The Arf/p53 Pathway in Cancer and Aging

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Abstract

Arf and p53 are regarded among the most relevant tumor suppressors based on their ubiquitous and frequent inactivation in human cancer. The Arf/p53 pathway protects cells against several types of damage and this is the basis of its tumor suppressor activity. Interestingly, aging is a process associated with the accumulation of damage derived from chronic stresses of small magnitude. In agreement with its damage protection role, it has been recently described that the Arf/p53 pathway not only protects mammalian organisms from cancer but also from aging. However, there is also evidence that p53, under certain circumstances, such as when constitutively active, can induce aging. We discuss here the current evidence linking the Arf/p53 pathway to the process of aging and present a unified model. [Cancer Res 2008;68(15):6031–4]

Background and Introduction

The ability of p53 to induce cell growth arrest and apoptosis is relatively well-understood, and its importance in tumor suppression is firmly established. p53 is a major sensor of stress whose main biological activity is to activate the transcription of a variety of genes involved, among other processes, in blocking cell proliferation and triggering apoptosis. The transcriptional activity of p53 is highly induced in response to many forms of cellular stress, including DNA damage, oncogene activation, hypoxia, and nutrient deprivation. The activity of p53 is finely tuned by posttranslational modifications, protein-protein interactions, and protein stabilization. Mdm2 has long been considered a major p53 regulator that inhibits p53 mainly through ubiquitination followed by proteasomal degradation (1). Mdm2 itself is transcriptionally activated by p53, thus creating a negative feedback loop that maintains p53 at very low levels in the absence of cellular damage. Upon stress, particularly DNA damage, both p53 and Mdm2 are phosphorylated by ATM/ATR and other kinases in a manner that prevents Mdm2 from interacting with p53, thus resulting in p53 stabilization (1). Another mechanism to stabilize p53 is by direct inhibition of Mdm2 ubiquitin ligase activity through its association with Arf (known as p14Arf in humans and p19Arf in mice).

Arf was originally identified as an alternative transcript of the Ink4a tumor suppressor locus (2). Its expression levels are normally low, but transcription of Arf is highly induced when oncogenes are introduced into normal cells. On this basis, Arf is regarded as a protein specialized in communicating to p53 what has been called "oncogenic stress," a term that encompasses the array of cell perturbations produced by oncogenes (3). The relevance of the Arf/p53 tandem is well-supported by genetic evidence in mice. A general observation in murine experimental systems is that the absence of Arf seriously compromises the activity of p53 and relieves the pressure to inactivate p53 during tumorigenesis (4). Similar observations have been made in some human cancers where the inactivation of Arf and p53 occurs in a mutually exclusive manner (5, 6). However, compared with murine cancers, human cancers may bear higher levels of DNA damage that could contribute to activate p53 independently of Arf (7, 8). Therefore, although in mice Arf seems to be critical for p53 tumor suppression, in humans, Arf may share this role with DNA damage signaling and perhaps with other signaling pathways. In any case, the Arf/p53 axis, regardless of its relative importance in human versus mouse, is a relevant mediator of the p53 tumor suppressor activity.

Activation of p53 elicits a cellular response that depends, in a complex manner, on the intensity of the triggering stress and on the cellular context. The most important cellular responses to p53 are as follows: (*a*) restoration of cellular homeostasis, for example, by a transient blockade of the cell cycle to allow for DNA repair; (*b*) cellular senescence; and (*c*) apoptosis (9). The latter two responses are terminal and essentially irreversible, thus preventing the damaged cell from further propagation. We have recently discussed senescence in detail (10); we emphasize here that cellular senescence should not be necessarily assimilated to aging. First, senescence is conceptually similar to apoptosis in that it prevents the propagation of damaged cells, thus restricting tissular damage; and, second, senescent cells may be efficiently cleared from tissues by phagocytes (11). Thus, it cannot be inferred that p53 activation is proaging simply based on the induction of cellular senescence. Below, we summarize the current available evidence supporting either a proaging or an antiaging activity of p53, and we try to present a coherent picture encompassing both sets of apparently conflicting observations.

