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Antagonistic pleiotropy and p53

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Introduction

Vertebrate species demonstrate great diversity in their maximum lifespans. These can range from just 8 weeks in the coral reef pygmy goby fish (Depczynski and Bellwood, 2005) to over 175 years in giant tortoises (de Magalhaes and Toussaint, 2002). There are even rare organisms, such as members of the genus *Hydra* (primitive freshwater invertebrates), that display no noticeable decline in fitness with age and are believed to be biologically immortal (Martinez, 1998). However, such cases of extreme longevity are rare in the wild as starvation or predation claim most animals before they have a chance to show discernable signs of aging. For this reason, it is generally assumed that genetics does not play a significant role in the aging process. However, a clear heritable component to aging has been described for a number of organisms, including humans (Antebi, 2007; Cournil and Kirkwood, 2001; Kenyon, 2005), and there is a growing list of so-called “longevity genes”.

The term “aging” is somewhat ambiguous and can refer to any change in an organism over time. For this discussion, we will define aging as *the decline in the ability of an organism to maintain tissue homeostasis over time*. At the molecular level, aging leads to the accumulation of cellular damage caused by various agents, such as reactive oxygen, nutritional stress, and spontaneous errors in DNA processing. Even the most basic unicellular organisms possess DNA repair machinery and antioxidant systems to protect from such damage. However, the maintenance and repair of damage to somatic cells requires significant metabolic resources (*i.e.* energy). The *disposable soma* theory of aging (Kirkwood, 1977) posits that, with a finite supply of energy, the body must compromise somatic maintenance in order to ensure reproductive success. This theory can explain why organisms that undergo rapid maturation and reach sexual maturity early in life pay for this advantage with imperfect cell and tissue repair and reduced longevity. George Williams’ *antagonistic pleiotropy* theory of aging (Williams, 1957) proposes that cellular damage and organismal aging are caused by pleiotrophic genes, or genes with multiple phenotypic effects. Most often, these genes increase the odds of successful reproduction early in life, but have deleterious effects later in life. The harmful effects of such genes often do not become apparent until after reproduction is complete, and as a result they cannot be eliminated by natural selection.

One gene that could exhibit antagonistic pleiotropy in humans is *p53*. *p53* confers protection against cancer (and death) by interrupting the abnormal proliferation of cells. This ability to interrupt cell proliferation, however, can be deleterious when it interferes with the proliferation of normal cells, such as stem cells, which are needed for tissue renewal as the organism ages.

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Are these deleterious effects of p53 later in life the unfortunate by-products of evolving tumor suppression mechanisms to ensure the survival of the species? We review how p53 regulates gene expression in response to signals that turn on its tumor suppressor activity. We reorient these genes within the context of aging and ask if changes to p53 that have beneficial effects on tumor suppression have deleterious effects on aging. This would argue that p53 exhibits antagonistic pleiotropy. Then, we use data from recently developed models of accelerated aging in mice to determine if the deleterious effects of p53 on aging are attributable to the *p53* gene or involve genes that have modifying effects on p53 activity but are evolving independently.

p53 and cancer

Human cancers have been recorded and studied for over 3500 years. Virtually every form of cancer contains clusters of affected families (Li, 1988), and yet the realization that cancer is a genetic disease was made only in the middle of the last century. Li-Fraumeni syndrome, an autosomal dominant disorder characterized by greatly increased susceptibility to a variety of neoplasms, is one of the earliest examples of cancer with a clear hereditary distribution. Genetic analyses of families affected with Li-Fraumeni syndrome revealed that mutations in the coding sequence of the p53 gene co-segregated with the disease phenotype (Srivastava et al., 1990). Similar p53 mutations have been found in a majority of sporadic cancer cases, (Hollstein et al., 1991) where both biallelic inactivation and single missense mutations are associated with a variety of cancer subtypes (Tokino and Nakamura, 2000). In mice, the loss of a single functional allele of p53 significantly increases the risk of cancer and virtually every mouse with homozygous deletion of p53 develops some form of tumor at a relatively young age (Donehower et al., 1992). These data from experimental models of cancer in mice and the very large number of tumors in humans demonstrate that *loss of p53 results in loss of tumor suppression*.

