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FORUM REVIEW ARTICLE

Redox Regulation of p53, Redox Effectors Regulated by p53: A Subtle Balance

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Abstract

Significance: Reactive oxygen species (ROS), generated by cells as side products of biological reactions, function as secondary messengers by impacting a host of cellular networks involved in maintaining normal homeostatic growth as well as pathological disease states. Redox-sensitive proteins, such as the tumor suppressor protein p53, are susceptible to ROS-dependent modifications, which could impact their activities and/or biological functions. ***Recent Advances:*** p53 is a transcription factor that controls a wide variety of target genes and regulates numerous cellular functions in response to stresses that lead to genomic instability. Thus, redox modifications of p53 could impact cell fate signaling and could have profound effects on pathways fundamental to maintaining cell and tissue integrity. ***Critical Issues:*** Recent studies present evidence that ROS function upstream of p53 in some model systems, while in others ROS production could be a downstream effect of p53 activation. ***Future Directions:*** In this review, we describe how ROS production regulates p53 activity and how p53 can, in turn, influence cellular ROS production. *Antioxid. Redox Signal.* 16, 1285–1294.

Introduction

THE P53 PROTEIN, first discovered in 1979 by Lane and Crawford (48), is a transcription factor encoded by the *p53* gene located on human chromosome 17p13.1. Upon activation by a variety of stress-related signals (hypoxia, heat shock, DNA damaging agents, *etc.*), p53 binds to DNA and transactivates genes encoding proteins responsible in cell cycle arrest, programmed cell death, and DNA repair. More than a dozen of genes have been identified as targets of p53 such as *p21*, cyclin G, *Fas/Apo1,* and *PIGs* (p53-induced genes) (86). p53 has been linked to the regulation of cell cycle progression and maintenance of genomic integrity since Baker *et al.* reported its tumor suppressor activity in colorectal carcinoma (8). Mutations of the *p53* gene and consequent alteration of the p53 protein structure are frequently observed in a wide variety of human cancer (9, 64, 70, 92). The p53 protein can be regulated by different post-translational modifications such as phosphorylation of serine and/or threonine residues (39), acetylation (67), ubiquitylation (54), or sumoylation (33) of lysine residues.

Redox homeostasis, the balance between cellular pro- and antioxidant status, is crucial for various physiological processes (7, 72). It controls the level of reactive oxygen species (ROS), derivatives of O_2, that are constantly formed as side products of biological reactions that use electron transfers, such as oxidative phosphorylation, oxidases, and reductases activity. Superoxide radical ($O_2^{\cdot -}$) is the primary derivative of O_2, which can be converted to hydrogen peroxide (H_2O_2), alkoxyl/peroxyl radical ($RO^{\cdot}/ROO^{\cdot}$), or peroxynitrite ($ONOOH/ONOO^-$) (27). Oxidative modification of the cysteine residues of p53 can cause conformational changes, which affects the function of the protein. Thus, p53 may undergo conformational changes in an oxidizing environment, which can influence its transcriptional activity and biological responses. In addition, it is recognized that ROS play a role as downstream effectors of p53, even if the mechanisms of the interactions between ROS and p53 are still elusive (20). The function of redox homeostasis in p53-regulated processes is a topic of great interest and the involvement of p53 in metabolic pathways and cell fate determination has been discussed in recent reports (34, 35). In this review, we will discuss the role of ROS as upstream regulators of p53 functions and, conversely, the impact of p53 on redox effectors and particularly its effect on mitochondrial ROS production.

Intracellular Sources of ROS

Mitochondrial generation of ROS

Under physiological conditions, mitochondria are a major intracellular source of ROS. Mitochondria are the site of

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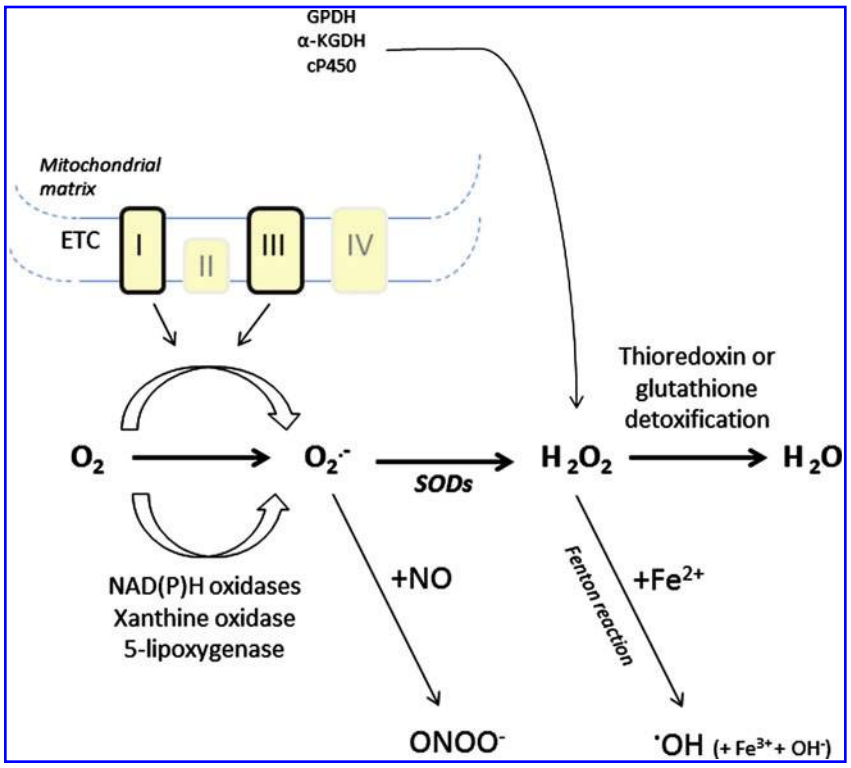

