Published in final edited form as:

Future Oncol. 2009 February ; 5(1): 117–130. doi:10.2217/14796694.5.1.117.

Watching the watcher: regulation of p53 by mitochondria

Aaron K Holley and

Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536, USA

Daret K St Clair[†]

Graduate Center for Toxicology, 454 HSRB, University of Kentucky, Lexington, KY 40536, USA,
Tel.: +1 859 257 3956, Fax: +1 859 323 1059, DSTCL00@uky.edu

Abstract

p53 has been referred to as the ‘guardian of the genome’ because of its role in protecting the cell from DNA damage. p53 performs its duties by regulating cell-cycle progression and DNA repair and, in cases of irreparable DNA damage, by executing programmed cell death. Mitochondria are an important target of transcription-dependent and -independent actions of p53 to carry out the apoptotic function. However, increasing evidence suggests that p53 activity is regulated by mitochondria. Cellular insults that alter mitochondrial function can have important consequences on p53 activity. In light of these new findings, the following review focuses on p53/mitochondria connections, in particular how reactive oxygen species generated at mitochondria regulate p53 activity. A better understanding of the mechanisms by which mitochondria regulate p53 may have an impact on our understanding of the development and progression of many diseases, especially cancer.

Keywords

mitochondria; p53; reactive oxygen species; skin cancer; ultraviolet radiation

p53 is a sequence-specific transcription factor that determines the fate of a cell [1,2]. p53 regulates the expression of an assortment of genes involved in various cellular functions, including cell-cycle regulation, apoptosis, DNA replication and repair, cell proliferation, cellular stress response and negative regulation of p53 [3,4]. p53 is an important tumor suppressor because of its role in regulating cell-cycle progression and cell death in response to cellular stress [5], and it is the most commonly mutated gene in cancer [6].

p53 normally exists in an inactive state at low concentrations in the cell, activated only when the cell is under stress, resulting in decreased degradation and rapid accumulation of p53. Stresses that activate p53 include heat shock, genetic or metabolic stress, and hypoxia [7], as well as stresses that lead to DNA damage (ionizing and UV radiation, depurination of bases, DNA base alkylation and reactive oxygen species [ROS]-induced damage) [8]. p53 protein levels are controlled largely by Mdm2, a ubiquitin ligase that targets p53 for degradation. In

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[†]Author for correspondence: Graduate Center for Toxicology, 454 HSRB, University of Kentucky, Lexington, KY 40536, USA, Tel.: +1 859 257 3956, Fax: +1 859 323 1059, E-mail: DSTCL00@uky.edu.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

response to cellular stress, p53/Mdm2 interactions diminish, decreasing p53 ubiquitination and leading to the build-up of p53 protein [9].

Protein stabilization is not sufficient for p53 to function properly. Post-translational modifications are important for activation of p53. For example, acetylation of lysine 120 on p53 by the histone acetyltransferase Tip60 is important for p53-induced cell-cycle arrest and apoptosis [10]. Treating neonatal rat cardiomyocytes with hydrogen peroxide results in apoptosis and correlates with increased phosphorylation of p53 at serine 15 [11]. Methylation of p53 at lysine 372 by Set9 methyltransferase leads to increased p53 stability and nuclear localization, resulting in increased expression of p53 target genes [12]. Various ROS and reactive nitrogen species (RNS) have also been shown to alter the expression and activity of p53. Other reviews discuss in detail these well-known features of p53 activation, stability and targets. In this review, we will focus on some lesser-known aspects of p53, with special emphasis on mitochondrial regulation of p53.

The mitochondrion is a membrane-bound organelle composed of an inner and outer membrane enclosing two distinct compartments: an intermembrane space between the two membranes and a matrix enclosed by the inner membrane [13,14]. The mitochondrion is the major site of ATP production in the cell through the process of oxidative phosphorylation. Oxidative phosphorylation is performed by five enzyme complexes embedded in the inner membrane: complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinol-cytochrome *c* oxidoreductase), complex IV (cytochrome *c*-oxygen oxidoreductase) and complex V (ATP synthase) [15]. The mitochondrion is also a major site for other diverse cellular functions [16,17], including various aspects of metabolism [18–23], cell-cycle control, apoptosis, signaling and stress response [18].

The mitochondrion is an important site of initiation and progression of apoptosis [24,25]. Many changes in the status of the mitochondrion initiate apoptosis. For example, altered mitochondrial membrane potential, membrane permeabilization and generation of ROS by the organelle are all key events that send cell death signals [26]. The mitochondrion also releases many molecules that carry out apoptosis. Cytochrome *c*, the protein that shuttles electrons between complex III and complex IV in oxidative phosphorylation [27] is an effector of apoptosis by interacting with cytosolic Apaf-1 to form the apoptosome and activate caspase-9. Smac/Diablo and Omi/Htr2 are proteins that interfere with the function of members of the inhibitor of the apoptosis (IAP) family. AIF and endonuclease G are released from the mitochondrion and enter the nucleus to participate in DNA degradation [28].

The mitochondrion is an important site for intracellular signaling [29]. This signaling is key for the coordination of mitochondrial and nuclear activity for various cellular events [30–33]. An important example is calcium-mediated mitochondrial signaling [34]. Different stresses on the mitochondrion (metabolic or genetic) result in increased cytosolic concentrations of calcium, leading to activation of the MAPKs ERK1/2 [35] and jun N-terminal kinase (JNK) [36], as well as the cAMP response element binding protein (CREB; through activation of calcium/calmodulin kinase IV) [37].

Many nuclear transcription factors have been identified in the mitochondrion [38,39], such as peroxisome proliferator-activated receptors (PPAR) [40], NF- κ B [41], retinoid receptor- α (RXR α) [42] and retinoic acid receptor (RAR) [43], as well as p53 [44,45]. Members of the steroid and thyroid hormone superfamily of nuclear receptors have also been discovered in the mitochondrion [46], including the triiodothyronine (T3) receptor [47], estrogen receptor- β (ER β) [48,49], androgen receptor (AR) [49] and glucocorticoid receptor- α (GR α) [48]. These receptors work to coordinate mitochondrial and nuclear functions for a variety of cellular

processes, including cell-cycle progression, metabolism and apoptosis [39,46,50]. For example, mitochondrial NF- κ B negatively regulates the expression of mitochondrial DNA-encoded cytochrome *c* oxidase III (COXIII) and cytochrome *b* in HT1080 cells after tumor necrosis factor- α treatment [41]. p53, a well-known nuclear transcription factor, also plays multiple roles in the mitochondrion through both transcription-dependent and -independent mechanisms.

Actions of p53 in the mitochondrion

p53 & oxidative phosphorylation/metabolism

One of the hallmarks of cancer is the ability for a cell to alter its metabolism from aerobic respiration to glycolysis, a process called the Warburg effect [51–53]. p53 is important in regulating the switch between glycolysis and aerobic respiration [54–56]. p53 can control aerobic respiration by directly regulating the expression of synthesis of cytochrome *c* oxidase 2 (SCO2), a protein responsible for the assembly of the cytochrome *c* oxidase (COX) complex in the mitochondrion. p53 directly regulates the expression of SCO2 through a p53 response element in the first intron of the *SCO2* gene. p53 stimulates luciferase activity in a dose-dependent manner in p53-null HCT116 cells transfected with a luciferase reporter plasmid containing a fragment of the *SCO2* gene that has a p53 response element and various concentrations of a p53 expression plasmid. Mutation of this p53 response element completely blocks the response of the reporter plasmid to p53. Over-expression of SCO2 in p53-deficient HCT116 cells increases mitochondrial oxygen consumption in a dose-dependent manner. Using parental HCT116 cells expressing p53, small-interfering RNA targeted against p53 results in decreased SCO2 protein expression and decreased oxygen consumption [57].

p53 is also involved in the regulation of glycolysis by controlling the expression of glycolytic enzymes. In mouse embryonic fibroblasts, overexpression of the glycolytic enzyme phosphoglycerate mutase (PGM) leads to increased glycolysis and immortalization. Expression of p53 in these cells results in decreased expression of PGM in a dose-dependent manner. Overexpression of PGM or a dominant-negative form of p53 results in decreased ROS production and oxidative DNA damage [58]. p53 also regulates the expression of TP53-induced glycolysis and apoptosis regulator (TIGAR) [59]. TIGAR expression leads to decreased rates of glycolysis, while siRNA targeted against TIGAR increases glycolysis rates. TIGAR overexpression induced by p53 protects cells from apoptosis, but not from cell-cycle arrest. Reduced glutathione concentrations lead to TIGAR protecting cells against ROS-mediated apoptosis, but not against apoptosis that does not require ROS generation. One mechanism by which TIGAR induces these effects is by dephosphorylating fructose-2,6-bisphosphate to fructose-6-phosphate and shuttling this intermediate through the pentose phosphate pathway, which results in the generation of reduced glutathione and reduces ROS levels in the cell.