Introduction of an additional copy of the p53 gene in mice has the opposite effect. Serrano and colleagues used bacterial artificial chromosome-based technology to insert a large fragment of DNA containing the entire p53 genomic region as a transgene. On a wild-type p53 background, these “Super-p53 mice” have three doses of the gene and an enhanced resistance to cancer compared to wild-type mice with only two doses (Garcia-Cao et al., 2002; Matheu et al., 2007). Data from this mouse model demonstrate that a *gain of p53 results in an increased level of tumor suppression*.

p53 and aging

The literature on p53 since it was first described in 1979 (DeLeo et al., 1979) is enormous (44,747 entries to date in PubMed), but only a small proportion of the most recent articles deals with the possibility that the activity of p53 as a tumor suppressor might be linked to aging and longevity. A phenotype of accelerated aging and reduced life span in mice was reported in 2002 in association with a mutant (“m”) allele of *p53* that could enhance the ability of p53 to act as a tumor suppressor (Tyner et al., 2002). The *p53^m* allele was the result of a gene targeting event in which the first six exons of full-length p53, as well as unknown upstream sequences, were deleted. Mice with one *m* allele and one normal allele of p53 (*p53^{+m}*) express the unique p53 *m* transcript in all tissues tested. The *m* transcript can be translated in vitro and codes for a 24 kDa carboxyl (C)-terminal fragment of p53 (Fig. 1D). Despite intense effort, the M protein has not been detected in any tissues from *p53^{+m}* mice. Expression of the full-length p53 protein in *p53^{+m}* mice is essentially equal to that of *p53^{+/-}* mice, but undergoes a higher and more prolonged increase in response to ionizing radiation (Tyner et al., 2002). This is reflected in increased resistance to experimentally-induced and spontaneous tumors compared to either *p53^{+/+}* or *p53^{+/-}* animals. Homozygosity for the *m* allele is lethal (Gentry and Venkatachalam, 2005), in contrast to loss of both wild-type alleles. This indicates that the *m* allele is not simply

a null allele and that the product of *m* acts through wild-type p53. Furthermore, it strongly argues against the possibility that genes other than p53 that were co-deleted in the recombination event play a major role in the phenotype of p53^{+/*m*} mice, but the possibility cannot be ruled out completely (Gentry and Venkatachalam, 2005; Vijg and Hasty, 2005).

When p53^{+/*m*} mice are young, they are indistinguishable from their p53^{+/+} littermates. However, at about 18 months of age, they begin to display progeroid symptoms, such as weight loss, lordokyphosis (hunched posture), and a general lack of vigor. Both mean and maximum life spans are significantly reduced. The mechanism underlying the accelerated aging and early death of p53^{+/*m*} mice is not yet known. One possibility is suggested by the observation that tissues from p53^{+/*m*} animals, including skin, subcutaneous fat, spleen, kidney, and bone display increased atrophy and a reduced capacity for repair compared to age-matched p53^{+/+} tissues. This simultaneous degeneration of several different tissues could reflect a more general effect of p53 on the organism's ability to maintain tissue homeostasis as could happen, for example, if stem cells were affected. A loss of regenerative capacity in these cells would be easily explained by hyperactivity of p53 at the promoters of cell cycle arrest genes, which then would limit cell proliferation and the production of replacement cells. In fact, p53^{+/*m*} hematopoietic stem cells (HSC) were less able than p53^{+/+} HSCs to regenerate peripheral blood cells when transplanted into mice chemically depleted of endogenous HSCs (Dumble et al., 2007; Tyner et al., 2002).

The protein generated from the p53 *m* allele lacks the activator, proline-rich, and DNA binding domains that make up the amino (N)-terminus and core of the full-length protein (Fig. 1A). This M protein, therefore, is unable to alter gene transcription on its own, but it can enhance the trans-activation activity of wild-type p53. Of the nine known natural isoforms of p53 (reviewed in (Murray-Zmijewski et al., 2006), the p53^{*m*} protein is most similar in structure to Δ133p53, which likewise contains only the tetramerization domain, basic domain, and part of the DNA binding domain (Fig. 1C). Several laboratories have shown that another naturally occurring short isoform of p53, which has been called ΔNp53, p53/47, Δ40p53, and p44 (Fig. 1B; we will use Δ40p53), is likewise capable of altering the activity of wild-type p53 (Courtois et al., 2002; Yin et al., 2002). In the mouse, Δ40p53 is made from the same RNA transcript as full length p53, but utilizes an isoform-specific internal ribosomal entry site to initiate translation at codon 41 in exon 4 (Ray et al., 2006). Δ40p53 is 44 kDa in size and is identical to full-length p53 except that it lacks the first activator domain in the N-terminus of the protein (Fig. 1A and B; reviewed in (Scrable et al., 2005). Increased dosage of Δ40p53 in mice with the normal complement of p53 alleles (p44Tg mice) causes premature aging similar to that of p53^{+/*m*} mice, with a mean lifespan of less than 16 months compared to over 20 months for non-transgenic controls (Maier et al., 2004).

