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FORUM ARTICLE

Interaction of p53 with Tumor Suppressive and Oncogenic Signaling Pathways to Control Cellular Reactive Oxygen Species Production

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Abstract

p53 is a crucial transcription factor with tumor suppressive properties that elicits its function through specific target genes. It constitutes a pivotal system that integrates information received by many signaling pathways and subsequently orchestrates cell fate decisions, namely, growth-arrest, senescence, or apoptosis. Reactive oxygen species (ROS) production in cells can play a key role in signal transduction, being able to trigger different processes as cell death or cell proliferation. Sustained oxidative stress can induce genomic instability and collaborates with cancer development, whereas acute enhancement of high ROS levels leads to toxic oxidative cell damage and cell death. Here, it has been considered p53 broad potential contribution through its ability to regulate selected key cancer signaling pathways, where ROS participate as inductors or effectors of the final biological outcome. Further, we have discussed how p53 could play a role in preventing potentially harmful oxidative state and cell proliferation by pro-oncogenic pathways such as PI3K/AKT/mTOR and WNT/β-catenin or under hypoxia state. In addition, we have considered potential mechanisms by which p53 could collaborate with signal transduction pathways such as transforming growth factor-β (TGF-β) and stress-activated protein kinases (SAPK) that produce ROS, to stop or eliminate uncontrolled proliferating cells. *Antioxid. Redox Signal*. 00, 000–000.

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Introduction

Reactive oxygen species production and regulation in cells

Complete reduction of molecular oxygen (O_2) (four electrons and four protons) produces water; however, its partial reduction produces O_2 intermediates that are highly reactive. One-electron reduction state of O_2 produces superoxide anion ($O_2^{-\bullet}$), two-electron reduction state produces hydrogen peroxide (H_2O_2), and three-electron reduction state produces hydroxyl radical ($^{\bullet}OH$). Hydrogen peroxide is a nonradical molecule that is more stable than superoxide anion or hydroxyl radicals. It can be produced through spontaneous superoxide radical dismutation and by superoxide dismutase (SOD) enzymes. All these highly reactive oxygen derivatives are collectively named reactive oxygen species (ROS). ROS are continuously produced in living cells as products or by-products of their normal metabolism (110, 146).

Excessive ROS generation can oxidize major cell macromolecules, thus compromising cell function: (i) ROS reaction with the amino acid cysteine can modulate the activity of transcription factors and other proteins (55), (ii) oxidation of nucleic acids (both DNA and RNA) strongly elevates mutation rates (25), and (iii) lipid peroxidation is considered the major damage caused by ROS to cell membrane, and its determination is a reliable sensor of oxidative stress (1, 110).

Until the late 1980s, ROS had been considered dangerous agents that must be kept under balance to avoid protein, DNA, and lipid modifications, which leads to an irreversible cell modification state or cell death (55). ROS can play different roles depending on their levels, being able to kill cells (136) or stimulate their proliferation through specific signaling pathways (153). ROS are able to participate both as upstream factors to activate a defined signaling cascade and as final pro-oxidant effectors, thus indicating that a subtle balance between ROS production and ROS elimination is required for the normal physiology of the cell.

ROS are mainly produced in the mitochondria during cellular respiration, which is required for oxidative phosphorylation to produce energy (ATP). Electrons can escape along the electron transport chain, most usually at complex I, II, or III, and generate ROS (149). In addition, a second physiological system important for ROS production is the NADPH oxidase (Nox) complex. Historically, the Nox system has been

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known as the source of ROS in phagocytes. In the last years, different Nox complexes have been identified as a key machinery to regulate cellular ROS production by different signaling pathways (12). The Nox complex is a membrane-associated multiprotein system that catalyzes molecular oxygen reduction using NADPH as electron donor and producing superoxide anion (85).

The cellular antioxidant system

ROS levels are regulated in cells through enzymatic and nonenzymatic antioxidant molecules. Nonenzymatic antioxidants or ROS scavengers are molecules that can neutralize oxygen free radicals by accepting or donating an electron to change their unpaired condition. This reaction converts the antioxidant in a free radical but with much less reactive potential than the neutralized free radical. Thiols, such as reduced glutathione (GSH) and thioredoxins (Trxs), ascorbic acid (vitamin C), and tocopherols (vitamin E) are the most important antioxidant molecules present in cells able to physiologically regulate the redox state. The enzymatic antioxidant defense is mainly composed by SODs, which eliminate superoxide anion; catalase (CAT), which converts hydrogen peroxide into oxygen and water; and, finally, glutathione peroxidase (GPX) and peroxiredoxins (Prx), which eliminate hydrogen and other peroxides (27, 104, 140).

ROS Are Key Components of p53 Function

p53 pathway and ROS

p53 is a key tumor suppressor protein that plays a pivotal role as node of several signaling pathways. p53 generates a coordinated cellular response to different stressing stimuli, mainly cell cycle arrest, senescence, or apoptosis (88). The relevance of p53 as tumor suppressor has been demonstrated by its inactivating high mutation rate found in tumors (115) and by the number of oncogenic viral proteins (87) or tumor-specific proteins (105, 160) that target p53 function.

Several publications support the notion that p53 is sensitive to ROS levels (10, 82, 95, 103, 152) as it is to other well-known stimuli such as DNA damage (72, 98) or oncogene activation (56, 100). Indeed, p53 is noteworthily required for the activation of a third of H_2O_2-inducible genes, indicating that p53 is the major regulator of H_2O_2 response in human cells (33).

Regulation of ROS levels by p53 displays opposite effects at its steady-state and activated-state levels. These effects are achieved either through direct regulation of pro- and antioxidant gene expression or through modulating the cellular metabolic pathways (91).

