**Radiotherapy-induced senescence and its effects on responses to treatment.**

*Antonella F. S. Tabasso[1,2], Donald J. L. Jones[1], George D. D. Jones[1] and Salvador Macip[2].*

[1]Leicester Cancer Research Centre, Leicester Royal Infirmary, University of Leicester, Leicester, UK. [2]Mechanisms of Cancer and Ageing Laboratory, Department of Molecular and Cell Biology, University of Leicester, Leicester, UK.

**Corresponding author:** Salvador Macip. Mechanisms of Cancer and Ageing Laboratory, Department of Molecular and Cell Biology, University of Leicester, University Road, Leicester, LE1 7RH, UK. sm460@le.ac.uk, Phone: +44 (0)116 229 7113. Fax: +44 (0)116 229 7123.

**ABSTRACT**

Radiotherapy is still a treatment of choice for many malignancies, often in combination with other strategies. However, its efficacy is limited by the dose that can be safely administered without eliciting serious side effects, as well as the fact that recurrence is common, particularly in large tumours. Combining radiotherapy with drugs that could sensitize cells to radiation and/or reduce the factors that promote the recovery of the surviving cancer cells is a promising approach. Ionizing radiation has been shown to induce senescence, and the accumulation of senescent cells creates a microenvironment that facilitates neoplastic growth. This provides a rationale to test the addition of anti-senescent drugs, some of them already available in the clinic, to radiotherapy protocols. Here, we discuss the relevance of radiotherapy-induced senescent cell accumulation and the potential interventions to minimize its negative effects.

**Keywords:** Senescence; Radiotherapy; Senolytics; Adjuvant therapy

## Introduction

Cancer has become the leading cause of death and the most important factor in lowering life expectancy in developed countries [1]. This is due to several factors, including an increasingly ageing population [2] and a reduction in the mortality caused by cardiovascular diseases and strokes [3]. In 2018, ~18.1 million new cases were diagnosed worldwide, and ~9.6 million deaths were caused by cancer [1]. Over half of all cancer patients will receive radiation, either alone or in combination with surgery or chemotherapy, in 40-60% of cases being given with curative intent as part or all of the treatment plan [4]. There have been constant improvements in radiotherapy practices and equipment, such as the change from external-beam radiotherapy (EBRT) to stereotactic body radiotherapy (SBRT) [5-7], which allows a much higher dose to be delivered to the tumour and therefore lead to an increase in survival for patients treated in this way [8-10]. For some cancers, overall survival (OS) rates of patients treated with radiotherapy or chemo-radiotherapy are becoming comparable to those after surgical resection, with lower costs involved. For non-small cell lung cancer (NSCLC), for example, 2 year survival for stage I cancers treated with radiotherapy is over 70% [8, 11] and for liver, the median OS at 18 months is around 60% [12], similar to the percentages obtained by surgery. Recurrence of disease is, however common, with nearly half of stage I NSCLC patients developing locoregional recurrence and around 20% presenting with distant metastases 2 years after treatment with SBRT [13, 14]. Interestingly, recurrence rates are higher in patients treated with SBRT than those whose tumours were surgically resected at the same stage [15]. One potential reason for the high recurrence of some cancers after radiotherapy could be the increase in senescent cells that can occur after treatment [16].

## The mechanisms of cellular senescence

Senescence is a cell autonomous state of permanent growth arrest, one of the main purposes of which is to prevent the proliferation of damaged cells [17]. Senescence can be triggered by several non-lethal stress signals and the type of damaging event is often used to name the different forms of the phenotype [18, 19]. Senescence-inducing damages include shortening of telomeres, which causes replicative senescence (RS) [20], activation of oncogenes, which leads to oncogene-induced senescence (OIS) [21, 22] and chemo- or radiotherapy treatment, which can lead to the induction of therapy-induced senescence (TIS) [23]. All types of non-replicative senescence are usually grouped under the name stress-induced premature senescence (SIPS) [24]. Regardless of the inducing stimulus, senescent cells share common activation and maintenance mechanisms, which are mostly related to the RB and p53 pathways (Figure 1) [25, 26].

Both the p53 protein levels and the activity of the pathway are increased when cells enter senescence. This can be due to the fact that, in response to senescence inducing signals, activity of TBX2, a transcription factor which represses the p14ARF promoter, is decreased [27]. This leads to a rise in expression of p14ARF, which sequesters the MDM2 E3 ubiquitin ligase, responsible for facilitating the proteasomal degradation of p53 [28]. In addition to this mechanism, senescence-inducing signals can increase the expression of promyelocytic leukemia protein (PML), which interacts with the acetyltransferases CBP and p300, forming the PML-acetyltransferase complex [29-31]. This complex is able to acetylate p53, thereby stimulating its activity. In response to both these activation mechanisms, p53 is able to induce the transcription of several genes, which can either cause or facilitate senescence [29] with the help of other signals, such as reactive oxygen species [32]. These include the CDK inhibitor p21 [33] and upstream kinase BTK, which reinforces the stability of p53 and enhances its senescence-related functions [34-36].

