Four faces of cellular senescence

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Cellular senescence is an important mechanism for preventing the proliferation of potential cancer cells. Recently, however, it has become apparent that this process entails more than a simple cessation of cell growth. In addition to suppressing tumorigenesis, cellular senescence might also promote tissue repair and fuel inflammation associated with aging and cancer progression. Thus, cellular senescence might participate in four complex biological processes (tumor suppression, tumor promotion, aging, and tissue repair), some of which have apparently opposing effects. The challenge now is to understand the senescence response well enough to harness its benefits while suppressing its drawbacks.

Introduction

Cellular senescence was formally described more than 40 years ago as a process that limited the proliferation (growth) of normal human cells in culture (Hayflick, 1965). This landmark paper contained two prescient statements. The first statement was "unlimited cellular division or . . . escape from senescent-like changes . . . can only be achieved by [somatic] cells which have . . . assumed properties of cancer cells." The second was "the [cessation of cell growth in culture] may be related to senescence [aging] *in vivo*." Thus, nearly half a century ago, the process now known as cellular senescence was linked to both tumor suppression and aging.

In the ensuing decades, we learned much about what causes cellular senescence and the nature of the senescent phenotype. Importantly, we are beginning to understand its physiological relevance. Recent data validate the early idea that cellular senescence is important for tumor suppression. The data now also strongly suggest that cellular senescence contributes to aging, and, further, that senescence-associated phenotypes can contribute to both tumor progression and normal tissue repair. They also offer insights into why, beyond the simple growth arrest, the complex senescent phenotypes may have evolved.

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Abbreviations used in this paper: ATM, ataxia telangiectasia mutated; DDR, DNA damage response; DNA-SCARS, DNA segments with chromatin alterations reinforcing senescence; IL, interleukin; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; SA-Bgal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype.

Cellular senescence: a primer

Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells that can divide encounter oncogenic stress. With the possible exception of embryonic stem cells (Miura et al., 2004), most division-competent cells, including some tumor cells, can undergo senescence when appropriately stimulated (Shay and Roninson, 2004; Campisi and d'Adda di Fagagna, 2007).

What causes cellular senescence? Senescence-inducing stimuli are myriad. We now know that the limited growth of human cells in culture is due in part to telomere erosion, the gradual loss of DNA at the ends of chromosomes (telomeres). Telomeric DNA is lost with each S phase because DNA polymerases are unidirectional and cannot prime a new DNA strand, resulting in loss of DNA near the end of a chromosome; additionally, most cells do not express telomerase, the specialized enzyme that can restore telomeric DNA sequences de novo (Harley et al., 1990; Bodnar et al., 1998). We also know that eroded telomeres generate a persistent DNA damage response (DDR), which initiates and maintains the senescence growth arrest (d'Adda di Fagagna et al., 2003; Takai et al., 2003; Herbig et al., 2004; Rodier et al., 2009, 2011). In fact, many senescent cells harbor genomic damage at nontelomeric sites, which also generate the persistent DDR signaling needed for the senescence growth arrest (Nakamura et al., 2008). DNA double strand breaks are especially potent senescence inducers (DiLeonardo et al., 1994). In addition, compounds such as histone deacetylase inhibitors, which relax chromatin without physically damaging DNA, activate the DDR proteins ataxia telangiectasia mutated (ATM) and the p53 tumor suppressor (Bakkenist and Kastan, 2003), and induce a senescence response (Ogryzko et al., 1996; Munro et al., 2004). Finally, many cells senesce when they experience strong mitogenic signals, such as those delivered by certain oncogenes or highly expressed pro-proliferative genes (Serrano et al., 1997; Lin et al., 1998; Zhu et al., 1998; Dimri et al., 2000). Notably, these mitogenic signals can create DNA damage and a persistent DDR due to misfired replication origins and

The Rockefeller University Press
J. Cell Biol. Vol. 192 No. 4 547–556
www.jcb.org/cgi/doi/10.1083/jcb.201009094

