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FORUM REVIEW ARTICLE

Role of MnSOD and p66shc in Mitochondrial Response to p53

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Abstract

Control of intracellular redox balance has emerged as a primary function of the p53 network, with crucial implications for tumor suppression, aging, and cell metabolism. Mitochondria are central to redox homeostasis, produce energy, and trigger apoptosis and senescence: not surprisingly, many "old" and "new" functions of p53 appear to be based in mitochondria. Genetic and biomolecular evidence indicates that generation of reactive oxygen species (ROS) in mitochondria can be a deliberate and finely regulated cell response on which signaling by environmental stressors, oncogenes, and nutrients converge. p53 orchestrates mitochondrial redox signaling by the coordinated control of at least two key effectors: the superoxide scavenger MnSOD, and the ROS generator p66shc. This review presents recent evidence and emerging questions regarding the p53–MnSOD–p66shc connection, and discusses how dissection of a circuitry comprising a tumor suppressor, an antioxidant, and a molecule regulating cell survival and mammalian lifespan can provide a framework to address important aspects related to the intricate connection between metabolism, aging, and cancer. *Antioxid. Redox Signal.* 15, 1715–1727.

Introduction

Over the last few years, the list of cellular processes and pathophysiological mechanisms that are directly or indirectly controlled by p53 (tumor protein of 53kD, TP53) has unceasingly grown, expanding from the largely established function of "guardian of genome," towards emerging fields of current biomedicine like cell metabolism function/dysfunction, stem cell biology, and aging. In parallel, massive new information has become available on the biochemical mechanisms lying upstream and downstream of p53, in the regulation and execution, respectively, of its numerous and complex biological actions (85).

While even a succinct description of these amazing advances is not in the scope of this review, it is interesting to note that a similar burst of knowledge has been experienced, in the same timeframe, by redox biology, with the novel concept that oxidant (oxygen and nitrogen-derived) species, universally known as genotoxic agents and mediators of cell damage, may also act as molecular messengers whereby signals that regulate cell proliferation and survival, migration, differentiation, and death are transduced inside the cell (40). In the midst of this ferment, it was almost unavoidable that p53 and "redox" cross each other's way.

The redox world is a strange kingdom that obeys the rule of contradiction: everything in this realm can appear black or white, good and bad in the same time, never straightforward. One may philosophically speculate that contradiction is inherent to redox systems, whereby for each molecule that is oxidized, one must be reduced. Anyway, p53 could not escape this "Yin and Yang" fate. p53 and ROS have surely emerged as a "versatile partnership"(53), but also as a very complex interplay, such that, for instance, p53 is activated, but also inhibited by ROS; and induces ROS, but also the antioxidant molecules that destroy them.

Nor is it of any help to focus, rather than on the entire issue of redox and p53, just on few actors of this plot and just one spot of the cellular stage. As in Russian nesting dolls, where one figure separates to reveal another figure of the same sort inside, in the same way complexity and bidirectionality recur, even if just considering, within the general theme of p53 and ROS, the relationship of p53 with one single antioxidant enzyme (MnSOD or SOD2) and one single prooxidant molecule (p66shc) in one single organelle, the mitochondrion. The present review focuses on this intriguing relationship, and on its potential implication in cancer and aging.

An Overview on p53 and Redox

In general terms, the relationship between p53 and the cellular redox system is biunivocal, in the sense that p53

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activity is regulated by redox-dependent signals, and in turn modulates the intracellular redox balance.

p53 response to oxidative stress is in part mediated by ROS damage to DNA that triggers a p53-dependent arrest-and-repair response. p53 phosphorylation by sensors of genotoxic damage such as ATM and DNA-PK contributes to p53 stabilization and increased transcriptional activity (85).

Phosphorylation/activation of p53 can however occur independent of DNA damage. PKCδ and p38MAPK are both redox-sensitive kinases that phosphorylate p53 in response to exogenous oxidative stress; in these cases, kinases are directly triggered by changes in the intracellular redox environment, transduced by, respectively, inactivation of tyrosine phosphatases and the dissociation of the p38 upstream kinase ASK1 (Apoptosis Stimulating Kinase 1) from its inhibitor thioredoxin (40) (Fig. 1). Additionally, AMP-activated protein kinase, a sensor of metabolic distress that activates p53, is itself sensitive to ROS and can contribute to ROS-dependent phosphorylation of p53 (42). Furthermore, hydrogen peroxide promotes p53 acetylation and p53-dependent cell death, in a fashion that can be attenuated by the deacetylase and longevity protein Sirt1 (49, 83). Interestingly, it has been reported that in cancer cells harboring wild-type p53, a significant fraction of the genes regulated by exposure to sublethal doses of oxidants are also p53 targets (23).

p53 is also subjected to direct redox regulation via oxidation of cysteine residues, organized in two clusters in the DNA binding domain; some of these residues are involved in copper coordination, and their oxidation, or copper sequestration by chelators, inhibits p53 transcriptional activity (32). Other cysteines out of this cluster (Cys 124, 141, and 182), have been found to be glutathionylated (*i.e.*, they form mixed disulphides with glutathione) under oxidative or genotoxic stress, again resulting in p53 inhibition (84). Accordingly, both the nuclear factor APE/Ref1 and thioredoxin, that maintain these residues in a reduced state in the nucleus, strongly activate p53-dependent transcription (33, 77). Of note, a similar dependence on reduced cysteines, which underlies the need of thiols in *in vitro* p53-DNA binding assays, has been previously described for "typical" redox sensitive transcription factors such as NF-κB and AP-1 (53). The meaning of this mechanism of regulation is not clear, but it may represent a feed-back signal to switch p53 off when intracellular ROS reach a critical threshold (see below); alternatively, oxidation of p53 may modulate protein shuttling between the nucleus and the cytoplasm; more attractively, oxidation of selective cysteine residues and the ensuing conformational change may shift p53 preference for different promoter regions, thereby contributing to channel p53 response towards either cell cycle arrest and DNA repair or apoptosis (14).

Accumulating evidence strongly suggests that p53 is a major regulator of cellular redox state. Initially, two independent groups reported that upon robust (adenovirus-mediated) overexpression of p53 in cancer cells, apoptosis was preceded by a significant increase of intracellular ROS (41, 72). Importantly, ROS scavengers were found to selectively inhibit p53-dependent apoptosis, with no effect on cell cycle arrest. Further biochemical analysis identified mitochondria as the likely target of p53-dependent oxidative stress, a finding also corroborated by gene profiling data indicating the upregulation by p53 of several redox-active enzyme, at least one of which, proline oxidase (PIG6) had a clear mitochondrial localization (72). Along similar lines, another mitochondrial oxidase, ferredoxin reductase (FDXR),

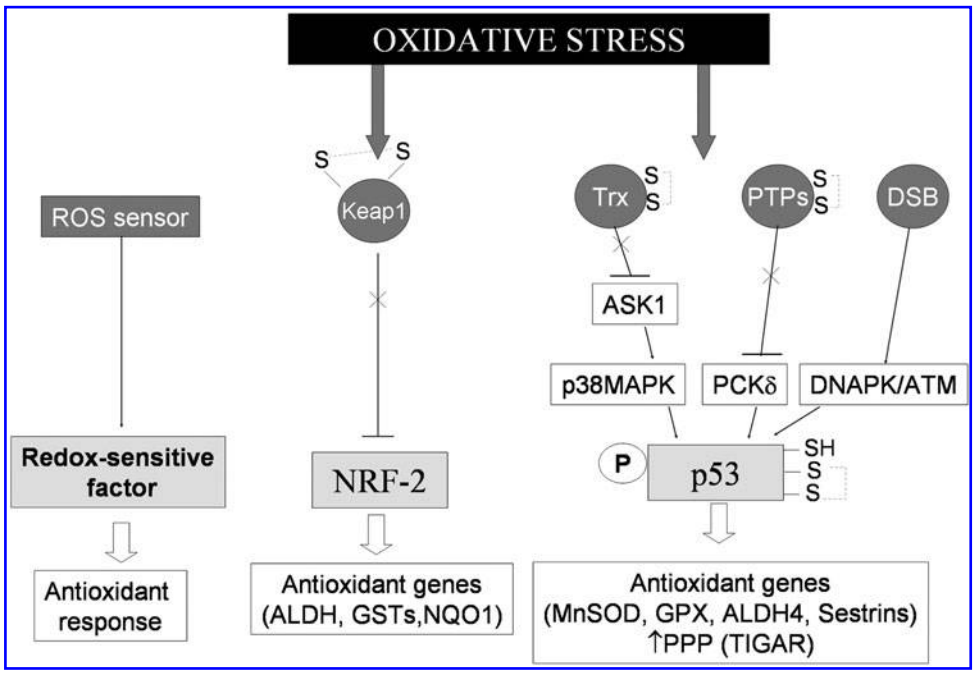

FIG. 1. p53 as an antioxidant factor. p53 antioxidant response can be viewed as a typical homeostatic circuitry. Such a circuitry requires that oxidative stress is detected by a ROS sensor, and translated into an antioxidant gene program by one or more sensor-regulated nuclear factors. A typical example is provided by the Keap-1/Nrf-2 signaling cassette, whereby oxidation-dependent dissociation of the inhibitor Keap1 allows nuclear translocation and transcriptional activity of the nuclear respiratory factor 2 (40). In the case of p53, phosphorylation/activation by p38MAPK, PKCδ, and ATM are triggered by ROS through, respectively, Trx, tyrosine phosphatases, and DNA damage (DSB = double strand breaks). Reversible cysteine oxidation in the DNA binding domain of p53, although inhibitory in general, may also fine tune the preference for some gene promoters. Redox switches upstream of p53 acetylation and sumoylation may also exist (not shown). ALDH, aldehyde dehydrogenase; GSTs, glutathione-S transferases; GPX, glutathione peroxidase; NQO1, NAD:ubiquinone oxidoreductase 1; PPP: pentose phosphate pathway.

was later reported to mediate the p53-dependent toxicity of 5-fluorouracyl on colon cancer cells (38). These findings, together with parallel evidence of ROS induction by PUMA and Bax, two other mitochondrial p53 targets, and with the fact that apoptosis *per se* generates mitochondrial ROS secondary to loss of cytochrome c (15), indicate in mitochondria a major source for oxidant species elicited during p53-dependent apoptosis.

