Cell

Cellular Senescence in Cancer and Aging

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DOI 10.1016/j.cell.2007.07.003

Cellular senescence, a state of irreversible growth arrest, can be triggered by multiple mechanisms including telomere shortening, the epigenetic derepression of the *INK4a/ARF* locus, and DNA damage. Together these mechanisms limit excessive or aberrant cellular proliferation, and so the state of senescence protects against the development of cancer. Recent evidence suggests that cellular senescence also may be involved in aging.

The process of cellular senescence was first described in a seminal study by Hayflick and Moorhead (1961) in which they observed that normal human fibroblasts were able to enter a state of irreversible growth arrest after serial cultivation in vitro; meanwhile cancer cells did not enter this growth arrest state and proliferated indefinitely. Hayflick and Moorhead (1961) presciently hypothesized the existence of cellular factors, whose loss through consecutive cell divisions limited the proliferation of normal cells. They went on to speculate that this "stopwatch" could be behind the process of organismal aging. Today, cellular senescence is considered a stress response triggered by a number of "counting mechanisms," such as telomere shortening, that are increasingly well understood at the molecular level. Importantly, the mechanisms underlying cellular senescence are involved in protection against cancer and also may be involved in organismal aging. It is important to emphasize here that although senescent cells in vitro may remain viable essentially indefinitely—albeit incapable of proliferation—the situation in vivo may be more complex. There are examples of in vivo senescent cells that may reside for years in the organism, such as the senescent melanocytes of moles or nevi (Michaloglou et al., 2005), and there are also examples of senescent cells that are rapidly removed by phagocytic cells, as in the case of senescent tumor cells in liver carcinomas (Xue et al., 2007). In the case of physiological aging, the increase in senescent cells is measurable but modest (Dimri et al., 1995). However, senescence may contribute to aging not only by net accumulation of senescent cells in tissues, but also by limiting the regenerative potential of stem cell pools. These two mechanisms—namely, accumulation of senescent cells and loss of stem cell function—probably contribute to aging simultaneously. Below, we review the molecular biology of the three main "Hayflick factors" (Figure 1; telomere loss, the accumulation of DNA damage, and derepression of the *INK4a/ARF* locus) and discuss their role in cancer protection and the current understanding of their involvement in aging.

Recording Cell Divisions by Telomere Loss

Studies of telomere length regulation provided the first molecular mechanism capable of counting cell divisions and implementing cell cycle arrest (Harley et al., 1990). Telomeres consist of repetitive DNA elements at the end of linear chromosomes that protect the DNA ends from degradation and recombination (Chan et al., 2001; de Lange, 2005). Due to the intrinsic inability of the replication machinery to copy the ends of linear molecules, telomeres become progressively shorter with every round of cell division (Blasco, 2005). Eventually, telomeres reach a

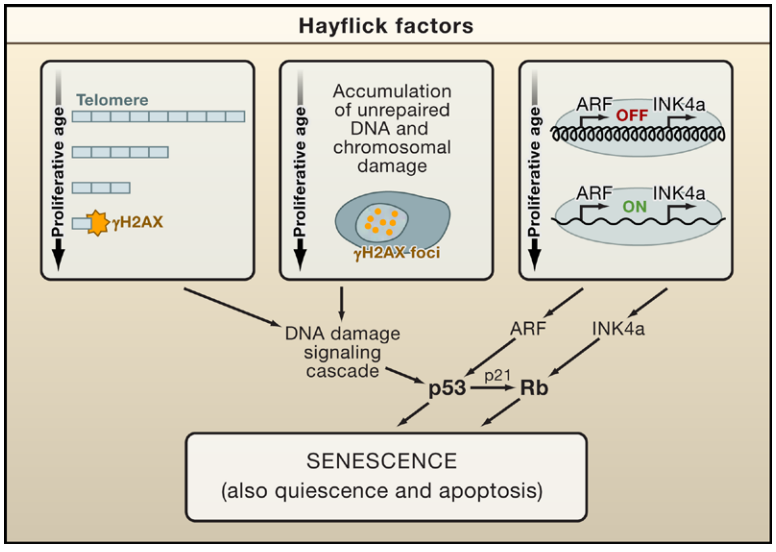

Figure 1. "Hayflick Factors" Record the Proliferative History of Cells and Tissues
The three best-known Hayflick factors—telomere shortening, accumulation of DNA damage, and derepression of the *INK4a/ARF* locus—are summarized together with their main effectors, the tumor suppressors p53 and retinoblastoma (Rb).

critically short length, behaving as double-stranded DNA breaks that activate the p53 tumor suppressor protein resulting in telomere-initiated senescence or apoptosis (de Lange, 2005; von Zglinicki et al., 2005). Telomerase is a ribonucleoprotein with DNA polymerase activity that elongates telomeres (Greider and Blackburn, 1985), but its level of activity in most adult tissues is not sufficient to compensate for the progressive telomere attrition that occurs with aging (Collins and Mitchell, 2002). The generation of telomerase-deficient mice demonstrated that telomerase is the main cellular activity responsible for maintaining telomere length (Blasco et al., 1997). Importantly, in the case of in vitro cultured cells, ectopic expression of telomerase was sufficient to prevent telomere shortening, and this resulted in immortalization of human fibroblasts, thus formally demonstrating that telomere exhaustion is a critical factor leading to cellular senescence (Bodnar et al., 1998).