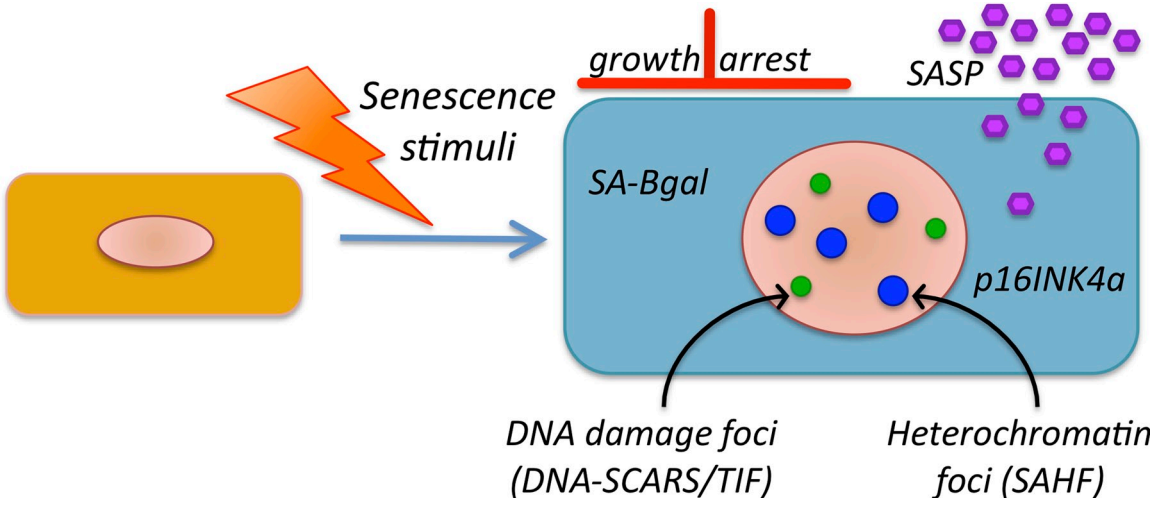

Figure 1. **Hallmarks of senescent cells.** Senescent cells differ from other nondividing (quiescent, terminally differentiated) cells in several ways, although no single feature of the senescent phenotype is exclusively specific. Hallmarks of senescent cells include an essentially irreversible growth arrest; expression of SA-Bgal and p16INK4a; robust secretion of numerous growth factors, cytokines, proteases, and other proteins (SASP); and nuclear foci containing DDR proteins (DNA-SCARS/TIF) or heterochromatin (SAHF). The pink circles in the nonsenescent cell (left) and senescent cell (right) represent the nucleus.

replication fork collapse (Bartkova et al., 2006; Di Micco et al., 2006; Mallette et al., 2007). Thus, many senescence-inducing stimuli cause epigenomic disruption or genomic damage.

Senescence can also occur, however, without detectable DDR signaling. "Culture stress," the natural and in vivo equivalent of which are unknown, causes a senescence arrest without significant telomere erosion (Ramirez et al., 2001). These stresses could include inappropriate substrata (e.g., tissue culture plastic), serum (most cells experience plasma, not serum, in vivo), and oxidative stress (e.g., culture in atmospheric O_2, which is hyperphysiological; Fusenig and Boukamp, 1998; Yaswen and Stampfer, 2002; Parrinello et al., 2003). Cells also senesce without a DDR upon loss of the Pten tumor suppressor, a phosphatase that counteracts pro-proliferative/pro-survival kinases (Alimonti et al., 2010). Additionally, ectopic expression of the cyclin-dependent kinase inhibitors (CDKis) that normally enforce the senescence growth arrest, notably p21WAF1 and p16INK4a, cause senescence without an obvious DDR (McConnell et al., 1998; Rodier et al., 2009).

What defines a senescent cell? Senescent cells are not quiescent or terminally differentiated cells, although the distinction is not always straightforward. No marker or hallmark of senescence identified thus far is entirely specific to the senescent state. Further, not all senescent cells express all possible senescence markers. Nonetheless, senescent cells display several phenotypes, which, in aggregate, define the senescent state (Fig. 1). Salient features of senescent cells are:

(a) The senescence growth arrest is essentially permanent and cannot be reversed by known physiological stimuli. However, some senescent cells that do not express the CDKi p16INK4a can resume growth after genetic interventions that inactivate the p53 tumor suppressor (Beauséjour et al., 2003). So far, there is no evidence that spontaneous p53 inactivation occurs in senescent cells (whether in culture or in vivo), although such an event is not impossible.

(b) Senescent cells increase in size, sometimes enlarging more than twofold relative to the size of nonsenescent counterparts (Hayflick, 1965).

(c) Senescent cells express a senescence-associated β-galactosidase (SA-Bgal; Dimri et al., 1995), which partly reflects the increase in lysosomal mass (Lee et al., 2006).

(d) Most senescent cells express p16INK4a, which is not commonly expressed by quiescent or terminally differentiated cells (Alcorta et al., 1996; Hara et al., 1996; Serrano et al., 1997; Brenner et al., 1998; Stein et al., 1999). In some cells, p16INK4a, by activating the pRB tumor suppressor, causes formation of senescence-associated heterochromatin foci (SAHF), which silence critical pro-proliferative genes (Narita et al., 2003). p16INK4a, a tumor suppressor, is induced by culture stress and as a late response to telomeric or intrachromosomal DNA damage (Brenner et al., 1998; Robles and Adami, 1998; Ramirez et al., 2001; te Poele et al., 2002; Jacobs and de Lange, 2004; Le et al., 2010). Moreover, p16INK4a expression increases with age in mice and humans (Zindy et al., 1997; Nielsen et al., 1999; Krishnamurthy et al., 2004; Ressler et al., 2006; Liu et al., 2009), and its activity has been functionally linked to the reduction in progenitor cell number that occurs in multiple tissues during aging (Janzen et al., 2006; Krishnamurthy et al., 2006; Molofsky et al., 2006).

