and enhance proliferation of pancreatic cancer cells through IL8 secretion and ERK1/2 and p38 MAPK signaling (28).

NFκB is involved in cell response to various stress stimuli including oxidative stress, heavy metals, proinflammatory cytokines, and infectious agents to promote prosurvival gene expression. Canonical pathway of NFκB activation is triggered by phosphorylation of IκB, NFκB inhibitor, with IκB kinases (IKK) resulted in IκB release and UPS-mediated degradation. IKK-dependent mechanism can underlie association between inflammation and cancer growth because H_2O_2-induced IKKβ Cys179 oxidation/S-glutathionylation reversed by Grx1 prevents TNFα-enhanced NFκB activation (46).

IKK-independent NFκB activation involves •NO-induced IκBα Tyr181 nitration (47) and Tyr42 phosphorylation through H_2O_2-induced c-Src oxidation during hypoxia/reoxygenation followed by IκBα dissociation from its complex with NFκB (48). During hypoxia, mitochondrial ROS promote HIF-1α expression and HepG2 hepatoma, SH-SY5Y neuroblastoma, and DLD-1 colon carcinoma cell survival via c-Src stimulation and NFκB activation (49). In addition, NFκB can be activated by •NO-induced Cys254 S-nitrosylation in caspase-like domain of c-FLIP, an inhibitor of TNFα, FasL, and TRAIL-induced apoptosis (50).

NFκB can act cooperatively with STAT3 that is phosphorylated/activated by JAK tyrosine kinases. RONS altered phosphorylation of JAK2 through growth hormone–associated •NO-induced Tyr1007/Tyr1008 nitration and JAK/STAT/Akt signaling (51). ROS generation by NADPH oxidase activates JAK2/STAT3 pathway and IL6 synthesis to induce autophagy of starved or rapamycin-treated HeLa cells (52). In addition, antineoplastic agent, BITC, can inhibit pancreatic carcinogenesis via apoptosis induction and proliferation/migration inhibition through ROS-induced downregulation of c-Myc and STAT3/phosphorylated STAT3 (53).

Another transcription factor, p53 tumor suppressor, triggers cell-cycle arrest and apoptosis, but can be also involved in maintaining cellular redox homeostasis via promoting expression of genes encoding MnSOD and GPx1 to prevent DNA damage, genomic instability, and accumulation of oxidized proteins. Elevated RONS inhibit wild-type p53 through Cys oxidation and Tyr nitration by $ONOO^-$ associated with creating tumor

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inflammatory microenvironment as observed in human malignant glioblastoma (54).

RONS-induced alterations in activities of another master regulator of antioxidant cell response, Nrf2, and its negative regulator, Keap1, also have been observed. Under nonstressed conditions, Nrf2 binds to Keap1 to undergo degradation through UPS-mediated pathway. Under oxidative stress conditions, Nrf2 releases from complex with Keap1, to translocate into the nucleus, where it heterodimerizes with small Maf protein and binds to antioxidant responsive element (ARE) of antioxidant genes (55). Mutations in *KEAP1* and *NRF2* genes disrupt Keap1–Nrf2 complex followed by persistent Nrf2 activation to create tumor cell prosurvival environment and anticancer drug resistance.

Keap1 inhibition due to oxidation of redox-sensitive Cys residues causes conformational changes in Nrf2-Keap1-Cul3 E3 ligase complex to prevent Nrf2 UPS-mediated degradation. Large Cys amount in Keap1 provides its role as a sensor to various electrophiles/oxidants named Nrf2 inducers (56). Keap1 exposure to RONS causes Cys226 oxidation and Cys226-Cys613 disulfide bonding (Table 1), while Cys151 oxidation triggers intermolecular disulfide bonding (57). Conserved Cys residues in Nrf2 itself are also critical to electrophile sensing to control Nrf2 binding to target ARE, UPS-mediated degradation, and coactivator recruitment (58).

