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REVIEW

The dual role of p53: DNA protection and antioxidant

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Abstract
The classical functions of p53 protein are those related to its role on DNA damage, cell growth arrest, senescence and apoptosis. For this reason it is called 'the guardian of the genome' and is considered one of the most important players in the development of cancer. However, more recently it has been show that p53 is not only involved in cancer, but also in ageing. p53 is stimulated by stress, which in turn results in the activation of a wide range of transcriptional targets. Low-intensity stress will activate p53 in a manner which results in antioxidant response, thus protecting against ageing because of its antioxidant function. On the contrary, high-intensity activation of p53 will result in an increase of oxidative stress by activation of p53-mediated pro-oxidant targets, thus increasing the rate of ageing, but protecting against cancer.

Keywords: *Anti-oncogenic protein, oxidative stress, ageing, cancer, antioxidant*

Introduction

Several groups reported in 1979 the finding of a protein (p53) that forms a complex with the SV40 tumour-virus oncoprotein [1,2]. Since then, the role of p53 has been widely studied and it is a very interesting protein because of its role as a tumour suppressor, but recently also because it has other important functions which we will review in this paper.

p53: A reminder

The classical functions of p53 protein are those related to its role in DNA damage, cell growth arrest, senescence and apoptosis [3] (see Figure 1). For this reason it is considered one of the most important players in the development of cancer [4].

DNA damage was the first stress signal to be identified as a modulator of p53 activity [4,5]. In fact, many kinds of DNA damage such as hypoxia [6], spindle poisons [7], telomere shortening [8], heat and cold shock [9,10], nutrient deficiency [11] and the mutational activation of some oncogenes [12–14] have been shown to activate p53 (see Figures 2A and B).

p53 is also involved in cell cycle arrest in G1, G2 and late G2 phases. G1 arrest is a prominent consequence of DNA damage and is induced in many cell types by expression of exogenous wild-type p53 [15], which interacts, among others, with the cdk inhibitor p21 (CIP1/WAF1) [16], the cyclins *Cln*G and *Cln*D1 [17] and the protein phosphatase WIP1 [18]. Activation of the DNA-damage cascade (ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3-related (ATR)-Chk2/1-p53) leads to cell cycle arrest in the G2 phase of the cell cycle [19]. There is also evidence for cell cycle controls in late G2 and early M phases that may involve p53 (see Figure 3).

Hayflick and colleagues [20,21] first formally described cellular senescence as the finite replicative life span of human fibroblasts in culture. Many kinds of oncogenic or stressful stimuli can induce a senescence response. Among them, the most important are DNA damage (telomere loss, DNA breaks, oxidative lesions) [22], over-expression of some oncogenes

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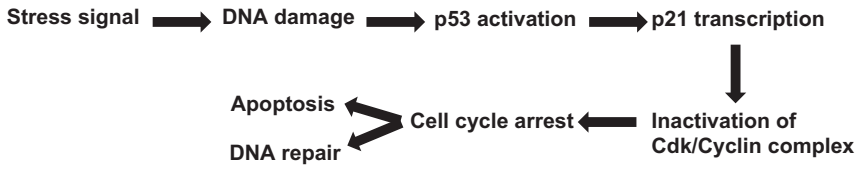

Figure 1. Classical functions of p53.

(activated components of the RAS-RAF-MEK signalling cascade) [23] or responses to epigenetic changes in chromatin organization (i.e. histone modifications) [24]. Multiple mechanisms are involved in p53-induced senescence, including stabilization of p53 by ADP ribosylation factor (ARF) which prevents ubiquitination and, thus, degradation of p53 [25]. Another example of p53-induced senescence is p53-mediated autophagy, which is activated during senescence and its activation is correlated with negative feedback in the PI3K-mammalian target of the rapamycin (mTOR) pathway [26,27] (see Figure 4).

