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Comprehensive Invited Review

Redox Regulation of Cell Survival

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ABSTRACT

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in regulation of cell survival. In general, moderate levels of ROS/RNS may function as signals to promote cell proliferation and survival, whereas severe increase of ROS/RNS can induce cell death. Under physiologic conditions, the balance between generation and elimination of ROS/RNS maintains the proper function of redox-sensitive signaling proteins. Normally, the redox homeostasis ensures that the cells respond properly to endogenous and exogenous stimuli. However, when the redox homeostasis is disturbed, oxidative stress may lead to aberrant cell death and contribute to disease development. This review focuses on the roles of key transcription factors, signal-transduction pathways, and cell-death regulators in affecting cell survival, and how the redox systems regulate the functions of these molecules. The current understanding of how disturbance in redox homeostasis may affect cell death and contribute to the development of diseases such as cancer and degenerative disorders is reviewed. We also discuss how the basic knowledge on redox regulation of cell survival can be used to develop strategies for the treatment or prevention of those diseases. *Antioxid. Redox Signal*. 10, 1343–1374.

I. REDOX BIOLOGY AND REGULATORY MECHANISMS

A. Redox homeostasis: ROS production and elimination

The redox system is essential in maintaining cellular homeostasis. Under physiologic conditions, cells maintain redox balance through generation and elimination of reactive oxygen/nitrogen species (ROS/RNS). ROS include radical species such as superoxide (O_2^{-}) and hydroxyl radical ($HO^{\cdot}$), along with nonradical species such as hydrogen peroxide (H_2O_2). RNS include nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$) (143). ROS are derived from oxygen, an obligate component of eukaryotic organisms. Reduction of molecular oxygen is the principal mechanism for ROS formation. The initial product, superoxide, results from the addition of a single

electron to molecular oxygen. Superoxide can be rapidly dismutated by superoxide dismutase (SOD), yielding H_2O_2 and O_2, which can be reused to generate superoxide radical. In the presence of reduced transition metals, H_2O_2 can be converted into the highly reactive hydroxyl radical $HO^{\cdot}$ (73).

Both exogenous and endogenous sources contribute to the formation of intracellular ROS/RNS. Exogenous sources include irradiation (*i.e.*, UV irradiation, x-ray, gamma-ray), atmospheric pollutants, and chemicals. For example, exposure to metabolites of polychlorinated biphenyls (PCBs) has been shown to increase ROS production in HL-60 cells (286). As illustrated in Fig. 1, a major endogenous source of cellular ROS is from the mitochondria, where O_2^- is generated by electron leakage from complex I and III of the electron-transport chain (177, 286). Microsomes and peroxisomes are also sources of ROS, primarily H_2O_2, whereas immune cells such as neutrophils and macrophages possess oxygen-dependent mechanisms to fight against invading microorganisms. Other endogenous sources of ROS include the membrane-associated NAD(P)H oxidase, cytochrome c oxidase, and xanthine oxidase. The presence of redox-active metals such as Fe and Cu also contributes to ROS generation. In the presence of Fe(II) and Fe(III), $HO^{\cdot}$ can be generated through the Fenton reaction or Haber–Weiss reaction (151). Similarly, $NO^{\cdot}$ generation occurs through specific nitric oxide synthase isozymes, including mitochondrial nitric oxide synthase (mtNOS), neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (93). NO can react with O_2^- to generate $ONOO^-$ (293).

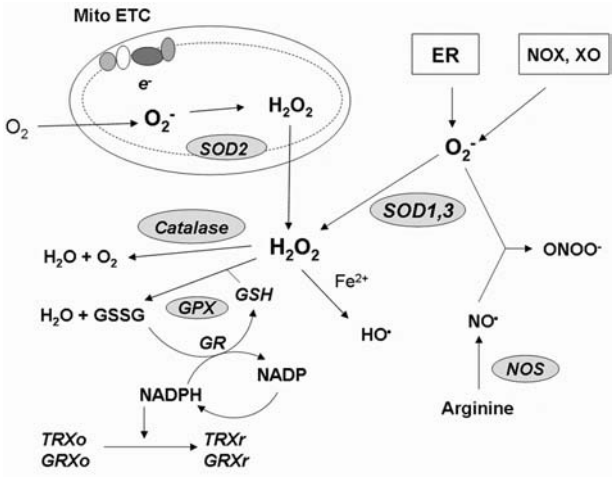

FIG. 1. Redox homeostasis. Major sites of cellular ROS generation include the mitochondrial electron transport chain (Mito ETC), the endoplasmic reticulum (ER) system, and the NAD(P)H oxidase (NOX) complex. Nitric oxide synthases (NOS) are key enzymes for production of NO. Major ROS-scavenging enzymes are highlighted in grey. GSH and NAPDH play roles in maintaining the reduced cellular redox state. GPX, glutathione peroxidase; GR, glutathione reductase; TRXo, thioredoxin (oxidized); TRXr, thioredoxin (reduced); GRXo, glutaredoxin (oxidized); GRXr, glutaredoxin (reduced); $HO^{\cdot}$, hydroxyl radical; $NO^{\cdot}$, nitric oxide; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase; GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH, reduced nicotinamide adenine dinucleotide phosphate; XO, xanthine oxidase.

Cells are equipped with enzymatic and nonenzymatic antioxidant systems to eliminate ROS/RNS and maintain redox homeostasis. A major class of enzymatic antioxidants, which catalyze the dismutation of O_2^- to H_2O_2, is known as superoxide dismutase (SOD). Multiple isoforms of SOD exist in different cellular compartments. SOD1 (CuZnSOD) is the major superoxide scavenger found in the cytoplasm, mitochondrial intermembrane space, nucleus, and lysosomes, whereas SOD2 (MnSOD) and SOD3 are found in the mitochondria and extracellular matrix, respectively (83). Further conversion of H_2O_2 to $H_2O + O_2$ occurs through the action of catalase, a heme-based enzyme that is normally localized in the peroxisome. Interestingly, catalase has extremely high substrate-turnover rates, scavenging ~6 million molecules of H_2O_2 per minute (309). H_2O_2 also can be converted to O_2 through coupled reactions with the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), catalyzed by glutathione peroxidase (GPX). Five isoforms of selenium (Se)-dependent GPXs are found in humans [for review, see (33)]. The reduction of hydroperoxides by wild-type GPXs is nearly 1,000-fold the rate found in mutated GPXs, which have a cysteine replacing selenocysteine at the active site (197). Glutathione peroxidase 1 (GPX1) is ubiquitously expressed and a major scavenger for H_2O_2 and lipid hydroperoxides. GPX2 is epithelium-specific and highly expressed in the gastrointestinal tract, whereas GPX3 is an extracellular glycosylated enzyme found in plasma. Interestingly, GPX3 can use thioredoxin and glutaredoxin in addition to GSH as electron donors to reduce a broad range of hydroperoxides. GPX4 is present in cytosolic, mitochondrial, and nuclear forms by alternative splicing, and is a major enzyme preventing oxidation of membrane phospholipids. A newly discovered GPX6 is localized preferentially in olfactory mucosa and embryonic tissue. Furthermore, enzymes such as glutathione S-transferases (GSTs) are known to have Se-independent peroxidase activity (279).

Nonenzymatic antioxidants, recognized to execute thiol–disulfide exchange reactions, also play a major role in maintaining cellular redox balance. In addition to being a cofactor of various antioxidant enzymes, GSH, which is the most abundant peptide in cells, possesses a plethora of functions. These include direct scavenging of $HO^{\cdot}$, singlet oxygen, and regeneration of other antioxidants such as vitamin C and E to their active forms (222). The thioredoxin system is another important thiol antioxidant consisting of thioredoxin (Trx) and thioredoxin reductase. Thioredoxin is a multifunctional selenoprotein containing two redox-active cysteines and a conserved active site (Cys-Gly-Pro-Cys) (27). Although many ROS are quenched by GSH through reaction with its thiol group, other thiol-containing proteins are also attacked by ROS, leading to their oxidation (184). Therefore, it is essential for cells to change these oxidized proteins to their reduced forms to maintain proper function. The thioredoxin system, in collaboration with the GSH system, plays an important role in reducing oxidized thiol-containing proteins. Similarly, the glutaredoxin (Grx) system, also with the CXXC conserved active site, functions to reduce protein disulfides. Grx1, Grx2, and Grx3 obtain their protein-reducing capacity from the GSH/glutathione reductase system, which is maintained by NADPH (123). Peroxiredoxins (Prxs) are a large family of proteins with cysteine-containing redox active centers (260). The six mammalian isoforms of Prxs are

classified into two groups: the two-cysteine peroxiredoxins (I–IV, V) and one-cysteine class (Prx VI). Peroxiredoxins use the peroxidatic cysteine (reactive center) to reduce hydroperoxides in a two-step reaction [for review, see (332)].

B. Oxidative stress and its consequences

The delicate balance between ROS generation and elimination is maintained by many complex mechanisms, and a dysfunction of any of these mechanisms could lead to alterations in cellular redox status. An increase in ROS production or a decrease in ROS-scavenging capacity due to exogenous stimuli or endogenous metabolic alterations can disrupt redox homeostasis, leading to an overall increase of intracellular ROS levels, or oxidative stress. Increased oxidative stress plays a crucial role in a variety of pathologic conditions including cancer, neurodegenerative diseases, and aging (310). Under normal physiologic conditions, the reactive nature of ROS/RNS at moderate levels allows their incorporation into the structure of macromolecules in a reversible fashion. Such reversible oxidative modifications play a critical role in regulating cellular function. However, under oxidative stress, excessive ROS/RNS constantly attack lipids, proteins, and DNA, leading to severe and irreversible oxidative damage.

Lipids are most susceptible to oxidative modification. Lipid peroxidation generates lipid radicals, which can further attack the subsequent lipid molecules and propagate as a chain reaction. The chain-reaction process consists of three stages: initiation, propagation, and termination [for review, see (98)]. Polyunsaturated fatty acid residues of phospholipids are attacked by a radical either at an internal position or near the end of the conjugated system, generating a peroxyl radical (309). Attack at an internal position allows the peroxyl radical to further undergo either a cyclization or metal-catalyzed reaction and produce reactive alkoxyl radicals. After cyclization, the fatty acid may form a hydroperoxide or undergo another cyclization, which produces aldehydes, including malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) (243). While MDA can further react with DNA bases, resulting in gene mutations, HNE reacts mostly with proteins, leading to significant functional alterations affecting signaling pathways. Oxidative damage of phospholipids can lead to cell death, not only through membrane damage but also through the lipid peroxidation product HNE. Attack of various proteins such as c-Jun N-terminal kinase (JNK) and caspase-3 activation was found to be a mechanism of cell death induced by lipid peroxidation (8).

Protein oxidation can be reversible or irreversible, depending on the target and the form of oxidative damage. The highly reactive $OH^{\cdot}$, generated through ionizing radiation or the Fenton reaction, and $ONOO^{-}$ are common reactive species that target proteins. Although all amino acid residues could be oxidized by ROS/RNS, certain side chains are particularly susceptible to oxidation. For instance, lysine, arginine, histidine, proline, and threonine are highly sensitive to metal-catalyzed oxidation (309). Oxidation of these side chains results in carbonyl derivatives, which can also be generated through glycation/glycoxidation pathways, lipid peroxidation, α-amidation, and glutamic acid pathways (257). Because a variety of mechanisms of protein oxidation can lead to formation of protein carbonyls, which are easily detectable, the level of protein carbonyls has been used as a quantitative marker of protein oxidation and oxidative stress (62). Sulfur-containing amino acids such as cysteine and methionine are also susceptible to either reversible or irreversible oxidation. Reversible oxidation of the sulfhydryl group includes intramolecular or intermolecular protein cross-linkages and glutathionylation (287). Irreversible protein oxidation includes nitrosylation of cysteine sulfhydryl groups, tyrosine, methionine, and tryptophan by $ONOO^{-}$. Nitration of tyrosine residues may inhibit its phosphorylation or adenylation, important for protein function (249). Severe oxidative stress can induce disulfide bond–mediated protein cross-linkage or secondary oxidative modifications such as adduct formation between oxidized proteins and lipid peroxides or glycation products. These products can generate aggregation of bulky protein complexes, which may inactivate both 26S and 20S proteosome, leading to accumulation of damaged proteins and cell death (247).

Compared with lipids and proteins, nuclear DNA may seem less susceptible to oxidative modifications because of its double-helix structure and the protective shield from histone and other coating proteins. However, oxidative nuclear DNA damage is detectable under various conditions. Thus, oxidized products of DNA bases such as 8-OHdG have been used as a marker for damage caused by oxidative stress (109). The correlation between oxidative DNA damage and various stages of carcinogenesis has been studied (305). DNA is subject to damage in nearly all of its components. Both purine and pyrimidine bases and the sugar backbone contain N and O as nucleophilic centers, which are highly susceptible to react with electrophiles, especially $OH^{\cdot}$. Furthermore, the double bonds within the bases are prime targets for $OH^{\cdot}$. Reactions are primarily centered at the C-5 and C-6 of pyrimidines and C-4 and C-8 of purines (31). $NO^{\cdot}$ and $ONOO^{-}$ have been found to react with DNA bases and induce single-strand breaks (305). Oxidative damage to the sugar backbone, through H abstraction, has been known to cause single-strand breaks and double-strand breaks (31). Unlike nuclear DNA, mitochondrial DNA (mtDNA) is more susceptible to oxidative damage, not only because of its close proximity to the major site of ROS generation (electron-transport chain), but also because of the limited capacity of mtDNA repair (129).

Under physiologic conditions, cellular DNA is constantly attacked by ROS. It has been estimated that in mammalian cells, $\sim 1.5 \times 10^5$ oxidative adducts in DNA per cell are found (15). As such, those hits may induce mutations and play a role in the evolution process. Moderate levels of DNA damage can trigger cell-cycle arrest and initiate DNA-repair processes that ensure DNA integrity. In contrast, excessive damage or failure in DNA repair can induce apoptosis (60). It is worth noting that oxidative modifications of lipids, proteins, or DNA play a crucial role in physiologic processes such as differentiation, maturation, and trafficking of intracellular vesicles (73). However, when the ROS/RNS levels are in excess, the biologic consequences are often deleterious. Therefore, regulation of ROS/RNS levels is critical in maintaining cellular homeostasis.

C. Redox-mediated mechanisms in regulation of cellular processes

Redox balance plays a critical role in maintaining the biologic process under normal conditions. However, disruption of

the balance due to an increase in ROS/RNS production or decrease in ROS-scavenging capacity may alter cellular functions. Alterations in ROS/RNS levels can modulate biologic activity through aberrant stimulation/suppression of certain signaling pathways and through direct modifications of biomolecules, especially proteins. The redox system can modify functions of proteins through regulating their expression, posttranslational modifications, and stabilities, as depicted in Fig 2.

1. Transcriptional regulation. At the synthesis level, expression of signaling proteins can be tightly controlled through the rate of gene transcription. A number of transcription factors contain redox-sensitive cysteine residues at their DNA-binding sites (107). Examples of the factors are NF-κB, AP-1, HIF-1α and P53. In most cases, thiol oxidation of these proteins would inhibit their DNA-binding activities (306). Under physiologic conditions, nuclear GSH plays a critical role in maintaining the reducing environment to prevent excessive oxidative modifications of nuclear DNA and to ensure proper gene transactivation (103). Furthermore, transcriptional co-activators such as CBP/p300 are equipped with histone acetylation activity, which is required to uncoil DNA structure, allowing accessibility of transcription factors to promoter regions of target genes (229). Interestingly, the enzyme histone deacetylase (HDAC), which reverses the acetylation process, was recently found to be redox sensitive (252). Thus, in addition to direct modification of the transcription factors, alteration in ROS/RNS level may regulate gene expression through modulation of chromatin remodeling.

