

Senescent Cells, Tumor Suppression, and Organismal Aging: Good Citizens, Bad Neighbors

Review

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Cells from organisms with renewable tissues can permanently withdraw from the cell cycle in response to diverse stress, including dysfunctional telomeres, DNA damage, strong mitogenic signals, and disrupted chromatin. This response, termed cellular senescence, is controlled by the p53 and RB tumor suppressor proteins and constitutes a potent anticancer mechanism. Nonetheless, senescent cells acquire phenotypic changes that may contribute to aging and certain age-related diseases, including late-life cancer. Thus, the senescence response may be antagonistically pleiotropic, promoting early-life survival by curtailing the development of cancer but eventually limiting longevity as dysfunctional senescent cells accumulate.

Multicellular organisms contain two fundamentally different cell types: postmitotic cells, which cannot divide, and mitotic (or mitotically competent) cells, which can divide. In many simple organisms—for example, the nematode *Caenorhabditis elegans* and fruit fly *Drosophila melanogaster*—postmitotic cells are the predominant, if not exclusive, cell type in the somatic tissues of adults. In complex animals such as mammals, by contrast, many somatic tissues contain mitotic cells. These tissues have an advantage over postmitotic tissues because they are capable of renewal, repair, and, in some cases, regeneration.

The evolution of renewable somatic tissues very likely afforded organisms increased longevity. In spite of this, renewable tissues—unlike postmitotic tissues—are susceptible to hyperproliferative disease, the most deadly of which is cancer (Hanahan and Weinberg, 2000). Why is this so? First, renewable tissues are generally repaired and replenished by cell proliferation, an early and essential step in the development of cancer. Second, DNA replication greatly increases the probability of acquiring, fixing, and propagating somatic mutations, a major driving force behind malignant tumorigenesis. Thus, as complex organisms with renewable tissues evolved, cancer posed a perpetual danger to the gains in longevity. This danger was offset by the coevolution of tumor suppressor mechanisms.

Tumor Suppression and Longevity

Most tumor suppressor genes can be classified into either of two broad categories, termed caretakers and gatekeepers (Kinzler and Vogelstein, 1997).

Caretaker tumor suppressors prevent cancer by pro-

tecting the genome from mutations. Caretakers generally act by preventing DNA damage and/or optimizing DNA repair. In addition to preventing cancer, genes that help maintain genomic integrity also prevent or retard the development of other aging phenotypes and age-related pathologies (Hasty et al., 2003). Thus, caretaker tumor suppressors are, in essence, longevity assurance genes.

Gatekeeper tumor suppressors, by contrast, prevent cancer by acting on intact cells—specifically, mitotic cells that are at risk for neoplastic transformation. Gatekeepers can virtually eliminate potential cancer cells by inducing programmed cell death (apoptosis). Alternatively, they can prevent potential cancer cells from proliferating by inducing permanent withdrawal from the cell cycle (cellular senescence). Although little is known about how cells choose between apoptotic and senescence responses, there is scant doubt that both responses are crucial for suppressing cancer (Campisi, 2001; Green and Evan, 2002). Thus, gatekeeper tumor suppressors promote longevity by preventing the development of cancer. However, gatekeeper functions are not risk free. The apoptotic and senescence responses they implement can have cumulative deleterious effects, and thus may also limit longevity by contributing to aging and late-life pathology (Campisi, 2003a).

How might gatekeeper tumor suppressors promote aging? In the case of apoptosis, this process could eventually deplete nonrenewable tissues of irreplaceable postmitotic cells and renewable tissues of proliferating or stem cell pools. The senescence response could likewise deplete tissues of proliferating or stem cell pools. In addition, as discussed further, senescent cells are dysfunctional and may actively disrupt normal tissues as they accumulate. Thus, gatekeeper tumor suppressor mechanisms may be an example of evolutionary antagonistic pleiotropy (reviewed in Kirkwood and Austad [2000]; Campisi, 2003b). Antagonistically pleiotropic genes or processes are those that benefit organisms early in life (e.g., by suppressing cancer) but are detrimental later in life (e.g., by compromising tissue function). This review considers the causes and consequences of the senescence response and the growing evidence that, at least in complex animals such as mammals, it can contribute to aging and age-related pathology.