ROS are involved in the p53 pro-apoptotic function

Transient cellular stress is able to activate p53-dependent checkpoints that lead to a reversible inhibition of cell cycle. During this time, specific machinery may be active to repair potential cell damage. Once repaired, cells can re-enter the cell cycle and p53 is downregulated by degradation (31, 54, 63). However, when the stress source persists and cell machinery fails in the repairing process, p53 remains activated and accumulated into the nucleus to induce a permanent inhibition of cell proliferation, mainly through apoptosis or cellular senescence (72, 94, 124, 132). ROS production is now considered a basic tool for p53 to induce cell death. Moreover, apoptosis triggered by p53 is dependent on ROS production and the release of pro-apoptotic factors from damaged mitochondria (118). p53 contributes to the increment of ROS level by inducing the expression of pro-oxidant genes involved in ROS-related pathways such as those encoded by *PIG3* (quinone oxidoreductase) (118), *POX* (proline oxidase, PIG6) (120), and ferredoxine reductase (*FDXR*) (92). In addition, a number of proteins encoded by p53-induced genes indirectly cause an increase in intracellular ROS levels. BH3-only proteins encoded by Bcl-2-associated X protein (*BAX*) (102), p53 upregulated modulator of apoptosis (*PUMA*) (107), and *NOXA* (111) genes are examples of p53-induced proteins involved in apoptosis mediated by ROS.

PUMA and BAX are required for p53-dependent apoptosis. The absence of BAX or PUMA strongly impairs ROS increment and p53-dependent apoptosis (95). Under hypoxia conditions, Noxa is involved in cell death through ROS generation (73). Apoptotic stimulation causes translocation of Noxa into the mitochondria and induction of mitochondrial outer membrane permeabilization by inactivating Bcl-2 proteins and/or activating pro-apoptotic members. Finally, $p21^{waf1}$, a well-known cell cycle regulator targeted by p53, has been linked to ROS-mediated effects. p21 overexpression causes ROS increment that could induce p53-dependent senescence or apoptosis (67). These data suggest that, at least in part, p53 induces apoptosis by ROS generation.

Activation of p53-dependent response also increases ROS levels by regulating the antioxidant manganese SOD (MnSOD) enzyme (37). One proposed mechanism indicates that MnSOD can be directly inhibited in the mitochondria by p53 through p53/MnSOD protein–protein interaction (166). In addition, p53 can affect MnSOD gene transcription by at least two mechanisms. SP1 and forkhead box O (FOXO) positively participate in MnSOD transcription. p53 controls MnSOD expression by targeting SP1 (34). Additionally, p53 upregulates the expression of p66shc, which is directly involved in mitochondrial ROS generation and apoptosis triggering (45, 147). Under oxidative stress, p66shc can inactivate FOXO transcription factor (108). This mechanism was proposed for p66shc-dependent downregulation of MnSOD expression (79).

Indirect control of ROS by p53 through metabolism regulation

TP-53-induced glycolysis and apoptosis regulator (*TIGAR*) is a p53 target gene activated by low levels of stress. TIGAR protein participates in the regulation of glycolysis and protects cells against oxidative stress (5). TIGAR hydrolyses fructose-2,6-bisphosphate (Fru-2,6-P_2); thus, as a consequence of TIGAR expression, Fru-2,6-P_2 level is reduced and glycolytic rate decreased. TIGAR also leads the redirection of glycolytic metabolic intermediates to the pentose phosphate pathway. Stimulation of this pathway results in accumulation of NADPH, needed for the scavenging of ROS by GSH. Therefore, inhibition of glycolysis and stimulation of pentose phosphate pathway by TIGAR reduce ROS levels and ROS-associated apoptosis (6).

Two different enzymes related to the creatine metabolism and fatty acid oxidation are directly regulated by p53, the creatine kinase, and the recently identified guanidinoacetate methyltransferase (GAMT) (66). Creatine and phosphocrea-

tine metabolism is involved in energy-generating pathways that play an essential role in the regulation of ATP homeostasis (158). Under metabolic stress there is an induction of GAMT dependent on p53. Consequently, creatine levels increase and a rise in intracellular ROS levels is observed. As p53 elicits apoptotic responses that are, to some degree, ROS dependent (95), ROS increased as a consequence of GAMT activation supports a new role for GAMT and creatine metabolism in p53-dependent apoptosis (66).

The glycolytic enzyme phosphoglycerate mutase (PGM) is regulated by physiological levels of p53. However, this regulation depends on cell type being PGM repressed by p53 in mouse embryo fibroblast (MEF) and positively regulated in muscle cells (80, 122). Loss of functional p53 protein in MEF increases PGM levels and enhances glycolysis and the Warburg effect (80). This limits ROS production by decreasing the requirement for mitochondrial respiration (46). In muscle cells, PGM regulation by p53 favors energy production by glycolysis and reduces ROS generation (122).

Transcription of synthesis of cytochrome c oxidase 2 (*SCO2*) gene is upregulated by basal levels of p53 (96). SCO2 is a chaperon involved in the assembly of the cytochrome c oxidase complex IV in the mitochondria (69). Disrupted expression of SCO2 in p53-deficient cells leads to low levels of O_2 consumption and energy obtained from glycolysis (165). Moreover, these cells generate reduced level of ROS (11), suggesting that constitutive levels of p53 sustain the basal level of ROS generated from the mitochondria (91).

The antioxidant role of p53

Contrary to activated p53, which increases ROS levels with pro-apoptotic purpose, the role of steady-state p53 is likely to avoid the exposure of normal cells to sub-lethal levels of ROS through the control of the antioxidant system components. This antioxidant activity of p53 is crucial for its tumor suppressor function since it protects the genome from ROS-induced DNA damage and genetic instability. In fact, downregulation of p53 causes DNA oxidation and elevated mutation rate, such mutations having been reverted by antioxidants (123). A diet supplemented with antioxidants prevented the development of spontaneous lymphoma commonly observed in p53-deficient mice and the development of lung cell carcinoma in xenograft bearing targeted p53 (123).

p53 antioxidant function is achieved through transcription of antioxidant genes such as *GPX1* (139), apoptosis-induced factor (*AIF*) (138), *PIG12* (microsomal glutathione transferase homolog) (118), aldehyde dehydrogenase (*ALDH4*) (163), and the members of the senstrin family *SESN1* and *SESN2*, which act as components of the Prx regeneration system (14). Therefore, steady-state level of p53 plays antioxidant functions and keeps physiological levels of ROS avoiding DNA oxidation and genetic instability, whereas in stressed conditions p53 increases ROS as part of its tumor suppressive repertory.