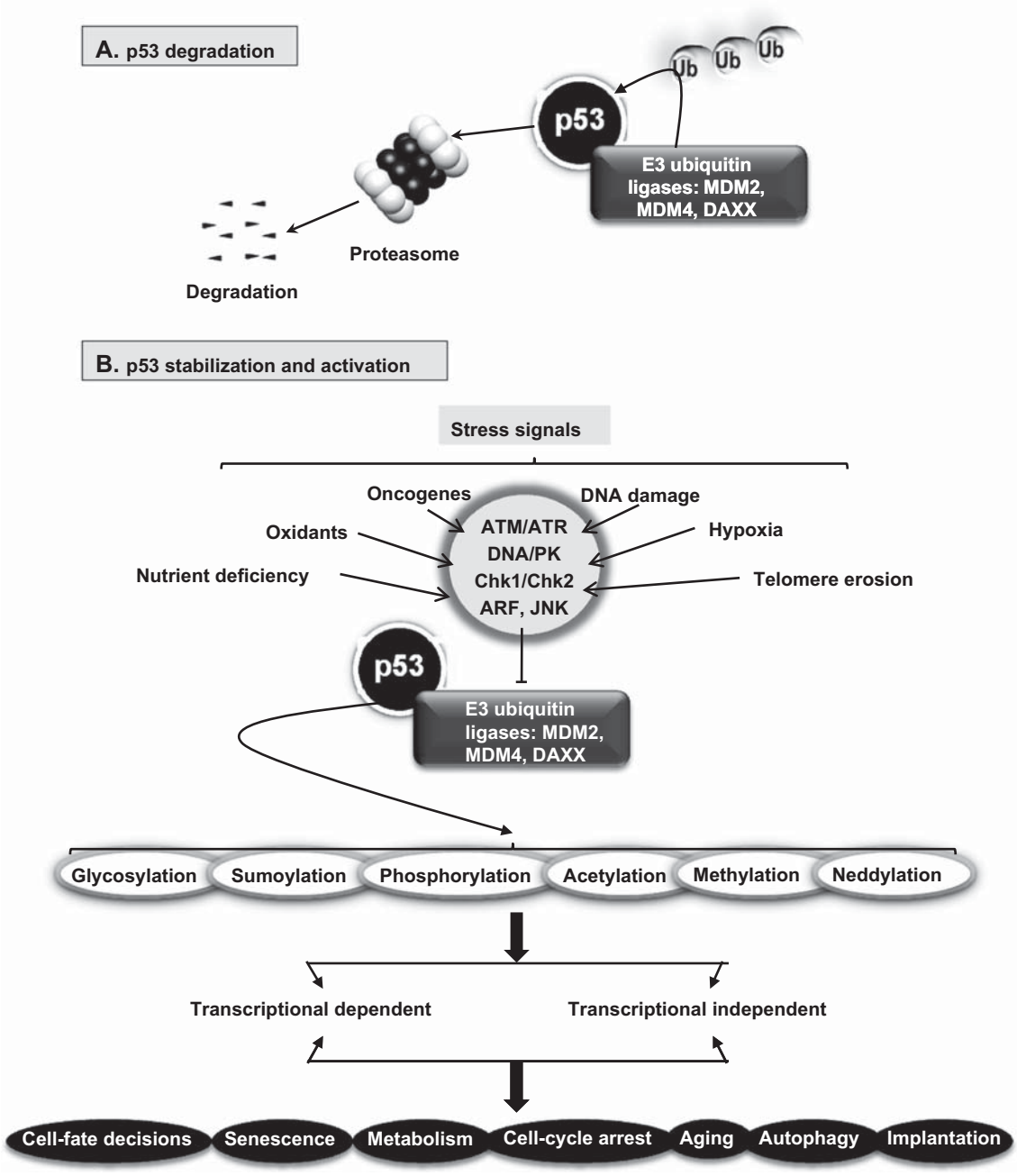

Figure 2. Scheme of p53 degradation (A), stabilization and activation (B).

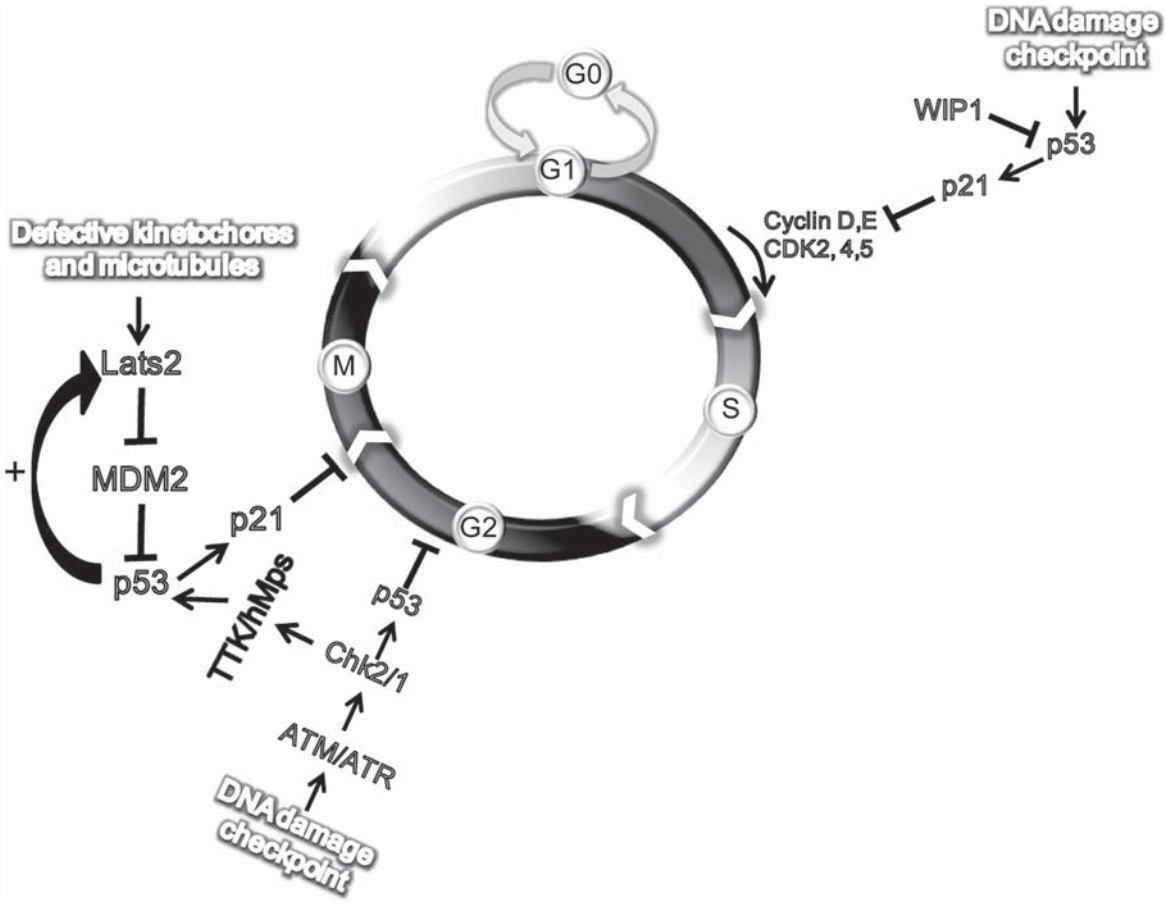

Figure 3. The role of p53 in cell growth.

p53 also induces programmed cell death by activation of pro-apoptotic target genes. Induction may depend on increased levels of p53 protein, cooperating with transcription factors such as NF-κB [28,29] or E2f, which share regulatory factors, such as ARF or MDM2 [30], p53-binding proteins like ASPP [31] or post-translational modifications with p53.

Regulation of p53 and main targets

Under basal condition, p53 is a short-lived transcription factor, which is kept at low levels through ubiquitylation and proteasomal degradation, mediated by several E3 ubiquitin ligases, such as MDM2 [32].

Classical models for the activation of p53 focus on three steps: p53 stabilization induced by ATM/ATR-mediated phosphorylation [33], sequence-specific DNA binding [34] and target gene activation by interacting with the general transcriptional machinery (see Figure 2B). Furthermore, a key step in the physiological activation of p53 appears to be its release from repression by factors such as MDM2 and MdmX [35,36].

Phosphorylation of p53 is classically regarded as the first crucial step of p53 stabilization. p53 is phosphorylated by a broad range of kinases, including those in the ATM/ATR/DNAPK cascade as well as Chk1/Chk2. N-terminal phosphorylation at Ser15 (mouse Ser18) and Ser20 (mouse Ser23) by ATM, ATR, DNA-PK, Chk1 and Chk2 has been considered to stabilize p53 by inhibiting the interaction between p53 and MDM2 (the negative regulator of p53) [32,37]. ARF binds and inhibits MDM2 that is normally present at low levels in the cell, but is highly induced at a transcriptional level when oncogenes are introduced into normal cells [38,39]. ARF is regarded as a protein specialized in communicating to p53, a process which has been called 'oncogenic stress'. This term encompasses the array of cell perturbations produced by oncogenes [40] (see Figure 5).