FIG. 1. Intracellular reactive oxygen species (ROS) production and detoxification. Various sources of ROS can be found in mammalian cells. Those sources can be separated in two classes: (i) the mitochondrial sources, such as complex I (NADH-ubiquinone oxidoreductase) and complex III (ubiquinol-cytochrome c oxidoreductase) of the electron transport chain, glycerophosphate dehydrogenase (GPDH), α-ketoglutarate dehydrogenase (α-KGDH), or cytochrome P450s; (ii) the nonmitochondrial sources, such as NAD(P)H oxidases, 5-lipoxygenase, or xanthine oxidase. In the cytosol, superoxide radical ($O_2^{\cdot -}$) can react with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$) and the Fenton reaction can lead to the formation of $^{\cdot}OH$. ROS production is countered by antioxidant systems such as superoxide dismutases (SODs), thioredoxin (Trx), or glutathione (GSH). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

oxidative phosphorylation that is carried out by four electron-transporting complexes and one H+-translocating ATP synthetic complex. During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors in redox reactions. During this process, some electrons may leak onto oxygen, resulting in the one-electron reduction of O_2 to generate superoxide ($O_2^{\cdot -}$) (29). Complex I (NADH-ubiquinone oxidoreductase) and complex III (ubiquinol-cytochrome c oxidoreductase) of the electron transport chain (ETC) are the main sources of $O_2^{\cdot -}$ in the mitochondria (Fig. 1). Superoxide can be toxic to the cells by its ability to target enzymes containing iron sulfur centers such as aconitase, succinate dehydrogenase, and NADH-ubiquinone oxidoreductase. Therefore, $O_2^{\cdot -}$ is rapidly removed by conversion to H_2O_2 in a reaction catalyzed by the superoxide dismutases (27, 75). There are three types of superoxide dismutases: a cytosolic dismutase (CuZnSOD/SOD1), an intra-mitochondrial manganese superoxide dismutase (MnSOD/SOD2), and an extracellular CuZn superoxide dismutase (CuZnSOD/SOD3) (56, 74). There are also other sources of ROS in mitochondria that are not directly linked to the ETC: glycerophosphate dehydrogenase (25), α-ketoglutarate dehydrogenase (88), or cytochrome P450s (37). ROS can regulate cellular functions by oxidative modification of key cysteine residues on important regulatory proteins, resulting in either activation or inactivation of these proteins.

Nonmitochondrial production of ROS

The superoxide anion can also be formed enzymatically by enzymes such as NAD(P)H oxidases, 5-lipoxygenase, or xanthine oxidase (Fig. 1). NADPH oxidases (Nox) were first studied in phagocytic leukocytes such as macrophages and neutrophils (46, 63). All Nox family members are transmembrane carriers that use NADPH as an electron source and molecular oxygen as an acceptor. These chemical reactions are invariably associated with the formation of $O_2^{\cdot -}$, which is produced when molecular oxygen, instead of the specific substrate, is reduced by the acquisition of an electron (53). NADPH oxidases function as electrogenic enzymes that pass electrons from the cytoplasm through a membrane into the extracytoplasmic space, either the cell exterior or intracellular compartments such as phagosomes or endosomes (43). Xanthine oxidase can generate $O_2^{\cdot -}$ when converting hypoxanthine into xanthine and xanthine into uric acid. Under physiological conditions, xanthine oxidase is responsible for a minor proportion of total intracellular ROS production; however, it could serve as a major source of oxidative stress under certain pathological states such as ischemia and reperfusion. 5-Lipoxygenase, an enzyme involved in the synthesis of pro-inflammatory leukotrienes and anti-inflammatory lipoxins, generates oxidized metabolites that have profound effects on cellular redox status (58). To that end, pharmacological inhibition of 5-lipoxygenase activity reduces $O_2^{\cdot -}$ production in human polymorphonuclear leukocytes induced by arachidonic acid treatment (85). Furthermore, the formation of $O_2^{\cdot -}$ and H_2O_2 in the cytosolic compartment can lead to the formation of other reactive oxygen/nitrogen derivatives. For example, production of $O_2^{\cdot -}$ can lead to the generation of $ONOO^-$ in a reaction mediated by nitric oxide (NO). H_2O_2 can also be transformed in the highly reactive hydroxyl radical ($^{\cdot}OH$) through Fenton reaction in the presence of iron (II) (Fig. 1).