2. Direct oxidative modification. At the posttranslational level, oxidative modification was found to be a major mechanism for redox regulation of protein functions (81). Multiple types of amino acids can be oxidatively modified, with various susceptibilities (27). Direct oxidation is mostly mediated by HO˙ and NO˙. Among those amino acids, sulfur-containing ones such as methionine and cysteine are preferential targets. Oxidative modifications of amino acid residues in a peptide may lead to structural and functional changes, ranging from a slight conformational change to a severe denaturation accompanied by fragmentation. The functional outcome of the oxidation depends on the types of modifications and the criticality of the modified amino acid in the protein function. It may lead to either activation or inhibition of the protein activities. Examples of common oxidative modification of proteins are illustrated in Fig. 3. Mild oxidative stress can induce modifications of Cys such as reversible glutathionylation (94), disulfide formation (4), and S-nitrosylation (292). These modifications are known to have regulatory roles in the function of many proteins such as TRX, p53, IκB, RAS, Akt, and protein tyrosine phosphatase. Conversely, a severe increase in oxidative stress likely promotes more-damaging types of modifications, such as sulfenic acid, sulfinic acid, and sulfonic acid formation (246). Protein carbonylation can occur through either direct oxidative attack (of Lys, Arg, Pro, Thr) or interaction between amino acids (such as Lys, Cys, His) and oxidation products of lipids and sugars. Protein carbonylation is often used as an indicator for protein oxidations, as it accumulates *in vivo* at high levels relative to other oxidative modifications and is readily detectable (59). Examples of proteins modified by carbonylation, which can impair their functions, include ANT, Hsp, and BCL2 (81). Besides Cys, Tyr is another attractive target for redox modification. Nitration of tyrosine by RNS yields nitrotyrosine, which causes the protein to lose its ability as a substrate for phosphorylation. Kinases such as JNK, p38MAPK, and PKC are targets for tyrosine nitration, which inhibits their activations (272). In contrast, reversible oxidation of a methionine residue

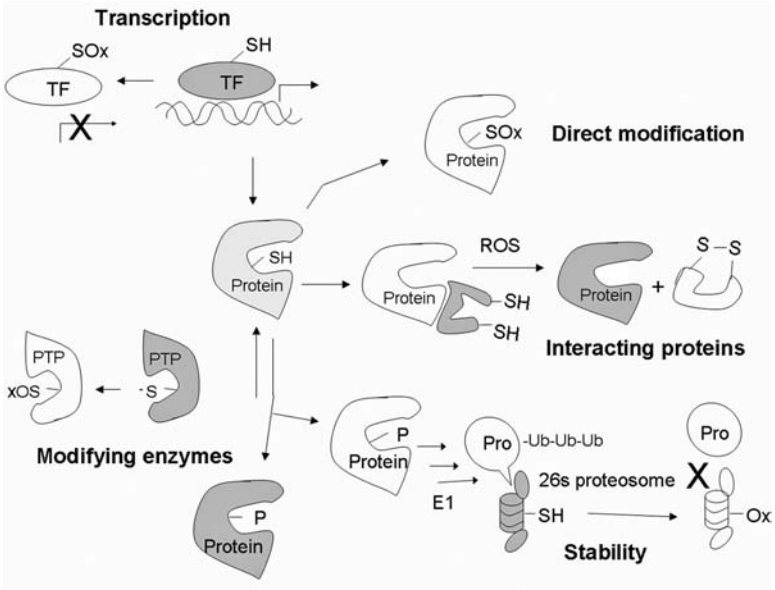

FIG. 2. Redox-mediated mechanisms that regulate protein functions. Protein expression can be regulated through redox modification of transcription factors. Oxidation of Cys at or near the DNA-binding site may disrupt the transactivation activity. Newly synthesized protein can be directly modified by oxidation of amino acids such as Cys, Tyr, and Met, resulting in alteration of the protein functions. Certain proteins are stabilized by their redox-sensitive interacting proteins. Modification of the interacting proteins can dissociate the complex and allow activation of the functional proteins. Posttranslational modifications such as phosphorylation can either activate or inhibit protein functions. Phosphatases, which are responsible for dephosphorylation, can be oxidatively inactivated, promoting phosphorylation of proteins. Stability of signaling proteins determines both the level and duration of their functional effects. Most proteins can be degraded through the ubiquitin–proteosome system. Ubiquitin-activating enzyme E1 and proteosome 26S and 20S can be inactivated under oxidative stress. TF, transcription factor; -SH, reduced thiol; SOx, oxidized thiol; PTP, protein tyrosine phosphatase; ub, ubiquitin. X, inhibition; white, inactive state; light grey, partially activated; dark grey, fully activated molecules.

in calmodulin (CaM) is essential for its function as a calcium-regulatory protein (23).

3. Regulation of redox-sensitive interacting proteins. Many proteins are stabilized by contact with others. Such protein–protein interactions may also modulate their functions, mostly being negative regulations of each other. Oxidative modification of the interacting partners can lead to dissociation of the protein complex, allowing activation of the free functional proteins (55) (see Fig. 2 for illustration). Examples of proteins whose function can be altered by such redox-sensitive mechanism include ASK1-TRX, JNK-GST, p53-JNK, and Nrf2-Keap1.

4. Regulation of redox-sensitive modifying enzymes. Posttranslational modification of proteins, especially by phosphorylation, has been known to be a critical regulatory mechanism for protein function. Phosphorylation of proteins may either lead to their activation or flag for degradation in a site-specific manner (137). Phosphorylation status is the outcome of the balance between kinases and phosphatases. Interestingly, whereas thiol oxidation of phosphotyrosine kinase (PTKs) leads to their activation, transient oxidation of protein tyrosine phosphatases (PTPases) inhibits their functions (48). Under physiologic conditions, because of its low pKa, the catalytic cysteine of active PTPases is in the thiolate anion form and thus susceptible to oxidative modification. Thiol modifications such as direct oxidation, inter- and intramolecular disulfide bridges, S-glutathionylation, and S-nitrosylation can all lead to inactivation of PTPases (119). The oxidative inhibition of PTPases consequently shifts the balance toward a phosphorylated state in target proteins. Besides PTPases, the lipid phosphatase PTEN and the low-molecular-weight phosphatase cdc25 can also be modified in a similar fashion (50).

5. Regulation of protein turnover. Stability of proteins can determine the extent of their functional effects. The rates of protein turnover can also be regulated by redox-mediated mechanisms. Many proteins are degraded by the proteosome system, whereas certain proteins are substrates of other proteases such as caspases. Under nonstressed conditions, ubiquitin and 26S proteosome play a crucial role in the degradation of misfolded/damaged proteins (256). Redox-mediated phosphorylation of IκB, Bcl-2, and p53 seems to increase the binding to their specific ubiquitin ligase E3 and to promote the proteosome-mediated degradation of these proteins. However, under oxidative stress, although the ubiquitin-activating enzymes (E1) and 26S proteosome are oxidatively inactivated, oxidized proteins may no longer be ubiquitinated and degraded (22). Instead, such oxidized products can be eradicated by the 20S proteosome in a ubiquitin-independent manner (285).

II. REDOX REGULATION OF SIGNALING PROTEINS AFFEECTING CELL DEATH AND SURVIVAL

The roles of ROS and antioxidant systems in regulation of cell survival are bifurcated. In general, ROS at low levels act as signaling molecules that promote cell proliferation and cell survival. In contrast, a severe increase in ROS can induce cell death. Previous studies suggest that regulation of signaling pathways by the redox system relies mostly on direct oxidative modifications of the redox-sensitive signaling proteins (143). However, recent evidence shed new light on the novel role of redox regulation in chromatin remodeling, which affects death/survival signals at the transcriptional level (252). Furthermore, posttranslational modifications of signaling proteins such as phosphorylation have recently been shown to be regulated in part through a redox-mediated mechanism (119). Oxidative modification of ubiquitin-proteosome or other proteases can also affect the turnover of signaling proteins (247). The following sections summarize redox regulation of cell survival through modulation of those factors at the transcription, signal transduction, and death-execution levels. Figure 4 illustrates an overview of the redox regulation at these levels and their crosstalk.

A. Redox regulation of cell survival at the transcription level

Intracellular redox homeostasis regulates the expression of multiple gene-encoded proteins affecting cell death and survival. In response to alterations in oxidative status, the transcription of those genes can be modulated in part through a redox control of transcription factors. Here, we focus on the roles of transcription factors NF-κB, AP-1, Nrf2, and HIF in cell survival and how the redox system regulates the functions of these factors.

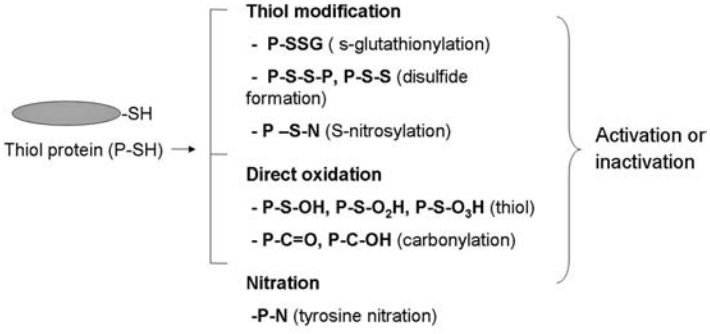

FIG. 3. Oxidative modification of proteins. Protein can be oxidatively modified by multiple types of modifications, and the consequence can be either activation or inactivation of protein functions. Cys in thiol proteins is a major target, which could be modified by reversible S-glutathionylation, disulfide formation, S-nitrosylation, or formation of sulfinic, sulfenic, and sulfonic acid derivatives. Nitration of Tyr is known to modulate the function of multiple kinases. P, protein.

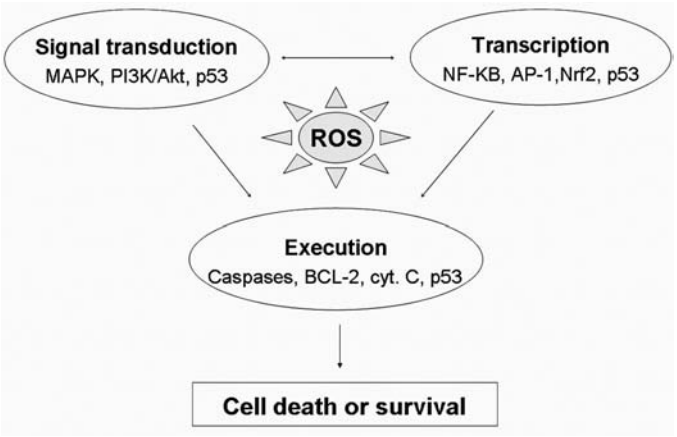

FIG. 4. Redox-sensitive signaling pathways for regulation of cell survival. The redox system can regulate the cell-fate decision through regulations of many functional proteins involving cell life-or-death decisions. Many of those signaling proteins are redox sensitive, which controls survival at the levels of signal transduction, transcriptional regulation, or execution. Examples of the key redox-sensitive molecules involved at each level are indicated. The possible crosstalk among these regulators/executors is indicated by arrows. Signal transduction may involve in transcriptional regulation, and p53 is a redox-sensitive molecule that affects cell survival at all three levels. Therefore, oxidative stress not only serves as a type of stimulus to trigger stress-response signal-transduction pathways, but also can modulate cell death/survival through direct oxidative modification of those signal molecules.

1. NF-κB. Nuclear factor kappa B (NF-κB) is a redox-sensitive transcription factor that coordinates regulators of immunity, inflammation, development, cell proliferation, and survival. In mammals, the NF-κB family consists of NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), c-Rel, and RelB. All members are characterized by the presence of the Rel homology domain (RHD). The RHD mediates DNA binding, dimerization between the family members, and the association of NF-κB dimers with the inhibitors of kappa B (IκB) (117). Normally, the NF-κB components are sequestered and inactivated by IκBs in the cytosol. A wide range of stimuli, including cytokines and ROS stress, are capable of activating NF-κB through activation of IκB kinase (IKK). Active IKK phosphorylates IκB, leading to dissociation of NF-κB from the inhibitor and the attraction of IκB to degradation by ubiquitin/proteasome system (96). Free NF-κB translocates to the nucleus, binds to DNA at the promoter region, and activates the transcription of target genes.

a. Role of NF-κB in cell survival. Active NF-κB controls cell survival through altering transcription of multiple genes as illustrated in Fig. 5. In response to oxidative stress, activation of NF-κB leads to elevated expression of (a) antiapoptotic Bcl-2 family members such as Bcl-xL and A1/Bfl-1; (b) the inactive homologue of caspase-8 ($FLIP_L$); (c) caspase inhibitors such as IAPs that directly prevent activation of caspases; (d) TNF receptor–associated factor TRAF1; (e) Gadd45, which inhibits JNK-mediated cell death; and (f) antioxidants such as Mn-SOD and ferritin heavy chain (FHC) (147).

b. Redox regulation of NF-κB. NF-κB has long been recognized as a redox-sensitive transcription factor. Experimental evidence suggests that ROS seem to have paradoxic effects on NF-κB regulation. ROS can either activate or inhibit NF-κB activity, depending on the level of ROS, types of stimuli, and cell types (140, 236). Moderate increase of ROS often leads to NF-κB activation, which requires sequential steps in the cytosol and nucleus. Conversely, severe increase of ROS could inactivate NF-κB, leading to cell death. As depicted in Fig. 6, multiple redox-mediated mechanisms can regulate NF-κB activity at various stages.

c. Redox regulation of nuclear NF-κB. Studies since the early 1990s demonstrated that the reduced form of nuclear NF-κB is required for its DNA binding. Oxidation of the redox-sensitive site Cys62 of the p50 subunit inhibits its ability to bind DNA (210, 298). Oxidation of p50 is reversible, and DNA binding can be restored through reduction by thioredoxin (204). Detailed studies revealed that nuclear NF-κB can be inactivated by several redox modifications including glutathionylation (244) and S-nitrosylation (200). Besides direct structural modification, DNA binding activity of NF-κB can be modulated by chromatin remodeling (252). Histone acetylation,

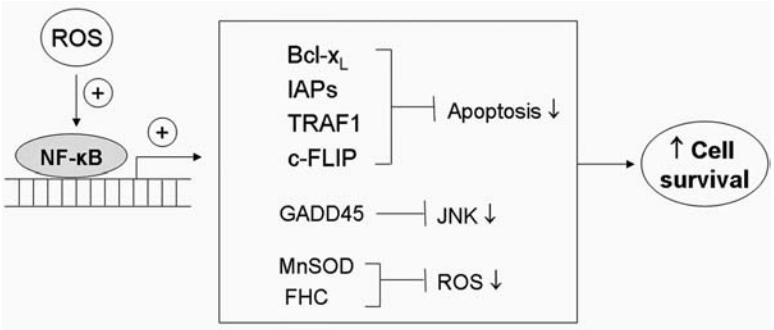

FIG. 5. Role of NF-κB in cell survival. NF-κB functions as a transcription factor regulating the expression of multiple genes. Activation of NF-κB by stimuli such as oxidative stress or cytokines promotes increased expression of antiapoptotic proteins such as Bcl-xL and XIAP, which suppress the execution phase of cell death. Induction of GADD45 leads to inhibition of JNK and prevents JNK-induced apoptosis. NF-κB also promotes the expression of antioxidant genes such as MnSOD, which plays a major role in scavenging mitochondria superoxide and in maintaining redox homeostasis. Overall, the activation of NF-κB by ROS leads to inhibition of apoptosis, redox rebalance, and enhanced cell survival.