While supraphysiological levels of p53 appear to tilt the intracellular redox balance towards a prooxidant and proapoptotic state, Sablina *et al.* surprisingly reported that in unstressed cancer cell lines or normal tissues, expressing low to normal level of p53, the p53 is essential to maintain the baseline expression of several antioxidant enzymes, including glutathione peroxidase 1 (GPX1), aldehyde dehydrogenase (ALDH4), and sestrins 1 and 2. Sestrins had been previously discovered by the same research group as a family of proteins modulated by p53 and required for the regeneration of overoxidized peroxiredoxins (Prxs) (75). Notably cellular levels of prooxidant p53 targets, like PUMA or Bax, were not increased by expression of low amounts of p53. In keeping with these findings, knock-down of endogenous p53 in these experimental systems was accompanied by an increase, rather than by a reduction, of intracellular ROS. These findings highlight a previously unsuspected antioxidant function of physiological levels of p53, which may contribute to tumor suppression by preventing oxidant-dependent DNA damage (75).

These observations have been confirmed and extended by Alice Carrier and colleagues, who identified the TP53 Induced Nuclear Protein 1 (TP53INP1, Inp) as one of the principal players in the antioxidant function of p53 (16). In fact, although no direct enzymatic activity has yet been demonstrated for this molecule, expression of TP53INP1 in p53-deficient cells lowers basal ROS to the level found in p53-proficient cells, while Inp -/- cells display elevated ROS and an antioxidant (N-acetyl-cysteine)-sensitive increase in cell proliferation. In addition, TP53INP1 deficiency increases oxidative stress-related lymphoma incidence and decreases survival of p53(+/-) mice, demonstrating that TP53INP1 antioxidant activity is relevant to tumor suppression by p53.

A further element of complexity in the interplay between p53 and the intracellular redox environment has emerged from recent breakthroughs on the physiological role of p53 in regulating cellular metabolism (86). p53 appears to promote mitochondrial respiration at the expense of glycolysis. This action, which is perfectly coherent with the Warburg effect [*i.e.,* with the fact that malignant cells (in which p53 is often lost or inactive) tend to use glycolysis instead of respiration even in the presence of oxygen] is in part due to the fact that p53 is needed for the optimal assembly of the mitochondrial respiratory chain. In particular, synthesis of cytochrome oxidase 2 (SCO2), encoding a molecule necessary for the import of the subunit II in the cytochrome oxidase complex, was identified by Hwang and colleagues as a target gene of p53; this molecule's concentration is critically decreased in the muscle of p53-/- mice, leading to reduced oxygen consumption and resistance to endurance in these animals (54). In addition, TIGAR (TP53-induced glycolysis and apoptosis regulator), another p53 target, inhibits glycolysis by reducing the cytosolic levels of fructose 2–6 bisphosphate, a potent allosteric activator of phosphofructokinase. Interestingly, these two effects, while cooperating in shifting the energy metabolism from glycolysis to respiration, are anticipated to modify the redox balance in opposite directions; in fact, while increased respiration leads in general to increased generation of oxygen species (4), ROS are actually downregulated by overexpression of TIGAR (8), due to glucose shunting toward the pentose phosphate pathway (PPP) and the increased generation of NADPH, a source of reducing equivalents that feeds major antioxidant buffers glutathione (GSH) and the thioredoxin/thioredoxin reductase system (40). Of note, metabolic actions of p53 appear, like the antioxidant ones, to be constitutive rather than stress-related.

Opposite effects of p53 on the intracellular redox state are not surprising since other examples exists of p53 exerting diverse responses depending on the intensity and persistence of the cellular stress p53 is triggered by (6, 75). The activation of an antioxidant program by low levels of p53 perfectly outlines a typical homeostatic circuitry, whereby oxidative stress, as detected by cellular redox sensors (in this case one of the many redox sensitive cascades leading to p53 stabilization/activation) activates a transcriptional regulator to increase antioxidant defenses and prevent further damage to DNA or other cellular components (Fig. 1). The p53-dependent antioxidant effect would therefore be part of a pro-survival, repair response to a nonlethal damage (6) [*i.e.,* a typical "caretaker" response (46)]. Instead, following robust and persistent genotoxic stress, prooxidant effects would dominate, due, in part, to the induction of prooxidant enzymes (some of which, like proline oxidase, are located in mitochondria), in part as a general consequence of mitochondrial permeability transition and loss of cytochrome c from the electron transport chain (ETC) (15).

It should be emphasized, however, that oxygen radicals not only mediate cellular damage, but also transduce intracellular signaling by growth factors, cytokines, hormones, and extracellular matrix (ECM) proteins (73). In particular, a reduction of cytosolic ROS occurs in cells subdued to antiproliferative conditions such as contact-inhibition (67) or cell detachment from the substrate (19), and antioxidants slow down the cell cycle in most cell models (35). It is thus tempting to imagine that p53-initiated antioxidant response may also contribute to cell cycle arrest, a crucial p53-dependent response, by downregulating ROS-mediated growth factor signaling (Fig. 2). Although in part speculative, this possibility is supported by the finding that p53 inhibits mTOR, a major effector of growth factor signaling, through sestrins, although a redox mechanism for this action has not been demonstrated (12). Most intriguingly, increased proliferation has been reported in fibroblasts lacking TP53INP1, the principal effector of p53 antioxidant function (16). In this perspective, p53 antioxidant action would participate in tumor suppression not only through a "caretaker" mechanism, by limiting DNA damage, but also as part of p53 "gatekeeping" activity (*i.e.,* by directly impinging on cell proliferative capacity), where growth arrest can be instrumental to genetic repair, or aimed at counteracting deregulated mitogenic signaling by activated oncogenes.

MnSOD: A Mitochondrial Antioxidant (?)

MnSOD (manganese-dependent superoxide dismutase, or SOD2) is a homotetrameric enzyme located in the mitochondrial

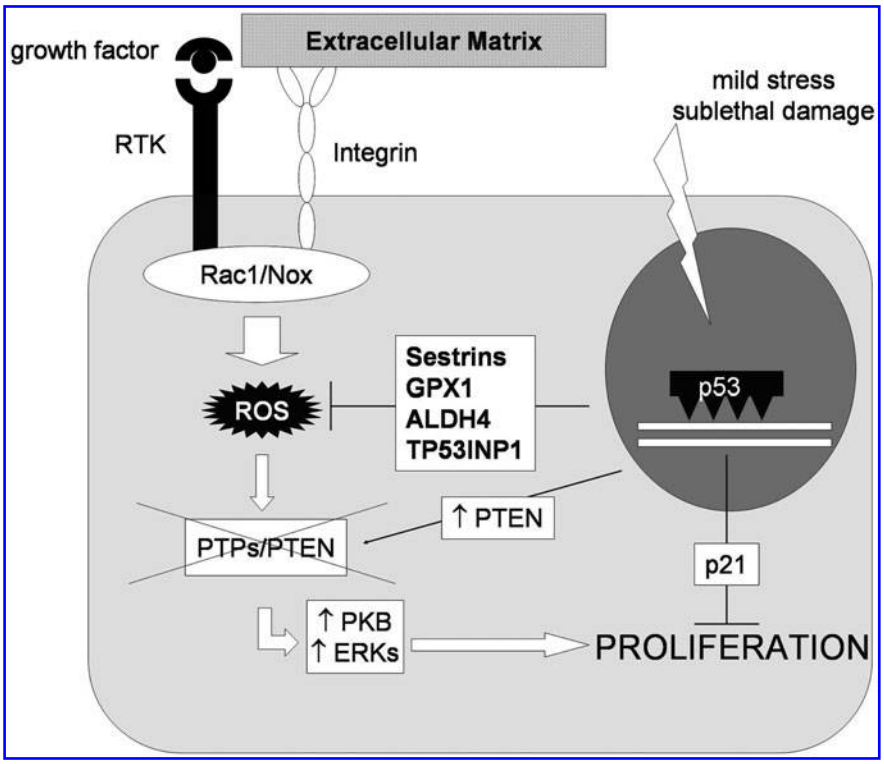

FIG. 2. p53-dependent antioxidant response likely contributes to cell cycle arrest. Induction of antioxidant enzymes by p53 is generally thought to prevent further oxidative damage to DNA. In a broader view, and in light for emerging roles of ROS in mitogenic signaling by growth factors and adhesion molecules, reduction of intracellular ROS may also contribute to arrest cell proliferation under sublethal cell damage. In this model, p53-induced antioxidants block ROS generated by RTK and adhesion molecules through Rac1 and NADPH oxidase (NOX), thus preventing oxidative inhibition of phosphotyrosine phosphatases (PTPs). Thus, protection of phosphatase activity, together with increased expression of PTEN, may reinforce, at an upstream level, the antimitogenic effect of the CDK inhibitor p21. ECM, extracellular matrix.

matrix where it catalyzes the dismutation of superoxide anion generated by one-electron reduction of molecular oxygen during respiration (28). Since MnSOD converts one oxygen radical, superoxide, into another reactive species, H_2O_2, its classification as an antioxidant enzyme is problematic, the net effect on cellular redox balance being strictly dependent on the coordinated action of peroxide scavengers located in the organelle (such as peroxiredoxin V and GPX) and in the cytosol (other peroxiredoxins, catalase).

