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Commentary

Accelerated senescence: An emerging role in tumor cell response to chemotherapy and radiation

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ABSTRACT

Treatment of malignancies with chemotherapeutic drugs and/or radiotherapy is designed to eliminate the disease by depriving the tumor cell of its reproductive potential. Frequently, the desired effect of cell killing is achieved through the promotion of apoptosis; however, accumulating evidence suggests that apoptosis may not be the exclusive or even primary mechanism whereby tumor cells lose their self-renewal capacity after radiation or drug treatment, particularly in the case of solid tumors. While failure to undergo apoptosis in response to chemotherapeutic drugs or radiation may represent a mechanism of drug and radiation resistance, particularly in the case of leukemias and lymphomas, it is gradually being recognized that in the case of solid tumors, loss of reproductive capacity can occur through alternative pathways including reproductive cell death or mitotic catastrophe, through autophagic cell death, and as described below, through a terminally arrested state similar to replicative senescence.

Studies building upon the phenomenon of replicative senescence in normal cells approaching the limit of their reproductive potential have identified a comparable senescence-like arrest as a component of the tumor cell response to chemotherapeutic drugs and radiation. This response, which has been termed "premature senescence", "senescence-like growth arrest", "stress-induced premature senescence", and "accelerated senescence", can also result from supraphysiological mitogenic signaling, sub-optimal culture conditions, and ectopic expression of oncogenes. Here, we will use the term "accelerated senescence" in our consideration of the morphological, biochemical, and molecular aspects of treatment-induced senescence, its relationship to classical replicative senescence, its prevalence in clinical specimens and the implications of accelerated senescence for the outcome of cancer therapy.

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1. Replicative senescence and limitations on the proliferative potential of normal cells

1.1. *Features of replicative senescence*

It has long been appreciated that somatic cells have a finite proliferative capacity, termed the ''Hayflick Limit'' [1] and that this mortal state is controlled by an ''internal clock'' [2]. When cultured cells reach their proliferative limit, they adopt an enlarged and flattened morphology, increased granularity, and a vacuole-rich cytoplasm, while remaining viable and metabolically active. This permanent growth arrested state is referred to as replicative senescence. Besides these classic morphological features that one typically associates with cellular senescence, there are a number of other biomarkers that together are supportive of a senescent state (Fig. 1). The most frequently used marker is Senescence-Associated β-galactosidase activity (SA-β-gal), which involves a simple histochemical staining procedure at pH 6.0 to detect the expanded lysosomal compartment in the perinuclear region of aged cells [3]. With advances in the biochemical characterization and increased mechanistic understanding of cellular senescence additional markers are emerging. Most notably are the altered expressions of a number of mediators of cell cycle checkpoints and DNA damage response. As presented below, this DNA damage response and repair signature is also intimately connected to telomere dysfunction and is a hallmark of both replicative and accelerated senescence. A number of distinct chromatin changes have also been associated with cellular senescence [4], but will not be incorporated into this review.

1.2. *Dysfunction telomeres as a trigger of replicative senescence*

Vertebrate telomeres, which lie at the ends of chromosomes, are composed of the repetitive, non-coding sequence TTAGGG and act as a buffer to provide chromosomal stability and prevent chromosomal unwinding [5]. Because traditional DNA polymerases fail to fully replicate to the end of linear chromosomes (the ''end replication problem''), telomeres shorten each time a cell goes through S phase [6]. In the 1990s, telomere attrition was firmly established as a primary trigger of replicative senescence [6,7]. This conclusion was based on studies in cells ectopically expressing telomerase, the enzyme that counteracts telomere shortening by elongating telomeres. Transfection of hTERT, the catalytic subunit of human telomerase, enables many, but not all cells to bypass the senescence barrier, conferring unlimited proliferative potential without inducing malignant transformation [8]. Despite the initial evidence suggesting that telomere length is the critical determinant in the induction of replicative senescence, it is becoming clear that preservation of the hierarchical structure of the telomere rather than telomere length *per se* is the more important element [9].

The primary function of the telomere is to provide a capping mechanism for the ends of linear chromosomes. This ''cap'' is a dynamic structure composed of numerous telomere binding proteins that collectively mask DNA ends from being recognized as damage, protect from inappropriate exonuclease digestion, and prevent end-to-end fusions [10]. The core constituent component of the telomeric cap is the Shelterin complex, which is composed of TRF1, TRF2, TIN2, RAP1, TPP1, and POT-1 and which stays complexed to telomeric DNA throughout the cell cycle [9].There are also numerous additional binding proteins, many implicated in DNA damage surveillance and repair [11], which associate more transiently to the telomere, and which could provide additional mechanistic-based markers of senescence.

Collectively, telomere binding proteins establish and maintain a telomere loop (or t-loop), in which the single-strand 3′ overhang folds back and invades the duplex DNA [9,12]. When this structure is compromised, for example by

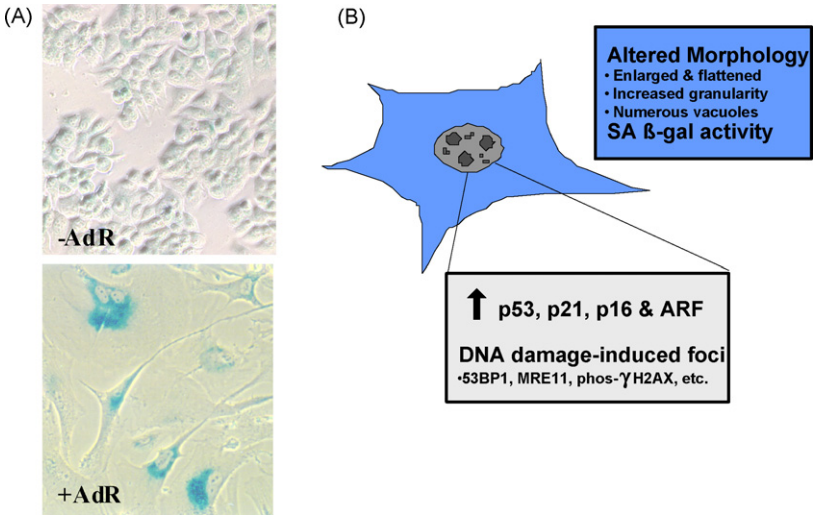

Fig. 1 – Features of senescent cells. (A) Breast tumor cells after treatment with Adriamycin show the characteristic senescence morphology (enlarged and flattened) and readily stain for the senescence-associated β-galactosidase marker (lower panel). The upper panel represents untreated MCF-7 cells stained for senescence-associated β-galactosidase. Note: both images are captured at the same magnification. (B) This schematic representation illustrates the cellular and molecular changes that typically occur during replicative senescence, many of which apply directly to accelerated senescence. An upward arrow indicates an increase in expression level and/or activity.

selectively removing TRF2 or POT-1 from telomeres, a high frequency of end-to-end fusions is observed [9,13,14]. The ultimate fate of a cell incurring such telomere dysfunction appears highly context dependent. Normal cells with intact cell cycle checkpoints tend to undergo senescence [15], while genomically unstable cancer cells or cancer-prone cells appear to die via apoptosis [16] or continue proliferating leading to increased genomic instability [17]. Although telomere dysfunction without telomere attrition can trigger senescence, this does not necessarily mean that replicative senescence is a telomere-length-independent process. Clearly, if telomeres are critically short, assembly and/or maintenance of a functional telomeric ''cap'' may not be possible, thus indirectly contributing to induction of senescence.