ROS in cell fate signaling

There is now overwhelming evidence that cellular redox status impacts cell fate decisions by regulating critical cellular processes such as growth and proliferation, gene expression, cell survival and death, and protein function. Traditionally,

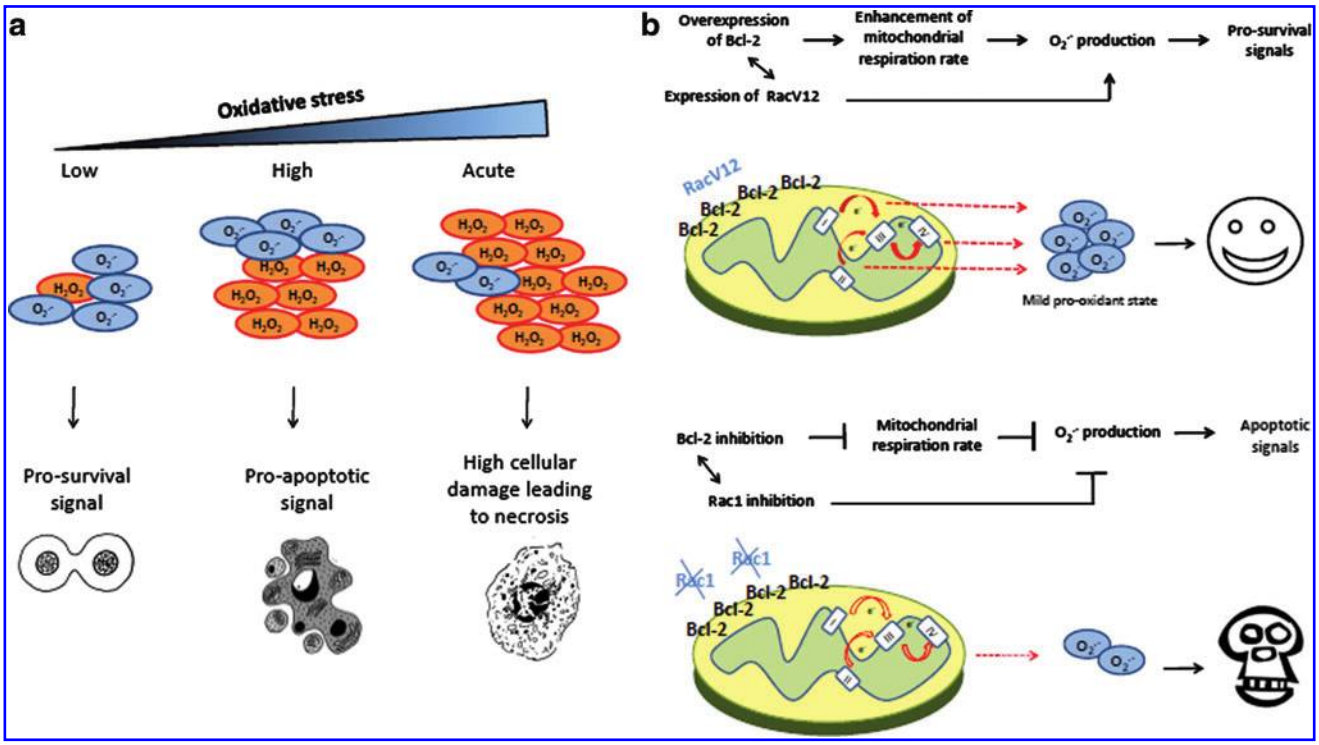

FIG. 2. Dual role of ROS in cell fate. Redox status is a key component in cell fate decisions and is highly dependent on the dose and the species of ROS involved in the process. **(a)** General role of ROS in cell fate. A mild pro-oxidant environment favors survival by triggering oncogenic signaling $O_2^{\cdot-}$-mediated, whereas a tilt in ROS levels dominated by hydrogen peroxide (H_2O_2) promotes apoptosis. Furthermore, an acute production of ROS generates high cellular macromolecules damages, which finally lead to necrosis. **(b)** Specific role of $O_2^{\cdot-}$ in cell survival: example of Rac1-Bcl-2 pathway. Enhancement of mitochondrial respiration rate is observed in Bcl-2-overexpressing cells and is followed by an increase in intracellular $O_2^{\cdot-}$ levels. This effect is mediated *via* an interaction between Rac1 and Bcl-2. The resultant pro-oxidant state promotes cell survival *via* inhibition of apoptosis or activation of survival signals. This effect is alleviated in cells upon exposure to Bcl-2 inhibitors or by inhibiting the interaction of Rac1 with Bcl-2 as well as upon gene silencing of *Rac1*. In a similar way, expression of the constitutively active form of Rac1 (RacV12) leads to a slight increase in intracellular $O_2^{\cdot-}$ level and inhibition of apoptosis. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