The p53-target gene, TP53-induced nuclear protein 1 (TP53INP1) (112, 143), is a key protein involved in the antioxidant function of p53 (21). TP53INP1 participates in a positive feedback loop to activate p53 through its direct interaction with homeodomain-interacting protein kinase-2 (HIPK2) and protein kinase C (PKC) kinases (142, 164). In addition, TP53INP1-deficient mice present increased susceptibility to colon tumor development when subjected to chronic inflammation (47), suggesting a tumor suppressor role for TP53INP1. Cano *et al.* demonstrated that TP53INP1 is induced upon oxidative stress in a p53-dependent fashion and that TP53INP1 deficiency correlates with cellular ROS production. Further, TP53INP1 expression fully neutralizes the ROS increment that naturally occurs in p53-deficient cells, suggesting that TP53INP1 is involved in the antioxidant function of p53 (21).

As mentioned throughout this work, p53 is activated by stimuli that can differ in nature, time, and concentrations. All this variants can produce different amounts of active p53 and different cellular outcomes. To analyze the potential behavior of p53 and its relationship with ROS production, it could be theoretically considered the existence of only three p53 cellular states: (i) basal p53, a physiologic concentration of p53 contained in unstressed cells, (ii) increased levels of p53, mainly caused by reversible stress situations, and (iii) high accumulation of active p53, caused by persistent and irreversible damage, which leads to senescence or apoptosis. As illustrated in Figure 1, and proposed by other authors (91), the ◀F1 level and activation state of p53 differentially regulates ROS. In unstressed cells, physiologic levels of p53 are necessary to keep ROS at safety levels through the control of glycolysis and antioxidant genes, probably to protect DNA integrity. Similarly, during reversible stress, activation of p53 could be involved in keeping controlled ROS production. However, when damaging stimuli persist, activated p53 is highly accumulated in cells and switches to induce a strong pro-oxidant state. This behavior is achieved by differential regulation of antioxidant *versus* pro-oxidant and pro-apoptotic genes, thus using ROS production to mainly induce apoptosis or senescence.

p53 as Potential Regulator of ROS Produced by Cancer Signaling Pathways

PI3K/AKT/mTOR pathway

PI3K/AKT/mTOR pathway is required for several cellular processes, including cell growth, survival, proliferation, and motility (22). Growth factor receptor–ligand interactions recruit and activate phosphoinositide 3 kinase (PI3K), which regulates PIP3 (phosphatidylinositol 3,4,5 triphosphate) production (36). PIP3 binds to pleckstrin homology domain of AKT, allowing its ◀AU2 translocation to the plasma membrane. There, AKT is phosphorylated and activated by phosphoinositide-dependent kinase I (PDK-1). AKT negatively regulates tuberous sclerosis proteins 1 and 2 (TSC1/TSC2) activity, allowing Ras homolog enriched in brain (Rheb) signaling to activate mammalian target of rapamycin (mTOR). In addition to TSC complex, phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a negative regulator of AKT signaling by inhibiting PI3K activity. Positive AKT signaling to mTOR stimulates cell proliferation by promoting biosynthesis of ribosomes, enhancing protein synthesis, and inhibiting autophagy (51, 128, 157).

Activated p53 can mainly control AKT/mTOR activation through transcription of negative regulators of this pathway. For example, p53 induces *PTEN* gene transcription (137) and PTEN counteracts PI3K activity and therefore AKT activation. Also, p53 controls AKT protein levels through a caspase-dependent mechanism (48). In addition, the protein products