Good Citizens—Cells Senesce in Response to Potential Cancer-Causing Events

Hayflick and colleagues first formally described cellular senescence as the finite replicative life span of human fibroblasts in culture. Because the cells underwent many divisions before arresting growth in a stable postmitotic state, this process is sometimes termed replicative senescence. Over the ensuing decades, it was discovered that proliferating cells reach the so-called Hayflick limit largely because repeated DNA replication in the absence of telomerase causes telomeres to pro-

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gressively shorten and eventually malfunction (Shay and Wright, 2000). Telomeres are the DNA sequence and proteins that cap the ends of linear chromosomes and prevent their fusion by cellular DNA repair processes. Because functional telomeres maintain the integrity and stability of the genome, they suppress the development of cancer. Cells that fail to senesce and proliferate despite dysfunctional telomeres develop chromosomal aberrations, which can result in malignant transformation (Artandi and DePinho, 2000). The senescence response, then, ensures that cells with dysfunctional telomeres permanently withdraw from the cell cycle, rendering them incapable of forming a tumor.

It is now apparent that many kinds of oncogenic or stressful stimuli can induce a senescence response. Foremost among these are certain types of DNA damage, including DNA breaks and oxidative lesions caused by environmental insults, genetic defects, or endogenous processes (DiLeonardo et al., 1994; Hasty et al., 2003; Samper et al., 2003). Cells evidently senesce when the damage is irreparable or threatens to overwhelm the DNA repair machinery. In addition, many normal cells senesce when they overexpress certain oncogenes, such as activated components of the RAS-RAF-MEK signaling cascade (Bringold and Serrano, 2000; Lundberg et al., 2000; Narita and Lowe, 2004). In these cases, it appears the supraphysiological mitogenic signals that result from the overexpression of the oncogenes are responsible for eliciting the senescence response. Consistent with this view, mutational activation of RAS without overexpression stimulates cell proliferation and transformation, not cellular senescence (Tuveson et al., 2004). Finally, cells can senesce in response to epigenetic changes to chromatin organization—for example, those caused by pharmacological agents or altered expression of proteins that modify DNA or histones (Neumeister et al., 2002; Bandyopadhyay and Medrano, 2003; Narita and Lowe, 2004). Such changes can alter the expression of protooncogenes or tumor suppressor genes and are a frequent occurrence among malignant tumors. Thus, the senescence response prevents the growth of cells that experience any one of an assortment of potentially oncogenic stimuli.

Many Stimuli, Two Pathways

Although diverse stimuli can induce a senescence response, they appear to converge on either or both of two pathways that establish and maintain the senescence growth arrest. These pathways are governed by the gatekeeper tumor suppressor proteins p53 and pRB (Bringold and Serrano, 2000; Lundberg et al., 2000; Campisi, 2001). Both these proteins are transcriptional regulators, and each lies at the heart of a pathway that includes a plethora of upstream regulators and downstream effectors (Sherr and McCormick, 2002). How do such diverse stimuli engage the p53 and pRB pathways? Are both pathways required to initiate the senescence response? And do both pathways maintain the senescent state? Answers to these questions are still fragmented and incomplete. Nonetheless, a consolidated, if not yet comprehensive, picture is emerging.

The p53 Pathway

p53 is a crucial mediator of cellular responses to DNA damage, including the senescence response (Wahl and Carr, 2001). Is the p53 pathway important for the senescence response to other stimuli?

It is well established that loss of p53 function delays or abrogates the replicative senescence of human cells (Itahana et al., 2001). This finding raised the possibility that dysfunctional telomeres resemble damaged (broken) DNA and thus trigger a p53-dependent DNA damage response. Indeed, recent data show that nuclear foci containing markers of DNA double strand breaks form at critically short or dysfunctional telomeres (d'Adda di Fagagna et al., 2003; Takai et al., 2003). Moreover, dysfunctional telomeres activate many components of the p53-mediated damage response, and the senescence response to dysfunctional telomeres requires integrity of the p53 pathway (Itahana et al., 2001; d'Adda di Fagagna et al., 2004). The p53 pathway is also important for the senescence response to overexpressed oncogenes such as activated RAS (Serrano et al., 1997; Ferbeyre et al., 2000; Pearson et al., 2000). The RAS mitogenic pathway signals through reactive oxygen species (ROS), generation of which is required for both the pathway's mitogenic effects (Irani et al., 1997) and ability to induce cellular senescence (Lee et al., 1999). Thus, overexpressed oncogenic RAS may also trigger a p53-dependent damage response, in this case by producing high levels of DNA-damaging ROS. However, oncogenic RAS can also induce p16, an activator of the pRB pathway, which provides a second barrier to the proliferation of potentially oncogenic cells (discussed below).