The Telomere Clock in Cancer Protection

The telomere clock limits not only the proliferation of normal noncancerous cells, but also the proliferation of those cells that are already on the road to neoplastic transformation. This is best illustrated by the fact that essentially all human cancers have acquired mechanisms to maintain telomeres, generally through the expression of high levels of telomerase (Stewart and Weinberg, 2006). Exceptionally, immortal human cell lines and tumors may maintain their telomeres in the absence of telomerase through a mechanism known as *a*lternative *l*engthening of *t*elomeres (ALT), which involves homologous recombination between telomeres (Muntoni and Reddel, 2005). These observations in human cancer strongly support the concept that telomerase is a tumorigenic factor that enables tumor progression. In agreement with this, mice deficient in telomerase activity are significantly resistant to cancer induced by a variety of genetic defects or carcinogenic treatments (Blasco, 2005). The two main exceptions to this general trend occur when lack of telomerase is combined with a deficiency in p53 (Chin et al., 1999) or with overexpression of the telomere-binding protein TRF2, which recruits the nuclease XPF to telomeres and degrades them (Blanco et al., 2007). Under these conditions, cells proliferate in the presence of rampant chromosomal aberrations, which in turn fuels the development of cancer (Artandi et al., 2000).

The final cellular outcome responsible for telomere-mediated protection against cancer involves senescence and apoptosis (Kelland, 2005; Shammas et al., 2005). Whether a cell senesces or undergoes apoptosis likely depends on both cell type and context. Moreover, preneoplastic cells are exposed not only to dysfunctional short telomeres but also to other potential triggers of senescence or apoptosis, such as oncogenic signaling. Recently, telomere-induced senescence has been shown to act as a tumor suppressor mechanism in telomerase-deficient mice expressing the oncogene Myc in B cells (Feldser and Greider, 2007). Short telomeres reduced the incidence of cancer in these animals even when apoptosis was blocked by overexpression of the antiapoptotic protein Bcl2. Examination of the lymph nodes of these apoptosis-resistant mice revealed clear signs of senescence induction, demonstrating that telomere-dependent senescence is an efficient mechanism to suppress cancer in vivo (Feldser and Greider, 2007).

The Telomere Clock in Aging

In humans, many studies have described an inverse correlation between telomere length and age in a variety of tissues and between telomere length and diseases associated with aging (Canela et al., 2007; Cawthon et al., 2003; Ogami et al., 2004; Panossian et al., 2003). Also, factors that may decrease longevity, such as psychological stress or obesity, decrease telomerase activity and telomere length (Epel et al., 2004; Valdes et al., 2005). In addition, several human premature aging syndromes, such as dyskeratosis congenita (DC) and aplastic anemia, are linked to mutations in telomerase or in proteins that directly affect telomerase activity and are characterized by a faster rate of telomere attrition with age (Mason and Bessler, 2004). Other premature aging syndromes, including those produced by mutations in DNA repair proteins such as NBS1 (Nijmegen breakage syndrome), MRE11 (Ataxia telangiectasia-like disorder), WRN (Werner syndrome), BLM (Bloom syndrome), ATM (Ataxia telangiectasia), and FANC (Fanconi anemia), are also characterized by an accelerated rate of telomere attrition and chromosomal instability (reviewed in Blasco, 2005). Additionally, it is important to note that the rate of telomere shortening is accelerated by oxidative damage (von Zglinicki and Martin-Ruiz, 2005). Taking into account that aging is associated with the accumulation of oxidative damage (see below), it is conceivable that telomere shortening could reflect not only the proliferative history of a cell, but also the accumulation of oxidative damage.

Further reinforcing the link between the telomere clock and aging, mice deficient in telomerase activity have short telomeres and age prematurely (Blasco et al., 1997; Lee et al., 1998). Remarkably, even the first generation of telomerase-deficient mice already has a shortened lifespan, which becomes shorter and shorter in subsequent generations (Garcia-Cao et al., 2006). Moreover, telomeres become measurably shorter in mice, particularly at very old ages, both in *Mus spretus* (Coviello-McLaughlin and Prowse, 1997) and in laboratory *Mus musculus* (unpublished data). Finally, mice overexpressing telomerase are prone to developing tumors (Canela et al., 2004; Gonzalez-Suarez et al., 2001), thus precluding direct demonstration of lifespan extension by telomerase; nonetheless, an increase in the lifespan of those few telomerase transgenic mice that do not develop cancer has been observed (Gonzalez-Suarez et al., 2005). Together, these observations suggest that telomere biology (including telomere length, telomere capping, and rate of attrition) has a strong influence on longevity both in humans and in mice.