severe oxidative stress induced by ROS such as $O_2^{\cdot-}$, H_2O_2, and their derivatives has been linked to cell and tissue injury/damage and death (15, 36); however, recent evidence strongly suggests that a slight pro-oxidant state endows cells with a survival advantage by serving as second messengers (69). In this regard, our work over the years provides evidence to support the hypothesis that a tight balance in the ratio of intracellular $O_2^{\cdot-}$ and H_2O_2 is crucial for cell fate determination (Fig. 2a) (16, 17). Indeed, a slight increase in intracellular $O_2^{\cdot-}$ serves as an oncogenic signal by inhibiting apoptotic execution and/or positively impacting pro-survival circuits (4). On the contrary, an increase in H_2O_2 suppresses cell proliferation by creating an intracellular milieu permissive for apoptotic signaling. (3, 40). Along similar lines, we linked the anti-apoptotic activity of the pro-oncogenic protein Bcl-2 to its ability to induce a slight increase in intracellular levels of $O_2^{\cdot-}$ (Fig. 2b) (14). Furthermore, our studies revealed that the small GTPase Rac1 was a novel binding partner of Bcl-2 and that expression of a constitutively active form of Rac1 (RacV12) was linked to a slight increase in intracellular $O_2^{\cdot-}$ level and inhibition of apoptosis (Fig. 2b) (68, 90). These data provide evidence that a slight increase in intracellular $O_2^{\cdot-}$ could favor oncogenesis by creating a conducive environment for cell growth and survival as well as evading death execution.

Redox Regulation of p53

Modifications of amino acids by ROS are important to control the activity of many transcription factors, including HIF-1 alpha, Sp1, NF-κB, and p53 (23, 49, 89). Various endogenous and exogenous molecules have been shown to modify the redox status of p53. These include, but are not limited to, glutathione (GSH), thioredoxin (Trx)/thioredoxin reductase (TrxR), and nitrating compounds.

The GSH system

GSH is a major cellular antioxidant whose thiol group is a potent reducing agent. It inactivates electrophilic compounds and peroxides *via* catalysis by glutathione S-transferases and also serves as a substrate for glutathione peroxidase (GPX), which removes H_2O_2 accumulated in the cells (87). GSH is a tripeptide that can exist either in an oxidized (GSSG) or in a reduced (GSH) state (Fig. 3a). Maintaining optimal GSH:GSSG ratios in the cell is critical to survival; therefore, a tight regulation of this system is necessary. Russo *et al.* first investigated whether COS-2 and Hep3B cells treated by diethylmaleate (DEM), an agent that increases the concentration of free radicals by depleting cellular stores of GSH, showed modification of p53 DNA binding or transcriptional activity.

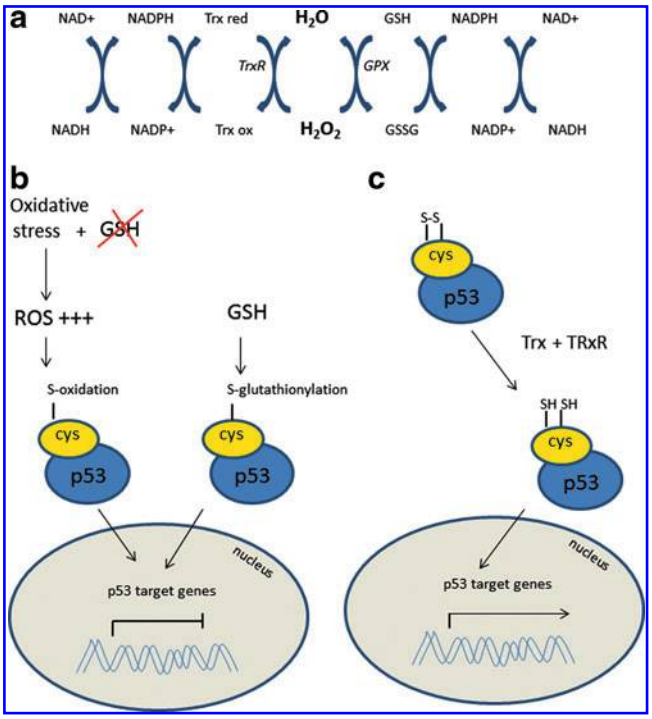

FIG. 3. Trx and GSH systems and their links with p53. Trx and GSH are involved in cellular detoxification by converting H_2O_2 into H_2O and constitute two major cellular antioxidants **(a)**. These two molecules are able to induce oxidative modifications of p53 cysteine residues, which can lead to conformational changes of the protein and inhibition of its function. Depletion of cellular store of GSH promotes increase of cellular oxidative stress, which can lead to p53 cysteine residue oxidation and loss of p53 transcriptional activity. Furthermore, glutathionylation of cysteines in p53 occurs *via* formation of a disulfide bond and can directly impair p53 transcriptional activity **(b)**. Trx reduces oxidized forms of p53, which leads to enhanced DNA binding and transcriptional activity **(c)**. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Indeed, DEM-induced oxidative stress significantly reduced p53 DNA-binding and activation of its specific reporter genes (79). More recently, Velu *et al.* showed that p53 was a substrate for glutathionylation under oxidative stress and that cysteine 141 was the most reactive cysteine residue (91). Furthermore, a molecular modeling study showed that glutathionylation of this residue inhibits p53-DNA association and also interferes with protein dimerization (94). The glutathionylated p53 was detected in the nucleus, which was associated with the elimination of p53 activities and oncogenesis. These results show that S-glutathionylation of p53 cysteine residue was associated with a loss of function of the protein (Figs. 3b and 4). Along similar lines, Richie *et al.* examined the effect of GSH on spontaneous tumor development by using p53 knock-out transgenic mice. They treated the mice with a GSH-specific inhibitor of GSH synthesis (buthionine sulfoximine [BSO]) or a cysteine and GSH precursor (1,2-oxothiazolidine-4-carboxylic acid or OTCA) and demonstrated that GSH levels were decreased up to 88% in the BSO-treated group. After 10 weeks, mice in both groups showed a high frequency of lymphomas (80%) and other tumors (38%). Furthermore, the incidence of colon tumor was seen to be increased by fivefold in the BSO-treated group compared to the control group, showing that GSH and p53 have contributory roles in colon carcinogenesis (77).