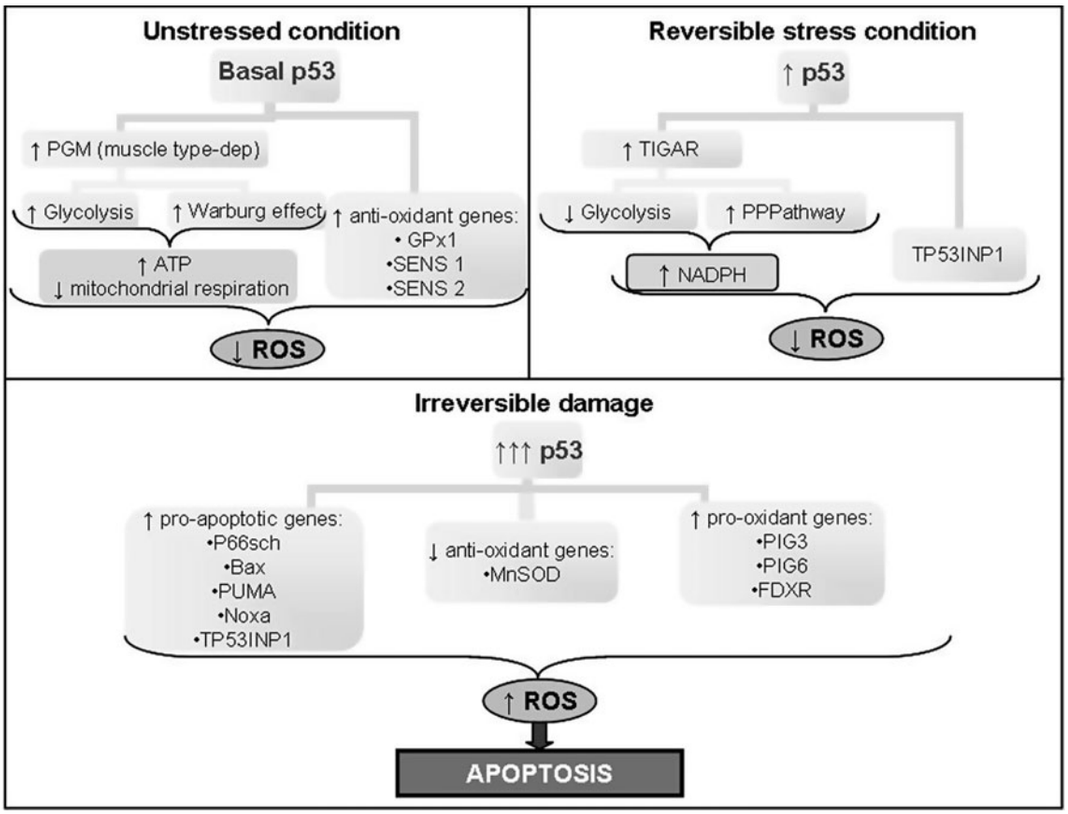

FIG. 1. Picture of how p53 could directly regulate reactive oxygen species (ROS) levels depending on its activation state. **(A)** In the absence of stressing stimuli p53 controls ROS levels. **(B)** At low cellular stress p53 could stimulate ROS clearance through different mechanisms. **(C)** High cellular stress induces strong p53 activation and the triggering of apoptosis and/or senescence through its target genes.

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of novel p53-target genes play key roles in the regulation of PI3K/AKT/mTOR signaling. SESN1 and SESN2 have been characterized as p53-target genes that are involved in AMPK/TSC2 complex formation, which in turns activates TSC2 and inhibits mTOR activation (13). In addition, Feng and coworkers (39, 88) demonstrated that p53 transcriptionally induces AMPK-β subunit and TSC2, that together with PTEN and IGF-BP3 form a p53-regulated network able to control the PI3K/AKT/mTOR signaling, in response to specific stress conditions.

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In contrast, growth factor-dependent activation of AKT/mTOR signaling includes p53 downregulation for full proliferating and survival activities. Phosphorylation of murine double minute 2 (Mdm2), the p53 ubiquitine E3 ligase, by AKT is required for the translocation of Mdm2 from the cytoplasm into the nucleus, where it can target p53 for inactivation and degradation (48), suggesting that the balance of specific signaling conditions could govern the reciprocal regulation of the p53 and AKT pathways.

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PTEN is sensible to ROS and its activity is negatively regulated through oxidation (24, 83). As a consequence, hyperactivation of AKT leads to uncontrolled cellular proliferation, and enhanced survival and growth (148, 153). In turn, AKT pathway activation induces ROS generation at nontoxic levels, which could be linked to its pro-proliferating/oncogenic function, keeping PTEN inactivated. AKT activity raises ROS levels by stimulating glycolysis and oxidative metabolism (117). Additionally, AKT induces ROS accumulation through direct phosphorylation and exclusion from the nucleus of the FOXO family of transcription factors, which are involved in transcriptional expression of antioxidant enzymes such as MnSOD, CAT, and SESN3 (Sesn3). This result indicates that uncontrolled AKT activation, which can counteract a wide range of pro-apoptotic stimuli, could not inhibit ROS-induced apoptosis (109). However, the same research group demonstrated that tumor cells displaying hyperactive AKT signaling also require forkhead box M1 (FOXM1) overexpression for cell proliferation (113). FOXM1 is a member of the Forkhead box transcription factors only expressed in proliferating cells and, similarly to FOXO, induces transcription of antioxidant enzymes. FOXM1 is implicated in tumorigenesis and contributes to both tumor initiation and progression (154). FOXM1 belongs to a short list of genes that were identified due to their differential expression in a number of solid tumors from different origin (116). In addition, FOXM1 is induced by H-ras expression in an ROS-dependent fashion, and FOXM1 expression is required to overpass oncogene-induced premature senescence. In this case, the main role of FOXM1 is to keep the ROS levels low, then counteracting their potential harmful oxidant effect (113). The mitogenic stimuli that induce AKT signaling also induce ROS production. FOXM1 expression is required in cells with uncontrolled AKT sig-

naling to promote cell proliferation and avoid ROS-induced senescence or cell death. In this scenario, it is possible to speculate that p53 could play a role in the balance between cancer cell proliferation and cancer cell depletion. If mitogen/AKT-induced ROS production occurs in tumor cells with enhanced expression of FOXM1 and nonfunctional p53, it is likely that these cells escape senescence and survive. On the other hand, if mitogen/AKT-dependent ROS production occurs in cells with limited FOXM1 expression and wt-p53, they would expectedly undergo senescence or apoptosis (Fig. 2).

WNT/β-catenin pathway

Canonical WNT signaling pathway initiates when WNT associates and induces frizzled/LRP complex formation to activate disheveled (Dvl), which in turns activates β-catenin. β-catenin translocates into the nucleus, where it binds the T-cell factor (TCF) transcription factor and induces TCF-dependent transcription through antagonizing the transcription inhibitor Gorucho (86). WNT/β-catenin axis plays a role in important biological processes such as embryogenesis, cell proliferation, cell adhesion, and stem cell maintenance (106). β-catenin regulation is very important for controlled cell proliferation, and, actually, deregulated WNT/β-catenin signaling is frequently found in cancer cells. In fact, β-catenin is controlled by a protein complex containing the tumor suppressor protein adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase-β (GSK-3β) (3, 53, 76). Dvl activates β-catenin through the inhibition of the β-catenin destruction complex (19, 89), which constitutively eliminates cytosolic β-catenin through GSK-3β-dependent phosphorylation, a signal for β-trCP-dependent β-catenin ubiquitination and degradation. In addition to APC, p53 tumor suppressor can also control β-catenin accumulation through a GSK-3β-independent way. p53 is a direct transcriptional activator of Siah-1, which forms a protein complex with stress-induced protein (SIP) and Ebi and induces β-catenin polyubiquintination and its subsequent proteasome-dependent degradation (93, 97). The p53 sibling, p63, is also able to negatively regulate WNT/β-catenin signaling by association to TCF (38), whereas, on the contrary, p73 induces TCF transcription (144, 151), indicating the specificity of the p53 family members in β-catenin signaling regulation.