Conceivably, telomere-driven aging could be the consequence of telomere shortening in stem cell pools, which in turn results in tissues with a high rate of

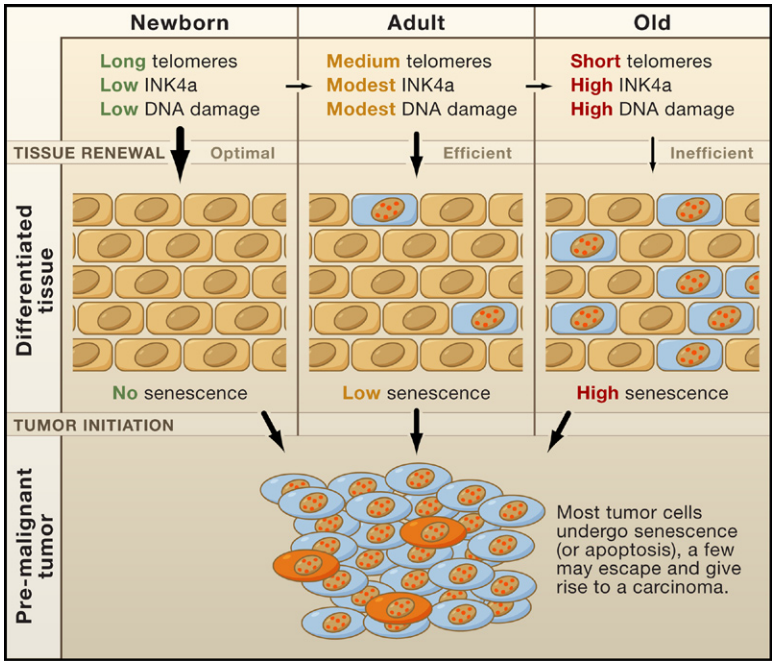

Figure 2. Senescence of Stem Cells and Committed Cells

Multiple mechanisms operate on stem cells and on committed cells to produce senescence. Telomere shortening, derepression of the *INK4a/ARF* locus, and the accumulation of DNA damage operate during the lifespan of the organism on stem cells (left side, from top to bottom), as well as on committed cells derived from the stem cells on their way to generating a differentiated tissue (from left to right). The final result is age-dependent loss of stem cell functionality (represented by a thin arrow in the top right) and an age-dependent increase in the number of senescent cells in differentiated tissues (represented as blue cells). Tumors may arise at any point in life, but the stress signals characteristic of tumors will engage the senescence program. This tumor-specific senescence constitutes an important barrier to tumor progression.

senescence, as well as a progressive exhaustion of the regenerative potential of stem cells (Figure 2). Together, these two factors—the accumulation of senescent cells and the loss of stem cell function—could result in impaired tissue function. Regarding the accumulation of senescent cells, there are few reports examining the accumulation of senescent cells in vivo due to telomere dysfunction. Abundant senescent cells (as measured by the widely used marker *s*enescence-*a*ssociated β-*g*alactosidase or SAβGal) have been reported in the liver of telomerase null mice (Satyanarayana et al., 2003). Independent of this study, a number of reports have obtained evidence for the presence of dysfunctional telomeres in aged tissues. Critically short or dysfunctional telomeres can be considered a particular form of DNA damage and, as such, they are marked with phosphorylated histone H2AX (γH2AX; d'Adda di Fagagna et al., 2003). Based on this, dysfunctional telomeres have been visualized as foci of γH2AX that colocalize with telomeres in cultured human cells and in tissues of aged mice and baboons, providing a marker of telomere loss (d'Adda di Fagagna et al., 2003; Garcia-Cao et al., 2006; Herbig et al., 2006). It should be noted, however, that not all the age-associated γH2AX foci are associated with telomeres (Sedelnikova et al., 2004; see below). Recently, p21Cip1, a cyclin-dependent kinase inhibitor activated by p53, has proved to be relevant in the signaling of critically short telomeres that leads to aging (Choudhury et al., 2007). Deletion of the gene encoding p21Cip1 prolongs lifespan and improves the general fitness of telomerase-deficient mice. These effects were observed in the absence of accelerated tumorigenesis, which contrasts with the situation in mice lacking p53 (Chin et al., 1999). In addition, given that p21Cip1 mediates p53-dependent senescence but not p53-dependent apoptosis, these results suggest that p53/p21Cip1-dependent senescence specifically mediates the proaging effect of short telomeres. A more recent addition to this pathway has been the discovery that the DNA mismatch repair machinery, in particular the DNA repair factor PMS2, is involved in triggering proliferation arrest and aging by telomere loss in a process that appears to be upstream of p21Cip1 (Siegl-Cachedenier et al., 2007). Together, these observations are important because they dissociate the proaging function of telomere shortening (PMS2- and p21Cip1-dependent) from the anticancer function of telomere shortening (p53-dependent but p21Cip1-independent).