Several aspects make MnSOD a rather unique piece of the intracellular redox puzzle.

First, MnSOD, unlike other mammalian antioxidant enzymes including superoxide dismutases 1 and 3, is a strongly inducible and finely regulated molecule. Interestingly, intracellular levels of MnSOD are responsive, even more than to oxidative stress *per se*, to cytokines and proinflammatory molecules (89), growth factors (5), and nutrients (48). Major transcriptional regulators downstream of these signals include NF-κB (90), SP-1, cAMP responsive element binding protein (CREB) (44), the forkhead family of transcription factors (48), PGC-1 alpha (79), HIF 2 alpha (76), and others including, of course, p53 (see below). Induction of MnSOD usually exerts a protective effect under the most diverse stressful conditions, most likely by interrupting the mitochondrial vicious circle whereby initial damage to the organelle promotes release of cytochrome c, which in turn determines electron leak from the ETC, formation of superoxide, and further mitochondrial damage (15). Accordingly, MnSOD upregulation promotes cell survival in response to TNF (88), anticancer drugs, neurotrophins (5), hyperglycemia (62), and mitochondrial poisons (45), to enumerate only a few of the reported examples. Additionally, MnSOD, which is downregulated in actively proliferating cells by the growth factor- and oncogene-mediated inhibition of the FoxO factors (48, 55), is induced by the same factors in quiescent cells, thereby providing protection from oxidative stress when PI3 kinase/Akt-dependent survival signals, normally activated by mitogens, are off-duty (13). The crucial importance of MnSOD is most relevantly underscored by the severe phenotype of mice homozygous for a MnSOD *null* allele, that die early after birth by dilated cardiomyopathy and systemic acidosis due to generalized mitochondrial failure (52), and by the milder but significant mitochondrial defects observed in adult heterozygous MnSOD +/− animals (87).

Another exceptionally interesting aspect of MnSOD biology is its altered expression in cancer cells. Early observations on the reduced expression of this enzyme in highly proliferating cells (63), together with the evidence of this enzyme being very low in some liver experimental tumors (10) and in human melanomas (20), have initially led to consider MnSOD as a potential tumor suppressor gene (11), and a loss of its expression, by genetic or epigenetic mechanisms (36), instrumental to carcinogenesis. This idea was also supported by mechanistic studies showing reduced experimental skin carcinogenesis in transgenic mice overexpressing MnSOD (93), as well as reduced proliferation of cultured cell lines genetically manipulated to express high amounts of the enzyme. Interestingly, in a few cases where the latter effect was further investigated, accumulation of hydrogen peroxide, presumably generated in excess with respect to cell scavenging capacity, emerged as the mechanism underlying MnSOD growth suppressive activity (51).

In keeping with this evidence, the finding that a MnSOD polymorphic variant affecting the mitochondrial signal peptide (Val-9Ala) was associated with breast carcinoma risk [and also with risk for prostate cancer (2, 43)] in combination with a diet poor in antioxidants, was also interpreted as evidence of a tumor suppressive activity of the enzyme, although

the real impact of this variant on enzyme import into mitochondria has not been unequivocally determined.

On the other hand, the widely documented activity of MnSOD as a survival protein suggests that this molecule may, under some circumstances, help cancer cells cope with their hostile environment and resist therapy, although maybe at the expense of a limited proliferative capacity. In keeping with this concept was the finding that the MnSOD content, while often reduced in many neoplasms, was instead strongly upregulated and had a negative prognostic value in some malignancies, like cervical carcinomas (59), gliomas (50), and colon carcinomas (39). Intriguingly, all these malignancies are known to harbor at high frequencies of alterations of the p53 tumor suppressor pathway.

p53 and MnSOD: "Ups and Downs" in Tumor Suppression

The functional correlation between MnSOD and p53 is probably the best exemplification of how complex and multifaceted the interplay between ROS and the p53 tumor suppressor pathway can be. Prompted by the frequent association between p53 accumulation (an immunohistochemical hallmark usually related to p53 mutation/inactivation) and elevated expression of MnSOD in human tumor samples (59, 74), our research group was the first to address the possible mechanistic link between these two events (65). Initial experiments on fibroblastoid cell lines from p53 wild-type or p53-/- mice, transformed *in vitro* by retroviral transduction of V12Ras and the adenoviral oncogene E1A, revealed in malignant fibroblasts lacking p53 a significantly higher expression of MnSOD and in parallel, a remarkable resistance to oxidant-dependent death triggered by serum deprivation, paraquat, or the anticancer drug Adriamycin. Moreover, overexpression of p53 in HeLa cells led to a reduction of MnSOD content as mRNA, protein, and activity, while overexpression of MnSOD significantly rescued HeLa cells from p53-induced replicative death. Importantly, elevated levels of MnSOD were also found in the liver of p53 -/- mice, indicating that negative control of MnSOD expression by p53 also occurs in normal tissues and thus in the presence of physiological amounts of this tumor suppressor (65).

These findings were largely confirmed by Drane and colleagues (27), who reported transcriptional downregulation of MnSOD by p53 in MCF-7 breast carcinoma cells, and protection from p53-induced cell death by overexpression of MnSOD in the same cell line. Additionally, these authors provided evidence for a reciprocal control between p53 and MnSOD, since forced expression of MnSOD led to a reduction of p53 mRNA. Collectively, results from these two studies clearly indicated that MnSOD downregulation is instrumental to induction of mitochondrial oxidative stress and apoptosis by p53, and by extension that elevation of MnSOD may counteract p53 activity and thus directly contribute to carcinogenesis.

A possible molecular basis for the negative control of MnSOD expression by p53 was proposed by Dhar and St Clair (24), who found that in human hepatoma HepG2 cells p53 is complexed with the SP-1 transcription factor at the MnSOD promoter, and that such interaction inhibited SP-1 dependent basal transcription of *MnSOD*. Again, knock-down of p53 increased MnSOD levels, indicating that "normal" levels of p53 are effective in MnSOD inhibition. Importantly, this model does not require the presence of a p53 binding site on the *MnSOD* promoter, a consensus sequence present in the human but not in rodent MnSOD genes (24).

Observations reported by Chathoo and colleagues on primary neurons (17) have extended the biological significance of MnSOD control by p53 from cancer to neurodegeneration, confirming that the presence of p53 on MnSOD promoter is associated with reduced expression of the enzyme, likely by recruitment of transcriptional repressors HDAC1 and N-CoR. In this cell model, inhibitory action of p53 on *MnSOD* (and other antioxidant genes) is restrained by the polycomb group protein and oncogene BMI1; accordingly, MnSOD levels are reduced, in a p53-dependent manner, in BMI1 -/- neuronal cells. Intriguingly, the same group has reported that CD133+ glioblastoma multiforme (GM) stem cells display elevated levels of BMI1(1); MnSOD levels are often elevated in GM (50), and, although not proven yet, such upregulation may involve BMI1 and contribute to BMI1-dependent increase in tumor "stemness" and malignancy.

In apparent contradiction with the above studies reporting MnSOD transcriptional repression by p53, Hussain and Harris identified, in an unbiased microarray screen, MnSOD as a p53-induced gene in human lymphoblasts, and confirmed their observation on normal and Li–Fraumeni syndrome (*i.e.*, p53-defective) human fibroblasts (37). They also found that MnSOD was co-induced with glutathione peroxidase (GPX1) but not with catalase, and concluded that hydrogen peroxide generated by MnSOD, insufficiently balanced by GPX, may contribute to p53-dependent apoptosis. Curiously, then, MnSOD upregulation was interpreted in this case as part of a pro-, rather than antioxidant response to p53 (37).

More recent work by Daret St Clair's group seems to have partially cleared this apparent contradiction (25). These authors found that re-expression of p53 into the mouse p53 -/- epithelial cell line PC3 modulated MnSOD gene in two opposite directions: low levels of p53 (*i.e.*, in the context of the "repair response") induced the enzyme, while high levels repressed its expression, presumably as part of a pro-oxidant and pro-apoptotic response. Accordingly, irradiation of another p53-proficient tumor cell line led to either MnSOD upregulation or downregulation according to the UVB dose. This model fits the scheme proposed by Sablina and colleagues (75), but at a higher level of complexity, since, in this case, p53 operates in opposite directions on the same gene (*SOD2*), through distinct molecular interactions. In particular, low levels of p53 appear to promote NF-κB (p65) binding to an intronic enhancer element of MnSOD, thus favoring its expression; but then at higher levels of p53, inhibitory interaction of the protein with the basal transcription factor SP-1 dominates, leading to reduced MnSOD transcription. Interestingly, increased expression of the NF-κB subunit p65 can overcome the suppressive effect of p53 on MnSOD expression, even when p53 is present at high levels (25).