(e) Cells that senesce with persistent DDR signaling harbor persistent nuclear foci, termed DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). These foci contain activated DDR proteins, including phospho-ATM and phosphorylated ATM/ataxia telangiectasia and Rad3 related (ATR) substrates (d'Adda di Fagagna et al., 2003; Herbig et al., 2004; Rodier et al., 2009), and are distinguishable from transient damage foci (Rodier et al., 2011). DNA-SCARS include dysfunctional telomeres or telomere dysfunction–induced foci (TIF; d'Adda di Fagagna et al., 2003; Takai et al., 2003; Herbig et al., 2004; Kim et al., 2004).

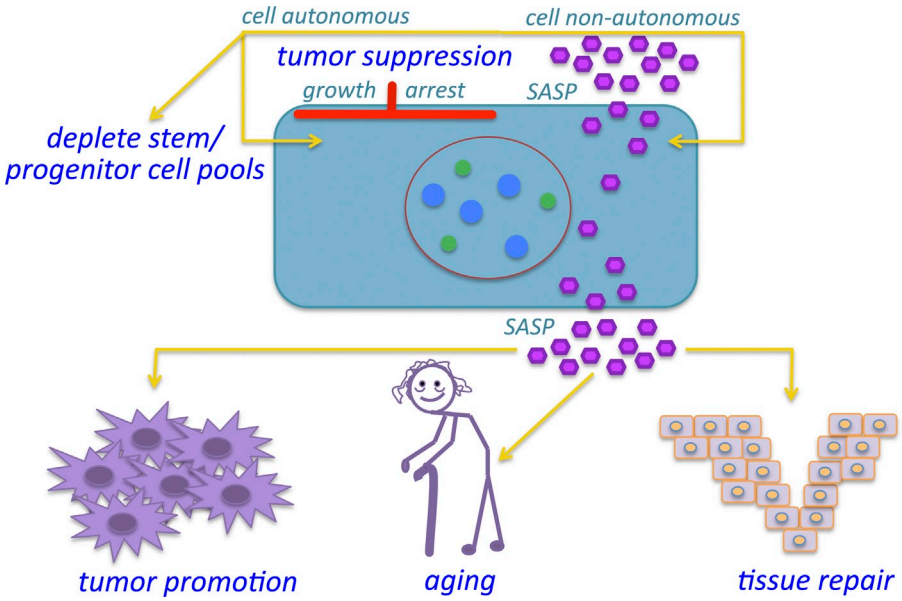

Figure 2. **Biological activities of cellular senescence.** Senescent cells arrest growth owing to cell autonomous mechanisms, imposed by the p53 and p16INK4a/pRB tumor suppressor pathways, and cell nonautonomous mechanisms, imposed by some of the proteins that comprise the SASP. The growth arrest is the main feature by which cellular senescence suppresses malignant tumorigenesis but can contribute to the depletion of proliferative (stem/progenitor) cell pools. Additionally, components of the SASP can promote tumor progression, facilitate wound healing, and, possibly, contribute to aging.

(f) Senescent cells with persistent DDR signaling secrete growth factors, proteases, cytokines, and other factors that have potent autocrine and paracrine activities (Acosta et al., 2008; Coppé et al., 2008, 2010; Kuilman et al., 2008). As we discuss later, this senescence-associated secretory phenotype (SASP) helps explain some of the biological activities of senescent cells.

Cellular senescence and tumor suppression

It is now clear that cellular senescence is a crucial anticancer mechanism that prevents the growth of cells at risk for neoplastic transformation.

The stimuli that elicit a senescence response all have the potential to initiate or promote carcinogenesis. Moreover, to form a lethal tumor, cancer cells must acquire a greatly expanded growth potential and ability to proliferate while expressing activated oncogenes (Hanahan and Weinberg, 2000), traits that are suppressed by the senescence program. Further, cellular senescence depends critically on two powerful tumor suppressor pathways: the p53 and pRB/p16INK4a pathways (Hara et al., 1991; Shay et al., 1991; Bond et al., 1994; Lin et al., 1998; Schmitt et al., 2002; Beauséjour et al., 2003; Collins and Sedivy, 2003; Oren, 2003; Herbig et al., 2004; Jacobs and de Lange, 2004; Ohtani et al., 2004; Chen et al., 2005; Campisi and d'Adda di Fagagna, 2007; Rodier et al., 2007). Both pathways integrate multiple aspects of cellular physiology to determine and orchestrate cell fate. In humans and mice, most, if not all, cancers harbor mutations in one or both these pathways. Moreover, defects in either pathway compromise cellular ability to undergo senescence, and greatly increase organismal susceptibility to cancer.