In senescent cells, the RB protein exists in its hypophosphorylated active form, which causes growth inhibition of the cell. Senescence-inducing signals are also able to reduce the activity of BMI-1, a protein that regulates chromatin structure and can silence the promoter of $p16^{INK4A}$ [37]. This reduction in activity leads to an upregulation of p16 [38]. Another mechanism for p16 activation in senescence is the increase in activity of E-26 transformation-specific (ETS). ETS is a transcription factor that is able to induce p16 [39]. In both these cases, the transcription of p16 leads to the inhibition of the CDKs that phosphorylate RB. This allows cell cycle arrest to be maintained for prolonged periods of time [28].

The p53 and pRB pathways interact at many different levels during the activation and maintenance of senescence. For example, when p21 is induced in response to p53 it can block the CDKs that usually inactivate RB [40, 41], as well as trigger an increase in oxidative stress that reinforces the arrest [42]. Similarly, RB can bind MDM2, thus preventing the degradation of p53 [28]. When either of these pathways is inactivated, senescence can be delayed or abrogated [30].

**Main features of senescent cells**

Senescent cells can be distinguished from normal or quiescent cells by a series of molecular markers and phenotypic changes [32, 43, 44]. These include a change in morphology, resulting in cells becoming enlarged and flattened with prominent single nucleoli and cytoplasmic granules; a change in chromatin organisation, resulting in the formation of senescence-associated heterochromatin foci (SAHFs, domains of nuclear DNA enriched for histone modifications that play a role in silencing proliferation genes); high lysosomal content; and changes in gene expression [45]. The induction of specific genes gives the cells the ability to secrete a number of factors, including proinflammatory cytokines, chemokines, growth factors and proteases, which are collectively known as the

senescence associated secretory phenotype (SASP) [46]. The SASP has different autocrine and paracrine functions, allowing the senescent cells to remain in communication with their microenvironment.

DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS, permanent foci of DNA damage and the associated proteins) are signs of the perpetuation of the DNA damage responses necessary to maintain growth arrest and are considered a feature of senescence as well [47]. Senescence associated ß-galactosidase activity (SA-ß-Gal) is often used to asses senescence, since the lysosomal ß-Gal enzyme's activity is higher in senescent cells than in proliferating cells due to an increase in lysosomal mass [48, 49]. Proteins involved in the senescence-associated growth arrest can also be used as markers, such as tumour suppressor genes that control functions related to the cell cycle (p16, p53, p21, $p27^{Kip1}$) [50]. A specific set of proteins, that associate with the plasma membrane, known as the senescent surfaceome, also contribute to defining senescent cells. These include ARMCX3, B2MG, PLD3, NTAL, LANCL1, VPS26A and DEP1, which can be used as selective markers for senescence enabling ease of detection of the phenotype [44, 51], as well as DPP4 [52] and SCAMP4 [53].

**Tumour suppressing properties of senescence**

The induction of senescence is crucial in avoiding cancer, as OIS is a powerful tumour suppressing mechanism that can prevent pre-neoplastic cells from progressing through the multi-step carcinogenesis process [25]. Bypassing senescence allows cells with potentially cancerous mutations to continue proliferating [54]. TIS can initially be beneficial to therapy, as cancerous cells that have not received a fatal dose of the treatment may go into senescence, thus preventing further proliferation [55-58]. The role of senescence in cancer therapy has recently become more prominent as it has become clear that for several cancer

types, such as lung and glioblastoma, senescence is preferentially induced over apoptosis after treatment with chemo- or radiotherapy [59-61]. In addition to this, some elements of the SASP can be beneficial to the slowing down or the prevention of tumour growth. The SASP of a senescent cell may contain anti-angiogenic elements such as maspin, which prevent the formation of new vasculature needed by the tumour to grow [62]. Interleukins can also help to reinforce senescence in damaged cells [50].

**The negative impact of TIS**

If senescent cells are not properly cleared by the immune system, their accumulation can have a detrimental effect on patient outcome. This is in part due to the antagonistic pleiotropy that elements of the SASP possess [63-65]. For instance, the same interleukins which are initially beneficial for tissue recovery, can begin to promote cancer through ras signalling and also play a significant role in promoting epithelial mesenchymal transition [66]. The SASP contains many pro-inflammatory cytokines and chemokines that, although initially aid in the recruitment of immune cells, can create a pro-inflammatory microenvironment that leads to tissue remodelling and regrowth of transformed cells [66-68]. In addition to this, VEGF in the SASP, can lead to the formation of new vasculature, thus promoting growth of the tumour [69]. Because of this, any neoplastic cells that survived therapy may be able to become cancerous in the microenvironment created by the SASP, and rapid recurrence of the tumour, with potentially more aggressive properties, may occur. There is evidence that suggests that up to around 10% of cells that display the characteristics of senescence are eventually able to evade TIS and re-enter the cell cycle by overexpressing the cyclin-dependent kinase Cdc2/Cdk1, thus contributing to the recurrence of the tumour [70, 71].