The Trx system

Trx are a family of small redox proteins that undergo NADPH-dependent reduction by TrxR and reduce oxidized cysteine groups on proteins (Fig. 3a). Oxidation of cysteine residues on proteins results in disulfide bond formation, thereby inducing a conformational change in the protein. The Trx/TrxR system has been shown to reverse the inactivating effect of oxidative stress on target proteins (6, 28). Similar to GSH molecule, Trx might play a direct or indirect role in regulating the redox status of p53.

Ravi *et al.* used antharacyclines, a class of ROS-producing antitumor drugs, to study the effect of Trx on p53. They showed that Trx increased p53-dependent apoptosis induced by daunomycin through elevated semiquinone production *via* redox cycling. Interestingly, using MCF-7 cells overexpressing Trx, they demonstrated significant induction of apoptosis upon daunomycin treatment, which was associated with intracellular generation of $O_2^{\cdot -}$ and increase in the expression and DNA binding activity of p53 (76).

It has been demonstrated that the covalent modification of TrxR by both endogenous and exogenous electrophiles causes the disruption of p53 protein conformation (62). Cassidy *et al.* reported that RKO colon cancer cells with damaged or deficient TrxR enzymatic activity by dint of electrophilic modification were less sensitive to electrophile-induced alteration in p53 conformation and apoptosis, compared to cells with normal TrxR activity. Transfection of TrxR-depleted cells with C-terminal mutants of TrxR lacking the catalytic selenocysteine led to the disruption of p53 conformation in a similar way to that induced by electrophiles in cells expressing wild-type TrxR (11). Furthermore, Stoner *et al.* demonstrated that p53 activity was unimpaired in yeast lacking *Trx1* and *Trx2* genes encoding cytosolic Trx. Subsequent analysis demonstrated that the inhibitory effect of TrxR was also deleted in yeast, suggesting that accumulation of oxidized Trx was necessary for p53 inhibition (81). These results provide evidence that formation of intramolecular disulfide bonds within p53 must be reduced by TrxR to facilitate the DNA binding activity of p53 (Figs. 3c and 4). To that end, Redox factor-1 (Ref-1) has been suggested as an intermediate in the inhibitory effect of oxidized Trx on p53 activity (42).

Reactive nitrogen species and p53

Reactive nitrogen species (NO or $ONOO^-$) can also modulate the redox status of p53 *via* nitration of critical tyrosine residues present in its DNA binding-domain (Fig. 4). $ONOO^-$ is produced by excess of NO and $O_2^{\cdot -}$ and can post-translationally modify proteins, in particular zinc finger family of transcription factors such as p53. Exposure of human glioma cells to exogenously added $OONO^-$ results in p53 aggregate formation, nitration of its tyrosine residues, as well as loss in DNA-binding ability (18). Subsequent studies showed that $OONO^-$ or 3-morpholinosydnonimine hydrochloride (SIN-1), a molecule that decomposes into NO and $O_2^{\cdot -}$ to form $OONO^-$, inhibits p53 function *in vitro*. In these malignant glioma cells, p53 transcriptional activity was dysregulated with a profound effect on downstream $p21^{WAF1}$ expression

