Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Review article: Biomedical intellegence | Published 17 January 2017 | doi:10.4414/smw.2017.14367
Cite this as: Swiss Med Wkly. 2017;147:14367

Aging tumour cells to cure cancer: "pro-senescence" therapy for cancer

Calcinotto Arianna, Alimonti Andrea

Institute of Research in Oncology, Bellinzona, Switzerland

Summary

Robust scientific evidence demonstrates that senescence induction in cancer works as a potent weapon to eradicate tumorigenesis. Therapies that enhance senescence not only promote a stable cell growth arrest but also work as a strong stimulus for the activation of the antitumour immune response. However, recent advances suggest that if senescent tumour cells are not cleared from the tumours, they may promote tumour progression and metastasis. In this article, we focus on concepts that are relevant to a pro-senescence therapeutic approach, including caveats, and we propose therapeutic strategies that involve the combined use of pro-senescence therapies with immunotherapies to promote the clearance of senescent tumour cells. In our opinion, these approaches may avoid potential negative effects of pro-senescence therapies and may also enhance the efficacy of currently available immunotherapies.

Key words: senescence, cell autonomous regulation of senescence in cancer, SASP, non-cell autonomous regulation of senescence in cancer, pro-senescence therapy for cancer, SASP reprogramming, immune cell subsets, myeloid-derived suppressor cells

Correspondence:
Andrea Alimonti, Istituto Oncologico della Svizzera Italiana, Via Vincenzo Vela, 6, CH-6500 Bellinzona, andrea.alimonti[at]ior.iosi.ch

Senescence

Senescence is a stable cell cycle arrest that limits the proliferative lifespan of human diploid cells, thereby promoting a gradual deterioration of the human body. In culture, normal cells can reach a maximum of 50 cell divisions before becoming senescent. This phenomenon, known as the "Hayflick limit", occurs as a result of progressive shortening of telomeres upon each cell division [1, 2]. In contrast to normal cells, one of the hallmarks of cancer cells is the capability to escape senescence, thus acquiring a limitless replicative potential that is the prelude to invasion, metastasis and additional features of malignancy [3]. However, cancer cells can undergo senescence if subjected to certain insults such as oncogenic stress, DNA damage and metabolic changes. This type of senescence response occurs immediately and also independently of telomere shortening, a phenomenon known as "premature" senescence [2, 4]. For instance, several anticancer chemotherapies and radiotherapies are known to induce senescence in both normal and cancer cells [5]. Similarly, changes in culture conditions that are associated with metabolic stress are also known to induce senescence [6].

In 2005, four papers reported that senescence can also occur in tumour cells *in vivo* as a consequence of overexpression of oncogenes or loss of tumour suppressor genes, demonstrating for the first time that senescence acts as a barrier against tumorigenesis [7–10]. Analysis of tumour samples from patients affected by prostate tumours, nevi or indolent lymphomas demonstrated that, whereas benign tumours accumulate markers of senescence, invasive cancers lack senescence [7–10]. Subsequent publications validated these findings in different types of tumour [11, 12].

Given the surprising discovery that senescence limits the development of cancer, we and others envisioned targeted therapies that selectively enhanced senescence in cancer cells used for the therapy of various tumours. This approach, named "pro-senescence" therapy for cancer, differs from the chemotherapy-induced senescence that affects both normal and cancer cells [13].

Pro-senescence therapy for cancer: a current challenge?

Several small molecule inhibitors that are currently in clinical development have been reported to induce senescence in cancer [9, 13, 14]. Among these compounds, inhibitors of the cyclin-dependent kinases CDK4/6 have been associated with a high percentage of responses in patients affected by breast cancer and are the most promising pro-senescence compounds currently being tested in the clinic [15]. These compounds promote cellular senescence by decreasing the phosphorylation of retinoblastoma protein (RB), thereby activating E2F transcription factors [15].