p53 & mitochondrial DNA repair

One function of p53 is to participate in the repair of DNA damaged by various stresses [1]. Participation of p53 in mitochondrial DNA repair has been identified in a variety of systems. Using liver mitochondria extracts from wild-type, heterozygous p53 (p53^{+/-}) and homozygous p53 knockout (p53^{-/-}) mice, de Souza-Pinto *et al.* [60] found that p53 participates in mitochondrial base excision repair by stimulating the nucleotide incorporation activity of DNA polymerase- γ . In HCT116 colorectal cancer cells, p53 stimulates both the glycosylase step of mitochondrial base excision repair (to remove the damaged base) and the incorporation step [19]. p53 increases DNA polymerase- γ nucleotide incorporation activity through direct interactions with the polymerase [61]. Nithipongvanitch *et al.* found that translocation of p53 to the mitochondria correlates with doxorubicin-induced oxidative mitochondrial DNA

damage in mouse cardiomyocytes, and that this mitochondrial DNA damage is significantly higher in p53^{-/-} mice [62].

p53 & apoptosis

Apoptosis is a highly regulated form of cell death in which single cells or small groups of cells are targeted to die. The cell first undergoes nuclear and cytoplasmic condensation, eventually leading to the formation of small membrane-bound fragments called apoptotic bodies. These apoptotic bodies are then phagocytosed by surrounding cells [63,64]. This process is important in a range of normal functions, including embryonic development and the immune system [65]. Apoptosis can be broadly divided into two pathways: the extrinsic and intrinsic pathways. The extrinsic pathway results from the cell receiving some external stimulus for cell death, while the intrinsic pathway is due to internal stresses [66], although cross-talk exists between these pathways [67,68]. Mitochondria are important for carrying out the intrinsic apoptotic pathway, and p53 is vital to the execution of this pathway through both transcription-dependent and -independent mechanisms [69].

p53 regulates the transcription of myriad proteins involved in apoptosis. p53 directly regulates the expression of Apaf-1, a scaffolding protein for caspase-9 activation [70], as well as survivin, a member of the IAP family of proteins [71,72]. p53 also controls apoptosis, in part, through transcriptional regulation of various members of the Bcl-2 family, an important family of proteins whose members are involved in both the execution and inhibition of apoptosis at mitochondria, including Bcl-2 [73,74], p53 upregulated modulator of apoptosis (PUMA) [75] and Bax [76,77].

p53 can also induce apoptosis in a transcription-independent manner by directly targeting the mitochondria of the cell [78,79]. Marchenko *et al.* showed that p53 translocates to the mitochondria very quickly (within 1 h) after γ -irradiation in various cell lines [45]. This translocation precedes cytochrome *c* release and changes in mitochondrial membrane potential. p53 also interacts directly with Bcl-2 and Bcl-xL to block their antiapoptotic activity and induce apoptosis [80,81]. p53 also interacts with the proapoptotic Bak [82,83], Bax [84,85] and PUMA [86] to induce apoptosis.

Manganese superoxide dismutase (MnSOD) is an important anti-oxidant enzyme found in mitochondria that protects cells from damage induced by ROS. Using E1A/Ras transformed fibroblasts that either express wild-type p53 (p53^{+/+}) or are p53 deficient (p53^{-/-}), Pani *et al.* [87] found that MnSOD expression and enzyme activity are much higher in p53^{-/-} cells compared with wild-type controls, and that these p53^{-/-} cells are more resistant to ROS-generating conditions (serum starvation or treatment with paraquat or adriamycin). The inverse relationship between p53 and MnSOD expression correlates with various cancer cell lines that express mutated p53 or have lower levels of p53 than corresponding normal tissues, suggesting that p53 negatively regulates MnSOD expression. Dhar *et al.* found that p53 blocks both constitutive- and TPA (12-*O*-tetradecanoyl-13-acetate)-stimulated *MnSOD* gene expression through interactions with Sp1 in the promoter region of the *MnSOD* gene [88]. p53-mediated repression of *MnSOD* gene expression was blocked by either lowering Sp1 levels with Sp1-specific siRNA or mutating Sp1 binding sites in the *MnSOD* basal promoter. Since MnSOD is important in protecting mitochondria from the damaging effects of ROS, it follows that suppression of MnSOD expression by p53 may be an important mechanism of p53-mediated increased ROS production from mitochondria.

Mitochondria & reactive oxygen/nitrogen species

Mitochondria are a major source of reactive oxygen species

ROS are a consequence of living in an oxygen-rich atmosphere. They are the products of oxygen metabolism [89] and can act as second messengers for cellular signaling [90–92]. ROS are involved in a variety of cellular processes, such as cell adhesion, immune response and apoptosis [93]. ROS exert their effects, in part, by altering the activity of many proteins, including many transcription factors [94,95], as well as diverse tyrosine and serine/threonine kinases [96] and mitogen-activated protein kinases [97,98]. While low levels of ROS are important for cell growth and differentiation [99], abnormal ROS production is involved in myriad diseases, including atherosclerosis, diabetes [93], various neurological disorders [100] and cancer [101–103].

Mitochondria are the principle source of ROS in the cell under physiological conditions, and superoxide radicals are the major ROS produced by this organelle. The two major superoxide-producing enzymes in mitochondria are complex I (NADH-ubiquinone oxidoreductase) [104–107] and complex III (ubiquinol-cytochrome *c* oxidoreductase) [108–110]. Superoxide radicals can be particularly damaging because they attack iron–sulfur centers in various enzymes in the mitochondria, resulting in the release of free iron cations. This free iron can catalyze the formation of hydroxyl radicals from hydrogen peroxide through the Haber–Weiss reaction (Figure 1) [111–113]. Superoxide radicals also participate in the formation of RNS. Superoxide radicals react with mitochondrial nitric oxide to form peroxynitrite [114], which can interact with and alter the activity of a variety of proteins in mitochondria, including complexes I, II and V of the electron transport chain and the matrix enzymes aconitase, glutathione peroxidase and MnSOD [115].

Ways to scavenge mitochondrial reactive oxygen species

Because of the toxic ROS produced from normal oxygen metabolism [89,116], cells are equipped with many enzymes that detoxify ROS, both within the mitochondria and in the cytoplasm (Figure 1) [117,118]. Superoxide dismutases (SODs) are the primary ROS detoxifying enzymes of the cell [119]. Three types of SOD exist in the cell: copper- and zinc-containing SOD (CuZnSOD), extracellular SOD and manganese-containing SOD (MnSOD) [120]. CuZnSOD is found primarily in the cytoplasm [120], although small amounts are also found in the intermembrane space of the mitochondria [121,122]. Extracellular SOD shares strong amino acid homology with CuZnSOD, contains Cu and Zn in its active site, and is localized to the extracellular region [123]. MnSOD is found exclusively in the mitochondrial matrix [121,122]. All three enzymes catalyze the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide [124].

Hydrogen peroxide is a ROS that has been shown to serve as a second messenger in cellular signaling [90–92]. The cell uses multiple enzyme systems to catalyze the decomposition of hydrogen peroxide into water and molecular oxygen [117]. One enzyme system that detoxifies hydrogen peroxide involves glutathione peroxidase. Two forms of GPX, GPX1 and phospholipid-hydroperoxide GPX (PHGPx) have been identified in mitochondria [125,126]. These enzymes catalyze the reduction of hydrogen peroxide to water using reduced glutathione, resulting in the formation of oxidized glutathione. Glutathione in mitochondria is regenerated by glutathione reductase [127]. Peroxiredoxins (PRX) are also used to detoxify hydrogen peroxide [128]. Two forms of peroxiredoxins have been identified in mitochondria: PRX III [129,130] and PRX V [131]. Using reduced thioredoxin, peroxiredoxins catalyze the decomposition of hydrogen peroxide to produce water and oxidized thioredoxin. Reduced thioredoxin is regenerated by mitochondrial thioredoxin reductase II [132,133]. Catalase is another important enzyme used by cells to decompose hydrogen peroxide [134,135]. Catalase

converts two molecules of hydrogen peroxide into two molecules of water and one molecule of oxygen; therefore, catalase works best with high concentrations of hydrogen peroxide. Catalase is found primarily in peroxisomes, where it decomposes hydrogen peroxide formed from the β -oxidation of fatty acids [136], although it is also found in the cytoplasm. Some controversy exists concerning the presence of catalase in mitochondria. Zhou and Kang [137] found catalase in the sarcoplasm, nucleus and peroxisomes, but not the mitochondria, of transgenic mice overexpressing catalase. On the other hand, Radi *et al.* [138] discovered catalase in the mitochondrial matrix of rat heart mitochondria, and a recent report by Salvi *et al.* [139] shows that catalase is found throughout different subcellular compartments, including mitochondria.

ROS/RNS are able to regulate p53

Redox modification of amino acid residues is an important mechanism for controlling the expression and transcriptional activity of various transcription factors [140]. These modifications include carbonylation of lysine, arginine, proline or threonine [141], in addition to nitration and phosphorylation of tyrosine [142,143] and various modifications of cysteine [141]. Transcription factors subject to regulation by the redox status of the cell include NF- κ B and AP-1 [140], as well as p53 [144–146]. Different ROS and RNS lead to modifications of p53 and alter its activity. Using recombinant human p53 protein, as well as the D54MG human glioblastoma cell line expressing wild-type p53, Cobbs *et al.* [147] found that treatment with peroxynitrite or NO-donating compounds leads to p53 tyrosine nitration, resulting in the formation of high-molecular-weight intermediates and loss of DNA binding activity. Increased p53 tyrosine nitration was also observed in MCF-7 human breast cancer cells treated with *S*-nitrosoglutathione [148]. Interestingly, low concentrations of these compounds increase p53 DNA-binding activity [148], while high concentrations decrease this binding activity [147–149]. Furukawa *et al.* [150] found that treatment of TIG-3 normal human fibroblast cells with sublethal doses of hydrogen peroxide leads to the accumulation of acetylated p53, increased expression of p21 and induction of cellular senescence through decreased activity of the NAD⁺-dependent deacetylase SIRT1.