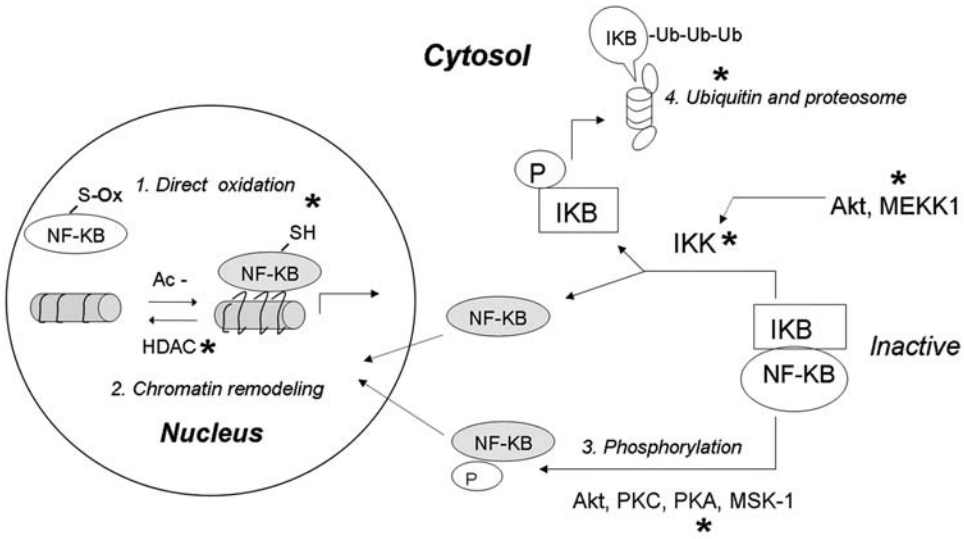

FIG. 6. Redox regulation of NF-κB. The function of NF-κB can be activated or inhibited through various redox-mediated mechanisms at multiple levels of the activation pathways. In the nucleus, direct oxidation of Cys in the DNA-binding domain can inhibit NF-κB–DNA-binding activity. In contrast, enzyme histone deacetylase (HDAC), which catalyzes the removal of an acetyl (Ac-) group from histone, can be inactivated by oxidative stress, allowing histone acetylation, chromatin uncoiling, and increased accessibility for NF-κB. In cytosol, activation of NF-κB can be regulated through phosphorylation of NF-κB itself or phosphorylation of its inhibitor IκB. Normally, NF-κB and IκB form a complex, which is sequestered in cytosol. Increased ROS can activate IκB-kinase (IκK) either directly through redox modification of IκK, or indirectly through activation of Akt and/or MEKK1, which then phosphorylates and activates IκK. Active IκK phosphorylates IκB and liberates active NF-κB from the complex to translocate to the nucleus. Phosphorylated IκB undergoes ubiquitination and degradation by proteosomes. Because the proteosome system is also redox sensitive, ROS can also regulate NF-κB activity by affecting the stability of IκB. Furthermore, phosphorylation of NF-κB by certain kinases may dissociate NF-κB from IκB and promote its nuclear translocation. *Grey*, Active forms of the proteins. *Major target molecules of redox regulation.

which is catalyzed by histone acetylase (HAT), uncoils the helical structure of DNA and exposes the binding sequence at the promoter regions, thus promoting NF-κB–DNA binding activity. Conversely, removal of the acetyl group from histone by histone deacetylase (HDAC) renders recoiling of DNA structure and prevents DNA binding by NF-κB. Acetylation of the RelA (p65) subunit of NF-κB was shown to increase DNA-binding activity, which can be reversed by HDAC (252). Interestingly, recent evidence suggests that HDAC activity can be inhibited by ROS. Oxidative inactivation of HDAC results in a shift of balance in favor of histone acetylation, which unwinds the DNA and promotes NF-κB activity (251). Further work suggested that inhibition of HDAC prevents apoptosis in leukemia cells by activation of NF-κB and downregulation of c-Jun kinase (57).

d. Cytoplasmic regulation of NF-κB. A key step in activation of NF-κB is the dissociation of NF-κB from IκB. Phosphorylation of either IκB or NF-κB subunits promotes such dissociations (236). Under certain conditions, hydrogen peroxide is able to activate NF-κB activity directly through phosphorylation/activation of IKK (144), or indirectly via MEKK-1 or Akt (196, 226). The transactivation of NF-κB induced by MEKK-1 or Akt was shown to be critical in mediating its antiapoptotic effect (225, 314). Conversely, in other system oxidants such as H_2O_2, arsenic, and lipid peroxidation product 4-HNE were found to inhibit IKK activity through direct oxidation, S-glutathionylation, or S-nitrosylation at Cys-179 of the IKK β-subunit (258, 259). Furthermore, MEKK-1 and Akt are redox sensitive. S-glutathionylation can inhibit their kinase activities (54, 219). It is worth noting that the oxidative activation or inhibition of IKK activity may or may not translate into corresponding changes in NF-κB activity. The overall consequences may be dependent on the different redox states of the cell types, the levels of the oxidants, and the durations of ROS exposure. For example, in human bronchial epithelial cells treated with H_2O_2, whereas the IKK activity was increased, leading to IκB phosphorylation, NF-κB–DNA-binding activity was inhibited. The unexpected result was attributed to an inhibitory effect on proteosomal degradation of IκB under oxidative stress (135). Besides phosphorylation of IκB by IKK, emerging evidence demonstrated that the NF-κB subunit may also be directly phosphorylated. Akt, protein kinase A (PKA), PKC ζ, mitogen and stress-activated kinase-1 (MSK-1), the 90-kDa ribosomal S6 kinase-1 (RSK-1), and casein kinase 2 (CK-2), can all phosphorylate RelA subunit. The phosphorylation of RelA can affect its binding affinity to IκB, to DNA and to the recruitment of essential cofactors (76, 236, 319, 344). Interestingly, these kinases were all redox sensitive, thus providing another layer for redox regulation of NF-κB.

2. AP-1. The AP-1 family of proteins represents an example of transcription factors whose functions involve control of both cell growth and apoptosis. Under certain conditions,

AP-1 activation could lead to cell death, whereas under other circumstances, AP-1 may promote cell proliferation and survival. The AP-1 family consists of several groups of basic leucine zipper domain (bZIP) proteins, including Jun (c-Jun, JunB, JunD), Fos (c-Fos, FosB, Fra-1, and Fra2), Maf (c-Maf, MafB, MafA, MafG/F/K, and Nrl), and ATF (ATF2, LRF1/ATF3, B-ATF, JDP1, JDP2) subfamilies (3). AP-1 proteins form heterodimers and bind to the target DNA sequence. Activation of AP-1 is regulated at both transcript and protein levels. The intracellular levels of c-jun and c-fos are controlled mainly by their transcription rates, which are tightly regulated by a variety of stimuli (278). The mitogen-activated protein kinase (MAPK) plays a major role in controlling activation of AP-1 proteins through phosphorylation. All three classes of MAPKs are involved in regulation of AP-1 activity (*i.e.*, c-jun is regulated mainly by JNK and ERK in some cell types). c-Fos is a substrate of ERK, and ATF2 is regulated by JNK and p38 kinases (146). JNK and p38 are both activated by stress stimuli.

a. Role of AP-1 in cell survival. AP-1 transcription factors are involved in both the induction and prevention of apoptosis, and the exact outcomes are highly tissue and developmental-stage specific (278). The pivotal role of c-Jun in cell survival was evidenced by embryonic lethality of mice lacking c-Jun, which is associated with prominent apoptosis of liver cells, leading to liver failure (121). Furthermore, by using sorbitol as an osmotic stressor, a recent study showed that c-Jun–deficient fibroblasts were more sensitive to osmotic stress-induced cell death, and downregulation of c-jun promoted cell death in c-Jun +/+ cells (334). These findings suggested a protective role of c-Jun against cell death. In contrast, studies in PC12 cells revealed a dual effect of c-Jun on cell death, depending on the stage of differentiation. In differentiated cells, c-Jun mediated induction of apoptosis, whereas in cells that were not yet differentiated, c-Jun exerted its cytoprotective effect (181). The role of c-Jun as an inducer of apoptosis was also seen in other systems. For example, overexpression of c-Jun was found to induce apoptosis in 3T3 fibroblast (26). Inhibition of c-Jun by antisense oligonucleotides is known to increase survival of lymphoid cells deprived of growth factor (53). Some studies suggested that the apoptosis-inducing effect of c-Jun may be triggered by JNK (71, 338). The role of the JNK signaling pathway in cell survival is discussed later in this section.

The mechanism by which c-Jun mediates cell survival or death seems to depend on the balance between the proapoptotic and antiapoptotic target-gene transcriptions and may be further regulated by p53 and p21 through their cell-cycle regulatory activity. *FasL*, *Bim*, and *Bcl3* are target genes of c-Jun. Induction of FasL and Bim may promote apoptosis, whereas upregulation of BCL3 by c-jun may potentiate its antiapoptotic function (278). It is the equilibrium between the positive and negative regulators of apoptosis that determines overall cell fate. This balance may be cell-type and stimulus dependent, as well as the integration of the effects from other transcription factors. Furthermore, c-Jun was shown to regulate the decision between p53-mediated cell-cycle arrest and apoptosis. A high level of c-Jun, which repressed p53-mediated p21 induction, was shown to prevent UV-induced growth arrest and shift most of p53 activity toward the induction of apoptosis (277). Interestingly, a recent report showed that Jun proteins (c-Jun, JunD, and JunB) upregulate antioxidant-responsive element (ARE)-mediated expression of antioxidant genes, such as thioredoxin, by associating with Nrf2 and Nrf1 and binding with ARE (318). This function may be important in the adaptive response to survive under oxidative stress.

b. Redox regulation of AP-1. Oxidative stress can activate c-Jun and ATF2 through phosphorylation by JNK and p38, respectively. Redox regulation of the JNK and p38 pathway is discussed later in this review. Like NF-κB, transcriptional activation of AP-1 can be regulated by chromatin remodeling (10, 211). Oxidative stress is known to promote AP1 activity through histone acetylation by inhibition of HDAC (251). Likewise, nitric oxide may suppress the DNA-binding activity of AP-1 through S-glutathionylation (158). Furthermore, the intracellular level of AP-1 can be regulated by redox-mediated mechanisms at the levels of transcription and protein turnover. Recent reports show that expression of c-Jun can be transcriptionally repressed by HDAC or proteosomally degraded through MEKK1-induced ubiquitination in response to osmotic stress. This downregulation of c-Jun plays an important role in apoptosis induction by oxidative stress (333, 334).

3. Nrf2. NF-E2–related factor 2 (Nrf2) is a member of p45 NF-E2–related proteins (p45 NF-E2, Nrf1, Nrf2, and Nrf3 (162, 163). The proteins in this family require a heterodimeric formation with small Maf proteins for DNA binding (215). Under normal conditions, Nrf2 localizes in the cytoplasm, where it interacts with the actin-binding protein, Kelch-like ECH-associating protein 1 (Keap1) (133). Keap1 functions as an adaptor of Cul3-based E3 ubiquitin ligase and targets Nrf2 for rapid degradation by the ubiquitin-proteasome (161). Oxidative stress and electrophiles are major activators of Nrf2 pathway. Dissociation of Nrf2 from Keap1 is a key step in activating Nrf2. The free Nrf2 translocates to the nucleus, heteromerizes with Maf(s), and binds to a *cis*-acting element known as antioxidant responsive element (ARE) or electrophile responsive element (EpRE) within the regulatory regions of many genes. Studies using Nrf2-deficient mice and microarray-based assays suggest that Nrf2 modulates transcription of almost 200 genes whose protein products function as antioxidants, phase II detoxification enzymes, proteosomes, heat-shock proteins, and glutathione-synthesis enzymes. These proteins all play a critical role in cellular defense against oxidative stress (132, 162).

a. Role of Nrf2 in cell survival. Nrf2 plays a critical role in protection against oxidative damage induced by acute injury, hyperoxia, nitrosative stress, ER stress, and exogenous prooxidative agents (43, 178, 189, 295). Nrf2 activation promotes cell survival under oxidative stress through multiple mechanisms. One major function is the transactivation of many antioxidant proteins, including heme oxygenase-1, ubiquitin/PKC-interacting protein A170, peroxiredoxin 1, the heavy and light chains of ferritin, catalase, glutathione peroxidase, superoxide dismutase, and thioredoxin (131). These proteins directly or indirectly scavenge free radicals and decrease the dose-dependent toxicity of ROS. Furthermore, Nrf2 regulates the synthesis of glutathione by controlling both the basal and inducible expression of genes encoding the heavy and light chains of glutamylcys-

teine synthetase (14). Because glutathione is not only the most abundant scavenger of ROS, but also the key controller of redox status of proteins affecting cell survival and death, the regulatory effect of Nrf2 on glutathione synthesis plays an important role in cell survival. Furthermore, Nrf2 was shown to modulate elimination of prooxidative electrophilic compounds through regulating expression of phase II detoxification enzymes such as glutathione-S-transferase (GST) and transporters such as multidrug resistance–associated protein 1/ATP-binding cassette transporter C. Direct roles of Nrf2 on cell survival and the death pathway are also evident. Nrf2 has been identified as an inhibitor of Fas-induced apoptosis (166, 213). In the absence of Nrf2, death-receptor–induced apoptosis was found to be enhanced. The cell death could be suppressed by supplementation of glutathione, suggesting that the antiapoptotic effect of Nrf2 was through elevating intracellular glutathione levels (166, 213).

Accumulation of unfolded polypeptides after oxidative stress could also trigger apoptosis. In response to unfolded protein stress, Nrf2 is a direct substrate of phosphorylation by PERK and acts as an effector of PERK-dependent cell survival (56). PERK is an ER transmembrane protein kinase that phosphorylates the subunit of translation-initiation factor 2 (eIF2a) in response to ER stress. Phosphorylation of eIF2a reduces the global translation, allowing cells sufficient time to correct the impaired protein folding (329). Induction of 26S proteosome and heat-shock proteins by Nrf2 facilitates the repair or elimination of the damaged proteins and thus protects cells from apoptosis (171).

b. Redox regulation of Nrf2. Association and dissociation of the Nrf2–Keap1 complex is considered as a key step in regulating Nrf2 activity. Multiple reactive Cys residues in Keap1 are targets of modifications by ROS and electrophiles. As illustrated in Fig. 7, sulfhydryl modifications dissociate Keap1 from Nrf2, allowing the translocation of Nrf2 to the nucleus, where it transactivates target-gene expression (70). Among the possible targeted residues for oxidation, Cys273 and Cys288 of Keap1 seem crucial for the ubiquitination-promoting activity. Therefore, the oxidative modification of Keap1 may also inhibit Keap1-mediated proteosomal degradation of Nrf2, allowing stabilization and nuclear accumulation of Nrf2 (163). In addition to targeting Keap1, oxidants and electrophiles can activate Nrf2 through phosphorylation by PKC and PERK. Phosphorylation of Nrf2 promotes its dissociation from Keap1, allowing the free Nrf2 to translocate to nucleus (56, 126). In the nucleus, Nrf2 can also be regulated at the step of DNA binding. Nrf2 cannot bind to the ARE without forming a heterodimer with one of the small Maf proteins (149, 215); therefore, the expression level of Maf protein likely regulates the Nrf2–DNA-binding capacity. Interestingly, expression of Maf can also be transcriptionally regulated by Nrf2/ARE itself, thus serving as an autoregulatory feedback mechanism (150). In addition to Maf, c-Jun and ATF-4 can heteromerize with Nrf2 and enhance Nrf2-DNA–binding activity (118). In contrast, Bach1, a transcriptional repressor of ARE/EpRE, can compete with Nrf2 to bind to the same DNA sequence, thus preventing Nrf2/ARE transcriptional activation. Recent study showed that oxidative stress can trigger nuclear accumulation of Bach1, leading to transcriptional suppression of ARE target genes (220).