Compounds that enhance the level of the tumour suppressor gene p53, such as MDM2 (mouse double minute 2 homolog) inhibitors and PRIMA-1 (proline-rich membrane anchor 1) analogues, have been reported to enhance senescence in tumour cells with normal and mutant p53 and are currently being tested in the clinic for the treatment of haematological and solid tumours [16–18]. Adenoviral delivery of p53, in tumour cells lacking this tumour suppressor gene, also triggers senescence [19]. Gendicine and H101, two p53-adenoviral vectors, have been approved in China in combination with chemotherapy for the treatment of head and neck squamous cell carcinoma. However, these vectors are not widely used for patients around the

world [20]. Another compound that enhances senescence by controlling p53 is dasatinib, an Src and c-Kit kinase inhibitor currently available for use in the clinic [21]. Imetelstat, which is an inhibitor of telomerase activity and controls telomere shortening, has also been shown to be associated with senescence activation in cancer cells [13]. Imetelstat has been recently shown to be effective in patients affected by myelofibrosis and essential thrombocytopenia [22]. Finally, our group has recently demonstrated that an antagonist of the type 2 C-X-C chemokine receptor (CXCR2) can enhance senescence in prostate cancers by blocking the recruitment of tumour infiltrating myeloid cells [23]. Clinical trials are ongoing to assess the efficacy of these compounds in patients affected by different tumours.

Many compounds that are currently being tested at the preclinical level are also promising pro-senescence therapies. Inhibitors of SirT1, a protein deacetylase that negatively regulates p53 function in cancer, induced senescence in preclinical tumour models [24]. MYC inhibitors can also drive a cellular senescence response [25]. The use of small molecules such as 10058-F4 derivatives [26] and RNA interference (RNAi) technologies [27, 28] provides strategies to target MYC for cancer therapy and are currently being tested at the preclinical level. Intriguingly, BET (bromodomain and extra-terminal motif) protein bromodomain inhibitors, such as JQ1, suppress MYC transcription, thereby enhancing senescence, and are a promising class of compounds currently in clinical trials in different cancer patients [29, 30]. From a therapeutic point of view, a current challenge for the development of optimal pro-senescence therapies is to identify compounds that activate senescence in advanced tumour cells without affecting normal cells. Preclinical evidence demonstrates that Skp2 (S-phase kinase-associated protein 2) inhibitors drive senescence in advanced p53 null prostate cancer by upregulating p27 without affecting normal cells [31]. Unpublished observations from our group demonstrates that a γ-secretase inhibitor elicits senescence in Pten;p53 double null prostate cancers by also enhancing p27 levels. Similarly, casein kinase 2 (CK2) inhibitors enhance senescence in phosphatase and tensin homolog (PTEN)-deficient breast and prostate cancer cells, whereas normal cells and wild-type PTEN tumour cells remain unaffected [14]. In MYC overexpressing lymphoma tumours, pharmacological inhibition of CDK2 induces senescence in tumour cells driven by MYC overexpression without affecting normal cells [32].

Taken together, these findings demonstrate that several compounds are capable of enhancing senescence in cancer and that cancer cell-selective induction of senescence represents a strong antitumour response.

Another challenge in the field of senescence therapy for cancer is the lack of clinically validated biomarkers for the identification of senescence in human tumours [33]. The prognostic use of senescence-associated-β-galactosidase (SA-β-galactosidase), a well characterised *in vitro* marker for senescence, has been tested in small trials evaluating the efficacy of neo-adjuvant chemotherapies [34, 35]. Results from these trials demonstrate that this marker increases upon treatment and predicts patient outcome. However, the use of SA-β-galactosidase alone as a unique marker of senescence has been criticised since it can lead to many false positives. Recent findings have identified of new markers of senescence with prognostic relevance [34, 36]. However, neither SA-β-galactosidase staining nor additional markers have been used so far in large clinical trials to evaluate the efficacy of pro-senescence compounds such as CDK4/6, MDM2 inhibitors or imetelstat. Thus, development of novel biomarkers that can accurately assess the occurrence of senescence in cancer patients is the need of the hour. This would help improve the stratification of patients who may respond to therapies that enhance senescence in cancer.