In contrast to the two strains of mice in which increased tumor suppression occurs in the context of decreased lifespan, Super-p53 mice undergo a normal course of aging and have normal longevity (Garcia-Cao et al., 2002). This discrepancy can be explained by differences in the regulatory sequences that surround the p53 coding region in p53^{+/*m*}, p44Tg, and Super-p53 mice. In Super-p53 mice, these sequences are identical to those at the endogenous p53 locus and p53 transgene expression is indistinguishable from that of endogenous p53. p53 is elevated only under circumstances of cellular stress, and (importantly) returns to low levels again when the stimulus is removed (Garcia-Cao 2002). In the case of p53^{+/*m*} and p44Tg mice, the sequences regulating p53 are not the same as the normal p53 gene. In the case of p53^{*m*}, a deletion has resulted in the juxtaposition of a new promoter and exon 7, and in p44Tg mice, an additional p53 coding unit has been generated in an entirely new chromosomal setting. As a result, the products of the mutant *m* allele or Δ40p53 are different not only in size, but also in how they are expressed under normal circumstances and when they are activated.

In all three strains, increased activation of p53 results in increased expression of *p21/Cip1/Waf1*, a p53 target gene (Matheu et al., 2007; Tyner et al., 2002; Maier et al., 2004). $\Delta 40p53$ has been found to enhance the trans-activation of other p53 targets as well, including *Mdm2* and *IGFBP-3*. The level of phosphorylation on p53 Ser15, a modification associated with activated p53 (Fiscella et al., 1993; Shieh et al., 1997), was also found to be elevated in p44Tg MEFs compared to MEFs derived from non-transgenic embryos (Maier et al., 2004). However, other p53-induced genes such as *Gadd45* and *PTEN* were found to be down-regulated in p44Tg cells, suggesting that $\Delta 40p53$ might alter p53 activity in a promoter-specific fashion. This hypothesis is further supported by fact that levels of the insulin-like growth factor-1 receptor (IGF-1R), which is normally transcriptionally suppressed by p53 (Werner et al., 1996), are elevated in p44Tg cells and tissues. Similar differential effects on repression and activation have been reported for human tumor-derived mutant forms of p53, which are defective at trans-repressing but not trans-activating p53 targets (Ryan and Vousden, 1998). This selectivity could be due at least in part to the tetrameric structure of the active p53 transcription factor in which, presumably, all monomers that have the tetramerization domain (Fig. 1, “TD”) participate (Nicholls et al., 2002). Inclusion of $\Delta 40p53$ or any of the other p53 isoforms lacking the N-terminal region (Bourdon et al., 2005; Courtois et al., 2002; Mills, 2005) would be expected to alter co-factor binding in much the same way as the glucocorticoid receptor, another multimeric transcription factor that has numerous isoforms (see (Yudt and Cidlowski, 2002) for a review). Isoforms lacking the N-terminus of p53 would also stabilize heterotetramers with full-length p53 by preventing proteolytic degradation mediated by Mdm2 (Michael and Oren, 2003), which binds to a site in the N-terminus of p53. The monomers themselves are much more stable than p53; for example, $\Delta 40p53$ has a prolonged half-life of 9.5 hours, 9 hours longer than full-length p53 (Lavigne et al., 1989). It is likely that $\Delta 40p53$, as well as the other p53 isoforms, are capable of altering p53 activity by additional mechanisms, and these are areas of very active research.

In summary, in both p53 models with negative life span effects, *a gain of p53 function results in increased tumor suppression and decreased longevity*. Super-p53 mice, on the other hand, have increased tumor suppression without deleterious consequences on life span and may, in fact, live longer than wild type mice (Matheu et al., 2007). We have looked at the molecular level to determine if this reflects antagonistic pleiotropy of p53 or aberrant activity of an unlinked gene that regulates p53.