1.3. *Replicative senescence is sensed as a permanent DNA damage response*

When telomeres become critically short and/or the telomere cap is compromised chromosome ends are exposed. In the case of aged human fibroblasts, the 3′ telomeric overhang is lost as senescence approaches [18], which one could envision being readily recognized as a DNA double strand break. Many of the mediators activated by critically short telomeres are indistinguishable from those observed early after DNA damage (Fig. 2), as described in sections pertaining to accelerated senescence below. In fact, ATM and ATR signaling kinases are recruited to uncapped telomeres and activated, leading to the phosphorylation of Ser-139 of histone H2AX molecules [19,20]. This phosphorylation event is believed to facilitate the assembly of nuclear foci (some localizing to telomeres in senescent cells) constituting numerous DNA repair factors, including phos-γ-H2AX, 53BP1, MDC1, NBS1 and phos-SMC1. The finding that these DNA damage induced foci assemble during replicative senescence and persist for months after growth arrest [20] suggests that maintenance of senescence depends on constant activity of signaling kinases. Also highly reminiscent of an early damage response, Chk1 and Chk2 become activated via phosphorylation, p53 is stabilized and $p21^{waf1/cip1}$ is stably upregulated in a cell that has undergone replicative senescence (as detailed in the next section). The concept that sustained signaling is required for the maintenance of a senescent state is further supported by the re-entry of senescent cells into the cell cycle when key sensors of DNA damage (i.e., Chk1/Chk2 and ATM/ATR) are blocked [21].

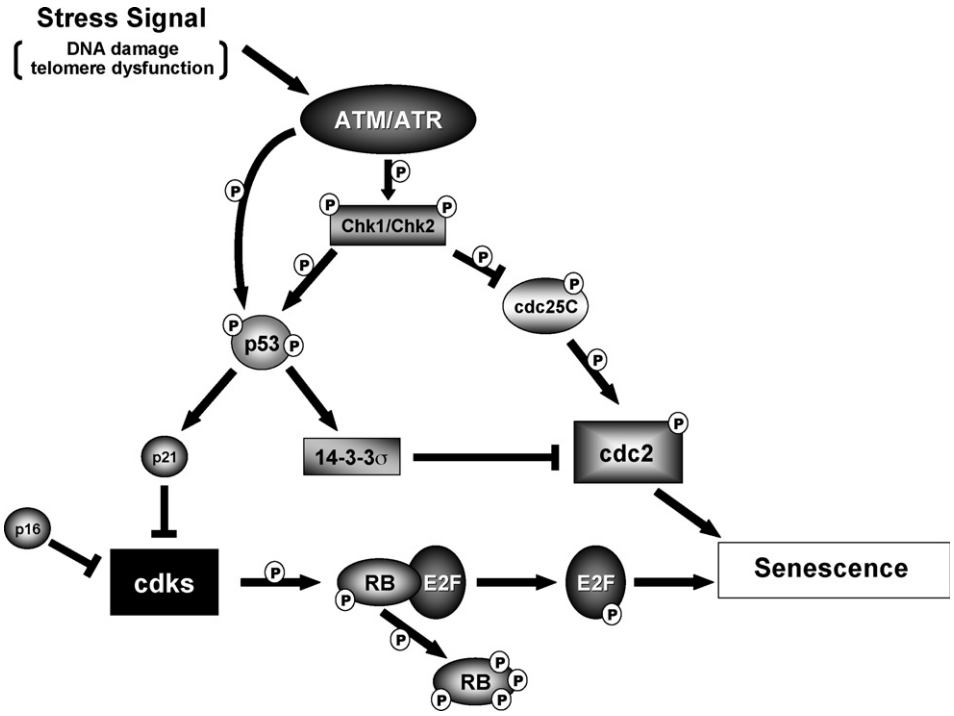

Fig. 2 – Senescence signaling pathways. As indicated in the text, senescence signaling pathways demonstrate extensive overlap with conventional growth arrest response signaling. The stress signal is initially detected by ATM/ATR, which activate the Chk1/Chk2 proteins and stabilizes p53, either directly or through Chk1/Chk2. $p21^{waf1/cip1}$ is induced either by p53 or by Chk1/Chk2 independently of p53. $p21^{waf1/cip1}$ interferes with the activity of cyclin dependent kinases (cdks), leading to dephosphorylation (activation) of Rb (or Rb family proteins, not shown), permitting the binding of Rb to the E2F family of transcription factors and thereby preventing their transactivation activity. p16 can also modulate this pathway independently of $p21^{waf1/cip1}$, although p16 does not appear to be involved in DNA damage induced accelerated senescence. In addition to $p21^{waf1/cip1}$, another central player appears to be cdc2, which can be inactivated through the suppression of cdc25C by Chk1/Chk2 or by p53 acting through 14-3-3σ. The basis for down-regulation of cdc2 levels is at present unknown but may be linked to the induction of $p21^{waf1/cip1}$.

2. Signaling elements that regulate replicative senescence

The one gene that is perhaps most strikingly implicated in replicative senescence is p53 [22]. *In vitro* data indicate that p53 binds to single-strand overhangs and cooperates with TRF2 in the formation of t-loop structures [23], suggesting a possible role for p53 in recognizing deprotected telomeres as damaged DNA. The replicative senescence resulting from telomere erosion is associated with enhanced phosphorylation of p53 at serines 15, 18 and 376 and reduced phosphorylation at serine 392 [24], sites that overlap with but do not match those for the DNA damage response to ionizing radiation or UV-induced damage. The p53 homologs p63 and p73 have also been implicated in the senescence response [25].

$p21^{waf1/cip1}$, a protein that inhibits a spectrum of cyclin dependent kinases, is an immediate downstream target of p53 [22,26], and is thought to be a critical element in the regulation of replicative senescence [27]. $p21^{waf1/cip1}$ mediated inhibition of the cyclin dependent kinases that regulate phosphorylation of the retinoblastoma protein, pRb, results in the binding of the dephosphorylated form of pRb to the transcription factor, E2F, and interference with the transactivation of genes necessary for the G1 to S transition [28]. However, there is at least one study suggesting that replicative senescence in normal fibroblasts is dissociable from the induction of $p21^{waf1/cip1}$ [29].

In addition to p53 and $p21^{waf1/cip1}$, there is extensive evidence in support of a role for p16 in replicative senescence [27]. Levels of p16 mRNA and protein were found to increase up to forty-fold in fibroblasts in the late stages of senescence; p16 was found to be complexed with both CDK4 and CDK6, thereby suppressing the respective kinase activities [30,31]. Inducible expression of p16 in a variety of normal and tumor cell types also resulted in a senescence response, which has also been shown to be, at least in part, dependent on functional Rb [32,33]. Replicative senescence can be delayed, although not prevented, by anti-sense p16 expression [34], strongly suggesting that other cyclin dependent kinase inhibitory proteins, such as $p21^{waf1/cip1}$ and $p27^{kip1}$, may provide alternative, compensatory pathways for a senescence response.

