

Hallmarks of senescence in carcinogenesis and cancer therapy

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Cellular senescence is a signal transduction program leading to irreversible cell cycle arrest. This growth arrest can be triggered by many different mechanisms including recognition by cellular sensors of DNA double-strand breaks leading to the activation of cell cycle checkpoint responses and recruitment of DNA repair foci. Senescence is initiated by the shortening of telomeres (replicative senescence) or by other endogenous and exogenous acute and chronic stress signals (STASIS: stress or aberrant signaling-induced senescence). The process of carcinogenesis involves a series of changes that allow tumor cells to bypass the senescence program. Nevertheless, tumor cells retain the capacity to undergo senescence. Treatment of tumor cells with many conventional anticancer therapies activates DNA damage signaling pathways, which induce apoptosis in some cells and senescence in others. Overexpression of tumor suppressors or inhibition of oncogenes can also induce rapid senescence in tumor cells. Senescent cells, while not dividing, remain metabolically active and produce many secreted factors, some of which stimulate and others inhibit the growth of tumors. The emerging knowledge about the pathways that lead to senescence and determine the pattern of gene expression in senescent cells may lead to more effective treatments for cancer.

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Introduction

The term ‘senescence’, originally defined as a series of cellular changes associated with aging, now refers more commonly to a signal transduction program leading to irreversible arrest of cell growth, accompanied by a distinct set of changes in the cellular phenotype. Senescence is a potent anticarcinogenic program, and the process of neoplastic transformation involves a series of events that allow cells to bypass senescence. Nevertheless, tumor cells have still retained the capacity to senesce. Most conventional anticancer therapies activate DNA damage signaling pathways, which induce

apoptotic cell death but in many instances cells do not die but rather undergo a senescence-like terminal growth arrest. In addition to chemotherapy and radiation therapy, both senescence and apoptosis can be induced by manipulating the expression of essential growth-regulatory genes. A fundamental area of recent investigations is to understand the diverse signaling pathways that cause cells, in some contexts, to undergo senescence and in other contexts initiate apoptotic programs. Another novel area is analysis of the roles of senescent cells and their secreted factors in tumor growth and treatment response. In the present overview, we will cover some of the emerging knowledge about these pathways and how this knowledge in the future may be translated into effective treatments for cancer.

Cellular senescence as a tumor-suppressor mechanism

Aging is one of the highest risk factors for cancer. It is generally believed that somatic cells in organisms with renewable tissues have evolutionarily conserved defense mechanisms that guard against unrestrained proliferation. In some instances, the cellular proliferation control pathways may be potent anticancer protection mechanisms (tumor-suppressor genes), so when there are sufficient acute stresses (or damage) cells immediately growth arrest or undergo cell death. Having cellular mechanisms to cause cells to stop growing or to die in the face of acute damage would be highly advantageous. In chronic situations where there are long periods of accumulation of subthreshold amounts of damage, cells continue to divide and accumulate multiple alterations (mutations) over many years (as well as progressively shorten the ends of their chromosomes, called telomeres). A hallmark of the theory of cancer is that the progressive accumulation of damage (epigenetic and genetic) leads to cells that have a growth advantage and thus within a given cell, multiple changes must occur for the success of the emerging tumor cell (Vogelstein and Kinzler, 1993). One could easily imagine that a major roadblock to cancer would be preventing the unlimited proliferation that characterizes almost all advanced cancers.

It would also be advantageous in long-lived organisms for cells to be limited in the maximal number of cell divisions permitted in order to ensure appropriate growth and DNA repair early in life, but not so many

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divisions that could lead to the early onset of diseases such as cancer (Wright and Shay, 2000, 2002). Therefore, selection for enhanced DNA repair and maintenance would be highly desirable during reproductive years, but this could be lost (or at least not selected for) in later life. The idea called 'antagonistic pleiotropy' in the simplest context would argue that what is good for an individual early in life could be bad or lead to undesirable consequences when old (Kirkwood, 1988; Kirkwood and Austad, 2000). In the 'natural environment' (less than 100 years ago) before antibiotics, vaccines, and modern medical interventions, most humans only lived for about 40 years and often died of infectious diseases. In our modern 'protected environment', humans are living on average twice as long and are dying of heart disease, cancer, and neurodegenerative disorders. Thus, normal cells in the context of genotoxic insults have innate and probably highly conserved defense mechanisms that initiate programs leading to growth arrest or apoptosis, perhaps in part as a mechanism to prevent cancer.

The Dr Jekyll and Mr Hyde of aging: do senescent fibroblasts prevent or promote cancer?

While there have been many theoretical and some experimentally derived lines of evidence to support the concept that senescent cells may represent a substantial barrier to cancer progression, recent results have also suggested that senescent stromal cells may actually promote the proliferation and tumorigenesis of mutant epithelial cells (Campisi, 2000, 2001; Krtolica *et al.*, 2001). Malignant tumors require a permissive tissue environment in order to survive, proliferate, and migrate. Most age-related cancers arise from the epithelial cells of renewable tissues. An often overlooked but essential component of epithelial tissue is the stroma, the subepithelial layer composed of extracellular matrix and several cell types (but mostly fibroblasts). There is increasing evidence that one change that occurs in tissues during aging is the accumulation of epithelial cells and fibroblasts that have undergone cellular senescence. Cellular senescence arrests proliferation in response to damage or stimuli that put cells at risk for neoplastic transformation. However, senescent cells also secrete growth factors as well as extracellular matrix components, matrix-degrading enzymes, and inflammatory cytokines that can disrupt tissue integrity and/or stimulate nearby cells to proliferate (Campisi, 2000, 2001). As a result, senescent fibroblasts admixed with transformed epithelial cells stimulate the growth of the latter in culture as well as in tumor models (Krtolica *et al.*, 2001). Thus, senescent stromal cells may in certain contexts create a pro-oncogenic tissue environment, leading to decline in tissue function that may in some cases synergize with oncogenic mutations to drive the rise in cancer incidence with age. While senescence may have evolved to initially protect humans against the

formation of early onset cancers, with humans living longer in modern times, again antagonistic pleiotropy may be the culprit.

Telomeres: a tale with many endings

Human chromosome ends are comprised of 15–20 kilobase pairs of the duplex repetitive TTAGGG tracts followed by a single-strand overhang on the 3' G-rich strand that ends in a lariat-like structure called a t-loop (Blackburn and Gall, 1978; Cooke and Smith, 1986; Moyzis *et al.*, 1988; Chong *et al.*, 1995; Broccoli *et al.*, 1997; Griffith *et al.*, 1999; Stansel *et al.*, 2001). Telomere-specific proteins bind directly to the single- and double-strand regions and form a complex providing a protective cap over the ends of the chromosomes that protects chromosome termini from degradation, recombination, and end-joining reactions (van Steensel *et al.*, 1998; Kim *et al.*, 1999; Li *et al.*, 2000; Bailey *et al.*, 2001; Baumann and Cech, 2001; de Lange, 2002). Functional telomeres are essential for continued cell proliferation. Owing to the incomplete replication of lagging strand DNA synthesis (Watson, 1972), telomeres progressively shorten in all somatic cells with each cell division (Olovnikov, 1973; de Lange *et al.*, 1990; Hastie *et al.*, 1990; Harley *et al.*, 1990; Lindsey *et al.*, 1991). When some telomeres are short, cells undergo a growth arrest called replicative senescence (Hayflick and Moorhead, 1961; Harley *et al.*, 1990; Shay and Wright, 2001). Cellular senescence in human fibroblasts occurs in two phases, mortality stages 1 and 2 (M1 and M2), and replicative senescence corresponds to the M1 stage. Cells that escape the M1 block of cell division divide until M2 (crisis), where the cells die, most likely through mitotic catastrophe, cell death that results from abnormal mitosis and occurs either through apoptosis or through nonapoptotic process that involves formation of multiple micronuclei.

In addition to progressive telomere shortening (leading to replicative senescence), telomere dysfunction can be caused by a change of state ('uncapping') that leads to a rapid induction of growth arrest (end fusions, fusion-bridge breakage leading to mitotic catastrophe). Thus, when the telomeric DNA sequence or structure is altered, or telomere proteins are mutated or depleted, cells undergo chromosome end associations and fusions leading to growth arrest or death (Makarov *et al.*, 1997; Shore, 1997; Wellinger and Sen, 1997; Lingner and Cech, 1998; Blackburn, 2000; Gasser, 2000; Dubrana *et al.*, 2001; Karlseder *et al.*, 2002; Smogorzewska and de Lange, 2002).

Putting stress on senescence: what is STASIS?

Cells can undergo an exogenously induced rapid G1 growth arrest that is different from the progression telomere shortening that causes replicative senescence (Sherr, 1998; Sherr and DePinho, 2000; Ramirez *et al.*,

2001; Drayton and Peters, 2002). This growth arrest is similar to replicative senescence since the cell cannot divide even if stimulated by mitogens. Also, as in the case of replicative senescence, these cells remain metabolically and synthetically active and show characteristic changes in morphology (Dimri *et al.*, 1995; Serrano *et al.*, 1997; Drayton and Peters, 2002). Both the telomere-based and nontelomere-based growth arrest may be due in part to repression of genes required for cell cycle progression and upregulation of growth-inhibitory genes (Figure 1). It has been demonstrated that these growth-inhibitory genes can also be activated in cell culture due to a variety of environmental stresses that have been termed premature senescence, culture shock, stasis, and stress-induced senescence (Chen and Ames, 1994; Serrano *et al.*, 1997; Ramirez *et al.*, 2001). This type of growth arrest has received a variety of names that are easily confused with replicative senescence so the term 'STASIS' (*stress or aberrant signaling-induced senescence*) has been suggested to convey the notion that cells engage a common senescence-like arrest mechanism in response to diverse signals (Drayton and Peters, 2002; Wright and Shay, 2002). Even though the artificial cell culture environment can activate STASIS, it is important to note that STASIS may be an evolutionary conserved mechanism that helps to guard cells against oncogenic insults.

