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Senescence; an endogenous anticancer mechanism

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1. ABSTRACT

Pre-malignant tumor cells enter a state of irreversible cell cycle arrest termed senescence (cellular senescence; CS). CS is a part of the aging program and involves multiple signaling cascades and transduction mechanisms. In general, senescence can be divided into replicative senescence and premature senescence. Replicative senescence (replicative CS) has been described for all metabolically active cells that undergo a spontaneous decline in growth rate. Notably, ectopic expression of telomerase holoenzyme (hTert) can prevent replicative CS. In cancer cells, premature senescence induced by oncogenes, named oncogene-induced senescence (oncogene induced CS; OIS), play an important role in preventing the development of cancer. Oncogene induced CS can be promoted by the loss of tumor suppressor genes, such as PTEN. Additionally, other interesting mechanisms, like selective microRNA expression, epigenetic modifications, or even stress conditions, are also able to activate the senescence program. Here, we will critically review the literature on the role of senescence in preventing the development of cancer and discuss the potential of senescence modulation for generating new molecular tools that could be explored as anticancer treatments.

2. INTRODUCTION

Cellular senescence (CS), which was first described in 1961 by Leonard Hayflick and Paul Moorhead, is a complex phenomenon characterized by irreversible growth arrest. This growth arrest is accompanied by changes in cellular structure, chromatin organization, and gene expression (*1*). Morphologically, senescent cells in culture grow in size, become flattened and show increased cytoplasmic granularity (*2*). In addition, a large increase in lysosome mass is observed upon the induction of senescence; the measurement of lysosomal β-galactosidase activity is a classical biomarker of senescence (*3*), as it could be observed in proliferating C6 rat glioblastoma cell treated with sodium butyrate, a drug that induces cellular senescence (Figure 1A). Chromatin remodeling, induced by proteins that bind to DNA, is another hallmark of senescence. One DNA-binding protein known to be involved in senescence is the heterochromatin protein 1 (HP1) family member HP1γ, which has been established as a marker for senescence-associated heterochromatin foci (SAHF) in human and murine cells (*4,5*).

T**able 1.** Types of senescence

Type of senescence[a]	Abbreviation	Mechanism	Reference
Replicative senescence	RS	Senescence dependent on telomere length and blocked by hTert expression.	(6 to 15)
Premature senescence	PS	Any senescence that is not replicative; includes SIPS, OIS and PICS.	(16 to 29)
Stress-induced premature senescence	SIPS	Senescence induced by several kinds of stresses, such as DNA damage, oxidative stress and culture conditions. Includes OIS and some aspects of PICS.	(16 to 29)
Oncogene-induced senescence	OIS	Senescence induced by oncogenes such as Ras^{G12V}, cMYC or $BRaf^{E600V}$; involves signaling pathways such as p38MAPK and the DNA damage response.	(16 to 23)
PTEN loss-induced cellular senescence	PICS	Shares several features with OIS but can also be induced through mechanisms that do not involve DDR or p53 signaling.	(24 to 29)

[a]"Types" do not refer to specific, independent mechanisms but rather to different terminologies used in the field of senescence.

Senescence itself is biochemically diverse, and the environmental/molecular signals that induce senescence are heterogeneous. Moreover, senescence can be divided into two major categories: replicative senescence (replicative CS; RS), which is normally induced after cells undergo a large number of divisions, and premature senescence (premature CS; PS), which results mainly from DNA damage and/or oncogenic signals. Several premature senescence sub-types have been defined, including stress-induced premature senescence (stress-induced premature CS; SIPS), oncogene-induced senescence (oncogene induced CS; OIS) and PTEN loss-induced cellular senescence (PTEN loss-induced CS; PICS) (Table 1).

Replicative senescence (replicative CS; RS) is characterized by an irreversible loss of replicative capacity and is associated with telomere dysfunction, as observed in the so-called Hayflick cell division limit (*6*). The Hayflick limit refers to the total number of divisions that a normal cell can undergo before arrest due to critical telomere shortening (*7*). As will be discussed later, telomeres and proteins that are necessary to maintain telomeric structure (e.g., telomerase) are fundamental components of the senescence pathway, and virtually all basic and applied research related to tumor development and senescence considers telomeres and their associated elements to be major targets for drug development.

Recently, many studies have shown that replicative CS is involved in tumor suppression activity and aging, with replicative CS acting as a barrier to cellular immortalization (*8*). In this context, it has been observed that many cell populations require replicative CS to maintain the balance between the rates of cell division and cell death and thus achieve tissue homeostasis (*9*). However, cell populations can tend toward an increase in cell division (tumorigenesis) or cell death (tissue aging) through the combined effects of three major factors: (i) cell lifespan, (ii) number of cell divisions (*10,11*), and (iii) stress caused by external factors (*30*). All of these factors are responsible, to different degrees, for an increase in molecular damage and mutations that cause genetic instability and could lead to tumor development or aging (*10*). It is important to note that the fate of each cell (tumor or aging) is strongly influenced by the cellular environment.

The ectopic expression of some oncogenes (e.g., Ras^{G12V}, B-Raf^{V600E} and c-MYC) in fibroblast cells induces senescence in a telomere-independent fashion and concomitantly activates the DNA damage response (DDR), which can thus be considered stress-induced premature CS (*16*). This form of senescence, called "oncogene-induced senescence" (oncogene induced CS), is displayed by a variety of cell types (*17*). Oncogene induced CS is rapidly activated when oncogenic stress is present, resulting in the death of neoplastic cells *in vitro* (*18*). However, the role of oncogene induced CS *in vivo* is not clear, although data gathered by some authors have shown that oncogene induced CS could also serve as an *in vivo* antitumoral mechanism. For example, the expression of mutant K-ras oncogene family members (e.g., K-rasG12V and N-rasG12D) can induce senescence in the mammary gland (19) or the bladder (20). Another oncogene, B-raf (a downstream effector of K-ras), induces senescence in melanocytes (*16, 21 23*).

The loss of PTEN causes an increase in PIP3, which in turn leads to the activation of Akt and other proteins. This process, termed PTEN loss-induced cellular senescence (PTEN-loss induced CS; PICS), has some interesting differences from the oncogene induced CS induced by Ras overexpression. While oncogene induced CS requires the hyper-replication response, which involves the DDR, PTEN-loss induced CS occurs even in the presence of S-phase blockers or ATM inhibitors, suggesting a fundamental difference between PTEN-loss induced CS and oncogene induced CS or replicative CS (*1*).

The link between oncogene induced CS and replicative CS is mainly determined by cell cycle-regulatory mechanisms (e.g., post-transcriptional and post-translational modifications) that are intimately involved in tumor progression (Figure 1B). In this sense, transcriptional factors (e.g., p53; Figure 1B) can act as key elements in oncogene induced CS and replicative CS by blocking the activity of different cyclins, CDCs, and CDKs proteins (Figure 1B), leading to a permanent cell cycle arrest, a hallmark of cellular senescence. It should be noted that different cyclin-CDC-CDK complexes found within the cell are cell cycle-dependent, but all lead to gene transcription induction by means of retinoblastoma (RB)-E2F complex (Figure 1B). However, the suppression of cyclin-CDC-CDK complexes by different transcriptional factors supports the notion that senescence can be activated independently of cell cycle's phase (Figure 1B). Notably, and due to the complexity of protein complexes involved, the identity of the molecular pathway that links oncogene induced CS and replicative CS is not known, despite intensive efforts in the last several years to understand the molecular basis of senescence. However, some molecular elements that are active during senescence have been described. For example, the activity of cyclin-dependent kinases (CDKs), proteins whose functions are strongly

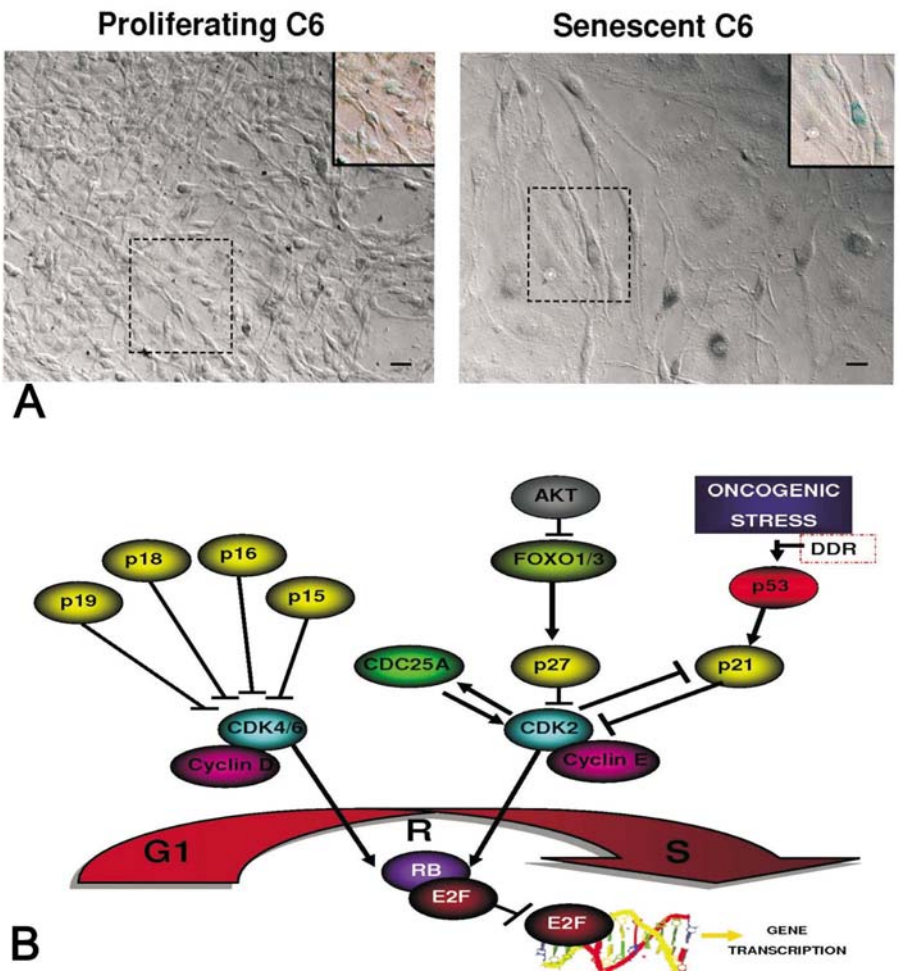

Figure 1. Phenotypic characteristics (A) and molecular mechanisms (B) associated with senescence. Both proliferating C6 rat glioblastoma cells and cells treated with sodium butyrate (A) display the hallmarks of senescence, which include the expression of β-galactosidase (senescence-associated β-galactosidase, SA-β-Gal; dotted box) and altered morphology (solid box). Cells were treated with sodium butyrate for 2 days in culture, followed by 10 days in drug-free medium (senescent C6 cells) and compared to control cells not exposed to sodium butyrate during the same time in culture (proliferating C6 cells). Cells were then stained with X-gal and visualized by optical microscopy (scale bar = 10 μm). Interestingly, the molecular pathways that lead to senescence are closely associated with the control of the different phases of cell cycle (B). In fact, many proteins that drive the cell toward senescence, such as p15, p16, p18, p19, p21, p27, and p53 (B) block the activity and gene expression of cyclins, cell division cycle (CDCs), and cyclin-dependent kinases (CDKs), resulting in cell cycle arrest and senescence. In addition, oncogene-induced senescence (OIS) leads to the activation of a DNA damage response (DDR) pathway (B) that in turn results in p53-dependent cell senescence.

associated with tumorigenic processes, is essential for cell cycle control and consequently senescence. CDKs and, therefore, cell cycle progression are controlled by specific phosphorylation and dephosphorylation mechanisms as well as by their interactions with specific proteins, including CDK inhibitors (*31,32*). Two families of CDK inhibitors have been described: the CDKN1 family [CDKN1A (p21), CDKN1B (p27) and CDKN1C (p57)] and the CDKN2 family [INK4A (p16), INK4B (p15), INK4C (p18), and INK4D (p19)] (for review, see *33*). Among these, p21, p27, and p16INK4A are the most directly involved in senescence induction.