The loss of stem cell function through telomere shortening has been extensively reported in a variety of tissues and experimental systems (Flores et al., 2006). On the one hand, short telomeres due to telomerase deficiency result in impaired stem cell functionality, defective tissue regeneration, and decreased tumorigenesis, while on the other hand telomerase overexpression has the opposite effects (Flores et al., 2005; Sarin et al., 2005). These findings suggest that telomere length and telomerase activity are important determinants of stem cell fitness and in this manner may influence cancer and aging. Despite this suggestive evidence from genetically manipulated mice, it still remains to be demonstrated whether the telomere shortening in stem cells occurs during physiological aging, and whether stem cells with critically short telomeres undergo senescence or apoptosis in vivo.

In summary, current knowledge indicates that telomere shortening is simultaneously a cancer protective mechanism and a proaging mechanism.

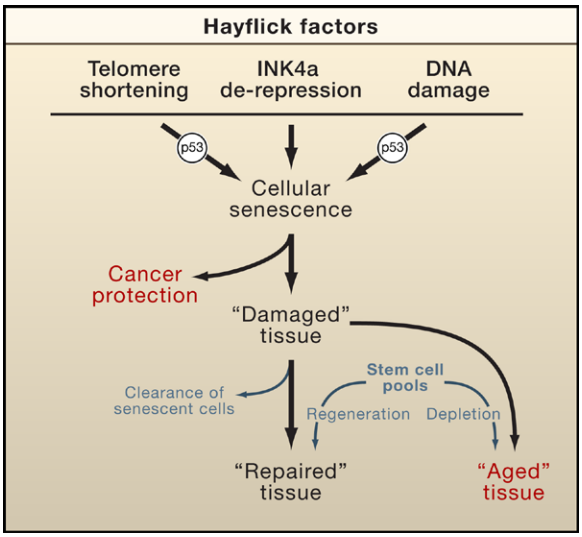

Figure 3. Cellular Senescence: Balancing Cancer and Aging
The main role of senescence in young/adult organisms is to provide cancer protection because the "damage" that senescent cells cause to tissues is efficiently repaired by the normal renewal processes. In mutant mice with severe constitutive DNA damage or DNA damage signaling (as in the case of permanently active p53), the rate of generation of senescent cells may outpace the renewal capacity of the stem cells (which themselves are also subject to constitutive DNA damage signaling), and tissues may age prematurely. This situation could also occur in old organisms.

Recording Mitogenic Stimulation by the *INK4a/ARF* Locus

The *INK4a/ARF* locus encodes two critical tumor suppressors, p16INK4 and ARF, which, in addition to their role in cancer, are important inducers of cellular senescence. Although sharing exons, the two proteins are encoded in different reading frames, and as a consequence they do not have any amino acid homology and possess different molecular functions (Figure 1). Whereas p16INK4a is an inhibitor of the cyclin-dependent kinases CDK4 and CDK6 and acts by imposing a G1 cell cycle arrest, ARF regulates p53 stability through inactivation of the p53-degrading ubiquitin ligase MDM2 (Gil and Peters, 2006; Kim and Sharpless, 2006). The *INK4a/ARF* locus is normally expressed at very low levels in most tissues in young organisms but becomes derepressed with aging (Krishnamurthy et al., 2004). The epigenetic regulators of the Polycomb family are responsible, at least in part, for the low expression levels of p16INK4a and ARF under normal conditions in young tissues (Bracken et al., 2007; Jacobs et al., 1999). In addition, numerous positive and negative transcriptional regulators have been reported, most of which have turned out to have relevance in cancer (Gil and Peters, 2006; Kim and Sharpless, 2006; Sharpless, 2005).

Soon after the discovery of p16INK4a (Serrano et al., 1993), and in the context of the search for factors that could explain cellular senescence, it was observed that p16INK4a levels accumulated progressively with the proliferative history of cells in culture. In the case of embryonic murine fibroblasts, senescence in culture occurs despite having long telomeres and high levels of telomerase, but this telomere-independent clock is abrogated in cells genetically deficient in the *INK4a/ARF* locus (Serrano et al., 1996). A wealth of data have subsequently supported a model in which cells in culture possess at least two independent clocks: one is telomere dependent and mostly registers the accumulated number of cell divisions; the other clock is INK4a/ARF dependent and mainly records the exposure of cells to mitogenic stimulation. It is possible to cancel the INK4a/ARF clock if culture conditions are finely tuned to avoid mitogenic overstimulation (Ramirez et al., 2001; Woo and Poon, 2004). Conversely, the INK4a/ARF clock can be accelerated by introducing high levels of expression of an oncogene, a phenomenon that has been called oncogene-induced senescence (Collado and Serrano, 2006; Serrano et al., 1997). Cancer protection through the activation of the *INK4a/ARF* locus by oncogenes is well documented and will not be discussed here; the interested reader is referred to recent comprehensive reviews (Gil and Peters, 2006; Kim and Sharpless, 2006; Sharpless, 2005).