Inactivation of p53 in at least some replicatively senescent human cells completely reverses the senescent growth arrest (Gire and Wynford-Thomas, 1998; Beausejour et al., 2003). In these cases, experimental manipulations that abolish p53 function cause postmitotic senescent cells to resume growth for many doublings, despite short telomeres, until widespread severe telomere dysfunction drives them into crisis (Beausejour et al., 2003), a state of acute genomic instability. Likewise, inactivation of the gene encoding p21, a target for p53 transactivation and inhibitor of cell cycle progression, causes cells to bypass telomere-dependent replicative senescence and enter crisis (Brown et al., 1997). At least in some cells, then, the induction of senescence by DNA damage, telomere dysfunction, and possibly oncogene overexpression converge on the p53 pathway, which is both necessary and sufficient to establish and maintain the senescence arrest (Figure 1A). Consequently, although the senescence growth arrest cannot be reversed by physiological signals, in such cells it can be reversed by loss of p53 function.

The pRB Pathway

Although p53 inactivation reverses the senescence arrest in some cells, it fails to do so in others (Sakamoto et al., 1993; Beausejour et al., 2003; Herbig et al., 2004). What distinguishes cells that remain senescent despite loss of p53 function from those that do not? The answer appears to be whether and to what extent cells express

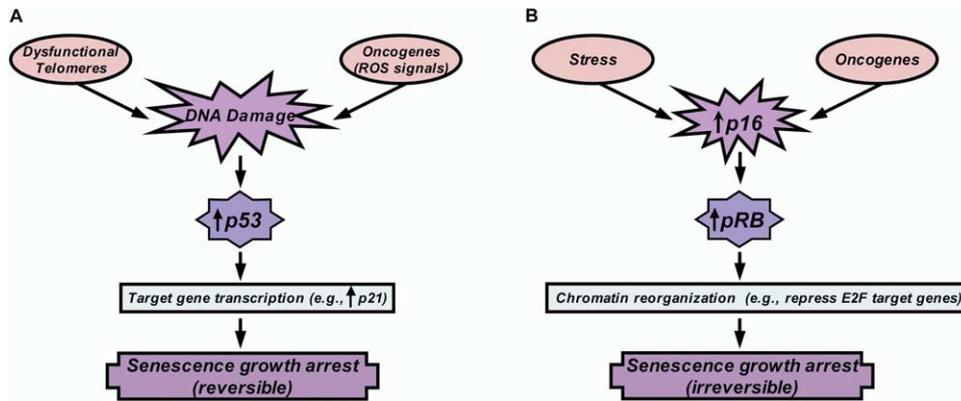


Figure 1. Roles of the p53 and p16/pRB Pathways in the Senescence Response

(A) The p53 pathway to senescence. DNA damage, dysfunctional telomeres, and genotoxic stress such as ROS produced by mitogenic signaling pathways activate the p53 damage response. The transcription of p53-dependent genes, including that encoding p21, induces a senescent-like growth arrest. This arrest cannot be reversed by physiological mitogens, but is reversible upon subsequent inactivation of p53. (B) The pRB pathway to senescence. Oncogenes and other types of stress induce p16, which activates pRB. pRB establishes repressive heterochromatin at loci containing E2F targets and possibly other growth-promoting genes. Once established, the pRB-mediated senescence arrest cannot be reversed by inactivating p53, pRB, or both.

the cell cycle inhibitor p16. p16 is a positive regulator of pRB and tumor suppressor in its own right (Sherr and McCormick, 2002). p16 is induced by a variety of stressful stimuli, including overexpression of oncogenes such as RAS and suboptimal culture conditions (Lowe and Sherr, 2003).

Some cells spontaneously reduce or silence p16 expression, often by promoter methylation. This frequently occurs during the long-term culture of human epithelial cells and occasionally in human fibroblast cultures but also can occur in cells residing in apparently normal human tissues (Holst et al., 2003; Itahana et al., 2003; Herbig et al., 2004). In such cells, then, the senescence response to DNA damage or dysfunctional telomeres depends primarily on the p53 pathway. However, at least some cells express p16 in vivo (Krishnamurthy et al., 2004), during long-term culture (Benanti and Galloway, 2004), and at elevated levels upon replicative senescence (Alcorta et al., 1996; Hara et al., 1996; Brenner et al., 1998; Stein et al., 1999; Itahana et al., 2003). In vivo (mouse model) studies indicate that p16 suppresses the development of spontaneous cancer (Sharpless et al., 2001). Cell culture studies indicate that p16 prevents the reversal of senescence by p53 inactivation (Beausejour et al., 2003) and is required for RAS-induced senescence (Brookes et al., 2002; Benanti and Galloway, 2004). Thus, the p16 tumor suppressor and presumably the pRB pathway it activates provide a formidable barrier to cell proliferation, which cannot be overcome by loss of p53 function.