Ionising radiation can also induce senescence in healthy endothelial tissue surrounding the tumour. This in itself can be detrimental for the patient, especially in the long term, as a build-up of senescent cells can lead to additional health problems, such as fibrosis [72] and cardiovascular disease [73, 74].

Finally, senescent cells also directly promote malignant phenotypes in neighbouring cells via the paracrine signalling of the SASP [65]. It is known that as the ratio of senescent cells to functional immune cells increases the role of senescent cells becomes more tumour-promoting, rather than tumour-suppressive [75]. Thus, while senescence can initially be beneficial both in suppressing the formation of tumours and in ensuring a successful treatment, accumulation of senescent cells can interfere with the therapy and encourage tumour regrowth.

**Targeting senescence to improve therapy outcomes**

The induction of senescent cells in normal tissue surrounding the tumour after radiotherapy has been found to have an impact on the overall survival and quality of life of the patient [72-74]. Since the build-up of senescent cells may pose a problem for radiotherapy treatments, efficient elimination of these cells could greatly improve outcomes. Thus, the use of drugs to eliminate senescent cells or prevent their accumulation could be an effective adjuvant strategy for radiotherapy. Consistent with this, the clearance of p16-positive cells has been shown to improve healthspan and delay tumorigenesis, in healthy animals [76]. Moreover, the treatment of irradiated cells with Cdc2 inhibitors was shown to prevent this evasion of senescence from occurring [71].

The use of senolytics, small molecules that are able to selectively induce apoptosis of senescent cells by inhibiting the cells' activation of anti-apoptotic and pro-survival mechanisms [77, 78], could also increase the efficiency of radiotherapy. There is growing

evidence to suggest that the use of senolytics in addition to more traditional cancer treatments may improve outcomes by reducing recurrence and increasing quality of life through the prevention of the side effects commonly associated with the build-up of senescent cells. The administration of senolytic agent ABT-263 to mice that had undergone chemotherapeutic treatment and in which TIS had been induced has been shown to allow for efficient clearance of senescent cells, which reduces the recurrence and metastasis of the tumour [79, 80]. ABT-263 has also been shown in mice to reverse pulmonary fibrosis, a common complication in thoracic cancer treated by radiation, in which senescent cells play an important role [81].

Panobinostat, a histone-deacetylase inhibitor (HDACi), can also act as a senolytic, as senescence is associated with histone deacetylation. Although HDACis alone have been shown to lead to poor clinical outcomes, in combination with more traditional cytotoxic drugs they have a potent anti-tumour efficacy in NSCLC and Head and Neck Squamous Cell Carcinoma [82]. Additionally, two new senolytics, the antibiotics Roxithromycin and Azithromycin, have been able to mitigate some of the damaging side effects of radiation, such as inflammation, fibrosis and hair loss. [84]. It remains to be seen whether these effects are mediated by a reduction in senescent cells. All these data together suggest that the protection of the cancer-surrounding normal tissue from TIS may drastically reduce the occurrence of radiation-associated complications and improve outcomes for the patient.

## Conclusions

There is a need to find cancer treatments that are more specific and have less side effects. Targeted therapies tend to elicit short-lived responses that often lead to recurrence. Thus, classic chemo and radiotherapy are still relevant therapeutic options and combination therapies, which may include any of the above, are becoming more prevalent. The addition of a targeted therapy that increases the therapeutic ratio may allow clinicians to reduce the dose

of chemo or radiotherapy, thus minimizing the impact on healthy cells. Part of this strategy may be to attack factors that influence neoplastic growth, even if they are not drivers of transformation. In this sense, controlling the tumour microenvironment may increase the effects of drugs or radiation and reduce the chances of relapse.

Senescent cells have been receiving increased attention lately due to the fact that they are potentially involved in many pathologies, including cancer [85]. Although senescence is an important tumour suppressor mechanism, when senescent cells accumulate negative pro-tumorigenic effects can occur. Thus, clearing them from tumours and surrounding healthy tissues may be a successful and beneficial adjuvant strategy in those cancers treatable with radiotherapy (Figure 2). This is especially important when considering that radiation often triggers senescence in normal and cancer cells. The fact that many senolytics (such as Panobinostat, Roxithromycin and Azithromycin) are already approved for treatment of other diseases could accelerate clinical trials, since they could easily be repurposed for use with radiation [82, 84]. Taking advantage of senolytics and other anti-senescent strategies in combination therapies could improve the overall outcome post-treatment and the survival of patients by reducing the chance of recurrence. This should be the subject of rigorous translational investigations, perhaps starting with Phase 1 studies amongst patients undergoing palliative treatments, such as that given when bone metastasis are present.