The INK4a/ARF Locus in Aging

Expression of both p16INK4a and ARF markedly increases with aging in the majority of rodent tissues (Krishnamurthy et al., 2004; Zindy et al., 1997). Studies performed in human tissues have also detected a clear age-dependent increase in the levels of p16INK4a in kidney and skin (Chkhotua et al., 2003; Ressler et al., 2006). Together, these studies make p16INK4a an excellent molecular marker of aging with on average a 30-fold induction in aged murine tissues (Krishnamurthy et al., 2004) and a 7-fold induction in human skin (Ressler et al., 2006). The molecular mechanisms responsible for the increased expression of p16INK4a and ARF during aging are not completely understood. Among the many known transcriptional regulators of the locus, two have been associated to the age-dependent induction of p16INK4a. In particular, the levels of the transcriptional factor Ets1, which is a positive regulator of p16INK4a (Ohtani et al., 2001), increase during mouse aging in parallel to the upregulation of p16INK4a (Krishnamurthy et al., 2004). Similarly, the levels of the Polycomb group member Bmi1, which is a potent repressor of p16INK4a (Jacobs et al., 1999), decrease with aging in human skin (Ressler et al., 2006). Another important mechanism that could link aging with p16INK4a is oxidative damage and the p38MAPK pathway (Iwasa et al., 2003; Wang et al., 2002). It has been recently observed in hematopoietic stem cells that reactive oxygen species activate the expression of the *INK4a/ARF* locus through p38MAPK, and this limits the functionality of these stem cells (Ito et al., 2006). Since oxidative damage increases with aging, it is conceivable that this could contribute to the age-dependent upregulation of the locus. Besides being a marker of aging, the question of whether p16INK4a is an

effector of aging remains open. Mice with increased levels of p16INK4a and ARF have a normal lifespan (Matheu et al., 2004), although it could be argued that in these mice the putative proaging effect of p16INK4a is cancelled by an antiaging effect of ARF (see below). On the other hand, mice with decreased levels of p16INK4a are tumor prone, and this precludes the analysis of aging (Krimpenfort et al., 2001; Sharpless et al., 2001).

As with telomere shortening, the proaging effects of p16INK4a can be the combined result of the accumulation of senescent cells in tissues and, perhaps of higher impact, the impairment of stem cell function and the ensuing defects in tissue regeneration (Figure 2). Regarding accumulation of senescent cells, it remains to be demonstrated that cells expressing high levels of p16INK4a correlate with senescent cells in aged tissues. Regarding the impairment of stem cell function, a connection between p16INK4a and stem cell biology has long been suspected based on the analysis of mice deficient in Polycomb genes (Valk-Lingbeek et al., 2004). This concept has received further support by a series of recent papers reporting age-induced expression of p16INK4a in adult stem cells and its association with impaired tissue regeneration of the pancreatic islets, hematopoietic system, and neuronal progenitors (Janzen et al., 2006; Krishnamurthy et al., 2006; Molofsky et al., 2006). Moreover, mice lacking p16INK4a have increased regeneration potential (Janzen et al., 2006; Krishnamurthy et al., 2006; Molofsky et al., 2006), whereas the opposite is true of mice with enhanced expression of p16INK4a (Krishnamurthy et al., 2006). Taken together, these observations point to p16INK4a contributing to the age-associated decline in tissue regeneration characteristic of mammalian aging. High levels of p16INK4a could result in suboptimal proliferation of the stem cells or even p16INK4a-induced senescence of the stem cells. A note of caution is warranted due to the fact that stem cell quiescence is also important to provide long-term regeneration potential, as it has been demonstrated for the cell cycle inhibitor p21Cip1 (Cheng et al., 2000). In this context, it is formally possible that although increased p16INK4a levels may impair tissue regeneration in models of acute tissue damage, it may not be detrimental for physiological longevity, as suggested by the analysis of mice with increased gene dosage of INK4a/ARF (Matheu et al., 2004). Recently, genetic variants in the *INK4a/ARF* locus have been linked to various age-associated diseases, including general frailty, heart failure, and type 2 diabetes (Helgadottir et al., 2007; McPherson et al., 2007; Melzer et al., 2007; Saxena et al., 2007; Scott et al., 2007; Zeggini et al., 2007). Much may be clarified by ascertaining whether these variants have increased or decreased expression of INK4a/ARF and how they behave in response to aging or mitogenic stimulation. An important question that remains to be elucidated is the precise mechanism responsible for the derepression of the *INK4a/ARF* locus in stem cells. An attractive hypothesis is that the derepression of the *INK4a/ARF* locus is intrinsically linked to the proliferation of stem cells and therefore is developmentally programmed to increase during aging.

In summary, the derepression of the *INK4a/ARF* locus is one of the main barriers to the outgrowth of cancer cells and likely contributes to aging in normal tissues.