Non-cell autonomous regulation of senescence in cancer

Senescent tumour cells remain stably arrested, but are also metabolically active and communicate with the surrounding tumour microenvironment. Indeed, in contrast to apoptotic cells, senescent cells secrete a number of growth factors, cytokines and proteases that impact on the tumour microenvironment. These secreted proteins constitute the so-called senescence-associated secretory phenotype (SASP). SASP components include inflammatory and immune-modulatory cytokines (e.g. interleukins 6 and 8: IL6 and IL8), growth factors (e.g. insulin-like growth factor-binding proteins: IGFBPs), cell surface molecules (e.g. tumour necrosis factor [TNF] receptors) and survival factors. The SASP can reinforce the senescence programme and influence the tumour microenvironment, impacting on the stroma and on the tumour immune cells [37, 38]. These effects can be either positive or negative, a phenomenon that has been described as the “double-edged sword” of the SASP [39]. For instance, IL8 and IL6, secreted by senescence cells, are needed for the induction and maintenance of senescence. IL8 is a known activator of the innate immune response through its binding to CXCR1 and CXCR2, and inhibition of CXCR2 promotes senescence bypass in oncogene-induced senescence (OIS) [40, 41]. Inhibition of IL6 or IL6 receptors also promotes senescence evasion in OIS [42]. In addition, recent evidence demonstrates that senescent cells can transmit senescence to normal and tumour cells by releasing IL1α. This phenomenon, called “paracrine” senescence, contributes to the tumour suppressive function of senescence in cancer [43] (fig. 1A). Moreover, senescent tumour cells have been described to promote through their SASP both the recruitment and activation of several immune subsets of the innate and adaptive tumour immune responses, including M1-like macrophages, natural killer (NK) cells, and T-helper 1 (Th1) lymphocytes. Such immune-infiltrates can restrain tumour progression by mediating the clearance of senescent tumour cells [44, 45]. Senescence cells can also promote the generation of an antigen-specific immune-surveillance against themselves [44]. Finally, recent findings demonstrate that Th1 lymphocytes are also capable of promoting senescence in tumour cells through the secretion of interferon-γ (IFN-γ) and TNF-α. Such cytokine-induced senescence strictly requires STAT1 (signal transducer and activator of transcription 1) and TNF receptor-1 signalling in addition to p16 INK4A [46]. Therefore, senescent tumour cells can, in principle, recruit T cells that can, in turn, propagate senescence in nonsenescent cells and mediate tumour clearance (fig. 1B). Macrophages secreting trans-

forming growth factor-β (TGF-β) have also been reported to induce senescence in cancer [47] (fig. 1B).