Central to the p53 master regulatory network are the target response element (RE) sequences [34]. In its role as a master regulator, the universe of genes subject to p53 control because they have RE extends across a diverse group of biological activities [41] that include DNA metabolism [42], apoptosis [43], cell cycle regulation [44], senescence [45], energy metabolism [46,47], angiogenesis [48], immune response [49], cell differentiation, motility and migration [50] and cell–cell communication [51]. One of the genes presenting RE is glutathione peroxidase 1, which has been shown to be regulated by p53 [52] (see below).

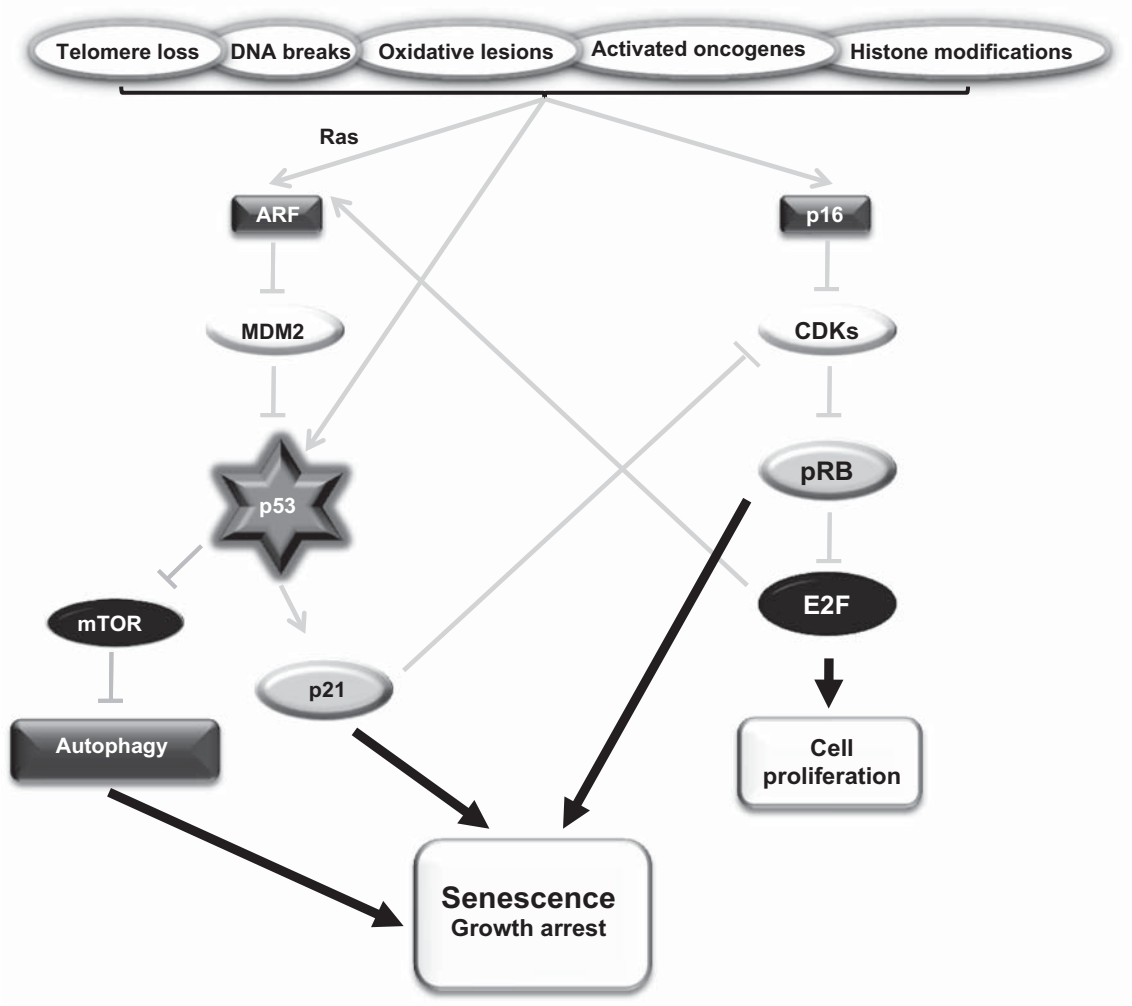

Figure 4. The role of p53 in senescence.

p53 in cancer and ageing

Cancer and ageing share convergent and divergent mechanisms [53]. On one side, both are caused by accumulation of damage; therefore, mechanisms which protect from cellular damage also protect from both cancer and ageing. On the other side, inhibition of cell proliferation would protect against cancer, but will exacerbate ageing. On the contrary, mechanisms which promote cell proliferation would inhibit ageing, but enhance cancer [53].

There are some mechanisms which could interfere with both cancer and ageing, such as oxidative stress and anti-oncogenic proteins.

Oxidative stress is one of the convergent mechanisms linking cancer and ageing. It is widely known that it causes damage to biomolecules such as lipids [54], proteins [55], DNA [56] and carbohydrates [57]. This cell damage is known to promote ageing [58] and also cancer, mainly because of the oxidative DNA damage [59]. Therefore, mechanisms which protect against oxidative stress will provide protection against cancer and ageing.

In cooperation with Dr Serrano (CNIO, Madrid), we found that p53 is related to cancer and ageing [40,60]. As mentioned, p53 is stimulated by stress, which in turn results in the activation of a wide range of transcriptional targets. The consequent cellular response depends on the type and intensity of the stress. Low-intensity stress activates p53 in a manner resulting in antioxidant response [60–63] and, therefore, it not only protects against cancer, but also against ageing because of its antioxidant function. On the contrary, high-intensity stress activates p53, resulting in an increase of oxidative stress by activation of pro-oxidant targets [64], thus increasing the rate of ageing.