DNA Damage

It is well established that DNA damage, in its multiple forms, contributes to the development of cancer. Therefore, we will focus here on the relation between DNA damage and aging. Early studies using in vitro cultured cells showed that the infliction of various forms of DNA damage triggers senescence (Parrinello et al., 2003; te Poele et al., 2002). Recent data suggest that this link could be more profound and that DNA damage could be a common causative agent underlying different forms of cellular senescence, including not only telomere dysfunction (see above), but also oncogene-induced senescence (Bartkova et al., 2006; d'Adda di Fagagna et al., 2003; Di Micco et al., 2006). Organismal aging is accompanied by significant increases in DNA mutations (Vijg, 2000), DNA oxidation (Hamilton et al., 2001), chromosome losses (Rehen et al., 2005; Weaver et al., 2007), and telomere-independent γH2AX foci (Sedelnikova et al., 2004). These and other observations have prompted researchers to consider DNA damage and DNA damage signaling as possible factors contributing to cellular senescence and to organismal aging. The age-dependent accumulation of DNA damage could result in the accumulation of senescent cells in aged tissues, and it could also diminish the functionality of stem cells (Figure 2).

The impact of particular DNA repair defects on cancer and aging has been the topic of excellent and comprehensive reviews (for example see Lombard et al., 2005). The numerous mouse models described with defects in DNA repair present a variety of phenotypes. Some are dramatically cancer prone (e.g., ATM null mice; Barlow et al., 1996) whereas others are minimally affected in their cancer susceptibility (e.g., Ku80 null mice; Lim et al., 2000); similarly, some age prematurely (e.g., ERCC1 null mice; Niedernhofer et al., 2006), whereas others do not; and, finally, the two phenotypes may or may not go together and may or may not correlate with premature cell senescence (reviewed in Lombard et al., 2005). At present, we lack a unified model to explain cancer and aging in all the available mouse models deficient in DNA repair, and this will most likely require a deeper understanding of the physiological consequences of each particular mutation. Nonetheless, we note some general tendencies. Those mutations impairing DNA damage signaling, but not DNA damage repair per se, such as ATM or Rad50, accumulate genomic instability because of their failure to notify the cell of DNA damage. This accumulation of "unnoticed" genomic instability results in increased propensity to cancer, whereas aging is not affected. On the other hand, those mutations that do not affect DNA damage signaling but affect DNA repair or

stability (such as ERCC1 null mice, BRCA1-hypomorphic mice, or ZMPSTE24 null mice; Cao et al., 2003; Niedernhofer et al., 2006; Varela et al., 2005) accumulate massive DNA damage that triggers strong protective responses that lead to premature aging in the absence of cancer. A consistent observation in many of the latter mouse models with constitutive high levels of DNA damage and premature aging is the fact that the premature aging is partially relieved by p53 deficiency, arguing that this phenotype is triggered as a consequence of the signaling of the damage rather than by the damage itself (Ferguson and Alt, 2001).

The existence of mutant mice that are deficient in DNA repair exhibiting premature senescence and progeroid phenotypes argues for the involvement of DNA damage-induced senescence in the process of aging. However, as a note of caution, it should be borne in mind that all of the above-mentioned mouse models have massive DNA damage that compromises cellular viability and, importantly, require an abnormally high level of cell renewal that could lead to loss of stem cell functionality (Figure 3). In our opinion, we still lack direct proof implicating physiological levels of DNA damage as a causative agent of aging. This would be clarified, for example, if mice with increased DNA damage repair age more slowly.

The Impact of p53 in Aging

The analysis of the effect of p53 in aging has revealed a dual role that seems to depend on the intensity of p53 activity. Mice genetically manipulated to express constitutively active mutant p53 have greater protection against tumor development than wild-type mice, while at the same time they show signs of premature aging (Maier et al., 2004; Tyner et al., 2002). In contrast, mouse models of increased wild-type p53 activity do not present premature aging. In particular, super-p53 mice, carrying an extra copy of the entire p53 gene, and thus under the control of its natural regulatory sequences, exhibit increased cancer protection while displaying normal longevity and overall fitness (Garcia-Cao et al., 2002). Concurring with these results, mice with a hypomorphic MDM2 allele, the major negative regulator of p53, present stronger p53-mediated activities that result in enhanced cancer protection without affecting aging (Mendrysa et al., 2006). An additional mouse model, super-INK4a/ARF mice, carrying an extra copy of the entire *INK4a/ARF* locus (being ARF an activator of p53), further supports the feasibility of getting extra cancer protection without the undesirable side effects of aging (Matheu et al., 2004). Moreover, increased p53 activity in super-p53 mice decreases the amount of telomere-damaged cells in aged mice (Garcia-Cao et al., 2006). Finally, in agreement with the antioxidant function of p53 (Sablina et al., 2005), increased activity of p53 by combination of extra gene dosage of p53 and ARF decreases the amount of oxidative damage in aged mice and delays aging (Matheu et al., 2007). Together, these observations indicate that the main role of p53 is to eliminate damaged cells, either by triggering their self-destruction (for example, by inducing apoptosis) or by pulling them out of the proliferative pool (by inducing senescence). We propose that under normal or moderate levels of damage, such as during physiological aging, the elimination of damage by p53 has a net antiaging effect that compensates for the possible proaging consequences of p53-dependent apoptosis or senescence. In contrast, uncontrolled activity of p53 or the presence of massive DNA damage, as in some of the DNA repair mutants mentioned above, results in excessive elimination of cells by p53 that exhausts the capacity of tissue regeneration leading to premature aging (Figure 3).