## ACKNOWLEDGEMENTS

This work was partially supported by the M.C. Andreu Memorial Fund. We thank Prof Paul Symonds for critically reviewing the manuscript. The authors declare no conflicts of interest.

**BIBLIOGRAPHY**

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.

[2] UK CR. Cancer Research UK. 2018.

[3] Mensah GA, Wei GS, Sorlie PD, Fine LJ, Rosenberg Y, Kaufmann PG, et al. Decline in Cardiovascular Mortality: Possible Causes and Implications. Circ Res. 2017;120:366-80.

[4] Institute NC. Radiation Therapy. 2018.

[5] Kelly P, Balter PA, Rebueno N, Sharp HJ, Liao Z, Komaki R, et al. Stereotactic body radiation therapy for patients with lung cancer previously treated with thoracic radiation. Int J Radiat Oncol Biol Phys. 2010;78:1387-93.

[6] Anwar M, Weinberg V, Chang AJ, Hsu IC, Roach M, 3rd, Gottschalk A. Hypofractionated SBRT versus conventionally fractionated EBRT for prostate cancer: comparison of PSA slope and nadir. Radiat Oncol. 2014;9:42.

[7] Kim MS, Choi C, Yoo S, Cho C, Seo Y, Ji Y, et al. Stereotactic body radiation therapy in patients with pelvic recurrence from rectal carcinoma. Jpn J Clin Oncol. 2008;38:695-700.

[8] Davis JN, Medbery C, Sharma S, Pablo J, Kimsey F, Perry D, et al. Stereotactic body radiotherapy for centrally located early-stage non-small cell lung cancer or lung metastases from the RSSearch((R)) patient registry. Radiat Oncol. 2015;10:113.

[9] Stish BJ, Pisansky TM, Harmsen WS, Davis BJ, Tzou KS, Choo R, et al. Improved Metastasis-Free and Survival Outcomes With Early Salvage Radiotherapy in Men With

Detectable Prostate-Specific Antigen After Prostatectomy for Prostate Cancer. J Clin Oncol. 2016;34:3864-71.

[10] Schanne DH, Nestle U, Allgauer M, Andratschke N, Appold S, Dieckmann U, et al. Stereotactic body radiotherapy for centrally located stage I NSCLC: a multicenter analysis. Strahlenther Onkol. 2015;191:125-32.

[11] Milano MT, Chen Y, Katz AW, Philip A, Schell MC, Okunieff P. Central thoracic lesions treated with hypofractionated stereotactic body radiotherapy. Radiother Oncol. 2009;91:301-6.

[12] Davis JN, Medbery C, 3rd, Sharma S, Danish A, Mahadevan A. The RSSearch Registry: patterns of care and outcomes research on patients treated with stereotactic radiosurgery and stereotactic body radiotherapy. Radiat Oncol. 2013;8:275.

[13] Zhao Q, Chen G, Ye L, Zeng Z, Shi S, He J. Clinical outcomes of stereotactic body radiotherapy for de novo pulmonary tumors in patients with completely resected early stage non-small cell lung cancer. Cancer Manag Res. 2018;10:6391-8.

[14] Spratt DE, Wu AJ, Adeseye V, Din SU, Shaikh F, Woo KM, et al. Recurrence Patterns and Second Primary Lung Cancers After Stereotactic Body Radiation Therapy for Early-Stage Non-Small-Cell Lung Cancer: Implications for Surveillance. Clin Lung Cancer. 2016;17:177-83 e2.

[15] van den Berg LL, Klinkenberg TJ, Groen HJ, Widder J. Patterns of Recurrence and Survival after Surgery or Stereotactic Radiotherapy for Early Stage NSCLC. J Thorac Oncol. 2015;10:826-31.

[16] Liao EC, Hsu YT, Chuah QY, Lee YJ, Hu JY, Huang TC, et al. Radiation induces senescence and a bystander effect through metabolic alterations. Cell Death Dis. 2014;5:e1255.

[17] Tasdemir N, Lowe SW. Senescent cells spread the word: non-cell autonomous propagation of cellular senescence. EMBO J. 2013;32:1975-6.

[18] Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev. 1994;8:2540-51.

[19] Toussaint O, Medrano EE, von Zglinicki T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp Gerontol. 2000;35:927-45.