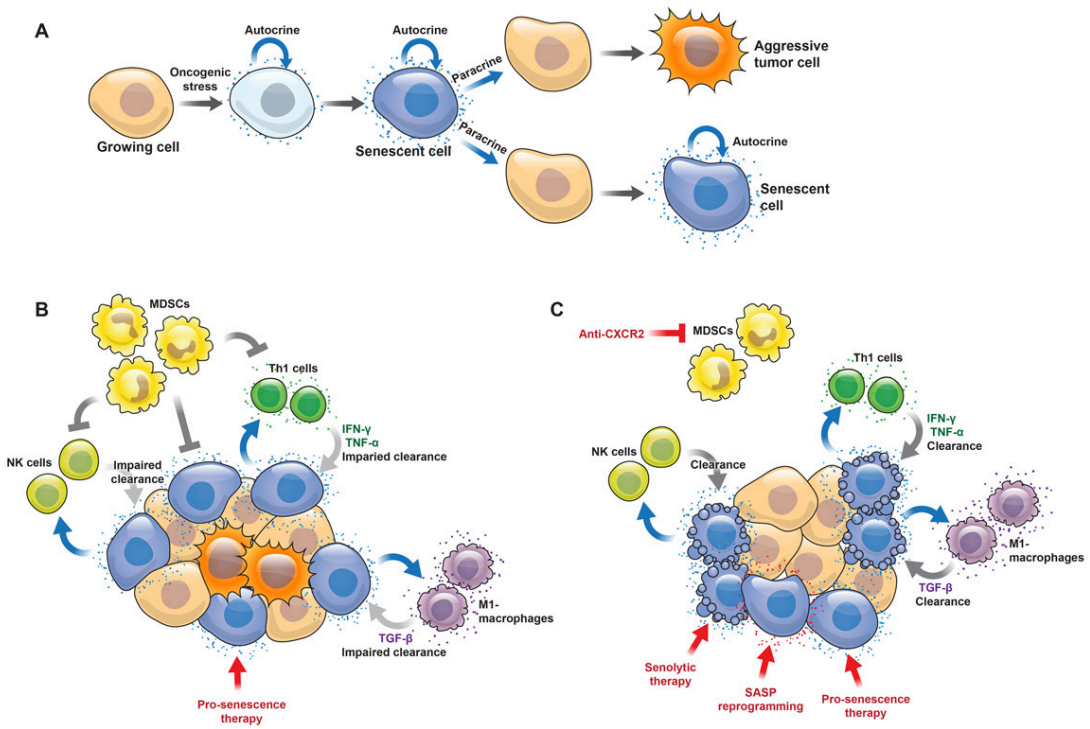
Paradoxically, senescence trough the SASP can also promote tumorigenesis by supporting the proliferation of neighbouring tumour cells and increasing tumour vascularisation [48]. IL8 and IL6 secreted by senescence cells can promote tumorigenesis, acting in a paracrine manner [42, 49]. TGF-β and IL1 secreted by senescent cells have been found to cooperatively support tumorigenesis through activation of the JAK/STAT, TGFβ/SMAD and IL1/NF-κB signalling pathways [50]. CXCL1 secreted by senescent fibroblasts can stimulate the growth of premalignant and malignant mammary epithelial cells [51]. Furthermore, matrix metalloproteinases (MMPs) secreted by senescent cells enhance the tumorigenicity of breast epithelial cells in xenografts models [52]. In addition, the SASP can hinder chemotherapy efficacy by inducing the activation of STAT3 in tumour cells [53]. Thus, factors secreted by senescent cells can exert both autocrine and paracrine effects, which can be either positive or negative (fig. 1A). Therefore, if senescent tumour cells are not removed from the tumours by the tumour immune response, the persistent stimulation of the SASP can promote cell proliferation, angiogenesis and epithelial–mesenchymal transition, thereby sustaining cancer progression [37].

Additional examples of non-cell autonomous regulation of senescence in cancer include sterile inflammation and the gut microbiota. Intriguingly, factors released by damaged tumour cells during sterile inflammation can promote senescence evasion in early tumour lesions through an unidentified mechanism [54]. Finally, metabolites secreted by the gut microbiota can promote senescence evasion and liver tumorigenesis [55]. These data demonstrate that senescence can be regulated in a non-cell autonomous manner at different levels, and by different factors.

SASP reprogramming as therapeutic strategy to enhance the tumour-suppressive power of senescence therapies

As discussed above, SASP has profound effects on the surrounding tumour microenvironment and it represents a promising target for cancer therapy. Several groups have recently proposed therapies that reprogram the SASP to enhance the tumour-suppressive role of senescence in cancer and restrain the negative effects of the SASP. For instance, we have recently shown that Stat3 regulates the