4. HIF. Hypoxia-inducible factor (HIF) is generally known as an important transcription factor that regulates cellular metabolism and cell survival under hypoxic stress (29). However, HIF can also be activated by nonhypoxic stimuli such as thrombin and $CoCl_2$ under normoxia (248). HIF is composed of an alpha and beta subunit (HIF-α and HIF-β). Three α isoforms have been described (HIF-1α, HIF-2α, HIF-3α), with HIF-1 α being most intensively studied (30). Active HIF requires heterodimeric formation of the two subunits, which then translocates to nucleus, binds to the hypoxia-response element (HRE), and associates with coactivators such as CBP/p300. The binding

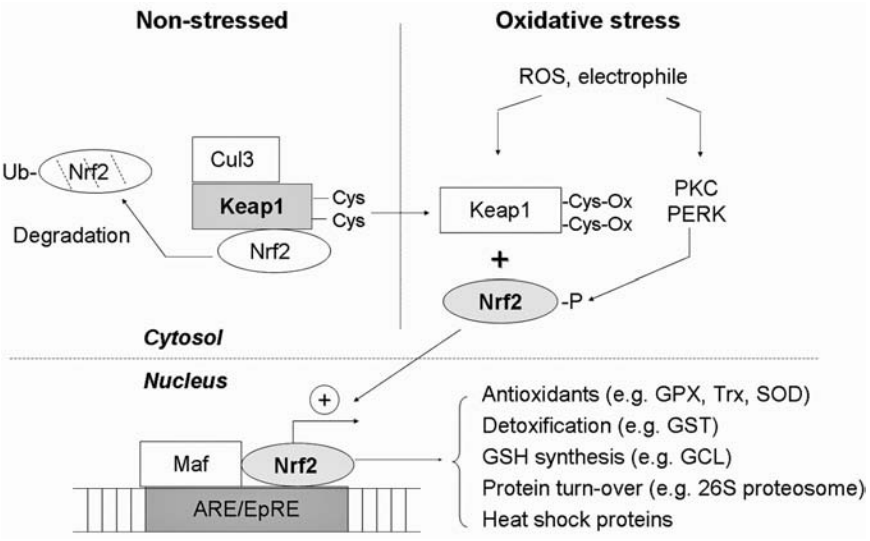

FIG. 7. Redox regulation of Nrf2. In unstressed cells, Nrf2 is sequestered in cytosol by Keap1, which functions as an adaptor for Cul3 (a ubiquitin E3 ligase) to target Nrf2 for ubiquitination and degradation. On oxidative stress or electrophilic stimuli, Nrf2 is activated via two mechanisms: (a) thiol oxidation of Keap1 and (b) phosphorylation of Nrf2 by kinases such as PKC or PERK. These cause release of Nrf2 from the inactive complex. The free Nrf2 is translocated to the nucleus, where it forms a heterodimer with Maf proteins and then binds to the antioxidant-responsive element or electrophile-responsive element (ARE/EpRE). The active binding triggers transcription of multiple target genes that encode antioxidants, glutathione synthesis enzymes, proteosomes, and heat-shock proteins. *Grey*, Active forms of the molecules. GPX, glutathione peroxidase; Trx, thioredoxin; SOD, superoxide dismutase; GCL, glutamylcysteine ligase, GST; glutathione-S-transferase.

results in activation or suppression of many genes involved in metabolism, angiogenesis, invasion/metastasis, and cell survival/death (29). The stability and activity of HIF1 can be regulated by oxygen-requiring hydroxylases [for review, see (270)]. Although HIF-β is constantly expressed regardless of oxygen levels, the stability of HIF-α is highly dependent on oxygen. Under atmospheric levels of oxygen (21%), the dioxygenase PHD (prolyl hydroxylase domain) hydroxylates HIF-1α on two proline residues. The hydroxylated HIF is then recognized by the von Hippel–Lindau (VHL) ubiquitin E3 ligase complex, which promotes the degradation of HIF-1α by the 26S proteosome (134). The hydroxylation of HIF requires iron in ferrous form (Fe^{2+}), oxygen, and 2-oxoglutarate as cofactors for the PHD catalytic activity (30). Under hypoxia, ferrous iron is converted to ferric form (Fe^{3+}), resulting in a decrease in HIF-α hydroxylation by PHD and subsequent stabilization of HIF-α (18). When the free HIF-1α binds to HIF-β and translocates to nucleus, another oxygen-dependent hydroxylase enzyme called factor-inhibiting HIF-1 (FIH-1) can regulate the DNA binding and transcriptional activity of HIF. Under normoxia, FIH-1 hydroxylates an N-terminal asparagines residue and renders HIF inactive by preventing the binding between HIF and its coactivators (175). Interestingly, genes encoding two isoforms of PHD proteins (*phd* 2 & 3) are HIF targets. Therefore, HIF can also be autoregulated under hypoxia by increased expression of its regulators. This response ensures a rapid and optimal degradation of HIF-α when the cells return to normoxia (30).

a. Role of HIF in cell survival. HIFs can act as both prosurvival and prodeath factors, depending on the stress conditions. Under most circumstances, HIF-1 actively contributes to adoptive responses to promote cell survival under hypoxia through transcriptional regulation of angiogenic factors and glycolytic enzymes (90). In tumor cells, HIF plays a major role in the metabolic switch that shunts glucose metabolites from mitochondria respiration to cytosolic glycolysis (Warburg effect) (194). HIF activation not only increases anaerobic glycolysis, but also attenuates mitochondrial respiration. The former occurs through upregulation of genes encoding glucose transporter (GLUT), glucokinases, aldolase A, and lactate dehydrogenase A (LDH-A), the enzymes that convert pyruvate to lactate. The latter occurs through the induction of pyruvate dehydrogenase kinase 1 (PDK1), which inhibits pyruvate dehydrogenase, the enzyme that converts pyruvate into acetyl-CoA in the mitochondria. These two phenomena were known to prevent the entry of pyruvate to the TCA cycle and shunt pyruvate toward lactate formation through glycolysis (29, 155). Recent study showed that silencing LDH-A resulted in a metabolic switch from glycolysis to the mitochondrial pathway and reduced tumor growth (82). These suggest that formation of lactate through glycolysis is important for tumor cell metabolism. Furthermore, it has been proposed that the increase in glycolysis mediated by HIF facilitates cell survival through maintaining ATP production and preventing the deleterious effect of ROS generated from mitochondrial respiration (155). Conversely, recent evidence suggests that HIF1 can also promote hypoxic cell death under certain conditions. It is proposed that active HIF may induce apoptosis though increased expression of several proapoptotic factors, including mitochondrial HGTD-P, Noxa, BNIP3, NIX, and IGFBP-3 (104). Thus, in response to hypoxia, HIF may promote or prevent cell death in a cell-type and stimulus-dependent manner.

b. Redox regulation of HIF. Under normoxia, a variety of stimuli [including growth factors such as IGF-1, hormones, vasoactive peptides such as thrombin, metal ions such as $CoCl_2$, H_2O_2, and certain NO donors such as S-nitrosoglutathione (GSNO) and NOC-18] are known to stabilize HIF, in part through increase ROS/RNS production (17, 100, 208). Interestingly, attenuation of ROS/RNS levels by antioxidants, such as NAC, ascorbate, and catalase, or genetic downmodulation of ROS-producing enzymes, such as NADPH oxidase 4 (NOX4), were found to decrease HIF1 expression under certain conditions (17, 153, 160, 276). At least three mechanisms have been proposed to explain how ROS/RNS stabilize HIF under normoxia (18). The first possibility is that the increased generation of $OH^{\cdot}$ from H_2O_2 through Fenton reactions, likely promotes the conversion of Fe^{2+} to Fe^{3+}. Because Fe^{2+} but not Fe^{3+} is required for active PHD activity, accumulation of ROS would lead to inactivation of PHD and consequently stabilization of HIF1 (248). This idea is supported by the findings that ROS accumulation by genetic loss of JunD–dependent antioxidant pathways leads to increased HIF1 activation through decreased availability of Fe^{2+} and attenuated activity of PHD (92). Furthermore, direct oxidative modifications such as S-nitrosylation of HIF or pVHL have been shown to cause stabilization of HIF (183, 234). Another putative mechanism of oxidative stabilization of HIF1 is that ROS/RNS activate multiple signaling pathways, such as PI3K/Akt and p38 MAPK, which may render PHD catalytically inactive (80, 148, 216).

Under hypoxic conditions, both ROS and RNS were found to inhibit HIF-1 DNA-binding activity and HIF-1 accumulation (34, 127). A study in HEK293 cells demonstrated that NO can destabilize HIF under hypoxic conditions through an increase in PHD-dependent degradation of HIF-1α (108). The author proposed that under hypoxia, NO inhibited mitochondrial respiration; thus, oxygen may be redistributed to other oxygen-dependent targets, such as PHD, and consequently promotes prolyl hydroxylation of HIF (108). Another study suggests that NO mediates destabilization of HIF under hypoxia through increased ROS production (40). *In vitro* HIF-1α–pVHL interaction assays demonstrated that a low level of ROS formation increased PHD activity and promoted ubiquitination and degradation of HIF (40). In addition to redox modulation of PHD, several lines of evidence suggest that a reducing condition is required for HIF-DNA–binding activity (152, 187, 325), and oxidative agents such as H_2O_2 can decrease HIF-DNA–binding activity and the expression of its target genes, such as EPO, aldolase A, and glucokinase (152). Taken together, these studies suggest the important role of the redox system in regulating HIF under both normoxia and hypoxia. However, further investigation is needed to provide a clear understanding of how ROS/RNS modulate HIF stability and activity under hypoxia.

B. Redox regulation of cell survival at the signal-transduction level

1. Mitogen-activated protein kinase. MAPK operates in a cascade fashion with a MAP kinase kinase kinase (MAPKKK) phosphorylating and activating a MAP kinase kinase (MAPKK), which then phosphorylates and activates a MAP ki-

nase (MAPK). The MAPK family consists of extracellular regulated kinases (ERK1/2), Jun N-terminal kinase (JNK), p38 kinase, ERK3/4, and the big mitogen-activated protein kinase 1 (BMK1/ERK5) pathways. The JNK and p38 kinase pathways are sometimes grouped together and referred to as the stress-activated protein kinases (SAPKs) (172).

a. Role of MAPK for cell survival under oxidative stress. Generally, ERK1/2 signaling promotes cell survival under mild oxidative stress, whereas SAPKs seem to induce cell death as a response to oxidative injuries (203). In response to oxidative stress, JNK and p38 activation can induce both intrinsic and extrinsic pathways of apoptosis and necrosis (206, 280). Induction of apoptosis by activated JNK involves direct phosphorylation of pro/anti-apoptotic BCL2 family members, transactivation of AP-1 (c-Jun and ATF2), and stabilization of p53. Phosphorylation of Bcl-2, Bcl-xL, and Mcl-1 by JNK is known to inhibit their antiapoptotic activities, whereas phosphorylation of Bim, Bmf, and Bad by JNK results in activation of those BH3-only proteins. The inhibition of antiapoptotic proteins and the activation of BH3-only proteins may promote translocation and activation of Bax/Bak, leading to mitochondria-mediated apoptosis (331). Furthermore, activation of JNK was shown to promote its dissociation from p53, leading to stabilization of p53. Active p53 in combination with AP-1 leads to Bid cleavage followed by the translocation of Bax protein to mitochondria and initiation of apoptosis (291). Although JNK and p38 activation can lead to cell death, the requirement of kinases seems to be cell-type and stimulus specific. An example is the critical role of p38 MAPK but not JNK in induction of apoptosis in keratinocytes by UVB irradiation (311). A recent study showed that the SAPK pathways can also induce apoptosis through the death-receptor pathway (280, 282). Conversely, JNK or p38 MAPK activation has been reported to have an antiapoptotic effect in malignant B and T lymphocytes, respectively (41, 191). The bifunctional role of the SAPK pathways in cell-fate decision may be dependent on different cell types and stimuli. Furthermore, the duration of the SAPK activation may dictate its consequences; that is, a transient activation may promote cell survival, whereas a sustained activation tends to induce apoptosis (203).

b. Redox regulation of MAPK. Multiple evidence shows that oxidative stress can activate the ERK pathway (301). This activation involves the stimulation of growth-factor receptor (tyrosine kinase receptor), which activates Ras, recruits Raf-1MAPKKK to the plasma membrane, and sequentially phosphorylates and activates MEK1/2 and ERK1/2. Redox modification can regulate ERK activation at the level of the tyrosine kinase receptor and the Ras activation. Autophosphorylation and activation of the tyrosine kinase receptor can be promoted by direct thiol-modification of the receptor (46). Furthermore, sustained activation of the receptor by oxidative stress could be obtained through oxidative inactivation of the phosphatases, the enzymes that dephosphorylate and inactivate the receptors (159). At the level of Ras activation, oxidative stress can modulate the function of Ras through thiol modifications. S-nitrosylation or glutathionylation of Ras is known to activate the protein directly and initiate the Ras-Raf-MEK/ERK cascade (174, 199).

The SAPK pathways are major transducers that signal cell death or survival in response to oxidative stress. In most circumstances, activation of SAPK pathways by ROS stress results in induction of apoptosis. The stress-response pathways are regulated at multiple levels, as illustrated in Fig. 8. The apoptosis-regulating signal kinase 1 (ASK1) is an important redox sensor for initiation of the SAPK signaling cascade. ASK1/MEKK5 is a ubiquitously expressed MAP kinase kinase kinase, which activates JNK and p38 MAP kinase pathways by Ser/Thr phosphorylation of their respective MKKs: MKK4/MKK7 for JNK and MKK3/MKK6 for p38 MAP kinases α/γ (291). Under nonstressed condition, ASK1 is sequestered by Trx in an inactive form (264). The association of thioredoxin with ASK1 *via* Cys32 or Cys35 of Trx appears to be necessary and sufficient to promote ASK1 ubiquitination and degradation, leading to abrogation of the ASK1 apoptotic activity. Because the binding between Trx and ASK1 requires a reduced form of Trx, the ASK-1/Trx complex can be dissociated through oxidative modifications. Apoptotic stimuli such as ROS and TNF induce oxidation of the critical cysteine residues in Trx and cause its dissociation from ASK1. The free ASK1 can then form a multimeric complex with active kinase activity (101, 186). A study using genetic deletion of ASK1 has confirmed that ASK1 activation is required for sustained activation

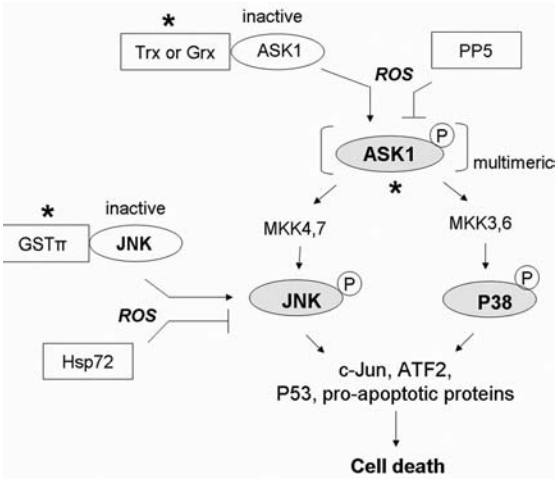

FIG. 8. Redox regulation of stress-responsive kinase (SAPK) signaling pathways. In most cases, activation of the SAPK pathway transduces an oxidative stress signal to cell death. Under nonstressed conditions, apoptosis-regulating signal kinase 1(ASK1) is inhibited by the reduced form of thioredoxin (Trx) or glutaredoxin (Grx). Increased oxidative stress causes oxidation of Trx and Grx and releases ASK1 to form an active multimeric complex with proper trans- or autophosphorylation. The activation of ASK1 subsequently leads to activation of c-Jun N-terminal kinase (JNK) and p38-MAPK, resulting in induction of cell death. JNK can also be inhibited by complex formation with glutathione S-transferase-π (GST-π) under nonstressed conditions, and can be activated by ROS in a similar fashion as ASK1. Negative regulatory molecules include the Ser/Thr phosphatase 5 (PP5), which inhibits ASK1 kinase activity by causing its dephosphorylation, and heat-shock protein 72 (Hsp72), which inhibits JNK activity. *Site of redox regulation. *Grey*, Active forms of the proteins.

of JNK/p38 MAPK and oxidative stress-induced apoptosis (297). Interestingly, recent work suggested that in response to H_2O_2, the active ASK1 seemed to undergo further oxidation *via* interchain disulfide bond formation, which maintained a sustained activation of JNK and induction of apoptosis (221). Besides Trx, glutaredoxin (Grx) has been reported to function as another negative regulator of ASK1 in a redox-sensitive and glutathione-dependent fashion (283). As a negative regulatory mechanism, activation of ASK1 can be inhibited through its binding with Ser/Thr phosphatase 5 (PP5). In response to oxidative stress, PP5 was found to dephosphorylate and inactivate ASK1 (212). At the level of JNK, recent studies showed that under nonstressed conditions, the π and μ classes of glutathione-S-transferase (GST) are negative regulators of JNK (1, 326). Under oxidative stress, suppression of JNK activity by GST can be reversed through dissociation of the GST/JNK complex and oligomerization of GSTπ (1). The oxidative liberation of JNK from GST resulted in induction of JNK-mediated apoptosis. In contrast, others reported that oxidative stress can inhibit JNK activity and apoptosis through induction of Hsp72 (238). RNS can oxidatively modify various SAPK pathways and provide differential effects. For example, S-nitrosylation of JNK1 and JNK2 was found to inhibit their activities (237), whereas tyrosine nitration of p38MAPK by peroxynitrite was shown to induce immediate activation of the kinase (269). The fairly complex effect of oxidative stress on SAPK pathways may be owing to the differential dose and duration of the stimuli and types of oxidative modifications.