How do telomeres trigger replicative senescence and is this different from STASIS?

Telomerase is a eukaryotic ribonucleoprotein complex (Greider and Blackburn, 1985; Morin, 1989; Blackburn, 1992; Lingner *et al.*, 1997; Nakamura *et al.*, 1997; Weinrich *et al.*, 1997; Nugent and Lundblad, 1998; Bryan and Cech, 1999) that helps to stabilize telomere length in human stem cells, reproductive cells (Wright *et al.*, 1996), and cancer cells (Kim *et al.*, 1994; Shay and Bacchetti, 1997). Telomerase functions by adding TTAGGG repeats onto the telomeres using its intrinsic RNA as a template for reverse transcription (Feng *et al.*, 1995). Telomerase activity is present in almost all human tumors but not in adjacent normal cells (Kim *et al.*, 1994; Shay and Bacchetti, 1997). The maintenance of telomere stability is almost universally required for the long-term proliferation of tumors (Shay, 1995; Shay and Wright, 1996a, b; Holt and Shay, 1999). Thus, escape from cellular senescence and becoming immortal by activating telomerase, or an alternative mechanism to maintain telomeres (Bryan *et al.*, 1995, 1997; Bryan and Cech, 1999) constitutes an additional step in oncogenesis that most tumors require for their ongoing proliferation. This makes telomerase a target not only for cancer diagnosis but also for the development of novel anticancer therapeutic agents.

The limited proliferative potential of normal human primary cells is caused by the lack of detectable or sufficient telomerase activity, and this leads to progressive telomere erosion with each replication (end-replication problem) and to the eventual deprotection of the telomere cap. The molecular mechanism(s) by which a single or a few short telomeres signal the growth arrest caused by replicative aging is starting to emerge (di Fagagna *et al.*, 2003; Takai *et al.*, 2003). In cells that have bypassed telomere-based replicative senescence as a result of loss of p53 function, an outstanding question has been how cells deal with the shortest telomeres during the extended lifespan period before cells enter crisis (Shay *et al.*, 1991; Wright and Shay, 1992, 1996). It is known in cells that reach telomere-based growth limits that ectopic expression of the catalytic subunit of telomerase (hTERT) is sufficient to bypass both the senescence and crisis growth arrest signals, indicating that telomeres are directly involved in both processes. However, in cells that growth arrest due to STASIS, hTERT does not directly immortalize cells. For example, the growth arrest elicited in primary fibroblasts in response to oncogenic Ras or Raf, oligonucleotides, inadequate culture conditions, or the limited proliferation of mouse embryonic fibroblasts with very long telomeres operates just as effectively in human cells expressing telomerase (Serrano *et al.*, 1997; Robles and Adami, 1998; Zhu *et al.*, 1998). This demonstrates that this type of growth arrest does not involve counting cell replications (e.g. telomere-based replicative senescence). Thus, the ability of hTERT to immortalize cells clearly distinguishes replicative senescence from premature senescence (STASIS) even though both processes share many similar characteristics.

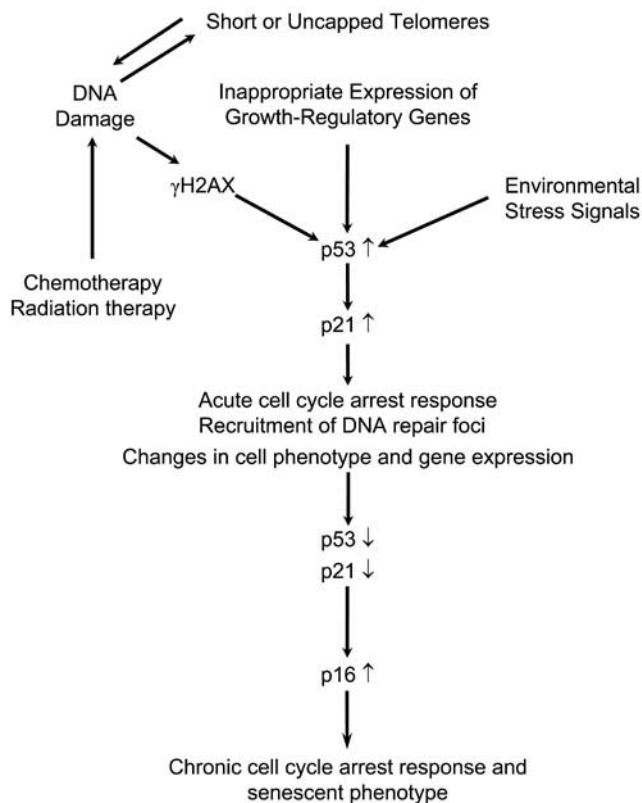


Figure 1 Induction of senescence in normal cells

Keeping the ends in check: γ -H2AX signaling and senescence

Telomere dysfunction is implicated in both aging and cancer (Harley, 1991; Holt *et al.*, 1997; Bodnar *et al.*, 1998; DePinho, 2000). Human cells can undergo only a limited number of cell divisions and, when at least some telomeres are short, cells exhaust their proliferative potential and undergo senescence (Harley *et al.*, 1990; Wright and Shay, 1992). However, little is currently known about the mechanisms cells use to respond to loss of telomere function. Indeed, the responses of cells to progressive telomere shortening (replicative senescence) versus acute uncapping of telomeres may not be similar. Since loss of telomere function can lead to cell cycle arrest or cell death (depending on the cellular context), one possibility is that DNA damage/repair responses may be involved in both programs.

It has recently been shown that DNA damage signals from telomeres induce typical DNA damage foci containing factors such as γ -H2AX, MRE11, NBS1, MDC1, 53BP1, RAD50, and BRCA1 at M1 senescence (di Fagagna *et al.*, 2003). It has also been shown that TIFs ('telomere-induced foci') are induced when telomeres are deprotected by expression of a dominant-negative TRF2 (double-stranded telomere binding protein) that causes loss of the single-stranded G-rich telomeric overhang (Takai *et al.*, 2003). Thus, experimental procedures that unfold the lariat-like telomere end protection lead to an 'immediate senescence' and this has led to the concept that the end-fusions and chromosome-breakage fusion cycles are the cause of senescence. Whether this rapid uncapping of telomeres reflects replicative senescence (M1) or crisis (M2) is not clear. It is known that rapid uncapping of telomeres induces a mitotic catastrophe, in which the end-fusions are rapidly converted into major chromosomal abnormalities including translocations, deletions, fragments, and gaps. What is not currently established is if the DNA damage signals from a few telomeres at replicative senescence (M1) is the same or a different phenomenon from uncapping most, if not all the telomeres.

A central unexplained aspect of the two-stage (M1/M2) model of senescence is the mechanism by which cells deal with their short telomeres between M1 and M2. If telomeres are sufficiently short to become deprotected at M1 and induce end-fusion events that lead to chromosome disruption, how is the cell able to divide for additional doublings (extended lifespan period) before significant cell death processes begin at M2? Is it simply a quantitative issue, in which a cell could tolerate a few chromosome fusions at M1 but only reaches M2 when the number of fusions becomes too large and cell death ensues, or are there qualitative differences in the state of deprotection between M1 and M2? Thus, remaining questions are how much DNA damage occurs between M1 and M2, do breakage-fusion cycles begin at M1, and overall how do cells continue to divide without dying in the presence of increasing DNA damage between M1 and M2.

While M1 senescence may be thought of as an initial protection against the development of cancer, it is now becoming clear that in the presence of other alterations (e.g. p53 inactivation), cells can tolerate the telomere associations activated at M1 and resume cell division, providing that the p16/pRB pathway has not been engaged (Beausejour *et al.*, 2003). A recent concept is that p16 may enable pRB to establish a chromatin state that once established is irreversible and no longer dependent on the presence of p16 or pRB (Narita *et al.*, 2003 and unpublished observations). An important function of pRB and associated family members is to recruit histone deacetylases (HDACs) to E2F-dependent promoters, deacetylating nearby histones and repressing gene expression. As cells transition from G1 to S, pRB becomes phosphorylated, freeing E2F to interact with histone acetyltransferases (HATs) to open chromatin structure and transactivate E2F-responsive genes important for G1 to S phase transition. The modification of histones by HATs and HDACs is reversible in quiescent cells but not in some types of senescent cells (Beausejour *et al.*, 2003). While this did not happen in all cell types and it was not confirmed that the cells where it occurred were truly senescent, modification of heterochromatin and associated proteins could potentially lead to the establishment of heterochromatic foci that are important in the initial tumor-suppressive properties of senescence.

What is not known at present is what determines the extent to which cells express p16 and if the p16/pRB-initiated chromatin state is truly irreversible (perhaps the number of γ -H2AX-induced repair foci may give some insights into this). However, increased knowledge of these separate pathways (M1 and M2) is likely to have important implications when initiating DNA damage responses as part of cancer therapy such as ionizing radiation and chemotherapy.

Of mice and men

If replicative aging evolved as a mechanism to limit the number of available cell divisions, and thus acts as a brake against the accumulation of the multiple mutations needed for a cell to become malignant, then a 70 kg man who lives for 80 years has to be approximately 14 000 times more resistant to cancer than a 0.2 kg rat that lives for 2 years $((70 \text{ kg}/0.2 \text{ kg}) \times (80 \text{ years}/2 \text{ years}) = 14 000)$. While mice and humans get about the same lifetime frequency of cancer (about 30%), there is also compelling evidence that rodent cells do not use the same mechanism as human cells to regulate the maximal number of cell divisions (Wright and Shay, 2001, 2002; Shay and Wright, 2001). Not only are mouse and rat telomeres 5–10 times longer than human cells, but many rodent tissues also have detectable telomerase (Shay and Wright, 2001; Wright and Shay, 2000).