[20] Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, et al. Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci U S A. 1992;89:10114-8.

[21] Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature. 2006;444:638-42.

[22] Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature. 2006;444:633-7.

[23] Shay JW, Roninson IB. Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene. 2004;23:2919-33.

[24] Toussaint O, Dumont P, Dierick JF, Pascal T, Frippiat C, Chainiaux F, et al. Stress-induced premature senescence. Essence of life, evolution, stress, and aging. Ann N Y Acad Sci. 2000;908:85-98.

[25] Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell. 1997;88:593-602.

[26] Dimri GP, Itahana K, Acosta M, Campisi J. Regulation of a senescence checkpoint response by the E2F1 transcription factor and p14(ARF) tumor suppressor. Mol Cell Biol. 2000;20:273-85.

[27] Jacobs JJ, Keblusek P, Robanus-Maandag E, Kristel P, Lingbeek M, Nederlof PM, et al. Senescence bypass screen identifies TBX2, which represses Cdkn2a (p19(ARF)) and is amplified in a subset of human breast cancers. Nat Genet. 2000;26:291-9.

[28] Bringold F, Serrano M. Tumor suppressors and oncogenes in cellular senescence. Exp Gerontol. 2000;35:317-29.

[29] Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, et al. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature. 2000;406:207-10.

[30] Campisi J. Cellular senescence as a tumor-suppressor mechanism. Trends Cell Biol. 2001;11:S27-31.

[31] Bandyopadhyay D, Okan NA, Bales E, Nascimento L, Cole PA, Medrano EE. Down-regulation of p300/CBP histone acetyltransferase activates a senescence checkpoint in human melanocytes. Cancer Res. 2002;62:6231-9.

[32] Macip S, Igarashi M, Berggren P, Yu J, Lee SW, Aaronson SA. Influence of induced reactive oxygen species in p53-mediated cell fate decisions. Mol Cell Biol. 2003;23:8576-85.

[33] Masgras I, Carrera S, de Verdier PJ, Brennan P, Majid A, Makhtar W, et al. Reactive oxygen species and mitochondrial sensitivity to oxidative stress determine induction of cancer cell death by p21. J Biol Chem. 2012;287:9845-54.

[34] Althubiti M, Rada M, Samuel J, Escorsa JM, Najeeb H, Lee KG, et al. BTK Modulates p53 Activity to Enhance Apoptotic and Senescent Responses. Cancer Res. 2016;76:5405-14.

[35] Rada M, Althubiti M, Ekpenyong-Akiba AE, Lee KG, Lam KP, Fedorova O, et al. BTK blocks the inhibitory effects of MDM2 on p53 activity. Oncotarget. 2017;8:106639-47.

[36] Rada M, Barlev N, Macip S. BTK: a two-faced effector in cancer and tumour suppression. Cell Death Dis. 2018;9:1064.

[37] Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature. 1999;397:164-8.

[38] Lundberg AS, Hahn WC, Gupta P, Weinberg RA. Genes involved in senescence and immortalization. Curr Opin Cell Biol. 2000;12:705-9.

[39] Ohtani N, Zebedee Z, Huot TJ, Stinson JA, Sugimoto M, Ohashi Y, et al. Opposing effects of Ets and Id proteins on p16INK4a expression during cellular senescence. Nature. 2001;409:1067-70.

[40] Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell. 2002;2:103-12.

[41] Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN. Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. Biochem J. 1998;332 ( Pt 1):43-50.

[42] Macip S, Igarashi M, Fang L, Chen A, Pan ZQ, Lee SW, et al. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. EMBO J. 2002;21:2180-8.

[43] Gonzalez LC, Ghadaouia S, Martinez A, Rodier F. Premature aging/senescence in cancer cells facing therapy: good or bad? Biogerontology. 2016;17:71-87.

[44] Althubiti M, Macip S. Detection of Senescent Cells by Extracellular Markers Using a Flow Cytometry-Based Approach. Methods Mol Biol. 2017;1534:147-53.

[45] Chandler H, Peters G. Stressing the cell cycle in senescence and aging. Curr Opin Cell Biol. 2013;25:765-71.

[46] Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol. 2013;75:685-705.

[47] Rodier F, Munoz DP, Teachenor R, Chu V, Le O, Bhaumik D, et al. DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. J Cell Sci. 2011;124:68-81.

[48] Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc. 2009;4:1798-806.

[49] 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 U S A. 1995;92:9363-7.

[50] Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev. 2010;24:2463-79.

[51] Althubiti M, Lezina L, Carrera S, Jukes-Jones R, Giblett SM, Antonov A, et al. Characterization of novel markers of senescence and their prognostic potential in cancer. Cell Death Dis. 2014;5:e1528.