Finally, all molecules that can modulate p53 activation/degradation would also interfere with cancer and ageing. One of these molecules is ARF, which has been shown to increase longevity and decrease cancer in mice [65].

p53 and ROS: A double-edge sword

By specific modulation of cellular ROS levels p53 can act as a double-edge sword. ROS act as both upstream

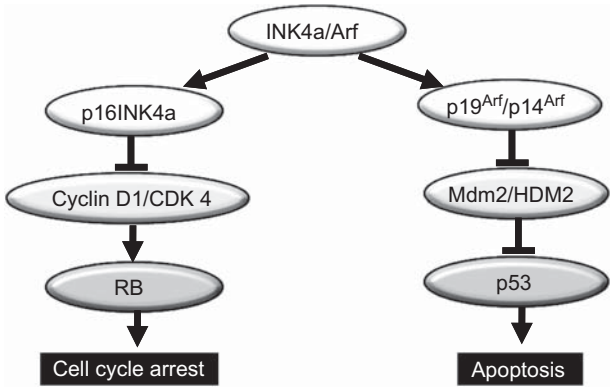

Figure 5. Arf as modulator of p53 activation.

signals that trigger p53 activation and downstream factors that mediate apoptosis [66]. Some of the p53 transcriptional target genes code for ROS-generating proteins and may be involved in cell death [67,68]. However, other genes up-regulated by p53, such as those regulating the expression of antioxidant enzymes [52,69], can act as antioxidants.

Cells contain a network of antioxidant defense mechanisms to reduce the risk of oxidative damage during periods of increased ROS production. The term *antioxidant* can be broadly defined as any substance that delays or prevents the oxidation of a substrate (i.e. all molecules found *in vivo*) [70]. Cells contain both enzymatic and non-enzymatic antioxidants that work as a complex unit to balance reactive oxygen species. Classical antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidases (GPX) and catalase. Additional antioxidant enzymes such as peroxiredoxins (Prx), glutaredoxin (Trx) and thioredoxin reductases (TrxR) also contribute to cellular protection against oxidation [71].

Given that both ROS and p53 participate in multiple redox-regulated cellular processes, there should be interactions between ROS and p53 and intersections between their signalling pathways. A microarray analysis of H_2O_2-treated human cells identified one-third of 48 highly H_2O_2-reponsive genes as targets of p53 [72]. In 2005 it was shown that the pro-oxidant function of p53 is tightly linked to release of mitochondrial ROS during stress-induced apoptosis [64]. When a cell is exposed to severe stress such as genotoxic stress, p53 can activate numerous genes which increase ROS generation, thus leading to apoptosis [60,73]. ROS generated by severe stress can further activate p53 in a positive feedback loop [74]. If, however, cells are exposed to light stress, basal activity of p53 leads to the activation of antioxidant genes, of which the most important are probably sestrins (see below). Thus, in non-stressed or physiologically-stressed cells p53 can act as an antioxidative through the induction of a set of antioxidant genes [64]. p53 activates a transcriptional programme that is particularly relevant when p53 is activated by mild stresses [75] such as those presumed to operate during physiological ageing [60]. An increase in inflammatory cytokines and a decrease in sex and growth hormones with age have been suggested to contribute to the ageing process [76,77]. Different studies have employed microarray technology to uncover changes in gene expression that accompany ageing of mice [78], rats [79], monkeys [80] and humans [81]. In 2007 it was demonstrated that genes that are activated by p53 or whose gene products are known to bind to p53 increase significantly with age, while the expression of genes that are known to inhibit p53 activity decline specifically in old muscle [82]. The concentration of 8-oxo-2 deoxyguanosine, a by-product of DNA oxidation, has been observed to increase with age in a variety of tissues [83]. One possible reason for the observed increase in p53 activity during ageing could be the elevated levels of DNA damage with age, although it is debatable [82]. Nevertheless, these observations support a role for a p53-mediated transcriptional programme in mammalian ageing [82].

p53 also transactivates a series of genes encoding pro-oxidant or redox active proteins, including ROS generating enzymes, NQO1 (quinone oxidoreductase) [67], proline oxidase (POX) [84], BAX, PUMA and p66Shc [66]. Up-regulation of these enzymes leads to oxidative stress and consequently to apoptosis [67,84,85]. Surprisingly, even though some antioxidant genes, e.g. PIG12 (microsomal glutathione transferase homologue) [67] and ALDH4 (aldehyde dehydrogenase 4) [86], are concomitantly up-regulated upon p53 over-expression, they are not able to reverse the apoptotic process and seem more like an adaptive response to p53-induced oxidative stress. More surprisingly, oxidative stress can result from the imbalanced induction of antioxidant enzymes by p53. In the human lymphoblast cell line TK6, Hussain et al. [69] found that over-expression of p53 increases cellular levels of MnSOD and GPX, but does not alter the level of catalase. The steady-state level of H_2O_2 may be increased by activating superoxide dismutase and not catalase. In the presence of iron, this usually results in an increased activity of the Haber-Weiss reaction and, thus, in increases in hydroxyl radicals which are indeed extremely harmful. However, the actual mechanism can be complex [87,88], with glutathione and NADPH possibly being involved [69,89].

In contrast to the pro-oxidant function of hyperphysiological levels of p53, physiological levels of p53 have a subtle but vital function in maintaining ROS at non-toxic levels through transactivation of antioxidant genes [64,90,91]. At physiological levels, p53 is required to maintain a normal basal transcription of antioxidant genes, including sestrins (see below) and GPx1 [64]. Interestingly, AIF (apoptosis-inducing factor), a pro-apoptotic protein by definition, has paradoxically been found to work as an antioxidant

enzyme under physiological conditions regulated by basal levels of p53 [91]. Suppression of p53 results in a significant decrease in the basal transcription of Sesn1, Sesn2 and GPx1, but does not affect the expression of the pro-oxidant genes BAX, NQO1 and PUMA [64]. This leads to an increase in ROS and subsequently to oxidative damage of DNA, whereas restoring physiological levels of p53 up-regulates the antioxidant enzymes and decreases the levels of ROS. Regulating antioxidant defense against ROS may be one of the tumour-suppressing mechanisms of p53. This mechanism seems to be general; its validity has been seen in multiple normal and carcinoma human cell lines as well as in p53-knockout mice [64].

In conclusion, p53-inducible genes can be broadly placed into two groups: those induced by low levels of stress (generally cell-cycle arrest or antioxidant targets) and those that are induced by higher levels of p53/stress (generally apoptotic targets) [64] (see Figure 6).