Effectors of p53 tumor suppression

p53 is a transcription factor that binds DNA in a sequence specific manner (Kern et al., 1992). The p53 protein is composed of several essential functional domains (Fig. 1). In addition to the TD mentioned previously, there is a central domain (DBD) that binds to specific DNA sequences (the so-called “p53 recognition element”) in p53 target genes; the region also contains recognition motifs for protein—protein binding to cofactors such as CBP/p300 (Avantaggiati et al., 1997; Lill et al., 1997), mSin3a (Murphy et al., 1999), or TFIID (Farmer et al., 1996). The N-terminus of p53 contains two activator domains (AD1 and AD2), which bind essential components of the transcription machinery. The C-terminus of the protein consists of the TD and a basic domain (BD), where multiple lysine residues are subject to acetylation and ubiquitination, as well as other post-translational modifications, that regulate p53 function. The function of p53 in tumor suppression involves the trans-activation or trans-repression of a large number of genes involved in cell cycle regulation and apoptosis. We will limit our discussion to two of these genes, the cyclin-dependent kinase (cdk) inhibitor p21 and the insulin-like growth factor receptor (IGF-1R).

p21/Cip1/Waf1

p21, alternatively referred to as Cdk-interacting protein-1 (CIP1) or wild-type p53-activated fragment-1 (WAF1), is a potent mediator of both cell cycle arrest and apoptosis. The role of p21 in apoptosis is currently poorly understood due to the highly variable effects of environment and cell type on the apoptotic response (Gartel and Tyner, 2002) and will not be dealt with further in this review. Although unstressed cells can produce p21 by p53-independent methods, p53 appears to be essential for the upregulation of p21 following DNA damage (Macleod et al., 1995). Genomic integrity is maintained in proliferating cells by a series of cell-cycle checkpoints, which transiently halt the cell cycle while the DNA damage is repaired. Checkpoint proteins like p21 can interact with cell cycle proteins, such as cyclin-dependent kinases (Cdks), and block the activity of cyclin/Cdk complexes. At low levels, p21 preferentially binds cdk4–6/cyclin D complexes and promotes progression to the S-phase of the cell cycle. However, when p21 levels are high, after DNA damage for example, it binds to cdk2/cyclin A-E complexes and prevents the exit from G1 into S (Chen et al., 1996; el-Deiry et al., 1994; Noda et al., 1994; Sherr and Roberts, 1999). p21 can also pause the cell cycle during S-phase by inhibiting proliferating cell nuclear antigen (PCNA), which is required for replicative DNA synthesis and DNA repair (Gartel and Radhakrishnan, 2005).

Mice lacking p21 develop normally (Deng et al., 1995), but demonstrate increased in spontaneous tumor development beginning at about 16 months of age (Martin-Caballero et al., 2001). p21 deletion accelerates the proliferation of a variety of normal cell types, including neuroblasts, forebrain stem cells, HSCs, hepatocytes, airway epithelial cells, and Sertoli cells (Blundell et al., 2004; Cheng et al., 2000; Holsberger et al., 2005; Kippin et al., 2005; Pechnick et al., 2008; Sheahan et al., 2007). However, although hematopoietic stem cells from p21-deficient mice are more mitotically active than HSCs from p21^{+/+} mice under normal homeostatic conditions, they fail to proliferate following exposure to cell cycle-specific myelotoxic injury and are less able to repopulate the hematopoietic system of irradiated recipient mice (Cheng et al., 2000). This is because p21 maintains HSCs in a quiescent (G₀) state (Cheng et al., 2000). In the absence of p21, stem cells enter the cell cycle as transit amplifying cells, proliferate, differentiate, perform their normal functions, and then die. Failing to self-renew, they ultimately exhaust their regenerative potential. On the other hand, cancer cells lacking p21 go through the same process, but, having lost the need to self-renew, continue to proliferate indefinitely (Waldman et al., 1995). These results demonstrate that, without p21, not only would tumor risk increase, but also tissue homeostasis would be impaired as p21-deficient stem cells are prematurely exhausted and the replacement cells they give rise to are depleted. Increased p21, on the other hand, would clearly be beneficial as a tumor suppressor mechanism. However, high levels of p21, although able to reduce the expansion of tumor cells, can also have the deleterious effect of inhibiting the proliferation of normal cells, for example lymphocytes (Khanna et al., 2005; Makri et al., 1998). Thus, *in addition to its beneficial effects on tumor suppression, increased p21 could have deleterious effects on life span* by blocking stem cell proliferation and preventing the generation of replacement cells needed to maintain tissue homeostasis.

The insulin-like growth factor receptor (IGF-1R)

The IGF-1R is a membrane-bound heterotetramer with ligand-induced tyrosine kinase capacity (LeRoith et al., 1995; Werner et al., 1989). It is constitutively expressed in most tissues and plays a central role in regulation of cell proliferation, growth, survival, differentiation, and cell motility (Larsson et al., 2005). IGF-1, and to a lesser extent, IGF-2, bind the extracellular portion of the IGF-1R and initiate a phosphorylation cascade which ultimately leads to activation of three major intracellular signaling pathways: the PI3K—AKT—mTOR/FOXO pathway; the Ras—Raf—MEK—ERK pathway; and the 14-3-3 proteins (Baserga, 2000).