Studies of human tissues and cancer-prone mice argue strongly that cellular senescence suppresses cancer in vivo. Premalignant human nevi and colon adenomas contained cells that express senescence markers, including SA-Bgal and DDR signaling; however, senescent cells were markedly diminished in the malignant melanomas and adenocarcinomas that develop from these lesions (Bartkova et al., 2005; Michaloglou et al., 2005). Likewise, in mouse models of oncogenic Ras expression or Pten deletion, senescent cells were abundant in premalignant lesions, but scarce in the cancers that eventually developed (Braig et al., 2005; Chen et al., 2005; Collado et al., 2005). Further, dismantling the senescence response by inactivating p53 caused a striking acceleration in the development of malignant tumors (Chen et al., 2005).

In addition, some tumor cells retain the ability to senesce (Shay and Roninson, 2004), and do so in vivo in response to chemotherapy (Schmitt et al., 2002; Coppé et al., 2010) or, in some tissues, after reactivation of p53 (Ventura et al., 2007; Xue et al., 2007). In these cases, the senescence response is associated with tumor regression. Of note, the regressing tumor elicits an inflammatory response that stimulates the innate immune system, which eliminates the senescent cells. As we discuss in subsequent sections, the generation of local inflammation may explain other biological activities of senescent cells.

Although it is clear that cellular senescence arrests incipient tumors at a premalignant stage, it is not clear how tumors eventually, albeit infrequently, emerge from these lesions. Do they arise from senescent cells in which mutations, epigenetic changes, or signals from the tissue reverse the senescence growth arrest? Or do they arise from nonsenescent cells in the premalignant lesion that are dormant or temporarily unable to proliferate and eventually bypass apoptosis or senescence? Whatever the case, the examples above, and a growing list of similar studies (Collado and Serrano, 2010), argue that cellular senescence restrains cancer by imposing a cell-autonomous block to the proliferation of oncogenically damaged/stressed cells (Fig. 2). It was surprising, then, to learn that senescent cells also can promote cancer progression. As discussed in the next section, this activity derives largely from cell nonautonomous mechanisms.

Cellular senescence and tumor promotion

At first glance, the idea that cellular senescence, an established anticancer mechanism, can promote cancer seems paradoxical.

However, the evolutionary theory of antagonistic pleiotropy stipulates that a biological process can be both beneficial and deleterious, depending on the age of the organism (Williams, 1957; Rauser et al., 2006). It is important to remember that cancer is primarily an age-related disease (Campisi, 2003; Balducci and Ershler, 2005). Age is the largest single risk factor for developing a malignant tumor, and cancer incidence rises approximately exponentially after about age 50 (in humans). In these respects, cancer is very similar to the degenerative diseases of aging.

The rationale for antagonistic pleiotropy rests on the fact that most organisms evolve in environments that are replete with fatal extrinsic hazards (predation, infection, starvation, etc.). Under these conditions, aged individuals are rare, and so selection against processes that promote late-life disability or disease is weak. That is, age-associated phenotypes, including age-related diseases, have escaped the force of natural selection. Thus, a biological process that was selected to promote fitness in young organisms (e.g., suppressing cancer) can be detrimental in aged organisms (promoting late-life disease, including cancer).

Why might cellular senescence be antagonistically pleiotropic? More specifically, how might the senescence response promote late-life cancer? There are as yet no definitive answers to these questions. However, recent evidence supports the idea that senescent cells can at least in principle fuel cancer, and provides a potential mechanism by which this might occur.

First, senescent cells increase with age in a variety of mammalian tissues (Dimri et al., 1995; Paradis et al., 2001; Melk et al., 2003; Erusalimsky and Kurz, 2005; Jeyapalan et al., 2007; Wang et al., 2009). It is not known whether this rise is caused by increased generation, decreased elimination, or both. Whatever the genesis, the age-related increase in senescent cells occurs in mitotically competent tissues, which, of course, are those that give rise to cancer.

Second, as noted in the section on what defines a senescent cell, senescent cells develop a secretory phenotype (SASP) that can affect the behavior of neighboring cells. Strikingly, many SASP factors are known to stimulate phenotypes associated with aggressive cancer cells. For example, senescent fibroblasts secrete amphiregulin and growth-related oncogene (GRO) α, which, in cell culture models, stimulate the proliferation of premalignant epithelial cells (Bavik et al., 2006; Coppé et al., 2010). Senescent cells also secrete high levels of interleukin 6 (IL-6) and IL-8, which can stimulate premalignant and weakly malignant epithelial cells to invade a basement membrane (Coppé et al., 2008). Further, senescent fibroblasts and mesothelial cells secrete VEGF (Coppé et al., 2006; Ksiazek et al., 2008), which stimulates endothelial cell migration and invasion (a critical step in tumor-initiated angiogenesis), and senescent fibroblasts and keratinocytes secrete matrix metalloproteinases (MMPs; Millis et al., 1992; Kang et al., 2003; Coppé et al., 2010), which facilitate tumor cell invasiveness. So, do senescent cells stimulate or inhibit tumorigenesis in vivo?