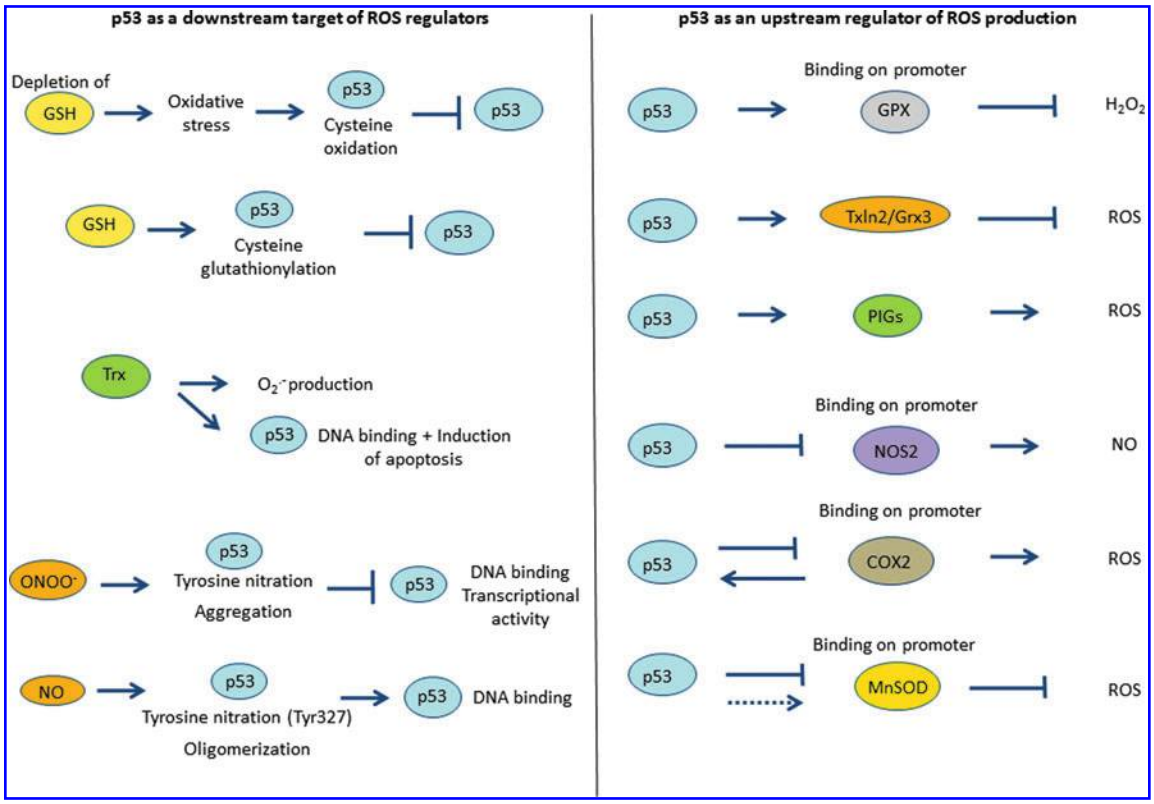

FIG. 4. Crosslinks between p53 and ROS/reactive nitrogen species pathways. p53 is at the center of an intricate network in which ROS are both upstream and downstream effectors. p53 expression is intrinsically redox-sensitive and can be modified by many redox regulators, such as GSH, Trx, and nitrating compounds ($ONOO^-$ or NO). In turn, p53 protein is able to transactivate or transrepresse genes involved in the production and regulation of ROS production, such as glutathione peroxidase (*GPX*), glutaredoxin 3 (*Grx3* also named *Txln2*), p53-induced genes (*PIGs*), the inducible nitric oxide synthase (*NOS2*), cyclooxygenase 2 (*COX2*), and the mitochondrial manganese superoxide dismutase (*MnSOD/SOD2*). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

(19). NO is a highly reactive radical that interacts directly with a variety of proteins to either activate or inhibit their function through oxidation of SH groups or nitrosylation of tyrosines residues (38). In a recent study, Yakovlev *et al.* investigated the role of tyrosine nitration in p53 activity using low concentration of the NO donor, diethylenetriamine NONOate (DETA NONOate), on MCF-7 cells. Treatment with the NO donor resulted in nitration on Tyr327, p53 oligomerization, nuclear accumulation, and increased DNA-binding activity without phosphorylation of Ser15 (93).

p53: A Transcriptional Regulator of Redox Effectors

p53 and ROS are both implicated in multiple physiological pathways. In order to better understand the relationship between p53 and ROS, it is fundamental to investigate how p53 expression and distribution influences intracellular ROS production. As a transcription factor, p53 can induce either transactivation or transrepression of its downstream targets. Among the redox-controlling genes specifically upregulated in a p53-dependent manner is *GPX*, *PIGs*, and glutaredoxin 3 (*Grx3*), while two other important redox modulators, cyclooxygenase 2 (COX2) and inducible nitric oxide synthase 2 (NOS2), are repressed upon p53 activation (Fig. 4).

GPX and Grx3

GPX is one of the primary cellular antioxidant enzymes that scavenge H_2O_2 and organic hydroperoxides using GSH as the hydrogen donor (Fig. 3a) (59). Tan *et al.* reported p53-binding site in the promoter of GPX. After determining a putative binding site by analysis of the sequence of the *GPX* gene, they showed that purified p53 protein or p53 obtained from nuclear extracts were binding to the promoter of GPX in U2-OS cells after exposure to the apoptosis-inducing agent, etoposide. Furthermore, p53 binding and transactivation of GPX promoter enhancement was blocked with a dominant negative form of p53 (84). These results, suggesting that GPX was a novel target of p53, were confirmed by a subsequent study conducted by Hussain *et al.* In their analysis, TK6 cells (p53 wild type) and WTK1 cells (p53 mutated) were exposed to doxorubucin and subjected to microarray analysis. The results showed an increase in the induction of GPX in TK6 cells compared with WTK1 cells, demonstrating that GPX was upregulated in a p53-dependent manner (41).