Senescence and Cancer

The antiproliferative power of the senescence response clearly places this process as a plausible tumor suppressor mechanism. Early on, senescence was found to be mediated by the two main tumor suppressor pathways of the cell, the ARF/p53 and the INK4a/RB pathways (see Figure 1; Gil and Peters, 2006; Kim and Sharpless, 2006). The demonstration of a senescent-like arrest mediated by p53 and p16INK4a after ectopic expression of oncogenic Ras in normal primary cells introduced the concept of oncogene-induced senescence as a mechanism to restrain the growth of potentially dangerous cells (Serrano et al., 1997). Loss of these tumor suppressors is essential for achieving oncogenic transformation of human cells in vitro (Hahn and Weinberg, 2002), and indeed these pathways are frequently disrupted in human cancer cells (Gil and Peters, 2006; Kim and Sharpless, 2006). An ongoing debate concerns the relative importance of ARF and p53 in oncogene-induced senescence. In vivo evidence using genetically manipulated mice indicates that ARF is the critical sensor that activates p53 during tumorigenesis in response to oncogenic signals (Christophorou et al., 2006; Efeyan et al., 2006). In contrast, other investigators using cultured human fibroblasts have concluded that the critical mediator that activates p53 in response to oncogenic signaling is the DNA damage signaling cascade triggered by the aberrant firing of replication origins (Bartkova et al., 2006; Di Micco et al., 2006; Mallette et al., 2007). This apparent conflict may reflect an intrinsic difference between human and mouse cells, or it could simply be due to differences in the experimental models employed.

The identification of a senescent response in vivo after oncogenic activation was reported only recently. Taking advantage of DNA microarrays to obtain the gene expression profiles of senescent cells, we identified markers of senescence that could then be used in histological sections (Collado et al., 2005). We observed that premalignant lesions in the lung (which developed in a conditional knockin mouse model of oncogenic K-RasV12) contained abundant senescent cells, whereas lung adenocarcinomas were almost completely devoid of cells positive for markers of oncogene-induced senes-

cence. These results have been extended to Raf, a crucial downstream effector for Ras-induced senescence, through the generation of a conditional BRAFV600E knockin mouse model (Dankort et al., 2007). Further experimental evidence for the occurrence of senescence during tumor progression has been independently provided by several groups. In humans, senescent cells were identified in benign lesions of the skin carrying oncogenic mutant BRAF (BRAFE600; Michaloglou et al., 2005); in neurofibromas from NF1 mutant patients, a genetic defect that leads to constitutively high levels of Ras activity (Courtois-Cox et al., 2006); and in benign lesions of the prostate (Chen et al., 2005). In mice, abundant senescent cells were identified in benign lesions of the prostate of mice lacking the tumor suppressor PTEN (Chen et al., 2005), in melanocytic lesions of UV-irradiated HGF/SF-transgenic mice (Ha et al., 2007), in lymphocytes and in the mammary gland from N-Ras transgenic mice (Braig et al., 2005; Sarkisian et al., 2007), and in hyperplasias of the pituitary gland of mice with deregulated E2F activity (Lazzerini Denchi et al., 2005). More importantly, genetic manipulations canceling the senescence response led to full-blown malignancy. Prostates from mice lacking PTEN developed adenocarcinomas when combined with p53 deficiency (Chen et al., 2005). Similarly, transgenic N-Ras animals developed malignant lymphoma after deletion of *Suv39H1*, a gene coding for a histone H3 lysine 9 methyl-transferase believed to be involved in the de novo formation of silent heterochromatin that takes part in senescence (Braig et al., 2005). Recently, by taking advantage of K-Ras-transgenic mice with adjustable levels of expression, it has been shown that moderate levels of Ras activity trigger mammary hyperplasias but are unable to produce tumors. Interestingly, higher levels of Ras (similar to those found in spontaneous tumors) trigger senescence and result in malignant tumors when the senescence-inducing pathways are disrupted (Sarkisian et al., 2007). In summary, there is now compelling evidence to associate senescent cells with premalignant stages of tumor development. These findings indicate that senescence restricts tumor progression and might potentially serve as a useful marker for tumor staging (Collado and Serrano, 2006). Despite the above-described advances, it still remains to be rigorously examined whether senescent cells in premalignant lesions share all the features that characterize in vitro senescent cells—in particular whether the proliferative arrest is irreversible. In this regard it is worth mentioning that some authors distinguish two types of senescence-like arrest in vitro—a reversible one mediated by p53 and an irreversible one mediated by the concomitant actions of the INK4a/Rb and p53 pathways (Beausejour et al., 2003).