Recently a new class of p53-regulated antioxidant proteins, sestrins, has emerged. Sestrins (Sesns) are a family of stress-inducible proteins that can function as antioxidants and inhibitors of mTORC1 [92,93]. Members of the family were named Sesns after Sestri Levante, a small town on the Ligurian coast of Italy where, during a human genetics course, researchers discovered the amino acid sequence homology of three proteins (Sesn1, Sesn2 and Sesn3) [94,95]. As antioxidants sestrins can control the activity of peroxiredoxins (Prxs) which scavenge ROS [63]. Sestrins are transcriptionally induced by oxidative stress. Both mammalian Sesn1 and Sesn2 are induced upon DNA damage in response to activation of p53 [94,96] and dSesn (Drosophila sestrin) is also induced upon radiation-induced DNA damage [92]. Sestrins can function as oxidoreductases *in vitro* and *in vivo*, leading to reactivation of oxidized Prxs [63]. Cysteine thiols in proteins are the most vulnerable targets of peroxides in cells and, based on their sensitivity to peroxides, thiol groups have been employed as redox sensors in biological systems [97,98]. The reaction of cysteinyl thiolates with hydrogen peroxide results in the formation of different oxidation forms, such as sulphenic acid (–SOH), sulphinic acid ($–SO_2H$), sulphonic acid ($–SO_3H$) and disulphide (–S-S–), including glutathione S-conjugate and disulphide S-oxides [99]. Prxs, a family of thiol-containing peroxidases conserved from bacteria to mammals [100], are major reductants of endogenously produced peroxides in eukaryotes [101]. In addition, Prxs catalyse decomposition of Reactive Nitrogen Species [63,102]. In contrast to catalases, which directly catalyse the decomposition of peroxides to water and oxygen, peroxiredoxins, which consume expensive cellular reducing equivalents to reduce peroxides, should be systematically regulated to conserve cellular reducing resources [98]. The eukaryotic 2-Cys Prxs have acquired increased sensitivity to inactivation by over-oxidation of their catalytic centre [103]. During background scavenging, the peroxidatic cysteine is oxidized to Cys-SOH, which forms a disulphide bridge with the resolving cysteine located in the other sub-unit of the Prx dimer. This disulphide bond is then reduced by thioredoxin [104]. However, because the formation of the resolving disulphide bond is slow, high concentrations of ROS cause further oxidation of the peroxidatic cysteine to Cys-SO_2H, yielding an inactive form of Prx that cannot be reduced by thioredoxin [100]. It has been demonstrated that sestrins are cysteine sulphinyl reductases required for regeneration of Prxs containing Cys-SO_2H [63]. However, recently

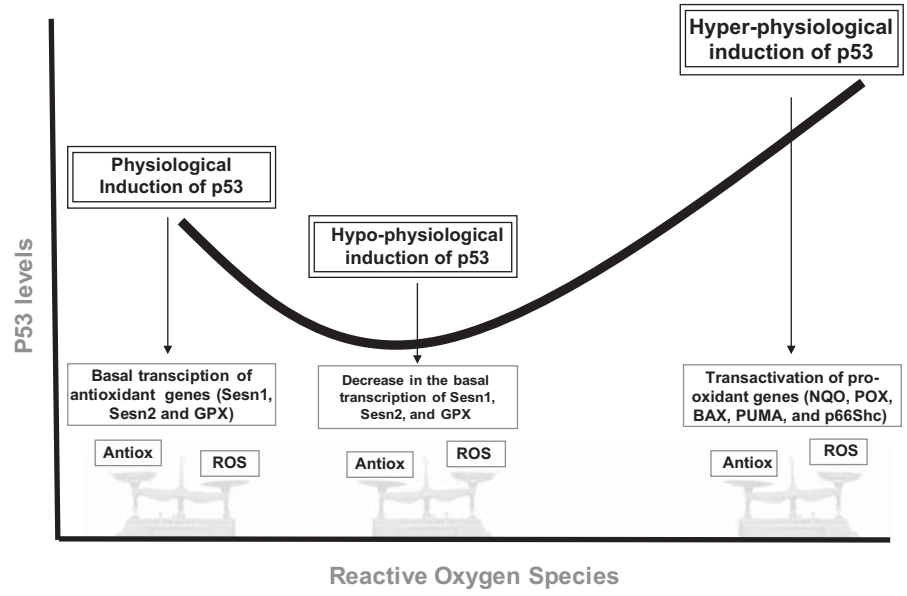

Figure 6. p53: a double edge sword.

Woo et al. [105] have questioned the role of sestrin 2 as a reductase for cysteine sulphinic acid of Prxs. Therefore, more research is needed to clarify the role of sestrin 2.

Modulation of sestrin expression by p53 might be part of an adaptive response mechanism that protects the cell against oxidative stress [63] (see Figure 7). On the other hand it has been recently demonstrated that increased sestrin abundance potentiates the activity of AMP-activated protein kinase (AMPK), thereby diminishing TORC1 activity [93]. Reduced TORC1 activity inhibits anabolic pathways including protein and lipid synthesis [106]. Shutdown of TORC1-dependent anabolism upon genotoxic stress is likely to be important for minimizing new protein and membrane synthesis saving the cell energy to promote DNA repair. Therefore, sestrin induction in response to DNA damage may contribute to the many tumour suppressor functions carried out by p53 [41]. Sestrins can suppress the growth of some cancer cell lines [94] and loss of Sesn2 makes immortalized cells more susceptible to oncogenic transformation [61]. The SESN1 (6q21) and SESN2 (1p35) loci are frequently deleted in a variety of human cancers [96], implicating loss of sestrins in tumour progression and suggesting that sestrin-dependent inhibition of TORC1 is critical for suppressing tumourigenesis induced by age-dependent accumulation of damaged DNA. Moreover, sestrins induce autophagy by inhibition of TORC1 [4,32,33]. Enhanced autophagy results in more efficient elimination of ROS-producing damaged mitochondria in stressed cells [27,28]. Finally, sestrin-induced activation of AMPK and inhibition of TORC1 can also reduce ROS production by increasing the efficiency of mitochondrial respiration [10,11]. Therefore, sestrins have a key role in maintaining cellular integrity and homeostasis during oxidative insults. Both ROS accumulation and TORC1 activation are associated with accelerated ageing and development of age-associated pathologies [93]. Thus, sestrins have been identified as a family of p53-inducible proteins that provide an antioxidant defense to protect cells from H_2O_2-induced damage [61,63], implicating that p53-induced sestrins may act as anti-ageing agents [60,92].

Concluding remarks

Thirty years ago, an antigen present in SV40 transformed cells was discovered. This protein had a molecular weight of ~53 KD and was named p53. Soon, it became apparent that p53 had such a powerful DNA protective effect.