Senescent cells can stimulate tumorigenesis in vivo. It is not yet known whether naturally occurring senescent cells stimulate the progression of naturally occurring tumors in vivo. However, senescent, but not nonsenescent, fibroblasts stimulate premalignant epithelial cells, which do not ordinarily form tumors, to form malignant cancers when the two cell types are co-injected into mice (Krtolica et al., 2001). Further, co-injection of senescent, but not nonsenescent, cells with fully malignant cancer cells markedly accelerates the rate of tumor formation in mice (Krtolica et al., 2001; Liu and Hornsby, 2007; Bhatia et al., 2008; Bartholomew et al., 2009; Coppé et al., 2010). Thus, at least in mouse xenografts, senescent cells have been shown to promote malignant progression of precancerous, as well as established cancer cells, in vivo (Fig. 2). In the future, a more critical test of the idea that senescent cells can promote the development of cancer, especially the progression of age-related cancers, will require strategies to eliminate senescent cells or effects of the SASP from cancer-prone tissues in vivo.

Although paracrine activities of many SASP proteins can promote phenotypes associated with malignancy, the SASP is complex and thus not all components are cancer promoting. For example, senescent keratinocytes secrete the anti-angiogenic factor maspin (Nickoloff et al., 2004). Further, senescent human melanocytes secrete IGFBP7, which induces senescence in a fraction of nonsenescent melanocytes and apoptosis in certain melanoma cell lines (Wajapeyee et al., 2008), at least in some cases (Decarlo et al., 2010). In addition, each SASP factor may have effects that depend on the cell and tissue context. For example, the IL-6, IL-8, and plasminogen activator inhibitor-1 (PAI-1) that are secreted by senescent fibroblasts can promote tumor suppression by reinforcing the senescence growth arrest induced by activated oncogenes or oxidative stress (Fig. 2; Kortlever et al., 2006; Acosta et al., 2008; Kuilman et al., 2008). However, IL-6 and IL-8 have also been shown to promote malignant tumorigenesis in cooperation with certain activated oncogenes (Sparmann and Bar-Sagi, 2004; Ancrile et al., 2007).

Cellular senescence and aging

Cancer is an age-related disease, but differs from most other age-related pathologies in at least one fundamental aspect. To form a lethal tumor, cancer cells must acquire new, albeit aberrant, phenotypes (Hanahan and Weinberg, 2000). In contrast, for most age-related diseases, normal cellular/tissue functions fail. Thus, most age-related pathologies are degenerative, whereas cancer can hardly be considered a degenerative disease. Does cellular senescence, then, contribute to aging and age-related diseases other than cancer? There is mounting, although not yet definitive, evidence that the answer to this question is yes.

Altered p53 function and aging. Among the more compelling evidence that senescent cells can drive degenerative aging pathologies are the phenotypes of transgenic mice with hyperactive p53. Several years ago, two landmark papers described mouse models in which constitutive expression of an artificially (Tyner et al., 2002) or naturally (Maier et al., 2004) truncated p53 protein resulted in chronically elevated p53 activity. These mice were exceptionally cancer-free, which was not surprising, as p53 is a critical tumor suppressor. What was surprising was their shortened life span and premature aging. Like all progeroid models, these mice did not completely phenocopy normal aging. Nonetheless, between the two models, the mice

showed premature degenerative changes, including loss of fertility, osteoporosis, sarcopenia, dermal thinning, loss of subcutaneous fat, reduced hair growth, and retarded wound healing. Notably, cells from these mice underwent rapid senescence in culture (Maier et al., 2004). Moreover, tissues from these mice rapidly accumulated senescent cells, and, in lymphoid tissue, the p53 response shifted from primarily apoptotic to primarily senescent in vivo (Hinkal et al., 2009). Thus, there was a strong correlation between excessive cellular senescence and premature aging phenotypes.

It should be noted that another mouse model of elevated p53 activity showed unusual cancer resistance but normal longevity, with no signs of premature aging (García-Cao et al., 2002). In this model, extra copies of the wild-type p53 locus were introduced into the mouse genome. So, rather than being constitutively expressed, p53 was regulated normally, reaching higher levels only upon activation. This heightened p53 activation synergized with other transgenes to extend mean life span (Matheu et al., 2004, 2007; Tomás-Loba et al., 2008), thus p53 can be pro-aging or pro-longevity, depending on the physiological context (de Keizer et al., 2010).

Other gene functions and aging. Other mouse models also suggest that cellular senescence can drive age-related pathologies other than cancer.