Retinoblastoma protein (Rb) is a major CDK target that, upon phosphorylation by CDKs, releases the transcription factor E2F, which in turn regulates several genes involved in the progression from G1 to S phase (*34,35*). The tumor suppressor protein p53 plays an important role in senescence induction by several oncogenes and stresses, but it is less important in replicative CS (*36*) (Figure 1B). Detailed descriptions of the roles of Rb and p53 in the different forms of senescence will be provided in the following sections.

However, it is noteworthy from a therapeutic point of view that cancer cells lacking functional Rb, p53, and other tumor suppressors retain the capacity for senescence induction, at least *in vitro*. This indicates that other proteins/mechanisms associated with senescence are

still functional. For example, the treatment of the osteosarcoma cell line SAOS-2 and the prostate cancer cell line DU145 with doxorubicin, an anthracycline-derived antitumor drug that promotes DNA damage by inducing crosslinks and oxidative stress (*37,38*), induced senescence in more than 50% of cells *in vitro* (*37,38*), even though these cell lines do not express p53 or Rb. In fact, recent data suggest that DNA damage could be a common causative agent that underlies several different forms of cellular senescence (Figure 1B), including not only telomere dysfunction but also oncogene induced CS (*39-41*).

Here, we will review the recent progress in elucidating the biology of cellular senescence. We will consider data from both basic and applied biomedical areas and discuss how this information is being used to develop new anticancer therapies and to understand the mechanisms of cancer progression.

3. JANUS'S FACES: SENESCENCE AND TELOMERIC (IN)STABILITY IN TUMORS

In Roman mythology, Janus is the two-faced god of beginnings and endings, transitions, gates, doors, doorways, and time. Janus symbolizes the progression from past to future, of one condition to another. Facing the passage of time and sensing the cumulative damages induced by metabolism and environment, all cells are prone to senescence and, ultimately, aging.

Telomeres, the structures present at the chromosome ends (*42*), have multiple roles in the maintenance of genomic integrity (*42*). Telomere shortening, or "erosion", in mammalian cells has been strongly associated with senescence (*32*) and protection against cancer. However, telomeres display a characteristic Janus face: instead of becoming senescent, cells can develop chromosomal instabilities due to telomere erosion and induce the development of tumors (*42, 43*).

Senescence and telomeres share a complex relationship, which has been covered in great detail in several previous reviews (*44, 45*). This review will focus on the potential applications of telomere shortening in cancer treatment and diagnostics.

3.1. Telomere shortening: cancer biomarker and/or antitumor target?

In eukaryotes, specifically vertebrates, telomeres are composed of short, hexameric, guanosine-rich repeat sequences (TTAGGG) that are located at chromosome ends and form a 3' single-stranded overhang. This overhang forms a structure that folds back into itself such that no free single-stranded DNA remains (Figure 2A) (*46-48*). The main function of telomeres is to protect chromosome ends against degradation (telomere erosion) and potential end fusion due to the gradual shortening of DNA upon each round of replication in mitotically active cells (*49,50*). Telomere erosion is variable from tissue to tissue and the degree of erosion depends on the age of the donor organism (*44,51-53*). Human telomeres are 5-15 kb in length (*47*) and lose approximately 2-4 kb of length over the lifespan of a cell (*54*).

Some cells can evade telomere erosion by expressing telomerase reverse transcriptase (TERT; Figure 2A), the major enzyme responsible for telomere extension during DNA replication. In a fibroblast model, hTERT-transduced cells had very long telomeres with an extended life span because replicative CS induction was blocked (*12*).

Interestingly, 85% of malignant tumor cells exhibit increased TERT expression (*13*), leading to the maintenance of telomeres and enabling continuous cell division (*14,15*) and supporting the hypothesis that high telomerase activity is important for tumorigenesis and/or tumor maintenance. Advanced-stage tumors display longer telomeres (*55*), suggesting that complete telomere elongation may be necessary to sustain a large number of cell divisions with telomere shortening in tumorigenesis (*56-57*). Another study of 86 patients with primary gastric adenocarcinoma confirmed the correlation of TERT reactivation with malignant progression compared with early gastrocarcinogenesis (*58*).

Direct *in vivo* evidence for the potential antitumor effect of telomere erosion was presented by Feldser and Cosme-Blanco (*59, 60*) and discussed by Sedivy (*61*). Knockout of the telomerase gene in mice expressing the Eμ-myc oncogene, an established model of Burkitt's lymphoma, significantly reduced tumor formation. This mechanism was not blocked by overexpression of Bcl2, suggesting that senescence, rather than apoptosis, is involved in the protective effect of the telomerase knockout. Additionally, KO animals presented strong senescence staining in lymph nodes compared to WT animals. The protective effect of telomerase KO required p53 (*59*). In parallel to these experiments, the use of a knock-in mouse model bearing the p53 allele $p53^{Arg172Pro}$, which is able to mediate the induction of senescence but not apoptosis, showed that spontaneous tumorigenesis is potently repressed by TERC KO. Surprisingly, chemically induced skin tumorigenesis is not blocked by Terc KO, indicating that telomerase-dependent senescence plays different roles depending on the tumorigenic stimulus (*60*).

Despite these data indicating the anti-tumorigenic role of short telomeres and senescence induction, short telomeres induce gross chromosomal rearrangements (GCRs) (*61*), a consequence of the formation of anaphase bridges and chromosome breakage, particularly if senescence is blocked. Repetition of the breakage-fusion-bridge cycle leads to aneuploidy and further chromosome fusions, loss of heterozygosity, and/or gene amplification (*62*). Although this hypothesis remains to be tested directly from an epidemiological perspective (*63*), one recent study has shown that both cancer incidence and cancer mortality are associated with telomere shortening. In this work, 92 out of 787 participants (11.7%) developed multiple cancer types, and a statistically significant inverse relationship between telomere length and cancer incidence and mortality was observed (*63*). This information is consistent

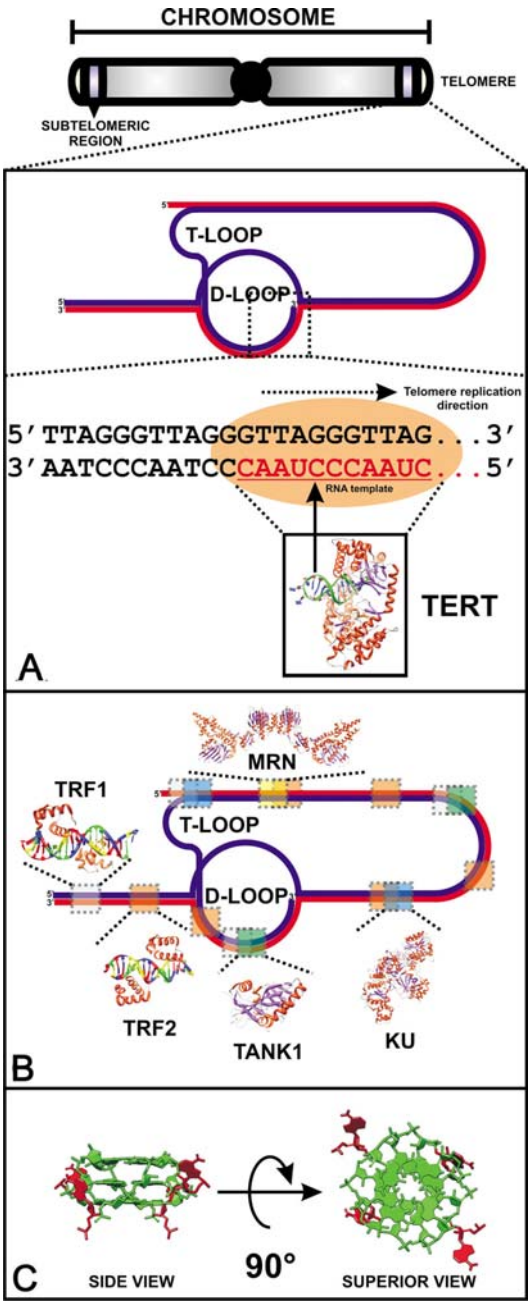

Figure 2. A schematic view of telomere structure (depicting T-loop and D-loop structures) and short hexameric repeat sequences (TTAGGG) (A). The synthesis and maintenance of these repeat sequences are mainly carried out by the telomerase reverse transcriptase [TERT; Protein Data Bank (PDB) number 3KYL), which contains in its three-dimensional structure (inset box) a short RNA molecule that is complementary to the telomere repeat sequence and allows its extension by DNA replication (A). The major protein complexes that participate in the maintenance of telomere structures (T-loop and D-loop; B), including guanosine (G)-quadruplex elements (C), are present in the extremities of the eukaryotic chromosome (yellow marks). Considering the data about telomere dynamics accumulated to date, it is clear that several proteins are necessary to conserve the secondary and tertiary structures of telomeres, including TRF1 and TRF2 (PDB numbers 1W0T and 1W0U, respectively), TANK1 (PDB number 2RF5), KU (PDB number 1JEQ), and the complex MRE11-RAD50-NBS1 (MRN; PDB number 3QG5), whose three-dimensional structures are depicted in (A). Other proteins (e.g., POT1, TIN2, RAP1, and TPP1) that are required for telomere structure maintenance are not shown in (B). Interestingly, many of these proteins combine to form different complexes (colored dotted boxes) that are responsible for particular aspects of telomere biology, such as capping induction or maintenance of G-quadruplexes (PDB number 2KBP). The three-dimensional structure of G-quadruplex DNA is shown from two different angles (C).

with previous evaluations of the correlation between shortened telomeres and different types of cancer (*64-67*). Therefore, the role of telomere erosion in both tumor suppression and tumor induction must be considered. The model that seems to emerge from these studies suggests that short telomeres are anti-tumorigenic during the early phases of tumor formation but can be pro-tumorigenic in late, established tumors in which senescence induction mechanisms are already lost, and the aneuploidy produced increases the tumor's heterogeneity.