Much more recent work by Serrano's group has shown its implication in ageing. Regulated overexpression of p53 protects against damage and prolongs longevity. Much of its anti-ageing activity is due to the fact that it behaves as an antioxidant (mediated by the recently discovered sestrins). The common biology between cancer and ageing and the involvement of p53 in both phenomena indicates that its activity may be exquisitely regulated.

Fine details of p53 regulation by physiological and nutritional manipulation promise interesting results regarding the biology of this remarkable protein.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

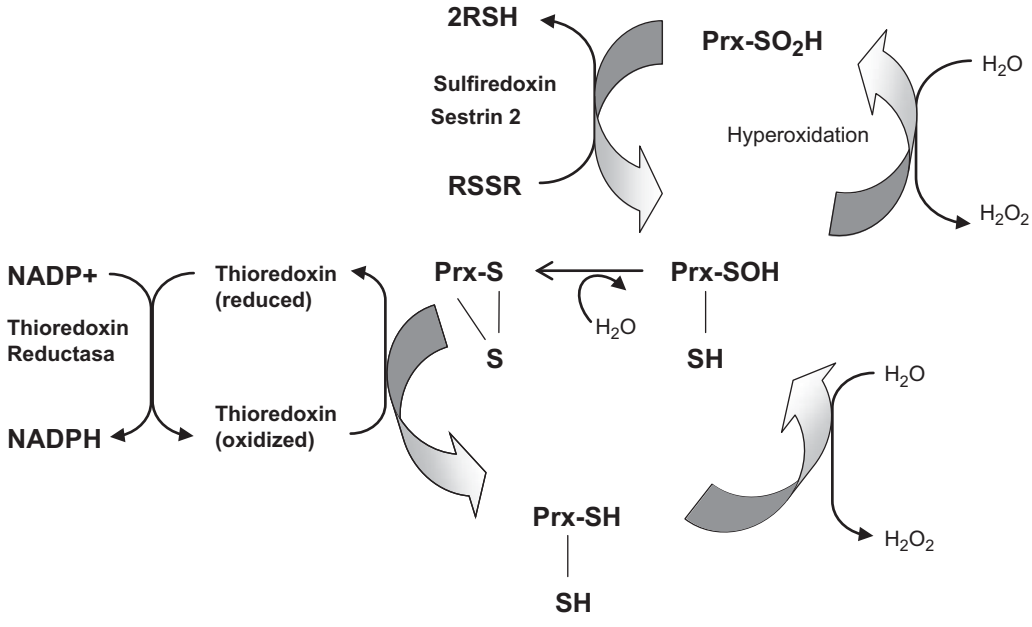

Figure 7. Role of sestrins as antioxidants.

References

[1] Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979;278:261–263.

[2] Linzer DI, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 1979; 17:43–52.

[3] Molchadsky A, Rivlin N, Brosh R, Rotter V, Sarig R. p53 is balancing development, differentiation and de-differentiation to assure cancer prevention. Carcinogenesis 2010;31:1501–1508

[4] Meek DW. Tumour suppression by p53: a role for the DNA damage response? Nat Rev Cancer 2009;9:714–723.

[5] Maltzman W, Czyzyk L. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol Cell Biol 1984;4:1689–1694.

[6] Sendoel A, Kohler I, Fellmann C, Lowe SW, Hengartner MO. HIF-1 antagonizes p53-mediated apoptosis through a secreted neuronal tyrosinase. Nature 2010;465:577–583.

[7] Ohno K, Ishihata K, Tanaka-Azuma Y, Yamada T. A genotoxicity test system based on p53R2 gene expression in human cells: assessment of its reactivity to various classes of genotoxic chemicals. Mutat Res 2008;656:27–35.

[8] Marion RM, Strati K, Li H, Murga M, Blanco R, Ortega S, Fernandez-Capetillo O, Serrano M, Blasco MA. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 2009;460: 1149–1153.

[9] Logan IR, McNeill HV, Cook S, Lu X, Meek DW, Fuller-Pace FV, Lunec J, Robson CN. Heat shock factor-1 modulates p53 activity in the transcriptional response to DNA damage. Nucleic Acids Res 2009;37:2962–2973.

[10] Dormoy-Raclet V, Markovits J, Malato Y, Huet S, Lagarde P, Montaudon D, Jacquemin-Sablon A, Jacquemin-Sablon H. Unr, a cytoplasmic RNA-binding protein with cold-shock domains, is involved in control of apoptosis in ES and HuH7 cells. Oncogene 2007;26:2595–2605.

[11] Ho E, Courtemanche C, Ames BN. Zinc deficiency induces oxidative DNA damage and increases p53 expression in human lung fibroblasts. J Nutr 2003;133:2543–258.

[12] Chang KW, Sarraj S, Lin SC, Tsai PI, Solt D. P53 expression, p53 and Ha-ras mutation and telomerase activation during nitrosamine-mediated hamster pouch carcinogenesis. Carcinogenesis 2000;21:1441–1451.

[13] Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene 2003;22:9030–9040.

[14] Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307–310.

[15] Amundson SA, Myers TG, Fornace AJ, Jr. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 1998;17:3287–3299.

[16] el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. Nat Genet 1992;1: 45–49.

[17] Niehans GA, Kratzke RA, Froberg MK, Aeppli DM, Nguyen PL, Geradts J. G1 checkpoint protein and p53 abnormalities occur in most invasive transitional cell carcinomas of the urinary bladder. Br J Cancer 1999;80:1175–1184.

[18] Song JY, Han HS, Sabapathy K, Lee BM, Yu E, Choi J. Expression of a homeostatic regulator, Wip1 (wild-type p53-induced phosphatase), is temporally induced by c-Jun and p53 in response to UV irradiation. J Biol Chem 2010;285: 9067–9076.

[19] Smits VA, Medema RH. Checking out the G(2)/M transition. Biochim Biophys Acta 2001;1519:1–12.

[20] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.

[21] Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res 1965;37:614–636.

[22] Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev 1994;8:2540–2551.

[23] Bringold F, Serrano M. Tumor suppressors and oncogenes in cellular senescence. Exp Gerontol 2000;35:317–329.

[24] Narita M. Cellular senescence and chromatin organisation. Br J Cancer 2007;96:686–691.

[25] Lin AW, Lowe SW. Oncogenic ras activates the ARF-p53 pathway to suppress epithelial cell transformation. Proc Natl Acad Sci USA 2001;98:5025–5030.