Oxidative stress–mediated Nrf2 expression is implicated in ERα-positive breast cancer cell survival and tamoxifen resistance (24). However, RONS-induced controlled pharmacologic Nrf2 upregulation can inhibit cancer cell proliferation and migration. Indeed, bardoxolone-methyl causes mitochondrial ROS-induced Nrf2 activation to downregulate glycolysis and oxidative phosphorylation, to decrease intracellular GSH level and inhibit MCF-7 cell migration (59). In addition, resveratrol, a natural phytoestrogen, significantly upregulates Nrf2 causing SOD3, NQO1, and OGG1 antioxidant gene expression, and inhibited E2-induced breast cancer cell migration and apoptosis induction (60). Different cell response to pharmacologic Nrf2 activation may be explained by interplay with other transcription factors and diverse mechanisms underlying regulation of Keap1-Nrf2 signaling in target gene- and inducer-dependent manner.

RONS and Protein Quality Control in Cancer

UPS- and lysosomal/autophagy-mediated protein degradation

ROS-induced carcinogenesis may be caused by UPS-mediated degradation of regulators of ROS-generating enzymes and intracellular effectors of signaling pathways. For example, EGF-mediated NoxO1 phosphorylation triggers formation of enzymatically active complex with NoxA1 and ROS production. In human colon cancer cells, NoxO1 and Grb2 interaction causes Cbl E3 ligase recruitment, leading to NoxO1 UPS-mediated degradation and decrease in Nox1 activity (61). Intracellular H_2O_2 accumulation led to UPS-mediated degradation of MKP-3, negative regulator of ERK1/2, contributing to ovarian cancer cell survival and chemoresistance (62). Furthermore, glutamate-induced oxidative stress and PKC-δ activation, aberrant ERK1/2 stimulation, increased tumorigenicity, and chemoresistance involve UPS-mediated MKP-1 degradation (63). Thus, targeting key oncoproteins to induce their RONS-mediated UPS-dependent degradation can serve as a basis for anticancer therapy.

Variety of tissue-specific proteasomes are targets for inhibitors used in anticancer therapy, although proteasome subtypes have different susceptibility to different inhibitors. Indeed, a proteasome inhibitor, b-AP15, causes distinct cellular response as compared with clinically used bortezomib and leads to oxidative stress- and endoplasmic reticulum stress–induced tumor cell apoptosis through JNK/AP-1–mediated signaling (64). Increased cancer cell resistance to existing proteasome inhibitors requires novel proteomic and drug discovery approaches.

Approximately 10%–20% of damaged proteins undergo degradation through lysosomal/autophagy pathway that plays a dual role in cancer cell death and survival being implicated in crosstalk between diverse cellular pathways including oncogene Ras-induced carcinogenesis, mitochondrial metabolism, redox status, and energy production (65). Generally, at initial cancer stages, autophagy inhibition is observed, while at advanced stages, autophagy is upregulated to provide cell survival and proliferation under oxygen/nutrient starved conditions. •NO negatively regulates autophagy initiation through Bcl-2 oxidation/S-nitrosylation to prevent UPS-mediated degradation and to inhibit apoptosis of malignant lung epithelial cells (66). However, Bcl-2 exposure to $O_2^{\bullet -}$ caused its downregulation and UPS-mediated degradation, suggesting a proapoptotic role of this ROS type (Fig. 1).

Mitochondrial ROS production depends on lysosomal ferritin degradation followed by redox-active iron release and cancer cell death in response to anticancer agent, artesunate (67). In addition, zinc protoporphyrin exhibits anticancer effects via induction of β-catenin lysosomal degradation followed by inhibition of Wnt/β-catenin signaling (68). Crosstalk between ROS generation and autophagy is an emerging challenge in targeting for anticancer chemotherapy in preclinical and clinical studies.