Considering the available data, innovative therapeutic strategies that employ telomere/TERT-based drugs with broad anticancer activities are abundant in the specialized literature. However, the unexpected side-effects of these drugs are dependent on the model chosen to study senescence and tumor treatment. This imposes a challenge for the development of new treatments, which will be the subject of this review. It is also important to emphasize that senescent cells can maintain long telomeric 3'-overhangs (*68,69*), suggesting that senescence can be induced in ways that are independent of telomere length or telomerase activity, as will be discussed below.

3.2. Telomere structure as a target for a potential therapeutic intervention

The leading cause of telomere erosion is the DNA replication process itself. During semiconservative replication, the DNA holoenzyme complex synthesizes a lagging strand made up of Okazaki fragments, with each fragment requiring a new RNA primer that is then extended by DNA polymerase. At the end of the chromosome, however, there is not enough DNA to serve as a template for another RNA primer, and the DNA replication machinery is unable to fill the gap between the final priming event and the end of the chromosome. Thus, the lagging strand is shorter than the original strand that was used as a template, producing a 3'-overhang (*7*). In 1999, electron microscopy studies suggested that the 3'-overhang can loop back and integrate into the duplex repeat tract, forming a lasso-like structure called the "T-loop" (Figure 2B). This hypothesis was confirmed by an analysis of telomeres in different organisms, which revealed that the 3'-single-stranded overhang can invade the double-stranded telomeric tracts, displacing the homologous strand of the same telomere (*70*).

In fibroblasts, the telomeric 3'-overhang and the telomeres work in a coordinated way to maintain an intact telomere structure and, consequently, chromosomal integrity and genome stability. When telomeres become too short to maintain the T-loop structure, tumorigenesis can be induced (*71*). Supporting this hypothesis, immortalized fibroblasts they presented short telomere and telomeric 3'-overhangs showed a high degree of dicentric chromosome induction, an indicator of end-to-end chromosome fusion (*71*). Therefore, a minimal length of telomeric 3'-overhang may be necessary to maintain a proper telomere end-capping structure and ensure genomic stability in normal cells (*72*).

One probable mechanism for the maintenance of telomeric 3'-overhangs is related to the presence of a secondary DNA structure termed G-quadruplex (Figure 2C), which is found in telomeres (*73-75*). The consecutive formation of G-quadruplex structures in single-stranded telomeric overhangs protects double-stranded DNA ends from being recognized as DSBs and protects against nuclease hydrolysis. Moreover, G-quadruplex structures are necessary for the effective packing of telomeric DNA into the protective capping state. However, the G-quadruplex and T-loop structures may provide conformational flexibility for chromosome ends in response to different environmental conditions.

Considering the importance of G-quadruplex and T-loop DNA in protecting telomeres against degradation (*73*), these DNA structures may be useful for the development of drugs to induce senescence in tumors, which will be reviewed further in this manuscript.

In addition to forming specialized DNA secondary structures, telomeres are bound by a multiprotein complex, known as shelterin or the telosome, that forms the end-capping structure. The shelterin complex includes tankyrase, telomeric repeat-binding factor 1 (TRF1), telomeric repeat-binding factor 2 (TRF2), TRF1-interacting protein 2 (TIN2), repressor-activator protein 1 (RAP1), protection of telomeres 1 (POT1), and TPP1 (formerly named PTOP/PIP1/TINT1) (*74*) (Figure 2B). Various experiments have been designed to investigate the functions of the telosome in the context of senescence.

The disruption of genes involved in telomeres/telosome function can lead to the induction of either apoptosis or senescence. For example, fibroblasts respond to POT1 depletion with a strong induction of senescence, which is independent of telomerase (*75*). The role of POT1 in protecting telomeres has been also evaluated in breast cancer cells exposed to anti-POT1 small-interfering RNAs (siRNAs) (*75,76*). POT1 knockdown leads to telomere dysfunction, activating apoptosis by positively modulating p53 and Bax expression and downregulating the expression of some anti-apoptotic genes, like Bcl-2.

TRF2 inhibition by a dominant-negative form of TRF2 (TRF2ΔBΔM) in mouse hepatocytes resulted in telomere dysfunction and the generation of GCRs, which induced p53-independent apoptosis and p53-dependent senescence. Interestingly, the overexpression of a different TRF2 dominant-negative mutant caused apoptosis in tumor cells but senescence in normal fibroblasts (*77*).

3.3. Telomerase, a key in cancer development: therapeutic perspectives

Considering the close association of TERT, telomere structure, and telosome length with senescence, it has been suggested that the development of drugs that alter the functionality of these molecules/DNA structures could lead to the development of anticancer strategies aimed at inducing telomere erosion and, therefore, senescence.

Currently, four major molecular tools are used to induce senescence in multiple tumor models: (i)

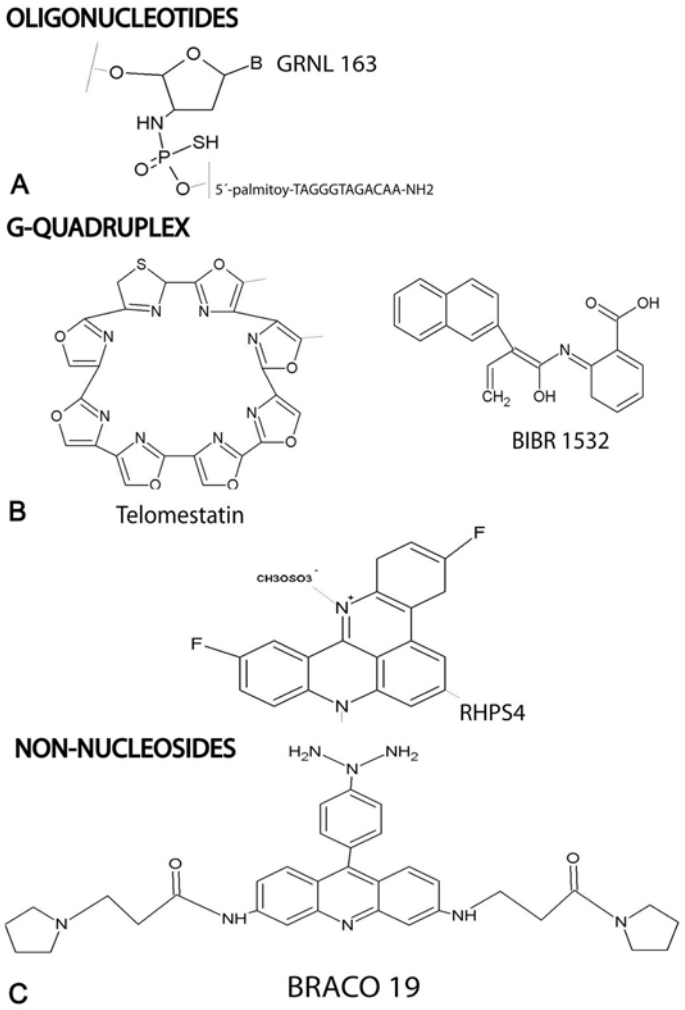

Figure 3. Molecular representations of representative compounds from different families of senescence-inducing drugs (A to C) used as telomere secondary structure stabilizers and TERT inhibitors. Please refer to the main text of the manuscript for additional details.

oligonucleotides (Figure 3A), (ii) G-quadruplex interacting agents (Figure 3B), (iii) non-nucleoside compounds (Figure 3C), and (iv) RNA interference (RNAi). These molecular tools will be discussed in detail below.

3.3.1. Oligonucleotides: a quasi-ideal molecular tool for senescence induction in tumor cells

The most potent and most frequently studied oligonucleotide used for TERT inhibition is GRN163L (Imetelstat). This molecule is a 13-mer oligonucleotide derived by palmitoylation of the parent molecule thiophosphoramidate GRN163 (Figure 3A) and is under clinical development by the Geron Corporation (Delaware, USA) (*78*). The DNA sequence of GRN163 is complementary to the mRNA sequence of hTERT, leading to the degradation of hTERT mRNA and thus limiting cell growth in multiple types of tumors (*79, 80*).

Initially, efficacy studies of GRN163 and GRN163L were conducted in mouse xenograft models representing a range of different human tumor types including lung (*81-84*), breast (*84-87*), liver (*88*), brain (*84, 89*) and hematological cancers, such as multiple myeloma and lymphoma (*90, 91*). The data collected from these animal models indicated that GRN163 and GRN163L have a strong TERT-inhibitory effect (*84*), sometimes blocking the induction of cancer metastases in the animals (*84, 86*).

Interestingly, the pharmacological inhibition of hTERT by GRN163 and/or GRN163L and the induction of senescence in tumors types are largely dependent upon telomere length and cell type. For example, little effect on growth was seen in U266 multiple myeloma cells (which exhibit long telomeres) (*87*) after 56 days of *in vitro* culture in the presence of GRN163, whereas growth inhibition was apparent in MM.1S multiple myeloma cells (which exhibit short telomeres) after only 28 days (*80, 90*). Other examples of the inverse correlation between the efficacy of GRN163 and telomere length in different tumor and biologic models, including in cancer stem cells (CSCs),

support the idea that long telomeres inhibit the activation of the senescence pathway. CSCs are naturally resistant to drug treatment (*91*), and TERT appears to regulate the clonogenic expansion of CSCs from multiple myelomas (*91*). The treatment of CSCs with GRN163L reduced cell proliferation *in vitro* five-fold after three weeks and 100-fold after five weeks, compared to controls. The administration of GRN163 *in vivo* resulted in significant survival of the animals compared to controls (*91*). Additionally, a recent preclinical assay suggests that GRN163L can cross the blood–brain barrier and inhibit telomerase in human glioblastoma cells, including glioblastoma CSCs, preventing cancer recurrence (reviewed in *92*). GRN163L is currently undergoing phase I and II clinical trials as a treatment for breast and lung cancers, myeloma, and chronic leukemia, all of which are driven in part by CSCs. An upcoming trial will test GRN136L in combination with paclitaxel and bevacizumab in breast cancer (*92*).

Despite the strong potential of oligonucleotides to induce senescence in different tumor types, this therapeutic approach has some major issues that impair its use *in vivo*. The lack of stability and bioavailability of oligonucleotides is an unsolved problem, although alternative approaches that use chemically modified oligonucleotides (e.g., peptide nucleic acids, or PNAs) are being developed. PNAs are modified oligonucleotides that contain a non-ionic backbone in which the deoxyribose linkages have been replaced by *n*-(2-amino-ethyl) glycine units, making PNAs resistant to degradation by endo- and exonucleases. PNAs bind to complementary nucleic acids with very high affinity (*93*), and these molecules are being evaluated as hTERT antagonists (*94, 95*). The cellular uptake of PNAs is very poor, requiring electroporation or the formation of DNA-PNA complexes that can be efficiently transfected by cationic lipids (*96,97*).