How does the p16/pRB pathway implement the senescence growth arrest? Although specific mechanisms are as yet unknown, the senescence response appears to result in a reorganization of chromatin, at least some aspects of which require pRB activity (Jacobs et al., 1999; Leung et al., 2001; Bandyopadhyay et al., 2002; Itahana et al., 2003; Narita et al., 2003; Zhang et al., 2003, 2005). Replicatively senescent cells develop dense foci of heterochromatin (Narita et al.,

2003; Zhang et al., 2005) which coincide with pRB-dependent heterochromatic repression of genes encoding cyclins and other positive cell cycle regulators (Bandyopadhyay et al., 2002; Narita et al., 2003). Many of these repressed genes are activation targets of E2F transcription factors (Narita et al., 2003), some of which are converted to transcriptional repressors when complexed with pRB (Trimarchi and Lees, 2002). Of special interest, once the pRB pathway is engaged, particularly by p16, the senescence growth arrest cannot be reversed by subsequent inactivation of p53, silencing of p16, or inactivation of pRB (Beausejour et al., 2003). Thus, once pRB establishes repressive chromatin at E2F target genes and possibly other loci, it appears that maintenance of the heterochromatic domains no longer requires p16 or pRB activity. These findings may help explain the remarkable stability of the senescence growth arrest. Thus, the p16/pRB pathway appears to be particularly important for ensuring that the senescence growth arrest is essentially irreversible and refractory to subsequent inactivation of p53, pRB, or both (Figure 1B).

Although the pRB pathway is essential for the transcriptional repression of loci in senescent cells, its role in the induction of gene expression (discussed further) at senescence is largely unknown. Because pRB/E2F complexes are usually repressive, they most likely do not directly regulate the genes that are highly expressed by senescent cells, although they could indirectly control such genes (by silencing a repressor, for example). Alternatively, the pRB pathway may be dispensable for senescence-associated increases in gene expression, despite its importance for the senescence growth arrest. Whatever the case, it is interesting that many of the genes that are upregulated in senescent cells are physically clustered (Zhang et al., 2003). This finding suggests that chromatin reorganization may also be responsible for upregulating gene expression in senescent cells, in these cases, however, by establishing a more open or euchromatic structure.

Independent or Dependent Senescence Pathways?

What is the relationship, if any, between the p53 and pRB pathways in regulating cellular senescence? There are of course molecular interactions that link these pathways (Sherr and McCormick, 2002), but many questions remain about whether and how these interactions factor into the senescence response. An important example is the induction of p21 expression by p53. p21 is a more global inhibitor of cyclin-dependent kinases than p16 and thus also causes hypophosphorylation and activation of pRB. Engagement of the p53 pathway should, therefore, engage the pRB pathway. Nonetheless, the consequences of pRB activation by p21 differ from that of activation by p16, at least in some respects. Specifically, p53 inactivation can induce proliferation in replicatively senescent human fibroblasts that express p21, but not those that express p16. Moreover, when ectopically overexpressed, p16 is more effective than p21 at inducing human fibroblasts to arrest with features of senescence (McConnell et al., 1998). In addition, upon replicative senescence, some human fibroblasts express either p21 or p16, but rarely both (Herbig et al., 2004).

These and other studies suggest there are notable distinctions between senescent states induced by the p53 and pRB pathways. Moreover, there is an emerging consensus that senescence occurs via one pathway or the other, with the p53 pathway mediating senescence due primarily to telomere dysfunction and DNA damage and the p16/pRB pathway mediating senescence due primarily to oncogenes, chromatin disruption, and various stresses (Wright and Shay, 2002; Collins and Sedivy, 2003; Ben-Porath and Weinberg, 2004). This may be the case. However, currently, there are no unambiguous, much less comprehensive, markers to define the senescent state. Therefore, it is difficult to know the extent to which senescent states induced by the p53 and pRB pathways are distinct (or similar). In addition, depending on the tissue and species of origin, cells may differ in both their senescent phenotype and the relative importance of the p53 or pRB pathways for the senescence response.