Inducing telomere shortening by knocking out telomerase in the mouse does not affect the number of doublings that occur before the explanted mouse

embryonic fibroblast (MEF) cells stop dividing in culture (Blasco *et al.*, 1997). In addition, since telomerase is detected in many rodent tissues, it does not explain why the MEF cells undergo a growth arrest after only 10–15 population doublings in cell culture. If telomere shortening is not inducing rodent ‘cellular senescence’, then what is? One possibility is that mouse and rat cells repair DNA damage much less efficiently than human cells (Hart and Setlow, 1974) and are thus much more sensitive to a variety of agents that produce oxidative stress (Kapahi *et al.*, 1999). This has led to the hypothesis that the foreign environment of tissue culture (in two dimensions, lacking most extracellular matrix molecules, exposed to 21% oxygen and unrestrained mitogen stimulations, as well as containing a mixture of nutrients, trace elements, hormones, and serum that may be inadequate) might produce a level of stress that can be tolerated by most human fibroblast strains, but which causes progressive damage and eventual growth arrest (STASIS) in mouse fibroblasts (Sherr and DePinho, 2000). In appropriate (or at least adequate) culture conditions, it has been shown that at least a subset of rat Schwann and oligodendrocyte precursor cells have limitless capacity to divide (Mathon *et al.*, 2001; Tang *et al.*, 2001). This is in contrast to MEFs from KU80 $-/-$, ATM $-/-$, or BRCA2 mutant mice, which are deficient in certain DNA damage responses (Barlow *et al.*, 1996; Nussenzweig *et al.*, 1996; Xu and Baltimore, 1996). These mutant mouse cells overexpress p53 and p21^{Waf1/Cip1} and undergo STASIS after only 3–4 doublings. Since these mice are viable, it is unlikely that their ‘premature senescence’ or STASIS in cell culture represents any limitation on division within the organism. A more reasonable explanation is that the reduced ability to correct the DNA damage produced by the culture environment leads to a more rapid activation of p53-dependent cell cycle checkpoints. The demonstration that telomere shortening is not involved, and that abrogation of ARF/p53 is sufficient for indefinite proliferation may explain why mouse cells ‘immortalize’ with such a high frequency compared to human cells.

The ability to obtain immortalized MEFs of the rodents in culture conditions (Loo *et al.*, 1987; Mathon *et al.*, 2001; Tang *et al.*, 2001) probably results from combining an environment that minimizes ‘stress’ while including enough of the right hormones and other factors to balance the stresses that do occur. In these studies (Mathon *et al.*, 2001; Tang *et al.*, 2001), the cultures are telomerase positive, remain diploid and, in contrast to most established rodent cell lines, retain normal cell cycle checkpoints in response to factors such as irradiation or the overexpression of Ras. The results of these investigations, demonstrating immortalization of rodent cells, support the interpretation that conventional mutation/tumor-protection mechanisms (DNA damage signaling, cell cycle checkpoint arrest, and repair pathways) are adequate in the short life of small organisms, and that replicative aging evolved to provide the many-fold increased protection needed by larger and longer-lived species (Shay and Wright, 2001; Wright and Shay, 2000). Thus, the indefinite growth of normal rat

precursor cells is probably not unique to particular cell types, but represents a fundamental biological difference between normal human cells (which count cell divisions) and rodent cells (which do not). Appreciating this difference is essential for designing appropriate animal models and interpreting experiments concerning the role of replicative aging, telomeres, and telomerase in aging and cancer.

What can mice without telomerase teach us about senescence?

Even though there is no evidence for a counting mechanism in the growth arrest of cultured mouse cells, similar to wild-type mouse cells, telomerase-negative MEFs stop dividing after only 10–15 doublings, long before significant telomere erosion occurs (Sherr and DePinho, 2000; Wright and Shay, 2000). Irrespective, telomerase knockout animal models can be useful to dissect the consequences of telomere dysfunction for cancer, and DNA repair, as well as the molecular mechanisms by which telomeres exert their protective function.

MEFs explanted into culture from telomerase knockout mice (generation 5–6) can growth arrest due to short telomeres, and rare survivors emerge permitting the cells to continue to grow by engaging an alternative lengthening of telomeres (ALT), telomerase-independent mechanism for telomere maintenance (Bryan *et al.*, 1995). Late-generation telomerase-deficient mice show severe proliferative defects leading to immune dysfunction and failure to reproduce (Blasco *et al.*, 1997; Lee *et al.*, 1998; Chin *et al.*, 1999; Rudolph *et al.*, 1999). While there is some chromosomal instability caused by telomere shortening in late-generation telomerase knockout mice, there is only a slightly increased incidence of tumor formation. Indeed, short telomeres (in the absence of other alterations) may actually suppress tumorigenesis (Greenberg *et al.*, 1999). For example, late-generation telomerase RNA-deficient (*Terc*^{-/-}) mice show significantly fewer skin tumors than wild-type controls upon chemical carcinogenesis of the skin (Gonzalez-Suarez *et al.*, 2000). This tumor-suppressor phenotype coincides with p53 upregulation in *Terc*^{-/-} papillomas that may sense short telomeres as damaged DNA (perhaps via γ -H2AX and recruitment of DNA repair foci) and thus contribute to growth arrest. In addition, mice deficient for telomerase and the INK4A locus (a locus which includes both p16 and p19ARF) do NOT make tumors (Greenberg *et al.*, 1999). Finally, when telomerase-deficient mice were crossed to *Apc*^{Min} mice, while there was an increased incidence of tumor initiation, these tumors did not progress past the early stages (Rudolph *et al.*, 2001). These results indicate that in a particular context, telomerase inhibition and telomere shortening may be an effective way to prevent tumor growth (e.g. telomere maintenance is required for the advanced growth of tumor cells). Thus, telomere dysfunction in the context

of normal replicative senescence probably does not cause cellular genomic instability and serves as a potent anticancer protection mechanism. In contrast, in a setting of alterations in the function of p53 and perhaps other tumor-suppressor genes, telomere dysfunction could lead to cancer initiation, but in the absence of telomerase these cells would be predicted to have limits on their proliferation and thus telomerase activation may actually be important in the later stages of cancer progression.

To further illustrate this point, almost 100% of animals deficient in telomerase and the p53 locus develop early onset lymphomas (about the same incidence as those with telomerase). This is a very different type of cancer compared to the constellation of carcinomas seen in humans, whose cells, in the course of transformation, become initially heterozygous and then functionally deficient in p53. More direct evidence that p53 may act as a sensor for short telomeres came from the study of *Terc*^{-/-} mice crossed to p53^{+/-} mice (Artandi *et al.*, 2000). In late-generation *Terc*^{-/-}/p53^{+/-} mice, a subset of cells lost the remaining wild-type p53 allele and instead of accumulating the typical early onset lymphomas, the mice developed late onset epithelial carcinomas (Artandi *et al.*, 2000), a pattern much more similar to those appearing in humans. This indicates that it takes an understanding of the pathways leading to human cancer in order to make a mouse model that resembles human disease. In humans, with heterozygosity of p53 and in the absence of telomerase, there may be extra rounds of cell proliferation, loss or mutation of the other p53 allele, critical shortening of telomeres, and then emergence of a telomerase-positive immortalized precancerous cell. This pathway to cancer can also occur if telomerase is upregulated prior to the loss of complete p53 function. The important point is that in the presence of p53 function, short telomeres are likely to provide a protective function against the development of cancer.

Overall, this suggests that p53 function is required for the growth arrest in MEFs, and for biochemical markers of senescence to show in normal human and mouse cells (Figure 1). This form of growth arrest reflects the accumulation of negative growth regulators (e.g. p16^{INK4a}, p21^{Cip1}), and factors (such as the senescence-associated (SA)- β galactosidase) induced during stress responses (Dimri *et al.*, 1995). These so-called senescence markers are rather nonspecific, and some of them (such as p21) are expressed in growth arrest situations that are neither replicative senescence nor STASIS. Phenotypes similar to replicative senescence can be produced by DNA damage and other stress responses, including genotoxic drugs (bleomycin or etoposide) overexpression of oncogenes (*H-ras* V12), or checkpoint factors (p16^{INK4a}). If replicative senescence (M1 in human cells) represents a DNA damage response due to too short telomeres, then any stimulus producing DNA damage might induce STASIS. Therefore, it is not surprising that the signaling pathway for growth arrest appears to be similar in replicative senescence and in damage-induced accelerated senescence (STASIS). This pathway (Figure 1) is initiated with the activation of

p53, which produces multiple effects on gene expression, including transcriptional activation of p21^{Waf1/Cip1}, a pleiotropic inhibitor of different cyclin/cyclin-dependent kinases (CDKs) (Dotto, 2000). p21 induction causes cell cycle arrest in senescent cells (Noda *et al.*, 1994; Alcorta *et al.*, 1996). The activation of p53 and p21 in senescent cells is usually transient, and the levels of both proteins decrease after the establishment of growth arrest (although they are still elevated above the basal levels found in proliferating cells). While p21 expression goes down, another CDK inhibitor, p16^{INK4a}, becomes constitutively upregulated, suggesting that p16 is responsible for the maintenance of growth arrest in senescent cells (Figure 1) (Alcorta *et al.*, 1996; Stein *et al.*, 1999).

The role of p53, p16, and telomerase in the maintenance of the senescent state has been recently investigated by Beausejour *et al.* (2003), who used lentiviral vectors to express different proteins in human fibroblasts and mammary epithelial cells that were cultured until senescence. Expression of telomerase did not reverse the growth arrest of senescent cells. A genetic inhibitor of p53, however, allowed some but not all of the senescent cultures to resume their growth, indicating that normally irreversible senescence may in fact be reversed by artificial genetic manipulations. The reversal of senescence by p53 inhibition, however, was achievable only in cultures with low levels of p16 and was blocked at high p16 levels. These results suggest that both p53 and p16 function in the maintenance of growth arrest in senescent human cells (Beausejour *et al.*, 2003).