One possible interpretation of these finding is that MnSOD is involved, unique among the other enzymes, in both pro- and antioxidant actions of p53, according to stress intensity and cell fate. However, mitochondrial compartmentalization of MnSOD and its capacity to generate hydrogen peroxide raise some unanswered questions. In the experience by others and ourselves, cellular ROS, as measured by the popular

fluorescent dye DCF-DA, are influenced by MnSOD in a nearly unpredictable way. This dye is in fact mostly sensitive to peroxides, and several reports indicate actually an increase of DCF-DA fluorescence in tumor cell line overexpressing MnSOD (51). Conversely, we have reported that elevated levels of MnSOD do not lead to major changes in overall cellular ROS in E1A/Ras transformed fibroblasts lacking p53 (65), while Dhar and St. Clair presented evidence that MnSOD knockdown elevates intracellular ROS in p53-deficient PC3 cells (25). However, these oxidants are unlikely to be superoxide, which is poorly detected by DCF-DA. Although it has been suggested that superoxide dismutation by MnSOD produces half the H_2O_2 compared to spontaneous, nonenzymatic dismutation, thus leading to an overall decrease of intracellular peroxide (80). This point needs to be further investigated and clarified.

Another issue, raised by the study of Dhar and St. Clair, deals with the lack of information on whether the "low" and "high" levels of p53 experimentally established by the authors actually correspond to a decrease and increase of intracellular ROS, respectively, as in the model proposed by Sablina *et al.* (75). This information is necessary to fit MnSOD in the two-state model (low p53 = antioxidant; high p53 = pro-oxidant) of p53 and ROS. Moreover, in at least three studies (17, 24, 65), absence of p53 in nonstressed cells/tissues leads, unlike for other antioxidants, to upregulation of MnSOD, a finding in conflict with the above "bidirectional" scheme. Thus, especially in light of the results by Hussain *et al.* showing overall cellular oxidation at the levels of p53 that induce MnSOD(37), the real role of MnSOD in the pro/antioxidant activity of p53 remains far from settled.

Notwithstanding these limitations, some unique aspects of the interplay between p53 and the mitochondrial MnSOD appear to be of high biological relevance.

One important implication of this interplay, is, of course, the fact that upregulation of MnSOD, a general antiapoptotic factor, may strongly contribute to resistance to senescence and apoptosis (and by extension to cancer chemo- and radiotherapy) displayed by p53 -/- cells; this has endless ramifications connecting to both tissue aging and oncogenic transformation (78), as well as to cancer therapy (68). For instance, as discussed below, ethanol intoxication, a condition characterized by abundant generation of mitochondrial ROS and thus reminiscent of an accelerated aging process, fails to induce hepatocyte apoptosis in the liver of p53 -/- mice likely due to increased levels of liver MnSOD, but leads in these mice to the early appearance of liver dysplasia (69).

Another interesting aspect of the p53/MnSOD relationship deals with the largely established evidence that MnSOD protects from p53-dependent apoptosis (25, 27, 65). Since a compartmentalization appears to exist in the redox activity of p53, whereby antioxidant function appears to be mainly cytosolic and nuclear, and the prooxidant one mostly centered in mitochondria (66), it is not too stretched to think that MnSOD may play a crucial role in cell decision-making towards repair/survival or death following p53 activation. In other words, the level of MnSOD may, rather than regulate the overall redox environment of the cell, set the threshold at which p53 activity turns from protective to proapoptotic. This idea is also in keeping with the finding that mitochondrial translocation of p53, an event heralding apoptosis, inhibits MnSOD (92) in a post-translational fashion (see below).

Inflammation promotes cancer in several clinical and experimental settings (22). Based on the finding of Dhar *et al.*, whereby NF-κB activation effectively counteracts MnSOD downregulation by p53 (25), an intriguing tumor-promoting circuitry can be envisaged whereby initiated cells bearing activated oncogenes, and thus bound to p53-dependent death or senescence (78), rather survive and further progress to malignancy due to NF-κB-dependent upregulation of MnSOD operated by factors and cytokines present in the inflammatory *milieu* (Fig. 3). Although speculative, this model could be tested in humans, for instance, by analyzing *SOD2* polymorphisms in the context of chronic inflammatory diseases and inflammation-related cancer.

p66: The Lesser The Better

p66shc was initially discovered as a long splice variant of the Src homology and collagen (Shc) adapters (molecules

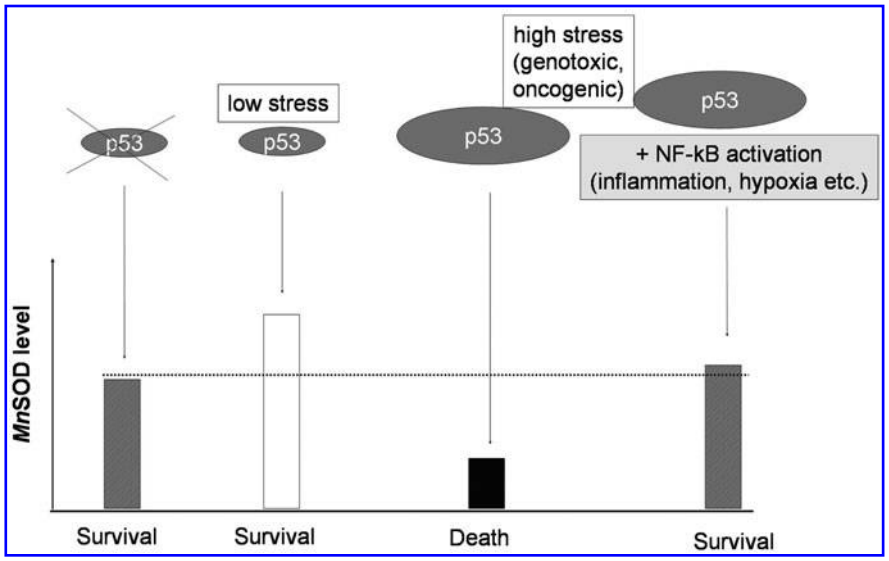

FIG. 3. Transcriptional regulation of MnSOD by p53 as a function of oxidative stress intensity and the inflammatory microenvironment. Elevated expression of MnSOD in the absence of p53 promotes cell survival; low levels of p53, as observed in normal, unstressed tissues, may further increase MnSOD levels, in the context of a general antioxidant response. However, during carcinogenesis, DNA damage and oncogenic signaling downregulate MnSOD thorough a robust activation of p53, thus promoting senescence or apoptosis. Environmental stimuli such as inflammatory cytokines and hypoxia can counteract MnSOD inhibition by upregulating NF-κB, thus allowing survival and expansion of cells harboring activated oncogenes. The *dotted line* indicates MnSOD baseline expression in the absence of p53.

connecting activated tyrosine kinase receptors to the Ras pathway) and received little attention until the late 1990's, when it was almost serendipitously observed that mice genetically deficient of this specific isoform (these mice were originally generated for cancer studies), lived significantly longer (30%) than their wild-type controls (56). This was indeed the first mutation leading to long lived and healthy (*i.e.*, not dwarf nor sterile) individuals ever described in mice and mammals in general.

In perfect agreement with Harman's free radical theory of aging that posits that chronic oxidative damage underlies tissue aging and limits lifespan (34), p66shc-/- mice appeared less prone than controls to systemic oxidative stress, and their cells remarkably resistant to oxidant-induced cell death *in vitro*, a phenotype somehow reminiscent of that conferred by p53 deficiency (56).

Moving from this evidence, Trinei and colleagues demonstrated that p66shc content is increased by p53 in oxidant-stressed cells, and is necessary for p53-dependent generation of ROS and cell death under the same experimental conditions (81). Interestingly, p66shc induction by p53 is not transcriptional, but rather due to increased stability of the p66 shc protein, which may explain why p66shc had not been previously identified in any systematic search for p53 targets (72).

Further characterization of the molecular cascade leading to generation of ROS by p66shc led to a number of surprising observations. A small fraction of p66shc, in fact, appears to translocate from the cytosol to mitochondria, where, as revealed by sophisticated micropotenziometric measurements, it directly transfers electrons from cytochrome c to molecular oxygen, thus generating hydrogen peroxide (29). Mitochondrial translocation of p66shc has been investigated in detail: PKC β, a protein kinase activated by oxidative stress, phosphorylates p66shc on serine 36, allowing its interaction with the, prolyl isomerase pin-1, which then physically translocates p66shc across the outer mitochondrial membrane (71). Thus, oxidative stress triggers p66shc phosphorylation and localization to mitochondria, where the molecule then generates hydrogen peroxide to initiate the apoptotic cascade. Several reports indicating resistance of p66-/- mice to a number of oxidative stress-related experimental pathologies (diabetic nephropathy, atherosclerosis, hindlimb ischemia, and alcohol-related liver damage) are consistent with this elegant model (21, 47). Even more interesting, lack of p66shc appears to dissociate p53 prooxidant and proapoptotic response from cell cycle arrest and other tumor suppressive functions. In fact, p66shc -/- mice are not prone to spontaneous or experimentally induced carcinogenesis, indicating that residual antioncogenic functions of p53 (including probably the induction of antioxidant enzymes), are effective in this mouse model (81).