The level of the IGF-1R is controlled by several tumor suppressors including WT-1, BRCA1, and p53 (Abramovitch et al., 2003; Werner et al., 1995; Werner et al., 1996). p53 suppresses IGF-1R activity by reducing endogenous levels of IGF-1R mRNA (Werner et al., 1996) and by stimulating the transcription of IGF binding protein-3 (IGFBP-3), which inhibits ligand-induced activation of the IGF-1R (Buckbinder et al., 1995). Loss of function mutations in p53 are associated with over-expression of the IGF-1R and an increased risk of tumorigenesis (Werner et al., 2000). High serum levels of the IGF-1 ligand are associated with an increased risk of prostate and breast cancers in human population studies (Renehan et al., 2004). Furthermore, *in vivo* over-expression of IGF-1R accelerates the rate of tumor development and produces more invasive and distant metastases in a mouse model of pancreatic cancer (Lopez and Hanahan, 2002).

Mutations that decrease the level of the IGF-1R or block signal transduction from the receptor are tumor suppressive, but are also significant effectors of lifespan extension. Mutations in the *daf-2* and *age-1* genes, which are the orthologs of mammalian IGF-1R and PI-3 kinase, respectively, can extend mean lifespan in the nematode *Caenorhabditis elegans* by as much as 100% (Friedman and Johnson, 1988; Kenyon et al., 1993). Mutations in the *Drosophila melanogaster* insulin receptor, *InR*, and in the insulin receptor substrate *CHICO* likewise cause significant improvements in longevity (Clancy et al., 2001; Tatar et al., 2001). Haploinsufficiency for the *Igf-1r* gene or deletion of *Irs2*, which codes for insulin receptor substrate-2, confer significant increases in life span in the mouse (Holzenberger et al., 2003; Taguchi et al., 2007). And in humans, a cohort study of elderly Dutch citizens revealed that genetic variations causing reduced insulin/IGF-1 signaling were beneficial for old age survival (van Heemst et al., 2005). These data indicate that *reduced IGF-1R or IGF-1 signaling, in addition to suppressing the growth of tumor cells, has beneficial effects on life span*. This is different from p21, where there are beneficial effects of increased p21 on tumor suppression, but deleterious effects of increased p21 on life span.

Trans-activation and trans-suppression by p53—molecular mechanisms

The p21 promoter contains five p53 binding sites and is directly regulated by p53 (el-Deiry et al., 1993; Ocker and Schneider-Stock, 2007; Xiong et al., 1993), as illustrated in Fig. 2A. Excess p53 activity results in elevated levels of p21. This has beneficial effects on tumor suppression, but can also have deleterious effects on the proliferation of stem cells, limit the maintenance of tissue homeostasis, and provoke aging. This could be an example of antagonistic pleiotropy caused by p53.

The *Igf-1R* promoter has no identifiable p53 recognition sites. p53 suppresses *Igf-1R* transcription by sequestering TBP, the TATA-box binding subunit of the general initiation factor TFIID. The interaction of p53 with TBP blocks the formation of transcription factor complexes on the *Igf-1R* promoter, repressing IGF-1R transcription (Fig. 2B) (Werner et al., 1996). In the absence of p53, TBP is free to initiate IGF-1R expression (Fig. 2C). Repression of IGF-1R expression by p53 is a powerful tumor suppressor mechanism that limits the growth of tumors by blocking signal transduction cascades that turn on RNA and protein synthesis. Because decreased activity of the insulin/IGF-1 receptor signaling cascade also promotes longevity, increased p53 activity at the IGF-1R promoter would increase tumor suppression and delay aging. In this case, therefore, the effect of p53 on gene activity would not appear to exhibit antagonistic pleiotropy.

The phenotype of p44Tg and p53^{+/m} mice is consistent with a model in which excess p53 enhances trans-activation of p21 (Fig. 2A) and elevated levels of p21 block the proliferation of both tumor cells and normal tissue stem cells. However, instead of enhancing trans-repression of the *Igf-1R* promoter (Fig. 2B), suppressing insulin/IGF signaling, and delaying aging, excess p53 is linked to loss of trans-repression (Fig. 2C), increased insulin/IGF

signaling, and accelerated aging. This would happen if p53 were altered in such a way that it could not interact normally with transcription complexes assembled on promoters of its target genes. It is difficult to reconcile with a model in which there was simply a change in p53 level.