Surprisingly, there have been only a few studies addressing the role of telomeres in rodent response to DNA-damaging agents (Goytisolo *et al.*, 2000; Wong *et al.*, 2000). These studies show that in late generation *Terc*^{-/-} mice irradiated with gamma-irradiation showed an enhanced mortality (Goytisolo *et al.*, 2000; Wong *et al.*, 2000). Their death appears to be induced by radiation toxicity to the gastrointestinal tract, lymphoid organs, and kidney (Goytisolo *et al.*, 2000), and might be due to radiation-induced mitotic catastrophe. Functional p53 is believed to be required for the enhanced response of late-generation *Terc*^{-/-} cell to DNA-damaging reagents (Lee *et al.*, 2001). These results may have important implications for human radiotherapy as treatment of patients with telomerase inhibitors could lead to telomere loss, which may affect the sensitivity of the tumors to radiotherapy depending on p53 status.

Telomerase: keeping the ends from meeting versus ALT: tying up loose ends

Almost all human primary cancers and tumor cell lines have telomerase activity, even though little is known about the transcriptional regulation of telomerase (Cong *et al.*, 2002). It is believed that most epithelial tissues have a telomerase-competent stem-like cell compartment and that it may be the dysregulation of this compartment that lead to the emerging carcinomas. While telomerase induction appears to be the preferred

pathway in the vast majority of human cancers, rare human cell lines and tumors that lack telomerase activity have been reported that are able to maintain or elongate their telomeres by alternative mechanisms, which have been termed ALT (Bryan *et al.*, 1995; Bryan *et al.*, 1997; Bryan and Cech, 1999). ALT only appears to occur in rare sarcomas that may arise from tissues that do not have well-recognized stem cell compartments. In mammalian ALT cells, DNA sequences are copied from telomere to telomere suggesting that ALT involves homologous recombination (Dunham *et al.*, 2000). In *Saccharomyces cerevisiae*, there are two homologous recombination pathways involved in ALT, RAD50, and RAD51 (Lundblad and Blackburn, 1993; Chen *et al.*, 2001). In addition, RecQ helicases (WRN and BLM in mammals) are required for ALT in yeast (Cohen and Sinclair, 2001). ALT can also be enhanced by eliminating the mismatch repair (inhibits homologous recombination) pathway in yeast (Rizki and Lundblad, 2001).

A potential concern about ALT is in the context of clinical use of telomerase inhibitors. Since ALT is present in other organisms, in experimentally derived human immortalized cell lines, and in some rare human cancers, it is possible that telomerase inhibitors could result in the emergence of drug-resistant telomerase-independent cancer cells. While this is certainly a possibility, there have been no published reports of telomerase-positive human tumor cells being experimentally converted to a telomerase-independent pathway using telomerase inhibitors. There is the possibility that advanced human malignancies may contain rare telomerase-independent variants. With the long-term use of telomerase-specific inhibitors, these cells may have a selective growth advantage. Thus, the outstanding question that remains to be determined is if inhibiting telomerase specifically will cause cancer-cell death. Only clinical trials will ultimately determine this.

Cajoling tumor cells into senescence: genetic approaches

Tumor cells carry a number of traits that trigger the program of senescence. While there is clearly a difference between telomere-based replicative senescence and what will be discussed in the remainder of this review in regard to tumor cells (STASIS), we will continue to use the generic term senescence since it is commonly used in the literature for both phenomena. Most tumor cells tend to have short telomeres, and they frequently express oncogenic mutant forms of Ras or Raf, as well as other constitutively upregulated mitogenic factors such as E2F1, overexpression of which induces senescence in normal cells. The process of carcinogenesis involves several events that inhibit the senescence response to these traits, such as activation of telomerase that counteracts telomere-mediated replicative senescence, as well as inactivation of tumor suppressors p53 or p16, which mediates a bypass of both STASIS and the M1 stage of replicative senescence.

The senescence-promoting and senescence-suppressing factors in tumor cells exist in a dynamic equilibrium, which is perhaps best illustrated by the report that overexpression of hTERT not only inhibits but – at very high levels – also promotes senescence (Gorbunova *et al.*, 2003). Many recent studies have demonstrated that a variety of stimuli can shift this equilibrium in favor of senescence, thereby stopping the growth of tumor cells. These results, which are described below, indicate that enhancement of the program of senescence in tumor cells provides a biologically justified approach to cancer therapy.

Early experiments with somatic cell fusion of tumor-derived ‘immortal’ cell lines with normal cells or with other tumor lines have demonstrated that the ability to senesce is dominant over immortality. As reviewed by Tominaga *et al.* (2002), these studies revealed four senescence-determining complementation groups. A senescence-determining gene has been identified so far for one of the complementation groups. This gene, located on chromosome 4 and termed MORF4, induces gradual onset of senescence (after 18–35 population doublings) when transfected into tumor cell lines of the same complementation group (Bertram *et al.*, 1999). While this may appear to be a cellular counting mechanism, it is unknown whether MORF4 affects telomeres or modulates the effects of cell culture that can mimic a counting mechanism that has been demonstrated for telomeres.

In contrast to the slow effect of MORF4, most other genetic manipulations that produce senescence in tumor cells act rapidly. The most striking example is provided by the inhibition of papillomavirus oncoproteins E6 and E7 that counteract senescence in cervical carcinoma cells by inhibiting p53 and Rb tumor suppressors, respectively. Expression of bovine papillomavirus protein E2, a negative regulator of both E6 and E7, rapidly induces stable growth arrest and the senescent phenotype in HeLa cells and human cervical carcinoma cell lines (Goodwin *et al.*, 2000; Wells *et al.*, 2000). The senescent phenotype is also induced by a small interfering RNA molecule targeting the bicistronic E6 and E7 mRNA (Hall and Alexander, 2003), or by the inhibition of E6 or E7 individually (DeFilippis *et al.*, 2003). Induction of senescence by E2 is not accompanied by telomere shortening (Goodwin *et al.*, 2000) and is not prevented by constitutive overexpression of telomerase (Goodwin and DiMaio, 2001), indicating that this form of senescence represents STASIS. E2-induced senescence is associated with p53 stabilization and p21 induction (Wells *et al.*, 2000) and can be prevented by using p21-inhibiting antisense oligonucleotides (Wells *et al.*, 2000). These results demonstrate that tumor cells are ‘primed’ to engage a senescence program as soon as their senescence-restraining mechanisms are suppressed.

Although telomerase induction is the best-known senescence-restraining mechanism, only a few studies have shown that telomerase inhibitors induce senescence in tumor cells (Kim *et al.*, 2003). Instead, most observations suggest that telomerase inhibition by small molecules (Herbert *et al.*, 1999) or by dominant-negative

mutants (Hahn *et al.*, 1999) produces cell death rather than senescence in tumor cell lines. While this may depend on the constellation of genetic and epigenetic changes in the tumor cell another possibility may involve telomerase itself. Recently it has been shown that there is an antiapoptotic function of telomerase, which may be independent of its effect on telomeres (Stewart *et al.*, 2002). This function is likely to act by preventing mitotic catastrophe that results from the fusion of chromosomes with abnormal telomeric structures.

Tumor cells readily develop the senescent phenotype as a result of forced overexpression of different tumor suppressors and some other senescence-associated growth-inhibitory genes. As reviewed in Roninson (2003), these genes include RB, p53, and two p53-related proteins (p63 and p73), and several CDK inhibitors (p21, p16, p57^{Kip2}, and p15^{Ink4b}). In addition to the natural CDK inhibitor proteins, a dominant-negative mutant of CDK2 was also shown to induce the senescent phenotype in a lung carcinoma cell line (Crescenzi *et al.*, 2003). Tumor cell senescence was also induced by a putative tumor-suppressor gene INI1 (Reincke *et al.*, 2003), by IGFBP-rP1, a member of the insulin-like growth factor binding protein family (Sprenger *et al.*, 2002), and by constitutively active mutants of two genes stimulating the MAPK pathway, including RAF-1 and MAPK kinase MKK6 (Ravi *et al.*, 1999; Haq *et al.*, 2002). Recently, the list of proteins that induce senescence in tumor cells received an unexpected addition: the best-known apoptosis suppressor Bcl2 (Crescenzi *et al.*, 2003). BCL-2 transfection was known to slow down cell growth in several tumor cell lines, and this effect is now shown to result from the induction of irreversible growth arrest and the senescent phenotype in a substantial fraction of Bcl2-expressing cells. Bcl2-induced senescence is associated with the induction of p27 and the resulting inhibition of CDK2; furthermore, p27 is required for Bcl2-induced tumor senescence. The authors proposed that downregulation of Bcl2, which has been observed during human carcinoma development, as well as the association of Bcl2 expression with a favorable prognosis, are related to the senescence-inducing ability of Bcl2 (Crescenzi *et al.*, 2003). The pro-senescence role of Bcl2 was also indicated by the report that expression of this protein accelerates Ras-induced senescence in human fibroblasts (Tombor *et al.*, 2003).

The ability of tumor cells to recover once the expression of a growth-inhibitory gene has been turned off has been investigated for p53, p21, and p16 tumor suppressors, expressed in tumor cells from regulated promoters (Sugrue *et al.*, 1997; Chang *et al.*, 2000a; Fang *et al.*, 1999; Day and Enders 2000). In all instances, the ability of the cells to grow and form colonies after the promoter shutoff was inversely related to the duration of expression of the tumor suppressor, with very few cells recovering after prolonged induction (4–5 days). The failure to recover, however, may result not only from permanent cell cycle arrest but also from cell death. The latter turned out to be the case for

HT1080 fibrosarcoma cells released after prolonged p21 expression from an inducible promoter (Chang *et al.*, 2000a). Essentially all of the released cells re-entered the cycle and replicated their DNA once p21 was turned off. Upon entering mitosis, however, most of the cells underwent mitotic catastrophe and either died or underwent senescence-like growth arrest in the interphase following the abnormal mitosis (Chang *et al.*, 2000a). While this observation is not necessarily extendable to other cases of tumor-suppressor-induced senescence, it raises a question whether the senescent phenotype resulting from the overexpression of a single growth-inhibitory protein can be equated with multifactorial senescence that develops as a result of telomere shortening or DNA damage.