3.3.2. G-quadruplex structure: an innovative approach to induce senescence in tumor cells

Recently, the discovery that telomere-disrupting agents can also inhibit TERT activity led to intensive research into G-quadruplex-stabilizing compounds. These telomere-disrupting agents interact with the TTAGGG repeats of telomeres and stabilize G-quadruplexes, inhibitingtelomere elongation and also hindering the ability of the telomeres to 'cap' and protect the ends of the chromosomes. One major advantage of these G-quadruplex stabilizers is that they induce cell death quickly (*98*).

Initial TERT inhibition via long-term exposure of human cancer cells to sublethal doses of G-quadruplex stabilizers induced progressive telomere shortening and replicative senescence (*99, 100*). However, several studies showed that G-quadruplex stabilizers, including RHPS4 (3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate; Figure 3B) and BRACO-19 (3,6,9-trisubstituted acridine compound; Figure 3C) , were able to induce a short-term anti-proliferative response that cannot be explained by TERT inhibition alone (*75*). Specifically, the observation that BRACO-19 and other G-quadruplex stabilizers lead to GCRs, together with the appearance of p16-associated senescence, led to the proposal that telomeres, rather than TERT, are the targets of G-quadruplex stabilizers (*101, 102*).

G-quadruplex stabilizers were also shown to inhibit the alternative lengthening of telomeres (ALT) mechanism in tumor cell lines (*103*). The ALT mechanism is found in a minority of tumors, where it compensates for the lack of TERT, increasing the length of telomeres and allowing indefinite cell proliferation (*104*).

Both the quinoline-based 115405 and RHPS4 G-quadruplex stabilizers inhibited growth in the GM847 cell line, an SV40-immortalized human fibroblast that displays the ALT phenotype (*105, 107*). Additionally, 2,6-pyridine-dicarboxamide derivatives were strongly selective for G-quadruplex structures, inducing an anti-proliferative effect in SAOS-2, a human osteogenic sarcoma cell line that maintains telomeres through the ALT mechanism in the absence of TERT activity (*75*). These findings further corroborated the hypothesis that the anti-proliferative effects of G-quadruplex stabilizers are largely independent of TERT activity (*105*).

In this context, two G-quadruplex stabilizers deserve attention: BRACO-19 and telomestatin (Figure 3B). BRACO-19 represents the first of a "second generation" of G-quadruplex stabilizers. It possesses nanomolar potency against TERT and low non-specific cytotoxicity, and it was shown to inhibit growth and induce senescence in a human breast cancer cell line (*105*). Significant antitumor activity *in vivo* was observed when BRACO-19 was administered after paclitaxel treatment of mice bearing a human tumor xenograft carcinoma (*105*). The second compound, telomestatin, shortens telomere repeat fragments, with concomitant displacement of POT1 and TRF2 from telomere sites, in cancer but not in normal cells (*106*). Based on this evidence, a consistent mechanism of action is now emerging for G-quadruplex stabilizers in tumor cells, which initially involves alteration of G-quadruplex-overhang structure followed by degradation through a DNA damage repair (DDR) pathway and the release of POT1 from telomeres. In this regard, telomestatin is classified as a telomere-disrupting agent rather than a TERT inhibitor. Numerous studies using telomestatin in a number of cancer cells lines have demonstrated that this compound is an effective anti-proliferative agent both *in vitro* and *in vivo* (*107*).

3.3.3. The use of non-nucleoside compounds to induce senescence in tumors

A variety of non-nucleoside drugs have been shown to inhibit telomerase, including epicatechin derivatives such as epigallocatechin gallate (EGCG), which strongly and directly inhibits telomerase (*108*). In the presence of nontoxic concentrations of EGCG, two representative human cancer cell lines, U937 monoblastoid leukemia and HT29 colon adenocarcinoma, showed lifespan limitations accompanied by telomere shortening, chromosomal abnormalities, and the expression of senescence-associated β-galactosidase activity (*109-111*). Another potent and specific inhibitor of telomerase, 2,3,7-

trichloro-5-nitroquinoxaline (TNQX), causes progressive telomere attrition followed by an increased incidence of chromosome abnormalities and the induction of senescence (*112*).

Another interesting small molecule is MKT077, a toxic rhodacyanine dye analogue, which preferentially accumulates in tumor cell mitochondria and inhibits telomerase. This molecule was used as a lead structure for the development of a potent telomerase inhibitor, designated FJ5002. Long-term cultivation of U937 human leukemia cells with subacute concentrations of FJ5002 resulted in population-doubling-dependent changes characterized by progressive telomerase shortening and senescence (*97*).

However, the best-studied senescence-inducing non-nucleoside is BIBR 1532 (Figure 3B), one of the most potent TERT inhibitors discovered thus far. The exposure of human cancer cells from different histological origins to BIBR 1532 led to progressive telomere shortening and inhibition of cell proliferation, independent of p53 gene status (*75*).

BIBR 1532 has been shown to directly target TERT core components. In addition, BIBR 1532 exhibits a non-competitive mode of inhibition, which is clearly distinct from the mechanism used by nucleoside compounds or antisense oligonucleotides. Furthermore, BIBR 1532 does not cause chain termination events but rather inhibits the formation of long reaction products, reducing the number of TTAGGG repeats added during each replication event (*113*). This mechanism of action suggests that BIBR 1532 impairs the elongation of telomere DNA after its initial extension to the 5′-end of the template. These steps are most likely unique to TERT due to its high activity in tumor cells, which could explain the high selectivity of the compound (*113*). BIBR 1532 has been shown to inhibit cell proliferation in lung, breast, fibrosarcoma and prostate cancer cells (*113*). Interestingly, proliferation arrest after a sustained period of BIBR 1532 treatment was observed in combination with hallmarks of senescence, including morphological, mitotic and chromosomal aberrations and altered patterns of gene expression (*97*).

3.3.4. RNA interference technology applied to the study of senescence and cancer

Small interfering RNA (siRNA) and short-hairpin RNA (shRNA) are usually used for effective gene-specific RNA silencing. The silencing response induced by siRNA is transient because siRNAs are stable for only 3-5 days in culture, which restricts their application in gene therapy. Nevertheless, shRNA can generate a long-term gene-silencing response. The first step used to apply shRNA in gene therapy involves its expression from plasmid vectors. These gene constructs utilize the RNA polymerase III promoters H1 or U6 to transcribe shRNA that will be processed into 21-bp siRNA by the enzyme Dicer. Subsequently, this treatment results in mRNA degradation and silencing of the cognate gene. By contrast, siRNA are small oligonucleotides that are transfected into target cells (*114*).

The modulation of TERT component expression using genetic constructs (e.g., antisense RNA) is a powerful tool to induce senescence in tumor cells. The advent of siRNA and shRNA technologies at the beginning of this century has allowed the study of basic and clinical aspects of tumors and senescence. The use of shRNA and siRNA targeting hTERT has been shown to inhibit cell proliferation, decrease telomerase activity, increase the number of cells arrested at the G_0/G_1 phase of the cell cycle, and attenuate the tumor growth of xenograft mouse models (*115*).

3.3.4.1. siRNA against TERT: When transient silencing can produce long lasting effects

Telomerase siRNAs targeted against regions of human telomerase caused efficient inhibition of telomerase expression, loss of telomerase activity and severe telomere shortening. Many cells that are deficient in TERT have no compensatory mechanism to maintain their cellular viability, eventually succumbing to apoptosis. Because cancer cells are so dependent on telomerase activity, a transient drop in telomerase expression can have irreversible effects on telomere length and therefore lead to senescence induction. This type of response was observed in tumors such as Barret's adenocarcinoma (*116*), hepatic cancer (*117*) and lung cancer, with siRNA treatment combined with doxorubicin in all these cases (*118*).

However, the most realistic model with which to study senescence in cancer cells uses a vector expressing shRNA directed against hTERT. The efficiency of stable TERT silencing is sometimes greater than that of transient silencing, e.g., in bladder and oral cancer models (*119, 120* respectively).

All of the TERT inhibitors described above, together with others found in the literature, are described in Table 2.

3.3.5. The dark side of TERT inhibitors

As discussed before, telomere length and the alternative mechanisms that maintain its structure (e.g., ALT), as well as the biologic model used to study the relationship between senescence and tumor inhibition, determine the positive or negative effect of a senescence-inducing treatment. The literature contains hundreds of experiments in which TERT inhibitors displayed no effect on tumor growth, especially in those cells that possess an active ALT mechanism (*137*)

Interestingly, ALT activation was not observed in cell culture experiments in which TERT-positive cell lines were treated with TERT inhibitors or transfected with dominant-negative TERT mutants (*138*), indicating that ALT is not a preferential mechanism to preserve telomere stability. However, *in vivo*, where large tumors may contain millions or billions of cells in different microenvironments, ALT could be induced in a small fraction of cells, thus leading to the development of more aggressive and resistant clones. The development of ALT inhibitors may therefore be necessary to counteract this resistance mechanism.

- **Table 2.** Major oligonucleotides/small molecules used to induce senescence in tumor cells and their molecular targets

Target	Oligonucleotides/small molecules	Senescence-inducing approach	Clinical trial (number identification)/tumor type[a]	References
Telosome (shelterin)	siRNA, shRNA, and Cre-lox system against POT1, TRF1, and TRF2 genes	Disruption of the telosome/shelterin complex	-	(75 to 78); (121 to 124)
Telomere structure	BRACO19, telomestatin, RHPS4, and SYUIQ-5	Disruption of telomere architecture; inhibition of TERT activity	-	(75); (98 to 107); (125 to 127)
Telomerase	GRNL163	Targeting the RNA template-region of TERT; blocking TERT expression and biogenesis	NCT00124189/Chronic lymphocytic leukaemia, NCT00594126, NCT00718601, NCT01242930/Multiple myeloma, NCT00310895/Solid tumor malignancies, NCT00510445/Lung cancer, NCT01137968/Non-small cell lung cancer, NCT00732056/Breast cancer, NCT01265927/Breast neoplasms	(78 to 102); (128 to 130)
Telomerase	BIBR1532	Selective binding to TERT; blocking TERT activity	-	(113);(131)
Telomerase	siTERT/shTERT	Downregulating TERT gene expression	-	116 to 120); (132)
Telomerase	Antisense oligonucleotides	Downregulating TERT gene expression	-	(133 to 136)

[a]Sources: http://www.clinical.trials.gov; Geron Corporation (http://www.geron.com).

In addition to the potential for selection for resistant cells, TERT inhibitors may also cause side effects in normal tissues. Fortunately, telomeres are longer in normal tissues than in most cancers, and treatments can be designed to end before telomere depletion occurs in normal tissues (*138*). Further studies examining this approach must be conducted to determine how to best protect tissues, such as intestine, epidermis, and hematopoietic tissue, in which stem cells and transit cells are constantly dividing at a high rate.