A number of recently published articles indicate that the WNT/β-catenin pathway is regulated by ROS and that oxidative stress causes a relevant change in β-catenin behavior. In the presence of ROS, β-catenin changes its classical nuclear partner TCF to FOXO or hypoxia-inducible factor (HIF) transcription factors (61). This scenario suggests a novel and flexible function for β-catenin in the regulation of transcription. The strength of this novel pathway is that both FOXO and HIF are regulated by ROS, and β-catenin plays a critical role in enhancing their transcriptional activation. This suggests that, while β-catenin activation through WNT signaling leads to β-catenin/TCF association and TCF-dependent gene regulation to induce cell proliferation, the presence of ROS induces a molecular switch to β-catenin/FOXO or β-catenin/HIF association to increase antioxidant and survival genes. FOXO transcription factor is induced by ROS and stimulates the transcription of key antioxidant enzymes such as MnSOD and CAT (81). In addition to β-catenin/FOXO cooperation during oxidative stress, new data suggest that β-catenin/TCF-dependent transcription should be shut-off for a complete antioxidant/survival cell program, since FOXO also represses TCF activity (62). Similarly, under hypoxia, β-catenin no longer associates to TCF but switches to form a complex and to enhance HIF1-dependent transcription, thus promoting cell survival and adaptation to hypoxia (70).

Under specific circumstances, sustained ROS levels could trigger p53 activation (depending on status, mutations, cell type, *etc.*), which could play a decisive role by switching the β-catenin-based survival program to p53-dependent apoptotic program. As mentioned above, p53 is able to regulate β-catenin through Siah-1 ubiquitin E3 ligase. In addition, a second p53-induced ubiquitin E3 ligase gene, *MDM2*, induces FOXO degradation (42). Similarly, p53 regulation could be proposed for β-catenin/HIF complex, since a number of evidence suggests that p53 negatively regulates HIF activity (2, 8, 29, 119, 129). Consequently, increased ROS levels and p53-dependent targeting of β-catenin or its transcription complexes could be a critical cell-fate decisive factor that changes the cellular program by shutting down cell survival signaling and promoting apoptosis (Fig. 3).

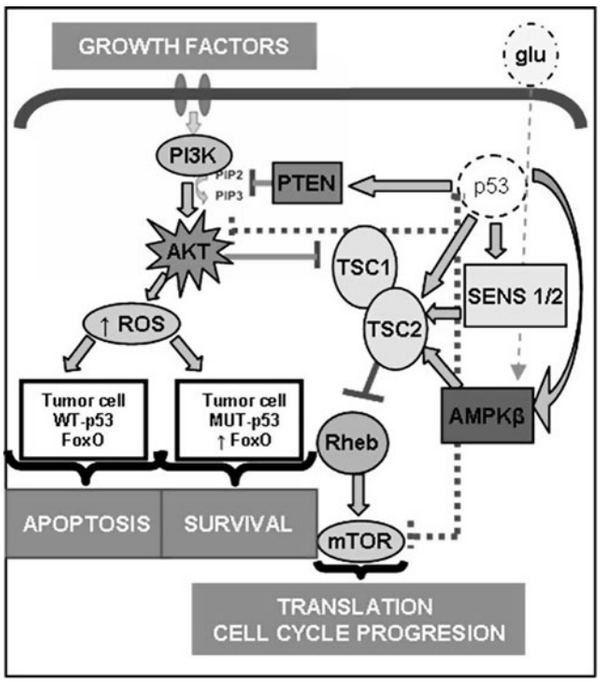

FIG. 2. Representation of the potential role of p53 in the phosphoinositide 3 kinase (PI3K)/AKT signaling pathway. Upon ROS production, p53 could act by inducing PTEN and SENS proteins to downregulate AKT to mammalian target of rapamycin (mTOR) signal. In addition, depending on p53 and forkhead box O (FoxO) status, tumor cells could undergo survival or apoptosis.

Hypoxia/HIF1 pathway

HIF is a family of transcription factors including HIF1, HIF2, and HIF3 that are activated when cells sense low

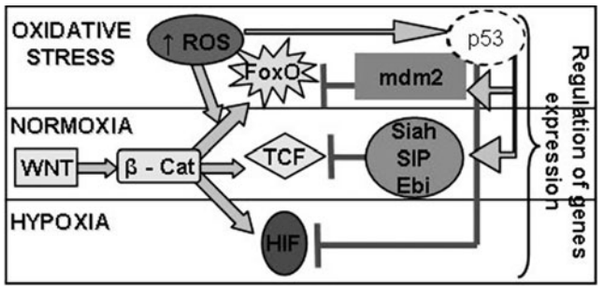

FIG. 3. Diagram of potential points for p53 to control β-catenin signaling under oxidative stress, normoxia, and hypoxia.