Shock therapy: damage-induced senescence in tumor cells

One could argue that genetic modifications that induce senescence in tumor cells are merely artificial laboratory manipulations. Tumor cells, however, are readily forced into senescence by various agents that are used in everyday clinical management of human cancers. Different classes of chemotherapeutic agents and ionizing radiation induce senescence-like morphological changes and SA- β -gal expression in cell lines derived from a variety of human solid tumors (Wang *et al.*, 1998; Chang *et al.*, 1999a; Michishita *et al.*, 1999; Park *et al.*, 2000; Suzuki *et al.*, 2001; Elmore *et al.*, 2002; Han *et al.*, 2002; Haq *et al.*, 2002; te Poele *et al.*, 2002). Within drug-treated cell populations, the senescent phenotype is specifically associated with those tumor cells that undergo terminal growth arrest after exposure to the drug (Chang *et al.*, 1999a, 2002). Among different classes of anticancer agents, the senescent phenotype is induced most strongly by agents that affect DNA structure, such as doxorubicin, aphidicolin, cisplatin, ionizing radiation, cytarabine, and etoposide, but much more weakly by microtubule-targeting drugs (taxol and vincristine) (Chang *et al.*, 1999a). Tumor cell senescence is also induced by TGF- β (Katakura *et al.*, 1999), antiestrogens (Christov *et al.*, 2003), and by so-called 'differentiating agents', including sodium butyrate (Terao *et al.*, 2001) and retinoids (discussed in a later section). Importantly, some of the 'immortal' tumor cell lines display the senescent phenotype in a substantial minority of the cells (10–20%) even without drug treatment (Chang *et al.*, 1999a), suggesting that tumor cell senescence could develop spontaneously. Such 'spontaneous senescence' could occur in response to subtle changes in the cell environment or could reflect shortening of telomeres below the critical length in some of the cells.

Induction of tumor cell senescence by chemotherapy (as detected by SA- β -gal staining) also occurs *in vivo* (Roninson, 2002a) as demonstrated in nude mouse xenografts of human tumor cell lines (Roninson *et al.*, 2001; Elmore *et al.*, 2002), in carcinogen-induced rat mammary tumors (Christov *et al.*, 2003), and in murine

Eμ-myc lymphoma, a transgenic model of B-cell lymphoma (Schmitt *et al.*, 2002). So far, there has been only one report investigating the senescence response to chemotherapy in clinical cancer. te Poele *et al.* (2002) stained archival material from frozen breast tumors of patients who had or had not received treatment with the CAF regimen (cyclophosphamide, doxorubicin and 5-fluorouracil) for SA-β-gal. Tumor sections of patients who had not received chemotherapy showed SA-β-gal staining in two of 20 (10%) cases, and this staining (possibly reflecting spontaneous senescence) was confined to isolated tumor cells. In contrast, 15 of 36 treated tumors (41%) showed SA-β-gal staining of extended areas. Since SA-β-gal enzymatic activity is unstable even in freshly frozen tissue samples, these numbers are likely to underestimate the extent of chemotherapy-induced senescence in clinical cancer. Remarkably, SA-β-gal staining was confined to tumor cells, whereas normal tissue was completely negative, suggesting that chemotherapy-induced senescence could be a specific response of tumor cells.

What are the mechanisms of damage-induced senescence in tumor cells? This response is not associated with telomere shortening and is not prevented by the overexpression of telomerase (Elmore *et al.*, 2002), indicating that it should be classified as STASIS rather than replicative senescence. On the other hand, p53, p21, and p16, which play a central role in normal cell senescence, are also implicated in the senescence of tumor cells. In murine *Eμ*-myc lymphoma, treatment-induced *in vivo* senescence becomes undetectable upon the knockout of either p53 or p16 (Schmitt *et al.*, 2002). Drug-induced *in vitro* senescence in HCT116 colon carcinoma cell line is decreased several-fold upon the knockout of either p53 or p21, and the same effect is observed in HT1080 fibrosarcoma cells upon the inhibition of p53 expression (Chang *et al.*, 1999b). A role for p53 and p16 is also suggested by the analysis of chemotherapy-induced senescence in clinical breast cancer, where SA-β-gal activity was analysed in parallel with tumor staining for p53 and p16 (te Poele *et al.*, 2002). SA-β-gal staining in breast cancer was significantly associated with low p53 staining (indicative of the lack of p53 mutations), and with strong staining for p16 (te Poele *et al.*, 2002).

On the other hand, it is also clear that treatment-induced senescence of tumor cells can occur in the absence of p16, p53, or p21. In particular, p16-deficient tumor cell lines, such as HT1080 and HCT116, show a strong senescence response both *in vitro* and *in vivo* (Chang *et al.*, 1999a; Roninson *et al.*, 2001), and 13% of the SA-β-gal-positive breast cancers did not stain for p16 (te Poele *et al.*, 2002). Furthermore, the inhibition or knockout of p53 or p21 in HCT116 or HT1080 cells does not fully abolish drug- or radiation-induced senescence, as determined by the presence of SA-β-gal-staining terminally growth-arrested cells (Chang *et al.*, 1999b). Furthermore, moderate doses of doxorubicin induced the senescent phenotype in p53-null Saos-2 cell line, in SW480 and U251 cells carrying mutant p53, and in HeLa and Hep-2

cell lines, where p53 function has been inhibited by papillomavirus protein E6 (Chang *et al.*, 1999a). Thus, p53 and p21 promote damage-induced senescence in tumor cells, but this response can also occur in the absence of p53 or p21, as well as p16, suggest that some other genes may also mediate damage-induced senescence of tumor cells.

The evidence described above suggests that p53, p21, and p16, as well as Bcl2, act as positive regulators of senescence in tumor cells, even if these genes are not absolutely required for tumor cell senescence. In considering the role of these regulators in the overall treatment outcome, it is important to bear in mind that these genes also regulate apoptosis, a physiological program stimulated by p53 and inhibited by Bcl2. p21 also inhibits apoptosis through both intracellular and paracrine mechanisms (Dotto, 2000; Roninson, 2002b). In addition to senescence and apoptosis, another major antiproliferative effect of DNA-damaging agents is cell death through mitotic catastrophe (Roninson *et al.*, 2001), which culminates either in apoptosis or in formation of nonviable cells with multiple micronuclei that die through a necrosis-like process or sometimes develop the senescent phenotype (Chang *et al.*, 1999b). Mitotic catastrophe is potentiated by deficiencies in cell cycle checkpoints. These checkpoints are regulated in part by p53 and p21, and therefore p53- and p21-deficient cells are more prone to mitotic catastrophe (and to apoptosis that develops as a consequence of abnormal mitosis). The mutual effects of apoptosis, senescence, and mitotic catastrophe in tumor response to treatment are illustrated by the studies where inhibition of apoptosis by various means was shown to increase mitotic catastrophe ending in micronucleation (Lock and Stribinskiene, 1996), senescence (Schmitt *et al.*, 2002; Rebbaa *et al.*, 2003), or both mitotic catastrophe and senescence (Ruth and Roninson, 2000).

Figure 2 illustrates the relationships between damage-induced senescence, mitotic catastrophe and apoptosis, as well as the effects of p53, p21, p16, and Bcl2 on these responses. The complexity of these relationships explains why changes in these genes have different effects on tumor response to treatment in different cellular contexts. For example, p53 inhibition, by counteracting the apoptotic program, produces damage resistance in cells that have a high propensity for apoptosis and also weakens the senescence response. The same mutations, however, disable cell cycle checkpoints and thereby promote mitotic catastrophe, increasing the sensitivity of cell lines with strong checkpoints but a weak apoptotic program (Chang *et al.*, 1999b). Analysis of these responses is further complicated by the existence of p53-independent mechanisms of apoptosis and senescence and by differential effects of genes such as Bcl2 on the growth of different cell lines. Thus, despite the undisputable significance of key regulatory genes in determining senescence and other damage responses, analysis of a small number of genes is generally insufficient to predict damage recovery in tumor cells.

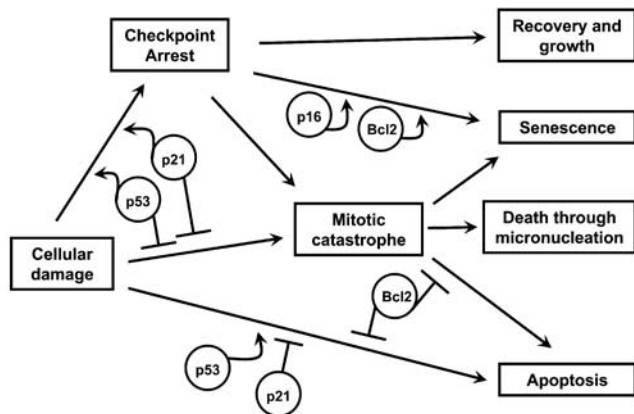


Figure 2 Responses of tumor cells to DNA damage and effects of key regulatory proteins

Changes in gene expression of senescent tumor cells: the return of Dr Jekyll and Mr Hyde

Large-scale profiling of changes in gene expression associated with doxorubicin-induced senescence in HCT116 colon carcinoma cells (which are wild type for p53 but deficient for p16) has revealed additional determinants and new biological aspects of damage-induced senescence (Chang *et al.*, 2002). HCT116 cells that either proliferated or became senescent 6–9 days after 1-day exposure to doxorubicin differ in their expression of specific clusters of genes with related biological functions. The majority of the genes that are strongly inhibited in senescent cells are involved in cell cycle progression, especially in mitosis and DNA replication. Analysis of HCT116 derivatives with homozygous knockout of p53 or p21 showed that inhibition of cell cycle progression genes was dependent on p53-mediated induction of p21 (Chang *et al.*, 2002). In fact, p21 induction alone was shown (in HT1080 fibrosarcoma) to be sufficient to shut off the same genes (Chang *et al.*, 2000b), by inhibiting transcription through negative regulatory elements in the promoters of such genes (Zhu *et al.*, 2002). Since p53- and p21-null cells are still able to undergo drug-induced senescence (albeit at a diminished rate) (Chang *et al.*, 1999b), inhibition of cell cycle progression genes does not appear to be an absolute requirement for this arrest (Chang *et al.*, 2002).