2. PI3K/Akt pathway. Signal transduction *via* PI3-kinases plays an important role in regulating cell growth, proliferation, survival, and motility. A moderate level of ROS activates PI3K signaling and promotes cell survival, whereas sustained oxidative stress may inhibit this pathway, allowing apoptosis to occur (182). The PI3K cascade is stimulated by phosphorylation of growth-factor receptor (tyrosine kinase receptor), which promotes its direct binding with PI3K or indirect binding through adapter proteins such as IRS docking protein for IGF-1 signaling. Activated PI3K converts the membrane phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] to the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3 or PIP_3]. PIP3 recruits Akt and 3′-phosphoinositide–dependent kinase-1 (PDK1) to the plasma membrane through its binding with pleckstrin homology (PH) domains of these proteins (315). Then the PDK1 can phosphorylate and activate Akt (22, 315). Akt, which can also be phosphorylated by the mTOR complex 2 (TORC2), regulates the functions of downstream targets through its Ser/Thr kinase activity (266). The activation of the PI3K/Akt pathway is tightly regulated by phosphatases, especially the reversion of PIP_3 back to PI(4,5)P_2 by phosphatase and tensin homologue (PTEN) and the inactivation of receptor tyrosine kinases by protein tyrosine phosphatases (PTPases) (68).

a. Role of PI3K/Akt in cell survival. Compelling evidence suggests that oxidative stress–induced activation of the PI3K/Akt pathway is crucial for cell survival (327). Paradoxically, under certain circumstances, phosphorylation of Akt seemed to play a proapoptotic role through induction of ROS production (105) or activation of the Fas-mediated death pathway (193). The dual effects of PI3K/Akt are likely the results of crosstalk with other signaling pathways, such as JNK and PKC (105). Survival signals from PI3K/Akt pathways were transduced mainly through the phosphorylation and inactivation of proapoptotic proteins such as BAD, caspase-9, P53, and forkhead transcription factor (FKHRL1), which targets FasL, Bim, IGFBP1, and Puma. Akt phosphorylates and activates IKK and cyclic AMP response element–binding protein CREB, resulting in elevated transcription of genes encoding antiapoptotic proteins such as Bcl-2, Bcl-Xl, and Mcl-1 (203). Furthermore, Akt also exerts its antiapoptotic function by phosphorylation and inhibition of ASK1 activity, which prevent stress-induced apoptosis (154). This is an example of crosstalk between PI3K/Akt and SAPK pathways in the regulation of cell survival.

b. Redox regulation of PI3K/Akt. As depicted in Fig. 9, several components of the PI3K/Akt signaling pathway are redox sensitive. Activation or inhibition of this pathway by the redox system is mainly through oxidative modification of Cys-dependent phosphatases (CDPs) and protein kinases. Although the oxidative inactivation of CDPs is known to be critical in activation of the pathways, redox modifications of protein kinases seems to inhibit the survival signaling. CDPs comprises of a large family of enzymes that share a conserved catalytic domain containing a highly reactive Cys residue. At physiologic pH, the Cys exists as a thiolate anion, which is required for its phosphatase activity. Oxidative modification of the Cys signif-

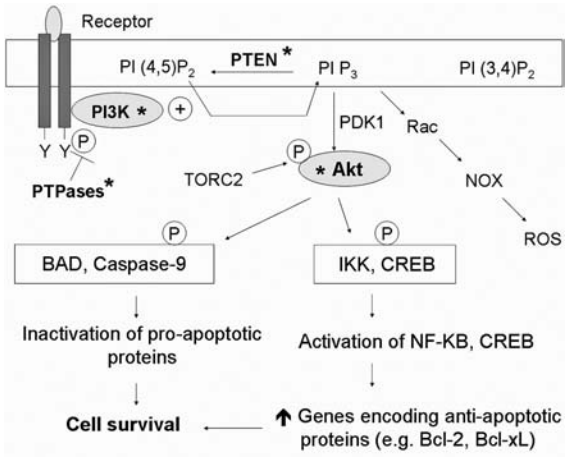

FIG. 9. Redox regulation of the PI3K/Akt signaling pathway. PI3K/Akt transduces the signal for cell survival mainly through phosphorylation of target molecules by Akt. This results in inactivation of proapoptotic proteins and activation of transcription factors, which target antiapoptotic proteins. Under oxidative stress, this pathway is activated by oxidative inactivation of phosphatases [*i.e.,* protein tyrosine phosphatases (PTPases) and PTEN], allowing constitutive activation of tyrosine kinase receptor and PI3K. However, direct oxidative modification of PI3K and Akt can result in their inactivation and compromise the survival signals. Furthermore, the PI3K/Akt pathway can also promote cellular production of ROS through activation of Rac and NADPH oxidase (NOX). *Site of redox regulation. *Grey*, Active forms of the proteins; TORC2, mTOR complex 2.

icantly inhibits the enzyme activities (265). CDPs that regulate PI3K/Akt signaling include PI3-phosphatase, PTEN, and PTPases. Oxidative inactivation of PTEN through an intramolecular disulfide bond or S-nitrosylation of the active Cys is known as an important mechanism of PI3K/Akt activation by oxidative stress (179, 342). The reduction of oxidized PTEN in cells appears to be mediated predominantly by the Trx system (180). Redox modification of PTEN was recently shown to be a mechanism to promote survival of cancer cells with mitochondrial dysfunction (240). Normally, active PTEN is maintained in a reduced state by the NADPH/Trx system. Therefore, a defect in mitochondrial respiration, which causes an increase in NADH and a decrease in NADPH, can lead to oxidative inactivation of PTEN and activation of the PI3K/Akt survival pathway (240). Besides the lipid phosphatase, protein phosphatases such as PTP1B, SHP-2, and TC45 are targets of ROS-mediated oxidation (179, 207). These phosphatases are negative regulators of receptor tyrosine kinases; therefore, their oxidative inactivations result in sustained activation of the receptor-mediated PI3K/Akt signaling (328).

Besides redox regulation of phosphatases, protein kinases such as PI3K and Akt can be modified by oxidation. In contrast to phosphatases, redox modification of the kinases results in downregulation of PI3K/Akt signals and a decrease in survival capacity. In response to oxidative stress, a disulfide bridge is formed between Cys297 and Cys311 in the kinase domain of Akt (219). Interestingly, the oxidation of Akt does not directly affect the kinase activity *in vitro*. Instead, it promotes the binding of Akt to protein phosphatase PP2A, leading to dephosphorylation and inactivation of Akt (219). The oxidative inactivation of Akt was shown to be reversible by GRX, which appears to exert its antiapoptotic effect through this mechanism (219). Recently, Akt was also shown to be inhibited by S-nitrosylation (340). At the level of PI3K, the p85 subunit of PI3K was shown to be a direct target for tyrosine nitration, leading to inactivation of the Akt survival pathways (77).

It is worth noting that receptor-mediated activation of PI3K can stimulate Rac-NAD(P)H oxidase (NOX), leading to increased generation of ROS (330). Recent study demonstrated that ROS produced by NOX may contribute to monocyte/macrophage cell survival through activation of Akt and inhibition of p38 MAPK pathways (328).

C. Redox regulation of cell survival at the cell death–execution level

The redox regulation of transcription factors and signal-transduction pathways may affect cell survival through a series of molecular processes to activate or inhibit the cell-death execution molecules. Under certain conditions, ROS stress may directly modulate the activity of these cell-death effectors. As shown in Fig. 10, several apoptotic effectors, such as caspases, Bcl-2, and cytochrome c, are redox-sensitive, and their functions can be significantly affected by cellular ROS.

1. Caspases. Caspases are evolutionarily conserved aspartate-specific, cysteine-dependent proteases. Caspases include large prodomain caspases such as caspase-1, -2, -4, -5, -9, -8, -10, -11, and -12, and small prodomain caspases including caspase-3, -6, -7, and -14. Activation of the large-domain caspases (initiator caspases) occurs by forming multimeric complexes [*i.e.*, apoptosome for caspase-9 and death-inducing signaling complex (DISC) for caspase-8]. Whereas the initiator caspases can be activated through proximity-induced autoacti-

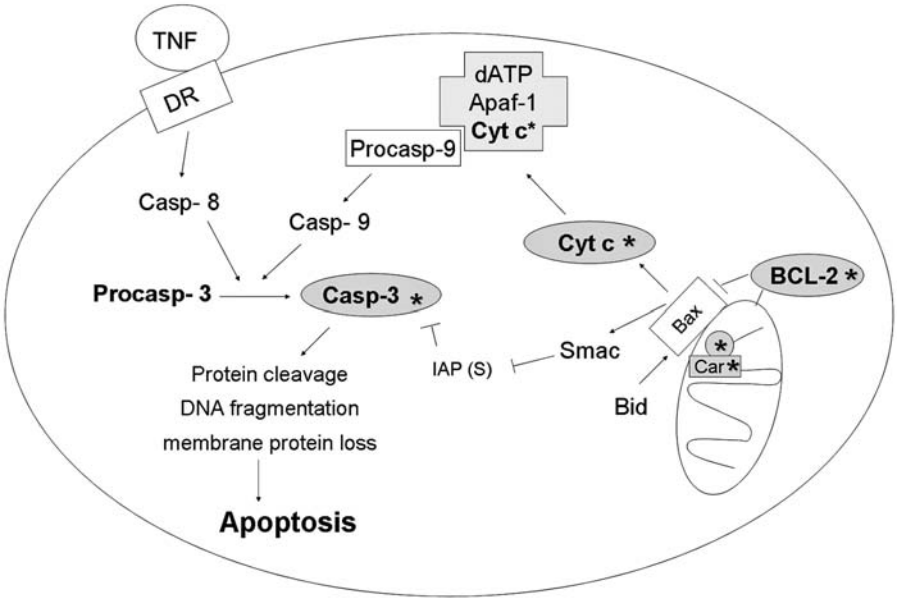

FIG. 10. Redox regulation at the execution level. Apoptosis can be triggered through extrinsic or intrinsic pathways. External stimuli such as TNF-α or Fas ligand binds to death receptor and transduces the signal into activation of caspase-8, leading to initiation of the extrinsic pathway. Intrinsic signals, such as DNA damage and oxidative stress, can transduce the death signal by causing release of cytochrome *c* from mitochondria to cytosol, followed by activation of caspase-9 through formation of apoptosome (Apaf-1, cytochrome c, pro-caspase 9, and dATP). Active caspase 8 and caspase 9 can further cleave procaspase-3, producing an active fragment of caspase-3, which cleaves its protein substrates such as PARP, resulting in apoptosis. To keep apoptosis in check, Bcl-2 family proteins play an important role in regulation of mitochondria membrane permeability and cytochrome *c* release. Antiapoptotic proteins such as Bcl-2 prevent apoptosis through both direct interaction with proapoptotic proteins such as Bax and indirect control of oxidative stress via maintenance of a reducing environment. *Grey**, Potential sites of redox regulation. Car, cardiolipin; Cyt c, cytochrome *c*; casp 3, caspase 3.

vation, effector caspases require proteolytic maturation (by the large-prodomain caspases) to be activated (281).

a. Role of caspases in cell death and survival. A hallmark of apoptosis is the activation of caspases, which requires sequential proteolysis of the initiator caspases and effector caspases. Apoptotic stimuli can trigger caspase activation either through the extrinsic (death receptor–mediated caspase-8 activation) or the intrinsic pathways (mitochondria-mediated caspase-9 activation). Activation of the caspase cascade ultimately leads to cleavage of a variety of substrates (*e.g*., PARP), DNA fragmentation, loss of membrane integrity, and cell death. Besides the critical role of caspases in apoptosis, emerging evidence suggests non-apoptotic functions of caspases, which includes an opposing role in promoting cell survival (173). An example is the requirement of caspase-8 and c-FLIP in NF-κB activation (288). Although oxidative stress is known to regulate the apoptotic activity of caspases, it is still unclear whether the prosurvival function of caspases can be regulated by redox modifications.

b. Redox regulation of caspases. As described earlier, oxidative stress can activate or inhibit caspases through signal-transduction pathways. Furthermore, the levels of oxidative stress can regulate the expression of FasL, which binds to its receptor and leads to caspase-8 activation (12). Increased endogenous levels of ROS, especially in the mitochondria, can stimulate the apoptotic machinery by promoting membrane permeabilization through mitochondrial permeability transition (MPT). The permeabilization of mitochondria may lead to a release of cytochrome *c* and an activation of caspase-9 through the formation of apoptosomes (6, 168, 177). In addition to the stimulation of the apoptotic apparatus, ROS can also directly affect the function of caspase proteins. The reduced state of Cys at the active site is required for the catalytic activity of caspases, and thus its function is redox sensitive. Caspases can be activated or inhibited by redox system, depending on the degree of the ROS stress (39). A study using various concentrations of H_2O_2 demonstrated that a low dose of H_2O_2 can activate caspases and induce apoptosis, whereas a high dose can cause oxidative inactivation of caspases, and the cells undergo necrosis (112). Several lines of evidence suggest that reducing environment is required for proper function of the Cys-containing active sites of caspases. Oxidative modifications of caspases, such as direct oxidation, glutathionylation, and S-nitrosylation, attenuated the proteolytic activities and inhibited apoptosis (112, 115, 156). Besides the regulation of its catalytic activity, a recent study with genetic silencing of Grx in endothelial cells showed that activation of caspase 3 and TNF-α–induced cell death can be suppressed by glutathionylation of procaspase-3 (235). This is because the glutathionylated procaspase 3 is less susceptible to cleavage by initiator caspase-8 (235). The important role of glutathione in modulating the activity of effector caspases may provide an explanation for the observation that the efflux of GSH is required for the execution of apoptosis (88, 95).

2. Bcl-2. Bcl-2 is the prototype of antiapoptotic BCL-2 family members (11). It contains BH1-BH4 domains and attaches to the outer mitochondrial membrane. Extensive studies highlight the importance of Bcl-2 in protecting cells against oxidative stress–induced apoptosis and its role in regulating redox signaling. These are evidenced by the suppression of lipid peroxidation and attenuated apoptosis observed in BCL-2–overexpressed cells, and the similar phenotypes between Bcl-2 knockout mice and mice exposed to chronic oxidative stress (122, 316). Furthermore, the increased amount of glutathione was observed in BCL-2–overexpressing cells, suggesting a role of BCL-2 in controlling the cellular redox status (79).

a. Role of Bcl-2 in cell survival. The antiapoptotic effects of Bcl-2 are observed at multiple levels. Bcl-2 can prevent mitochondrial membrane permeabilization through direct interaction with proapoptotic Bax/Bak or BH3-only proteins (6). Furthermore, Bcl-2 was shown to prevent ROS-induced mitochondrial permeability transition pore opening in certain experimental models (167). Besides its direct antiapoptotic role, Bcl-2 also functions to maintain redox homeostasis by regulating glutathione and NADPH levels (324). Interestingly, because the redox environment of the nucleus has an effect on the accessibility of transcription factors to their targets, nuclear GSH compartmentalization controlled by Bcl-2 is thought to play a role in regulating the transcription of genes encoding certain mitochondria proteins such as fatty acid–binding proteins (FABPs), VDAC, and UCP (324). These proteins may directly or indirectly modulate mitochondria-induced apoptosis. Interestingly, recent work showed that conformational change of Bcl-2 through its binding with Nur77 can convert Bcl-2 to a proapoptotic protein (185).

b. Redox regulation of Bcl-2. The function of Bcl-2 can be regulated through redox-sensitive signaling. Mild oxidative stress is shown to induce the expression of Bcl-2 through activation of transcription factors such as NF-κB, as an adaptive response to promote cell survival (42). In contrast, in response to oxidative stress, JNK can phosphorylate and inhibit Bcl-2 function, allowing apoptotic processes to occur (11, 65). Besides regulation at the signal-transduction level, recent evidence suggests that Bcl2 can be directly affected by oxidative stress. For example, oxidative carbonylation of Bcl-2 by NO has been shown to be an important mechanism of NO-induced apoptosis in insulin-secreting cells (38). Furthermore, inactivation of ERK1/2 was found to cause proteosomal degradation of Bcl-2 in a redox-dependent manner (32).