Figure 1: Combinatorial approach to enhance the efficacy of pro-senescent therapy. (A) Oncogenic stress drives growing cells into senescence. Senescent cells actively communicate with their microenvironment through the SASP. Depending on the composition of the SASP, secreted factors can either drive both autocrine and paracrine induction of senescence, or enhance the aggressiveness of neighbouring tumour cells. **(B) Senescence induction in tumours treated with pro-senescence therapy.** Within the tumour microenvironment, senescent tumour cells through the SASP can promote both the recruitment and activation of several immune populations, including M1-macrophages, NK cells, and Th1 cells. Such tumour-infiltrating immune subsets can restrain tumour progression by mediating the clearance of senescent tumour cells, and also promoting senescence. Conversely, MDSCs limit senescence induction in the tumour microenvironment by blocking senescence induction and/or anti-tumour immunity. **(C) Optimisation of pro-senescence therapies for cancer.** Immunotherapies may enhance tumour clearance in tumours treated with pro-senescence therapies. Pharmacological reprogramming of the SASP may increase the anti-tumour immune response in tumours upon treatment with pro-senescence therapies. Senolytic therapies may remove senescence tumour cells in tumours where senescence surveillance is impaired, to avoid negative effects induced by the SASP. Anti-CXCR2 treatment limits MDSC recruitment in the tumour, favouring senescence induction and/or antitumour immunity.Ochre cells represent growing cells; light-blue cells show cells undergoing to senescence; blue cells represent senescent cells; orange cells depict aggressive tumour cells.CXCR = C-X-C chemokine receptor; IFN = interferon; MDSC = myeloid-derived suppressor cells; NK = natural killer; SASP = senescence-associated secretory phenotype; TGF = transforming growth factor; Th1 = type 1 helper T cells; TNF = tumour necrosis factor



SASP of Pten-loss induced cellular senescence (PICS). In Pten null senescent tumours, Stat3 activation promotes an immunosuppressive tumour microenvironment that impairs senescence surveillance. However, pharmacological inhibition of Janus kinase 2 (JAK2) in these tumours induces the reprogramming of the SASP, thus leading to an antitumour immune response that promotes the clearance of senescence tumour cells [56]. The SASP is also controlled by mTOR (mechanistic target of rapamycin). Indeed, mTOR inhibitors reduced SASP by differentially regulating the translation of the MAP kinase-activated protein kinase MK2 through 4EBP1 (eukaryotic translation initiation factor 4E-binding protein 1) [57]. Although mTOR inhibition prevented the pro-tumorigenic effects of the SASP *in vivo*, it also interfered with the induction of paracrine senescence and senescence surveillance, two important tumour suppressive arms of senescence [57]. In another study, rapamycin, a well-known mTOR inhibitor, also suppressed the ability of senescent fibroblasts to stimulate prostate tumour growth in mice by attenuating the SASP [58]. Therefore, JAK2 and mTOR inhibitors could be used in the clinic to decrease some of the negative effects of the SASP in tumours with therapy-induced senescence. The SASP is also regulated by BRD4, one of the bromodomain and extra-terminal motif (BET) proteins. Inhibition of BRD4 by JQ1 blocked the SASP in a model of OIS, thereby affecting senescence surveillance *in vivo* [59]. Therefore, although previous evidence demonstrated that JQ1 induces senescence in cancer [29, 30], it also affects the removal of senescence cells from tumours and should be combined with compounds that reactivate senescence surveillance or that selectively kill senescence tumour cells (senolytic therapy). Simvastatin, a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor known to attenuate inflammation, has also been recently identified as a modulator of the SASP. Interestingly, *in vitro* evidence demonstrates that simvastatin suppresses breast cancer cell proliferation induced by senescent cells [60]. Altogether, these studies highlight the importance of strategies aiming at the SASP reprogramming as potential cancer therapies. Thus, identification of compounds that can attenuate the "dark side" of the SASP without affecting its tumour suppressive function could be used in the clinic to enhance the therapeutic efficacy of pro-senescence compounds.