4. OXIDATIVE STRESS, DNA DAMAGE, AND SENESCENCE: A PHYSIOLOGICAL LINK TO GENETIC INSTABILITY

4.1. The DNA damage response and senescence

The DNA damage response mechanism comprises a series of biochemical pathways that are activated in the presence of different types of lesions induced by chemical compounds and/or ionizing and non-ionizing radiation. Many DNA lesions are capable of altering DNA structure and function, resulting in the loss of genetic information and leading to cell senescence or even loss of viability over time (*139,140*). Moreover, the loss of specific DNA sequences or GCRs induced by DNA damage are one of the driving forces of tumorigenesis (*140*).

A variety of DNA lesions have been described, including single base or nucleotide modifications (e.g., oxidized purines and pyrimidines), single-strand breaks (SSBs), interstrand crosslinks (ICLs) and double-strand breaks (DSBs) (*141-143*). Of these lesions, ICLs and DSBs are considered to be the most damaging to the genome (*143*). One major mechanism that leads to the formation of ICLs and DSBs is oxidative damage, which is caused by reactive oxygen species (ROS) generated by an imbalance between ROS production and degradation by antioxidant systems (*144*). ROS generate mainly SSBs that can progress to DSBs. Interestingly, it was demonstrated that DSBs accumulate during senescence and aging as a consequence of decreased DDR (*145*), leading to GCR. GCR is also a leading cause of tumorigenesis, and a strong correlation between aging and tumor development has been established by different authors (*146-148*).

To protect the genome from DNA damage and the loss of genetic information, all cells use a complex network of proteins and signaling molecules termed the DNA damage response (DDR) pathway. The main function of the DDR is to sense the different types of DNA lesions and mount a cell-wide response that includes the modulation of cell cycle transitions and transcriptional processes and the stimulation of DNA repair pathways (*149*). Both responses are coordinated at the molecular level by three major classes of proteins: (i) sensor proteins that recognize abnormally structured DNA and initiate the signaling response; (ii) transducers, which amplify the signal; and (iii) effector proteins that are present in numerous downstream pathways and repair the damage in an error-free or error-prone fashion (*149*). The DDR mechanism is also necessary to evaluate potential damage that could arise during DNA replication (*150*).

The diverse nature of DNA lesions led to the evolution of different DNA repair pathways that act downstream of the DDR. In general, these DNA repair pathways are classified as (i) excision mechanisms, which encompass the base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) pathways; (ii) recombinational mechanisms, which are normally recruited in response to SSBs and/or DSBs and include homologous recombination (HR), single-strand annealing (SSA) and non-homologous end joining (NHEJ); and (iii) direct repair, which encompasses photorepair (mediated by photolyases), DNA alkylation repair, and the

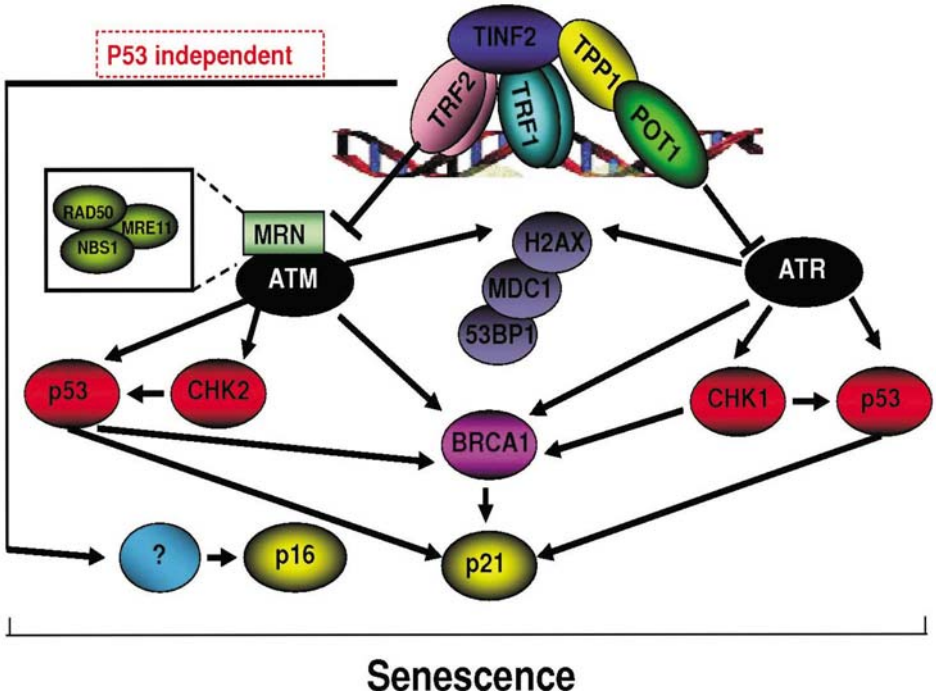

Figure 4. Schematic representation of senescence-induced DNA damage response (DDR). The progressive telomere shortening or the presence of uncapped telomeres initiates the DDR, resulting in the activation of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia (ATR) DNA-damage sensor proteins. The activation of ATM and ATR leads to the phosphorylation of the downstream kinases CHK1 and CHK2, as well as p53. Phosphorylated p53 transcriptionally upregulates genes that mediate cellular senescence and inhibit tumorigenesis. Depending on how telomeres are uncapped, the removal of telomeric-repeat binding factor 2 (TRF2) preferentially engages an ATM-dependent checkpoint, whereas removal of POT1 preferentially engages ATR. Although less well-understood, telomere dysfunction could also activate the p16 pathway and inhibit cellular proliferation.

direct reversion of damaged bases (*149*). Many of these DNA repair mechanisms are strongly interconnected. Some important DDR-associated proteins that act as sensors and/or transducers in the DNA repair pathways include the ataxia telangiectasia-mutated (ATM) and ATM-Rad3-related (ATR) protein kinases, BRCA1, BRIT1, the checkpoint kinases CHK1 and CHK2, p53, and the histone variant γ-H2AX. These sensor/transducers seem to be essential for the control of senescence, especially for regulating telomere maintenance. Thus, the discovery that DDR-associated sensors and transducers are important for normal telomere maintenance has yielded important insights into which molecules sense short telomeres and signal to modulate telomerase action and eventually induce senescence (Figure 4).

It is important to note that all telomeres are maintained in a stabilized, functional form by a process termed "telomere capping", which is promoted by the action of telomerase. However, telomeres can be converted to a non-functional state through a reverse process, termed "telomere uncapping", that is triggered by low telomerase activity and results in the activation of the DDR (Figure 4). Four distinct structural components contribute to telomere capping: (1) the higher-order telomeric DNA-protein complex, whose overall length (i.e., the number of telomeric repeats) dictates whether telomerase or nucleases can access telomeric DNA; (2) the protein complexes bound to the terminal repeats; (3) the DNA-protein complex at the single-stranded, G-rich DNA extension of the telomere, which is likely important for preventing the DNA damage response and regulating the cell cycle-dependent structure of the telomere; and (4) telomerase itself (*43*). Telomere uncapping results in a progressive shortening of telomeres over time, leading to replicative senescence by a p53-dependent mechanism (Figure 4). This p53 response to critically shortened telomeres is a result of the activation of the DDR (*151*); the shortening of telomeres results in their uncapping and subsequently their recognition as damaged DNA (*151*). Thus, under cellular conditions in which telomeres are uncapped, the cellular responses that occur are symptomatic of DNA damage, such as cell cycle arrest or cell death (*43*). Telomeric DNA is then also subjected to the molecular processes that are normally applied to DSBs within chromosomes: end-to-end fusions, degradation, and recombinational events, which in some cases fuse telomeric ends. Certain molecular changes at telomeres that compromise capping can also unleash unregulated telomerase action at the uncapped telomere, in contrast to the normally tight regulation of its action on capped telomeres (*43*).

Interestingly, cancer cells tend to have short telomeres but elevated telomerase expression, and the induction of telomere capping protects cancer cells against the DDR and, consequently, senescence (*43*). This

induction is probably related to the formation of higher-order structures at telomere sites that recruit the hTERT complex, and it blocks the DDR mechanism. However, in an uncapped telomere, the DDR is activated and sensor/transducer DNA damage-associated proteins, such as ATM and the MRN complex (MRE11-RAD50-NBS1 proteins; Figure 4), which acts together with effector proteins from recombination repair pathways (*43*), are recruited. Additionally, ATM is required to modulate telomere structure, either to facilitate telomerase access and/or to create a suitable substrate for the enzyme (*43*). In this context, telomere uncapping can cause GCRs (e.g., dicentric chromosomes) that lead to DDR activation by modulating ATM activity. Finally, activated ATM signals to the p53 and p21/SD11/C1P1 pathways, resulting in cell senescence (Figure 4) (*153*). In capped telomeres, ATM signaling is repressed by TRF2, whereas the single-stranded telomeric DNA-binding protein POT1 blocks the activation of the ATR kinase (Figure 4) (*154*). Depletion of POT1a/b induces an ATR kinase response that leads to the accumulation of DNA damage factors at chromosome ends and the activation of the effector kinases Chk1 and Chk2 (*154*). This DDR is persistent because the repair of the damaged telomeres by NHEJ is repressed by TRF2, which remains associated with telomeres despite the removal of POT1a and -b (Figure 4) (*156*).

POT1a/b deletion in cells lacking a functional p53 pathway was shown to cause polyploidization, producing cells with 4n, 8n, and 16n DNA content. In addition, it was observed that the resulting GCRs in p53-deficient cells could initiate breakage-fusion-bridge cycles that promoted the main genomic alterations observed in cancer cells: loss of heterozygosity, gene amplification, and nonreciprocal translocations (*156,157*). Polyploidization promotes the activation of telomerase, resulting in some chromosome loss (*158*) but mainly stabilizing the resulting new genome structures. Finally, the cell progeny exhibit extensively altered subtetraploid genomes on which selection for the most malignant clone can take place.

4.2. Chemotherapy and senescence

Many studies have evaluated the effects of chronic exposure to standard chemotherapeutic agents on cultured human cancer cells. The data demonstrate that cancer cells undergo senescence when exposed to a wide variety of DNA-damage drugs, especially when the cells are exposed to topoisomerase II inhibitors, such as etoposide, camptothecin, and doxorubicin (*159*).
VP-16, or etoposide, produces a senescence-like phenotype both *in vitro* (*160*) and *in vivo* (161) in a p53-dependent fashion. However, one study showed that 40–60% of p53-null lung cancer cells exposed to VP-16 also became senescent (*160*).

Camptothecin is able to induce the senescence response in tumor cells that are $p53^{+/+}$ and $p21^{waf1/cip1+/+}$ (*162*) by downregulating the expression of *CDC2*. Although the senescence response to camptothecin can be blocked in p53-null and p16-deficient human non–small cell H1299 carcinoma cells, this escape from stress-induced premature CS can be disrupted by Cdc2/Cdk1 kinase inhibitors or by knockdown of Cdc2/Cdk1 (*163*), suggesting that senescence induced by antitumor agents is strongly dependent on the genetic aspects of tumor cells.

Another antitumor drug that induces accelerated senescence in some tumor types is cisplatin. The DNA lesions generated by cisplatin are composed primarily of intrastrand crosslinks (ICLs), and it is likely that cisplatin induces senescence through both p53-dependent and -independent pathways (*164*). It is unclear whether cisplatin or other platinum-based drugs affect telomere length or TERT activity (*165*).