3. Cytochrome c. Cytochrome c is a small-molecular-weight heme-containing protein, which participates in an electron transfer from complex III to complex IV in mitochondrial electron-transport chain. Because of the nature of cytochrome *c* as a reversible electron donor/acceptor, this protein is highly redox-sensitive. Cytochrome *c* locates in inner mitochondria membrane and interacts with the membrane phospholipid cardiolipin (233). The release of cytochrome *c* is considered a hallmark of mitochondrial-mediated apoptosis (99).

a. Role of cytochrome c in cell survival. Under physiologic conditions, cytochrome *c* plays an essential role in oxidative phosphorylation and production of ATP, which is the major energy source for biologic reactions. Generation of superoxide in mitochondria occurs mainly through electron leakage from

complex I and complex III. Therefore, cytochrome *c*, which accepts an electron from complex III and donates it to cytochrome *c* oxidase, also functions to prevent the electron outflow and superoxide generation. Because of this function, cytochrome *c* plays a critical role in keeping ROS generation at a low level, optimal for cell survival (343). Apoptotic stimuli trigger mitochondrial membrane permeabilization and promote the release of cytochrome *c* from mitochondria to cytosol, where it acts as an apoptotic inducer. As shown in Fig. 10, the released cytochrome *c* forms a complex (apoptosome) with procaspase-9, Apaf-1, and ATP or dATP, leading to activation of caspase-9 and the downstream caspase cascade (168). Furthermore, the loss of cytochrome *c* from the respiratory chain leads to electron leakage from complex III and increased mitochondrial generation of superoxide radicals (343). This may explain the increase of ROS level observed during apoptosis, even when the stimuli are not prooxidants. In addition to ROS generation from the electron leakage, direct oxidation of cytochrome *c* by p66Shc has recently been proposed as a novel mechanism of mitochondrial ROS generation during apoptosis (97). On proapoptotic signals, a mammalian adapter protein p66Shc can be liberated from its putative inhibitory complex in mitochondria. The active p66Shc then oxidizes cytochrome *c* and promotes the formation of ROS (97). The ROS increase mediated by cytochrome *c* may amplify the apoptotic signals and accelerate the death-execution process.

Besides its function as an electron carrier, cytochrome *c* may act as a peroxidase (13). During the cell-death process, the released cytochrome *c* in the cytosol binds and exerts a peroxidase activity on plasma membrane lipids, especially phosphatidyl serine, leading to lipid peroxidation (141). This structural modification of the plasma membrane can lead to exposure of the signals recognized by the macrophage to engulf the cell corpse (13).

b. Redox regulation of cytochrome c. Cytochrome *c* is tightly regulated by the redox system at multiple levels. In the mitochondria, the interaction between cytochrome *c* and cardiolipin plays a critical role in maintaining cytochrome *c* in its proper location and prevents its release to the cytosol (233). The possible role of cardiolipin in the release of cytochrome *c* from mitochondria during apoptosis has recently been discussed (233). Cardiolipin is sensitive to lipid peroxidation, and the oxidized cardiolipin will lose its binding affinity to cytochrome *c*. Therefore, under normal conditions, the cardiolipin–cytochrome *c* complex is stabilized by the ROS-scavenging effect of the mitochondrial glutathione and mitochondrial membrane glutathione peroxidase 4 (mtGPX4) (253). Interestingly, a novel role of cytochrome *c* as a cardiolipin peroxidase has recently been found (142). When ROS stress exceeds the capacity of the glutathione system or when the mitochondrial GSH pool is depleted, excessive ROS can activate the peroxidase activity of cytochrome *c*, leading to cardiolipin peroxidation (142). Oxidized cardiolipin loses its binding affinity to cytochrome *c*, allowing cytochrome *c* to be dissociated and released to cytosol. This interesting finding raises a novel concept that the redox status of cytochrome *c* can autoregulate the localization of cytochrome *c*. It is worth noting that the effect of ROS/RNS on the cardiolipin peroxidase activity of cytochrome *c* is species specific. For example, H_2O_2 is a very important cofactor for the peroxidase reaction, whereas the physiologic concentration of NO has been shown to effectively inhibit the peroxidase activity of cytochrome *c* (323).

The release of cytochrome *c* requires not only its dissociation from cardiolipin but also the permeabilization of the mitochondrial membrane. This provides another level of its redox regulation. The increased mitochondrial ROS and the disruption of the electron-transport chain can cause the collapse of the mitochondrial transmembrane potential ($\Delta\Psi m$). This may result in permeabilization of the mitochondrial membrane, which allows cytochrome *c* to be released from the mitochondria. The mechanism of membrane permeabilization has been extensively reviewed (168). Although low levels of NO can inhibit the peroxidase activity of the cytochrome *c*/cardiolipin complex, a recent study showed that a direct nitrosylation of mitochondrial cytochrome *c* promotes its release to the cytosol (271).

Once cytochrome *c* is released to the cytosol, it can induce apoptosis only if it is in an oxidized form. Under physiologic conditions, the presence of high levels of cytoplasmic GSH keeps the released cytochrome *c* in an inactive (reduced) state, thus functioning as a fail-safe mechanism if cytochrome *c* is released from mitochondria. If the redox status of the cell is disturbed, however, perhaps in the presence of hydrogen peroxide or depletion of GSH or both, the cellular redox status will be shifted toward the oxidized state, and cytochrome *c* will be active, allowing caspase activation and apoptosis to proceed (114).

D. Integration of redox-sensitive signaling pathways in the regulation of cell survival

1. Crosstalk between signaling pathways. Redox regulation of the key factors affecting cell death/survival is often bifurcated (*i.e.*, the same protein can either be activated or inhibited by redox alteration). Whether the consequence of oxidative stress will lead to cell survival or death is likely dependent on the integration of those redox-sensitive signals. This is exemplified by the crosstalk between the PI3K/Akt and SAPK pathways. As shown in Fig. 11, both pathways can be activated by oxidative stress. Activation of the PI3K/Akt pathway can activate nuclear translocation of NF-κB mainly through phosphorylation of the IKK or NF-κB subunit (314). Activated NF-κB induces transcription of multiple genes to promote cell survival (147). In contrast, activation of ASK1 may lead to phosphorylation of JNK and JNK-induced cell death (297). Under physiologic conditions, cell survival is a favorable process, and survival mechanisms are activated to inhibit the cell-death signals at multiple levels. For instance, Akt can phosphorylate and inhibit the ASK1 cascades (154), whereas NF-κB inhibits JNK-induced apoptosis through increasing the transcription of JNK inhibitor (GADD45, XIAP) (61, 147). Studies using knockout IKK or RelA cells showed that, in the absence of NF-κB activation, ROS stress induces JNK activation, leading to cell death (91, 145). Severe oxidative stress inhibited NF-κB signaling, which allowed not only sustained JNK activation but also amplification of ROS stress due to decreased transcription of antioxidants (35). These results suggest that the crosstalk between NF-κB and JNK signaling is important in determining the final cell fate in response to oxidative stress. In

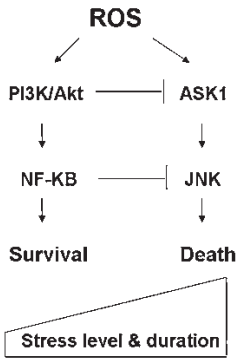

FIG. 11. Integration of redox signaling. An increase of reactive oxygen species (ROS) can result in either cell survival or cell death, depending on the integration of the proapoptotic and antiapoptotic signals. The levels and durations of ROS stress determine the activation or inhibition of each signal-transduction pathway. The crosstalk between PI3K/Akt and stress-responsive MAPK pathway (SAPK) serves as an example. A low level or transient increase of ROS can activate PI3K/Akt, leading to cell survival through NF-κB. The predominant survival signals of the Akt and NF-κB pathways prevent cell death by inhibiting ASK1 and JNK, respectively. However, a high level of ROS causes a sustained activation of the ASK1-JNK cascade and inactivation of PI3K/Akt and NF-κB due to protein oxidation, leading to cell death.

general, transient activation of JNK signaling by mild ROS stress leads to predominant antiapoptotic signals and cell survival, whereas sustained activation of JNK by severe ROS stress would be proapoptotic. The integration of NF-κB and JNK pathways in signaling apoptosis and cell survival in response to oxidative stress was recently reviewed (35, 224).

2. Role of p53. Besides the crosstalk between multiple signaling pathways, certain factors such as p53, which is a redox-sensitive molecule, can regulate cell survival at multiple levels of signaling. Under normal physiologic conditions, p53 protein has a short half-life. It is maintained at a low level by MDM2-mediated inactivation and ubiquitin/proteasome degradation (116, 169, 198, 230). With certain stimuli, such as oxidative stress and DNA damage, p53 is stabilized by posttranslational modification and translocates to the nucleus. p53 serves as a master transcription factor to activate the expression of proteins involved in maintaining genomic stability and cellular homeostasis. As a tumor suppressor, p53 exerts its genome guardian effect by controlling various cell-cycle checkpoints and regulating DNA-damage repair, senescence, and apoptotic machineries (9). Recent studies suggested a novel function of p53 in maintaining redox homeostasis through regulating energy metabolism, mitochondrial biogenesis, and the expression of antioxidant enzymes (21). Loss of p53 function contributes to the development of many types of human cancer (284). A number of studies suggest that p53 plays an important role in controlling cell fate through regulation of cellular ROS level (28, 69, 300).

a. P53 serves as an antioxidant to maintain redox homeostasis and normal cell survival. Recent study revealed that under physiologic conditions or mild ROS stress, p53 is activated to induce transcriptions of multiple antioxidant molecules such as SESN1 and SESN2 and GPX1, which function to decrease ROS level and to promote cell survival (263). Suppression of p53 by siRNA caused an increase in cellular ROS, which can be completely reversed by the use of antioxidant *N*-acetylcysteine (NAC) (263). Splenocytes and thymocytes of Trp53$^{-/-}$ mice also exhibited increased ROS compared with that of the wild-type mice. Furthermore, a recent report showed that the p53 tumor suppressor also affects cellular redox homeostasis by inhibiting glycolysis and stimulating mitochondrial bioenergetics through transcriptional activation of TIGAR (TP53-induced glycolysis and apoptosis regulator) and the SCO2 (synthesis of cytochrome *c* oxidase 2), respectively (20, 202). TIGAR, a homologue of fructose-2,6-biphosphatase, functions to inhibit phospho-fructokinase activity and reduce fructose-2,6-bisphosphate levels, resulting in an inhibition of glycolysis. This causes a shift toward the pentose phosphate pathway, leading to the production of NADPH. The reducing equivalents of NADPH can be used to regenerate major cellular antioxidant GSH, which in turn promotes the scavenging of ROS, genomic stability, and prevention of cancer. Thus, TIGAR seems to function as a checkpoint to regulate glycolysis negatively (102). SCO2 is a nuclear gene that encodes a copper-binding protein required for the assembly of cytochrome *c* oxidase II (CO II) subunits of complex IV in the respiratory chain. Disruption of the SCO2 gene in wt-p53 cancer cells caused the metabolic switch toward glycolysis, as exhibited in p53-deficient cells (202). Interestingly, using new technology to increase an extra copy of p53 and Arf genes, Matheu and colleagues also confirm the redox modulating function of p53 *in vivo* (201) . They found that the super Arf/p53 (s-Arf/p53) mice, which have higher stimulated expression of p53, exhibited an increase in glutathione and SESN1/SESN2 level, along with a decrease in ROS, lipid peroxidation, and oxidative damage to protein and DNA (201). Taken together, activation of p53 can decrease ROS stress, maintain redox homeostasis, and promote cell survival through transcriptional regulation of antioxidant enzymes, TIGAR, and SCO2, which regulate activities of glycolysis and mitochondrial electron-transport chain.

b. Role of p53 in cell death. P53 controls cell fate through several mechanisms, depending on the magnitude of the stress. High levels of ROS cause phosphorylation and stabilization of p53 protein, which often exhibits proapoptotic function under such conditions. Whereas a low level of stress induces p53 to upregulate the expression of genes encoding ROS-scavenging enzymes, high level of stress induces p53 to upregulate genes encoding prooxidant such as PIG3, p66^{Shc}, and proline oxidase (97, 261, 263). Furthermore, p53 is also known to increase gene expression of proapoptotic proteins such as BAX, BBC3 (Puma) and decrease gene expression of antiapoptotic proteins such as Bcl-2 (45). As illustrated in Fig. 12, these observations suggest that the very same p53 protein can control ROS levels and cell fate by transcriptional regulation of different sets of genes in response to different intensities of stress. Interestingly, when cells are under severe stress, activated p53 can also directly interact with ARE-containing promoters and suppress Nrf2-dependent transcription of antioxidant response genes such as x-CT, NQO1, and GST-α1, resulting in an elevation of ROS and apoptosis (84). Reactivation of p53 in p53-deficient tumors can cause complete tumor regression, suggesting a proapoptotic role of p53 (336). Thus, p53 exerts antioxidant function in cells under moderate (physiologically relevant) levels of ROS stress, but exhibits prooxidant function in the severely damaged cells (263). Besides transcriptional regulation,

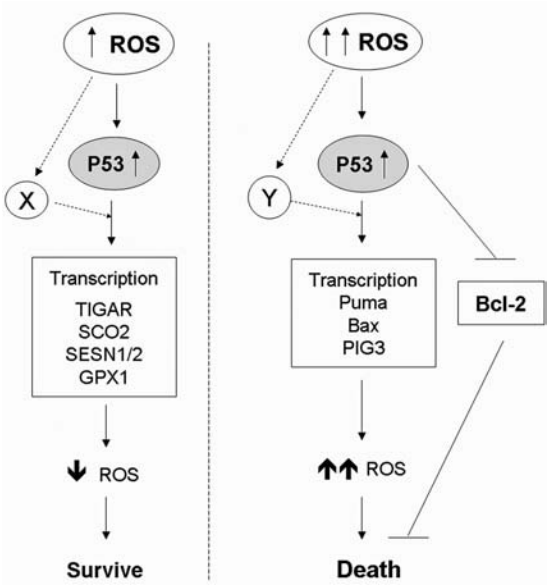

FIG. 12. Regulation of cell fate by p53. Different levels of ROS stress activate p53, leading to various outcomes. Activation of p53 by a low level of ROS stress promotes cell survival, whereas severe ROS stress activates p53 and causes cell death. It is possible that the activated p53 might interact with different factors (indicated as "X" and "Y") under different levels of ROS stress, leading to activation of distinct sets of target genes (see text for details).

p53 also modulates cell-survival pathways through direct interaction and inhibition of antiapoptotic protein Bcl-2 (299).

c. Redox regulation of p53. Although p53 functions as a transcription factor to control the expression of several redox-regulating molecules, p53 itself is redox sensitive. p53 is a zinc-binding protein containing 10 cysteine residues susceptible to ROS oxidation. The mechanisms that regulate the redox status of p53 remain to be fully elucidated, but much evidence suggests that p53-DNA–binding ability can be strongly inhibited by oxidation and nitrosylation. *In vitro* experiments revealed that only the reduced form of p53 can specifically bind to the target DNA, whereas direct oxidation or S-glutathionylation of Cys can block its binding to DNA and transactivation activity (63, 110, 317). Further study suggested that the redox status of p53 can be controlled by redox-sensitive thioredoxin, thioredoxin reductase, and redox factor-1 (Ref-1) (111). Besides thiol oxidations, p53 protein can be oxidatively modified and inactivated through protein nitration by $ONOO^-$ in human glioblastoma cells (51, 52). The redox-sensitive nature of p53 protein suggests that this molecule may act as a redox sensor in mammalian cells, as OxyR in bacterial system. Therefore, it is possible that a loss of p53 function, as observed in many types of cancer cells, may disable this important redox homeostasis mechanism and allow cancer cells to escape senescence and cell death. It is worth noting that not only the redox system can regulate the function of p53 through direct oxidative modifications, but the indirect activation of p53 through its interacting partners such as ATM or MDM-2 also provides another layer of redox regulation of p53 (44, 120).

III. ROLE OF REDOX REGULATION OF CELL SURVIVAL IN PATHOGENESIS OF DISEASES

As described in previous sections, the redox system plays a crucial role in regulating cell survival. Disruption of redox homeostasis will result in a deregulation of apoptosis associated with various diseases, including cancer, degenerative diseases, and aging. Increased oxidative stress can either promote cell survival or induce cell death, depending on the cellular context. Genetically unstable cells can adapt to live with the stress by adjusting the level of ROS to the extent that promotes cell survival, leading to development of cancer. In contrast, normal or aging cells that failed to maintain redox balance are prone to oxidative stress–induced cell death. This may act as a pathogenic mechanism of degenerative diseases. Figure 13 shows the overall roles of redox regulation in cell survival and the pathologic process of diseases.