The induction of senescence has been shown to be associated with DNA damage signaling and is related to activation of ATM and p53, where ATM regulates p53 phosphorylation and stabilization in the DNA damage response pathway [35]. In this context, there is evidence that telomere dysfunction signals senescence through ATM upstream of p53 and $p21^{waf1/cip1}$ [36]. Furthermore, cells lacking ATM function fail to respond to TRF2 inhibition in the same manner as ATM functional cells (i.e. through senescence which is succeeded by cell death) [37].

The Chk2 protein acts downstream of ATM to induce p53 and thereby promote senescence [38], although there is evidence for a p53 *independent* function of Chk2 in promoting senescence through induction of $p21^{waf1/cip1}$ [39,40]. The activated forms of both Chk1 and Chk2 have been detected as a consequence of telomere shortening induced senescence, although a direct cause and effect relationship has not been established [21]. Finally, the possibility remains that the ATR/Chk1 axis is capable of promoting senescence in cells lacking functional ATM [36].

The involvement of ATM, Chk1/Chk2, p53, $p21^{waf1/cip1}$and pRb in both the G1 arrest and senescence response pathway raises the intriguing question of how the cell discriminates between signals for replicative senescence and those for more conventional growth arrest associated with quiescence or differentiation.

The reader is also referred to a relatively recent review on the signals and pathways activating cellular senescence [41].

3. The accelerated senescence response to chemotherapy and radiotherapy

Accelerated senescence is characterized by the rapid induction of a permanent growth arrested state with many of the same morphological and biochemical features described above for replicative senescence. One notable exception is that accelerated senescence of cancer cells is not p16-dependent, as this cyclin dependent kinase inhibitor is silenced in the majority of cancer cell lines that readily undergo senescence in response to DNA damage [42]. While a variety of cellular stresses have been shown to promote accelerated senescence in normal and malignant cells, including hydrogen peroxide [43], UV- and γ-irradiation [44], TGF-β [45], and overexpression of oncogenes such as Ha-Ras [46], this review will focus exclusively on ionizing radiation and chemotherapeutic drugs used in the treatment of cancer.

Many studies have evaluated the biological fate of cultured human cancer cells following either acute or chronic exposure to standard chemotherapeutic agents (Table 1). Collectively, these data demonstrate that cancer cells derived from solid tumors readily undergo senescence when exposed to a wide variety of DNA-damaging drugs. One particular focus has been on topoisomerase poisons, mainstay chemotherapeutic agents, which interfere with the ability of topoisomerases to re-ligate DNA, including Adriamycin (doxorubicin), etoposide (VP-16) and camptothecin.

3.1. *Topoisomerase poisons*

Isogenic derivatives of established human cancer cell lines have provided a valuable tool for the direct testing of the dependency of accelerated senescence on specific proteins. In the case of p53 and $p21^{waf1/cip1}$(Fig. 2), p53 wild-type HCT-116 colon cancer cells and their p53 null and $p21^{waf1/cip1}$ null homologous knockouts, as well as p53 wild-type MCF-7 breast cancer cells and MCF-7/HPV-E6 cells, where p53 is targeted for ubiquitin-mediated degradation, have been particularly useful. Data from numerous experiments using different DNA damaging agents (Table 1) make a compelling case that p53, via its downstream effector, $p21^{waf1/cip1}$, greatly facilitates induction of accelerated senescence in human cancer cells. Conversely, when p53 is functionally inactivated, cells are destined for an alternate cell fate, most notably apoptotic cell death [47,48]. Interestingly, we have recently observed senescence in residual subpopulations of p53 mutant or p53 null breast tumor cells that survived the initial cell killing by Adriamycin (unpublished observations).

There is accumulating evidence to support a fundamental role for $p21^{waf1/cip1}$ in accelerated senescence [49,50]. Early studies demonstrated that a knockdown of $p21^{waf1}$ conferred susceptibility to drug-induced apoptosis rather than growth arrest [51]. Src expression blocks senescence induced by

Table 1 – Induction of cell fate in human cancer cells following chemotherapy or radiation

Cell line	p53 status	Modality	Cell fate	References
MCF-7	+	AdR	Senescence + MC[a]	[47,49,50]
	+	SN-38, IR	Senescence	[48,54,106]
MCF-7 hTERT	+	AdR, IR[b]	Senescence	[47]
MCF-7 HPV-16 E6	–	AdR	Apoptosis ≫ senescence	[47]
	–	IR	Apoptosis ≫ senescence	[48]
MDA-MB231	–	AdR	Apoptosis ≫ senescence	[47]
	–	IR	Apoptosis ≫ senescence	[48]
HCT116	+	AdR	Senescence + MC	[49,50]
	+	VP-16, SN-38, camptothecin, cisplatin	Senescence	[49,54,57,65,97,107]
HCT116 p53–/–	–	AdR, VP-16	Apoptosis + MC ≫ senescence	[49,97]
	–	SN-38, cisplatin	Apoptosis ≫ senescence	[49,54,97,107]
	–	Camptothecin	Apoptosis	[57]
HCT116 p21–/–	+	AdR, VP-16, SN-38, IR, camptothecin	Apoptosis	[54,57,65,97,107]
	+	Cisplatin	Apoptosis ≫ senescence	[97]
H1299	–	VP-16	Senescence	[56]
	–	IR	Apoptosis	[108]
H1299 + p53	+	IR	Senescence	[108]
Glioblastoma	+	SN-38, IR	Senescence	[58,109]
Glioblastoma p53–/–	–	IR	Apoptosis	[109]

[a] MC: mitotic catastrophe.
[b] Gewirtz, Holt, and Elmore, unpublished observations.

Adriamycin, at least partially by preventing the induction of $p21^{waf1/cip1}$, forcing the cells to undergo mitotic catastrophe [52]. There is additional evidence of mitotic catastrophe *coincident* with senescence, suggesting that the growth arrest and cell death responses could occur simultaneously [53].

Another topoisomerase II poison, VP-16, has been shown to produce a senescence-like phenotype both *in vitro* [49] and *in vivo* [54]. The cumulative data confirms a role for p53 and $p21^{waf1/cip1}$ in the induction of senescence since apoptosis was the primary response in one study of cells with mutant p53 [55]. To add some contradiction to these conclusions, a single study showed that 40–60% of the population of p53 null lung cancer cells exposed to VP-16 demonstrated senescence [56].

p53 and $p21^{waf1/cip1}$ are also implicated in the senescent response to camptothecin [57] and SN-38 [58], the active metabolite of irinotecan. Both compounds induce a senescence response in p53 +/+ and $p21^{waf1/cip1}$ +/+ cells but cause cell death with either p53 or $p21^{waf1/cip1}$ knocked out. Interestingly, down-regulation of cdc2 (cdk1) is observed in cells exposed to camptothecin [57] as well as in senescing lung cancer cells [56] and in breast tumor cells where senescence is induced by doxorubicin [59], raising the possibility that this protein is a potential regulator of accelerated senescence. Again, in contrast to these observations, a robust accelerated senescence response to camptothecin was observed in non-small cell H1299 lung carcinoma cells null for both p53 and p16 [56], again suggesting some unique characteristics of this specific cell line.