Proaging Activity of p53

Two mouse models have unveiled a proaging activity of p53 (12, 13). These two mouse models share expression of a truncated form of p53 that lacks the Mdm2-binding domain, thereby releasing p53 from its main negative regulator. Another particularity of these two mouse models is the fact that p53 is not under its natural transcriptional control, although the contribution of this factor to the final phenotypes remains to be established. Interestingly, these mice show cancer protection and accelerated aging. The premature aging of mice carrying Mdm2-insensitive p53 has been explained by the excessive loss of cells due to the exacerbated and constitutive activation of p53, which could have its most deleterious effects on adult stem cells (14).

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A proaging activity of p53 has also been unveiled by studying mice with constitutively high levels of endogenous damage, such as those deficient in BRCA1 or those with a defect in the nuclear envelope that recapitulates the human progeroid Hutchinson-Gilford syndrome (15, 16). These mice undergo premature death in association with rampant cellular senescence, and both phenotypes are largely alleviated in the absence of p53 (15, 16). Together with the mouse models discussed above, it can be concluded that permanent activation of p53, either by loss of Mdm2 regulation or by constitutive DNA instability, is deleterious, exhausts the renewal capacity of tissues, and results in premature aging (Fig. 1, *far left*).

Antiaging Activity of p53

Other investigations, including our own, have generated genetically manipulated mice with a modest increase in normally regulated p53. This is the case of mice carrying transgenic alleles consisting of large genomic segments containing the intact p53 gene (17) or the intact Arf gene (18), or mutant mice with partially reduced Mdm2 activity (19). It is important to emphasize that in all these mice, p53 retains its natural regulation, yet its levels are moderately increased. These mice show increased cancer protection and, interestingly, do not suffer from premature aging. Moreover, the presence of the transgenic p53 allele in mice deficient in telomerase does not aggravate the accelerated aging of telomerase-deficient mice (20).

In an attempt to further increase the activity of p53 in mice, we recently combined in the same mouse strain the transgenic p53 allele and the transgenic Arf allele to generate doubly transgenic Arf/p53 mice (21). Combination of these two alleles had a significant cooperative effect on the cancer resistance of these mice when compared with the single transgenic mice, further supporting the functional connection between Arf and p53 in tumor suppression (21). Remarkably, the concomitant increase of Arf and p53 resulted in a measurable and significant increase in median longevity, which was increased by 16%, a magnitude of importance when compared with other mouse models of increased longevity (21). It must be emphasized that the effect on longevity was genuinely due to delayed aging and not to cancer protection because when longevity was compared among the mice that did not die of cancer, the doubly transgenic Arf/p53 mice still presented the same increased longevity (21).

We have also generated mice carrying two copies of the transgenic Arf allele (that is, homozygous transgenic Arf/Arf mice), which in total carry four alleles of Arf (the two endogenous alleles and the two transgenic alleles). Interestingly, these mice not only have an increased resistance to cancer compared with singly transgenic Arf mice, but they also present an extended average life span and delayed aging.[3] Arf is known to possess p53-independent activities (22) and it remains to be elucidated whether the antiaging activity of Arf is partially or completely mediated by p53. A compounding issue is the fact that the Arf transgenic allele also includes the Ink4a gene (21). As we have discussed elsewhere (10), Ink4a could be a proaging gene and consequently the antiaging activity of Arf in our transgenic mice could be partially counterbalanced and, hence, underestimated. In any case, increasing the dosage of Arf above a certain level can measurably delay aging.

[3] Our unpublished data.

A number of additional observations have recently supported a positive association between p53 activity and antiaging protection, or, conversely, between decreased p53 activity and aging. Mice with an engineered mutation in Serine 18 (p53-Ser18Ala), a residue phosphorylated upon p53 activation, are partially unprotected from cancer and, importantly, present an accelerated aging (23). Also, the ability of p53 to respond to stress declines significantly with aging, and the time of onset of this decline correlates with the life span of mice; thus, long-lived mouse strains retain p53 activity longer than short-lived mouse strains (24). Finally, diverse mutations that increase longevity in the worm *Caenorhabditis elegans* also increase p53 activity (25). Altogether, this new evidence supports a scenario where p53 actively participates in postponing aging. We propose that p53 is at the crossroads of cancer and aging, simultaneously contributing both to cancer protection and to aging protection, and thus providing a rationale for the coevolution of cancer resistance and longevity across species.