[52] Kim KM, Noh JH, Bodogai M, Martindale JL, Yang X, Indig FE, et al. Identification of senescent cell surface targetable protein DPP4. Genes Dev. 2017;31:1529-34.

[53] Kim KM, Noh JH, Bodogai M, Martindale JL, Pandey PR, Yang X, et al. SCAMP4 enhances the senescent cell secretome. Genes Dev. 2018;32:909-14.

[54] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-74.

[55] Ewald JA, Desotelle JA, Wilding G, Jarrard DF. Therapy-induced senescence in cancer. J Natl Cancer Inst. 2010;102:1536-46.

[56] Zurgil U, Ben-Ari A, Atias K, Isakov N, Apte R, Livneh E. PKCeta promotes senescence induced by oxidative stress and chemotherapy. Cell Death Dis. 2014;5:e1531.

[57] Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, et al. Skp2 is oncogenic and overexpressed in human cancers. Proc Natl Acad Sci U S A. 2001;98:5043-8.

[58] Liu Y, Hawkins OE, Su Y, Vilgelm AE, Sobolik T, Thu YM, et al. Targeting aurora kinases limits tumour growth through DNA damage-mediated senescence and blockade of NF-kappaB impairs this drug-induced senescence. EMBO Mol Med. 2013;5:149-66.

[59] Hotta K, Tabata M, Kiura K, Kozuki T, Hisamoto A, Katayama H, et al. Gefitinib induces premature senescence in non-small cell lung cancer cells with or without EGFR gene mutation. Oncol Rep. 2007;17:313-7.

[60] Joyner DE, Bastar JD, Randall RL. Doxorubicin induces cell senescence preferentially over apoptosis in the FU-SY-1 synovial sarcoma cell line. J Orthop Res. 2006;24:1163-9.

[61] Jeon HY, Kim JK, Ham SW, Oh SY, Kim J, Park JB, et al. Irradiation induces glioblastoma cell senescence and senescence-associated secretory phenotype. Tumour Biol. 2016;37:5857-67.

[62] Nickoloff BJ, Lingen MW, Chang BD, Shen M, Swift M, Curry J, et al. Tumor suppressor maspin is up-regulated during keratinocyte senescence, exerting a paracrine antiangiogenic activity. Cancer Res. 2004;64:2956-61.

[63] Giaimo S, d'Adda di Fagagna F. Is cellular senescence an example of antagonistic pleiotropy? Aging Cell. 2012;11:378-83.

[64] Rodier F, Campisi J. Four faces of cellular senescence. J Cell Biol. 2011;192:547-56.

[65] Campisi J, Andersen JK, Kapahi P, Melov S. Cellular senescence: a link between cancer and age-related degenerative disease? Semin Cancer Biol. 2011;21:354-9.

[66] Ancrile B, Lim KH, Counter CM. Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Genes Dev. 2007;21:1714-9.

[67] Floyd RA, Hensley K, Jaffery F, Maidt L, Robinson K, Pye Q, et al. Increased oxidative stress brought on by pro-inflammatory cytokines in neurodegenerative processes and the protective role of nitrone-based free radical traps. Life Sci. 1999;65:1893-9.

[68] Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature. 2011;479:547-51.

[69] Coppe JP, Kauser K, Campisi J, Beausejour CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem. 2006;281:29568-74.

[70] 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.

[71] 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.

[72] Citrin DE, Shankavaram U, Horton JA, Shield W, 3rd, Zhao S, Asano H, et al. Role of type II pneumocyte senescence in radiation-induced lung fibrosis. J Natl Cancer Inst. 2013;105:1474-84.

[73] Borghini A, Gianicolo EA, Picano E, Andreassi MG. Ionizing radiation and atherosclerosis: current knowledge and future challenges. Atherosclerosis. 2013;230:40-7.

[74] Stewart FA, Seemann I, Hoving S, Russell NS. Understanding radiation-induced cardiovascular damage and strategies for intervention. Clin Oncol (R Coll Radiol). 2013;25:617-24.

[75] Naylor RM, Baker DJ, van Deursen JM. Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin Pharmacol Ther. 2013;93:105-16.

[76] Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature. 2016;530:184-9.

[77] Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015;14:644-58.

[78] Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med. 2015;21:1424-35.

[79] Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med. 2016;22:78-83.

[80] Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov. 2017;7:165-76.

[81] Pan J, Li D, Xu Y, Zhang J, Wang Y, Chen M, et al. Inhibition of Bcl-2/xl With ABT-263 Selectively Kills Senescent Type II Pneumocytes and Reverses Persistent Pulmonary Fibrosis Induced by Ionizing Radiation in Mice. Int J Radiat Oncol Biol Phys. 2017;99:353-61.