The modification of cysteine is vitally important to redox regulation of proteins [141,151]. Modifications of cysteine residues include oxidation of the sulfhydryl group to sulphenic, sulphinic or sulphonic acids and formation of *S*-nitrosothiols and *S*-thiolation through the production of inter-/intra-protein disulfide bonds or mixed disulfides with glutathione [141]. Examples of cysteine modification can be found in many transcription factors, including NF- κ B and AP-1 [152,153]. Cysteine residues are also essential for the redox regulation of p53 function [154]. For example, pyrrolidine dithiocarbamate, a strong pro-/anti-oxidant, blocks UV-induced build-up of p53 protein and expression of the p53 target gene *MDM2* in various fibroblast cell lines through increased cysteine oxidation [155]. Oxidation of Cys277 in p53 is responsible for changes in DNA sequence binding specificity. 24 h after UVC irradiation of WS-1 human fibroblasts, p53 bound to response elements that contain thymine that contacts Cys277. Reduction of p53 using dithiothreitol (DTT) results in increased binding to cytosine-containing p53 response elements [156]. Velu *et al.* have reported glutathionylation of p53 after treatment with various anticancer drugs (cisplatin, doxorubicin, amsacrine and vincristine), as well as different sources of exogenous ROS (hydrogen peroxide, diamide and *tert*-butyl hydroperoxide) [157]. Glutathionylation occurs in tandem with p53 phosphorylation, and results in decreased p53 DNA binding activity due to modification of cysteine residues at the DNA-binding domain that interfere with both DNA-binding and dimer formation.

The redox status of p53 is fundamentally important to its DNA-binding activity [158]. Reduced p53 preferentially binds to supercoiled DNA, while oxidation of p53 decreases this favored binding [158–160]. Once oxidation of p53 occurs, reduction with DTT does not restore DNA

binding [159]. The effect of oxidation on p53 DNA binding activity is due, in part, to the release of Zn ions from the DNA-binding domain, and the presence of excess Zn ions partially protecting p53 from the irreversible decrease in DNA binding induced by oxidizing agents [158,159]. Redox status also affects sequence-specific DNA binding of p53. In the WS-1 human fibroblast cell line, treatment with UVC radiation leads to changes in the redox status of p53 and a time-dependent decrease in p53 binding to a *GADD45* sequence, while binding to sequences for *p21^{WAF1/CIP1}* and *Bax* genes is not affected [156].

Redox status also affects p53 conformation and oligomer formation. p53 reduced by DTT contains five free thiol groups, while oxidized p53 contains only four free thiols, suggesting that oxidized p53 contains a disulfide bond. Addition of DTT to oxidized p53 results in increased DNA binding. The formation of this disulfide bond in oxidized p53 results in distinct conformational changes compared with reduced p53. Reduced p53 binds to DNA as a tetramer, while the majority of oxidized p53 exists as monomers, dimers and various other high-molecular-weight oligomers (as determined by atomic force microscopy) [161].

Many oxidants and anti-oxidants have been shown to carry out their actions through p53. Liu *et al.* found that sulfur-containing anti-oxidants, such as *N*-acetylcysteine, induce apoptosis by increasing the expression of p53 [162]. Selenium-containing anti-oxidants also activate p53. Treatment of H1299 human lung cancer cells with selenomethionine (SeMet) leads to reduction of cysteine residues. The transcription factor Ref1 proved to be important in SeMet-induced cysteine reduction. SeMet treatment also leads to the increase of p53 DNA binding and the protection of mouse embryonic fibroblasts from UV radiation [163]. Other selenium-containing compounds, including methyl-selenic acid and sodium selenite, cause phosphorylation of various serine and threonine residues of p53 that are involved in p53-mediated apoptosis [164]. The oxidant pyrrolidine dithiocarbamate, on the other hand, inhibits UV-induced translocation of p53 to the nucleus and transactivation of p53-responsive genes by oxidizing key p53 cysteine residues [155].

Various p53 gene mutations [165,166] and polymorphisms [167–170] have been reported, and the resulting changes in the p53 protein may affect intracellular localization and redox modification of the protein. For instance, a polymorphism at codon 72 results in either proline or arginine at position 72 in the p53 protein. This polymorphism does not affect the ability of p53 to regulate gene expression, but it does affect intracellular localization. The Arg72 polymorphism has greater mitochondrial localization of p53 than the Pro72 variant has. This difference in localization between the variants is due to increased interaction of Arg72 with MDM2 and CRM1, which increases p53 ubiquitination and nuclear export. The Arg72 variant also shows increased interaction with the mitochondrial import proteins GRP75 and Hsp60 [171]. The effects of this polymorphism, or others, on ROS regulation of p53 would provide important insights into the effects of oxidative stress on p53 stabilization, localization and activity.

Since mitochondria are the major source of ROS in the cell [111–113], and ROS can modify the activity of p53 [144–146], it follows that alterations in mitochondrial functions that affect ROS formation may also affect p53. In particular, changes in mitochondrial membrane potential (MMP) affect p53 activity. In MOLT-3 human leukemia cells, treatment with oligomycin (an ATP synthase inhibitor) causes a decrease in mitochondrial membrane potential and an increase in mitochondrial ROS production, resulting in decreased etoposide-induced p53 accumulation and expression of p53 target genes, as well as decreased apoptosis [172]. Treatment of JB6 mouse epithelial cells with TPA (12-*O*-tetradecanoylphorbol-13-acetate) causes a rapid accumulation of p53 and localization to both the nucleus and mitochondria (outer membrane and matrix). p53 interacts directly with MnSOD and leads to a decrease in superoxide-scavenging activity of MnSOD. p53 mitochondrial localization leads to decreased

MMP and increased apoptosis. Treatment with MnTE-2-PyP⁵⁺ (a MnSOD mimetic) has only a small effect on mitochondrial p53 localization, but it abrogates p53 nuclear translocation and expression of Bax [173]. Cyclosporin A (CsA; an agent that blocks the mitochondrial permeability transition pore) blocks TPA-induced p53 translocation to mitochondria in JB6 cells by hindering TPA-mediated decreases in MMP and complex I activity [174].

The mitochondrion is an important sensor of oxygen in the cell. Changes in oxygen concentration elicit changes in mitochondrial activity, resulting in an adaptive response by the cell [175]. For example, in embryonic chick cardiomyocytes, a decrease in atmospheric oxygen concentration results in an increase in ROS production (demonstrated by increase in dichlorofluorescein fluorescence) and a simultaneous decrease in contractile motion. Treatment with various anti-oxidants (2-mercaptopyrionyl glycine and 1,10-phenanthroline) or inhibitors of oxidative phosphorylation (rotenone and TTFA) blocks the increase in dichlorofluorescein fluorescence, suggesting that ROS are generated from the mitochondria [176]. Mitochondrial ROS generated as a result of hypoxia leads to accumulation of p53 protein in MCF-7 human breast cancer cells [177]. In human ML-1 cells, hypoxic conditions lead to accumulation of p53 at the outer membrane of mitochondria [178]. Thus, it is possible that ROS initiated in mitochondria may cause p53 translocation to mitochondria and enhance oxidative stress in mitochondria by mechanisms that are both dependent and independent of its transcription function, thereby amplifying its apoptotic pathway. Potential effects of changes in mitochondrial ROS/RNS production on p53 localization and activity are summarized in Figure 2.

Implications for skin cancer

The effects of mitochondrial function (in particular, ROS generation) on p53 activity may have important implications for the development and progression of skin cancer, especially for UV radiation-induced carcinogenesis. It has been known for some time that UV radiation causes skin cancer [179]. While the role of UVB (short-wave) radiation in the development of skin cancer is well established, an increasing number of studies have demonstrated that UVA (long-wave) radiation is also capable of inducing skin cancer. For example, Agar *et al.* [180] found more UVA fingerprint mutations in the basal layer of various human skin samples than UVB mutations, suggesting that UVA may act as a carcinogen in skin stem cells. In the immortalized human skin keratinocyte cell line HaCaT, UVA exposure causes double-strand DNA breaks and increases formation of micronuclei. UVA exposure results in tumorigenic transformation in HaCaT cells, leading to the formation of squamous cell carcinomas in nude mice [181]. Low doses of UVA cause cell-cycle progression through pathways involving AKT, epidermal growth factor receptor, cyclin D1 and the metalloprotease ADAM17 (a disintegrin and metalloprotease 17) in HaCaT cells [182].

UV radiation is capable of inflicting both direct and indirect DNA damage through various mechanisms. UV can induce damage through direct absorption by DNA bases, resulting in the formation of cyclopurimidine dimers. UV can also cause indirect DNA damage through various chromophores. These chromophores can absorb UV radiation and either transfer this energy to the DNA bases (type I photosensitized reaction) or to molecular oxygen, with the resulting ROS damaging the DNA (type II photosensitized reaction) [183]. Different types of UV cause various forms of DNA damage: UVB produces more direct DNA damage, while UVA induces more indirect (oxidative) DNA damage, especially the formation of 7,8-dihydro-8-oxoguanine (8-oxoG) [183,184].