3.2. *Cisplatin*

Cisplatin, an antitumor drug that produces both intrastrand and interstrand cross links in DNA, has also been shown to promote accelerated senescence; however, the involvement of p53 is inconclusive, as senescence has been observed in cells both with and without functional p53 [49,56,62]. Likewise, it is unclear as to whether platinum based drugs have a uniform impact on telomere lengths or telomerase activity [60–62].

3.3. *Ionizing radiation*

The cellular response to ionizing radiation is complex and varied, encompassing a spectrum ranging from growth arrest to apoptotic cell death, reproductive failure or mitotic catastrophe, and autophagy [63,64]. As shown in Table 1 and Fig. 2, and highly reminiscent of the trends seen following DNA damaging chemotherapeutics, ionizing radiation triggers a widespread p53-mediated senescence response in cancer cells [63,65] that is closely associated with the induction of $p21^{waf1/cip1}$ [65]. Data are also accumulating to indicate that irradiation-induced senescence is not limited to cancer cells in that normal cells also undergo radiation-induced accelerated senescence [66]. When p53 function in tumor cells is compromised, radiation-induced senescence is abrogated, and in some cases delayed apoptosis ensues [48], similar to what occurs with drug-induced accelerated senescence. In addition, we and others have shown that ionizing radiation treatment is independent of p16 in the accelerated senescence response [48,49]. In this context, it should be noted that p16 has been implicated in long-term maintenance of senescence [67].

Finally, it is important to emphasize that senescence appears to mediate the impact of ionizing radiation on self-renewal capacity, having shown a close correspondence between the extent of radiation-induced senescence and radiation sensitivity [68]. More recently, ionizing radiation-induced accelerated senescence was again linked to p53 function, where the loss of p53 function that prevented

senescence also abrogated the cytotoxicity of radiation treatment [69]. An excellent review of accelerated senescence in response to ionizing radiation has been published recently [70].

3.4. Other non-DNA damaging agents

Microtubule poisons such as the vinca alkaloids, vincristine, vinblastine, and taxol do not directly target DNA but have been shown in some studies to induce reactive oxygen species [71,72], which may cause telomere damage directly or indirectly. There are some reports of a relatively modest p53-dependent senescence-like response [49] and others showing a rapid accelerated senescence response to discomerdolide [73], again dependent upon p53 and $p21^{waf1/cip1}$. We have also found that mutant p53 cells undergo apoptosis as well as autophagy (but not senescence) in response to treatment with a novel microtubule poison, JG-03-14 [74], while residual surviving p53 wild-type MCF-7 breast tumor cells are senescent, again implicating p53 and/or $p21^{waf1/cip1}$ in the promotion of a senescence response. Overall, however, senescence does not appear to be a primary component of the tumor cell response to microtubule poisons. The connection between senescence and antimetabolites is tenuous, with a few studies implicating p53, c-jun, and p16 in response to cytarabine [49], hydroxyurea [75], and/or methotrexate [76].

4. Telomeres and telomerase in accelerated senescence

Since cancer cells typically have relatively short telomeres [77], and telomere attrition contributes to induction of replicative senescence [6,7], it is logical to assume that cancer cells senescing in response to chemotherapy and/or ionizing radiation involved a telomere-length-dependent process. Through ectopic expression of hTERT as a means to elongate telomeres, it proved feasible to directly test the telomere length dependency on the kinetics and frequency of induction of accelerated senescence. Adriamycin and irradiation-induced senescence was observed with the same frequency and timing in parental cells and in cells expressing exogenous hTERT [47,48], where telomeres are substantially elongated and telomerase activity is maintained even after the onset of senescence. In the case of Adriamycin, induction of accelerated senescence temporally preceded the suppression of telomerase activity [47]. Moreover, inhibition of telomerase in parental MCF-7 cells in the absence of drug treatment did not result in an immediate senescence phenotype, but rather the continued growth of the cells with telomere shortening (unpublished observations). Taken together, these data suggest that suppression of telomerase after Adriamycin is a *consequence* of exiting the cell cycle rather than a cause of senescence.

There is increasing evidence for the conclusion that antitumor drugs do not promote senescence through direct effects on telomerase activity. Cytostatic concentrations of a number of antitumor drugs, including etoposide, daunorubicin, cisplatin, and irinotecan, failed to suppress telomerase in leukemic cells [78]. Similarly, no effects of doxorubicin, bleomycin, methotrexate or melphalan on telomerase activity were observed in human testicular cancer cells [60], while VP-16 failed to induce alterations in hTERT or telomerase activity in human pancreatic cancer cells undergoing senescence [79]. With regard to cisplatin, one study failed to detect suppression of telomerase activity [62], while another reported down-regulation of telomerase activity after cisplatin treatment [60], suggesting some cell type specificity. Cisplatin has been reported to promote telomere loss prior to apoptosis in HeLa cells, presumably due to the formation of telomeric intrastrand cross links and induction of telomere shortening after a single round of DNA replication [61]. Consequently, the relationship between telomeres, telomerase, and the senescence-promoting effects of cisplatin remains to be defined.

For ionizing irradiation, widespread senescence has been observed without a detectable reduction in telomerase activity [44,48], further discounting telomere shortening as a trigger of accelerated senescence. While the induction of telomerase activity has been observed in some irradiated cell types [80], this likely reflects either a transient phenomenon unrelated to the promotion of senescence or the use of supraclinical doses of radiation coupled with extensive cell death, suggesting that the changes in telomerase likely reflect cytotoxicity unrelated to senescence. Collectively, these studies strongly suggest that the shortening of already short telomeres is not the trigger for accelerated senescence after exposure to chemotherapeutic drugs or ionizing radiation.

Since standard chemotherapeutic agents and ionizing radiation cause DNA strand breaks, it is possible that proximal breaks in the telomeres or subtelomeric regions are sensed as critically short, damaged DNA and consequently senescence is induced. This may be the case where cisplatin was shown to promote telomere loss [61], or where VP-16, but not Adriamycin, caused direct telomere breaks [81]. Moreover, no differences in overall telomere length were detected via a telomere length restriction (TRF) analysis after either Adriamycin exposure [47] or irradiation [44,48]. However, recognizing the limited sensitivity of this technique and the fact that only a few critically short telomeres are sufficient to trigger senescence [82], a cytogenetic approach showed that Adriamycin and ionizing radiation cause a high frequency of end-associated abnormalities [47,48]. Thus, the observed accelerated senescence after treatment with chemotherapeutic agents or irradiation is at least partially caused by telomere dysfunction in the absence of net global telomere loss.

The lack of direct involvement of telomeres and telomerase in the radiation response is addressed, in part, by data suggesting that neither elongated telomeres nor increased telomerase activity serve to protect cells from radiation [83], similar to what we observe for Adriamycin [47]. In contrast, shortened telomeres increase radiation susceptibility [84,85], which is not entirely unexpected given the inherent genomic instability associated with short telomeres. Two independent studies in mice support the importance of telomere dysfunction. One shows that loss of telomerase activity failed to influence radiation sensitivity while telomere dysfunction accelerated mortality in irradiated mice [86]. The other demonstrated that shortened telomeres enhanced sensitivity to the toxicity of radiation in the GI tract, lymphoid tissues, and kidney [84]. Taken together, these studies indicate that critically shortened telomeres enhance susceptibility to cell

death in response to radiation-induced DNA damage, likely through the disruption of telomere structure and function (i.e. telomere dysfunction).