Even though cancer cells have partially lost the capacity to signal senescence or apoptosis, these responses can conceivably be engaged by exogenous chemotherapeutic drugs. Many of the chemotherapeutic drugs currently used in the clinic or under investigation act by inflicting severe DNA damage to the tumor cell and correspondingly trigger cellular senescence in vitro. Interestingly, there is evidence that they can also trigger senescence of cancer cells in real tumors, and it is possible that senescence could contribute to the success of chemotherapy (Roberson et al., 2005; te Poele et al., 2002). In this sense, chemotherapeutic intervention aimed to trigger senescence might prove effective. Genetic demonstration of this notion has been provided recently using two elegantly designed mouse models. Tumors initiated by loss of p53 can be eliminated by p53 restoration and, at least for some tumor types, such as hepatocarcinomas or soft-tissue sarcomas, tumor regression is achieved through senescence (Ventura et al., 2007; Xue et al., 2007). This implies that the signals that would activate p53 to induce senescence might still be present in the tumor. Thus, small molecule inhibitors aimed at restoring normal p53 signaling could prove effective in controlling cancer. Significantly, tumor regression by senescence induced after p53 restoration proceeded through an innate immune response that led to clearance of the senescent cells (Xue et al., 2007). Thus, senescent cells can be cleared in vivo, at least in the context of tumor suppression. Whether this is also the case during aging remains to be investigated, and it would imply that the higher abundance of senescent cells in aged tissues (see above) reflects an increase in the generation of senescent cells rather than their net accumulation over the years. Hopefully, future research will clarify whether therapies that induce senescence are useful for treating cancer and will determine their effect on normal cells and in aging.

Senescence and Aging

The association between cellular senescence and organismal aging is highly suggestive of a causal link between these two processes, although establishing a direct causative relationship is challenging. To start with, the factors that trigger senescence also trigger apoptosis and quiescence, making it difficult to dissect the contribution of each of these responses to aging. An additional complexity, already mentioned at the beginning of this review, is that senescence may impact on aging through two nonexclusive and possibly concomitant mechanisms (Figure 2). First, accumulation of senescent cells in tissues may reach a point that compromises functionality, and, second, senescence may limit the regenerative potential of adult stem cells (a limitation that may be produced as well by quiescence or apoptosis of stem cells). In one extreme, aging could be produced by the net accumulation of senescent cells; in the other extreme, the accumulation of senescent cells per se could be harmless, and aging could result primarily from the exhaustion of the regenerative potential of stem cells. Finally, and in relation to the above, it remains to be determined whether adult stem cells undergo senescence, quiescence, or apoptosis upon exhaustion of their proliferative potential.

It is an incontrovertible fact that cancer is more frequent at advanced ages. The simplest explanation for this is that old organisms have accumulated more genetic and epigenetic aberrations than young organisms, but this does not say anything about the intrinsic susceptibility (that is, apart from the accumulation of mutations) of young and old organisms to develop cancer. The progressive action of the Hayflick factors throughout life poses important questions regarding the link between cancer and aging. Conceivably, it could be argued that because the Hayflick factors become progressively activated with time, this should result not only in aging but also in cancer protection. For example, genetically manipulated mice with short telomeres (Blasco, 2005) or with higher levels of p16INK4a (Matheu et al., 2004) are cancer resistant. In this sense, aged organisms, by having shorter telomeres, higher levels of p16INK4a, or higher levels of DNA damage signaling to p53, should be more resistant to oncogene-driven proliferation than young organisms. According to this view, the higher incidence of cancer at old ages simply reflects the time needed for the accumulation of oncogenic mutations. Alternatively, it has been observed that senescent cells, in contrast to normal cells, provide a better milieu and stromal support for cancer cells (Krtolica et al., 2001). According to this notion, aged organisms are more cancer prone by the combination of two factors: the accumulation of oncogenic mutations and the favorable environment for cancer growth. Addressing these issues will be of great importance in understanding the link between cancer and aging.

Great progress has been made in the last few years in understanding how cells limit their proliferative potential and in linking these mechanisms to cancer protection and aging. Future research will clarify whether therapies that induce senescence are useful for cancer treatment, and determine their effect on aging. Conversely, treatments that inhibit senescence in healthy individuals might slow aspects of aging.

ACKNOWLEDGMENTS

M.A.B. and M.S. are funded by the CNIO, the Spanish Ministry of Education and Science, the E.U. (projects PROTEOMAGE and INTACT to M.S. and projects INTACT, TELOSENS, ZINCAGE, RISC-RAD, and MOL CANCER MED to M.A.B.), and a Josef Steiner Cancer Research Award 2003 to M.A.B.

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