Oxidative stress plays an important role in the development and progression of skin cancer [185]. Various carcinogens are activated by ROS or generate ROS as a part of their mechanism of cancer initiation and/or promotion [186]. Different studies have shown that UVA inflicts

DNA damage through the production of ROS, especially singlet oxygen, leading to 8-oxoG formation [187,188]. Oxidative damage caused by UVA is not limited to DNA. In HaCaT cells, UVA also induces lipid peroxidation. Interestingly, a low-dose rate produces more oxidative damage than a high-dose rate does [189].

p53 is activated in response to UV-induced DNA damage [179,190]. However, UVA and UVB have different effects on p53. In human primary neonatal skin fibroblasts, activation of p53 (as indicated by Ser15 phosphorylation) is less pronounced in UVA- than in UVB-irradiated cells [191]. In a study of the effects of UV radiation on melanoma cells [192], WM239 cells (expressing wild-type p53) were more sensitive to both UVA- and UVB-induced apoptosis than SK-MEL-28 cells were, which express a mutant form of p53. These studies suggest the possibility that UVA and UVB, through different mechanisms, may act together to induce skin cancer.

Mitochondria are an important target of UV radiation. UVA irradiation has been shown to cause decreased MMP in the HL-60 cell line [193]. Gniadecki *et al.* [194], on the other hand, found UVA does not alter MMP but increases ROS production through complexes II and III of the mitochondrial electron transport chain. In HaCaT cells, UVB irradiation results in an increase in ROS levels and mitochondrial membrane depolarization, starting 6- and 12-h post-irradiation, respectively. These changes correlate with an increase in p53 levels, starting 12-h post-irradiation [195]. These results suggest that p53 accumulation might be a result of UV-mediated alterations in mitochondrial activity.

Conclusion

p53 is vital to the life of a cell, regulating many activities that maintain genomic stability and cell viability through both transcription-dependent and -independent mechanisms. The mitochondrion is an important target of p53 activity. While p53 regulates many mitochondrial functions, the mitochondrion can also regulate p53 accumulation and activity through ROS-mediated mechanisms. As mitochondrial activity is altered by UV exposure, control of p53 by the mitochondrion may prove to be important in UV-induced skin carcinogenesis, and an understanding of this relationship might lead to improved skin cancer therapies and chemoprevention.

Future perspective

To better understand the mechanisms by which mitochondrial ROS regulate p53 stability and activity, many questions remain to be answered. Various ROS are produced by mitochondria, including superoxide, hydrogen peroxide and peroxynitrite. Post-translational modifications of p53 are important for the stabilization, as well as the activation, of p53. Do these ROS alter p53 activity by directly modifying p53 protein or by altering the activity of certain proteins that interact with and/or alter p53, such as MDM2, molecular chaperones, or even other members of the p53 family (p63 and p73)? How does p53 get to mitochondria without mitochondrial targeting sequences? Once p53 migrates to mitochondria, does it undergo further ROS-mediated modifications that alter its activity within the organelle? Post-translational modifications have diverse effects on p53 activity, with some modifications increasing and others decreasing p53 activity. Do the same conditions that cause p53 to migrate to mitochondria also cause p53 to later migrate to the nucleus? Does p53 mitochondrial localization alter the activity of mitochondria in such a way that a different set of signals causes p53 nuclear translocation?

Finally, what roles do p53 gene polymorphisms and mutations play in ROS-induced p53 mitochondrial translocation? For instance, the Arg72 variant of p53 has a greater affinity toward mitochondrial translocation than the Pro72 variant has. Do other polymorphisms or

mutations also modify p53 subcellular localization, thereby affecting p53-induced cell-cycle regulation or apoptosis? *p53* polymorphisms and mutations may have two effects on p53 that make them more susceptible to ROS-mediated modification. First, changes in the amino acid sequence of p53 introduced by mutations and/or polymorphisms may alter the conformation of p53 in such a way that reactive amino acids are available for modification. Second, these mutations and polymorphisms may themselves be reactive toward either direct modification by ROS or some ROS-mediated post-translational modification.

Executive Summary

Actions of p53 at mitochondria

- p53 controls the expression of genes that regulate oxidative phosphorylation (*SCO2*) or are involved in pathways that feed into oxidative phosphorylation (*PGM* and *TIGAR*).
- p53 maintains mitochondrial genome stability by directly interacting with proteins involved in mitochondrial base excision repair.
- p53 controls mitochondria-centered apoptosis in two ways: p53 regulates the expression of genes that participate in apoptosis (transcription-dependent actions); and p53 translocates to mitochondria and physically interacts with mitochondrial proteins that carry out apoptosis (transcription-independent actions).

Mitochondria & reactive oxygen and nitrogen species

- Mitochondria are a major source of reactive oxygen species (ROS) through superoxide radical formation at complexes I (NADH-ubiquinone oxidoreductase) and III (ubiquinol-cytochrome *c* oxidoreductase).
- Mitochondria contain several enzymes that detoxify ROS (manganese superoxide dismutase, glutathione peroxidase and peroxiredoxin).
- p53 oligomerization and DNA-binding activity is altered by its redox status through changes in conformation, changes in zinc binding at the DNA-binding domain and oxidation of key cysteine residues.
- Changes in mitochondrial function directly impact p53 activity by altering ROS levels.

Implications for skin cancer

- UV irradiation causes increased oxidative stress, in part, through increased mitochondrial ROS production and changes in mitochondrial membrane potential.
- UV irradiation results in accumulation of p53 due to increased ROS production and decreased mitochondria membrane potential.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nat. Rev. Cancer* 2002;2:594–604. [PubMed: 12154352]

2. Vousden KH. Outcomes of p53 activation - spoilt for choice. *J. Cell Sci* 2006;119:5015–5020. [PubMed: 17158908]
3. Tokino T, Nakamura Y. The role of p53-target genes in human cancer. *Crit. Rev. Oncol. Hematol* 2000;33:1–6. [PubMed: 10714958]
4. Ko LJ, Prives C. p53: puzzle and paradigm. *Genes Dev* 1996;10:1054–1072. [PubMed: 8654922]
5. Oren M. Decision making by p53: life, death and cancer. *Cell Death Differ* 2003;10:431–442. [PubMed: 12719720]
6. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994;54:4855–4878. [PubMed: 8069852]
7. Prives C, Hall PA. The p53 pathway. *J. Pathol* 1999;187:112–126. [PubMed: 10341712]
8. Harris SL, Levine AJ. The p53 pathway: positive and negative feedback loops. *Oncogene* 2005;24:2899–2908. [PubMed: 15838523]
9. Momand J, Wu HH, Dasgupta G. MDM-2 - master regulator of the p53 tumor suppressor protein. *Gene* 2000;242:15–29. [PubMed: 10721693]
10. Tang Y, Luo J, Zhang W, Gu W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol. Cell* 2006;24:827–839. [PubMed: 17189186]
11. Long X, Goldenthal MJ, Marin-Garcia J. Oxidative stress enhances phosphorylation of p53 in neonatal rat cardiomyocytes. *Mol. Cell. Biochem* 2007;303:167–174. [PubMed: 17457521]
12. Chuikov S, Kurash JK, Wilson JR, et al. Regulation of p53 activity through lysine methylation. *Nat. Rev. Cancer* 2004;432:353–360.
13. Ernster L, Schatz G. Mitochondria: a historical review. *J. Cell Biol* 1981;91(3):227s–255s. [PubMed: 7033239]
14. Frey TG, Mannella CA. The internal structure of mitochondria. *Trends Biochem. Sci* 2000;25:319–324. [PubMed: 10871882]
15. Hatefi Y. The mitochondrial electron transport chain and oxidative phosphorylation system. *Annu. Rev. Biochem* 1985;54:1015–1069. [PubMed: 2862839]
16. McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr. Biol* 2006;16:R551–R560. [PubMed: 16860735]
17. Logan DC. The mitochondrial compartment. *J. Exp. Bot* 2006;57:1225–1243. [PubMed: 16556626]
18. Manoli I, Alesci S, Blackman MR, Su YA, Rennert OM, Chrousos GP. Mitochondria as key components of the stress response. *Trends Endocrinol. Metab* 2007;18(5):190–198. [PubMed: 17500006]
19. Chen D, Yu Z, Zhu Z, Lopez CD. The p53 pathway promotes efficient mitochondrial DNA base excision repair in colorectal cancer cells. *Cancer Res* 2006;66(7):3485–3494. [PubMed: 16585172]
20. Bowsher CG, Tobin AK. Compartmentation of metabolism within mitochondria and plastids. *J. Exp. Bot* 2001;53(356):513–527. [PubMed: 11373301]
21. Newsholme P, Bender K, Kiely A, Brennan L. Amino acid metabolism, insulin secretion and diabetes. *Biochem. Soc. Trans* 2007;35(5):1180–1186. [PubMed: 17956307]
22. Leverve XM. Mitochondrial function and substrate availability. *Crit. Care Med* 2007;35:S454–S460. [PubMed: 17713393]
23. Eaton S, Bartlett K, Pourfarzam M. Mammalian mitochondrial β -oxidation. *Biochem. J* 1996;320:345–357. [PubMed: 8973539]
24. Spierings D, McStay G, Saleh M, et al. Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. *Science* 2005;310:66–67. [PubMed: 16210526]
25. Gulbins E, Dreschers S, Bock J. Role of mitochondria in apoptosis. *Exp. Physiol* 2003;88:85–90. [PubMed: 12525857]
26. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998;281:1309–1312. [PubMed: 9721092]
27. Scheffler, IE. Mitochondria. NY, USA: Wiley-Liss, Inc.; 1999.
28. Adams JM. Ways of dying: multiple pathways to apoptosis. *Genes Dev* 2003;17:2481–2495. [PubMed: 14561771]