At present, there are a number of phenomena and some apparent paradoxes that indicate gaps in our knowledge about how the p53 and pRB pathways function to implement the senescence response. For example, mouse cells are thought to rely primarily on the p53 pathway for both replicative and telomere-dependent senescence (Harvey et al., 1993; Smogorzewska and de Lange, 2002). However, as discussed below, the replicative senescence of mouse cells is due primarily to oxygen toxicity (Parrinello et al., 2003), which can confound assessment of senescent states in mouse cells. Nonetheless, some human fibroblasts express very little p16 and thus also rely primarily on the p53 pathway for the senescence response; however, such cells senesce in response to overexpressed oncogenic RAS (Beausejour et al., 2003) which is thought to require p16 activity (Brookes et al., 2002; Huot et al., 2002). In these cells, the high levels of ROS produced by RAS (Irani et al., 1997) may trigger senescence by the p53 pathway.

Likewise, telomere-dependent replicative senescence is thought to depend primarily on the p53 pathway (Ramirez et al., 2001; Herbig et al., 2004). Nonetheless, exten-

sive telomere uncapping (caused by expression of a mutant telomere binding protein) induces p16 in some human cells (Jacobs and de Lange, 2004), consistent with p16 responding to a variety of stresses, including acute genotoxic stress. Of course, extensive telomere uncapping may induce a stress response that does not occur when cells undergo telomere-dependent replicative senescence, which is thought to entail only a few dysfunctional telomeres (Zou et al., 2004). This possibility might explain why some strains of replicatively senescent cells are either p16 positive (presumably induced by culture stress) or p21 positive (presumably induced by telomere dysfunction) and why the p16-positive cells are devoid of foci containing markers of DNA damage. On the other hand, in some human fibroblasts, replicative senescence is marked by the sequential rise in p21 expression, followed by a decline in p21 and concomitant rise in p16 (Stein et al., 1999). Thus, signaling from the p53 to p16/pRB pathway might occur in some cells, with the p53-generated signal being transient. Alternatively, or in other cells, the p16 pathway may somehow sense the genomic stress of dysfunctional telomeres and arrest proliferation prior to significant telomere dysfunction. This possibility may explain why human epithelial cells that have silenced p16 or human fibroblasts that are genetically deficient in p16, replicatively senesce with signs of genomic instability or a precrisis genomic state (Romanov et al., 2001; Brookes et al., 2004).

Of Mice and Humans

Many murine cells undergo only a few doublings in culture, despite long telomeres and expression of telomerase. These cells most likely arrest because standard culture conditions cause oxidative stress, which human cells resist much more effectively than mouse cells (Parrinello et al., 2003). When cultured at physiological oxygen tension, murine fibroblasts can proliferate indefinitely. They do so with intact p53 function and initially low levels of p16. Eventually, however, p16 levels rise, yet proliferation continues. The mechanisms that allow murine cells to proliferate despite high levels of p16 are unknown, but may entail adaptive responses (upregulation of cyclin-dependent kinase activities, for example). These mechanisms may explain another difference between rodent and human cells: in contrast to the senescence arrest of human fibroblasts (Beausejour et al., 2003), the senescence arrest of murine fibroblasts can be reversed by inactivating pRB (Sage et al., 2003). Thus, rodent cells may more readily deploy mechanisms to overcome irreversible effects of the p16/pRB pathway, such as formation of repressive heterochromatin. Primary human fibroblasts and epithelial cells also express increasing levels of p16 during culture (Reznikoff et al., 1996; Brenner et al., 1998; Rheinwald et al., 2002; Benanti and Galloway, 2004). It is not known whether human cells can also adapt to high levels of p16 expression. Nonetheless, there appears to be a threshold level that renders human cells susceptible to RAS-induced senescence (Benanti and Galloway, 2004) and beyond which human cells cannot proliferate.

Are the p53 and pRB pathways regulated in fundamentally different ways in rodent and human cells? Be-

cause many studies of rodent cell senescence are confounded by the severe oxidative stress of standard culture conditions and many studies employ fibroblasts or one of a small number of epithelial cell types, it is possible there are fewer differences than currently assumed. Nonetheless, there remain many intriguing questions regarding how the p53 and pRB pathways are regulated and how they impact the development of cancer and rate of aging. If senescent cells contribute to organismal aging, are their detrimental effects due to activities of the p53 or pRB pathway (or both)? Does the induction of p16 by culture stress reflect its induction by exogenous and endogenous stress *in vivo*? Most important, do senescent cells contribute to aging or age-related disease? There are no definitive answers to these questions, but recent studies have provided some partial answers.