5. Are replicative and accelerated senescence separate pathways?

The early discoveries that replicative senescence is dependent on the activity of the tumor suppressor p53, the cyclin dependent kinase inhibitory protein $p21^{waf1/cip1}$ and dephosphorylation of pRb, hinted that senescence is highly reminiscent of the DNA damage response pathway leading to G1 arrest (Fig. 2). This is in fact intuitive since exposed ends of linear chromosomes would be sensed as double strand breaks. As discussed above, the list of telomere-associated proteins has grown, with many of these (i.e., Ku70, Ku80, MRE11, NBS1, RAD50) previously being implicated in the sensing and/or repair of DNA damage.

As expected, prominent and numerous DNA damage-induced foci form in cancer cells after exposure to ionizing radiation [87] or standard chemotherapeutic agents (unpublished data). To date, it is unclear the proportion of these foci that are localized to dysfunctional telomeres, but our preliminary data indicate that the vast majority of DNA damage-induced foci assembling in response to Adriamycin exposure do not overlap with a telomere marker, possibly representing widespread interstitial DNA damage (unpublished data) [88]. However, some co-localization is evident (unpublished data), favoring the existence of a classic early DNA damage response at the telomere prior to induction of accelerated senescence.

It appears that the signaling pathways promoting replicative and accelerated senescence (at least in response to DNA damaging treatment modalities) are more similar than different. For both, dysfunctional telomeres represent one element that can trigger a common DNA damage response signaling pathway culminating in a prolonged (perhaps permanent) growth arrested state. The speed with which the process occurs is really the defining feature: for replicative senescence, gradual telomere attrition due to the ''end replication problem'' [2] eventually leads to the inability to maintain a functional, protective telomere cap; for DNA damage-induced accelerated senescence, rapid telomere dysfunction occurs in a telomere-length-independent manner [47,48]. However, the pathways integrating the early DNA damage sensors, mid-point transducers, and downstream effectors appear very similar (Fig. 2).

6. Accelerated senescence as a barrier to tumor growth and disease recurrence

Numerous studies have relied exclusively on the *in vitro* response of cancer cells to chemotherapeutic agents and irradiation, raising the question of whether senescence might merely be a tissue culture artifact. One limitation to assaying senescence *in vivo* is the paucity of senescence-associated markers. While the identification of senescence in cells is often based on the distinct cellular morphology together with senescence-associated β-galactosidase (SA-β-gal) activity at pH 6.0 [3], this SA-β-gal marker has no known functional basis. Furthermore, in order to detect activity, the histochemical staining procedure requires frozen, not routinely processed specimens. While this may impose a minor constraint for tissue availability, the more significant limitation is the poor resolution typically achieved when histochemically staining a frozen tissue section. With this said, there is a growing number of studies reporting SA-β-gal activity in clinical specimens, many of which appear to be complementary to the *in vitro* accumulated data. Assessing the staining of p53, p16 and SA-β-gal in 20 archival breast tumor resections of patients treated neo-adjuvantly with chemotherapy, it was observed that: (1) DNA damage is able to trigger senescence in tumor cells *in vivo*; (2) SA-β-gal activity was limited to tumors expressing wild-type p53; and (3) breast cancers with undetectable SA-β-gal activity tended to express mutant p53 and low levels of p16 [54]. Because p16 has been implicated in the maintenance of senescence in tumors [67], and since many tumors lack functional p16 [42], the ability to recover proliferative capacity after a transient senescence arrest may be related to the absence of sustained growth arrest mediated through p16.

Additional studies have reported SA-β-gal activity in treated tumor samples [56], while SA-β-gal expressing cells were detected in benign tissues as well [89]. The two-stage model of cellular senescence states that cells must overcome Mortality Stage 1 (M1/Senescence), usually by inactivation of tumor suppressors, and then bypass Mortality Stage 2 (M2/Crisis) in order to immortalize [90]. Extrapolating upon this model, it is possible that the senescent cells within these benign lesions are unable to overcome M1/Senescence, thus providing a senescence-based tumor suppressive mechanism at a pre-malignant level.

It is uncertain as to whether accelerated senescence and cell death are mutually exclusive, sequential, or coincident events or whether senescence can itself be considered a form of reproductive cell death. Various approaches to suppress telomerase activity and/or induce senescence, including telomere shortening, have subsequently resulted in apoptosis or mitotic cell death, with evidence of senescing cell undergoing mitotic catastrophe [91] or senescence accompanied by necrosis [69]. In contrast, studies in mice suggest that senescence is triggered when apoptosis is blocked by over-expression of Bcl-2 [92] and that senescence appears to confer resistance to apoptosis [54]. Similarly, a blockade to Adriamycin-induced apoptosis resulted in a switch to senescence [93]. Of note, sensitivity to Adriamycin was unaltered.

While studies using clinical specimens have been important for validating the tendency for cancer cells to undergo senescence in response to DNA damage, they have provided little insight into the biological significance of such a cell fate. Here, murine models have provided an experimental platform to explore genetic mechanisms of treatment response *in vivo*. Studies using the Eμ-myc transgenic mouse prone to Burkitt's-like B-cell lymphomas, in which apoptosis has been compromised via bcl-2 over-expression, have demonstrated that these lymphomas retain the ability to undergo senescence in response to cyclophosphamide with a complete cessation of cellular proliferation [92]. Perhaps most importantly, mice bearing senescent lymphomas survived for an extended period

of time despite a large tumor burden, indicating the capacity of a senescence response to suppress tumor growth. In the absence of p53, lymphomas formed rapidly and displayed chemotherapy resistance [54], further validating both *in vitro* and clinical data implicating p53 as a critical mediator of accelerated senescence. More recently, reversible RNAi was employed to control p53 expression in a chimeric liver cancer mouse model to definitively show that brief reactivation of p53 in p53-deficient tumors can produce complete tumor regression due predominantly to induction of senescence [94]. In complementary studies, restoration of endogenous p53 expression led to tumor regression in sarcomas through the promotion of senescence [95]. Additionally, using two distinct murine models, it was shown that cellular senescence can limit tumor growth and may contribute to improved long-term survival of the host [94,95]. The studies by Xue et al. [94] clearly implicate the innate immune system in mediating the regression of the senescent tumor cells. However, long-term clinical data on the prognostic impact stratified by the ability *versus* inability to undergo senescence in response to chemotherapy are not yet available.

7. Accelerated senescence: a dead-end or detour?

Historically, senescence has been defined as a permanent growth arrested state, despite the fact that technical difficulties remain in the discrimination of a truly irreversible condition and a reversible long-term growth arrested state. *In vitro* data indicating that senescent cells can re-enter the cell cycle [21,56,59,67] casts some doubt on this ''permanent'' growth arrested state. This proliferative recovery has been achieved, however, by selectively inactivating critical mediators of DNA damage checkpoints (i.e., CHK1/CHK2, ATM/ATR, p53, pRB, cdc2/cdk1), which is arguably a highly contrived scenario. We have reported that a clonal MCF-7 population expressing high levels of cdc2/cdk1 kinase evaded Adriamycin-induced senescence [59]. This clone was refractory to re-exposure of Adriamycin and several other DNA damaging chemotherapeutic agents, but not taxol. Collectively these data indicate that a DNA damage signaling pathway must be constitutively active in order to keep cells in a senescent state. When the DNA damage response is impaired or inactivated, evasion of senescence may result; a transient state of senescence is unlikely to be sufficient for promoting tumor cell death.