A. Aberrant prolonged cell survival leads to cancer

Cancer cells often exhibit increased generation of ROS compared with normal cells (294). Although the exact mechanisms responsible for the intrinsic ROS stress in cancer cells remain unclear, several lines of evidence suggest that ROS production is induced after the expression of genes associ-

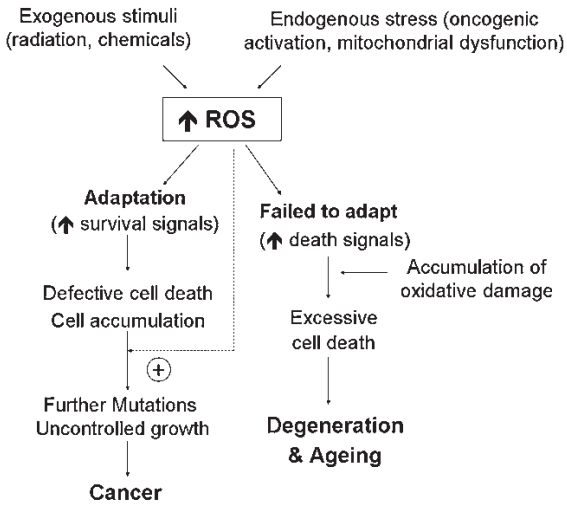

FIG. 13. Disturbance of redox homeostasis and pathogenesis of diseases. Alterations in redox homeostasis by exogenous stimuli or endogenous stress or both can result in increased oxidative stress with elevated cellular ROS. The ability to adapt to such ROS stress determines the overall fates of the cells. A successful adaptation to increased survival signals in combination with further ROS-mediated mutations and loss of critical regulatory mechanisms lead to defective cell death and aberrant proliferation. These may contribute to development of cancer. A failure in adaptation to the ROS stress while the cells accumulate oxidative DNA, protein, and lipid damage product may result in excessive cell death, leading to degenerative disorders and aging.

ated with tumor transformation, such as *Ras*, *Bcr-Abl*, and *c-Myc* (16). Not only ROS production seems to be increased in cancer cells, but the levels of antioxidants also were shown to be profoundly altered in the malignant cells (228). These suggest that redox balance is impaired in cancer cells. The intrinsic oxidative stress in cancer cells is thought to play an important role not only in cell proliferation (125) and genetic instability (250), but also in evasion of cell death. Increased ROS stress in cancer cells may activate survival pathways (130), disrupt cell-death signaling (242), and evade senescence (47). Because high levels of oxidative stress can kill cells, the cells that are equipped with flexible machinery have a higher possibility of adapting and surviving the ROS stress. Genomic instability is likely the key mechanism that cancer cells use for that purpose. The loss of functional p53 is thought to play a pivotal role in the adaptation process. Prolonged cell survival, together with increased proliferation, metastasis, and angiogenesis, are known to be required for development of cancer (113). As illustrated in Fig. 14, oncogene activation, dysfunction of redox signaling, and loss of p53 can all lead to disruption of redox homeostasis, resulting in increased cell proliferation and survival, and can contribute to the development of cancer.

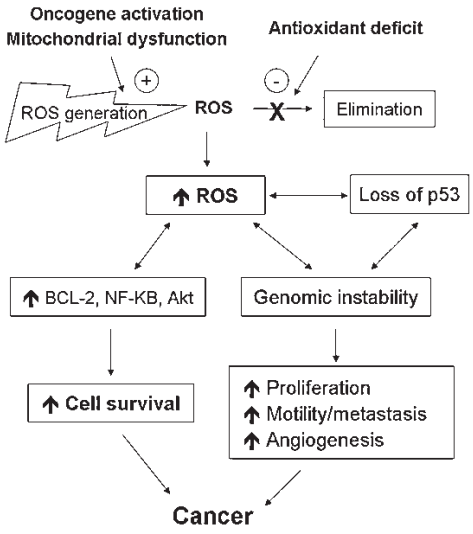

FIG. 14. Redox alterations and cancer development. Disturbance of redox homeostasis by an increase in ROS production (due to oncogenic stimulation, mitochondrial dysfunction, etc.) or a decrease in ROS elimination (due to a deficit or dysfunction of the antioxidant system) can lead to elevated ROS. The increased ROS stress can induce DNA mutations and genetic instability, including a loss of tumor-suppressor genes such as p53. The loss of p53 function can in turn further contribute to mitochondrial dysfunction, ROS generation, and genomic instability, forming a vicious cycle. ROS stress may also induce the expression of prosurvival factors and certain ROS-scavenging proteins, which would enable the cells to adapt and survive under oxidative stress. The ROS-induced mutations and genetic instability further enhance the chance for selection of cells with malignant phenotypes (an increase in proliferation, survival capacity, cell motility, and angiogenesis), leading to development of cancer.

1. Oncogene activation

a. Ras. Ras is a guanine nucleotide triphosphatase (GTPase) that functions as a molecular switch in a large network of intracellular signaling pathways. Constitutive activation of Ras, either by overexpression or by mutation, is very common in various human cancers. Although sharing a high degree of sequence identity, the active mutation rates of three different Ras genes (H-Ras, K-Ras, and N-Ras) are tissue- and tumor-type specific. It has been shown that Ras expression promotes ROS production. In the *H-Ras*^{v12}-transformed NIH3T3 fibroblasts cells; large amounts of superoxide were generated through pathways involving flavoprotein and Rac1, which activated NADPH oxidase–mediated ROS generation (130). Further study by conditional deletion of Rac1 confirmed that Rac1 function is required for *Ras*-mediated tumorigenesis, and loss of Rac1 causes a substantial reduction in cell proliferation (157). Besides the direct activation of ROS-production machinery, a recent study showed that *Ras* oncogenic signal also induced repression of the antioxidant gene SESN1 (165), resulting in a shift of redox balance toward increased ROS levels. Certain regulators of cell survival, such as PI3K/Akt, NF-kB, and c-Jun/AP-1, were shown to be influenced by the *Ras* oncogenic signal, which plays a major role in the transformation process (335). Although the increase in ROS production induced by *Ras* can transform some immortalized nontumorigenic cells to malignant cells, *Ras* transformation also provoked premature senescence in primary cells in the Em-N-Ras transgenic mouse model (275). These observations suggest that the increased ROS triggered by Ras can induce either cellular senescence or malignant transformation, depending on the cellular context. A study using doxycycline-inducible *Ras* transgenic mice showed that long-term low-level induction of *K-Ras* resulted in tumor formation after evasion of the senescence checkpoint, whereas high-levels of *K-Ras* activation led to upregulation of tumor suppressors and cellular senescence (267).

Besides the extent of ROS production induced by the oncogenic signal, the adaptation process for cell survival seems to be influenced by the ROS-scavenging systems. As shown in a genetically defined human ovarian cancer *H-Ras*^{v12} model, *Ras*-transformed cells, which had increased O_2^- and H_2O_2 levels, were shown to have an upregulation of multiple antioxidants such as SOD2 and peroxiredoxin 3, compared with their nontumorigenic parental cells (341). This study also suggested that the enhanced antioxidant capability serves as an important mechanism to evade apoptosis induced by ROS stress. This was evidenced by the increased resistance to H_2O_2 induced cell death observed in the *Ras*-transformed cells (341). Furthermore, a recent study demonstrated that the *Ras*-transformed cells were more sensitive to depletion of glutathione, leading to massive ROS accumulation and preferential cell death (302). These suggested a critical role of antioxidants in cell survival. It is conceivable that maintaining redox homeostasis in a high dynamic state (active ROS scavenging to counteract increased ROS generation) may be an adaptation mechanism used by cancer cells to survive under *Ras* oncogenic stress.

b. c-Myc. Myc is a helix-loop-helix leucine zipper transcription factor that regulates the expression of many genes involved in normal cell growth, proliferation, apoptosis, and me-

tabolism. Abnormal expression of the *c-Myc* protooncogene can lead to aberrant activation of its downstream pathways and deregulation of the chromatin state, subsequently causing genomic instability. Thus, it has been implicated in a wide spectrum of human cancers. Significant increases in H_2O_2 and double-strand breaks of DNA were detected after *c-Myc* activation (85, 308). Such ROS stress is reversible by antioxidant NAC (308) and vitamin C (139). The role of *c-Myc* in cell survival is rather complicated. Downregulation of *c-Myc* has been shown to sensitize cancer cells to cisplatin or radiation-induced apoptosis (24, 36), suggesting a prosurvival role of *c-Myc* against oxidative stress. In contrast, overexpression of *c-Myc* was shown to enhance serum-deprivation–induced apoptosis (296). This occurred through excessive accumulation of ROS, which inhibited NF-κB–mediated transactivation of SOD2 (296). Based on these observations, it is likely that the life/death decision under *c-Myc* expression is mediated through a differential dose effect of ROS. Studies using inducible *c-Myc* in melanoma cells revealed that apoptosis induced by downregulation of *c-Myc* was associated with cellular depletion of GSH. The change of GSH level after altered *c-Myc* expression occurred through the transcriptional control of the glutathione-synthesis enzyme (19). This indicates that the survival effect of *c-Myc* may be regulated by redox homeostasis. The upregulation of the glutathione antioxidant synthesis by *c-Myc* may represent an adaptive mechanism to survival under oxidative stress.

c. Bcr-Abl. Philadelphia (Ph)-positive leukemia cells contain a chromosomal translocation (t9/22) that results in a fusion of *Bcr* and *c-Abl* genes. The chimeric BCR-ABL tyrosine kinase is oncogenic and is responsible for the development of chronic myelogenous leukemia (CML), a relatively common adult leukemia. In experimental systems, the expression of *Bcr-Abl* completely abrogated growth factor dependence and transformed primary hematopoietic cells (58). Further study revealed that BCR-ABL exerts its antiapoptotic effects by activating multiple pathways, including RAS, PI3K/Akt, NF-κB, and STAT, leading to activation of antiapoptotic factors such as Bcl-xL (86). Compared with quiescent, nontransformed hematopoietic cells, *Bcr-Abl* transformed cells also have increased intracellular levels of ROS and decreased protein-tyrosine phosphatases (PTPases) (268). Treatment of *Bcr-Abl*–expressing cells with reducing agents such as NAC or PDTC decreased the ROS level and attenuated protein tyrosine phosphorylation, likely through activation of PTPases. This suggests a positive-feedback regulation between ROS generation and BCR-ABL activation (268). Furthermore, *Bcr-Abl*–induced ROS stress may induce DNA double-strand breaks. An inadequate repair of the damaged DNA may cause mutations and genetic instability, thus promoting the progression of CML (227).

2. Loss of functional p53. An activation of oncogenes and a defect in antioxidant systems can increase oxidative stress, which may contribute to tumorigenesis. However, the transformation process might not occur if the redox sensor p53 is still capable of maintaining redox homeostasis and protecting genome integrity. The role of p53 as a tumor suppressor and how oxidative stress modulates its function and contributes to tumor development were recently discussed (124). Cells and mice with defective p53 exhibited increased ROS stress, high mutagenesis, and increased tumor growth rate, which can be delayed by antioxidant NAC. Reactivation of p53 in p53-deficient tumors can cause a complete tumor regression (7). Furthermore, recent work demonstrated that s-Arf/s-p53 mice (which have increased levels of Arf and p53) show decreased ROS levels and reduced oxidative DNA damage (201). These mice seemed resistant to *H-Ras*^{V12} and E1A oncogenic transformation (201). The incidences of both sporadic cancer and carcinogen-induced cancer (fibrosarcomas and papillomas) were significantly decreased in the s-Arf/s-p53 mice (201). Taken together, this evidence suggests that p53 plays a key role as tumor suppressor, and that its functions to maintain redox homeostasis and genomic stability plays an important role in suppressing tumor formation.

3. Aberrant expression of antioxidant enzymes. Maintaining redox homeostasis is essential for cell survival. Alterations in the antioxidant system could induce redox imbalance and promote the development of cancer. Most evidence linking a deficit in antioxidant capacity to cancer development came mainly from animal studies in which antioxidant molecules were either knocked out or overexpressed. Then, the tumor incidence in the genetically altered animals was compared with those in the wild-type animals.

a. Superoxide dismutase (SOD). More than 30% of SOD1$^{-/-}$ mice developed liver tumors, and >70% of the mice developed tumor nodules (78). Further study showed that the mutation frequency of the SOD1-deficient mice was significantly increased. The mutation types of the mice were mainly GC to AT transversions and GC to AT transitions, which was consistent with mutations induced by oxidative stress (37). Heterozygous SOD2$^{+/-}$ mice, which exhibited a 50% reduction in SOD levels, appeared normal but had mitochondrial oxidative damage and decreased mitochondrial membrane potential (312). Furthermore, significantly elevated levels of 8-oxo-2-deoxyguanosine (8-oxo-dG) in nuclear and mitochondrial DNA and premature induction of apoptosis were observed (164). Tumor incidence, particularly for lymphoma and pituitary adenoma, increased 100% in old SOD$^{+/-}$ mice compared with the wild-type mice. Interestingly, transgenic mice (SOD3^{TG}) with skin-specific overexpression of SOD3 exhibited a decrease in DNA damage and a 50% reduction in skin tumor formation in a chemically induced carcinogenesis model treated with DMBA/TPA.

b. Glutathione peroxidase (GPX) and peroxiredoxin (Prx). Transgenic mice overexpressing GPX-1 or coexpressing GPX-1 and SOD1 were found to have an increased incidence of tumorigenesis in a DMBA/TPA two-stage skin carcinogenesis model (195). This suggests that a precise redox homeostasis is essential and that overexpression of GPX-1 might disturb the redox balance and contribute to cancer development. Prx1 knock-out mice revealed higher levels of ROS and an increased predisposition to cancer (217). Furthermore, Prx1 seems to abrogate *c-Myc*–mediated transformation through interaction with its transcriptional regulatory domain (217), suggesting the potential role of Prx1 as a tumor suppressor.

B. Diseases with excessive cell death: aging and degenerative disorders

One characteristic of aging is the loss of cellularity and the gradual decline of tissue function (304). This may be caused by progressive senescence of postmitotic tissues, likely due to chronic damage by ROS (218). Multiple evidence suggests the involvement of redox imbalance in the aging process. For example, increased NO level and nitrosative stress were shown to induce protein misfolding and neuronal cell death, which may play a role in development of brain aging and neurodegenerative conditions (223). Mutations of the antioxidant gene *SOD1* were linked to 20% of cases of the familial amyotrophic lateral sclerosis (ALS), an age-dependent degenerative disorder of motor neurons in cortex, brainstem, and spinal cord (64, 262). Furthermore, mice lacking *SOD1* exhibited premature aging and neuromuscular dysfunction. The aged mice have elevated oxidative damage to lipid, protein, and DNA, which are associated with increased apoptosis and reduced life span (78). In addition to the increase of ROS associated with aging, impairments in antioxidant capacity were often observed. For instance, the level of glutathione and the expression of glutathione-synthesis enzymes were shown to be decreased in aged mice and rats (188, 255). Recent study suggested that this phenomenon may be caused by attenuation of Nrf2/ARE transcriptional activity (289). Studies of normal aging, of genetic mutations that cause disease, and of environmental factors that affect disease risk have revealed multiple mechanisms underlying how excessive oxidative stress can cause neuronal cell death. For example, accumulation of oxidative DNA, protein, and lipid products; accumulation of self-aggregating proteins such as amyloid β-peptide, tau, α-synuclein, and huntingtin; oxidative perturbation of lipid metabolism and disruption of calcium homeostasis were shown to induce mitochondria-mediated apoptosis of neurons in several neurodegenerative disorders [for review, see (205)]. Furthermore, ROS-mediated activation of insulin-receptor signaling is known to cause impairment in autophagy, which has been associated with altered life span and decline in cognitive functions (75).