Switching from trans-suppressor to trans-activator

The interactions that p53 makes with other proteins, including other transcription factors and co-factors, are dependent on post-translational modifications to the p53 protein (extensively reviewed in (Bode and Dong, 2004; Lu, 2005; Toledo and Wahl, 2006). There are currently nine recognized types of post-translational modifications to p53: phosphorylation, acetylation, ubiquitination, glycosylation, ribosylation, methylation, sumoylation, neddylation, and redox regulation. These modifications are transiently conferred to p53 following environmental stresses, such as DNA damage, hypoxia, or nucleotide depletion. The best studied are the phosphorylated residues, which include a cluster of serines in the N-terminus (Fig. 1A, yellow symbols) associated with p53 activation and scattered sites on serines and threonines in other domains (reviewed in (Toledo and Wahl, 2006).

In the C-terminus, there is a series of lysine residues (Fig. 1A, green symbols) where acetyl groups can be added by several different histone/protein acetyl transferases (HATs): CBP/p300 (Gu and Roeder, 1997), p300/CBP associated factor (PCAF) (Liu et al., 1999), hMOF (Sykes et al., 2006), or TIP60 (Tang et al., 2006), with each HAT targeting specific residues in response to different kinds of cellular stress. For example, acetylation of Lys320 by CBP/p300 promotes the interaction of p53 with high-affinity binding sites regulating genes that support cell cycle arrest and survival. Acetylation of Lys373 by the same enzyme, on the other hand, targets p53 to low-affinity binding sites, which are found in the promoters of proapoptotic genes that initiate cell death (Knights et al., 2006). Deacetylation of these and the other acetylated residues in the C-terminus of p53 is performed by histone deacetylase (HDAC)1-containing complexes (Juan et al., 2000) or by members of the silent information regulator-2 (SIR2) family of NAD⁺ dependent HDACs (Luo et al., 2000; Vaziri et al., 2001).

There are several ways by which acetylation can alter the activity of p53. First, acetylation causes a conformational change in the protein that exposes the DNA-binding domain, thereby allowing p53 to bind target promoters (Gu and Roeder, 1997; Sakaguchi et al., 1998). Second, acetylation prevents ubiquitination by Mdm2 and stabilizes the protein, thereby promoting p53 activation (Liu et al., 1999). Finally, the combinatorial nature of multiple lysine residues and the modifications each combination makes to the conformation of p53 confers specificity on the choice of promoter p53 can regulate. Acetylation of p53 has been reported to increase the trans-activation capacity of p53 while at the same time reducing its trans-repression functions. The interaction of p53 with HATs such as CBP/p300 and PCAF is critical for trans-activation (Avantaggiati et al., 1997; Lill et al., 1997), while the association of p53 with HDACs seems to be necessary for gene trans-suppression but not trans-activation. Repression also requires an intact N-terminus (Borger and DeCaprio, 2006; Murphy et al., 1996) and the co-repressor mSin3a, which mediates the interaction of p53 with HDAC complexes (Murphy et al., 1999).

The differential effect of acetylation on suppression and activation by p53 was demonstrated for the first time on the promoters of the *p21* and *stathmin* genes (Murphy et al., 1999; Murphy et al., 1996). DNA damage by adriamycin (doxorubicin) significantly induced expression of *p21* while it suppressed expression of *stathmin*. Trichostatin A (TSA), a potent HDAC inhibitor, completely blocked DNA damage-induced trans-repression of *stathmin* (Murphy et al., 1999), but had little effect on trans-activation of *p21*. This difference can be explained in terms of the effect of TSA on HATs and HDACs. TSA treatment enhances the interaction between p53 and HATs, causing the level of acetylated p53 to rise and stabilizing the transcription complex (increased trans-activation). At the same time, TSA blocks the

association of p53 with HDAC complexes, inhibiting deacetylation and preventing p53 from interrupting an active transcription complex (decreased trans-suppression). TSA has a similar effect on the p53-repressed genes *Map4* (Murphy et al., 1999) and *Igf-1R* (Kavurma et al., 2007).