Glutaredoxins are a group of redox enzymes that are reduced by the oxidation of GSH. A recent study has described that Grx3, also named Txnl2, is a p53 target and is upregulated after NGF stimulation (10). Furthermore, Txln2/Grx3 has

been shown to be involved in growth and metastasis regulation through redox mechanisms. Of note, knockdown of Txln2 in breast cancer cells resulted in an increase in ROS production, which correlated with inhibition of proliferation *in vitro* and inhibition of tumorigenesis *in vivo* (73). Moreover, Cheng *et al.* demonstrated that Grx3 is ubiquitously expressed in adult mice and embryos and its expression is induced under oxidative stress. $Grx3^{-/-}$ mouse embryonic fibroblasts had impaired growth and cell cycle progression (12).

p53-induced genes

PIGs were first discovered by Polyak *et al.* in a screen for genes induced by p53 during p53-induced apoptosis. In this study, Polyak *et al.* predicted that many of these genes encoded proteins that impacted cellular redox status, including two ROS-generating enzymes: PIG3, a homolog of the quinone oxidoreductase (NQO1), and PIG6, a homolog of the proline oxidase (POX) (71). In line with this, more recent reports strongly suggest that PIG3 and PIG6 are involved in the intracellular generation of ROS and ROS-mediated apoptosis (65, 78). However, PIG3 is not only restricted to the apoptotic response but also plays a role during p53-mediated growth arrest. It has recently been shown that depletion of PIG3 sensitized cells to DNA damage-inducing agents and impaired DNA repair (30, 51). Donald *et al.* showed that p53 was controlling PIG6/POX expression and consequent ROS production. POX induces ROS production when catalyzing the conversion of proline to pyrroline-5-carboxylate. When POX was conditionally expressed in p53-negative DLD-1 colon cancer cells, proline-dependent generation of ROS required the induction of POX (24). Furthermore, a recent study published by Abbas *et al.* has demonstrated a correlation between ROS production and PIGs overexpression in an *in vivo* model of p53 lacking apoptosis-inducing ability (hypomorphic allele p53R172P encoded by $p53^{515C}$). In this study, they showed that $Mdm2^{-/-}$ $p53^{515C/515C}$ new-born mice had high ROS levels in whole bone marrow in comparison to $Mdm2^{+/-}$ $p53^{515C/515C}$ animals. These pups also presented higher PIG1, PIG8, and PIG12 levels compared with the control animals. These data give strong evidence that p53, *via* its role on PIGs, can be a key player in ROS production *in vivo via* its regulation by Mdm2 (1).

Nitric oxide synthase and cyclooxygenase

NOS catalyze the synthesis of NO from L-arginine, oxygen, and NADPH. High concentrations of the free radical NO can induce DNA damage and apoptosis in many cell types; therefore, regulation of NOS activity is fundamental to preserve cells integrity. Forrester *et al.* demonstrated that the expression of wt p53 in various human tumor cell lines or in murine fibroblasts resulted in the down-regulation of NOS2, the inducible form of NOS, through inhibition of its promoter (31). Later on, the same team showed that NOS2 was upregulated in p53 knock-out mice (5). Altogether, these results demonstrate that p53 is a transrepressor of NOS2 expression and attenuates NO production in a regulatory negative feedback loop.

Cyclooxygenase 2 (COX2), which catalyzes the conversion of arachidonic acid to prostaglandin, has been shown to generate ROS and has been linked to neuronal oxidative stress (44, 47). Similar to NOS, COX2 expression has been linked to p53 activity; COX2 protein and mRNA levels were markedly suppressed by wt p53 as well as the activity of the COX2 promoter was reduced by 85% in response to p53 (82). Thus, p53 might also play a role in modulating oxidative stress through its regulation of COX2. Interestingly, it should be pointed out that p53 and COX2 can reciprocally regulate each other (13, 52).

p53 and Regulation of Mitochondrial ROS Production

In addition to its role in response to cellular stress and DNA damage, p53 has been described as a modulator of mitochondrial respiration (60) and mitochondrial ROS production (45). In p53 null mouse and p53 knock-down fibroblasts, both the mitochondrial DNA and the mitochondrial mass are decreased. This is correlated with a disruption of cellular ROS homeostasis, characterized by a reduction in $O_2^{\cdot -}$ levels and an increase in H_2O_2 (50). This could be interesting from the standpoint of the pro-apoptotic function of p53 as a tilt in the intracellular $O_2^{\cdot -}$: H_2O_2 in favor of H_2O_2 serves to sensitize cells to apoptotic stimuli, thereby suggesting an oncosuppressor role of this ROS species. Interestingly, oncogenic proteins, such as Rac and Bcl-2, promote cell survival by increasing intracellular $O_2^{\cdot -}$, thereby shifting the critical ratio in favor of $O_2^{\cdot -}$. As p53 can sequester Bcl-2 *via* physically interacting with it, it remains to be seen whether the effect on cell death signaling is indeed a function of a change in the intracellular $O_2^{\cdot -}$:H_2O_2 ratio in favor of H_2O_2.

In addition, Achanta *et al.* demonstrated that in response to oxidative mtDNA damage, p53 translocated to the mitochondria, where it interacted with mtDNA polymerase γ and enhanced the DNA replication function of the enzyme. As some components of the ETC are encoded by mtDNA and that a well-controlled functioning of the ETC is necessary to prevent spurious generation of $O_2^{\cdot -}$, these data suggest a direct role for p53 in the regulation of mitochondrial ROS production (2).