The p53 Pathway and Aging

Recent findings suggests that p53, despite being a crucial tumor suppressor, also contributes to aging and does so at least in part by enhancing the senescence response. Evidence for this idea comes from the phenotype of genetically modified mice in which p53 function was enhanced either by low-level expression of an artificially truncated p53 protein (Tyner et al., 2002; Maier et al., 2004) or modest overexpression of a naturally occurring short isoform (Maier et al., 2004). In both mouse models, the short p53 proteins were N-terminally truncated, and the mice continued to express full-length p53. p53 functions as a tetramer, so the short p53 proteins were thought to form mixed tetramers with full-length p53, resulting in constitutive enhancement of at least some p53 activities. Consistent with the short forms hyperactivating p53 and p53 being a tumor suppressor, both mouse strains were remarkably resistant to cancer. However, even though cancer is a major cause of death in laboratory mice, these mice had a reduced life span and showed signs of accelerated aging.

How might constitutively hyperactive p53 accelerate aging? Cells from these modified mice were more susceptible to both p53-mediated apoptosis (Tyner et al., 2002) and p53-mediated senescence (Maier et al., 2004). Of particular interest, the hyperactive p53 upregulated components the IGF1 signaling pathway and the enhanced IGF1 signaling induced a senescence response, presumably by mechanisms similar to those utilized by activated the RAS-RAF-MEK pathway. Although not definitive, these findings are consistent with the idea that senescent cells can contribute to organismal aging. They also suggest one potential mechanism—induction of cellular senescence—by which the evolutionarily conserved insulin/IGF1 signaling pathway (Rincon et al., 2004) may drive aging in complex organisms.

These findings also suggest there may be a cost (accelerated aging) to enhanced protection from cancer. Arguing against this idea are mouse models carrying one or two transgenic copies of the entire TP53 locus (Garcia-Cao et al., 2002; Matheu et al., 2004) or one copy of the entire INK4a/ARF locus (Matheu et al., 2004). These mice showed resistance to spontaneous

and induced tumorigenesis without apparent shortening of life span or accelerated aging, although the sample sizes used for the life span and aging analyses were small. Because p53 and ARF were expressed from their natural promoters and subject to physiological regulation, it is possible that p53 (and possibly ARF) accelerates aging when chronically active but not when normally regulated. The question remains as to whether endogenous p53 contributes to phenotypes of normal aging. At least in rodents, the expression of ARF increases with age (Krishnamurthy et al., 2004), suggesting that cells with chronic p53 activity might accumulate. An interesting, but difficult-to-answer question is if p53 (or ARF)-deficient mice did not die of cancer, would they have an increased life span or show retarded aging of tissues that contain mitotic cells?

The p16/pRB Pathway and Aging

There is no evidence yet that enhanced pRB function, analogous to the enhanced p53 function conferred by truncated p53 proteins, accelerates aging. There is, however, intriguing evidence that, similar to p19/ARF, the levels of p16 expression and incidence of p16-positive cells increase with age in many mouse and rat tissues (Zindy et al., 1997; Krishnamurthy et al., 2004). Moreover, p16 expression increased to substantially greater extents than ARF expression, making it a conceivable biomarker of aging. Are p16-positive cells senescent cells? Very possibly. p16 expression paralleled that of senescence-associated β -galactosidase (SA- β -gal), an activity associated with the senescent phenotype (Dimri et al., 1995). Of particular importance, caloric restriction, which retards aging in rodents and other species, attenuated the accumulation of p16-positive cells (Krishnamurthy et al., 2004). In addition, expression of Ets-1, a transcriptional activator of p16 (Ohtani et al., 2001), correlated with p16 expression, although absolute levels of Ets-1 did not necessarily increase with age (Krishnamurthy et al., 2004). This finding raises the possibility that aging increases the sensitivity of the INK4a/ARF locus to transcriptional activation.

Because p16 activates the pRB pathway and induces senescence, these studies support the idea that the pRB pathway, like the p53 pathway, may drive aging phenotypes by inducing cellular senescence.