An alternative mechanism to link p66shc with oxidative stress, cell death, and organismal aging has been proposed by Nemoto and Finkel (61). These authors demonstrated that p66shc promotes the redox-dependent inactivation of FOXO3a, a member of the *Forkhead* transcription factor family that is regulated by insulin and operates as a positive genetic determinant of longevity throughout the evolutionary scale (30). Importantly, p66shc-dependent inactivation of FOXO3a requires ROS and results in the reduced FOXO3a-dependent

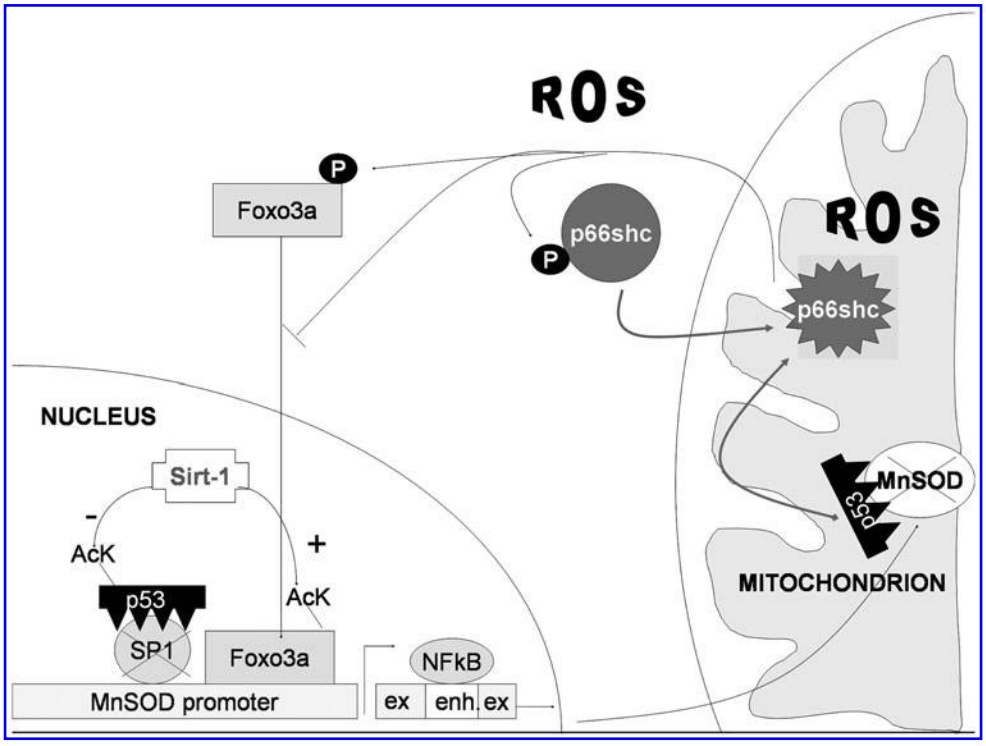

FIG. 4. A p53–MnSOD–p66shc network controls the level of mitochondrial ROS. In response to apoptogenic stimuli, p53 inhibits MnSOD through at least two distinct mechanisms: a) by preventing SP-1 binding to the MnSOD promoter; b) by translocation to mitochondria and interaction with MnSOD. p53 also increases, through mechanisms not yet clarified, the expression of p66shc. p66shc generates ROS (hydrogen peroxide) that can cross the mitochondrial membrane and inactivate FoxOs by phosphorylation-dependent nuclear exclusion, thus further decreasing MnSOD expression. Note that p66shc phosphorylation and translocation to mitochondria is in turn increased by oxidative stress. This self-amplifying cascade is counteracted by Sirt1 that inhibits p53 and activates FoxO3a by deacetylating the two factors in the nucleus. Other p53-dependent sources of mitochondrial ROS distinct from p66shc are not indicated for simplicity. In the SOD2 promoter, *enh* indicates an intronic enhancer that binds NF-κB.

expression of ROS scavengers catalase and MnSOD (48, 61). In other words, p66shc translates lethal oxidative stress into a genetic program that leads to reduced antioxidant defenses and further mitochondrial damage. Although somehow circular, this model has the merit of incorporating in one single circuitry three previously unrelated determinant of longevity (*i.e.*, forkhead proteins, p66shc, and antioxidants/ROS; Fig. 4). Moreover, since p53 induces p66shc, the p66-FOXO axis may contribute to MnSOD downregulation by p53.

Along similar lines of investigation, Burgering and colleagues have shown that oxidative stress induces a FOXO3a-dependent antioxidant response that includes MnSOD, and requires that this factor is deacetylated by Sirtuin 1, another genetic determinant of longevity in model organisms and in mammals (30, 82). Interestingly, sirtuins control also the p53 pathway, since Sirt1-dependent deacetylation inhibits p53 and attenuates p53-dependent apoptosis (83).

Thus, diverse aging-related molecular players and signaling pathways appear to regulate, at different levels, mitochondrial generation of ROS and the level of mitochondrial antioxidant defenses. These multiple interactions are obviously relevant to cellular response to p53, and by extension to the intricate connection between p53, aging, and cancer.

Genetics and Mitochondrial Pro/Antioxidants. Alcohol as a Model

Studies on alcohol-dependent liver damage performed by our research group have contributed to test the relevance of the p53–MnSOD–p66shc axis in a human disease-related context. Ethanol intoxication leads to a rise of mitochondrial ROS, in part due to increased availability of reduced NADH that is reoxidized by mitochondria, in part by largely unknown molecular mechanisms leading to electron leak, mainly at the level of the respiratory Complex I; (3) with this respect, ethanol recapitulates, in an amplified fashion, the mitochondrial oxidative derangement proposed by Harman as the "clock" of aging (34). Our studies initially showed that alcohol-induced hepatotoxicity involves p53, and that livers of p53 -/- mice display reduced levels of apoptosis induced by ethanol. In keeping with the notion that escape from apoptosis promotes malignant transformation, however, we found that ethanol consumption leads in p53 -/- mice to the early appearance of dysplastic changes, heralding liver carcinogenesis (69). These changes correlated with elevated levels of MnSOD in the liver of p53-/- mice.

p66-/- mice fed with ethanol in the drinking water were also resistant to alcohol-induced liver pathologic changes (mainly steatosis), compared to their p66shc-normal controls; again, resistance correlated with a higher expression of MnSOD in the liver tissue, and in isolated hepatocytes exposed to ethanol *in vitro*. However, unlike in the p53-/- model, p66shc-/- mice did not develop liver cancer following alcohol treatment, confirming that tumor suppressive capacity of p53 is largely preserved in the absence of p66shc. An important corollary of this finding is that MnSOD, that likely contributes to ethanol-induced carcinogenesis in p53-/- mice by preventing apoptosis of alcohol-initiated cells, is obviously not sufficient to sustain hepatocyte transformation in the context of p66shc absence.

In the same study, biochemical analysis of human hepatoma HepG2 cells engineered to overexpress p66shc (HepG2–p66) allowed us to confirm that p66shc negatively regulates the expression of MnSOD at a transcriptional level, and that this effect is mediated by phosphorylation/inhibition of FOXO3a. Accordingly, in HepG2–p66 cells, mitochondrial generation of ROS in response to ethanol was increased, and paralleled by a more rapid and dramatic drop of the mitochondrial potential. These and other observations (31) thus support the relevance of the p66shc–FOXO3a–MnSOD cascade in pathophysiological conditions sustained by mitochondrial accumulation of ROS.

p53, p66shc, and MnSOD: How to Make ROS in Mitochondria

The pro-oxidant effects of p53 in mitochondria involve much more complicated molecular interactions than it was probably thought at the beginning. We have recently proposed that p53, p66shc, and MnSOD coordinately regulate mitochondrial oxidative stress (70). In this model (Fig. 4), high proapoptotic levels of p53 induce p66shc (81), thus favoring H_2O_2 generation at the ETC (29); peroxide may diffuse to cytosol where it triggers the redox-dependent inhibition of FOXO3a (61), leading to reduced expression of ROS scavengers including MnSOD (47, 48), and to further oxidative stress. In parallel, p53 reduces MnSOD gene transcription by inhibiting SP-1, but acts also on MnSOD protein by binding to the enzyme in mitochondria (92). This pro-oxidant network is counteracted by Sirt-1 that increases MnSOD levels in two ways: by deacetylating and inhibiting p53 (83), and by directly activating FOXO3a (82). Of note, this action may contribute to the evolution-conserved capacity of Sirt1 to delay aging (4).

While consistent with much published evidence, this model raises several questions. How does p53 act on p66shc? Do these two proteins physically interact? Since one fraction of p53 translocates to mitochondria as an early event in the apoptotic cascade (92), it is attractive to speculate that p53 may increase p66 levels by binding to it in the organelle; interestingly, both p53 and p66 are phosphorylated by PKC (71, 85) and interact with the prolyl isomerase Pin1 (71, 91). Does Pin1 facilitate the p66–p53 *rendez-vous* in mitochondria? Since p53 does bind and inhibit MnSOD, a trimolecular interaction would not be too surprising. A much simpler alternative is that p53 activates p66shc by a redox mechanism (*i.e.*, through p53 capacity to elicit ROS); ROS facilitate p66shc translocation to mitochondria (71), but may also stabilize the protein. This possibility implies that p66shc does not account for the entire prooxidant capacity of p53 (72).