oxygen levels (hypoxia). Generally, HIF-induced gene products help to sustain the supply of oxygen to tissues and to enhance cell survival during severe oxygen deprivation. The archetype of HIFs is HIF1 that in hypoxia is responsible for mediating relevant processes for cancer development such as glucose metabolism and angiogenesis. It is generally accepted that HIF1 expression is associated to tumor adaptation to hypoxia and survival, and correlates with tumor resistance to cancer treatments and poor prognosis (121). HIF1 consists of an oxygen-sensitive HIF1α subunit that heterodimerizes with the HIF1β subunit to bind DNA. The classical HIF1 target genes under hypoxia are vascular endothelial growth factor (*VEGF*), erythropoietin (*EPO*), glucose transporter 1 (*GLUT1*), and phosphoglycerate kinase 1 (*PGK1*) (145). Under normal oxygen levels (normoxia) HIF1α is unstable since it is continuously degraded by PHD/VHL/proteasome system. Prolyl hydroxylases domains (PHDs) are oxygenases able to hydroxylate critical proline residues of HIF1α. von Hippel-Lindau (VHL) protein recognizes and associates to hydroxylated HIF1α targeting it for ubiquitination and proteasome degradation. Under hypoxia, HIF1α is not hydroxylated since, due to lack of oxygen, PHD oxygenases are not functional and therefore HIF1α levels increase. Stabilized HIF1α shuttles into the nucleus, where it associates to HIF1β and starts transcription. Other proteins such as arrest-defect-1 protein (ARD1) and OS-9 have been also involved in the regulation of HIF1α stability *via* VHL complex. In addition, VHL-independent pathways that are able to govern HIF1α degradation have been identified. p53 is a potent inhibitor of HIF1α and could regulate HIF1α expression at least by two different mechanisms, one involving Mdm2 (119) and the other involving eukaryotic translation initiation factor 2 a (eIF2a) phosphorylation (161).

Hypoxia condition induces ROS production by mitochondrial complex III and stabilizes HIF1α (4). ROS are able to inhibit PHDs, thus keeping HIF1α unhydroxylated and free of VHL-dependent degradation (20). In addition, low O_2 concentration induces accumulation of p53 (50), which plays a central role in hypoxia-induced apoptosis (52). In fact, hypoxia promotes the selection of tumor cells harboring mutant p53 (49).

Prolonged hypoxia or very low O_2 concentration (0.2% O_2) enhances ROS and both p53 and HIF1α protein levels. In this case, p53 can regulate HIF1 activity by competing for p300, which can enhance p53 and HIF1 transcriptional activity (8, 129). p53 transactivation function is not required for HIF1 signaling attenuation or hypoxia-induced apoptosis, since transcriptionally inactive p53 retains its ability to bind p300 and to inhibit HIF1 (9).

Transforming growth factor-β/SMADs pathway

Transforming growth factor-β (TGF-β) regulates cell proliferation, differentiation, and apoptosis, playing important roles in development, tissue homeostasis, and disease.

TGF-β is a key ligand in cancer cell signaling that, upon binding to serine/threonine kinase receptors (TGFBRs), activates intracellular SMAD effectors, as well as other signaling proteins. Activated SMADs translocate into the nucleus, where they specifically associate with transcription factors and regulate gene expression (133). Among the wide range of TGF-β activities, this cytokine controls cell growth and apoptosis, being considered in specific tissues, a tumor suppressor protein (135).

TGF-β plays a suppressive role in the liver. Impaired TGF-β response is associated with hepatocarcinogenesis, indicating that intact TGF-β signaling pathway is necessary to avoid cell transformation (127). TGF-β mediated cell death in hepatocytes requires the production of ROS (7, 57, 58). After TGF-β treatment, mitochondrial ROS levels increase. This occurs in part through the depletion of intracellular GSH, since TGF-β downregulates *GCLC* gene expression, which encodes the catalytic subunit of the glutamate cysteine ligase (GCL) (41). In addition, TGF-β negatively regulates MnSOD expression (59), a key enzyme that eliminates superoxide anion. Therefore, downregulation of GSH and MnSOD could account for the increased mitochondrial ROS observed after TGF-β treatment of hepatocyte-derived cells. In addition, extramitochondrial ROS are produced by an NADPH oxidase-like system (see Introduction section). Upregulation of the rac-independent NADPH oxidase isoform 4 (NOX4) by TGF-β is required for sustained ROS production and TGF-β-induced cell death (23), whereas TGF-β activation of rac and the rac-dependent NOXs could be involved in an earlier and transient induction of ROS.

In 2003, Piccolo's group demonstrated a cross talk between TGF-β signaling and p53 (26): p53 collaborates with TGF-β by interacting with SMAD2 and SMAD3, thus acting selectively on a subset of TGF-β responsive promoters that also contain the DNA consensus sequence for p53 binding. Regarding cell cycle arrest, TGF-β induces transcription of the cyclin-dependent kinases (Cdk) inhibitor, $p21^{waf1}$, the first reported p53 induced gene. TGF-β-dependent cell cycle arrest is impaired in p53-deficient MEF (135).

Besides, p53 could collaborate with the TGF-β response not only through Smad interaction but also by enhancing cell death through ROS production. Oxidative damage could induce a canonical p53-dependent induction of ROS (*i.e.*, mitochondrial translocation of Bax, cytochrome c release, increased production of ROS, and loss of mitochondrial transmembrane potential) independently of NOX complex activation. In fact, in rat liver cells, TGF-β induces p53 and its target gene *BAX* (141), which is linked to mitochondrial ROS production. Further, TGF-β induces apoptosis by the p53 sibling, α-p73 (159), supporting the hypothesis that p53 or its family members could be involved in enhancing the oxidation-dependent apoptosis triggered by TGF-β in liver cells (Fig. 4).

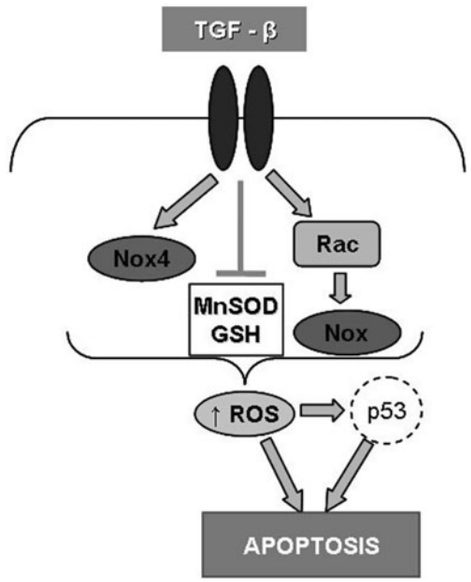

FIG. 4. Diagram depicting a potential collaborative role of p53 in hepatic cells. Upon transforming growth factor-β (TGF-β)-induced ROS production, p53 could be activated to collaborate in TGF-β-induced apoptosis.