Cellular Senescence and Aging

p16, and to a lesser extent ARF, join the extremely short list of markers that are expressed predominantly, although not exclusively, by senescent cells (reviewed in Krtolica and Campisi [2002]). The combined results from use of both p16 and SA- β -gal indicate that cells with characteristics of senescence accumulate with age in multiple tissues from both humans and rodents. Moreover, these cells are present at sites of certain age-related pathologies, including atherosclerotic lesions, skin ulcers and arthritic joints, as well as benign and preneoplastic hyperproliferative lesions in the prostate and liver (reviewed in Krtolica and Campisi, 2002). Thus, senescent cells appear to be present at the expected times and places.

Bad Neighbors—Senescent Cells Alter Tissue Structure and Function

How might senescent cells promote aging phenotypes or age-related pathology? Because tissues have a fairly constant number of cells, the accumulation of nondividing senescent cells may compromise tissue renewal or repair. In addition, among the genes that are upregulated by the senescence response (Shelton et al., 1999; Chang et al., 2000; Zhang et al., 2003), many encode secreted proteins that can alter the tissue microenvironment thus alter tissue structure and function. Both possibilities are viable, but at present evidence for the latter possibility is strongest.

The factors that are secreted by senescent cells vary depending on the cell type. Among the major mammalian cell types, the most thoroughly studied with regard to the senescent secretory phenotype are fibroblasts, which synthesize and maintain the stromal support for virtually all renewable epithelial tissues. Senescent fibroblasts secrete high levels of several matrix metalloproteinases, epithelial growth factors, and inflammatory cytokines (reviewed in Krtolica and Campisi [2002]). In many ways, the secretory phenotype of senescent fibroblasts resembles that of fibroblasts undergoing a wounding response, which entails local remodeling of the tissue structure (Grinnell, 2003). The wounding response also entails local inflammation, a frequent occurrence in aging tissues and proposed initiating or causative factor in a variety of age-related diseases, including atherosclerosis and cancer (Longo and Finch, 2003). The senescent secretory phenotype also resembles that of fibroblasts associated with some carcinomas. These carcinoma-associated fibroblasts are components of the so-called reactive stroma, which facilitates the progression of epithelial cancers (Olumi et al., 1999; Park et al., 2000; Tlsty and Hein, 2001). Thus, senescent cells might contribute to aging and age-related pathology by stimulating chronic tissue remodeling and/or local inflammation, which would compromise tissue structure and function (Figure 2A). In addition, senescent cells might stimulate the proliferation of cells that harbor preneoplastic mutations (Figure 2B).

Recent findings suggest that senescent fibroblasts can disrupt the functional and morphological differentiation of epithelial cells, at least in three-dimensional cultures of mammary epithelial cells (Parrinello et al., 2005). In these models, senescent fibroblasts perturbed alveolar morphogenesis and reduced milk protein expression by normal mammary epithelial cells. They also stimulated aberrant branching morphogenesis by normal breast epithelial cells, owing in large measure to their secretion of a specific matrix metalloproteinase (MMP3). This finding suggests that senescent stromal cells might promote the development of hyperplastic epithelial lesions in vivo. In addition, even apparently normal tissues harbor cells with potentially oncogenic mutations, and the incidence of mutant cells increases with age (Jonason et al., 1996; Dolle et al., 2002). Further, many cells with oncogenic mutations are kept in check by the tissue microenvironment (Park et al., 2000). It is possible, therefore, that changes in the microenvironment caused by senescent cells can fuel the growth and progression of such cells toward malignancy. Indeed, there is mounting evidence that senes-

cent fibroblasts create a local tissue environment that promotes the growth of initiated or preneoplastic epithelial cells both in culture and in vivo (Krtolica et al., 2001; Roninson, 2002; Dilley et al., 2003). Thus, senescent cells can, at least in principle, contribute to age-related changes in tissue structure and function, as well as the development of age-related pathology, including late-life cancer.

A more speculative, but potentially important, consequence of cellular senescence may be its impact on stem cells and their niches. Stem cells, of course, are prime targets for carcinogenesis. Embryonic stem cells, whether human or rodent, express high levels of telomerase and thus do not undergo replicative senescence in culture or in vivo (Odorico et al., 2001; Miura et al., 2004). However, many adult stem or progenitor cells do not proliferate indefinitely, whether mouse or human in origin (Geiger and Van Zant, 2002; Chen, 2004; Park et al., 2004; Villa et al., 2004). Whether this limited replicative potential is due a complete lack of telomerase activity, lack of sufficient telomerase activity, or telomere-independent senescence or cell death due to nontelomeric damage or stress most likely depends on the tissue and species of origin. Whatever the case, the ability of stem cells to undergo senescence (and apoptosis, for that matter) appears to be an important mechanism for preventing cancer (Boulanger and Smith, 2001; Serakinci et al., 2004). On the other hand, cellular senescence could negatively impact stem cells in two ways. First, it could deplete tissues of stem or progenitor cell pools. From mouse models, such as mice genetically engineered to lack telomerase activity, and human conditions, such as the hereditary telomerase deficiency syndrome dyskeratosis congenita, it is clear that replicative exhaustion of proliferating pools can result in some features of premature aging, including increased cancer incidence (Rudolph et al., 1999; Wong and Collins, 2003). Second, the presence of senescent cells, whether stem cells themselves or surrounding cells in stem cell niches, could disrupt the stem cell microenvironment. The senescent microenvironment in turn could alter the proliferation, differentiation, and/or mobilization the resident stem cells.