One example is conditional ablation of a single allele encoding the p53-related protein p63, which caused extensive cellular senescence and multiple age-related pathologies (Keyes et al., 2005). Another example is mice that express a hypomorphic form of the mitotic checkpoint protein BubR1. These mice experience genotoxic stress, which induced widespread cellular senescence and several age-related degenerative pathologies; further, genetic manipulations that attenuated (p16INK4a deficiency) or exacerbated (deficiency in p19ARF) the senescence response also attenuated or exacerbated the pathology (Baker et al., 2008). Likewise, a mouse model of Hutchinson-Gilford progeria syndrome (HGPS), a childhood premature aging syndrome caused by aberrant lamin A processing, developed phenotypes that overlap with those of HGPS children and do not include cancer; cells from these mice showed chronic DDR signaling, chronic p53 activation, and cellular senescence (Varela et al., 2005). Further, administration of drugs such as statins and aminobisphosphonates reduced DDR signaling in the cells, and also alleviated some of the progeroid symptoms in the mice (Varela et al., 2008). In all these (and other) models of both accelerated and normal aging, it is important to note that the crucial roles for the p53 and/or p16INK4a/pRB pathways are not singular. There is mounting evidence that these pathways interact and modulate each other (Zhang et al., 2006; Leong et al., 2009; Su et al., 2009; Yamakoshi et al., 2009).

Finally, mouse models without obvious activated DDR signaling also suggest that senescent cells can drive aging phenotypes. One example is mice that lack CHIP (carboxy terminus of Hsp70-interacting protein), a chaperone/ubiquitin ligase that helps eliminate damaged proteins. CHIP-deficient animals rapidly accumulate senescent cells, and rapidly develop age-related phenotypes, including thin skin and loss of adiposity and bone density (Min et al., 2008). Likewise, mice that lack the circulating hormone Klotho age prematurely (Kuro-o et al., 1997). The primary cause of the progeroid phenotypes of Klotho-deficient mice is not known, but Klotho mediates calcium-regulated parathyroid hormone secretion (Imura et al., 2007), stimulates FGF signaling in the kidney (Urakawa et al., 2006), and dampens WNT signaling (Liu et al., 2007); the unopposed WNT signaling in Klotho-deficient mice is associated with the premature senescence of progenitor cells in several tissues.

A short retreat from the senescence-centric view of aging. Although these mouse models and other findings indicate a strong association between aging phenotypes and pathologies and cellular senescence, other processes undoubtedly also contribute to aging and age-related disease. One such process is cell death. For example, in one of the mouse models of constitutive p53 activity, there was also excessive p53-dependent apoptosis, which was also proposed to contribute to the progeroid phenotypes shown by these mice (Tyner et al., 2002). In addition, some cells in aging organisms simply lose functionality, which certainly also contributes aging phenotypes. Neurons, for example, lose the ability to form synapses, despite cell bodies remaining viable, which is an important component of many neurodegenerative pathologies (Esiri, 2007). Likewise, cardiomyocytes lose synchronicity of gene expression, which almost certainly affects heart function (Bahar et al., 2006).

How might senescent cells promote age-related pathologies? There are three possible scenarios by which senescent cells might drive aging.

First, as suggested by at least one of the defects shown by Klotho-deficient mice (Liu et al., 2007), cellular senescence can deplete tissues of stem or progenitor cells. This depletion will compromise tissue repair, regeneration, and normal turnover, leading to functional decrements (Drummond-Barbosa, 2008).

Second, the factors that senescent cells secrete affect vital processes–cell growth and migration, tissue architecture, blood vessel formation, and differentiation–and so are tightly regulated. The inappropriate presence of these factors can disrupt tissue structure and function. For example, the MMP3 secreted by senescent fibroblasts inhibits the morphological and functional differentiation of breast epithelial cells (Parrinello et al., 2005) and can promote tumor growth (Liu and Hornsby, 2007).

Third, the SASP includes several potent inflammatory cytokines (Freund et al., 2010). Low-level, chronic, "sterile" inflammation is a hallmark of aging that initiates or promotes most, if not all, major age-related diseases (Franceschi et al., 2007; Chung et al., 2009). Chronic inflammation can destroy cells and tissues because some immune cells produce strong oxidants. Also, immune cells secrete factors that further alter and remodel the tissue environment, which can cause cell/tissue dysfunction and impair stem cell niches. Inflammatory oxidative damage can also initiate carcinogenesis, and the inflammatory milieu can promote cancer by suppressing immune surveillance and/or stimulating malignant phenotypes (Allavena et al., 2008; Grivennikov et al., 2010). Thus, senescent cells might fuel cancer and other age-related pathologies by the same mechanism (the SASP; Fig. 2).