Doxorubicin-induced senescence was also found to involve upregulation of multiple genes with reported growth-inhibitory functions, including several tumor suppressors that are silenced during carcinogenesis but become reactivated in senescent tumor cells (Chang *et al.*, 2002). Coinduction of multiple growth inhibitors in senescent cells was also observed in BrdU-induced senescence of HeLa cells (Suzuki *et al.*, 2001) and in retinoid-induced senescence (Dokmanovic *et al.*, 2002). Genes induced in senescent HCT116 cells encode intracellular growth-inhibiting proteins p21, BTG1, BTG2, EPLIN (epithelial protein lost in neoplasm), as well as secreted growth inhibitors Maspin (a member of

serpin family that inhibits tumor invasion and angiogenesis), MIC-1 (a member of the TGF- β family), IGF-binding protein 6 (IGFBP-6), and amphiregulin (Chang *et al.*, 2002). Induction of these genes suggests that senescent cells may have paracrine growth-inhibitory activity. Such activity was in fact demonstrated in conditioned media and coculture assays with drug-treated cells, where the induction of secreted tumor-suppressing proteins was reported to be mediated by p53 (Komarova *et al.*, 1998). Analysis of p53-deficient HCT116 cells showed, however, that senescence-associated growth inhibitors are induced by doxorubicin even in the absence of p53, although the induction of some of these genes is delayed or diminished in p53-null cells (Chang *et al.*, 2002). Sustained induction of multiple growth inhibitors can explain the growth arrest of senescent cells even in the absence of p16 or p53. Furthermore, overexpression of secreted antimetastatic proteins by senescent tumor cells suggests that these cells provide a reservoir of tumor-suppressing factors that could contribute to the long-term success of chemotherapy.

Drug-induced senescence of HCT116 cells involves upregulation not only of growth inhibitors but also of secreted factors with the opposite, tumor-promoting activity. Examples of such proteins include extracellular matrix components Cyr61 (which has mitogenic and angiogenic functions) and prosaposin (antiapoptotic factor), transforming growth factor TGF α , and several proteases that may potentially contribute to metastatic growth (Chang *et al.*, 2002). Some of these proteins and other tumor-promoting factors were also shown to be induced in response to p21 expression and to produce paracrine antiapoptotic and mitogenic activities (Chang *et al.*, 2000b). Some of the p21-inducible genes have also been implicated in age-related diseases other than cancer, such as Alzheimer's disease, atherosclerosis, and arthritis (Chang *et al.*, 2000b). p21 knockout in HCT116 cells diminishes or delays the induction of some of these genes by doxorubicin, but it does not prevent their induction completely (Chang *et al.*, 2002), thus suggesting that expression of tumor-promoting factors by senescent cells is mediated in part but not entirely by p21. Indeed, our recent experiments show that most of the effects of p21 on the induction of gene expression can also be reproduced by other CDK inhibitors, such as p16 and p27 (BD Chang, J Poole and IBR, in preparation). The paracrine effects of p21 induction mimic the tumor-promoting activities that are present not only in normal senescent fibroblasts (Krtolica *et al.*, 2001) but also in tumor-associated stromal fibroblasts (Elenbaas and Weinberg, 2001). As discussed elsewhere (Roninson, 2002b), all the treatments known to activate the tumor-promoting functions of stromal fibroblasts also lead to p21 induction, suggesting that p21 or other CDK inhibitors could be responsible for the paracrine tumor-promoting functions of stromal fibroblasts.

In summary, global expression profiling of damage-induced senescence in tumor cells has provided an explanation for the ability of such cells to undergo senescence in the absence of p16 and p53, since senescent

cells upregulate concurrently many other growth inhibitors. Many genes induced in senescent cells encode secreted proteins that can affect the growth of neighboring nonsenescent tumor cells, as well as have systemic effects on the organism (see Roninson (2003) for a detailed list of senescence-associated growth-regulatory genes). Some of these factors have a tumor-suppressive function and should be beneficial in the process of cancer treatment, but other proteins secreted by senescent cells promote the growth of tumors or the development of age-related diseases. Induction of the latter proteins is mediated to a large extent by p21 and other CDK inhibitors. Relative expression of different biological classes of senescence-associated genes is likely therefore to determine whether tumor senescence would have a mostly positive or a mostly negative effect on the outcome of treatment. This concept is diagrammatically illustrated in Figure 3 (from Roninson, 2003).

Retinoid-induced senescence: hiding Mr Hyde

Can the positive effects of tumor senescence (permanent growth arrest of tumor cells and secretion of tumor-suppressing factors) be separated from the disease-promoting activities of senescent cells? Analysis of the retinoid-induced senescence of tumor cells suggests that such separation is possible. Retinoids are natural and synthetic derivatives of vitamin A, which regulate cell growth and differentiation through their effects on gene expression, mediated by the binding of these compounds to retinoid receptors that act as regulators of transcription. Retinoids stop tumor cell growth not only by inducing differentiation or apoptosis but also by producing stable growth arrest with all the phenotypic features of senescence but without the expression of any markers of cell differentiation (Roninson and Dokmanovic, 2002). This effect of retinoids has been observed in malignant and premalignant human and rat breast carcinoma cells, *in vitro* and *in vivo* (Chang *et al.*, 1999a; Christov *et al.*, 2003), and in a neuroblastoma cell line (Wainwright *et al.*, 2001). In addition, very low concentrations of 9 and 13-*cis* retinoic acid have been shown to prevent the spontaneous immortalization of a breast epithelial cells derived from a Li-Fraumeni

patient (methylated for p16 and heterozygous for p53) (Herbert *et al.*, 2001).

Retinoid-induced senescence in MCF-7 breast carcinoma cells was characterized by cDNA microarray analysis and found to involve upregulation of several intracellular and secreted growth inhibitors (Dokmanovic *et al.*, 2002), some of which were the same or related to genes that are upregulated in doxorubicin-induced (Chang *et al.*, 2002) or BrdU-induced (Suzuki *et al.*, 2001) senescence. A survey of genes induced by retinoids in different tumor cell types showed that retinoids induce many other intracellular and secreted growth inhibitors, most of which are also known to be upregulated in senescent cells (Roninson and Dokmanovic, 2002), indicating that induction of multiple senescence-associated growth inhibitors is a general mechanism of the antiproliferative effect of retinoids. In contrast to damage-induced senescence, however, no tumor-promoting factors or genes implicated in age-related diseases were identified as induced in retinoid-induced senescence of MCF-7 cells (Dokmanovic *et al.*, 2002). Furthermore, retinoid treatment decreases rather than increases p21 expression in MCF-7 cells (Zhu *et al.*, 1997), providing a likely reason for noninduction of disease-associated genes. Interestingly, retinoid treatment also decreases p21 expression in a subline of neuroblastoma cells where it induces senescence, whereas it increases p21 in another subline that undergoes differentiation rather than senescence upon retinoid treatment, suggesting that p21 may act as a switch between differentiation and senescence in retinoid-treated cells (Wainwright *et al.*, 2001). Hence, retinoid-induced senescence appears to be associated with paracrine tumor-suppressive functions but not the disease-promoting side effects of damage-induced senescence.

Implications of senescence for cancer therapy

As reviewed above, the programs of replicative senescence and STASIS are important anticarcinogenic mechanisms, and therefore genetic or epigenetic changes that allow cells to escape senescence are a necessary part of the process of neoplastic transformation. Nevertheless, tumor cells remain prone to senescence, and they undergo it in response to chemotherapy or radiation, as well as spontaneously. Senescence, along with mitotic catastrophe and apoptosis, is a major antiproliferative effect and a determinant of the long-term success of cancer therapy. In contrast to cell death, however, senescence leaves tumor cells alive and physiologically active. Senescent cells within the tumor can produce secreted factors with both tumor-promoting and tumor-suppressing activities. Hence, the presence of senescent cells in the tumor and the balance of biologically active proteins produced by such cells should have significant prognostic implications. As illustrated in Figure 3, more aggressive cancers may be associated not only with the lack of senescence but also with the presence of

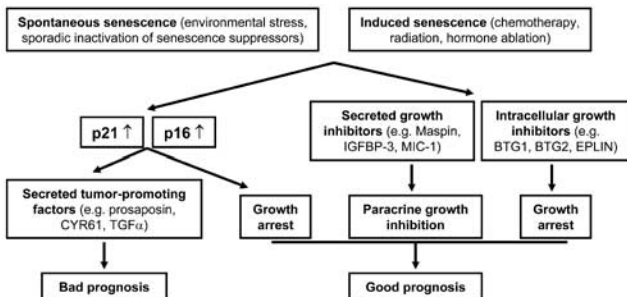


Figure 3 Tumor senescence and its consequences, reproduced with permission from Roninson (2003)

senescent cells that express tumor-promoting factors and CDK inhibitors that induce them. In contrast, tumors containing senescent cells that express high levels of secreted growth inhibitors but few tumor-promoting factors should have a more favorable prognosis.

Although the breast cancer study of te Poele *et al.* (2002) is the only report that directly addresses the induction of senescence during chemotherapy in patients' tumors, indirect observations in radiation therapy suggest that senescence may be a primary mode of treatment response. In particular, complete regression of prostate cancers was reported in some patients to take more than a year after radiation treatment (Cox and Kline, 1983), whereas regression of desmoid tumors took up to 2 years (Bataini *et al.*, 1988). This slow response seems most consistent with radiation-induced senescence. The predictions of the model in Figure 3 are in agreement with the results of many clinical and preclinical studies in prostate cancer and some other malignancies (Roninson, 2003). In particular, senescence-associated growth inhibitors (such as Maspin and IGFBP-3) correlate with good prognosis, whereas p16 and p21 are associated in many cases with unfavorable prognosis, despite the tumor-suppressing nature of these genes (Roninson, 2003). The recent finding that the antiapoptotic protein Bcl2 can induce senescence may also explain the paradoxical observations that expression of this established oncogene is a marker of good prognosis in several cancers (Crescenzi *et al.*, 2003). With the identification of multiple senescence-associated tumor-suppressing and tumor-promoting proteins, it should now be possible to determine the relationship between the expression of these proteins, tumor staging, and treatment outcome.