The impact of accelerated senescence on the effectiveness of chemotherapy and radiotherapy is not fully understood [96], but it is likely to be dependent on whether the senescence arrest is followed by cell death, possibly through the involvement of cellular and humoral immune responses. In part, this may also be related to the ability of the cell to repair damaged DNA. The extended period of growth arrest associated with accelerated senescence is likely to provide an opportunity for DNA damage repair. Depending on the genetic background of the tumor cell in question, this interval of repair may be indefinite (i.e. a sustained arrest) or may be succeeded by apoptotic or mitotic cell death. As indicated above, in some cases, accelerated senescence may be permissive for recovery of growth potential, particularly in tumors that have lost functional p16 protein. We and others have shown that a subpopulation of cells treated with either drugs or radiation recovers proliferative capacity [48,56,59,97], suggesting that cells failing to senesce demonstrated outgrowth, although we have not determined the source of the recovered cell population. If accelerated senescence proves to be a quantitatively substantive component of the response of malignancies to chemotherapy or radiation treatment, it is possible that cells that escape from senescence could ultimately contribute to tumor regrowth and recurrence of malignancies.

The mechanism by which senescent cells contribute to disease recurrence may extend beyond the possibility of reversing cellular senescence and re-entering the proliferative pool. Senescent cells, while growth arrested, remain metabolically active [98]. Since senescent cells synthesize a number of soluble factors with diverse biological activities [99], their presence may influence the fate of neighboring cells. It has been demonstrated that cells senescing in response to chemotherapy secrete proteins with anti-apoptotic, mitogenic, and angiogenic activities [50], suggesting paracrine tumor promoting effects. Conversely, senescent cells may inhibit tumor growth by secreting growth inhibitory substances [100], senescence-promoting factors [101], or death [102]. While irradiation and chemotherapy bystander effects have been well-documented [103,104], there is likely to be utility in exploring the possibility that senescence bystander effects may act to amplify drug action in naïve cells that are not directly exposed to antitumor drugs [105].

8. Concluding remarks

Overall, it has become evident that accelerated senescence must be considered a critical component of the tumor cell response to various modes of stress imposed by chemotherapeutic drugs and radiation. Furthermore, senescence appears to be sufficient to promote tumor regression, at least in experimental animal model systems. Accelerated senescence clearly plays a role in the drug and radiation treatment response in patients, although the relative contributions of the different modes of cell death (including apoptosis, autophagy and mitotic catastrophe) remain to be defined. At the cellular level, this same question relating to the involvement of different modes of cell death is under active investigation by a number of laboratories, including our own. There are a host of additional questions to be addressed. One is how the cell distinguishes between signals leading to senescence versus those for conventional growth arrest, given the extensive overlap between the proteins associated with both pathways. Another clinically relevant issue is whether accelerated senescence is truly an irreversible form of growth arrest accompanied by loss of self-renewal capacity (i.e. cells that are reproductively ''dead''), or whether senescence might actually provide a temporary refuge for dormant tumor cells that could at some subsequent time recover proliferative capacity, contributing to disease recurrence. Alternatively, our findings relating to a senescence bystander effect suggests that cells undergoing senescence could promote a similar response in naïve untreated cells, amplifying the impact of drug treatment.

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REFERENCES

[1] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.
[2] Hayflick L. The illusion of cell immortality. Br J Cancer 2000;83:841–6.
[3] Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 1995;92:9363–7.
[4] Hemann MT, Narita M. Oncogenes and senescence: breaking down in the fast lane. Genes Dev 2007;21:1–5.
[5] Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci USA 1988;85:6622–6.
[6] Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345: 458–60.
[7] Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279: 349–52.
[8] Jiang XR, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, et al. Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nat Genet 1999;21:111–4.
[9] de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 2005;19:2100–10.
[10] Deng Y, Chang S. Role of telomeres and telomerase in genomic instability, senescence and cancer. Lab Invest 2007;87:1071–6.
[11] Matulic M, Sopta M, Rubelj I. Telomere dynamics: the means to an end. Cell Prolif 2007;40:462–74.
[12] Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, et al. Mammalian telomeres end in a large duplex loop. Cell 1999;97:503–14.
[13] Loayza D, de Lange T. POT1 as a terminal transducer of TRF1 telomere length control. Nature 2003;423:1013–8.
[14] Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature 2007;448:1068–71.
[15] Smogorzewska A, de Lange T. Different telomere damage signaling pathways in human and mouse cells. EMBO J 2002;21:4338–48.
[16] Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 1999;283:1321–5.
[17] Callen E, Surralles J. Telomere dysfunction in genome instability syndromes. Mutat Res 2004;567:85–104.
[18] Stewart SA, Ben-Porath I, Carey VJ, O'Connor BF, Hahn WC, Weinberg RA. Erosion of the telomeric single-strand overhang at replicative senescence. Nat Genet 2003;33: 492–6.
[19] Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 2003;3:155–68.
[20] von Zglinicki T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson SP. Human cell senescence as a DNA damage response. Mech Ageing Dev 2005;126:111–7.
[21] d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature 2003;426:194–8.
[22] Sugrue MM, Shin DY, Lee SW, Aaronson SA. Wild-type p53 triggers a rapid senescence program in human tumor cells lacking functional p53. Proc Natl Acad Sci USA 1997;94:9648–53.
[23] Stansel RM, Subramanian D, Griffith JD. P53 binds telomeric single strand overhangs and T-loop junctions in vitro. J Biol Chem 2002;277:11625–8.
[24] Webley K, Bond JA, Jones CJ, Blaydes JP, Craig A, Hupp T, et al. Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. Mol Cell Biol 2000;20:2803–8.
[25] Jung MS, Yun J, Chae HD, Kim JM, Kim SC, Choi TS, et al. p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. Oncogene 2001;20:5818–25.
[26] el-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, et al. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 1994;54: 1169–74.
[27] Zhang H. Molecular signaling and genetic pathways of senescence: its role in tumorigenesis and aging. J Cell Physiol 2007;210:567–74.
[28] Bartek J, Lukas J. Pathways governing G1/S transition and their response to DNA damage. FEBS Lett 2001;490:117–22.
[29] Wyllie F, Haughton M, Bartek J, Rowson J, Wynford-Thomas D. Mutant p53 can delay growth arrest and loss of CDK2 activity in senescing human fibroblasts without reducing p21(WAF1) expression. Exp Cell Res 2003;285:236–42.
[30] Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc Natl Acad Sci USA 1996;93: 13742–7.
[31] Sandhu C, Peehl DM, Slingerland J. p16INK4A mediates cyclin dependent kinase 4 and 6 inhibition in senescent prostatic epithelial cells. Cancer Res 2000;60:2616–22.
[32] Dai CY, Enders GH. p16 INK4a can initiate an autonomous senescence program. Oncogene 2000;19:1613–22.
[33] Steiner MS, Wang Y, Zhang Y, Zhang X, Lu Y. p16/MTS1/INK4A suppresses prostate cancer by both pRb dependent and independent pathways. Oncogene 2000;19: 1297–306.
[34] Duan J, Zhang Z, Tong T. Senescence delay of human diploid fibroblast induced by anti-sense p16INK4a expression. J Biol Chem 2001;276:48325–31.
[35] Zhang X, Li J, Sejas DP, Pang Q. The ATM/p53/p21 pathway influences cell fate decision between apoptosis and senescence in reoxygenated hematopoietic progenitor cells. J Biol Chem 2005;280:19635–40.
[36] Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell 2004;14:501–13.
[37] Karlseder J, Hoke K, Mirzoeva OK, Bakkenist C, Kastan MB, Petrini JH, et al. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. PLoS Biol 2004;2:E240.