The relative importance of p66shc in p53-dependent generation of ROS is indeed another burning question. The finding of Trinei and colleagues that p53-induced ROS are nearly abolished in p66 -/- cells (81) is difficult to reconcile with a role for other p53-induced prooxidant mitochondrial factors such as proline oxidase or ferredoxin reductase. One possibility is that p66shc operates also upstream of p53 (*i.e.*, contributes to its redox activation under oxidative stress and indirectly favors the p53-dependent expression of other prooxidant enzymes). In this respect, it is interesting that blockade of ROS after p53 translocation to mitochondria decreases p53 migration to the nucleus (92), suggesting that a "first wave" of mitochondrial ROS may be instrumental to the triggering of a full-blown p53 prooxidant response. If so, p66shc and MnSOD inhibi-

tion (through the FoxO pathway) may have a critical role in this event.

Finally, how relevant is direct generation of ROS *versus* inhibition of antioxidant scavenger in the pro-oxidant activity of p66shc? While both mechanisms are probably true and operate in a coordinated fashion, it should be noted that genetic ablation of p66shc does not rescue early lethality of MnSOD -/- mice (Pani G, unpublished); this finding, while excluding a significant contribution of p66shc to the severe consequences of complete MnSOD deficiency, may also suggest that the "antioxidant" effect of p66shc deletion requires, as a downstream event, the upregulation of MnSOD, and is thus ineffective in the absence of this enzyme.

Clarification of these questions promises to complete our picture of how p66 and MnSOD contribute to mitochondrial oxidative and apoptotic responses to p53.

Conclusive Considerations—p53, Aging, and Cancer: All About Energy?

Deliberate generation of ROS in mitochondria certainly represents an effective strategy to trigger apoptosis (57). An interesting point is whether this represents the main "function" (if any) of mitochondrial ROS; recently discovered metabolic roles of p53 may in fact suggest a different interpretation (7). We know that p53 promotes mitochondrial respiration (54) and that this function can be stimulated by the AMP kinase, a sensor of cellular de-energization (42). Another metabolic sensor, Sirt1, regulates p53 function, and inhibits its activity when intracellular NADH (*i.e.*, the main mitochondrial fuel) is low, and NAD+ high (83). If stimulation of respiration in abundance of nutrients or under ATP shortage represents a main "housekeeping" function p53 function, then generation of mitochondrial ROS by p53 may have originally been just the by-product of a "metabolic burst", and induction of antioxidants likely a homeostatic strategy to circumvent metabolic oxidative stress (75). This property of p53 may have subsequently been incorporated into a proapoptotic response. Following this line of thinking, we learn that p66shc also appears to stimulates mitochondrial metabolism (60). Additionally, p66-generated ROS regulate insulin signaling (9). Is this also true for p53? Both p53 (58) and p66 (72a) exacerbate insulin resistance in the context of obesity and nutrient overload, indicating that mitochondrial ROS may represent a general feed back signal to regulate nutrient signaling.

The idea that p53 and p66 translate nutrient availability into mitochondrial generation of oxygen species has again important implication for cancer, aging mechanisms, and stem cell biology. Oncogenic transformation strongly activates p53 up to levels certainly compatible with its pro-oxidant and proapoptotic functions (64, 78). Preference for glycolysis may thus represent a cancer cell strategy to avoid excess prooxidant activity by p53. Interestingly, glycolysis is typical of both normal and cancer stem cells, together with low intracellular ROS and elevated MnSOD content (26). Thus, inactivation of p53 may promote cancer cell stemness, in part, by lowering metabolic activity and ROS in mitochondria.

Seen from a different angle, under the selective pressure of a pro-oxidant environment as is typical of senescent tissues, loss of "normal" stem cells may promote the expansion of stem cells with reduced mitochondrial ROS and elevated MnSOD (Fig. 5); these cells are likely to have defects in the p53 pathway, and may easily evolve to cancer, thus explaining why age is the main risk factor for malignancies. Evidence that excess nutrients accelerate proliferation and exhaustion of hemopoietic stem cells via ROS (18), while instead calorie restriction prolongs mammalian lifespan (4), obviously support these speculative views.

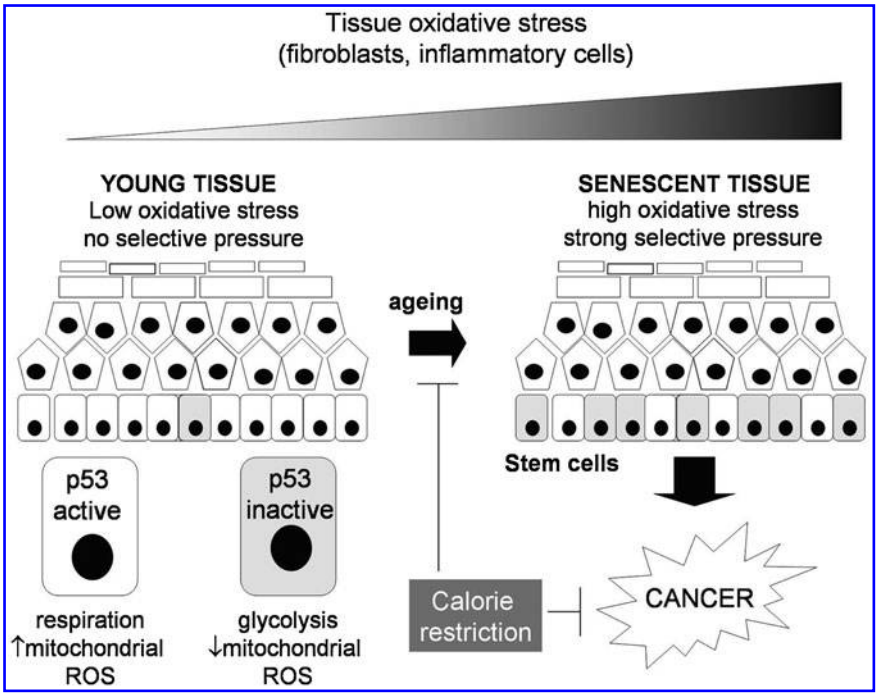

FIG. 5. p53-regulated mitochondrial ROS link cell metabolism, tissue senescence, and cancer. In a young epithelial tissue with a low level of oxidative stress, cell renewal is normally maintained by "normal" stem cells, with little selective advantage for p53-mutated cells. In a senescent tissue, exhaustion of normal stem cells (*white rectangles*) and an increased oxidative pressure favor cells with low respiratory rate and higher mitochondrial antioxidants that are likely to have an inactive p53 pathway (*gray rectangles*). Although resistance to oxidative stress and to mitochondrial damage is not *per se* sufficient to cause cancer (as demonstrated by normal tumor incidence in p66shc-/- mice), positive selection of p53-deficient cells in this context leads to increased probability of malignancy. Calorie restriction may prevent both aging and cancer by reducing the mitochondrial oxidative burden.

No article can cover all the possible implications of the redox and metabolic properties of p53. Yet, just one part of the story (like the one regarding the p53–p66–MnSOD "connection") is probably sufficient to communicate the sense of both an overwhelming complexity and a slow but exciting disclosure. Time will tell whether interpreting the p53 network from a redox standpoint will help to clear contradictions or change current paradigms on this fascinating molecule; big questions surely will not change aging, cancer, and how to fight them.