Mitogen-activated protein kinase pathway

Mitogen-activated protein kinase (MAPK) signaling pathway consists of a hierarchical order of protein kinases that respond to a variety of stimuli, from growth factors to different harmful agents or insults. Signaling modules comprise a MAP3K (usually the sensor) that phosphorylates an MAP2K, and this one phosphorylates an MAPK. Depending on the stimulus, a specific MAP3K is activated to spread the signaling to a definite MAP2K and MAPK. Eukaryotic cells have different MAPK pathways (28). For example, growth factors stimulate the extracellular regulated protein kinases (ERK) MAPK pathway, whereas the stress-activated protein kinases (SAPK) MAPK pathway is usually activated by environmental stresses. c-Jun N-terminal kinase (JNK) and p38-MAPK are MAPKs grouped in the SAPK subfamily. Therefore, ERK pathway is generally associated to a positive regulation of cell proliferation, whereas SAPKs (p38 and JNK) are more frequently activated to induce cell cycle arrest, senescence, or cell death.

Changes in ROS levels affect both ERK and SAPK signaling; however, p53 could most likely play a central role in ROS/SAPK-induced senescence or apoptosis. As two major signaling pathways, p53 and MAPK are reciprocally controlled: MAPK signaling regulates p53 activity and p53 activation can control the MAPK phosphorylation cascade (155). A number of studies show that upon a variety of stresses, p38-MAPK can activate p53 by phosphorylation on specific key serine residues (*i.e.*, Ser15, 20, 46, 389) and that generally p53 activation is determinant for the induction of cell cycle arrest, senescence, or apoptosis (15, 32, 60, 64, 74, 75, 77, 125, 134, 167). In addition, p73 is also phosphorylated, stabilized, and activated through p38-MAPK (126). Similar to p38-MAPK, JNK activation leads to specific substrate phosphorylation, including p53 (17, 18, 43, 44, 101).

One of the most studied mechanisms for ROS-dependent activation of MAPKs is the one involving the MAP3K apoptosis signaling-regulating kinase (ASK1), which in its inactive form is bound to a reduced Trx. Increased ROS levels oxidize Trx causing its dissociation from ASK1 and the initiation of ASK1 signaling to JNK and p38-MAPK cascades. However, ROS oxidize JNK phosphatases at their critical cysteine, thus inducing sustained JNK kinase activity (71). Supporting a key role of p53 in JNK signaling pathway, upon oxidative insults, JNK phosphorylates p53 at serine 15 to induce cell death in liver cells (30). H-Ras oncogene expression induces p53 activation (114, 132) and intracellular ROS raising (68, 84). In a recent publication by Nebreda's lab (35), it was demonstrated that p38-MAPK protein behaves as a specific sensor of H-ras-induced ROS and that p38 activation is required to inhibit tumor initiation. Berberine induces apoptosis in liver cancer cell through ROS increment, ASK1/SAPK phosphorylation, and p53 induction (65). All these data suggest that SAPK-induced p53 activation could play a critical role in the execution of cell fate, namely, cell cycle arrest, senescence, or cell death upon oxidative stress.

On the contrary, low doses of H_2O_2 can promote cell growth (16) and activate growth factor receptors and ERK signaling (78, 99). Therefore, in addition to a possible contribution of p53 in ROS-activated SAPK signaling to stop cell proliferation, it is also conceivable that p53 could play a role by interfering with the ROS-dependent ERK pathway activation, which promotes cell proliferation. H-ras oncogene activation increases ROS levels and activates ERK1/2, which transcriptionally induces cyclin D1 production. Association of Cdk4/6 with cyclin D1 activates the Cdk kinase activity that is necessary for retinoblastoma protein (pRb) hyperphosphorylation and E2F transcription factor 1 (E2F1) release. Therefore, ERK1/2 plays a critical role in E2F1 activation to complete the G1 to S cell cycle transition.

p53 can control MAPKs signaling, since it is able to transcriptionally induce a series of phosphatases that negatively regulate MAPKs: wild-type p53-induced phosphatase (Wip1), MAP kinase phosphatase 1 (MKP1), PAC1/dual-specificity phosphatase 2 (DUSP2), and DUSP5 (40, 90, 150, 162). Through PAC1/DUSP2 expression, p53 inhibits MAPKs activation and induces apoptosis in response to serum deprivation and oxidative stress (90). This is also true for E2F-induced apoptosis, which directly upregulates PAC1/DUSP2 expression to inhibit ERK activation and promotes cell death (156). DUSP5 is a specific ERK phosphatase whose mRNA increases after p53 expression (150) and upon ROS production in human fibroblasts (130). A mechanism for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced breast cancer carcinogenesis relies on targeting p53 expression and therefore downregulating DUSP5 protein levels. Repressed p53 activation and increased ERK activity may contribute to cancer promotion by this strong oxidant compound (131) (Fig. 5). ◀F5

Concluding Remarks

Depending on their levels, ROS can play an important role as strong oxidant molecules able to eliminate cells or as second messengers able to start specific signal transduction frequently associated to cell proliferation, survival, and malignant transformation. The p53 transcription factor plays

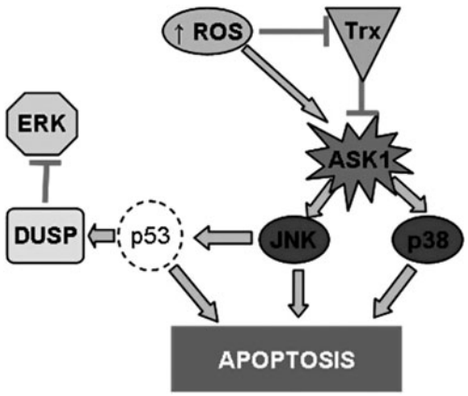

FIG. 5. Schematic diagram of how p53 could be involved in apoptosis triggering by mitogen-activated protein kinase (MAPKs). c-Jun N-terminal kinase (JNK) and p38 that are activated by ROS also phosphorylate p53. In this way p53 could cooperate in ROS/MAPK-induced cell death.

a critical role as a potent tumor suppressor, and in part, this role is linked to its ability to keep cellular redox homeostasis.