Tipping the Balance

The antagonistically pleiotropic effects of senescent cells suggest that aging is, at least in part, a consequence of gatekeeper tumor suppressor mechanisms. Moreover, because senescent cells can disrupt the morphological and functional differentiation of normal cells, the efficacy of promising therapies for age-related diseases, such as stem cell replacement, may be compromised by the presence of senescent cells. Of more immediate concern, many DNA-damaging chemotherapeutic agents induce tumor cells to senesce, thereby creating a concentrated core of senescent cells that can have both local and systemic effects (Roninson, 2003). Is it possible, then, to minimize or abrogate the deleterious effects of senescent cells? A complete answer to this question will require a much better understanding of why cells undergo senescence rather than

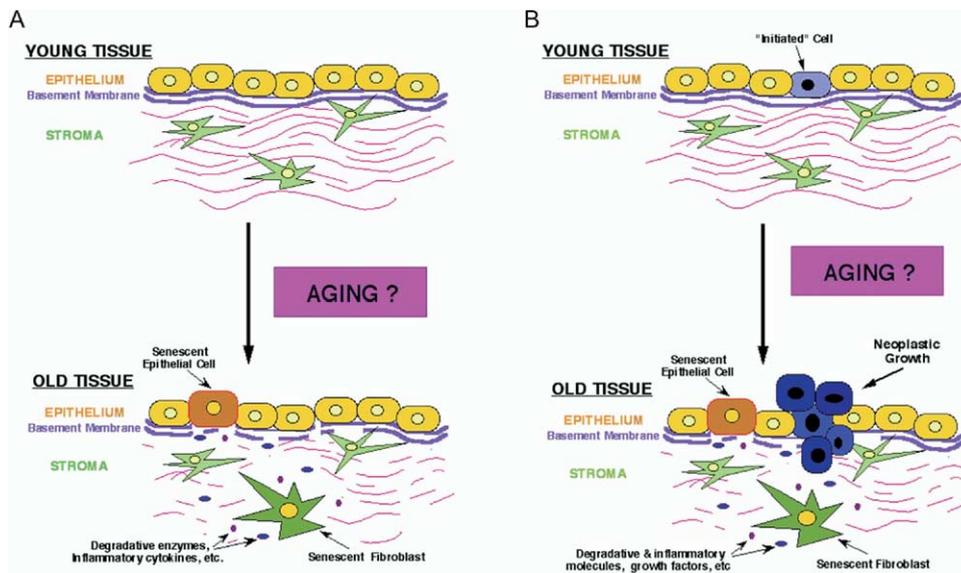


Figure 2. Senescent Cells May Contribute to Aging and Age-Related Pathology, Including Late-Life Cancer

(A) Senescent cells may disrupt normal tissue structure and function. Shown is a prototypical epithelium, in contact with a basement membrane and supporting stroma. As senescent cells accumulate with age, they produce degradative enzymes and inflammatory cytokines, which can disrupt the tissue structure and consequently decrease tissue function.

(B) Senescent cells may promote cancer progression. Both senescent cells and cells with preneoplastic mutations (initiated cells) accumulate with age, as does the probability of both occurring in close proximity. When this occurs, molecules secreted by senescent cells may create a permissive microenvironment that allows the proliferation of preneoplastic cells.

apoptosis, how the senescent phenotype is regulated, and particularly how genes encoding secreted factors are upregulated as a consequence of the senescence response. In the interim, however, it may be possible to inhibit portions of the senescent secretory phenotype to abrogate selected effects, such as inhibiting matrix metalloproteinases selectively abrogated the derangement of mammary branching morphogenesis by senescent fibroblasts (Parrinello et al., 2005).

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