References

- Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D and Barrett JC. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 13742–13747.
- Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L and DePinho RA. (2000). *Nature*, **406**, 641–645.
- Bailey SM, Cornforth MN, Kurimasa A, Chen DJ and Goodwin EH. (2001). *Science*, **293**, 2462–2465.
- Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D and Wynshaw-Boris A. (1996). *Cell*, **86**, 159–171.
- Bataini JP, Belloir C, Mazabraud A, Pilleron JP, Cartigny A, Jaulerry C and Ghossein NA. (1988). *Am. J. Surg.*, **155**, 754–760.
- Baumann P and Cech TR. (2001). *Science*, **292**, 1171–1175.
- Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P and Campisi J. (2003). *EMBO J.*, **22**, 4212–4222.
- Bertram MJ, Berube NG, Hang-Swanson X, Ran Q, Leung JK, Bryce S, Spurgers K, Bick RJ, Baldini A, Ning Y, Clark LJ, Parkinson EK, Barrett JC, Smith JR and Pereira-Smith OM. (1999). *Mol. Cell. Biol.*, **19**, 1479–1485.
- Blackburn EH. (1992). *Annu. Rev. Biochem.*, **61**, 113–129.
- Blackburn EH. (2000). *Nature*, **408**, 53–56.
- Blackburn EH and Gall JG. (1978). *J. Mol. Biol.*, **120**, 33–53.
- Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA and Greider CW. (1997). *Cell*, **91**, 25–34.
- Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S and Wright WE. (1998). *Science*, **279**, 349–352.
- Broccoli D, Smogorzewska A, Chong L and de Lange T. (1997). *Nat. Genet.*, **17**, 231–235.
- Bryan TM and Cech TR. (1999). *Curr. Opin. Cell Biol.*, **11**, 318–324.
- Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA and Reddel RR. (1997). *Nat. Med.*, **3**, 1271–1274.
- Bryan TM, Englezou A, Gupta J, Bacchetti S and Reddel RR. (1995). *EMBO J.*, **14**, 4240–4248.
- Campisi J. (2000). *In vivo*, **14**, 183–188.
- Campisi J. (2001). *Exp. Gerontol.*, **36**, 607–618.
- Chang BD, Broude EV, Dokmanovic M, Zhu H, Ruth A, Xuan Y, Kandel ES, Lausch E, Christov K and Roninson IB. (1999a). *Cancer Res.*, **59**, 3761–3767.
- Chang BD, Broude EV, Fang J, Kalinichenko TV, Abdryshitov R, Poole JC and Roninson IB. (2000a). *Oncogene*, **19**, 2165–2170.
- Chang BD, Swift ME, Shen M, Fang J, Broude EV and Roninson IB. (2002). *Proc. Natl. Acad. Sci. USA*, **99**, 389–394.

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- Chang BD, Watanabe K, Broude EV, Fang J, Poole JC, Kalinichenko TV and Roninson IB. (2000b). *Proc. Natl. Acad. Sci. USA*, **97**, 4291–4296.
- Chang BD, Xuan Y, Broude EV, Zhu H, Schott B, Fang J and Roninson IB. (1999b). *Oncogene*, **18**, 4808–4818.
- Chen Q and Ames BN. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 4130–4134.
- Chen Q, Ijima A and Greider CW. (2001). *Mol. Cell. Biol.*, **21**, 1819–1827.
- Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider CW and DePinho RA. (1999). *Cell*, **97**, 527–538.
- Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P and de Lange T. (1995). *Science*, **270**, 1663–1667.
- Christov KT, Shilkaitis AL, Kim ES, Steele VE and Lubet RA. (2003). *Eur. J. Cancer*, **39**, 230–239.
- Cohen H and Sinclair DA. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 3174–3179.
- Cong YS, Wright WE and Shay JW. (2002). *Microbiol. Mol. Biol. Rev.*, **66**, 407–425 table.
- Cooke HJ and Smith BA. (1986). *Cold Spring Harb. Symp. Quant. Biol.*, **51** (Part 1), 213–219.
- Cox JD and Kline RW. (1983). *Int. J. Radiat. Oncol. Biol. Phys.*, **9**, 299–303.
- Crescenzi E, Palumbo G and Brady HJ. (2003). *Biochem. J.*, **375**, 263–274.
- Day CY and Enders GH. (2000). *Oncogene*, **19**, 1613–1622.
- de Lange T. (2002). *Oncogene*, **21**, 532–540.
- de Lange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM and Varmus HE. (1990). *Mol. Cell. Biol.*, **10**, 518–527.
- DeFilippis RA, Goodwin EC, Wu L and DiMaio D. (2003). *J. Virol.*, **77**, 1551–1563.
- DePinho RA. (2000). *Nature*, **408**, 248–254.
- di Fagagna FD, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, von Zglinicki T, Saretzki G, Carter NP and Jackson SP. (2003). *Nature*, **426**, 194–198.
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I and Pereira-Smith O. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 9363–9367.
- Dokmanovic M, Chang BD, Fang J and Roninson IB. (2002). *Cancer Biol. Ther.*, **1**, 24–27.
- Dotto GP. (2000). *Biochim. Biophys. Acta*, **1471**, M43–M56.
- Drayton S and Peters G. (2002). *Curr. Opin. Genet. Dev.*, **12**, 98–104.
- Dubrana K, Perrod S and Gasser SM. (2001). *Curr. Opin. Cell Biol.*, **13**, 281–289.
- Dunham MA, Neumann AA, Fasching CL and Reddel RR. (2000). *Nat. Genet.*, **26**, 447–450.
- Elenbaas B and Weinberg RA. (2001). *Exp. Cell Res.*, **264**, 169–184.
- Elmore LW, Rehder CW, Di X, McChesney PA, Jackson-Cook CK, Gewirtz DA and Holt SE. (2002). *J. Biol. Chem.*, **277**, 35509–35515.
- Fang L, Igarashi M, Leung J, Sugrae MM, Lee SW and Aaronson SA. (1999). *Oncogene*, **18**, 2789–2797.
- Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC and Yu J. (1995). *Science*, **269**, 1236–1241.
- Gasser SM. (2000). *Science*, **288**, 1377–1379.
- Gonzalez-Suarez E, Samper E, Flores JM and Blasco MA. (2000). *Nat. Genet.*, **26**, 114–117.
- Goodwin EC and DiMaio D. (2001). *Cell Growth Differ.*, **12**, 525–534.
- Goodwin EC, Yang E, Lee CJ, Lee HW, DiMaio D and Wang ES. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 10978–10983.
- Gorburnova V, Seluanov A and Pereira-Smith OM. (2003). *J. Biol. Chem.*, **278**, 7692–7698.
- Goytisolo FA, Samper E, Martin-Caballero J, Finnon P, Herrera E, Flores JM, Bouffler SD and Blasco MA. (2000). *J. Exp. Med.*, **192**, 1625–1636.
- Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider CW and DePinho RA. (1999). *Cell*, **97**, 515–525.
- Greider CW and Blackburn EH. (1985). *Cell*, **43**, 405–413.
- Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H and de Lange T. (1999). *Cell*, **97**, 503–514.
- Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson M and Weinberg RA. (1999). *Nat. Med.*, **5**, 1164–1170.
- Hall AH and Alexander KA. (2003). *J. Virol.*, **77**, 6066–6069.
- Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, Hendrickson EA, Balan KV, Pantazis P and Wyche JH. (2002). *J. Biol. Chem.*, **277**, 17154–17160.
- Haq R, Brenton JD, Takahashi M, Finan D, Rottapel R and Zanke B. (2002). *Cancer Res.*, **62**, 5076–5082.
- Harley CB. (1991). *Mutat. Res.*, **256**, 271–282.
- Harley CB, Futcher AB and Greider CW. (1990). *Nature*, **345**, 458–460.
- Hart RW and Setlow RB. (1974). *Proc. Natl. Acad. Sci. USA*, **71**, 2169–2173.
- Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK and Allshire RC. (1990). *Nature*, **346**, 866–868.
- Hayflick L and Moorhead PS. (1961). *Exp. Cell Res.*, **37**, 585–621.
- Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW and Corey DR. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 14276–14281.
- Herbert BS, Wright AC, Passons CM, Wright WE, Ali IU, Kopelovich L and Shay JW. (2001). *J. Natl. Cancer Inst.*, **93**, 39–45.
- Holt SE and Shay JW. (1999). *J. Cell Physiol.*, **180**, 10–18.
- Holt SE, Wright WE and Shay JW. (1997). *Eur. J. Cancer*, **33**, 761–766.
- Kapahi P, Boulton ME and Kirkwood TB. (1999). *Free Radic. Biol. Med.*, **26**, 495–500.
- Karlseder J, Smogorzewska A and de Lange T. (2002). *Science*, **295**, 2446–2449.
- Katakura Y, Nakata E, Miura T and Shirahata S. (1999). *Biochem. Biophys. Res. Commun.*, **255**, 110–115.
- Kim JH, Kim JH, Lee GE, Kim SW and Chung IK. (2003). *Biochem. J.*, **373**, 523–529.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL and Shay JW. (1994). *Science*, **266**, 2011–2015.
- Kim SH, Kaminker P and Campisi J. (1999). *Nat. Genet.*, **23**, 405–412.
- Kirkwood TB. (1988). *Ciba Found. Symp.*, **134**, 193–207.
- Kirkwood TB and Austad SN. (2000). *Nature*, **408**, 233–238.
- Komarova EA, Diatchenko L, Rokhlin OW, Hill JE, Wang ZJ, Krivokrysenko VI, Feinstein E and Gudkov AV. (1998). *Oncogene*, **17**, 1089–1096.
- Krtolica P, Parrinello S, Lockett S, Desprez PY and Campisi J. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 12072–12077.
- Lee HW, Blasco MA, Gottlieb GJ, Horner JW, Greider CW and DePinho RA. (1998). *Nature*, **392**, 569–574.
- Lee KH, Rudolph KL, Ju YJ, Greenberg RA, Cannizzaro L, Chin L, Weiler SR and DePinho RA. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 3381–3386.
- Li B, Oestreich S and de Lange T. (2000). *Cell*, **101**, 471–483.