A possible mechanism that could connect DNA damage signaling and senescence induction by antitumor drugs is the activation of a complex cytokine network by means of promyelocytic leukemia protein (PM) in the tumor (*166*). This cytokine network includes the proinflammatory interleukins IL-6 and IL-8 and involves the reorganization and/or multiplication of a specific nuclear compartment. It has been demonstrated that cytokine signaling pathways are involved in drug-induced senescence (*167*), and chemotherapy-induced senescence can occur in neighboring cells through the so-called "bystander" effect (*168*). Work by Hubackova *et al.* (*169*) indicated that exposure of human normal and cancer cells to genotoxic drugs, including camptothecin and etoposide, results in increased PML transcript levels and activated JAK/STAT signaling. Both endogenous PML transcript levels and PML promoter-driven luciferase activity were suppressed by chemical inhibition or RNAi-mediated knockdown of JAK1 kinase, revealing a key role for JAK1-controlled signaling in PML transcription induced by genotoxic stress. Furthermore, in contrast to oncogene induced CS, in which PML expression is controlled by p53, this work demonstrated that cells expressing a dominant-negative allele of p53 also display a PML response to genotoxic drugs (*169*).

5. ONCOGENE EXPRESSION AND SENESCENCE INDUCTION IN TUMOR CELLS

5.1. Oncogene-induced senescence: primary barrier to cancer prevention and/or treatment

The early stages of cancerous transformation feature neoplastic transition followed by an accumulation of mutations that produce more aggressive cells, which are further selected by the tissue and/or tumor microenvironment (*170*). The neoplastic transition is characterized by an increase in the expression of oncogenes, which control different biological processes, such as cell proliferation and apoptosis. Oncogenes can be activated by GCRs, as a consequence of clastogenesis, or by gene structural alterations, such as fusion (*171, 172*), juxtaposition of enhancer elements (*172, 173*) or gene amplification. Translocations and mutations can occur during the initiating events that lead to tumorigenesis (*174*), whereas gene amplification is usually associated with tumor progression (*172*).

Oncogenic mutations typically cause excessive cell proliferation, leading to the disruption of normal tissue

Table 3. Oncogenes and tumor suppressor proteins that regulate senescence in tumor cells

Oncogene	Biological processes	References
H-ras^{G12V}	RAS signaling cascade	(176), (177)
K-ras^{G12V}	RAS signaling cascade	(176), (177)
N-ras^{G12D}	RAS signaling cascade	(176), (177)
BRAF^{V600E}	Promotes RAS signaling cascade	(178), (179)
c-Myc	Ras signaling effector; gene transcription inducer and chromatin remodeling factor	(180), (181)
RAF	RAS signaling cascade	(182)
AKT	PI3K/AKT signaling cascade	(183)
STAT5	Promotes JAK/STAT pathway	(184)
E2F1	Associates with retinoblastoma protein (pRB) in a cell-cycle-dependent manner; regulates cell proliferation	(185)
E2F3	Promotes G1 to S phase transition; transcription factor	(186)
Runx	Transcription inducer and chromatin remodeling factor	(187)
CDC6	Replication licensing factor; promotes S phase progression	(188)
Tumor supressor		
p53^{VP16}	Pleiotropic activity in cell cycle	(189),(190)
RB	Regulate cell cycle proliferation by selective bind to E2F family protein	(190), (191)
CDKN2A (p16INK4a)	Cyclin-dependent kinase inhibitor: inhibits G1 progression	(192), (193)
CDKN1A (p21Cip/Waf)	Cyclin-dependent kinase inhibitor; inhibits G1 progression	(194), (195)
CDKN2B (p15INK4B)	Cyclin-dependent kinase inhibitor; inhibits G1 progression	(196)
NF1	Downregulates RAS signaling	(197)
PTEN	Downregulates PI3K/Akt/mTOR signaling	(198)
VHL	Target HIF for degradation	(199)

microanatomy and impaired tissue function. Moreover, oncogenes have pleiotropic functions in the cell and can be broadly classified into seven groups (*172*): (i) transcription factors, (ii) chromatin remodelers, (iii) growth factors, (iv) growth factor receptors, (v) signal transducers, (vi) apoptosis and (vii) cell cycle regulators.

Notably, nearly three decades ago, it was observed that normal cells are refractory to oncogenic cancer initiation, indicating that non-tumor cells probably have mechanisms that override the proliferative activity of oncogenes (*16,175*) by promoting entry into senescence. Interestingly, oncogenes like Ras, Raf, E2Fs, Stat5, and AKT (Table 3) can induce cellular senescence in normal cells by activating diverse tumor supressors (Table 3). This mechanism is called oncogene-induced senescence, or oncogene induced CS. An interesting model for oncogene induced CS is the skin nevus. Nevi are characterized by the presence of slightly altered melanocytes, with variable sizes and shapes. (*200,201*). The high frequency of B-Raf mutations in common nevi is of special interest for the study of oncogene induced CS because active mitotic cells are rare or absent in this tissue, despite the potentially pro-proliferative signaling activated by B-Raf. In turn, the expression of endogenous B-raf in melanocytes resulted in growth arrest that does not require p16^{ink4a}. A murine knockin model of B-Raf targeted to cutaneous melanocytes induced benign tissue proliferation that did not generate melanomas over a period of 15 to 20 months. However, when combined with PTEN silencing, all animals developed metastasis in short periods of time, indicating that oncogene induced CS is dependent on this tumor suppressor (*202*).

5.2. Ras and B-raf: an oncogenic paradox that leads to senescence

The high complexity of the signal activation/repression mediated by oncogenes makes a full understanding of oncogene induced CS difficult. However, two major oncogenes widely studied in the context of oncogene induced CS, Ras and B-Raf, activate a common pathway (MEK/ERK) but induce senescence in tumors through different mechanisms (Figures 5A-C).

Cells that overexpress Ras consistently exhibit several signs of an activated DNA replication checkpoint, such as a high fraction of cells arrested in S phase, activation of the ATR pathway and preferential loss of heterozygosity at fragile sites. Oncogenic Ras expression leads to the overexpression of CDC6, increased numbers of active replicons and oncogene induced CS (Figure 5A). Oncogenic stress mediated by Ras has been shown to induce genome instability after a single round of DNA replication (*21*), while CDC6 overexpression is sufficient to induce DDR activation and senescence (*22*).

Another mechanism of senescence induction by Ras is mediated by the loss of the tumor suppressor NF1 (neurofibromatosis 1; Figure 5B), which results in sustained Ras activation, hypersensitivity to growth factors, and immortalization. However, in normal human diploid fibroblasts, the loss of NF1 triggers a transient activation of Ras and its effectors, followed by a dramatic suppression of these signals to lower than baseline levels. Moreover, these cells become senescent, indicating that the ultimate response to the aberrant activation of Ras pathway is a dramatic termination of Ras signaling at many levels, followed by a cellular response designed to eliminate the proliferative potential of cells that harbor oncogenic Ras (*203*). Furthermore, this sensitivity is determined not by a single biochemical event but rather by the coordinated output of cell type-specific signaling networks (*203*).

B-Raf (Figure 5C) can also results in oncogene induced CS by a different mechanism based on cytokine secretion. B-Raf induces the transcription factor C/EBP-β, which activates interleukin-6 (IL-6), forming a positive feedback loop. The C/EBP-β-IL6 feedback loop in turn activates the tumor suppressor p15^{ink4b} and an inflammatory network that includes IL-8. IL-6/IL-8 acts in concert, in a

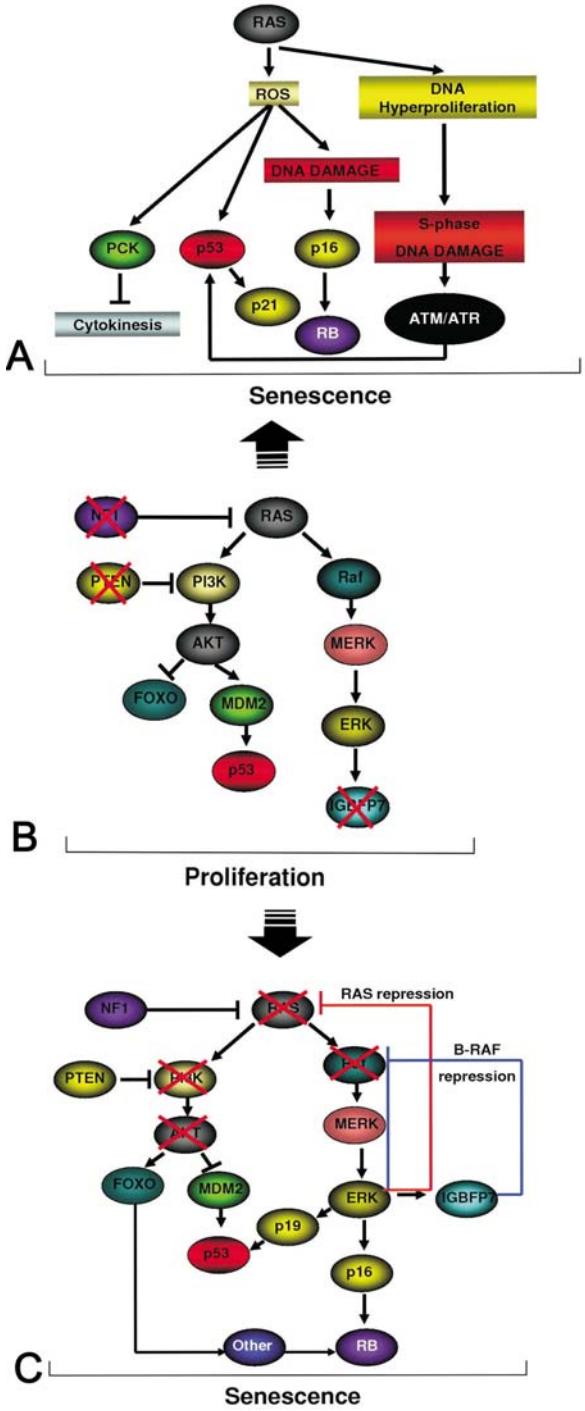

Figure 5. Model of oncogene-induced senescence (OIS) by Ras regulation. In (A), strong Ras activation can lead to DNA damage through ROS generation and/or DNA hyper-replication, which have been suggested to induce OIS by a p53-dependent mechanism. In cells that are insensitive to OIS (B), the Ras-associated pathway leads to hyperactivation of well-known effector proteins that induce cell proliferation (e.g., PI3K and RAI) and their downstream targets (e.g., AKT, MDM2, p53, MERK, ERK), thus promoting tumorigenesis. On the other hand, activated RAS leads to growth arrest as a result of potent negative feedback that abrogates ERK and PI3K signaling (C). In this sense, a negative feedback signal generated by ERK represses the RAS pathway (red line; C), while the IGFP-7 pathway becomes activated and represses B-raf, resulting in OIS via p16 (blue line; C) or p53 via p19. Despite the fact that the biochemical mechanisms of these three models differ, they are not mutually exclusive, and an overlap of pathways is observed in different biological models.

cell-autonomous manner, to leads to oncogene induced CS. IL-1a, a multifunctional cytokine and component of the "senescence-associated secretory phenotype" (SASP), was reported to be the upstream regulator of the senescence-associated IL-6/IL-8 cytokine network (*16*). Notably, DDR signaling was able to initiate and maintain cytokine secretion in a p53-independent and PRB-independent manner (*23*). The demonstration that BRAFV600E-linked oncogene induced CS displays a mosaic $p16^{ink4a}$ expression pattern indicates that $p16^{ink4a}$ is not required for senescence induction (*23*).