Cause or effect? Evidence that senescent cells drive aging remains circumstantial. The classical approach to

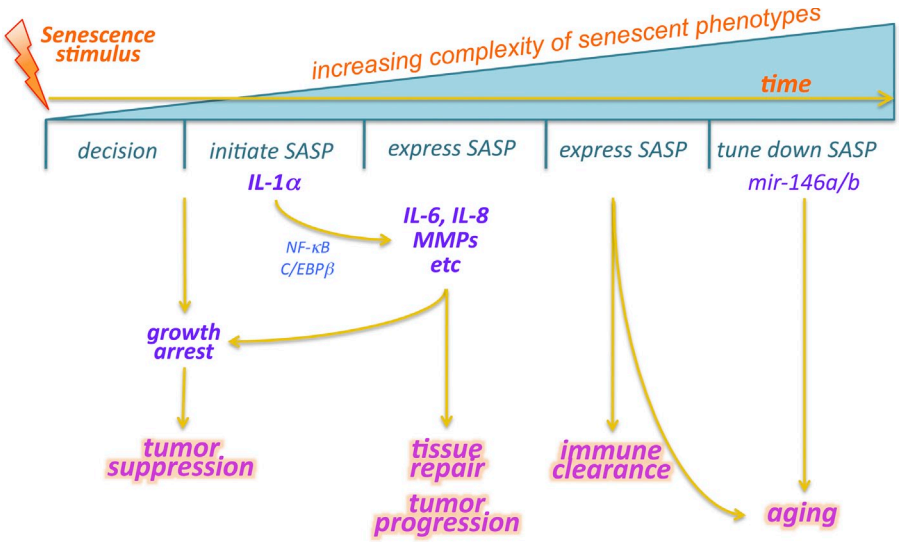

Figure 3. **Temporal organization of the senescent phenotype.** Upon experiencing a potentially oncogenic insult, cells assess the stress and must "decide" whether to attempt repair and recovery, or undergo senescence. After an interval (decision period), the length of which is imprecisely known, the senescence growth arrest becomes essentially permanent, effectively suppressing the ability of the stressed cell to form a malignant tumor. One early manifestation of the senescent phenotype is the expression of cell surface–bound IL-1α. This cytokine acts in a juxtacrine manner to bind the cell surface–bound IL-1 receptor, which initiates a signaling cascade that activates transcription factors (NF-κB, C/EBPβ). The transcription factors subsequently stimulate the expression of many secreted (SASP) proteins, including increasing the expression of IL-1α and inducing expression of the inflammatory cytokines IL-6 and IL-8. These positive cytokine feedback loops intensify the SASP until it reaches levels found in senescent cells. SASP components such as IL-6, IL-8, and MMPs can promote tissue repair, but also cancer progression. Some SASP proteins, in conjunction with cell surface ligands and adhesion molecules expressed by senescent cells, eventually attract immune cells that kill and clear senescent cells. A late manifestation of the senescent phenotype is the expression of microRNAs (mir-146a and mir-146b), which tune down the expression IL-6, IL-8, and possibly other SASP proteins, presumably to prevent the SASP from generating a persistent acute inflammatory response. Despite this dampening effect, the SASP can nonetheless continue to generate low-level chronic inflammation. The accumulation of senescent cells that either escape or outpace immune clearance and express a SASP at chronic low levels is hypothesized to drive aging phenotypes. Thus, senescent cells, over time (yellow line), develop a phenotype that becomes increasingly complex (blue triangle), with both beneficial (tumor suppression and tissue repair) and deleterious (tumor promotion and aging) effects on the health of the organism.

demonstrating cause and effect in biology—eliminate a gene or process, and determine the phenotype—cannot be applied in this case. Organisms in which cells fail to undergo senescence do not live longer; rather, they die prematurely of cancer (Rodier et al., 2007). Another approach might be to engineer mice in which senescent cells can be eliminated as they are formed. Although this feat has not yet been accomplished, recent short-term manipulations in mice revealed another surprising aspect of the senescence response: a role in tissue repair.

Cellular senescence and tissue repair

From even a cursory perusal of factors that comprise the SASP, it is obvious that many are important for tissue repair: growth factors and proteases that participate in wound healing, attractants for immune cells that kill pathogens, and proteins that mobilize stem or progenitor cells. Thus, the SASP may serve to communicate cellular damage/dysfunction to the surrounding tissue and stimulate repair, if needed. Two recent studies support this idea.

Upon acute liver injury in mice, hepatic stellate cells initially proliferate and secrete ECM components, which produce a fibrotic scar that eventually resolves. Shortly after the proliferative stage, stellate cells in the injured liver undergo senescence (Krizhanovsky et al., 2008). This senescence response is accompanied by a decline in ECM production and, as expected from the SASP, increased secretion of several MMPs, which are known to degrade ECM proteins. This finding suggested that the senescence response helps resolve the fibrotic scar. Consistent with this idea, when stellate cells are compromised for their ability to undergo senescence (because of deficiencies in p53 or p16INK4a and p19ARF), mice developed severe fibrosis after acute liver injury.