- Lindsey J, McGill NI, Lindsey LA, Green DK and Cooke HJ. (1991). *Mutat. Res.*, **256**, 45–48.
- Lingner J and Cech TR. (1998). *Curr. Opin. Genet. Dev.*, **8**, 226–232.
- Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V and Cech TR. (1997). *Science*, **276**, 561–567.
- Lock RB and Stribinskiene L. (1996). *Cancer Res.*, **56**, 4006–4012.
- Loo DT, Fuquay JI, Rawson CL and Barnes DW. (1987). *Science*, **236**, 200–202.
- Lundblad V and Blackburn EH. (1993). *Cell*, **73**, 347–360.
- Makarov VL, Hirose Y and Langmore JP. (1997). *Cell*, **88**, 657–666.
- Mathon NF, Malcolm DS, Harrisingh MC, Cheng L and Lloyd AC. (2001). *Science*, **291**, 872–875.
- Michishita E, Nakabayashi K, Suzuki T, Kaul SC, Ogino H, Fujii M, Mitsui Y and Ayusawa D. (1999). *J. Biochem. (Tokyo)*, **126**, 1052–1059.
- Morin GB. (1989). *Cell*, **59**, 521–529.
- Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL and Wu JR. (1988). *Proc. Natl. Acad. Sci. USA*, **85**, 6622–6626.
- Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB and Cech TR. (1997). *Science*, **277**, 955–959.
- Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ and Lowe SW. (2003). *Cell*, **113**, 703–716.
- Noda A, Ning Y, Venable SF, Pereira-Smith OM and Smith JR. (1994). *Exp. Cell Res.*, **211**, 90–98.
- Nugent CI and Lundblad V. (1998). *Genes Dev.*, **12**, 1073–1085.
- Nussenzweig A, Chen C, da CSV, Sanchez M, Sokol K, Nussenzweig MC and Li GC. (1996). *Nature*, **382**, 551–555.
- Olovnikov AM. (1973). *J. Theor. Biol.*, **41**, 181–190.
- Park JI, Jeong JS, Han JY, Kim DI, Gao YH, Park SC, Rodgers GP and Kim IH. (2000). *J. Cancer Res. Clin. Oncol.*, **126**, 455–460.
- Ramirez RD, Morales CP, Herbert BS, Rohde JM, Passons C, Shay JW and Wright WE. (2001). *Genes Dev.*, **15**, 398–403.
- Ravi RK, McMahan M, Yangang Z, Williams JR, Dillehay LE, Nelkin BD and Mabry M. (1999). *J. Cell Biochem.*, **72**, 458–469.
- Rebbaa A, Zheng X, Chou PM and Mirkin BL. (2003). *Oncogene*, **22**, 2805–2811.
- Reincke BS, Rosson GB, Oswald BW and Wright CF. (2003). *J. Cell Physiol.*, **194**, 303–313.
- Rizki A and Lundblad V. (2001). *Nature*, **411**, 713–716.
- Robles SJ and Adami GR. (1998). *Oncogene*, **16**, 1113–1123.
- Roninson IB. (2002a). *Drug Resist. Update*, **252**, 1–5.
- Roninson IB. (2002b). *Cancer Lett.*, **179**, 1–14.
- Roninson IB. (2003). *Cancer Res.*, **63**, 2705–2715.
- Roninson IB, Broude EV and Chang BD. (2001). *Drug Resist. Update*, **4**, 303–313.
- Roninson IB and Dokmanovic M. (2002). *J. Cell Biochem.*, **1**–12.
- Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C and DePinho RA. (1999). *Cell*, **96**, 701–712.
- Rudolph KL, Millard M, Bosenberg MW and DePinho RA. (2001). *Nat. Genet.*, **28**, 155–159.
- Ruth AC and Roninson IB. (2000). *Cancer Res.*, **60**, 2576–2578.
- Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM and Lowe SW. (2002). *Cell*, **109**, 335–346.
- Serrano M, Lin AW, McCurrach ME, Beach D and Lowe SW. (1997). *Cell*, **88**, 593–602.
- Shay JW. (1995). *Mol. Med. Today*, **1**, 378–384.
- Shay JW and Bacchetti S. (1997). *Eur. J. Cancer*, **33**, 787–791.
- Shay JW, Pereira-Smith OM and Wright WE. (1991). *Exp. Cell Res.*, **196**, 33–39.
- Shay JW and Wright WE. (1996a). *Curr. Opin. Oncol.*, **8**, 66–71.
- Shay JW and Wright WE. (1996b). *Trends Genet.*, **12**, 129–131.
- Shay JW and Wright WE. (2001). *Science*, **291**, 839–840.
- Sherr CJ. (1998). *Genes Dev.*, **12**, 2984–2991.
- Sherr CJ and DePinho RA. (2000). *Cell*, **102**, 407–410.
- Shore D. (1997). *Trends Biochem. Sci.*, **22**, 233–235.
- Smogorzewska A and de Lange T. (2002). *EMBO J.*, **21**, 4338–4348.
- Sprenger CC, Vail ME, Evans K, Simurdak J and Plymate SR. (2002). *Oncogene*, **21**, 140–147.
- Stansel RM, de Lange T and Griffith JD. (2001). *EMBO J.*, **20**, 5532–5540.
- Stein GH, Drullinger LF, Soulard A and Dulic V. (1999). *Mol. Cell. Biol.*, **19**, 2109–2117.
- Stewart SA, Hahn WC, O'Connor BF, Banner EN, Lundberg AS, Modha P, Mizuno H, Brooks MW, Fleming M, Zimonjic DB, Popescu NC and Weinberg RA. (2002). *Proc. Natl. Acad. Sci. USA*, **99**, 12606–12611.
- Sugrue MM, Shin DY, Lee SW and Aaronson SA. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 9648–9653.
- Suzuki T, Minagawa S, Michishita E, Ogino H, Fujii M, Mitsui Y and Ayusawa D. (2001). *Exp. Gerontol.*, **36**, 465–474.
- Takai H, Smogorzewska A and de Lange T. (2003). *Curr. Biol.*, **13**, 1549–1556.
- Tang DG, Tokumoto YM, Apperly JA, Lloyd AC and Raff MC. (2001). *Science*, **291**, 868–871.
- te Poele RH, Okorokov AL, Jardine L, Cummings J and Joel SP. (2002). *Cancer Res.*, **62**, 1876–1883.
- Terao Y, Nishida J, Horiuchi S, Rong F, Ueoka Y, Matsuda T, Kato H, Furugen Y, Yoshida K, Kato K and Wake N. (2001). *Int. J. Cancer*, **94**, 257–267.
- Tombor B, Rundell K and Oltvai ZN. (2003). *Biochem. Biophys. Res. Commun.*, **303**, 800–807.
- Tominaga K, Olgun A, Smith JR and Pereira-Smith OM. (2002). *Mech. Ageing Dev.*, **123**, 927–936.
- van Steensel B, Smogorzewska A and de Lange T. (1998). *Cell*, **92**, 401–413.
- Vogelstein B and Kinzler KW. (1993). *Trends Genet.*, **9**, 138–141.
- Wainwright LJ, Lasorella A and Iavarone A. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 9396–9400.
- Wang X, Wong SC, Pan J, Tsao SW, Fung KH, Kwong DL, Sham JS and Nicholls JM. (1998). *Cancer Res.*, **58**, 5019–5022.
- Watson JD. (1972). *Nat. New Biol.*, **239**, 197–201.
- Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB and Morin GB. (1997). *Nat. Genet.*, **17**, 498–502.
- Wellinger RJ and Sen D. (1997). *Eur. J. Cancer*, **33**, 735–749.
- Wells SI, Francis DA, Karpova AY, Dowhanick JJ, Benson JD and Howley PM. (2000). *EMBO J.*, **19**, 5762–5771.
- Wong KK, Chang S, Weiler SR, Ganesan S, Chaudhuri J, Zhu C, Artandi SE, Rudolph KL, Gottlieb GJ, Chin L, Alt FW and DePinho RA. (2000). *Nat. Genet.*, **26**, 85–88.
- Wright WE, Piatyszek MA, Rainey WE, Byrd W and Shay JW. (1996). *Dev. Genet.*, **18**, 173–179.
- Wright WE and Shay JW. (1992). *Exp. Gerontol.*, **27**, 383–389.

- Wright WE and Shay JW. (1996). In *Modern Cell Biology Series*, Holbrook NJ, Martin GR (eds) Wiley-Liss Inc.: New York, pp 153–167.
- Wright WE and Shay JW. (2000). *Nat. Med.*, **6**, 849–851.
- Wright WE and Shay JW. (2001). *Curr. Opin. Genet. Dev.*, **11**, 98–103.
- Wright WE and Shay JW. (2002). *Nat. Biotechnol.*, **20**, 682–688.
- Xu Y and Baltimore D. (1996). *Genes Dev.*, **10**, 2401–2410.
- Zhu H, Chang BD, Uchiumi T and Roninson IB. (2002). *Cell Cycle*, **1**, 59–66.
- Zhu J, Woods D, McMahon M and Bishop JM. (1998). *Genes Dev.*, **12**, 2997–3007.
- Zhu WY, Jones CS, Kiss A, Matsukuma K, Amin S and De Luca LM. (1997). *Exp. Cell Res.*, **234**, 293–299.