[82] Samaraweera L, Adomako A, Rodriguez-Gabin A, McDaid HM. A Novel Indication for Panobinostat as a Senolytic Drug in NSCLC and HNSCC. Sci Rep. 2017;7:1900.

[83] Marampon F, Gravina GL, Festuccia C, Popov VM, Colapietro EA, Sanita P, et al. Vitamin D protects endothelial cells from irradiation-induced senescence and apoptosis by modulating MAPK/SirT1 axis. J Endocrinol Invest. 2016;39:411-22.

[84] Ozsvari B, Nuttall JR, Sotgia F, Lisanti MP. Azithromycin and Roxithromycin define a new family of "senolytic" drugs that target senescent human fibroblasts. Aging (Albany NY). 2018;10:3294-307.

[85] Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol. 2014;15:482-96.

**FIGURES**

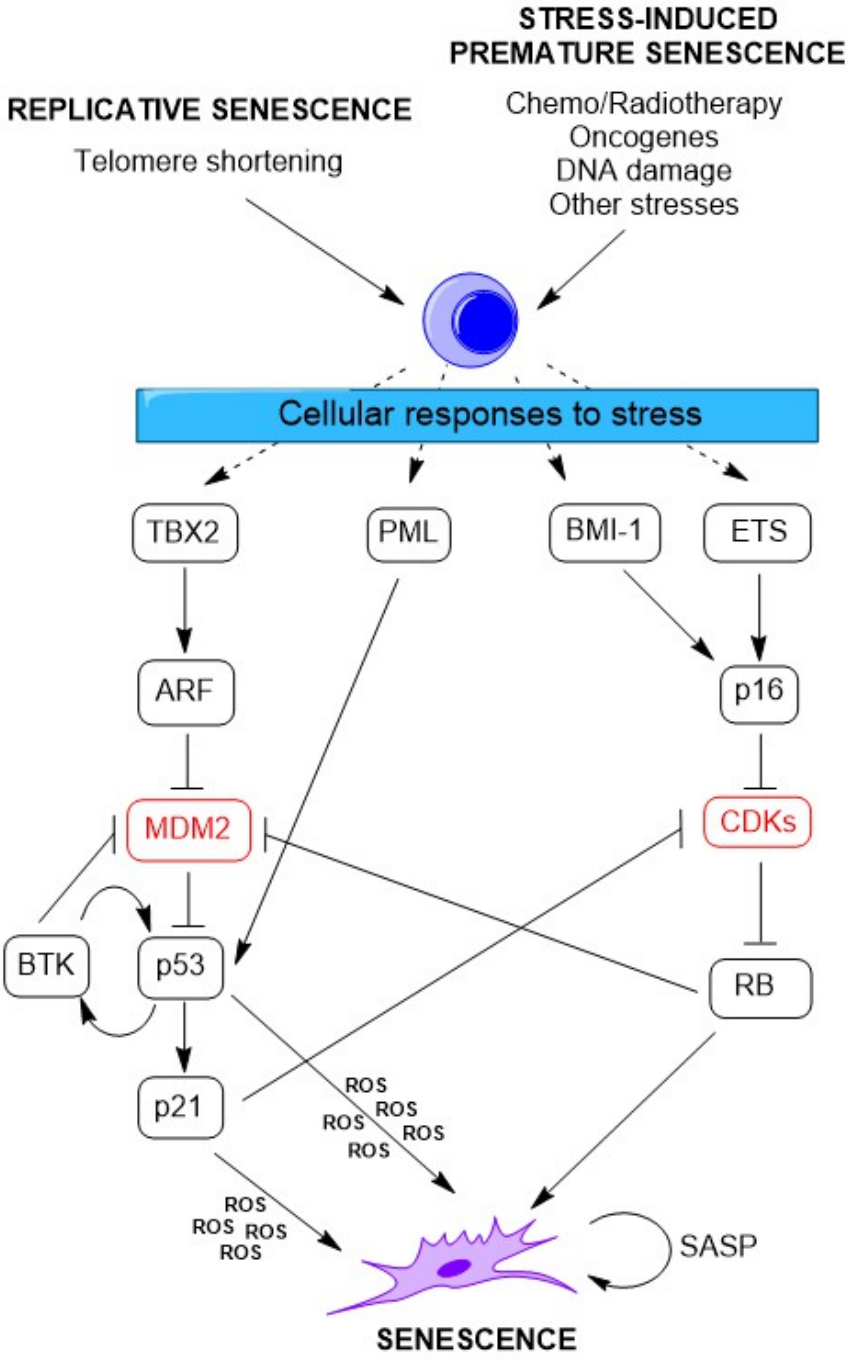


**Figure 1. Summary of the main pathways involved in senescence.** Senescence is induced in response to different damage signals that activate the cellular responses to stress. Different effectors in the p53 and RB pathways trigger the phenotype and block inhibitory proteins (in red). The induction of senescence is reinforced by biochemical mechanisms, such as the increase in reactive oxygen species (ROS) and the autocrine effects of SASP.