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References

1. Abdouh M, Facchino S, Chatoo W, Balasingam V, Ferreira J, and Bernier G. BMI1 sustains human glioblastoma multiforme stem cell renewal. *J Neurosci* 29: 8884–8896, 2009.
2. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, and Shields PG. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res* 59: 602–606, 1999.
3. Bailey SM and Cunningham CC. Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. *Free Radic Biol Med* 32: 11–16, 2002.
4. Balaban RS, Nemoto S, and Finkel T. Mitochondria, oxidants, and aging. *Cell* 120: 483–495, 2005.
5. Bedogni B, Pani G, Colavitti R, Riccio A, Borrello S, Murphy M, Smith R, Eboli ML, and Galeotti T. Redox regulation of cAMP-responsive element-binding protein and induction of manganese superoxide dismutase in nerve growth factor-dependent cell survival. *J Biol Chem* 278: 16510–16519, 2003.
6. Bensaad K and Vousden KH. Savior and slayer: The two faces of p53. *Nat Med* 11: 1278–1279, 2005.
7. Bensaad K and Vousden KH. p53: New roles in metabolism. *Trends Cell Biol* 17: 286–291, 2007.
8. Bensaad K, Tsuruta A, Selak MA, Vidal MNC, Nakano K, Bartrons R, Gottlieb E, and Vousden KH. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 126: 107–120, 2006.
9. Berniakovich I, Trinei M, Stendardo M, Migliaccio E, Minucci S, Bernardi P, Pelicci PG, and Giorgio M. p66Shc-generated oxidative signal promotes fat accumulation. *J Biol Chem* 283: 34283–34293, 2008.
10. Borrello S, De Leo ME, and Galeotti T. Defective gene expression of MnSOD in cancer cells. *Mol Aspects Med* 14: 253–258, 1993.
11. Bravard A, Sabatier L, Hoffschir F, Ricoul M, Luccioni C, and Dutrillaux B. SOD2: A new type of tumor-suppressor gene? *Int J Cancer* 51: 476–480, 1992.
12. Budanov AV and Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* 134: 451–460, 2008.
13. Burgering BM and Medema RH. Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. *J Leukoc Biol* 73: 689–701, 2003.
14. Buzek J, Latonen L, Kurki S, Peltonen K, and Laiho M. Redox state of tumor suppressor p53 regulates its sequence-specific DNA binding in DNA-damaged cells by cysteine 277. *Nucleic Acids Res* 30: 2340–2348, 2002.
15. Cai J and Jones DP. Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome c loss. *J Biol Chem* 273: 11401–11404, 1998.
16. Cano CE, Gommeaux J, Pietri S, Culcasi M, Garcia S, Seux M, Barelier S, Vasseur S, Spoto RP, Pebusque MJ, Dusetti NJ, Iovanna JL, and Carrier A. Tumor protein 53-induced nuclear protein 1 is a major mediator of p53 antioxidant function. *Cancer Res* 69: 219–226, 2009.
17. Chatoo W, Abdouh M, David J, Champagne MP, Ferreira J, Rodier F, and Bernier G. The polycomb group gene Bmi1 regulates antioxidant defenses in neurons by repressing p53 pro-oxidant activity. *J Neurosci* 29: 529–542, 2009.
18. Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, and Zheng P. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. *J Exp Med* 205: 2397–2408, 2008.
19. Chiarugi P, Pani G, Giannoni E, Taddei L, Colavitti R, Raugei G, Symons M, Borrello S, Galeotti T, and Ramponi G. Reactive oxygen species as essential mediators of cell adhesion: The oxidative inhibition of a FAK tyrosine phosphatase is required for cell adhesion. *J Cell Biol* 161: 933–944, 2003.
20. Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, and Trent JM. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc Natl Acad Sci USA* 90: 3113–3117, 1993.
21. Cosentino F, Francia P, Camici GG, Pelicci PG, Luscher TF, and Volpe M. Final common molecular pathways of aging and cardiovascular disease: Role of the p66Shc protein. *Arterioscler Thromb Vasc Biol* 28: 622–628, 2008.
22. Coussens LM and Werb Z. Inflammation and cancer. *Nature* 420: 860–867, 2002.
23. Desaint S, Luriau S, Aude JC, Rousselet G, and Toledano MB. Mammalian antioxidant defenses are not inducible by H2O2. *J Biol Chem* 279: 31157–31163, 2004.
24. Dhar SK, Xu Y, Chen Y, and St Clair DK. Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide dismutase gene expression. *J Biol Chem* 281: 21698–21709, 2006.
25. Dhar SK, Xu Y, and St Clair DK. Nuclear factor kappa-B- and specificity protein 1-dependent p53-mediated bi-directional regulation of the human manganese superoxide dismutase gene. *J Biol Chem* 285: 9835–9846, 2010.
26. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, and Clarke MF. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 458: 780–783, 2009.
27. Drane P, Bravard A, Bouvard V, and May E. Reciprocal down-regulation of p53 and SOD2 gene expression. Implication in p53 mediated apoptosis. *Oncogene* 20: 430–439, 2001.

28. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64: 97–112, 1995.
29. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, and Pelicci PG. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* 122: 221–233, 2005.
30. Guarente L and Kenyon C. Genetic pathways that regulate aging in model organisms. *Nature* 408: 255–262, 2000.
31. Haga S, Terui K, Fukai M, Oikawa Y, Irani K, Furukawa H, Todo S, and Ozaki M. Preventing hypoxia/reoxygenation damage to hepatocytes by p66(shc) ablation: Up-regulation of anti-oxidant and anti-apoptotic proteins. *J Hepatol* 48: 422–432, 2008.
32. Hainaut P and Mann K. Zinc binding and redox control of p53 structure and function. *Antioxid Redox Signal* 3: 611–623, 2001.
33. Hanson S, Kim E, and Deppert W. Redox factor 1 (Ref-1) enhances specific DNA binding of p53 by promoting p53 tetramerization. *Oncogene* 24: 1641–1647, 2005.
34. Harman D. The biologic clock: The mitochondria? *J Am Geriatr Soc* 20: 145–147, 1972.
35. Havens CG, Ho A, Yoshioka N, and Dowdy SF. Regulation of late G1/S phase transition and APC Cdh1 by reactive oxygen species. *Mol Cell Biol* 26: 4701–4711, 2006.
36. Hitchler MJ, Wikainapakul K, Yu L, Powers K, Attatippaholkun W, and Domann FE. Epigenetic regulation of manganese superoxide dismutase expression in human breast cancer cells. *Epigenetics* 1: 163–171, 2006.
37. Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, Ichimiya M, Sengupta S, Mechanic L, Okamura S, Hofseth LJ, Moake M, Nagashima M, Forrester KS, and Harris CC. p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res* 64: 2350–2356, 2004.
38. Hwang PM, Bunz F, Yu J, Rago C, Chan TA, Murphy MP, Kelso GF, Smith RA, Kinzler KW, and Vogelstein B. Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. *Nat Med* 7: 1111–1117, 2001.
39. Janssen AM, Bosman CB, Sier CF, Griffioen G, Kubben FJ, Lamers CB, van Krieken JH, van de Velde CJ, and Verspaget HW. Superoxide dismutases in relation to the overall survival of colorectal cancer patients. *Br J Cancer* 78: 1051–1057, 1998.
40. Janssen–Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG, and van d, V. Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic Biol Med* 45: 1–17, 2008.
41. Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, and Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc Natl Acad Sci USA* 93: 11848–11852, 1996.
42. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ, and Thompson CB. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell* 18: 283–293, 2005.
43. Kang D, Lee KM, Park SK, Berndt SI, Peters U, Reding D, Chatterjee N, Welch R, Chanock S, Huang WY, and Hayes RB. Functional variant of manganese superoxide dismutase (SOD2 V16A) polymorphism is associated with prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer study. *Cancer Epidemiol Biomarkers Prev* 16: 1581–1586, 2007.
44. Kim HP, Roe JH, Chock PB, and Yim MB. Transcriptional activation of the human manganese superoxide dismutase gene mediated by tetradecanoylphorbol acetate. *J Biol Chem* 274: 37455–37460, 1999.
45. Kiningham KK, Oberley TD, Lin S, Mattingly CA, and St Clair DK. Overexpression of manganese superoxide dismutase protects against mitochondrial-initiated poly(ADP-ribose) polymerase-mediated cell death. *FASEB J* 13: 1601–1610, 1999.
46. Kinzler KW and Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 87: 159–170, 1996.
47. Koch OR, Fusco S, Ranieri SC, Maulucci G, Palozza P, Larocca LM, Cravero AA, Farre' SM, De SM, Galeotti T, and Pani G. Role of the life span determinant P66 (shcA) in ethanol-induced liver damage. *Lab Invest* 88: 750–760, 2008.
48. Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, and Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419: 316–321, 2002.
49. Kume S, Haneda M, Kanasaki K, Sugimoto T, Araki S, Isono M, Isshiki K, Uzu T, Kashiwagi A, and Koya D. Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation. *Free Radic Biol Med* 40: 2175–2182, 2006.
50. Landriscina M, Remiddi F, Ria F, Palazzotti B, De Leo ME, Iacoangeli M, Rosselli R, Scerrati M, and Galeotti T. The level of MnSOD is directly correlated with grade of brain tumours of neuroepithelial origin. *Br J Cancer* 74: 1877–1885, 1996.
51. Li S, Yan T, Yang JQ, Oberley TD, and Oberley LW. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res* 60: 3927–3939, 2000.
52. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, and Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 11: 376–381, 1995.
53. Liu B, Chen Y, and St Clair DK. ROS and p53: A versatile partnership. *Free Radic Biol Med* 44: 1529–1535, 2008.
54. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, and Hwang PM. p53 regulates mitochondrial respiration. *Science* 312: 1650–1653, 2006.
55. Medema RH, Kops GJ, Bos JL, and Burgering BM. AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. *Nature* 404: 782–787, 2000.
56. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, and Pelicci PG. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309–313, 1999.
57. Migliaccio E, Giorgio M, and Pelicci PG. Apoptosis and aging: Role of p66Shc redox protein. *Antioxid Redox Signal* 8: 600–608, 2006.
58. Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, Nojima A, Nabetani A, Oike Y, Matsubara H, Ishikawa F, and Komuro I. A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat Med* 15: 1082–1087, 2009.
59. Nakano T, Oka K, and Taniguchi N. Manganese superoxide dismutase expression correlates with p53 status and local