Dysfunction of molecular chaperone machinery

Additional mechanism underlying ROS-induced carcinogenesis is disruption of molecular chaperone machinery, which leads to impairment in cellular protein quality control. Indeed, HGF-induced ROS generation in HepG2 cells causes decrease in free thiol group amount in HSP60 and protein disulfide isomerase (PDI) resulted from HSP60 Cys442 and PDI Cys18 S-glutathionylation followed by ERK1/2 phosphorylation/activation and HCC cell migration (69). In addition, HSP70 is expressed at very low concentrations in mitochondria of normal cells, being abundant in mitochondria of tumor cells to play key roles in stress-inducible pathways. HSP70 inhibition causes multifaceted mitochondrial dysfunction, impaired mitochondrial integrity, loss of mitochondrial membrane potential, reduced oxygen consumption, oxidative phosphorylation, and ATP production (70).

Mitochondria of tumor cells substantially differ from mitochondria of nontransformed cells in metabolism, energy and ROS production, and membrane permeability. HSP90, a key player of protein quality control machinery, compartmentalizes in mitochondria to provide mitochondrial reprogramming and apoptosis suppression. This is achieved via inhibition of AMPK, preservation of cytoskeletal dynamics, and release of FAK from its complex with FIP200, autophagy initiator, to promote cancer cell adhesion and metastasis (71).

Because induction of HSP synthesis protects cancer cells from oxidative stress, effective HSP inhibitor–based anticancer therapy is of great concern. For example, ascorbate/menadione ROS-generating system used to induce cancer cell death exerts its action through complex mechanisms including glycolysis inhibition, dysregulation of calcium homeostasis, and impairment of HSP chaperoning functions (72). Additional strategy is an oxidative

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stress–induced HSP cleavage to cause degradation of their client proteins and cancer cell death (73).

Cytoskeletal disruption

Under physiologic conditions, cytoskeleton dynamics provides multiple cellular functions including migration, adhesion, and endocytosis. However, oxidative stress causes disruption in cytoskeleton assembly and dynamics, which facilitates cancer cell motility and aggressive behavior. RONS-induced cytoskeleton protein modifications change microtubule stability in a wide range of cancers. For example, treatment of pancreatic cancer cells with high H_2O_2 concentrations caused rapid loss of intracellular actin microfilaments and microtubules accompanied by increased NFκB activity and morphologic changes (74).

Cytoskeletal proteins, actin and vimentin, contain Cys and Met residues susceptible to oxidation. Cys oxidation/S-glutathionylation and both Cys and Met carbonylation (Table 1) in actin monomers control cytoskeleton dynamics (75). H_2O_2 significantly reduces cell adhesion on fibronectin, laminin, and collagen, and decreases FAK and paxillin and α5β1, αvβ3, β1 integrin levels (76). These changes associate with increased NFκB activity, and actin and vimentin microfilament reorganization.

Another mechanism underlying regulation of cytoskeleton organization may involve proteolysis and protein fragmentation. Indeed, RhoA, a member of Ras-related small GTPases, which regulates actin microfilament assembly and cytoskeleton dynamics, undergoes proteolysis/cleavage in response to oxidative stress (77). Oxidative stress–induced cytoskeletal disruption correlates with poor prognosis and chemoresistance, especially, in patients with solid and hematologic cancers.

Conclusion and Perspectives

Understanding cancer complexity requires elucidating molecular mechanisms underlying alterations in cellular functioning during carcinogenesis. An association of RONS generation with human cancer initiation and progression occurs via several mechanisms including oxidative/nitrosative modifications of key redox-sensitive residues in enzymes, intracellular effectors of signal transduction pathways, transcription factors, molecular chaperones, cytoskeleton, and proteasomal/lysosomal proteins. However, data on RONS-induced protein modifications during cancer growth are not complete; more investigations are required in this field. Novel redox proteomic approaches are being developed for high-throughput screening of redox-sensitive proteins to benefit discovery of novel molecular targets for anticancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Reactive Oxygen and Nitrogen Species–Induced Protein Modifications: Implication in Carcinogenesis and Anticancer Therapy

Nurbubu T. Moldogazieva, Sergey V. Lutsenko and Alexander A. Terentiev

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