6. TWO DIFFERENT FACES OF SENESCENCE: INDEPENDENT MECHANISMS OF CLASSICAL PATHWAYS OF AGING IN CANCER

6.1. Premature-induced cellular senescence: Another form of senescence

Like oncogenes, the loss of tumor suppressors can also trigger senescence in mouse and human cells. While p53 is necessary for the function of several senescence inducers, its loss does not induce senescence. In contrast, the loss of PTEN induces senescence is several cell types (*204-207*). PTEN encodes a phosphatase that catalyzes the conversion of the membrane lipid PIP3 to the PI3K substrate PIP2 and negatively regulates the PI3K–AKT–mTOR pathway. This phosphatase is commonly mutated or lost in many cancer types (*208-212*).

PICS (PTEN loss-induced cellular senescence; PTEN-loss induced CS) shares several features with oncogene induced CS but is independent of the classical senescence induction that requires DDR activation and/or telomere erosion (*24*). It was shown that MEFs deficient in PTEN undergo senescence accompanied by an induction of p53 (*25*). However, in contrast to other forms of senescence, this increase in p53 was mediated mTOR-mediated translation regulation rather than the DDR (*24, 26*). Additionally, deletion of the gene encoding ARF (which promotes MDM2 degradation and leads to p53 accumulation) at the Cdkn2a locus *in vivo* fails to prevent PTEN-loss induced CS in prostate tumorigenesis, showing that ARF is dispensable for this type of senescence (*27*).

CDH1, an activator of the anaphase-promoting complex/cyclosome (APC), contributes to mitotic exit and G_1 maintenance by targeting cell cycle proteins for degradation (*20*). Nuclear PTEN activates APC–CDH1 to regulate the degradation of its targets. The observation that CDH1-null cells are refractory to PTEN-mediated growth suppression positions this protein as a mediator of PTEN-loss induced CS (*28*). However, in cancer models with PTEN loss, CDH1 is separated from the cyclosome proteins, producing an accumulation of ETS2 (substrate of CDH1). EST2 is an important transcription factor for INK4A, leading to upregulation of this CDK inhibitor and subsequent senescence induction (*29*).

Another interesting feature of PTEN-loss induced CS is that it can be induced in quiescent or growth-arrested cell types (24), whereas oncogene induced CS normally occurs only in mitotically active cells. Cancer-initiating cells (CICs) naturally arrest in a quiescent state, contribute to the maintenance of the tumour, and fail to be targeted by current therapeutic protocols (*23*) and might escape the activation of oncogene induced CS, but could be susceptible to PTEN-loss induced CS (*reviewed in 24*). An additional difference between oncogene induced CS and PTEN-loss induced CS is that the latter is activated over a much shorter time (*24*).

6.2. The SKP2 pathway induces senescence independent of p53 activation

Skp2 is a component of the Skp2–SCF complex that acts as an E3 ubiquitin ligase of the CDK inhibitor p27 and other substrates (*214, 215*). There is evidence that SKP2 may act as an oncogene (*217*), and this protein is frequently overexpressed in non-Hodgkin's lymphomas (*217*), mucosal epithelial dysplasias and squamous cell carcinomas (*216*).

The inactivation of Skp2 reduces tumorigenesis by inducing cellular senescence only under oncogenic conditions. Remarkably, this senescence response is triggered in a $p19^{Arf}$–p53-independent manner (*27*). Pharmacological inactivation of Skp2 may therefore represent a general approach towards a 'pro-senescence' therapy for cancer prevention and treatment.

7. EPIGENETICS TOOLS APPLIED TO TUMOR-INDUCED SENESCENCE: STATE-OF-THE-ART

Epigenetic modifications also play a crucial role in tumorigenesis. By definition, epigenetic modifications are heritable changes in genes and/or proteins that do not alter the primary DNA sequence. The best-known epigenetic marks are methylation, acetylation, ubiquitination, sumoylation and phosphorylation.

These marks can directly regulate gene expression by altering the chromatin state, facilitating the addition or removal of methyl and acetyl groups in chromatin. While chromatin methylation results in heterochromatin formation, acetylation leads to euchromatin formation. A special group of enzymes named DNA methyltransferases (DNMTs) are responsible for the transfer of the methyl mark to the 5′-carbon position in cytosine bases at CpG dinucleotide residues *(217)*. However, the transfer of the methyl group to the cytosine residue may occur improperly, causing DNA mutations that can lead to tumor development (*218*). It should be noticed that epigenetic mechanisms are also responsible for the repression of different sets of genes through their association with a specific complex called the polycomb repressive complex (PRC1) (*219*). As an example of the importance of these complexes, the histone methyltransferase EZH2 (enhancer of zeste homolog 2) in the PRC2 and PRC3 complexes plays a key role by serving as a recruit platform for the DNMTs and is therefore crucial to establishing gene expression silencing and epigenetic memory (*221*).

Senescence is also associated with errors in the epigenetic machinery (*218*). The loss of DNMT1 (and, therefore, global DNA methylation), particularly in

heterochromatic zones, could lead to senescence in adult organisms (*221*). Interestingly, it has been hypothesized that DNMT3B could hypermethylate DNA sequences that are not normally methylated, leading to mutations in local genes and tumor initiation (*221*). Also, during aging, some genes in CpG islands are found to be hypermethylated, whereas repetitive sequences in heterochromatin are hypomethylated (*222*). In this scenario, global gene expression could be severely compromised, again promoting tumorigenesis. Furthermore, it has been proposed that senescence can disrupt the epigenetic organization of heterochromatin, leading to disordered gene transcription and causing DNA damage (*222*). On a related note, heterochromatin loss can reduce the activity of the NURD complex, which is composed of the histone deacetylases HDAC-1 and HDAC-2 and the ATPases CHD3 and CHD4. Reduced NURD activity may cause many defects in chromatin structure and integrity (*222*) and thus activate the DDR. The DDR can lead to further rearrangement of the epigenetic mechanisms, causing alterations in chromatin integrity and, therefore, gene misregulation and cancer (*222*).

The NAD-dependent deacetylase sirtuin-1 (SIRT1) is also downregulated during aging and senescence (*221, 222*). However, in some tumor cells, SIRT1 is commonly found to be upregulated, demonstrating the interplay between senescence and cancer development (*222*). Moreover, in estrogen-dependent breast cancers, estrogen receptors can act as oncogenes by facilitating the age-related increase methylation at the promoter of the tumor suppressor RASSF1A (*223*). Likewise, in tobacco users, many tumor suppressor genes were found to be hypermethylated (*223*). However, in other types of lung cancers, the DNMT1 gene is found to be downregulated by a solution containing nicotine (*224*). Therefore, changes in methylation patterns (hypermethylation or hypomethylation) could lead to tumor progression.

Interestingly, epigenetic mechanisms are associated with the induction of cell senescence in human and mouse tumor cells (*225*). The CDK inhibitor p16 can suppress the development of spontaneous cancers by making the senescence triggered by p53 inactivation irreversible. p16 can be silenced by the methylation of its promoter (*226*). Thus, some DNMT inhibitors, such as 5-azacytidine and decitabine (5'-aza-2'-deoxycytidine), were proposed as potential treatments for cancer (*227*).

Another epigenetics-associated family of proteins, termed INGs, was found to be downregulated in many types of cancer (*228*). INGs interact with p53 in the p53-dependent response to cell senescence and apoptosis (*228*). This again implies a link between senescence and chromatin remodeling; INGs participate in the epigenetic regulation of gene expression by interacting with HDACs and proteins with histone acetyltransferase (HAT) activity such as p300, CBP, PCF, and TRRAP (*228*).

8. MICRORNAS AND THEIR ROLE IN SENESCENCE

Micro-RNAs (miRNAs) are a novel class of non-protein coding genes that play an important role in the post-transcriptional regulation of gene expression (*229*). These miRNAs are produced from PolII-transcribed primary RNA transcripts by several processing steps. The final step in the miRNA pathway is the loading of one RNA strand into the RNA-induced silencing complex (RISC) (*229*). Mature miRNAS are short non-coding sequences that range in size from 19 to 22 nucleotides and are highly conserved. miRNAs regulate protein expression by binding the 3' untranslated region (UTR) of an mRNA (*229*). The ability of miRNAs to regulate a variety of target genes allows them to induce changes in multiple pathways, and miRNAs are involved in diverse processes, including development (*230*), apoptosis, proliferation and differentiation (*231*).

Recent studies have shown that miRNA expression profiles differ between normal and tumor tissues (*232*). Interestingly, the downregulation of subsets of miRNAs is a common finding in some tumors (*233*), and the discovery that miRNA silencing could revert the tumorigenic phenotype revealed a novel regulatory mechanism in cancer proliferation (*234*). The evidence for a regulatory role for the miR-34 family of miRNAs in senescence is growing and has stemmed from the investigation of p53 and its role in senescence. p53 regulates miR-34a; members of the miR-34 family of genes contain p53-binding sites in their promoters, which are conserved among humans and rodents. In turn, miR-34a increases the activity of p53 by reducing the expression of sirtuin 1 (SIRT1), which interacts with p53 and deacetylates p53 at Lys382 in a NAD^+-dependent manner. This deacetylation decreases p53-mediated transcriptional activation and thus reduces the expression of downstream proteins, such as p21Cip1 (*235, 236*)**.** Also, miR-34a can induce senescence and suppress cell proliferation through downregulation of the E2F pathway in human colon cancer cells regardless of p53 status, leading to the upregulation of the p53/p21Cip1 pathway (*237*). Moreover, miR-34a is upregulated after activation of the B-RAF oncogene. miR-34a also induces senescence through repression of v-myc (*238*).

Another target that induces cellular senescence mediated by miR-30 is LIN28 (a homolog of lin-28 in *Caenorhabditis elegans*) in embryonic stem cells and cancer cells. It is important to note that LIN28 functions as an oncogene to promote malignant transformation and tumor progression (*239*) Another miRNA involved in senescence, miR-449a, induces senescence by suppressing Rb phosphorylation by directly repressing the upstream regulatory factors of Rb, such as cyclin D1 (CCND1), HDAC1, cyclin-dependent kinase 6 (CDK6), and cell division cycle 25 homolog A (CDC25A) (*240*). A recent study has shown that miR-449a is downregulated in prostate cancer, indicating that this miRNA regulates cell growth and viability in part by repressing HDAC-1 expression (*241*).