Manipulation of the tumour immune response for pro-senescence therapy for cancer

Recent evidence from our laboratory demonstrates that tumour-infiltrating myeloid cells promote prostate tumour progression by opposing senescence *in vivo* [23]. In another paper, we have also demonstrated that these Gr1+ myeloid cells are myeloid-derived suppressor cells (MDSCs) because they suppress the recruitment and activation of T cells [56]. MDSCs are a phenotypically heterogeneous cell population that has common biological activity. Cancer formation promotes the migration of MDSCs from the bone marrow to the tumour through the release of several soluble factors that induce myeloid cells trafficking, proliferation, and differentiation [61]. MDSCs mediate senescence evasion in cancer cells through the secretion of IL1 receptor antagonist (IL1RA) into the tumour microenvironment. Indeed, IL1RA blocks the IL1R signalling that is required for PICS. Interestingly, patients with high IL1RA tumour levels did not respond to chemotherapy-induced senescence (docetaxel) and showed a short disease-free survival compared with patients with normal IL1RA levels. Taken together these findings demonstrate that senescence in cancer can be antagonised in a non-cell-autonomous manner by a subset of tumour-infiltrating immune cells. Importantly, we have also shown that treatment with CXCR2 antagonists potentiates senescence by inhibiting MDSC recruitment [23].

An intriguing aspect of the role of MDSCs in cancer is that these cells also have prognostic relevance in cancer patients and affect tumorigenesis not only by blocking senescence but also by additional mechanisms [62]. Several studies demonstrate that the number of circulating MDSCs correlate with poor prognosis in patients affected by head and neck, melanoma, breast, lung and prostate cancers [62–64]. MDSCs, together with regulatory T cells (Tregs) and tumour-associated macrophages, are the main immune subsets responsible for immunosuppression in the tumour microenvironment. Their suppressive activity is mediated by a variety of mechanisms, mostly involving arginase, inducible nitric oxide synthase (iNOS), reactive oxygen species, TGF-β, IL10, and prostaglandin E2. This suppressive activity results in tolerance to cytotxic T lymphocytes (CTLs), which thus lose their effector function at the tumour site [61, 65].

In addition, MDSCs are also involved in a whole array of nonimmunological functions, such as the promotion of angiogenesis, tumour local invasion and metastases. Indeed, MDSCs produce MMPs that can support tumour cell invasion by directly promoting tumour angiogenesis and lymphangiogenesis [66–69]. Several chemotherapies can suppress the MDSC count and it is postulated that this may be critical to benefit from such treatments [70, 71]. However, following anticancer treatments, the frequency of MDSCs does not decline to the level seen in tumour-free mice and healthy human subjects. Moreover, tumour recurrence after several treatments correlates with re-expansion of MDSCs [72]. Therefore, treatments that decrease the trafficking or function of myeloid cells in the tumours may not only enhance the efficacy of pro-senescence therapies but also limit the additional pro-tumorigenic features of MDSCs. Thus, our findings have paved the way for the development of treatments that combine different immunotherapies with pro-senescence compounds, and novel trials are ongoing to validate the relevance of these findings in patients affected by various tumours.

Dual targeting of senescence and the tumour immune response for cancer therapy

Accumulating experimental evidence lends weight to the concept that both cell-autonomous and non-cell-autonomous mechanisms can account for senescence evasion. Consequently, treatments that elicit senescence induction or inhibit senescence evasion in tumours are fundamental to limiting tumour progression. As discussed above, a plethora of pro-senescence compounds with different specificities are currently under evaluation in the clinic. A step forward will be to devise multiple targeted therapies that simultaneously or subsequently target senes-

cence tumour cells and the tumour microenvironment. Because cellular senescence has been associated with the activation of anti-tumour mechanisms, mainly mediated by the SASP and the tumour immune response [37], it is reasonable to hypothesise that the combination with immunotherapy approaches would be the most effective (fig. 1C). A recent report demonstrates that T-cell-activating therapies based on CD137 antibodies enhance the efficacy of pro-senescence compounds in a xenograft model of melanoma [73]. Recent advances in immunotherapy for cancer involve the clinical use of immune checkpoint inhibitors. These therapies have improved the median survival and long-term durable responses of patients affected by various tumours [74]. However, the long-term clinical benefit is limited in a number of patients. This further highlights the importance of combined therapies to improve the survival of treated patients. We believed that pro-senescence therapies might be introduced into the clinic also as an adjuvant regimen to increase the efficacy of immune checkpoint inhibitors. However, preclinical evidence is still needed to support such a claim, and trials are currently ongoing in different laboratories to validate this hypothesis.