As mitochondria play a role in ROS production and apoptosis, the role of mitochondrial ROS in aging has been extensively investigated (192). In a study of transgenic mice with catalase targeted to peroxisome, nucleus, or mitochondria, the catalase transgene targeting mitochondria was designed by removing the peroxisomal localization signal along with the initial methionine and the addition of the mitochondrial localization signal to the amino terminal to target catalase expression to the mitochondria. Similarly, a nuclear-localization sequence was added to the amino terminus of peroxisomal catalase for nuclear expression. Only the mice overexpressing mitochondria-targeted catalase exhibited a 5-month increase in life span. These mice also exhibited a decrease in H_2O_2 level and in mtDNA damage (273). This study suggested the important role of mitochondrial H_2O_2 in aging. Another study found that the activity of aconitase, a redox-sensitive mitochondrial enzyme, was severely inhibited by excess superoxide and hydroxyl radicals in aging cells (339). Mice lacking $p66^{Shc}$, an adapter protein that promotes ROS generation in mitochondria, were shown to have longer life span. This work supported the causative role of mitochondrial ROS in aging (209). In addition to ROS, mitochondrial dysfunction may also lead to development of aging in a redox-independent manner. In a study using homozygous mutation in mitochondrial DNA replication enzyme (DNA polymerase-γ), the mutator mice had a reduced life span of ~46 weeks on average (303). The mice developed premature onset of the aging-related phenotypes, which included weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, reduced fertility, and heart enlargement (303). Nevertheless, accumulation of mtDNA mutations seemed not to be associated with increased oxidative stress in this model (170).

Surprisingly, a recent report showed that although an 11-fold increase in mitochondrial point mutations has been observed with age, a mitochondrial mutator mouse was able to sustain a 500-fold higher mutation burden than normal mice, without obvious features of accelerated aging (320). The author concluded, based on this work, that the mitochondrial mutations do not limit the life span of the mice. Apparently, further studies are needed in this area.

Recent evidence suggests a crucial role of p53 in prevention of aging. As described earlier, s-Arf/p53 mice not only showed a decrease in ROS level and increased resistance to oxidative DNA damage, but also showed a 9-month delay in aging. However, mice harboring s-Arf or s-p53 alone did not show an increase in life span. These observations suggest that p53 may require Arf to be stabilized and to exert its antiaging effect.

In another study, $p53^{+/-}$ mice lacking exon 1 to 6 of the p53 gene were generated through an aberrant gene-targeting event in embryonic stem cells (307). Despite only having one wild-type allele, the mice showed a premature aging phenotype, but seemed not prone to cancer development (307). It would be interesting to examine the cellular redox status in these mice.

IV. THERAPEUTIC STRATEGIES BASED ON REDOX REGULATION OF CELL SURVIVAL

Alterations in redox homeostasis can promote cell death or cell survival, depending on the magnitude of the stimuli. Because the redox alteration may contribute to development of diseases, the dose-dependent effects of ROS may provide an opportunity for potential clinical applications in therapeutics and prevention of diseases. As described previously, the final outcomes of cell fates (survival or death) under oxidative stress depend largely on the levels and types of ROS. Also, the functional status of cellular antioxidant systems and the redox-sensitive survival-signaling pathways can significantly influence the cell-fate decision. Therefore, the redox-based therapeutic/preventive strategies should include manipulations of redox homeostasis and modulations of the redox-sensitive factors that regulate cell survival and apoptosis.

A. Manipulating redox homeostasis

The principle of redox homeostasis and the strategies to modulate redox dynamics are illustrated in Fig. 15. Under physio-

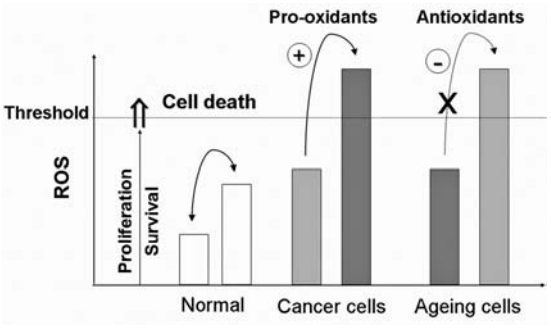

FIG. 15. Redox homeostasis and strategies to modulate redox dynamics for potential therapeutic applications. Under physiologic conditions, normal cells maintain redox homeostasis by controlling the proper balance between ROS generation and elimination. The redox dynamics may fluctuate within a tolerable range. An increase of ROS may promote cell proliferation and survival, as in the case of many cancer cells. However, when the increase of ROS reaches a critical level (the threshold), it may overwhelm the cellular antioxidant capacity and trigger the cell-death process. Chronic ROS stress may cause accumulation of damage to a level that induces cell death. This is thought to be a mechanism contributing to neural degenerative diseases and aging. For therapeutic purposes, it is possible to use agents that promote ROS generation or inhibit the cellular antioxidant system to trigger cancer cell death by pushing the ROS above the threshold level. In contrast, antioxidants may be used to prevent cells from oxidative damage and delay aging and the neurodegenerative process.

logic conditions, normal cells maintain redox homeostasis by controlling the proper balance between ROS generation and elimination. ROS are a double-edge sword. A moderate increase of ROS may promote cell proliferation and survival. However, when the increase of ROS reaches a certain level (the threshold), it may overwhelm the cellular antioxidant capacity and trigger the cell-death process. Therefore, cells with higher basal ROS generation (as in the case of cancer cells) would be more dependent on the antioxidant system and more vulnerable to further oxidative stress–inducing agents. A further increase of ROS stress by using exogenous ROS-generating agents or drugs that disable the endogenous antioxidant system may preferentially increase ROS above the threshold level in cancer cells, leading to cell death. In contrast, normal cells may be able to tolerate better such exogenous ROS stress because of their low basal ROS outputs and normal metabolic regulations. Prolonged accumulation of ROS-induced damage in neurons and other normal cells may result in cell death, leading to aging or degenerative disorders. Reducing ROS levels with proper antioxidants may be a useful strategy to prevent or delay these pathologic processes.

1. Pro-oxidants as a therapeutic strategy for cancer. A defect in apoptosis plays a major role in development of most cancers. A moderate increase of ROS is known to activate survival pathways and inhibit apoptosis in cancer cells. Thus, increased ROS in cancer have been viewed as an adverse event. However, because severe increases of ROS can cause lethal damage and kill the cells, it is possible to use ROS-generating agents or compounds that abrogate the antioxidant system to further increase ROS in cancer cells to a level that triggers cell death (for review see refs. 89 and 239). Such ROS-mediated therapeutic strategies have been tested in various experimental systems with promising results (128). Because toxic side effects in normal tissue is a major problem in clinical treatment of cancer by using cytotoxic drugs, new agents with high therapeutic selectivity are urgently needed. Because cancer cells exhibit increased ROS compared with normal cells, this redox difference provides a biochemical basis for development of new therapeutics with high selectivity. In the *Ras*-transformed ovarian cancer model, it was recently shown that the oncogenic transformed cells exhibited increased ROS stress and were more dependent on the glutathione antioxidant system to maintain homeostasis and survive. Disruption of glutathione by a natural compound, β-phenylethyl isothiocyanate (PEITC), was shown to preferentially increase ROS stress and selectively kill the malignant cells *in vitro* with minimal toxicity to their nontumorigenic parental cells (302). *In vivo*, treatment with PEITC prolonged survival of mice bearing Ras-transformed ovarian cancer (302). These results demonstrated the critical role of redox homeostasis in regulation of cancer cell survival and cell death and suggest that it is possible to kill cancer cells preferentially through ROS-mediated mechanism. Because ROS stress is prevalent in cancer cells, the ROS-based approach may have broad therapeutic applications.

Defective apoptosis in cancer not only promotes disease progression but also confers resistance to many therapeutic agents (138). Because alterations of multiple redox regulators such as Trx, GST, GPX1, and Prx were observed in drug-resistant cancer cells (241), it was speculated that an altered redox homeostasis may play a role in the drug-resistance mechanism, and that manipulation of the redox system may be a useful strategy to overcome the problem of apoptosis resistance in tumor cells (242). Recent work showed that a compound known as TDZD-8 (4-benzyl, 2-methyl, 1,2,4-thiadiazolidine, 3,5 dione) can induce depletion of thiols and rapid accumulation of ROS and selectively kill leukemic cells expressing stem-cell marker, with minimal toxicity to normal hematopoietic stem cells (106). Because tumor stem cells are thought to be the subpopulation of cells highly resistant to chemotherapy and play a critical role in disease relapse after treatment, the potency of the prooxidative compound in removing those cells underscores a key role of the redox system in regulating survival of stem cells and highlights the promising therapeutic potential of using redox-based strategy in cancer treatment.

2. Antioxidants for prevention of degenerative diseases. Because accumulation of ROS was known to promote excessive or premature cell death, leading to aging and neural degenerative diseases, the use of antioxidants in prevention of aging has been established for decades. This involves the use of both supplementation of natural ROS scavengers and treatment with exogenous antioxidants [for review see (75)]. For example, cysteine supplementation in addition to the normal protein diet has shown significant beneficial effects on several parameters relevant to aging, including skeletal muscle functions (74). *N*-acetylcysteine (NAC), a glutathione precursor, has been shown to protect against oxidative stress–induced neuronal death and thus might delay neurogenerative diseases

(5). Melatonin, a physiologic hormone and antioxidant, seems to have a protective effect against neurodegenerative diseases, especially Alzheimer's disease (245). Phenolic compounds such as resveratrol are potent antioxidants and have been reported to be protective against neuronal apoptosis associated with the pathogenesis of Alzheimer's disease (72). Experimental and clinical studies showed that ebselen (PZ51), a GPX mimetic that can inhibit lipid peroxidation and decrease iNOS expression, was shown to suppress cell death of cortical neurons and prevent cerebral ischemia (stroke) in clinical trials (290, 337). Mitochondria-targeted antioxidants such as MitoQ and MitoVitamin E were developed as pharmaceutical products for prevention of stroke and cardiovascular disease (321). GSNO, a physiologic metabolite of glutathione (GSH) and NO, was shown to be several-fold more potent than GSH in protecting cells against oxidative stress caused by peroxynitrite. In rats, GSNO therapy was proven to be neuroprotective in cerebral ischemia (254).

In addition to the pharmacologic interventions, epidemiologic studies have shown that regular consumption of diets rich in antioxidants and antiinflammatory agents, such as those found in fruits and vegetables, may reduce the risk of developing age-related neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (176). These suggest that maintaining redox homeostasis may prevent degenerative diseases.

It is worth noting that, although accumulation of ROS is thought to play a role in development of cancer, the benefit of using antioxidants as cancer chemopreventive agents is somewhat controversial. An early study showed that certain antioxidants may enhance the therapeutic efficacy of chemotherapy in a colorectal cancer model (49). Antioxidants may also mitigate the adverse effects of radiation therapy (214). However, compounds such as carotenoids, tocopherols, and ascorbate derivatives may act as antioxidants or prooxidants, depending on doses (274). The use of β-carotene and vitamin A in lung-cancer prevention trials showed no chemopreventive effect and might even increase the risk of lung cancer incidence and mortality in smokers (231, 232). Recent evidence suggests that antioxidants such as NAC may blunt the therapeutic activity of chemotherapeutic agents, including cisplatin and paclitaxel, against tumor cells (2, 67). Because ROS stress can either promote cell survival or induce cell death, depending on dosage and duration, cautions should be exercised in using antioxidants and prooxidants to modulate cellular redox, with full consideration given to the time- and dose-dependent nature of such modulations.

B. Modulating redox-sensitive signaling molecules

Besides manipulating ROS level by using prooxidants or antioxidants, cell death and survival can also be modulated by targeting redox-sensitive signaling molecules at the signal-transduction, transcription, or death-execution levels (241). For example, at the level of signal transduction, JNK activation was found to be associated with ROS-induced neuronal death in Parkinson's and Alzheimer's disease (25). Chemical inhibitors of this signaling pathway have proven to be effective *in vivo* to reduce brain damage and some of the symptoms of arthritis in animal models (25). At the level of execution, proapoptotic or antiapoptotic factors, such as caspases and Bcl-2, are attractive targets for cancer therapies. Pharmacologic inhibitors, mimetics, activators, and anti-sense oligonucleotides targeting these molecules are of potential therapeutic utility (87). Because molecular pathways of apoptosis are excessively activated in aging and neurodegenerative disorders, pharmacologic/genetic inhibitions of apoptotic players such as Fas, caspases, and p53 are emerging strategies to prevent or retard the degenerative diseases (322). At the level of transcription, redox-sensitive transcription factors regulating expression of multiple antioxidants, such as NF-κB and Nrf2, are of particular interest. Since activation of the prosurvival NF-κB pathway was shown to play a role in cancer development induced by carcinogens and oncogenic viruses, a variety of NF-κB inhibitors such as curcumin have been under clinical evaluation for use in cancer prevention and treatment. New agents targeting the proteasome, IKK, and other upstream molecules involved in NF-κB activation seem to show anticancer activity in clinical or preclinical studies (313). The fact that Nrf2 can be activated not only by oxidative stress, but also by electrophilic compounds such as isothiocyanates, provides therapeutic and preventive opportunities. For example, increased Nrf2 transactivation is considered a major mechanism for the cancer chemopreventive effects of isothiocyanates (136). Furthermore, a recent study suggests the essential roles of PI3K and PKC signaling in the activation of the Nrf2/ARE in the absence of general oxidative stress (190). Because ARE-targeted genes were known to play a protective role against apoptosis in cortical neuron (66), compounds activating Nrf2 *via* PI3K and PKC signaling would likely prevent neuronal cell death. This may provide a new basis for development of chemopreventive agents for degenerative diseases.

V. CONCLUDING REMARKS

Cellular redox systems control the functions of multiple signaling proteins affecting cell survival at the levels of signal transduction, transcriptional regulation, and cell-death execution. Oxidative stress can either enhance cell survival or promote cell death, depending on the magnitude and duration of the stress and the genetic background and redox states of the cells. Oxidative stress not only serves as a type of stimulus to trigger stress-response signal-transduction pathways but also can modulate cell death/survival through direct oxidative modifications of the execution molecules. Under physiologic conditions, the balance between production and elimination of ROS ensures the proper maintenance of cellular metabolism and other functions. The final decision, whether the cells will survive or die, is the overall outcome of the integration of signals from redox-sensitive factors and other regulatory mechanisms. However, when the redox homeostasis is disturbed by either oncogenic activation, mitochondrial dysfunction, or accumulation of oxidative stress, cells that acquire genetic instability may adapt to survive under the stress by acquiring mutations to attenuate programmed cell death. The defective cell death in conjunction with increased cell proliferation, angiogenesis, and metastatic potentials can promote the development of cancer. Conversely, accumulation of oxidative-damage products and

failure to adapt to ROS stress may result in excessive cell death, leading to degenerative disorders and aging. Strategies to modulate cellular redox status, either by prooxidants and antioxidants or by affecting redox-sensitive signaling pathways, may have significant clinical applications in disease treatment and prevention. Logical combinations of ROS-modulating agents and compounds that affect redox-sensitive signaling pathways may further enhance therapeutic activity and selectivity. A comprehensive understanding of the redox biology underlying the disease processes and the mechanisms of action of pro-oxidants and antioxidants is essential for developing effective therapeutic strategies.

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ABBREVIATIONS

ARE, antioxidant responsive element; ASK1, apoptosis-regulating signal kinase 1; EpRE, electrophile responsive element; ER, endoplasmic reticulum; ERK, extracellular regulated kinase; GPX, glutathione peroxidase; GRX, glutaredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; HAT, histone acetylase; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; HNE, 4-hydroxy-2-nonenal; $HO^{\cdot}$, hydroxyl radical; H_2O_2, hydrogen peroxide; Hsp, heat-shock protein; IκB, inhibitor of NF-κB; JNK, c-Jun-N-terminal kinase; Keap1, Kelch-like ECH-associating protein 1; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MPT, mitochondrial permeability transition; NAC, *N*-acetylcysteine; NADPH, nicotinamide adenine dinucleotide phosphate (reduced); NF-κB, nuclear factor kappa B; $NO^{\cdot}$, nitric oxide; NOS, nitric oxide synthase; NOX, NAD(P)H oxidase; Nrf2, NF-E2–related factor 2; O_2^-, superoxide; $ONOO^-$, peroxynitrite; Prx, peroxyredoxins; PTEN, phosphatase and tensin homologue; PTK, phosphotyrosine kinase; PTPase, protein tyrosine phosphatase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAPK, stress-activated protein kinases; SCO2, synthesis of cytochrome *c* oxidase 2; SOD, superoxide dismutase; TIGAR, TP53-induced glycolysis and apoptosis regulator; TRX, thioredoxin; ub, ubiquitin; XO, xanthine oxidase.

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