A genetic screen designed to identify new miRNAs characterized by their ability to bypass senescence induced by oncogenic Ras (oncogene induced CS) led to the identification of miR-372 and miR-373 (*242*). This study was performed in partially immortalized IMR90 fibroblasts. These miRNAs are considered to be novel oncogenes, participating in the development of human testicular germ cell tumors by blocking the p53 pathway and promoting tumorigenic growth in the presence of wild-type p53. Importantly, miR-373 is able to induce the formation of foci in soft-agar assays, demonstrating its transforming capability. By contrast, the introduction of miR-34a and miR-34b/c into primary human diploid fibroblasts induces cellular senescence (*243*). Tumor cells also show signs of senescence after the introduction of ectopic miR-34a (*244*). Downregulation of miR-138 is associated with overexpression of telomerase and the acquisition of malignant behavior in human anaplastic thyroid carcinoma cell lines (*245*). Therefore, it is expected that miR-138 would be useful as a diagnostic tool and might be a target for the development of new strategic treatments for specific kinds of carcinomas, as has already been suggested for miR-378 (*246*).

The Polycomb complex is regulated by microRNAs that induce senescence, of which miR-128a directly targets the Bmi-1 oncogene (polycomb ring finger oncogene; BMI1), increasing the expression of $p16^{INK4A}$ and the production of reactive oxygen species (ROS), which promote cellular senescence in medulloblastoma cell lines (*247*). Recently, it has been reported that this tumor suppressive miRNA (miR-128a) is downregulated in medulloblastomas (*248*), glioblastomas (*249*) and acute myeloid leukemia (*250*), suggesting that this miRNA plays an important role in these types of cancer.

9. AN EVOLUTIONARY FOCUS ON SENESCENCE: AN ENDOGENOUS ANTICANCER MECHANISM?

For the past 50 years, two theories on the ultimate cause of aging, the mutation accumulation theory and the antagonistic pleiotropy theory, have dominated evolutionary discussions of senescence.

The mutation accumulation theory proposes that aging occurs due to cumulative deleterious mutations in germline cells that are only expressed during the later stages of an organism's life (*251*). Aging is thus able to occur even in an immortal population by the accumulation of these age-specific mutations over successive generations (reviewed in *252*). Antagonistic pleiotropy centers on genetic effects that enhance fitness early in life but depress fitness late in life (*253*). Such genetic alterations are able to spread because the force of selection is stronger earlier in life because more individuals are alive and, more importantly, reproductive, at this stage than at later ages. Overall, both of these theories view aging as a collateral effect of the adaptation process (*252, 253*).

The mutation accumulation theory and the antagonistic pleiotropy theories are hypothetico-deductive in nature (*254*), meaning that when first conceived they were deduced from assumed laws or premises rather than from empirical observations. These hypotheses have been adopted as gerontological paradigms largely due to a lack of alternatives rather than for any compelling evidential reasons (*255*).

The mutation accumulation theory assumes that, in the wild, most organisms die before they reach old age. For this reason, deleterious age-specific alleles can build up and eventually cause aging. In the light of current knowledge, however, this theory is untenable as an explanation of how the telomere system evolved. The telomere system is complex, hierarchical, integrated and finely regulated, and it is implausible that such sophistication could be the result of unselected mutation accumulation. Because the mutation accumulation theory is an untenable explanation of replicative senescence, the only other mainstream alternative is antagonistic pleiotropy. Here, one must argue that replicative senescence confers an adaptive advantage earlier in life, and that aging is an incidental late-life side effect of the program (*252*). Not surprisingly, then, this is the position taken by the majority of evolutionary gerontologists. It has been proposed that the primary function of the telomere system is its role as a natural defense against cancer (*256*). The basic idea here is that telomere attrition restrains tumor growth by limiting the replicative capacity of transformed cells. Once the maximum number of doublings has been reached, telomeres induce cell senescence, thereby permanently removing such cells from the cell cycle. In this way, telomerase repression, by allowing telomere attrition, acts as a barrier to uncontrolled proliferation (*257*). Under antagonistic pleiotropy, the later effects of replicative senescence (i.e., aging) are seen as secondary side effects—effects that have been allowed to persist because selection at older ages is weak (*252*).

There is abundant evidence to support the hypothesis that telomere-induced cell senescence is instrumental in the suppression of cancer, and this proposal is uncontroversial (*258*). Tumorigenic human cells often lose the functionality of key players in senescence induction, such as p53, p21, p27, and ARF, among others (*258*), and one of the hallmarks of malignancy is the ability to overcome replicative senescence by the reactivation of telomerase (reviewed in *252*). Replicative senescence (unless subverted) is an effective barrier to malignant transformation. Therefore, telomere-induced cell senescence has a function outside aging, and this function is adaptive. In mice, the deletion of one p53 allele reduces median survival to 70 weeks and homozygous deletion to 50 weeks, whereas wild type mice have a median life expectancy of around 110 weeks. Human patients with the loss of one allele of p53 have an increased incidence of several cancers from an early age (*259*).

However, there are also examples for which this prediction seems to be patently false. Telomerase activity is high in the somatic tissues of organisms that do not appear to age, such as the rainbow trout and the lobster (*252*). Libertini states (*252*) that the low cancer risk in these

organisms is evidenced by the fact they show negligible senescence. High levels of telomerase activity have also been found in several long-lived bird species, including Leach's storm petrels, again suggesting that high telomerase activity does not correlate with high cancer risk (*260*). The situation in mammals is similar; the longest-lived species within the Rodentia, such as the naked mole rat and the grey squirrel, have high telomerase activity in their somatic tissue (*261*). However, the forced overexpression of telomerase in mice reduces their lifespan due to increased cancer occurrence, but this phenotype can be reversed by the overexpression of p53, suggesting that in organisms with high telomerase activity, other genetic events may have occurred to counteract the pro-cancer effects of high telomerase activity (*262*).

When telomere uncapping occurs, telomeres from different chromosomes begin to fuse, causing genomic instability. This instability disrupts the expression of genes involved in growth control, which ultimately leads to tumorigenesis (*263*). Thus, the idea that telomerase activity removes a barrier to oncogenic risk is only half of the story; it may remove one barrier, but it simultaneously erects another. This fact is not often discussed in the mainstream literature on aging. In fact, tumors are mostly caused by the accumulation of mutations, in general due to DNA damage, which is not biologically unavoidable. Thus, a greater investment in DNA repair mechanisms can produce a much longer-lived organism. Humans, for example, live much longer than mice because, among other differences, they have much more efficient DNA maintenance and repair mechanisms, a trait which is genetically determined and thus evolutionarily malleable (*15*). Embryonic stem cells also have lower mutation frequencies, due to their superior ability to minimize oxidative stress. As soon as cells begin to differentiate, however, these repair mechanisms are downregulated (*264*). This shows that mutations are not inevitable and that organisms are able to cut the risk of cancer in other ways (commented in 2). Oncogene induced CS can co-evolve with DNA damage responses (*265*) as an antitumor mechanism to avoid cancer initiation. However, the senescence-promoting process, when it does not end in actual senescence, can lead to a trail of genetically unstable cells, which potentially can contribute to tumorigenesis (*266*). If this system was selected first and foremost as a cancer defense strategy, it is not clear why selection has not altered this post-secretory phenotype, or at least caused the immune system to efficiently remove such cells from the tissue, as it does for apoptotic cells. However, this argument is not applicable to all cancer types; for example, nevi, as described above, are a case in which senescence is clearly an endogenous anticancer mechanism.

Senescence is, in fact, an intricate process, involving the sequential activation of multiple molecular mechanisms and subjected to selective pressures that have proven necessary for the establishment and maintenance of the phenotype. New questions have been developed by evolutionary biologists together with oncologists: for example, is senescence a tissue-specific endogenous anticancer mechanism? Can cellular senescence promote or repress cancer progression dependent on specific microenvironments? Senescence as an endogenous anticancer mechanism is a mystery of aging with multiple paths remaining to be explored.

10. SUMMARY AND PERSPECTIVES

Cellular senescence is becoming a fundamental concept in tumorigenesis and cancer therapeutics. Whether telomerase-dependent replicative CS, DDR-dependent oncogene induced CS or DDR-independent PTEN-loss induced CS, all "types" of senescence seem to play an important role in preventing different types of cancers. Understanding the molecular mechanisms of senescence activation and, most importantly, escape, will allow the design of therapies aimed at (re)activating senescence in cancer cells. Many small compounds and/or genetic approaches to induce senescence in cancer cells are in development, and these senescence-associated therapies show excellent selectivity and low cytotoxicity for normal tissues. Importantly, the therapeutic potential of senescence induction strongly relies on the irreversibility of this mechanism.

Notably, many traditional anticancer therapies (e.g., alkylating anticancer drugs, ionizing radiation) have been reported to induce senescence in different tumor types, but this mechanism has never been deeply explored. Moreover, the combination of traditional anticancer treatments with senescence-inducing drugs is still in its infancy, which calls for more studies *in vitro* and *in vivo*. Thus, the direct activation or enhancement of senescence induced by classical cancer therapies has a great potential to improve cancer therapeutics.

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Abbreviations: RS: replicative senescence, hTERT: catalytic subunit of the telomerase holoenzyme, HP1: heterochromatin protein 1, SAHF: senescence-associated heterochromatin foci, SIPS: stress-induced premature senescence, OIS: oncogene-induced senescence, CDKs: cyclin-dependent kinases, pRb: retinoblastoma-associated protein, TERT: telomerase reverse transcriptase, DSBs: DNA double-strand breaks, TRF1: telomeric repeat-binding factor 1, TRF2: telomeric repeat-binding factor 2, TIN2: TRF1-interacting protein 2, RAP1: repressor-activator protein 1, POT1: protection of telomeres 1, TRF2ΔBΔM: small-dominant-negative form of TRF2, CSCs: cancer stem cells, PNAs: peptide nucleic acids, BRACO-19: 3,6,9-trisubstituted acridine compound, RHPS4: 3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate), ALT: alternative lengthening of telomeres, DDR: DNA damage response, EGCG: epigallocatechin gallate, siRNA: small interfering RNA, shRNA: short-hairpin RNA, TNQX: trichloro-5-nitroquinoxaline, GCR: gross chromosomal rearrangements, BER: base excision repair, NER: nucleotide excision repair, MMR: mismatch repair, HR: homologous recombination, SSA: single strand annealing, NHEJ: non-homologous end joining, ATM: ataxia telangiectasia-mutated, ATR: ATM-Rad3-related, CHK1: checkpoint kinase 1, CHK2: checkpoint kinase 2, DNMTs: DNA methyltransferases, PRC1: polycomb repression complex 1, SIRT: NAD-dependent deacetylase sirtuin-1, miRNAs: micro-RNAs.

Key Words: Cancer, Replicative Senescence, Premature Senescence, Telomere Erosion, DNA Damage, Chemotherapy, Epigenetics, Evolution, Review

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