The mechanisms underlying physiologic aging are still under investigation, the free radical theory of aging being one of the oldest and more thoroughly investigated. Recently, it has been shown that basal levels of p53, in the absence of severe stress, upregulate several antioxidant genes, most notably Sestrins 1 and 2, whose products decrease the intracellular levels of reactive oxygen species. This translates into a reduced rate of aging-associated accumulation of oxidative damage (26). In agreement with this, we observed that doubly transgenic Arf/p53 mice are protected against oxidative stress (21), and this could explain, at least to some extent, the antiaging activity of the Arf/p53 tandem. A related process that may contribute to the longevity of the doubly transgenic Arf/p53 mice is a most efficient elimination of cells. Indeed, the accumulation of DNA damage (visualized as nuclear foci of γH2AX) with aging is slowed in doubly transgenic Arf/p53 mice compared with single-transgenic or wild-type mice (21).

In summary, a variety of evidence in animal models supports the concept that the Arf/p53 tandem, in addition to its well-established cancer protection activity, also provides antiaging activity, probably by alleviating the load of age-associated damage (Fig. 1, *far right*).

Genetic Evidence in Humans

A separate mention must be made of the evidence obtained in humans linking p53 to aging. Polymorphic variations that result in codon changes can cause important alterations in protein activity. Regarding p53, much attention has been devoted to the analysis of a frequent polymorphism that affects codon 72 of human p53. Compared with the most common variant p53-Arg72 (Arginine), carriers of the p53-Pro72 (Proline) have a higher risk of cancer, yet, if not succumbing to cancer, enjoy an extended longevity (27). At face value, these correlations suggest that lower p53 activity translates into postponed aging. However, analyses of the mechanisms underlying these observations present a complex picture. Molecular studies *in vitro* have shown that p53-Arg72 has higher apoptotic potential than p53-Pro72 (28, 29); but, on the other hand, p53-Pro72 is more active than p53-Arg72 in inducing cell cycle arrest and senescence (30, 31). Therefore, it appears that the proarrest/prosenescence variant p53-Pro72 is associated with increased longevity, whereas the proapoptotic variant p53-Arg72 is associated with decreased longevity. In light of the recently