[26] Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavare S, Arakawa S, Shimizu S, Watt FM. Autophagy mediates the mitotic senescence transition. Genes Dev 2009; 23:798–803.

[27] White E, Lowe SW. Eating to exit: autophagy-enabled senescence revealed. Genes Dev 2009;23:784–787.

[28] Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. Nature 2000;404:892–897.

[29] Shetty S, Gyetko MR, Mazar AP. Induction of p53 by urokinase in lung epithelial cells. J Biol Chem 2005;280:28133–28141.

[30] Polager S, Ginsberg D. p53 and E2f: partners in life and death. Nat Rev Cancer 2009;9:738–748.

[31] Samuels-Lev Y, O'Connor DJ, Bergamaschi D, Trigiante G, Hsieh JK, Zhong S, Campargue I, Naumovski L, Crook T, Lu X. ASPP proteins specifically stimulate the apoptotic function of p53. Mol Cell 2001;8:781–794.

[32] Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature 1997;387:296–299.

[33] Meulmeester E, Pereg Y, Shiloh Y, Jochemsen AG. ATM-mediated phosphorylations inhibit Mdmx/Mdm2 stabilization by HAUSP in favor of p53 activation. Cell Cycle 2005;4: 1166–1170.

[34] Menendez D, Inga A, Resnick MA. The expanding universe of p53 targets. Nat Rev Cancer 2009;9:724–737.

[35] Kruse JP, Gu W. Modes of p53 regulation. Cell 2009;137: 609–622.

[36] Meek DW, Anderson CW. Posttranslational Modification of p53: cooperative integrators of function. Cold Spring Harb Perspect Biol 2009;1:a000950.

[37] Appella E, Anderson CW. Post-translational modifications and activation of p53 by genotoxic stresses. Eur J Biochem 2001;268:2764–2772.

[38] Gonzalez S, Klatt P, Delgado S, Conde E, Lopez-Rios F, Sanchez-Cespedes M, Mendez J, Antequera F, Serrano M. Oncogenic activity of Cdc6 through repression of the INK4/ARF locus. Nature 2006;440:702–706.

[39] Matheu A, Klatt P, Serrano M. Regulation of the INK4a/ARF locus by histone deacetylase inhibitors. J Biol Chem 2005;280: 42433–42441.

[40] Matheu A, Maraver A, Serrano M. The Arf/p53 pathway in cancer and aging. Cancer Res 2008;68:6031–6034.

[41] Vousden KH, Prives C. Blinded by the light: the growing complexity of p53. Cell 2009;137:413–431.

[42] Helton ES, Chen X. p53 modulation of the DNA damage response. J Cell Biochem 2007;100:883–896.

[43] Vousden KH. Outcomes of p53 activation–spoilt for choice. J Cell Sci 2006;119:5015–5020.

[44] Kastan MB, Zhan Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ, Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 1992;71: 587–597.

[45] Garbe JC, Holst CR, Bassett E, Tlsty T, Stampfer MR. Inactivation of p53 function in cultured human mammary

epithelial cells turns the telomere-length dependent senescence barrier from agonescence into crisis. Cell Cycle 2007; 6:1927–1936.
[46] Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM. p53 regulates mitochondrial respiration. Science 2006;312:1650–1653.
[47] Green DR, Chipuk JE. p53 and metabolism: inside the TIGAR. Cell 2006;126:30–32.
[48] Menendez D, Krysiak O, Inga A, Krysiak B, Resnick MA, Schonfelder G. A SNP in the flt-1 promoter integrates the VEGF system into the p53 transcriptional network. Proc Natl Acad Sci USA 2006;103:1406–1411.
[49] Taura M, Eguma A, Suico MA, Shuto T, Koga T, Komatsu K, Komune T, Sato T, Saya H, Li JD, Kai H. p53 regulates Toll-like receptor 3 expression and function in human epithelial cell lines. Mol Cell Biol 2008;28:6557–667.
[50] Qin Q, Baudry M, Liao G, Noniyev A, Galeano J, Bi X. A novel function for p53: regulation of growth cone motility through interaction with Rho kinase. J Neurosci 2009;29: 5183–5192.
[51] Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: a novel function of the p53 protein. Cancer Res 2006;66: 4795–4801.
[52] Tan M, Li S, Swaroop M, Guan K, Oberley LW, Sun Y. Transcriptional activation of the human glutathione peroxidase promoter by p53. J Biol Chem 1999;274:12061–12066.
[53] Serrano M, Blasco MA. Cancer and ageing: convergent and divergent mechanisms. Nat Rev Mol Cell Biol 2007;8: 715–722.
[54] Cheeseman KH, Slater TF. An introduction to free radical biochemistry. Br Med Bull 1993;49:588–603.
[55] Davies KJA. Protein damage and degradation by oxygen radicals. General aspects. J Biol Chem 1987;262:9895–9901.
[56] García de la Asunción J, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, Sastre J, Viña J. Mitochondiral glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J 1996;10:333–338.
[57] Borel JP, Monboisse JC, Bellon G. Inflammation, collagene et radicaux libres ocygénés. Med Sci 1988;5:304–311.
[58] Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;2:298–300.
[59] De Flora S, Izzotti A, D'Agostini F, Balansky RM. Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis 2001;22:999–1013.
[60] Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, Flores JM, Vina J, Blasco MA, Serrano M. Delayed ageing through damage protection by the Arf/p53 pathway. Nature 2007;448:375–379.
[61] Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell 2008; 134:451–460.
[62] Lee JH, Budanov AV, Park EJ, Birse R, Kim TE, Perkins GA, Ocorr K, Ellisman MH, Bodmer R, Bier E, Karin M. Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. Science;327:1223–1228.
[63] Budanov AV, Sablina AA, Feinstein E, Koonin EV, Chumakov PM. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. Science 2004;304:596–600.
[64] Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. Nat Med 2005;11:1306–1313.
[65] Matheu A, Maraver A, Collado M, Garcia-Cao I, Canamero M, Borras C, Flores JM, Klatt P, Vina J, Serrano M. Anti-aging activity of the Ink4/Arf locus. Aging Cell 2009;8:152–161.
[66] Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. Free Radic Biol Med 2008;44:1529–1535.
[67] Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature 1997;389:300–305.
[68] Macip S, Igarashi M, Berggren P, Yu J, Lee SW, Aaronson SA. Influence of induced reactive oxygen species in p53-mediated cell fate decisions. Mol Cell Biol 2003;23: 8576–8585.
[69] 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, Harris CC. p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. Cancer Res 2004;64: 2350–2356.
[70] Halliwell B, Gutteridge JM. The definition and measurement of antioxidants in biological systems. Free Radic Biol Med 1995;18:125–126.
[71] Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008;88:1243–1276.
[72] Desaint S, Luriau S, Aude JC, Rousselet G, Toledano MB. Mammalian antioxidant defenses are not inducible by H2O2. J Biol Chem 2004;279:31157–31163.
[73] Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc Natl Acad Sci USA 1996;93: 11848–11852.
[74] Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol 2002; 192:1–15.
[75] Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol 2007;8:275–283.
[76] Roubenoff R. Catabolism of aging: is it an inflammatory process? Curr Opin Clin Nutr Metab Care 2003;6:295–299.
[77] Pedersen M, Bruunsgaard H, Weis N, Hendel HW, Andreassen BU, Eldrup E, Dela F, Pedersen BK. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. Mech Ageing Dev 2003;124:495–502.
[78] Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. Science 1999;28:1390–1393.
[79] Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, Barazzoni R, Nair KS. Effects of caloric restriction on mitochondrial function and gene transcripts in rat muscle. Am J Physiol Endocrinol Metab 2002;283:38–43.
[80] Kayo T, Allison DB, Weindruch R, Prolla TA. Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. Proc Natl Acad Sci USA 2001;98:5093–5098.
[81] Welle S, Brooks AI, Delehanty JM, Needler N, Bhatt K, Shah B, Thornton CA. Skeletal muscle gene expression profiles in 20–29 year old and 65–71 year old women. Exp Gerontol 2004;39:369–377.
[82] Edwards MG, Anderson RM, Yuan M, Kendziorski CM, Weindruch R, Prolla TA. Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. BMC Genomics 2007;8:80.
[83] Hamilton ML, Van Remmen H, Drake JA, Yang H, Guo ZM, Kewitt K, Walter CA, Richardson A. Does oxidative damage to DNA increase with age? Proc Natl Acad Sci USA 2001;98: 10469–10474.
[84] Rivera A, Maxwell SA. The p53-induced gene-6 (proline oxidase) mediates apoptosis through a calcineurin-dependent pathway. J Biol Chem 2005;280:29346–29354.
[85] Esteve JM, Mompó J, García de la Asunción J, Sastre J, Asensi M, Boix J, Vina JR, Viña J, Pallardó FV. Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies in vivo and in vitro. FASEB Journal 1999;13: 1055–1064.
[86] Yoon KA, Nakamura Y, Arakawa H. Identification of ALDH4 as a p53-inducible gene and its protective role in cellular stresses. J Hum Genet 2004;49:134–140.