recurrence of cervical carcinoma treated with radiation therapy. *Cancer Res* 56: 2771–2775, 1996.
60. Nemoto S, Combs CA, French S, Ahn BH, Fergusson MM, Balaban RS, and Finkel T. The mammalian longevity-associated gene product p66shc regulates mitochondrial metabolism. *J Biol Chem* 281: 10555–10560, 2006.
61. Nemoto S and Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452, 2002.
62. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
63. Oberley LW and Oberley TD. Role of antioxidant enzymes in cell immortalization and transformation. *Mol Cell Biochem* 84: 147–153, 1988.
64. Palmero I, Pantoja C, and Serrano M. p19ARF links the tumour suppressor p53 to Ras. *Nature* 395: 125–126, 1998.
65. Pani G, Bedogni B, Anzevino R, Colavitti R, Palazzotti B, Borrello S, and Galeotti T. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. *Cancer Res* 60: 4654–4660, 2000.
66. Pani G, Bedogni B, Colavitti R, Anzevino R, Borrello S, and Galeotti T. Cell compartmentalization in redox signaling. *IUBMB Life* 52: 7–16, 2001.
67. Pani G, Colavitti R, Bedogni B, Anzevino R, Borrello S, and Galeotti T. A redox signaling mechanism for density-dependent inhibition of cell growth. *J Biol Chem* 275: 38891–38899, 2000.
68. Pani G, Colavitti R, Bedogni B, Fusco S, Ferraro D, Borrello S, and Galeotti T. Mitochondrial superoxide dismutase: A promising target for new anticancer therapies. *Curr Med Chem* 11: 1299–1308, 2004.
69. Pani G, Fusco S, Colavitti R, Borrello S, Maggiano N, Cravero AA, Farre SM, Galeotti T, and Koch OR. Abrogation of hepatocyte apoptosis and early appearance of liver dysplasia in ethanol-fed p53-deficient mice. *Biochem Biophys Res Commun* 325: 97–100, 2004.
70. Pani G, Koch OR, and Galeotti T. The p53-p66shc-manganese superoxide dismutase (MnSOD) network: A mitochondrial intrigue to generate reactive oxygen species. *Int J Biochem Cell Biol* 41: 1002–1005, 2009.
71. Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, Contursi C, Minucci S, Mantovani F, Wieckowski MR, Del SG, Pelicci PG, and Rizzuto R. Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* 315: 659–663, 2007.
72. Polyak K, Xia Y, Zweier JL, Kinzler KW, and Vogelstein B. A model for p53-induced apoptosis. *Nature* 389: 300–305, 1997.
72a. Ranieri SC, Fusco S, Panieri E, Labate V, Mele M, Tesori V, Ferrara AM, Maulucci G, De Spirito M, Martorana GE, Galeotti T, and Pani G. Mammalian life-span determinant p66shcA mediates obesity-induced insulin resistance. *Proc Natl Acad Sci USA* 107: 13420–13425, 2010.
73. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
74. Ria F, Landriscina M, Remiddi F, Rosselli R, Iacoangeli M, Scerrati M, Pani G, Borrello S, and Galeotti T. The level of manganese superoxide dismutase content is an independent prognostic factor for glioblastoma. Biological mechanisms and clinical implications. *Br J Cancer* 84: 529–534, 2001.
75. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, and Chumakov PM. The antioxidant function of the p53 tumor suppressor. *Nat Med* 11: 1306–1313, 2005.
76. Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ, Marck BT, Matsumoto AM, Shelton JM, Richardson JA, Bennett MJ, and Garcia JA. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. *Nat Genet* 35: 331–340, 2003.
77. Seemann S and Hainaut P. Roles of thioredoxin reductase 1 and APE/Ref-1 in the control of basal p53 stability and activity. *Oncogene* 24: 3853–3863, 2005.
78. Serrano M, Lin AW, McCurrach ME, Beach D, and Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88: 593–602, 1997.
79. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, and Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127: 397–408, 2006.
80. Teixeira HD, Schumacher RI, and Meneghini R. Lower intracellular hydrogen peroxide levels in cells overexpressing CuZn-superoxide dismutase. *Proc Natl Acad Sci USA* 95: 7872–7875, 1998.
81. Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, Milia E, Padura IM, Raker VA, Maccarana M, Petronilli V, Minucci S, Bernardi P, Lanfrancone L, and Pelicci PG. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene* 21: 3872–3878, 2002.
82. van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, and Burgering BM. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J Biol Chem* 279: 28873–28879, 2004.
83. Vaziri H, Dessain SK, Ng EE, Imai SI, Frye RA, Pandita TK, Guarente L, and Weinberg RA. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* 107: 149–159, 2001.
84. Velu CS, Niture SK, Doneanu CE, Pattabiraman N, and Srivenugopal KS. Human p53 is inhibited by glutathionylation of cysteines present in the proximal DNA-binding domain during oxidative stress. *Biochemistry* 46: 7765–7780, 2007.
85. Vousden KH and Prives C. Blinded by the light: The growing complexity of p53. *Cell* 137: 413–431, 2009.
86. Vousden KH and Ryan KM. p53 and metabolism. *Nat Rev Cancer* 9: 691–700, 2009.
87. Williams MD, Van RH, Conrad CC, Huang TT, Epstein CJ, and Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J Biol Chem* 273: 28510–28515, 1998.
88. Wong GH, Elwell JH, Oberley LW, and Goeddel DV. Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* 58: 923–931, 1989.
89. Wong GH and Goeddel DV. Induction of manganous superoxide dismutase by tumor necrosis factor: Possible protective mechanism. *Science* 242: 941–944, 1988.
90. Xu Y, Kiningham KK, Devalaraja MN, Yeh CC, Majima H, Kasarskis EJ, and St Clair DK. An intronic NF-kappaB element is essential for induction of the human manganese superoxide dismutase gene by tumor necrosis factor-alpha and interleukin-1beta. *DNA Cell Biol* 18: 709–722, 1999.

91. Zacchi P, Gostissa M, Uchida T, Salvagno C, Avolio F, Volinia S, Ronai Z, Blandino G, Schneider C, and Del SG. The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. *Nature* 419: 853–857, 2002.
92. Zhao Y, Chaiswing L, Velez JM, Batinic–Haberle I, Colburn NH, Oberley TD, and St Clair DK. p53 translocation to mitochondria precedes its nuclear translocation and targets mitochondrial oxidative defense protein-manganese superoxide dismutase. *Cancer Res* 65: 3745–3750, 2005.
93. Zhao Y, Xue Y, Oberley TD, Kiningham KK, Lin SM, Yen HC, Majima H, Hines J, and St Clair D. Overexpression of manganese superoxide dismutase suppresses tumor formation by modulation of activator protein-1 signaling in a multistage skin carcinogenesis model. *Cancer Res* 61: 6082–6088, 2001.

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Abbreviations Used

ALDH4 = aldehyde dehydrogenase 4
AMPK = AMP activated protein kinase
APE-Ref1 = AP endonuclease 1/redox factor 1
ASK1 = apoptosis stimulating kinase 1
ATM = ataxia teleangectasia mutated
CREB = cAMP responsive element binding
DCF-DA = dichlorofluorescein diacetate
DNA-PK = DNA dependent protein kinase
DSB = double strand breaks
ECM = extracellular matrix
ETC = electron transport chain
GPX1 = glutathione peroxidase 1
HIF = hypoxia induced factor
NF-κB = nuclear factor kappa B
NQO1 = NAD:ubiquinone oxidoreductase 1
Nrf-2 = nuclear respiratory factor 2
PIG = p53 induced gene
PPP = pentose phosphate pathway
ROS = reactive oxygen species
SCO2 = synthesis of cytochrome oxidase 2
SHC = Src homology and collagen
SOD2 = superoxide dismutase 2
SP-1 = specificity protein 1
TIGAR = TP53 Induced Glycolysis and Apoptosis Regulator;
TNF = tumor necrosis factor
TP53INP1 = TP53 induced nuclear protein 1

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2. Jie He, Zhengtang Qi, Yuhui Su, Qiang He, Jingxia Liu, Lu Yu, Omar S. Al-Attas, Tajamul Hussain, Edgardo Tan De Rosas, Liu Ji, Shuzhe Ding. 2012. Pifithrin-μ increases mitochondrial COX biogenesis and MnSOD activity in skeletal muscle of middle-aged mice. *Mitochondrion* . [CrossRef]
3. M Y Kang, H-B Kim, C Piao, K H Lee, J W Hyun, I-Y Chang, H J You. 2012. The critical role of catalase in prooxidant and antioxidant function of p53. *Cell Death and Differentiation* . [CrossRef]
4. José M. Matés, Juan A. Segura, Francisco J. Alonso, Javier Márquez. 2012. Oxidative stress in apoptosis and cancer: an update. *Archives of Toxicology* . [CrossRef]
5. Magdalena Lebiedzinska, Agnieszka Karkucinska-Wieckowska, Aleksandra Wojtala, Jan M. Suski, Gyorgy Szabadkai, Grzegorz Wilczynski, Jakub Wlodarczyk, Catia V. Diogo, Paulo J. Oliveira, Jan Tauber, Petr Ježek, Maciej Pronicki, Jerzy Duszynski, Paolo Pinton, Mariusz R. Wieckowski. 2012. Disrupted ATP synthase activity and mitochondrial hyperpolarisation-dependent oxidative stress is associated with p66Shc phosphorylation in fibroblasts of NARP patients. *The International Journal of Biochemistry & Cell Biology* . [CrossRef]
6. Laura Vera-Ramirez, MCarmen Ramirez-Tortosa, Patricia Perez-Lopez, Sergio Granados-Principal, Maurizio Battino, José L. Quiles. 2012. Long-term effects of systemic cancer treatment on DNA oxidative damage: the potential for targeted therapies. *Cancer Letters* . [CrossRef]
7. Versha Banerji, Spencer B. Gibson. 2012. Targeting Metabolism and Autophagy in the Context of Haematologic Malignancies. *International Journal of Cell Biology* **2012**, 1-9. [CrossRef]