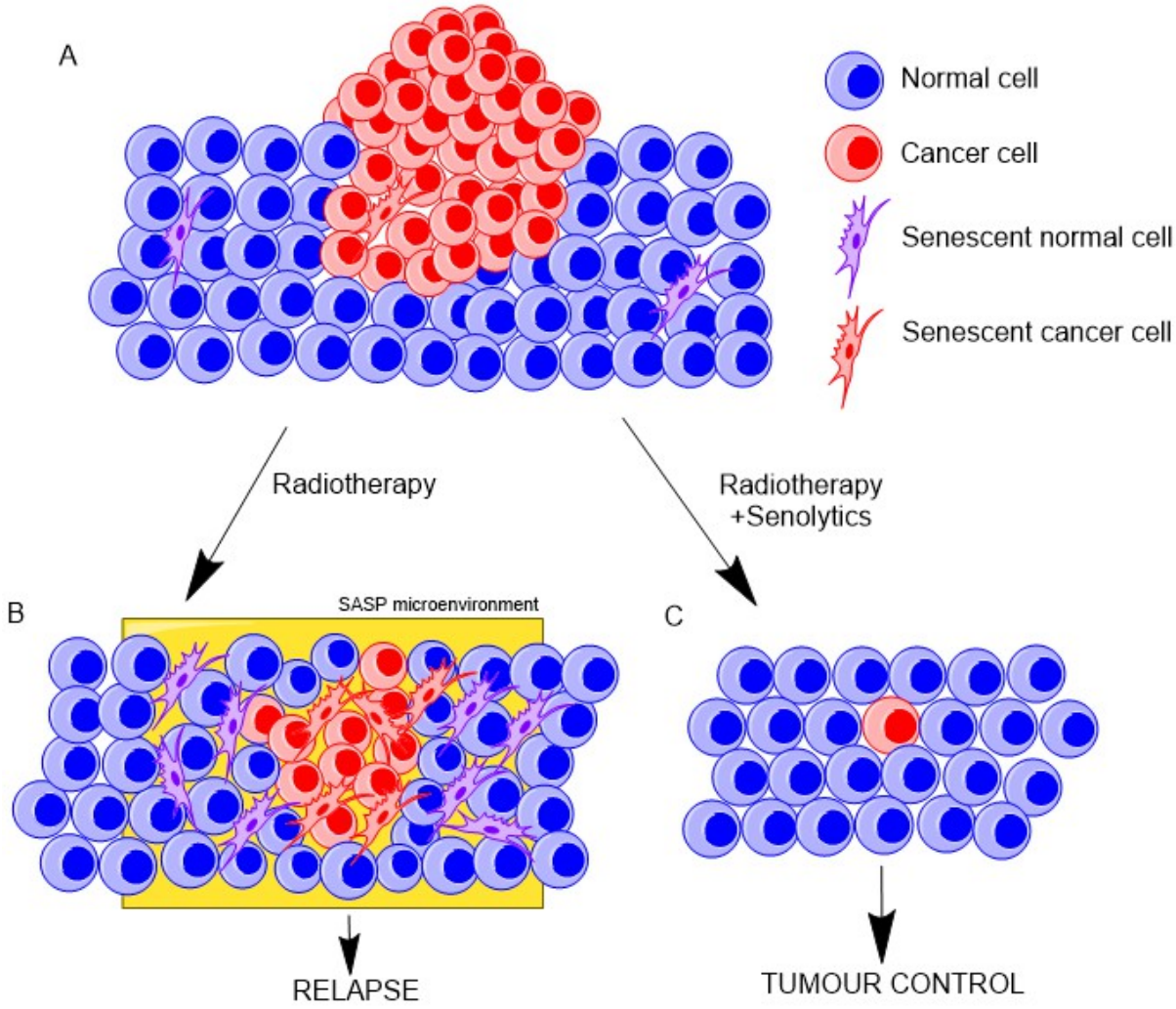


**Figure 2. The potential benefits of senolytics as adjuvants in radiotherapy.** Senescence can occur spontaneously in both normal and cancer cells (A). However, radiotherapy increases the presence of senescent cells in the tumour and the surrounding tissue, leading to the establishment of a microenvironment that facilitates the growth of any surviving cancer cells and, eventually, the relapse of the tumour (B). This is mostly mediated by the SASP, which can also induce senescence on its own. The addition of senolytics as adjuvants in radiotherapy would eliminate senescent cells and could thus prevent the negative effects of the SASP, keeping any cancer cells that survived the treatment in a non-permissive environment (C). This would result in an improved management of the tumour.