29. Goldenthal MJ, Marin-Garcia J. Mitochondrial signaling pathways: a receiver/integrator organelle. *Mol. Cell. Biochem* 2004;262:1–16. [PubMed: 15532704]
30. Cannino G, Di Liegro CM, Rinaldi AM. Nuclear-mitochondrial interaction. *Mitochondrion* 2007;7:359–366. [PubMed: 17822963]
31. Owusu-Ansah E, Yavari A, Mandal S, Banerjee U. Distinct mitochondrial retrograde signals control the G1-S cell cycle checkpoint. *Nat. Genet* 2008;40:356–361. [PubMed: 18246068]
32. Travan A, Wong JMS, Xu D, Sopta M, Ingles CJ. Altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. *J. Biol. Chem* 2001;276:4020–4027. [PubMed: 11054416]
33. Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. *Annu. Rev. Biochem* 2007;76:701–722. [PubMed: 17227225]
34. Nicholls DG. Mitochondria and calcium signaling. *Cell Calcium* 2005;38:311–317. [PubMed: 16087232]
35. Luo Y, Bond JD, Ingram VM. Compromised mitochondrial function leads to increased cytosolic calcium and to activation of MAP kinases. *Proc. Natl Acad. Sci. USA* 1997;94:9705–9710. [PubMed: 9275188]
36. Biswas G, Adebajo OA, Freedman BD, et al. Retrograde Ca^{2+} signaling in C2C12 skeletal myocytes in response to mitochondrial genetic and metabolic stress: a novel mode of inter-organelle crosstalk. *EMBO J* 1999;18(3):522–533. [PubMed: 9927412]
37. Arnould T, Vankoningsloo S, Renard P, et al. CREB activation induced by mitochondrial dysfunction is a new signaling pathway that impairs cell proliferation. *EMBO J* 2002;21:53–63. [PubMed: 11782425]
38. Psarra AMG, Solakidi S, Sekeris CE. The mitochondrion as a primary site of action of regulatory agents involved in neuroimmunomodulation. *Ann. NY Acad. Sci* 2006;1088:12–22. [PubMed: 17192553]
39. Psarra AM, Sekeris CE. Nuclear receptors and other nuclear transcription factors in mitochondria: regulatory molecules in a new environment. *Biochim. Biophys. Acta* 2008;1783:1–11. [PubMed: 18062929]
40. Casas F, Domenjoud L, Rochard P, et al. A 45 kDa protein related to PPAR γ 2 induced by peroxisome proliferators, is located in the mitochondrial matrix. *FEBS Lett* 2000;478:4–8. [PubMed: 10922459]
41. Cogswell PC, Kashatus DF, Keifer JA, et al. NF- κ B and I κ B α are found in the mitochondria. Evidence for regulation of mitochondrial gene expression by NF- κ B. *J. Biol. Chem* 2003;278(5):2963–2968. [PubMed: 12433922]
42. Casas F, Daury L, Grandemange S, et al. Endocrine regulation of mitochondrial activity: involvement of truncated RXR α and c-Erb A α 1 proteins. *FASEB J* 2003;17:426–436. [PubMed: 12631582]
43. Berdanier CD, Everts HB, Hermoyian C, Mathews CE. Role of vitamin A in mitochondrial gene expression. *Diabetes. Res. Clin. Pract* 2001;54:S11–S27. [PubMed: 11733105]
44. Katsumoto T, Higaki K, Ohno K, Onodera K. Cell-cycle dependent biosynthesis and localization of p53 protein in untransformed human cells. *Biol. Cell* 1995;84:167–173. [PubMed: 8720437]
45. Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J. Biol. Chem* 2000;275(21):16202–16212. [PubMed: 10821866] ■ This article is particularly interesting as it describes p53 translocation to mitochondria in response to stress, and its role in apoptosis.
46. Psarra AM, Sekeris CE. Steroid and thyroid hormone receptors in mitochondria. *IUBMB Life* 2008;60(4):210–223. [PubMed: 18344181]
47. Morrish F, Buroker NE, Ge M, et al. Thyroid hormone receptor isoforms localize to cardiac mitochondrial matrix with potential for binding to receptor elements on mtDNA. *Mitochondrion* 2006;6(3):143–148. [PubMed: 16730242]
48. Solakidi S, Psarra AMG, Sekeris CE. Differential distribution of glucocorticoid and estrogen receptor isoforms: localization of GR β and ER α in nucleoli and GR α and ER β in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. *J. Musculoskelet. Neuronal Interact* 2007;7:240–245. [PubMed: 17947807]
49. Solakidi S, Psarra AMG, Nikolaropoulos S, Sekeris CE. Estrogen receptors α and β (ER α and ER β) and androgen receptor (AR) in human sperm: localization of ER β and AR in mitochondria of the midpiece. *Hum. Reprod* 2005;20:3481–3487. [PubMed: 16123086]

50. Gavrilova-Jordan LP, Price TM. Actions of steroids in mitochondria. *Semin. Reprod. Med* 2007;25:154–164. [PubMed: 17447205]
51. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer* 2004;4:891–899. [PubMed: 15516961]
52. Bui T, Thompson CB. Cancer's sweet tooth. *Cancer Cell* 2006;9:419–420. [PubMed: 16766260]
53. Kim JW, Dang CV. Cancer's molecular sweet tooth and the Warburg effect. *Cancer Res* 2006;66:8927–8930. [PubMed: 16982728]
54. Assaily W, Benchimol S. Differential utilization of two ATP-generating pathways is regulated by p53. *Cancer Cell* 2006;10(1):4–6. [PubMed: 16843260]
55. Ma W, Sung HJ, Park JY, Matoba S, Hwang PM. A pivotal role for p53: balancing aerobic respiration and glycolysis. *J. Bioenerg. Biomembr* 2007;39:243–246. [PubMed: 17551815]
56. Corcoran CA, Huang Y, Sheikh MS. The regulation of energy generating metabolic pathways by p53. *Cancer Biol. Ther* 2006;5:1610–1613. [PubMed: 17204863]
57. Matoba S, Kang JG, Patino WD, et al. p53 regulates mitochondrial respiration. *Science* 2006;312:1650–1653. [PubMed: 16728594]
58. Kondoh H, Leonart ME, Gil J, et al. Glycolytic enzymes can modulate cellular life span. *Cancer Res* 2005;65:177–185. [PubMed: 15665293]
59. Bensaad K, Tsuruta A, Selak MA, et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 2006;126:107–120. [PubMed: 16839880]
60. de Souza-Pinto NC, Harris CC, Bohr VA. p53 functions in the incorporation step in DNA base excision repair in mouse liver mitochondria. *Oncogene* 2004;23:6559–6568. [PubMed: 15208669]
61. Achanta G, Sasaki R, Feng L, et al. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA pol- γ . *EMBO J* 2005;24:3483–3492.
62. Nithipongvanitch R, Ittarat W, Velez JM, Zhao R, St Clair DK, Oberley TD. Evidence for p53 as guardian of the cardiomyocyte mitochondrial genome following acute adriamycin treatment. *J. Histochem. Cytochem* 2007;55(6):629–639. [PubMed: 17312011]
63. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am. J. Pathol* 1995;146:3–15. [PubMed: 7856735]
64. Afford S, Randhawa S. Demystified ... apoptosis. *J. Clin. Pathol. Mol. Pathol* 2000;53:55–63.
65. Schwartzman RA, Cidlowski JA. Apoptosis: the biochemistry and molecular biology of programmed cell death. *Endocr. Rev* 1993;14(2):133–151. [PubMed: 8325248]
66. Reed JC. Mechanisms of apoptosis. *Am. J. Pathol* 2000;157(5):1415–1430. [PubMed: 11073801]
67. Roy S, Nicholson DW. Cross-talk in cell death signaling. *J. Exp. Med* 2000;192(8):F21–F25. [PubMed: 11034612]
68. Basu A, Castle VP, Bouziane M, Bhalla K, Halder S. Crosstalk between extrinsic and intrinsic cell death pathways in pancreatic cancer: synergistic action of estrogen metabolite and ligands of death receptor family. *Cancer Res* 2006;66(8):4309–4318. [PubMed: 16618756]
69. Moll UM, Zaika A. Nuclear and mitochondrial apoptotic pathways of p53. *FEBS Lett* 2001;493:65–69. [PubMed: 11286997] • This review describes many of the transcriptional-dependent and -independent actions of p53 in apoptosis.
70. Robles AI, Bemmels NA, Foraker AB, Harris CC. APAF-1 is a transcriptional target of p53 in DNA damage-induced apoptosis. *Cancer Res* 2001;61:6660–6664. [PubMed: 11559530]
71. Mirza A, McGuirk M, Hockenberry TN, et al. Human survivin is negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway. *Oncogene* 2002;21:2613–2622. [PubMed: 11965534]
72. Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J. Biol. Chem* 2002;277(5):3247–3257. [PubMed: 11714700]
73. Halder S, Negrini M, Monne M, Sabbioni S, Croce CM. Down-regulation of bcl-2 by p53 in breast cancer cells. *Cancer Res* 1994;54:2095–2097. [PubMed: 8174112]
74. Miyashita T, Harigai M, Hanada M, Reed JC. Identification of a p53-dependent negative response element in the *bcl-2* gene. *Cancer Res* 1994;54:3131–3135. [PubMed: 8205530]
75. Nakano K, Vousden KH. *PUMA*, a novel proapoptotic gene, is induced by p53. *Mol. Cell* 2001;7:683–694. [PubMed: 11463392]