The use of senolytic therapies may also enhance the efficacy of pro-senescence therapies by removing senescence cells from the tumour [75]. Senolytic therapies may be administered concomitantly with or after pro-senescence compounds to decrease potential negative side effects of the SASP in tumours where the tumour immune clearance does not take place. As recently reported, senescent tumour cells rely on pro-survival networks and are therefore more susceptible to the inhibition of these pathways. For instance, Bcl-2/Bcl-x inhibitors may be used in combination with pro-senescence compounds to enhance the efficacy of pro-senescence therapy [76]. Since senescent tumour cells also undergo to metabolic reprogramming, pharmacological inhibition of specific metabolic demands may be used to promote the clearance of senescent cells in tumours treated with pro-senescence therapies. Such an approach has been successfully tested in a model of lymphoma but it still remains to be validated in additional tumour models [77]. In conclusion, we believe that pro-senescence therapy for cancer is a promising new therapeutic strategy and that in the future novel, therapies based on senescence induction in cancer will be the standard of care for the treatment of cancer patients.

Disclosure statement

No financial support and no other potential conflict of interest relevant to this article was reported.

References

1 Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25(3):585–621. doi: http://dx.doi.org/10.1016/0014-4827(61)90192-6. PubMed.

2 Courtois-Cox S, Jones SL, Cichowski K. Many roads lead to oncogene-induced senescence. Oncogene. 2008;27(20):2801–9. doi: http://dx.doi.org/10.1038/sj.onc.1210950. PubMed.

3 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. doi: http://dx.doi.org/10.1016/j.cell.2011.02.013. PubMed.

4 d'Adda di Fagagna F. Living on a break: cellular senescence as a DNA-damage response. Nat Rev Cancer. 2008;8(7):512–22. doi: http://dx.doi.org/10.1038/nrc2440. PubMed.

5 Ewald JA, Desotelle JA, Wilding G, Jarrard DF. Therapy-induced senescence in cancer. J Natl Cancer Inst. 2010;102(20):1536–46. doi: http://dx.doi.org/10.1093/jnci/djq364. PubMed.

6 Sherr CJ, DePinho RA. Cellular senescence: mitotic clock or culture shock? Cell. 2000;102(4):407–10. doi: http://dx.doi.org/10.1016/S0092-8674(00)00046-5. PubMed.

7 Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, et al. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature. 2005;436(7051):660–5. doi: http://dx.doi.org/10.1038/nature03841. PubMed.

8 Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature. 2005;436(7051):725–30. doi: http://dx.doi.org/10.1038/nature03918. PubMed.

9 Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, et al. Tumour biology: senescence in premalignant tumours. Nature. 2005;436(7051):642. doi: http://dx.doi.org/10.1038/436642a. PubMed.

10 Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005;436(7051):720–4. doi: http://dx.doi.org/10.1038/nature03890. PubMed.

11 Courtois-Cox S, Genther Williams SM, Reczek EE, Johnson BW, McGillicuddy LT, Johannessen CM, et al. A negative feedback signaling network underlies oncogene-induced senescence. Cancer Cell. 2006;10(6):459–72. doi: http://dx.doi.org/10.1016/j.ccr.2006.10.003. PubMed.

12 Gewinner C, Wang ZC, Richardson A, Teruya-Feldstein J, Etemadmoghadam D, Bowtell D, et al. Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. Cancer Cell. 2009;16(2):115–25. doi: http://dx.doi.org/10.1016/j.ccr.2009.06.006. PubMed.