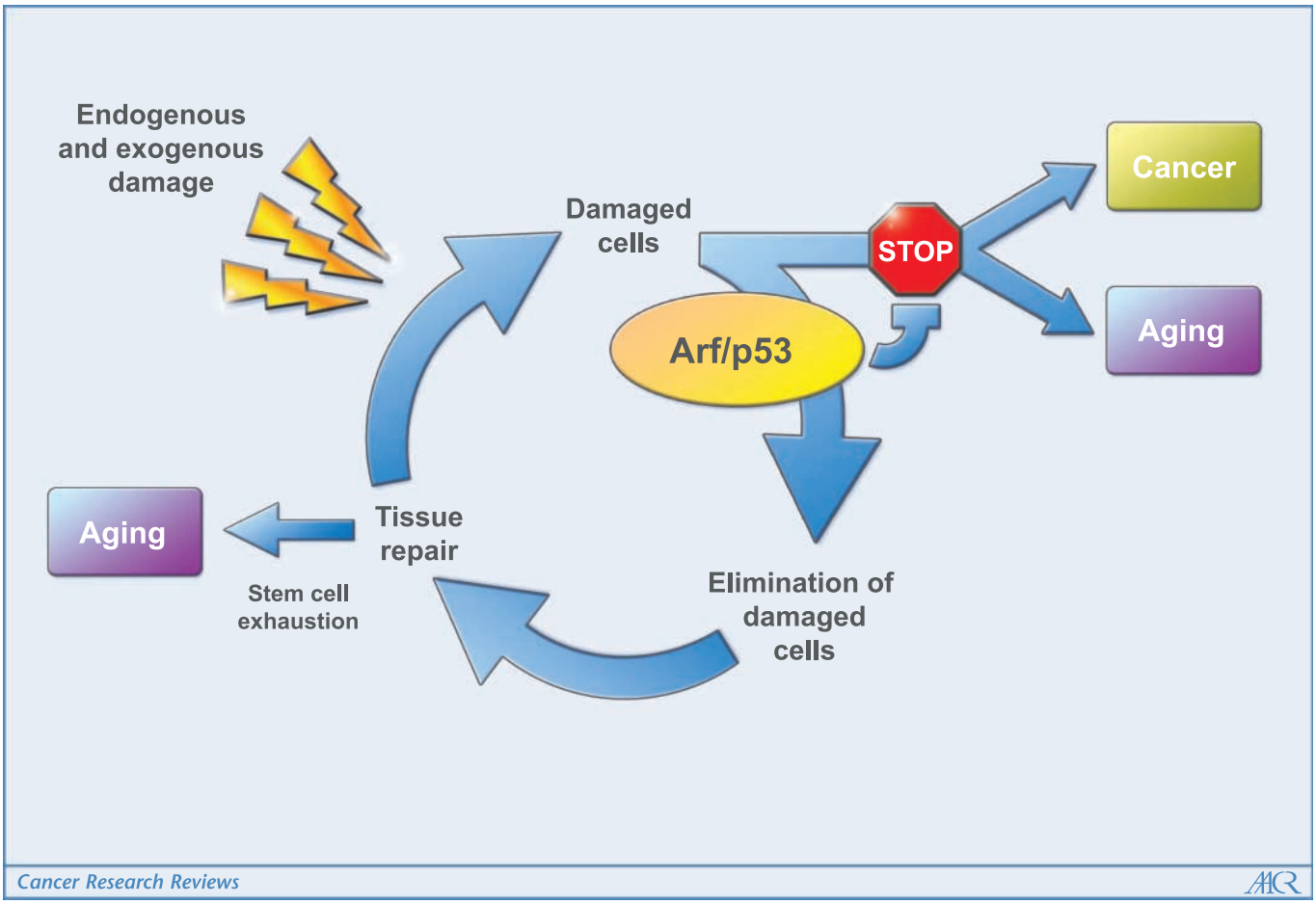

Figure 1. The Arf/p53 pathway in cancer and aging. Cancer and aging are both initiated by the accumulation of cellular damage. The Arf/p53 pathway plays a central role in the detection and elimination of damaged cells. Accordingly, modest and regulated increments in Arf/p53 activity are antiaging (*far right*). However, the elimination of damaged cells implies tissue regeneration and, if the Arf/p53 pathway is constitutively active, it may eventually lead to a premature exhaustion of the stem cell pools, thus accelerating aging (*far left*).

discovered antioxidant targets of p53 (see above), it would be interesting to compare the relative antioxidant activity of the two p53 variants at codon 72 and, more generally, a genome-wide understanding of the transcriptional programs triggered by them. Along these lines and with a larger scope, the transcriptional programs triggered by the various p53 splicing isoforms (32), as well as how they are affected by physiologic aging remain to be determined. Another piece to this puzzle is the observation that, contrary to expectations (16), cells from patients with Hutchinson-Gilford premature aging disorder do not have enhanced p53 activity (33).

Polymorphisms at the human Mdm2 gene promoter have also been found that result in higher levels of Mdm2 and, hence, a lower activity of p53 and an increased risk of certain cancers (34), but their relation to aging has not yet been explored. Finally, polymorphisms in the vicinity of the Arf gene have been linked to aging (35), although the direct implication of Arf, or alternatively a neighbor gene, such as Ink4a, remains to be determined, as well as their effect on cancer susceptibility.

In summary, the above-described observations indicate that the evidence linking the Arf/p53 tandem to human aging (either contributing to aging or delaying aging) is still inconclusive. Future studies will undoubtedly clarify this exciting area of research.

A Unified Picture

Genetic evidence in mice indicates that deregulated constitutive activation of p53 accelerates aging, whereas modest increases of regulated Arf/p53 activity are antiaging. These two sets of observations are not in conflict per se because it is conceivable that the effects of Arf/p53 could be beneficial or detrimental for aging depending on their intensity and regulation (or lack of regulation). Constitutive activation of Arf/p53 may produce unscheduled and excessive cell death or cell senescence, which may eventually exhaust the regenerative potential of tissues, thus accelerating aging. In contrast, a modestly increased, but otherwise normally regulated, Arf/p53 pathway may decrease the damage associated with the aging process, thus postponing aging (Fig. 1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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