The absence of wt-p53 in mice is associated to unbalanced cellular redox state, increased ROS levels, genetic instability, and predisposition to tumor development. Such tendency to tumor development was prevented by a diet supplemented with antioxidants (48). This observation unequivocally indicates a direct relationship between p53 and ROS. However, this carcinogenic effect of ROS is more likely associated to specific activation of signal transduction pathways than to unspecific cellular oxidation.

Most of the different signaling pathways considered herein may be activated under different circumstances and cell types. Although the cancer development process is not normally attributed to a single and specific defect in cells, inactivation of p53 could affect more than one (ROS-related) signaling pathway, giving widespread effects that predispose cells to undergo tumor transformation. In addition, p53 is crucial for efficient response to cancer treatment since most of them are based on DNA damage. Again, an important part of p53 relevance relies on its ability to stop proliferation in stress conditions by regulating pro-oncogenic pathways as described here for the AKT and β-catenin signaling. p53 itself is under redox regulation, and its activation determines cell fate through its target genes. In unstressed condition, p53 make efforts to maintain low ROS levels. However, when the cell has suffered irreversible damage, p53 triggers the apoptosis program and makes use of ROS to eliminate such harmfully damaged cells through a subset of pro-oxidant target genes with pro-apoptotic function.

p53 regulates the cellular redox state not only directly, but also indirectly through its ability to crosstalk with other signaling pathways relevant to cancer development. Here we have discussed and hypothesized how p53 could be involved in the regulation of different signaling pathways that are known to have a relevant role in cancer and ROS regulation. This wide view of the importance of ROS on p53 tumor suppressive function aspires to highlight an important side of p53 function, potentially relevant for the development of novel p53-based cancer therapy.

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Author Disclosure Statement

No competing financial interests exist.

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AU2 ▶

Abbreviations Used

AIF = apoptosis-induced factor
AKT =
ALDH4 = aldehyde dehydrogenase
APC = adenomatous polyposis coli
ARD1 = arrest-defect-1 protein
ASK1 = apoptosis signaling-regulating kinase
BAX = Bcl-2-associated X protein
CAT = catalase
Cdk = cyclin-dependent kinases
CK1 = casein kinase 1
DUSP2/5 = dual-specificity phosphatase 2/5
Dvl = disheveled
E2F1 = E2F transcription factor 1
eIF2a = eukaryotic translation initiation factor 2 a
EPO = erythropoietin
ERK = extracellular regulated protein kinases
FDXR = ferredoxine reductase
FOXO/FOXM1 = forkhead box O/M1
Fru-2,6-P_2 = fructose-2,6-bisphosphate
GAMT = guanidinoacetate methyltransferase
GCL = glutamate cysteine ligase
GLUT1 = glucose transporter 1
GPX1 = glutathione peroxidase 1
GSH = reduced glutathione
GSK-3β = glycogen synthase kinase-β
HIF1 = hypoxia-inducible factor
HIPK2 = homeodomain-interacting protein kinase-2
JNK = c-Jun N-terminal kinase
MAPK = mitogen-activated protein kinase
Mdm2 = murine double minute 2
MEF = mouse embryo fibroblast
MKP1 = MAP kinase phosphatase 1
MnSOD = manganese superoxide dismutase
mTOR = mammalian target of rapamycin
Nox = NADPH oxidase oxidase
PDK-1 = phosphoinositide-dependent kinase I
PGK1 = phosphoglycerate kinase 1
PGM = phosphoglycerate mutase
PHD = prolyl hydroxylases domain
PI3K = phosphoinositide 3 kinase
PIG12 = microsomal glutathione transferase homolog
PIG3 = quinone oxidoreductase
PIP3 = phosphatidylinositol 3,4,5 triphosphate
PKC = protein kinase C
POX = proline oxidase
pRb = retinoblastoma protein
Prx = peroxiredoxins
PTEN = phosphatase and tensin homolog deleted on chromosome ten
PUMA = p53-upregulated modulator of apoptosis
Rheb = Ras homolog enriched in brain
ROS = reactive oxygen species
SAPK = stress-activated protein kinases
SCO2 = synthesis of cytochrome c oxidase 2
SESN1/2 = senstrin family 1/2
SIP = stress-induced protein
SOD = superoxide dismutase
SMAD = ◀ AU2
TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin
TCF = T-cell factor
TGF-β = transforming growth factor-β
TIGAR = TP-53-induced glycolysis and apoptosis regulator
TP53INP1 = TP53-induced nuclear protein 1
Trx = thioredoxin
TSC1/2 = tuberous sclerosis proteins 1/2
VEGF = vascular endothelial growth factor
VHL = von Hippel-Lindau
Wip1 = wild-type p53-induced phosphatase
WNT = ◀ AU2

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AU1: Please expand WNT, IGF-BP3, LRP, and SMAD.
AU2: Please define AKT, WNT, and SMAD.
AU3: ATK has been changed to AKT. Please confirm.
AU4: Kindly check that the address of correspondence is OK as given.
AU5: Please mention the degrees of the corresponding author.
AU6: Labels are not found in Fig. 1. Please check.