Similarly, in a mouse model of cutaneous wound healing, the ECM protein CCN1 is highly expressed and important for cell migration, differentiation, and survival. Surprisingly, healing wounds accumulated senescent fibroblasts and myofibroblasts; this did not occur in mice in which wild-type CCN1 alleles were exchanged for mutant alleles encoding CCN1 proteins that cannot bind fibroblasts (Jun and Lau, 2010). Wounds in the mutant mice were excessively fibrotic, but the fibrosis was reversed upon topical application of wild-type CCN1 protein; topical CCN1 induced cellular senescence and the expected expression of MMPs, which presumably helped resolve the fibrosis in the wound.

Together, these studies suggest that cellular senescence, although undoubtedly an important tumor suppressive response, is not simply a failsafe mechanism that is redundant to apoptosis. Rather, the senescence response may also be necessary for resolving normal tissue damage (Fig. 2). This new senescence-associated function in tissue repair suggests that the growth arrest was selected during evolution to suppress tumorigenesis, and possibly excessive cell proliferation or matrix deposition during wound repair. In contrast, the SASP most likely was selected to allow damaged cells to interact with the tissue microenvironment. In addition, some SASP components may have been selected to reinforce the senescence growth arrest.

Four faces of cellular senescence

So, how does cellular senescence participate in four complex processes (tumor suppression, tumor promotion, aging, and tissue repair), some of which have apparently opposing effects? We envision the senescent phenotype progressing through temporally regulated steps that orchestrate its activities (Fig. 3).

In cultured cells synchronously induced to senesce by ionizing radiation, the senescent growth arrest establishes rapidly, generally within 24–48 h (DiLeonardo et al., 1994; Rodier et al., 2009, 2011). Cells given a nonsenescence-inducing ionizing

radiation dose recover after 24 h, but those given a senescing dose do not. Thus, there must be a decision period during which oncogenically stressed cells "decide" to senesce. This decision, of course, precludes that cell from developing into a cancer (Fig. 3).

Among the earliest events after the growth arrest, at least in culture, is expression of IL-1α (Orjalo et al., 2009). This cell surface–bound cytokine activates the transcription factors nuclear factor κB (NF-κB) and C/EBPβ (Orjalo et al., 2009), which are required for the expression of many SASP proteins (Fig. 3; Acosta et al., 2008; Kuilman et al., 2008; Freund et al., 2010). Some of these second-wave SASP proteins reinforce the growth arrest, whereas others facilitate tissue repair and drive cancer progression (Fig. 3).

We imagine these activities antecede the expression of proteins that permit the immune system to clear senescent cells (Fig. 3). Senescent cells express surface-bound ligands and adhesion molecules that target them for attack by natural killer and other immune cells (Krizhanovsky et al., 2008), although it is not known when these proteins are expressed relative to the SASP. Because senescent cells increase with age, either clearance is incomplete (and so senescent cells gradually accumulate) or aged individuals generate senescent cells faster than their immune system can handle, or both (Fig. 3).

Finally, in culture, senescent cells eventually express two microRNAs, mir-146a and mir-146b (Bhaumik et al., 2009), which comprise a negative feedback loop to dampen NF-κB activity (Taganov et al., 2006; Freund et al., 2010). Mir-146a/b increase many days after IL-6 and IL-8 are maximally secreted (Fig. 3), and only in cells that secrete very high levels (Bhaumik et al., 2009). Thus, these microRNAs are an example of another layer of complexity in the regulation of long-term senescence phenotypes, one that "tunes down" the SASP should it reach very high levels (Fig. 3). The induction of these miRNAs may prevent the SASP from generating persistent acute (robust) inflammation, which, unlike low-level chronic inflammation, is designed to be self-limiting (Taganov et al., 2006). Despite their induction, however, the inflammatory response can persist, albeit at a low chronic level, and we speculate that it can drive the chronic pathologies associated with aging.

From cell culture phenomenon to orchestrator of tumor suppression, cancer, wound healing, and aging, cellular senescence has a rich history, marked by unexpected complexity. Some aspects of its physiological significance remain conjecture, and several aspects of its regulation remain enigmatic. As biologists further unravel the foundations and consequences of cellular senescence, they will likely reveal a deepening complexity and additional surprises.

We thank members of our laboratories and many colleagues for discussions that helped the preparation of this manuscript.

F. Rodier is supported by grants from the Institut du Cancer de Montréal (René Malo Initiative for Innovative Research) and Cancer Research Society (CRS). J. Campisi is supported by grants from the US National Institutes of Health (National Institute on Aging, National Cancer Institute) and the Department of Energy. The authors declare no commercial affiliations and no conflicts of interest.

Submitted: 17 September 2010
Accepted: 20 January 2011

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