[38] Gire V, Roux P, Wynford-Thomas D, Brondello JM, Dulic V. DNA damage checkpoint kinase Chk2 triggers replicative senescence. EMBO J 2004;23:2554–63.
[39] Aliouat-Denis CM, Dendouga N, Van den Wyngaert I, Goehlmann H, Steller U, van de Weyer I, et al. p53-independent regulation of $p21^{Waf1/Cip1}$ expression and senescence by Chk2. Mol Cancer Res 2005;3:627–34.
[40] Chen CR, Wang W, Rogoff HA, Li X, Mang W, Li CJ. Dual induction of apoptosis and senescence in cancer cells by Chk2 activation: checkpoint activation as a strategy against cancer. Cancer Res 2005;65:6017–21.
[41] Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. Int J Biochem Cell Biol 2005;37:961–76.
[42] Liggett Jr WH, Sidransky D. Role of the p16 tumor suppressor gene in cancer. J Clin Oncol 1998;16:1197–206.
[43] Duan J, Duan J, Zhang Z, Tong T. Irreversible cellular senescence induced by prolonged exposure to H_2O_2 involves DNA-damage-and-repair genes and telomere shortening. Int J Biochem Cell Biol 2005;37:1407–20.
[44] Gorbunova V, Seluanov A, Pereira-Smith OM. Expression of human telomerase (hTERT) does not prevent stress-induced senescence in normal human fibroblasts but protects the cells from stress-induced apoptosis and necrosis. J Biol Chem 2002;277:38540–9.
[45] Katakura Y, Nakata E, Miura T, Shirahata S. Transforming growth factor beta triggers two independent-senescence programs in cancer cells. Biochem Biophys Res Commun 1999;255:110–5.
[46] Yaswen P, Campisi J. Oncogene-induced senescence pathways weave an intricate tapestry. Cell 2007;128:233–4.
[47] Elmore LW, Rehder CW, Di X, McChesney PA, Jackson-Cook CK, Gewirtz DA, et al. Adriamycin-induced senescence in breast tumor cells involves functional p53 and telomere dysfunction. J Biol Chem 2002;277:35509–15.
[48] Jones KR, Elmore LW, Jackson-Cook C, Demasters G, Povirk LF, Holt SE, et al. p53-dependent accelerated senescence induced by ionizing radiation in breast tumour cells. Int J Radiat Biol 2005;81:445–58.
[49] Chang BD, Xuan Y, Broude EV, Zhu H, Schott B, Fang J, et al. Role of p53 and $p21^{waf1/cip1}$ in senescence-like terminal proliferation arrest induced in human tumor cells by chemotherapeutic drugs. Oncogene 1999;18:4808–18.
[50] Chang BD, Swift ME, Shen M, Fang J, Broude EV, Roninson IB. Molecular determinants of terminal growth arrest induced in tumor cells by a chemotherapeutic agent. Proc Natl Acad Sci USA 2002;99:389–94.
[51] Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, et al. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. J Clin Invest 1999;104:263–9.
[52] Vigneron A, Roninson IB, Gamelin E, Coqueret O. Src inhibits adriamycin-induced senescence and G2 checkpoint arrest by blocking the induction of $p21^{waf1}$. Cancer Res 2005;65:8927–35.
[53] Roninson IB, Broude EV, Chang BD. If not apoptosis, then what? Treatment-induced senescence and mitotic catastrophe in tumor cells. Drug Resist Updat 2001;4:303–13.
[54] te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. DNA damage is able to induce senescence in tumor cells in vitro and in vivo. Cancer Res 2002;62:1876–83.
[55] Chiu CC, Li CH, Ung MW, Fuh TS, Chen WL, Fang K. Etoposide (VP-16) elicits apoptosis following prolonged G2-M cell arrest in p53-mutated human non-small cell lung cancer cells. Cancer Lett 2005;223:249–58.
[56] Roberson RS, Kussick SJ, Vallieres E, Chen SY, Wu DY. Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. Cancer Res 2005;65:2795–803.
[57] Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, et al. Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. J Biol Chem 2002;277:17154–60.
[58] Wang Y, Zhu S, Cloughesy TF, Liau LM, Mischel PS. p53 disruption profoundly alters the response of human glioblastoma cells to DNA topoisomerase I inhibition. Oncogene 2004;23:1283–90.
[59] Elmore LW, Di X, Dumur C, Holt SE, Gewirtz DA. Evasion of a single-step, chemotherapy-induced senescence in breast cancer cells: implications for treatment response. Clin Cancer Res 2005;11:2637–43.
[60] Burger AM, Double JA, Newell DR. Inhibition of telomerase activity by cisplatin in human testicular cancer cells. Eur J Cancer 1997;33:638–44.
[61] Ishibashi T, Lippard SJ. Telomere loss in cells treated with cisplatin. Proc Natl Acad Sci USA 1998;95:4219–23.
[62] Wang X, Wong SC, Pan J, Tsao SW, Fung KH, Kwong DL, et al. Evidence of cisplatin-induced senescent-like growth arrest in nasopharyngeal carcinoma cells. Cancer Res 1998;58:5019–22.
[63] Chu K, Teele N, Dewey MW, Albright N, Dewey WC. Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. Radiat Res 2004;162:270–86.
[64] Demasters G, Di X, Newsham I, Shiu R, Gewirtz DA. Potentiation of radiation sensitivity in breast tumor cells by the vitamin D3 analogue, EB 1089, through promotion of autophagy and interference with proliferative recovery. Mol Cancer Ther 2006;5:2786–97.
[65] Mirzayans R, Scott A, Cameron M, Murray D. Induction of accelerated senescence by gamma radiation in human solid tumor-derived cell lines expressing wild-type TP53. Radiat Res 2005;163:53–62.
[66] Meng A, Wang Y, Van Zant G, Zhou D. Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells. Cancer Res 2003;63:5414–9.
[67] Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, et al. Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J 2003;22:4212–22.
[68] Podtcheko A, Ohtsuru A, Namba H, Saenko V, Starenki D, Palona I, et al. Inhibition of ABL tyrosine kinase potentiates radiation-induced terminal growth arrest in anaplastic thyroid cancer cells. Radiat Res 2006;165:35–42.
[69] Lehmann BD, McCubrey JA, Jefferson HS, Paine MS, Chappell WH, Terrian DM. A dominant role for p53-dependent cellular senescence in radiosensitization of human prostate cancer cells. Cell Cycle 2007;6:595–605.
[70] Suzuki M, Boothman DA. Stress-induced premature senescence (SIPS)—influence of SIPS on radiotherapy. J Radiat Res (Tokyo) 2008;49:105–12.
[71] Alexandre J, Hu Y, Lu W, Pelicano H, Huang P. Novel action of paclitaxel against cancer cells: bystander effect mediated by reactive oxygen species. Cancer Res 2007;67:3512–7.
[72] Ramanathan B, Jan KY, Chen CH, Hour TC, Yu HJ, Pu YS. Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res 2005;65:8455–60.
[73] Klein LE, Freeze BS, Smith 3rd AB, Horwitz SB. The microtubule stabilizing agent discodermolide is a potent inducer of accelerated cell senescence. Cell Cycle 2005;4:501–7.
[74] Arthur CR, Gupton JT, Kellogg GE, Yeudall WA, Cabot MC, Newsham IF, et al. Autophagic cell death, polyploidy and