The differential effect of acetylation on p53 function can explain how $\Delta 40$ p53 causes both an increase in expression of the *Igf-1R* and an increase in the expression of *p21* in mice. This is illustrated in Fig. 3, where S represents a serine residue in the N-terminus and P its phosphorylated form, and K1 and K2 represent two different acetylation sites in the C-terminus of p53. In addition, K2 represents a site where acetylation requires prior phosphorylation in order to be acetylated. In this model, acetylation at K1 turns the *Igf-1R* promoter on, and acetylation at K2 induces expression of *p21*. In Fig. 3A, phosphorylation of S15 promotes acetylation of K2 and induces expression of *p21*. *Igf-1R* expression is turned off because K1 is deacetylated. In Fig. 3B, acetylation of K1 turns on *Igf-1R* expression. *p21* is not induced because S15 is dephosphorylated and K2 is deacetylated. In Fig. 3C, the conditions for acetylation to occur at both sites are met and both promoters are activated. In $\Delta 40$ p53, the critical phosphorylation site at S15 that stimulates the interaction of p53 with p300/CBP and facilitates the acetylation of certain lysine residues in the C-terminus (Lambert et al., 1998; Dumaz and Meek, 1999) is missing. In tetramers with full-length p53, therefore, $\Delta 40$ p53 monomers would change the availability of substrates for post-translational modifications that determine the ability of p53 to bind to a specific promoter. In response to an event that phosphorylates S15, like genotoxic stress, acetylation at sites dependent on S15 phosphorylation, like K2 in Fig. 3, would be disrupted. If K2 is the site where acetylation prevents p53 from blocking transcription of the *Igf-1R* gene, trans-repression would be lost and the promoter would be activated (de-repressed). Sites like K1, where acetylation is independent of S15 phosphorylation, however, would be unaffected. If K1 acetylation induces *p21* expression, then even in the presence of $\Delta 40$ p53, induction would occur. The overall result would be loss of *Igf-1R* trans-repression and gain of *p21* trans-activation, which is indeed the case with p44Tg mice and cells (Maier et al., 2004). Therefore, we favor the idea that the deleterious effects on aging in these mice are due changes in post-translational modifications that interfere with the plasticity of p53 in transcription complexes. Furthermore, the association of the loss of plasticity with changes in $\Delta 40$ p53 suggests that each p53 isoform must be precisely regulated in order to maintain normal p53 function over the lifetime of the organism.

How p53 acetylation status “ages”

If changes in post-translational modifications can affect how p53 trans-activates and trans-represses its target promoters, then age-associated changes in the enzymes responsible for modifying p53 could have deleterious effects on aging similar to those caused by $\Delta 40$ p53. One of the enzymes responsible for the acetylation state of p53 is the NAD⁺-dependent deacetylase SIRT1. The level of SIRT1 is regulated by a feedback loop with p53 in which p53 binds to and represses the *SIRT1* promoter and SIRT1 deacetylates and inactivates p53 (Cohen et al., 2004; Nemoto et al., 2004). This feedback loop is completed by activated HATs, which re-acetylate and activate p53 under conditions of genotoxic or oxidative stress (Saunders and Verdin, 2007). *SIRT1* is the human homologue of *Sir2*, which codes for an enzyme that links replicative aging to nutrient availability in *S. cerevisiae*. Under conditions of nutrient deprivation, yeast cells stop dividing and enter a quiescent state that allows them to survive until nutrients become available again. Increased expression of SIR2 promotes increased replicative life span in yeast (Kaeberlein et al., 1999), and its orthologues, Sir2.1 in *C. elegans* and dSir2 in *D. melanogaster*, promote increased longevity in worms (Tissenbaum and Guarente, 2001) and flies (Rogina and Helfand, 2004). We have recently reported that mammalian cells undergoing replicative senescence, the phenomenon by which primary cells in culture lose their ability to divide (Hayflick and Moorhead, 1961), show a decline in SIRT1

(Sasaki et al., 2006). In human fibroblasts, the levels of both HDAC1 and SIRT1 vary inversely with donor age (Kim et al., 2004). Low SIRT1 activity in human cells has been found to increase p53 acetylation, increase the expression of *p21* mRNA, and cause premature replicative senescence (Ota et al., 2007).

In vivo, the level of SIRT1 in organs with high mitotic activity, such as the testis and thymus, declines with age (Sasaki et al., 2006). In mice exhibiting accelerated aging (such as p44Tg mice), this decrease takes place more rapidly, and in long-lived mice (such as GHRKO mice) it occurs more slowly than in mice that age normally (Sasaki et al., 2006). Emerging data suggest that manipulating SIRT1 levels in mice might increase longevity. Transgenic mice over-expressing SIRT1 appear to age like mice subjected to a regimen of calorie restriction (Bordone et al., 2007) and SIRT1 activators can ameliorate the detrimental effects on life span caused by the consumption of excess fat (the so-called “western diet”) (Baur et al., 2006). These data indicate that increased SIRT1 is associated with longer life span and decreased SIRT1 with shorter life span.