[87] Liochev SI, Fridovich I. Copper, zinc superoxide dismutase and H2O2. Effects of bicarbonate on inactivation and oxidations of NADPH and urate, and on consumption of H2O2. J Biol Chem 2002;277:34674–34678.
[88] Liochev SI, Fridovich I. The effects of superoxide dismutase on H2O2 formation. Free Radic Biol Med 2007;42: 1465–1469.
[89] Jones DP. Disruption of mitochondrial redox circuitry in oxidative stress. Chem Biol Interact 2006;163:38–53.
[90] Tomko RJ, Jr, Bansal P, Lazo JS. Airing out an antioxidant role for the tumor suppressor p53. Mol Interv 2006;6:23–25, 2.
[91] Stambolsky P, Weisz L, Shats I, Klein Y, Goldfinger N, Oren M, Rotter V. Regulation of AIF expression by p53. Cell Death Differ 2006;13:2140-2149.
[92] Lee JH, Bodmer R, Bier E, Karin M. Sestrins at the crossroad between stress and aging. Aging (Albany NY) 2010;2: 369–374.
[93] Lee JH, Budanov AV, Park EJ, Birse R, Kim TE, Perkins GA, Ocorr K, Ellisman MH, Bodmer R, Bier E, Karin M. Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. Science 2010;327:1223–1228.
[94] Budanov AV, Shoshani T, Faerman A, Zelin E, Kamer I, Kalinski H, Gorodin S, Fishman A, Chajut A, Einat P, Skaliter R, Gudkov AV, Chumakov PM, Feinstein E. Identification of a novel stress-responsive gene Hi95 involved in regulation of cell viability. Oncogene 2002;21:6017–6031.
[95] Peeters H, Debeer P, Bairoch A, Wilquet V, Huysmans C, Parthoens E, Fryns JP, Gewillig M, Nakamura Y, Niikawa N, Van de Ven W, Devriendt K. PA26 is a candidate gene for heterotaxia in humans: identification of a novel PA26-related gene family in human and mouse. Hum Genet 2003;112: 573–580.
[96] Velasco-Miguel S, Buckbinder L, Jean P, Gelbert L, Talbott R, Laidlaw J, Seizinger B, Kley N. PA26, a novel target of the p53 tumor suppressor and member of the GADD family of DNA damage and growth arrest inducible genes. Oncogene 1999;18:127–137.
[97] Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. Science 2006;312:1882–1883.
[98] Lim JC, Choi HI, Park YS, Nam HW, Woo HA, Kwon KS, Kim YS, Rhee SG, Kim K, Chae HZ. Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity. J Biol Chem 2008;283:28873–28880.
[99] Jacob C, Giles GI, Giles NM, Sies H. Sulfur and selenium: the role of oxidation state in protein structure and function. Angew Chem Int Ed Engl 2003;42:4742–4758.
[100] Wood ZA, Poole LB, Karplus PA. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. Science 2003;300:650–653.
[101] Seaver LC, Imlay JA. Hydrogen peroxide fluxes and compartmentalization inside growing Escherichia coli. J Bacteriol 2001;183:7182–7189.
[102] Chen L, Xie QW, Nathan C. Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates. Mol Cell 1998;1: 795–805.
[103] Yang KS, Kang SW, Woo HA, Hwang SC, Chae HZ, Kim K, Rhee SG. Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid. J Biol Chem 2002;277: 38029–38036.
[104] Chae HZ, Uhm TB, Rhee SG. Dimerization of thiol-specific antioxidant and the essential role of cysteine 47. Proc Natl Acad Sci USA 1994;91:7022–7026.
[105] Woo HA, Bae SH, Park S, Rhee SG. Sestrin 2 is not a reductase for cysteine sulfinic acid of peroxiredoxins. Antioxid Redox Signal 2009;11:739–745.
[106] Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev 2004;18:1926–1945.

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