senescence induced in breast tumor cells by the substituted pyrrole JG-03-14, a novel microtubule poison. Biochem Pharmacol 2007;74:981–91.
[75] Park JI, Jeong JS, Han JY, Kim DI, Gao YH, Park SC, et al. Hydroxyurea induces a senescence-like change of K562 human erythroleukemia cell. J Cancer Res Clin Oncol 2000;126:455–60.
[76] Hattangadi DK, DeMasters GA, Walker TD, Jones KR, Di X, Newsham IF, et al. Influence of p53 and caspase 3 activity on cell death and senescence in response to methotrexate in the breast tumor cell. Biochem Pharmacol 2004;68: 1699–708.
[77] Shay JW, Wright WE. Telomerase therapeutics for cancer: challenges and new directions. Nat Rev Drug Discov 2006;5:577–84.
[78] Akiyama M, Horiguchi-Yamada J, Saito S, Hoshi Y, Yamada O, Mizoguchi H, et al. Cytostatic concentrations of anticancer agents do not affect telomerase activity of leukaemic cells in vitro. Eur J Cancer 1999;35:309–15.
[79] Kusumoto M, Ogawa T, Mizumoto K, Ueno H, Niiyama H, Sato N, et al. Adenovirus-mediated p53 gene transduction inhibits telomerase activity independent of its effects on cell cycle arrest and apoptosis in human pancreatic cancer cells. Clin Cancer Res 1999;5:2140–7.
[80] Hyeon Joo O, Hande MP, Lansdorp PM, Natarajan AT. Induction of telomerase activity and chromosome aberrations in human tumour cell lines following X-irradiation. Mutat Res 1998;401:121–31.
[81] Yoon HJ, Choi IY, Kang MR, Kim SS, Muller MT, Spitzner JR, et al. DNA topoisomerase II cleavage of telomeres in vitro and in vivo. Biochim Biophys Acta 1998;1395:110–20.
[82] Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 2001;107:67–77.
[83] Rubio MA, Kim SH, Campisi J. Reversible manipulation of telomerase expression and telomere length. Implications for the ionizing radiation response and replicative senescence of human cells. J Biol Chem 2002;277:28609–17.
[84] Goytisolo FA, Samper E, Martin-Caballero J, Finnon P, Herrera E, Flores JM, et al. Short telomeres result in organismal hypersensitivity to ionizing radiation in mammals. J Exp Med 2000;192:1625–36.
[85] Rubio MA, Davalos AR, Campisi J. Telomere length mediates the effects of telomerase on the cellular response to genotoxic stress. Exp Cell Res 2004;298:17–27.
[86] Wong KK, Chang S, Weiler SR, Ganesan S, Chaudhuri J, Zhu C, et al. Telomere dysfunction impairs DNA repair and enhances sensitivity to ionizing radiation. Nat Genet 2000;26:85–8.
[87] Ward IM, Minn K, Jorda KG, Chen J. Accumulation of checkpoint protein 53BP1 at DNA breaks involves its binding to phosphorylated histone H2AX. J Biol Chem 2003;278:19579–82.
[88] Bradshaw PS, Stavropoulos DJ, Meyn MS. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. Nat Genet 2005;37:193–7.
[89] Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, et al. Tumour biology: senescence in premalignant tumours. Nature 2005;436:642.
[90] Wright WE, Shay JW. The two-stage mechanism controlling cellular senescence and immortalization. Exp Gerontol 1992;27:383–9.
[91] Podtcheko A, Namba H, Saenko V, Ohtsuru A, Starenki D, Meirmanov S, et al. Radiation-induced senescence-like terminal growth arrest in thyroid cells. Thyroid 2005;15:306–13.
[92] Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, et al. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002;109:335–46.
[93] Rebbaa A, Zheng X, Chou PM, Mirkin BL. Caspase inhibition switches doxorubicin-induced apoptosis to senescence. Oncogene 2003;22:2805–11.
[94] Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007;445:656–60.
[95] Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, et al. Restoration of p53 function leads to tumour regression in vivo. Nature 2007;445:661–5.
[96] Schmitt CA. Cellular senescence and cancer treatment. Biochim Biophys Acta 2007;1775:5–20.
[97] Chang BD, Broude EV, Dokmanovic M, Zhu H, Ruth A, Xuan Y, et al. A senescence-like phenotype distinguishes tumor cells that undergo terminal proliferation arrest after exposure to anticancer agents. Cancer Res 1999;59:3761–7.
[98] Shay JW, Roninson IB. Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene 2004;23:2919–33.
[99] Mathon NF, Lloyd AC. Cell senescence and cancer. Nat Rev Cancer 2001;1:203–13.
[100] Morgan WF, Sowa MB. Non-targeted bystander effects induced by ionizing radiation. Mutat Res 2007;616:159–64.
[101] Sedelnikova OA, Nakamura A, Kovalchuk O, Koturbash I, Mitchell SA, Marino SA, et al. DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. Cancer Res 2007;67:4295–302.
[102] Chhipa RR, Bhat MK. Bystander killing of breast cancer MCF-7 cells by MDA-MB-231 cells exposed to 5-fluorouracil is mediated via Fas. J Cell Biochem 2007;101:68–79.
[103] Djordjevic B. Bystander effects: a concept in need of clarification. Bioessays 2000;22:286–90.
[104] Little JB. Cellular radiation effects and the bystander response. Mutat Res 2006;597:113–8.
[105] Di X, Bright AT, Bellott R, Gaskins E, Robert J, Holt S, et al. A chemotherapy-associated senescence bystander effect in breast cancer cells. Cancer Biol Ther 2008;7.
[106] DeMasters GA, Gupta MS, Jones KR, Cabot M, Wang H, Gennings C, et al. Potentiation of cell killing by fractionated radiation and suppression of proliferative recovery in MCF-7 breast tumor cells by the Vitamin D3 analog EB 1089. J Steroid Biochem Mol Biol 2004;92: 365–74.
[107] Hayward RL, Macpherson JS, Cummings J, Monia BP, Smyth JF, Jodrell DI. Antisense Bcl-xl down-regulation switches the response to topoisomerase I inhibition from senescence to apoptosis in colorectal cancer cells, enhancing global cytotoxicity. Clin Cancer Res 2003;9:2856–65.
[108] Suzuki K, Mori I, Nakayama Y, Miyakoda M, Kodama S, Watanabe M. Radiation-induced senescence-like growth arrest requires TP53 function but not telomere shortening. Radiat Res 2001;155:248–53.
[109] Quick QA, Gewirtz DA. An accelerated senescence response to radiation in wild-type p53 glioblastoma multiforme cells. J Neurosurg 2006;105:111–8.