The significance of these findings is that they link p53 activity and acetylation state with the level of SIRT1 and age. The effect of age on p53 or SIRT1, for example, might explain why the level of *Igf-1R* mRNA is higher in the hippocampus of old (29-month old) rats compared to young (2-month old) rats (Stenvers et al., 1996) or why *Igf-1R* expression is higher in senescent human IMR-90 fibroblasts compared to early passage cells (Chen et al., 2005). If post-translational modifications that modulate p53 activity change with time, then age can alter how p53 can and does respond to changes in the environment.

Summary and Model

Increased longevity is associated with increased SIRT1 (in yeast, worms, and flies) and decreased IGF-1R (in worms, flies, and mice). However, tumor suppression by p53 is enhanced by decreased SIRT1 (because acetylated p53 induces p21, for example) and decreased IGF-1R. This means that changes that decrease SIRT1 (and increase p53 acetylation) decrease the risk of developing tumors, but increase the risk of aging (Fig. 4). The deleterious effects of p53 on aging are not attributable solely to the p53 gene, but involve at least one other gene (SIRT1) that has modifying effects on p53 activity but is evolving independently.

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Figure 1. p53 protein isoform structure

The amino acid positions denoted above each isoform correspond to the murine protein sequence. Arrows mark the regions containing important sites for phosphorylation in the N-terminus or acetylation in the C-terminus for each isoform. Functional domains are coded by the following colors: activator domain 1 (AD1), yellow; activator domain 2 (AD2) and the proline-rich domain (PRD), red; DNA-binding domain (DBD) and nuclear localization signal (NLS), blue; tetramerization domain (TD), grey; and the C-terminal basic domain (BD), green.

(A) Full-length p53 initiates at codon 1.

(B) Δ40p53 initiates at codon 41 and is lacking most of AD1.

(C) Δ133p53 initiates at codon 133 and lacks both AD1 and AD2, as well as the PRD and part of the DBD.

(D) The mutant protein p53^m initiates at codon 246. It is similar in structure to the naturally-occurring Δ133p53 isoform, but lacks an additional 113 amino acids from the DBD.

Figure 2. Target gene activation and suppression by p53 (see text for full description)

The p53 protein is represented in red and post-translational modifications to the protein are shown with yellow (phosphorylated serine) or green (acetylated lysine) symbols.

A) p53 binds directly to the *p21* promoter (grey) as a tetramer to activate p21 expression. Phosphorylation of the N-terminus and acetylation of K2 in the C-terminus of p53, as well as direct binding with the HAT CBP (orange) is required for this interaction.

B) p53 binds to TBP (the TATA binding subunit of TFIID; purple) and prevents transcription complex assembly on the *Igf-1R* promoter. This suppresses IGF-1R expression (OFF).

C) Acetylation of p53 on a different lysine residue (K1) prevents p53 from interacting with TBP and allows transcription of the IGF-1R (ON).

Figure 3. Acetylation has differential effects on p53 trans-activation and transsuppression (see text for full description)

The p53 protein is represented in red and post-translational modifications to the protein are shown with yellow (phosphorylated serine) or green (acetylated lysine) symbols.

A) Following N-terminal phosphorylation of p53, K2 is acetylated. This activates *p21* and suppresses *Igf-1R* expression.

B) In the absence of N-terminal p53 phosphorylation, K1 is acetylated. This results in normal (basal) *p21* expression, but loss of trans-suppression (increased expression) of the *Igf-1R*.

C) When both K1 and K2 of p53 are acetylated, there is expression of both *p21* and the *Igf-1R*.

Figure 4. Coordinate regulation of cell proliferation by p53 and SIRT1

Model illustrating the relationship between p53 protein level, acetylation status, aging and tumor suppression. The activity of p53 (x -axis) is affected by both the level of the protein (**blue**) and its acetylation status (**red**). Cell proliferation (y -axis) is a function of p53 activity. Cell proliferation is high when p53 activity is low, either because the protein is scarce or because it is inactivated by SIRT1. In this situation, the risk of cancer is high. Conversely, when p53 activity is elevated due to high levels of acetylated protein, proliferation is too low to maintain tissue homeostasis and longevity is compromised. When SIRT1 activity is diminished, for example during aging, p53 will remain in its acetylated and activated state, so that even low levels of protein can lead to deleterious effects on gene expression.