76. Thornborrow EC, Manfredi JJ. The tumor suppressor protein p53 requires a cofactor to activate transcriptionally the human BAX promoter. *J. Biol. Chem* 2001;276(19):15598–15608. [PubMed: 11278953]
77. Thornborrow EC, Patel S, Mastropietro AE, Schwartzfarb EM, Manfredi JJ. A conserved intronic response element mediates direct p53-dependent transcriptional activation of both the human and murine *bax* genes. *Oncogene* 2002;21:990–999. [PubMed: 11850816]
78. Murphy ME, Leu JI, George DL. p53 moves to mitochondria. A turn on the path of apoptosis. *Cell Cycle* 2004;3(7):836–839. [PubMed: 15190209]
79. Moll UM, Wolff S, Speidel D, Deppert W. Transcription-independent pro-apoptotic functions of p53. *Curr. Opin. Cell Biol* 2005;17:631–636. [PubMed: 16226451]
80. Mihara M, Erster S, Zaika A, et al. p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell* 2003;11:577–590. [PubMed: 12667443]
81. Deng X, Gao F, Flagg T, Anderson J, May WS. Bcl2's flexible loop domain regulates p53 binding and survival. *Mol. Cell. Biol* 2006;26(12):4421–4434. [PubMed: 16738310]
82. Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat. Cell Biol* 2004;6(5):443–450. [PubMed: 15077116]
83. Pietsch EC, Perchiniak E, Canutescu AA. Oligomerization of BAK by p53 utilizes conserved residues of the p53 DNA binding domain. *J. Biol. Chem* 2008;283(30):21294–21304. [PubMed: 18524770]
84. Chipuk JE, Kuwana T, Bouchier-Hayes L, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 2004;303:1010–1014. [PubMed: 14963330]
85. Schuler M, Bossy-Wetzel E, Goldstein JC, Fitzgerald P, Green DR. p53 induces apoptosis by caspase activation through mitochondrial cytochrome c release. *J. Biol. Chem* 2000;275(10):7337–7342. [PubMed: 10702305]
86. Chipuk JE, Bouchier-Hayes L, Kuwana T, Newmeyer DD, Green DR. PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. *Science* 2005;309:1732–1735. [PubMed: 16151013]
87. Pani G, Bedogni B, Anzevino R, et al. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. *Cancer Res* 2000;60:4654–4660. [PubMed: 10969820]
88. Dhar SK, Xu Y, Chen Y, St Clair DK. Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide dismutase gene expression. *J. Biol. Chem* 2006;281:21698–21709. [PubMed: 16740634]
89. Fridovich I. The biology of oxygen radicals. *Science* 1978;201:875–880. [PubMed: 210504]
90. Forman HJ, Torres M, Fukuto J. Redox signaling. *Mol. Cell. Biochem* 2002;234–235:49–62.
91. Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. *Am. J. Physiol. Cell Physiol* 2004;287:C246–C256. [PubMed: 15238356]
92. Rhee SG, Chang TS, Bae YS, Lee SR, Kang SW. Cellular regulation by hydrogen peroxide. *J. Am. Soc. Nephrol* 2003;14:S211–S215. [PubMed: 12874433]
93. Droge W. Free radicals in the physiological control of cell function. *Physiol. Rev* 2002;82:47–95. [PubMed: 11773609]
94. Kabe Y, Ando K, Hirao S, Yoshida M, Handa H. Redox regulation of NF- κ B activation: Distinct redox regulation between the cytoplasm and the nucleus. *Antioxid. Redox Signal* 2005;7(3–4):395–403. [PubMed: 15706086]
95. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J* 1996;10:709–720. [PubMed: 8635688]
96. Poli G, Leonarduzzi G, Biasi F, Chiarotto E. Oxidative stress and cell signaling. *Curr. Med. Chem* 2004;11:1163–1182. [PubMed: 15134513]
97. Wang X, Martindale JL, Liu Y, Holbrook NJ. The cellular response to oxidative stress: influences of mitogen-activated protein kinase signaling pathways on cell survival. *Biochem. J* 1998;333:291–300. [PubMed: 9657968]

98. Schafer M, Schafer C, Ewald N, Piper HM, Noll T. Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia. *Circ. Res* 2003;92:1010–1015. [PubMed: 12690038]
99. Boonstra J, Post JA. Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene* 2004;337:1–13. [PubMed: 15276197]
100. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J. Carcinog* 2006;5:14–21. [PubMed: 16689993]
101. Storz P. Reactive oxygen species in tumor progression. *Front. Biosci* 2005;10:1881–1896. [PubMed: 15769673]
102. Behrend I, Henderson G, Zwacka RM. Reactive oxygen species in oncogenic transformation. *Biochem. Soc. Trans* 2003;31(6):1441–1444. [PubMed: 14641084]
103. Gius D, Spitz DR. Redox signaling in cancer biology. *Antioxid. Redox Signal* 2006;8(7–8):1249–1252. [PubMed: 16910772]
104. Verkaar S, Koopman WJH, van Ernst-de Vries SE, et al. Superoxide production is inversely related to complex I activity in inherited complex I deficiency. *Biochim. Biophys. Acta* 2007;1772:373–381. [PubMed: 17289351]
105. Genova ML, Ventura B, Giuliano G, et al. The site of production of superoxide radical in mitochondrial complex I is not a bound ubiquinone but presumably iron-sulfur cluster N2. *FEBS Lett* 2001;505:364–368. [PubMed: 11576529]
106. Dlaskova A, Hlavata L, Jezek P. Oxidative stress caused by blocking of mitochondrial complex I H^+ pumping as a link in aging/disease vicious cycle. *Int. J. Biochem. Cell Biol* 2008;40:1792–1805. [PubMed: 18291703]
107. Yen HC, Oberley TD, Gairola CG, Szweda LI, St Clair DK. Manganese superoxide dismutase protects mitochondrial complex I against adriamycin-induced cardiomyopathy in transgenic mice. *Arch. Biochem. Biophys* 1999;362(1):59–66. [PubMed: 9917329]
108. Kakkar P, Singh BK. Mitochondria: a hub of redox activities and cellular distress control. *Mol. Cell. Biochem* 2007;305:235–253.253 [PubMed: 17562131] • Good review describing the generation of radical oxygen species (ROS) and radical nitrogen species in mitochondria and the role mitochondria play in various cellular processes.
109. Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. *Biochem. J* 2001;353:411–416. [PubMed: 11139407]
110. Han D, Antunes F, Canali R, Rettori D, Cadenas E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J. Biol. Chem* 2003;278(8):5557–5563. [PubMed: 12482755]
111. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol., Cell Physiol* 2004;287:817–833.
112. Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease, and ageing. *Trends Biochem. Sci* 2000;25:502–508. [PubMed: 11050436]
113. Raha S, Robinson BH. Mitochondria, oxygen free radicals, and apoptosis. *Am. J. Med. Genet* 2001;106:62–70. [PubMed: 11579426]
114. Hoye AT, Davoren JE, Wipf P, Fink MP, Kagan VE. Targeting mitochondria. *Acc. Chem. Res* 2008;41(1):87–97. [PubMed: 18193822]
115. Radi R, Cassina A, Hodara R, Quijano C, Castro L. Peroxynitrite reactions and formation in mitochondria. *Free Radic. Biol. Med* 2002;33(11):1451–1464. [PubMed: 12446202]
116. Fridovich I. Oxygen toxicity: a radical explanation. *J. Exp. Biol* 1998;201:1203–1209. [PubMed: 9510531]
117. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. *Biochemistry Mosc* 2005;70:200–214. [PubMed: 15807660]
118. Koehler CM, Beverley KN, Leverich EP. Redox pathways of the mitochondrion. *Antioxid. Redox Signal* 2006;8:813–822. [PubMed: 16771672]
119. Fridovich I. Superoxide dismutases. An adaptation to a paramagnetic gas. *J. Biol. Chem* 1989;264(14):7761–7764. [PubMed: 2542241]