Another way by which p53 could regulate cellular ROS production is *via* its ability to modulate antioxidant genes expression, such as the mitochondrial *MnSOD/SOD2*. A few recent studies have investigated the link between p53 activity and MnSOD expression. Pani *et al.* demonstrated that E1A-Ras-transformed fibroblasts lacking p53 showed increased MnSOD expression in comparison to wild-type controls. This is associated with a higher resistance to apoptosis induced by serum deprivation or by treatment with ROS-generating agents, such as paraquat and adriamycin. Furthermore, transfection of HeLa cells with wt p53 led to a drop in MnSOD mRNA level and activity (66). Taken together, these results describe MnSOD as a downstream target of p53, which can be specifically downregulated by the tumor suppressor. In addition, Drane *et al.* showed that p53 repression of MnSOD was mediated *via* an effect on the gene promoter. Indeed, using a plasmid plucSOD2 in which the luciferase reporter gene was placed under the control of SOD2 promoter, they showed that luciferase activity was three times higher in MCF-7 cells expressing wt p53 than those expressing mutant p53 (26). Further studies have supported these findings and demonstrated that p53, NF-κB, and Sp1 were acting as co-factors for MnSOD activation (21).

In contrast to the repressive effect of p53 on MnSOD expression, a positive effect of p53 on MnSOD has also been reported. To that end, arsenite-induced apoptosis of human leukemia cells resulted in a slight upregulation of MnSOD, which was dependent on intracellular ROS-mediated activation of the MAP kinase, extracellular signal-regulated kinase (Erk2).

After its nuclear translocation, Erk2 was responsible for the phosphorylation of p53 dowsntream *MnSOD* gene induction (55). It has been postulated that this bi-modal effect of p53 on MnSOD could be a function of the level of activation of p53. For example, in PC3 human prostate cancer cell line low level of p53 increases *MnSOD* gene transcription in a p65-dependent and NF-κB-dependent manner, but, on the other hand, robust activation of p53 abrogated p65-driven transcription of MnSOD resulting in its downregulation (22). Taken together, these results indicate that the effect of p53 on MnSOD expression remains ambiguous and further investigations are needed to elucidate the nature of the crosstalk between p53 and MnSOD. Analysis of the domains involved in p53-MnSOD interaction could clarify the complex role of p53 in MnSOD regulation and, thus, its involvement in mitochondrial ROS production.

The ROS regulatory activity of p53 at the mitochondria could be compromised by pro-apoptotic proteins of the Bcl-2 family such as Bax and PUMA that induce mitochondria leading to ROS production by a less efficient ETC (80). Interestingly, both these pro-oxidant proteins (Bax and PUMA) are transcriptional targets of p53 (57, 61). Furthermore, p66Shc, another downstream target of p53, under pro-apoptotic stress generates H_2O_2 production utilizing reducing equivalents of the mitochondrial ETC through the oxidation of cytochrome c. Finally, this leads to opening of the mitochondrial permeability transition pore and to apoptosis (32).

Conclusion

In this review, we have summarized the results of recent studies focused on exploring the interactions between p53 and ROS pathways. Indeed, as p53 and ROS participate in many cellular processes, it has become evident that there is a close crosstalk between these two signaling networks. p53 has been shown to transactivate genes implicated in the regulation of cellular redox status. That p53 can directly or indirectly impact antioxidant genes such as *GPx, SOD2,* sestrins, and aldehyde dehydrogenase-4, as well as prooxidant genes such as *PIGs*, is rather intriguing and clearly underscores the wide spectrum of cellular proteins and signaling circuits impacted by this remarkable transcription factor. It has been proposed that these genes can be regulated at different stages after p53 induction; antioxidant genes, such as *GPX*, activated at an early time point (83) would be part of an initial protective response against oxidative stress, whereas prooxidant genes, such as *PIGs*, would be activated as a late response and leading to ROS generation (30). Thus, p53 has a definite role in redox homeostasis but is also a target of ROS and susceptible to redox modification at its cysteine or tyrosine residues. These modifications can directly affect the stability, DNA-binding, and transactivation potential of the protein. However, p53-mediated ROS generation is a function of its level of activity as well as subcellular localization, and this phenomenon is regulated by a feedback loop because ROS alter p53 subcellular localization and transcriptional activity.

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Abbreviations Used

BSO = buthionine sulfoximine
COX2 = cyclooxygenase 2
DEM = diethylmaleate
Erk = extracellular signal-regulated kinase
ETC = electron transport chain
GPX = glutathione peroxidise
Grx3 = glutaredoxin 3
GSH = glutathione
GST = glutathione-S-transferase
H_2O_2 = hydrogen peroxide
MnSOD = manganese superoxide dismutase
NO = nitric oxide
NOS2 = inducible nitric oxide synthase
$O_2^{\cdot -}$ = superoxide radical
$^{\cdot}OH$ = hydroxyl radical
$ONOO^-$ = peroxynitrite
PIGs = p53 induced genes
POX = proline oxidase
Ref-1 = redox factor 1
ROS = reactive oxygen species
Trx = thioredoxin
TrxR = thioredoxin reductase

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