120. Zelko IN, Mariani TJ, Felz RJ. Superoxide dismutase multigene family: a comparison of CuZn-SOD (*SOD1*), Mn-SOD (*SOD2*), and EC-SOD (*SOD3*) gene structure, evolution, and expression. *Free Radic. Biol. Med* 2002;33(3):337–349.349 [PubMed: 12126755] ▪ This review is a good source of information on the types of superoxide dismutases that exist in the cell and their regulation.
121. Okado-Matsumoto A, Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver. Cu,Zn-SOD in mitochondria. *J. Biol. Chem* 2001;276(42):38388–38393. [PubMed: 11507097]
122. Weisiger RA, Fridovich I. Mitochondrial superoxide dismutase. Site of synthesis and intramitochondrial localization. *J. Biol. Chem* 1973;248(13):4793–4796. [PubMed: 4578091]
123. Hjalmarsson K, Marklund SL, Engstrom A, Edlund T. Isolation and sequence of complimentary DNA encoding human extracellular superoxide dismutase. *Proc. Natl Acad. Sci. USA* 1987;84:6340–6344. [PubMed: 3476950]
124. Fridovich I. Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem* 1995;64:97–112. [PubMed: 7574505]
125. Esworthy RS, Ho YS, Chu FF. The *Gpx1* gene encodes mitochondrial glutathione peroxidase in the mouse liver. *Arch. Biochem. Biophys* 1997;340:59–63. [PubMed: 9126277]
126. Maiorino M, Scapin M, Ursini F, Biasolo M, Bosello V, Flohe L. Distinct promoters determine alternative transcription of gpx-4 into phospholipid-hydroperoxide glutathione peroxidase variants. *J. Biol. Chem* 2003;278(36):34286–34290. [PubMed: 12819198]
127. Kelner MJ, Montoya MA. Structural organization of the human glutathione reductase gene: determination of correct cDNA sequence and identification of a mitochondrial leader sequence. *Biochem. Biophys. Res. Commun* 2000;269:366–368. [PubMed: 10708558]
128. Oberley TD, Verwiebe E, Zhong W, Kang SW, Rhee SG. Localization of the thioredoxin system in normal rat kidney. *Free Radic. Biol. Med* 2001;30:412–424. [PubMed: 11182297]
129. Chang TS, Cho CS, Park S, Yu S, Kang SW, Rhee SG. Peroxiredoxin III, a mitochondrion-specific peroxidase, regulate apoptotic signaling by mitochondria. *J. Biol. Chem* 2004;279(40):41975–41984. [PubMed: 15280382]
130. Shibata E, Nanri H, Ejima K, et al. Enhancement of mitochondrial oxidative stress and up-regulation of antioxidant protein peroxiredoxin III/SP-22 in the mitochondria of human pre-eclamptic placentae. *Placenta* 2003;24:698–705. [PubMed: 12828928]
131. Seo MS, Kang SW, Kim K, Baines IC, Lee TH, Rhee SG. Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. *J. Biol. Chem* 2000;275:20346–20354. [PubMed: 10751410]
132. Lee SR, Kim JR, Kwon KS, et al. Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. *J. Biol. Chem* 1999;274(8):4722–4734. [PubMed: 9988709]
133. Choi JH, Kim TN, Kim S, et al. Overexpression of mitochondrial thioredoxin reductase and peroxiredoxin III in hepatocellular carcinoma. *Anticancer Res* 2002;22(6A):3331–3335. [PubMed: 12530083]
134. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. *Cell. Mol. Life Sci* 2004;61:192–208. [PubMed: 14745498]
135. Zamocky M, Furtmuller PG, Obinger C. Evolution of catalases from bacteria to humans. *Antioxid. Redox Signal* 2008;10(9):1527–1547. [PubMed: 18498226]
136. Schrader M, Fahimi HD. Peroxisomes and oxidative stress. *Biochim. Biophys. Acta* 2006;1763:1755–1766. [PubMed: 17034877]
137. Zhou Z, Kang YJ. Cellular and subcellular localization of catalase in the heart of transgenic mice. *J. Histochem. Cytochem* 2000;48(5):585–594. [PubMed: 10769042]
138. Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA. Detection of catalase in rat heart mitochondria. *J. Biol. Chem* 1991;266(32):22028–22034. [PubMed: 1657986]
139. Salvi M, Battaglia V, Brunati AM, et al. Catalase takes part in rat liver mitochondria oxidative stress defense. *J. Biol. Chem* 2007;282(33):24407–24415. [PubMed: 17576767]
140. Oktyabrsky ON, Smirnova GV. Redox regulation of cellular functions. *Biochemistry Mosc* 2007;72:132–145.145 [PubMed: 17367290] ▪ This is a very good review concerning the production of ROS in the cell, cellular anti-oxidant defenses, and the role of ROS in myriad cellular functions.

141. Ghezzi P. Oxidoreduction of protein thiols in redox regulation. *Biochem. Soc. Trans* 2005;33(6): 1378–1381. [PubMed: 16246123]
142. Yeo WS, Lee SJ, Lee JR, Kim KP. Nitrosative protein tyrosine modifications: biochemistry and functional significance. *BMB Rep* 2008;41:194–203. [PubMed: 18377722]
143. Monteiro HP, Arai RJ, Travassos LR. Protein tyrosine phosphorylation and protein tyrosine nitration in redox signaling. *Antioxid. Redox Signal* 2008;10:843–889. [PubMed: 18220476]
144. Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. *Free Radic. Biol. Med* 2008;44:1529–1535. [PubMed: 18275858] ■ This important review covers the redox regulation and modification of p53 by ROS and their effects on p53 activity.
145. Marshall HE, Merchant K, Stamler JS. Nitrosation and oxidation in the regulation of gene expression. *FASEB J* 2000;14:1889–1900. [PubMed: 11023973]
146. Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Radic. Biol. Med* 1996;21(3):335–348. [PubMed: 8855444]
147. Cobbs CS, Samanta M, Harkins LE, Gillespie GY, Merrick BA, MacMillan-Crow LA. Evidence for peroxynitrite-mediated modifications to p53 in human gliomas: possible functional consequences. *Arch. Biochem. Biophys* 2001;394(2):167–172. [PubMed: 11594730]
148. Chazotte-Aubert L, Hainaut P, Ohshima H. Nitric oxide nitrates tyrosine residues of tumor-suppressor p53 protein in MCF-7 cells. *Biochem. Biophys. Res. Commun* 2000;267:609–613. [PubMed: 10631110]
149. Cobbs CS, Whisenant TR, Wesemann DR, Harkins LE, Van Meir EG, Samanta M. Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. *Cancer Res* 2003;63:8670–8673. [PubMed: 14695179]
150. Furukawa A, Tada-Oikawa S, Kawanishi S, Oikawa S. H₂O₂ accelerates cellular senescence by accumulation of acetylated p53 via decrease in the function of SIRT1 by NAD⁺ depletion. *Cell. Physiol. Biochem* 2007;20:45–54. [PubMed: 17595514]
151. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. *Biochem. Pharmacol* 2006;71:551–564. [PubMed: 16337153]
152. Klatt P, Molina EP, de Locaba MG, et al. Redox regulation of c-Jun DNA binding by reversible S-glutathionylation. *FASEB J* 1999;13:1481–1490. [PubMed: 10463938]
153. Pineda-Molina E, Klatt P, Vazquez J, et al. Glutathionylation of the p50 subunit of NF-κB: a mechanism for redox-induced inhibition of DNA binding. *Biochemistry* 2001;40:14134–14142. [PubMed: 11714266]
154. Rainwater R, Parks D, Anderson ME, Tegtmeyer P, Mann K. Role of cysteine residues in regulation of p53 function. *Mol. Cell. Biol* 1995;15(7):3892–3903. [PubMed: 7791795]
155. Wu HH, Momand J. Pyrrolidine dithiocarbamate prevents p53 activation and promotes p53 cysteine residue oxidation. *J. Biol. Chem* 1998;273(30):18898–18905. [PubMed: 9668066]
156. Buzek J, Latonen L, Kurki S, Peltonen K, Laiho M. Redox state of tumor suppressor p53 regulates its sequence-specific DNA binding in DNA-damaged cells by cysteine-277. *Nucleic Acids Res* 2002;30(11):2340–2348. [PubMed: 12034820]
157. Velu CS, Niture SK, Doneanu CE, Pattabiraman N, Srivenugopal KS. Human p53 is inhibited by glutathionylation of cysteines present in the proximal DNA-binding domain during oxidative stress. *Biochemistry* 2007;46:7765–7780. [PubMed: 17555331]
158. Hainaut P, Milner J. Redox modulation of p53 conformation and sequence-specific DNA binding *in vitro*. *Cancer Res* 1993;53:4469–4473. [PubMed: 8402615] ■ Demonstrates how redox modification of p53 affects its DNA binding activity.
159. Fojta M, Kubcarova T, Vojtesek B, Palecek E. Effect of p53 protein redox states on binding to supercoiled and linear DNA. *J. Biol. Chem* 1999;274(36):25749–25755. [PubMed: 10464313]
160. Fojta M, Pivonkova H, Brazdova M, Nemcova K, Palecek J, Vojtesek B. Investigations of the supercoil-selective DNA binding of wild type p53 suggest a novel mechanism for controlling p53 function. *Eur. J. Biochem* 2004;271:3865–3876. [PubMed: 15373832]
161. Sun XZ, Vinci C, Makmura L, et al. Formation of disulfide bond in p53 correlates with inhibition of DNA binding and tetramerization. *Antioxid. Redox Signal* 2003;5(5):655–665. [PubMed: 14580323]

162. Liu M, Pelling JC, Ju J, Chu E, Brash DE. Antioxidant action via p53-mediated apoptosis. *Cancer Res* 1998;58:1723–1729. [PubMed: 9563490]
163. Seo YR, Kelley MR, Smith ML. Selenomethionine regulation of p53 by a ref1-dependent redox mechanism. *Proc. Natl Acad. Sci. USA* 2002;22:14548–14553. [PubMed: 12357032]
164. Smith ML, Lancia JK, Mercer TI, Ip C. Selenium compounds regulate p53 by common and distinctive mechanisms. *Anticancer Res* 2004;24(3a):1401–1408. [PubMed: 15274301]
165. Petitjean A, Achatz MIW, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene* 2007;26:2157–2165. [PubMed: 17401424]
166. Bolshakov S, Walker CM, Strom SS, et al. p53 mutations in human aggressive and nonaggressive basal and squamous cell carcinomas. *Clin. Cancer Res* 2003;9:228–234. [PubMed: 12538474]
167. Murphy ME. Polymorphic variants in the p53 pathway. *Cell Death Differ* 2006;13:916–920. [PubMed: 16557270]
168. Pietsch EC, Humbey O, Murphy ME. Polymorphisms in the p53 pathway. *Oncogene* 2006;25:1602–1611. [PubMed: 16550160]
169. Pezeshki A, Sari-Aslani F, Ghaderi A, Doroudchi M. p53 codon 72 polymorphism in basal cell carcinoma of the skin. *Pathol. Oncol. Res* 2006;12(1):29–33. [PubMed: 16554913]
170. Bendesky A, Rosales A, Salazar AM, Sordo M, Peniche J, Ostrosky-Wegman P. p53 codon 72 polymorphism, DNA damage and repair, and risk of non-melanoma skin cancer. *Mutat. Res* 2007;619:38–44. [PubMed: 17403527]
171. Dumont P, Leu JI, Pietra ACD 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat. Genet* 2003;33:357–365.365 [PubMed: 12567188] • The authors show the differences in apoptotic potential between a proline and arginine variants at codon 72 of p53. Part of the difference in apoptotic potential between the variants is the greater ability for the arginine variant to translocate to mitochondria.
172. Karawajew L, Rhein P, Czerwony G, Ludwig WD. Stress-induced activation of the p53 tumor suppressor in leukemia cells and normal lymphocytes requires mitochondrial activity and reactive oxygen species. *Blood* 2005;105:4767–4775. [PubMed: 15705792]
173. Zhao Y, Chaiswing L, Velez JM, et al. p53 translocation to mitochondria precedes its nuclear translocation and targets mitochondrial oxidative defense protein-manganese superoxide dismutase. *Cancer Res* 2005;65(9):3745–3750.3750 [PubMed: 15867370] •• Shows that p53 transcriptional activity is affected by mitochondrial ROS. Treatment of cells with a MnSOD mimetic prevented TPA-induced transcription of p53 target gene *bax*.
174. Liu J, St Clair DK, Gu X, Zhao Y. Blocking mitochondrial permeability transition prevents p53 mitochondrial translocation during skin tumor promotion. *FEBS Lett* 2008;582:1319–1324. [PubMed: 18358838]
175. Chandel NS, Schumacker PT. Cellular oxygen sensing by mitochondria: old questions, new insight. *J. Appl. Physiol* 2000;88:1880–1889.1889 [PubMed: 10797153] •• The authors demonstrate that mitochondrial ROS produced as a result of hypoxia leads to increased p53 stability.
176. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J. Biol. Chem* 1998;273(19):11619–11624. [PubMed: 9565580]
177. Chandel NS, Vander Heiden MG, Thompson CB, Schumacker PT. Redox regulation of p53 during hypoxia. *Oncogene* 2000;19:3840–3848. [PubMed: 10951577]
178. Sansome C, zaika A, Marchenko ND, Moll UM. Hypoxia death stimulus induces translocation of p53 protein to mitochondria. Detection by immunofluorescence on whole cells. *FEBS Lett* 2001;488:110–115. [PubMed: 11163756]
179. Molho-Pessach, V.; Lotem, M. Ultraviolet radiation and cutaneous carcinogenesis. In: Tur, E., editor. *Current Problems in Dermatology*. Switzerland: Karger, Basel; 2007. p. 14-27.
180. Agar NS, Halliday GM, Barnetson RS, Ananthaswamy HN, Wheeler M, Jones AM. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis. *Proc. Natl. Acad. Sci.USA* 2004;101:4954–4959. [PubMed: 15041750]

181. Wischermann K, Popp S, Moshir S, et al. UVA radiation causes DNA strand breaks, chromosomal aberrations and tumorigenic transformation in HaCaT skin keratinocytes. *Oncogene* 2008;27(31): 4269–4280. [PubMed: 18372922]
182. He YY, Council SE, Feng L, Chignell CF. UVA-induced cell cycle progression is mediated by a disintegrin and metalloprotease/epidermal growth factor receptor/AKT/cyclin D1 pathways in keratinocytes. *Cancer Res* 2008;68:3752–3758. [PubMed: 18483258]
183. Runger TM, Kappes UP. Mechanisms of mutation formation with long-wave ultraviolet light (UVA). *Photodermatol. Photoimmunol. Photomed* 2008;24:2–10. [PubMed: 18201350]
184. Pfeifer GP, You YH, Besaratinia A. Mutations induced by ultraviolet light. *Mutat. Res* 2005;571:19–31. [PubMed: 15748635]
185. Trouba KJ, Hamadeh HK, Amin RP, Germolec DR. Oxidative stress and its role in skin disease. *Antioxid. Redox Signal* 2002;4:665–673. [PubMed: 12230879]
186. Sander CS, Chang H, Hamm F, Elsner P, Thiele JJ. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *Int. J. Dermatol* 2004;43:326–335. [PubMed: 15117361]
187. Phillipson RP, Tobi SE, Morris JA, McMillan TJ. UV-A induces persistent genomic instability in human keratinocytes through an oxidative stress mechanism. *Free Radic. Biol. Med* 2002;32(5): 474–480. [PubMed: 11864787]
188. Hazane F, Sauvaigo S, Douki T, Favier A, Beani JC. Age-dependent DNA repair and cell cycle distribution of human skin fibroblasts in response to UVA irradiation. *J. Photochem. Photobiol. B, Biol* 2006;82:214–223.
189. Shorrocks J, Paul ND, McMillan TJ. The dose rate of UVA treatment influences the cellular response of HaCaT keratinocytes. *J. Invest. Dermatol* 2008;128:685–693. [PubMed: 17762856]
190. Latonen L, Laiho M. Cellular UV damage responses - Functions of tumor suppressor p53. *Biochim. Biophys. Acta* 2005;1755:71–89. [PubMed: 15921859]
191. Kappes UP, Luo D, Potter M, Schulmeister K, Runger TM. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cancers. *J. Invest. Dermatol* 2006;126:667–675. [PubMed: 16374481]
192. Zhang H. p53 plays a central role in UVA and UVB induced cell damage and apoptosis in melanoma cells. *Cancer Lett* 2006;244:229–238. [PubMed: 16504377]
193. Tada-Oikawa S, Oikawa S, Kawanishi S. Role of ultraviolet A-induced oxidative DNA damage in apoptosis via loss of mitochondrial membrane potential and caspase-3 activation. *Biomchem. Biophys. Res. Commun* 1998;247:693–696.
194. Gniadecki R, Thorn T, Vicanova J, Petersen A, Wulf HC. Role of mitochondria in ultraviolet-induced oxidative stress. *J. Cell. Biochem* 2000;80:216–222. [PubMed: 11074592]
195. Paz ML, Maglio DHG, Weill FS, Bustamante J, Leoni J. Mitochondrial dysfunction and cellular stress progression after ultraviolet B irradiation in human keratinocytes. *Photodermatol. Photoimmunol. Photomed* 2008;24:115–122. [PubMed: 18477129]

Figure 1. Major sources of reactive oxygen species in mitochondria and methods of detoxification
Superoxide radicals are produced at complexes I and III. Superoxide is dismutated to hydrogen peroxide by MnSOD. Hydrogen peroxide is then decomposed to water using GPx or PRx.
Complex I: NADH-ubiquinone oxidoreductase; complex II: succinateubiquinone oxidoreductase; complex III: ubiquinol-cytochrome *c* oxidoreductase; complex IV: ferrocytochrome *c*-oxygen oxidoreductase; complex V: ATP synthase; cyt *c*: Cytochrome *c*; CuZnSOD: Copper–zinc superoxide dismutase; GPx: Glutathione peroxidase; GSH: Reduced glutathione; GSSG: Oxidized glutathione; GSR: Glutathione reductase; MnSOD: Manganese superoxide dismutase; PRx: Peroxiredoxin; Q: Coenzyme Q; TRx: Thioredoxin; TRxR: Thioredoxin reductase; VDAC: Voltage-dependent anion channel.

Figure 2. Model for the effects of mitochondrial reactive oxygen species production on p53 localization and activity

Under cellular stress conditions, mitochondrial dysfunction occurs (1), leading to increased ROS production (2). ROS stimulate p53 activation (3) and localization to the mitochondrial matrix and outer membrane (4), where p53 interacts with different proteins to either rescue the cell from the insult or to kill the cell. Mitochondrial p53 further stimulates ROS production through increased mitochondrial dysfunction, promoting a feed-forward mechanism between ROS production and p53 mitochondrial localization, as well as p53 nuclear translocation and altered expression of p53 target genes (5). Blocking mitochondrial ROS production by various methods (6) prevents ROS-stimulated p53 translocation to the nucleus. MnSOD: Manganese superoxide dismutase; MMP: Matrix metalloproteinase.