13 Nardella C, Clohessy JG, Alimonti A, Pandolfi PP. Pro-senescence therapy for cancer treatment. Nat Rev Cancer. 2011;11(7):503–11. doi: http://dx.doi.org/10.1038/nrc3057. PubMed.

14 Kalathur M, Toso A, Chen J, Revandkar A, Danzer-Baltzer C, Guccini I, et al. A chemogenomic screening identifies CK2 as a target for pro-senescence therapy in PTEN-deficient tumours. Nat Commun. 2015;6:7227. doi: http://dx.doi.org/10.1038/ncomms8227. PubMed.

15 O'Leary B, Finn RS, Turner NC. Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol. 2016;13(7):417–30. doi: http://dx.doi.org/10.1038/nrclinonc.2016.26. PubMed.

16 Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013;13(2):83–96. doi: http://dx.doi.org/10.1038/nrc3430. PubMed.

17 Peng X, Zhang MQ, Conserva F, Hosny G, Selivanova G, Bykov VJ, et al. APR-246/PRIMA-1MET inhibits thioredoxin reductase 1 and converts the enzyme to a dedicated NADPH oxidase. Cell Death Dis. 2013;4(10):e881. doi: http://dx.doi.org/10.1038/cddis.2013.417. PubMed.

18 Bykov VJ, Issaeva N, Selivanova G, Wiman KG. Mutant p53-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute database. Carcinogenesis. 2002;23(12):2011–8. doi: http://dx.doi.org/10.1093/carcin/23.12.2011. PubMed.

19 Shi J, Zheng D. An update on gene therapy in China. Curr Opin Mol Ther. 2009;11(5):547–53. PubMed.

20 Chen GX, Zhang S, He XH, Liu SY, Ma C, Zou XP. Clinical utility of recombinant adenoviral human p53 gene therapy: current perspectives. Onco Targets Ther. 2014;7:1901–9. doi: http://dx.doi.org/10.2147/OTT.S50483. PubMed.

21 Dos Santos C, McDonald T, Ho YW, Liu H, Lin A, Forman SJ, et al. The Src and c-Kit kinase inhibitor dasatinib enhances p53-mediated targeting of human acute myeloid leukemia stem cells by chemotherapeutic agents. Blood. 2013;122(11):1900–13. doi: http://dx.doi.org/10.1182/blood-2012-11-466425. PubMed.

22 Tefferi A, Lasho TL, Begna KH, Patnaik MM, Zblewski DL, Finke CM, et al. A Pilot Study of the Telomerase Inhibitor Imetelstat for Myelofibrosis. N Engl J Med. 2015;373(10):908–19. doi: http://dx.doi.org/10.1056/NEJMoa1310523. PubMed.

23 Di Mitri D, Toso A, Chen JJ, Sarti M, Pinton S, Jost TR, et al. Tumour-infiltrating Gr-1+ myeloid cells antagonize senescence in cancer. Nature. 2014;515(7525):134–7. doi: http://dx.doi.org/10.1038/nature13638. PubMed.

24 van Leeuwen I, Lain S. Sirtuins and p53. Adv Cancer Res. 2009;102:171–95. doi: http://dx.doi.org/10.1016/S0065-230X(09)02005-3. PubMed.

25 Yetil A, Anchang B, Gouw AM, Adam SJ, Zabuawala T, Parameswaran R, et al. p19ARF is a critical mediator of both cellular senescence and an innate immune response associated with MYC inactivation in mouse model of acute leukemia. Oncotarget. 2015;6(6):3563–77. doi: http://dx.doi.org/10.18632/oncotarget.2969. PubMed.

26 Huang MJ, Cheng YC, Liu CR, Lin S, Liu HE. A small-molecule c-Myc inhibitor, 10058-F4, induces cell-cycle arrest, apoptosis, and myeloid differentiation of human acute myeloid leukemia. Exp Hematol. 2006;34(11):1480–9. doi: http://dx.doi.org/10.1016/j.exphem.2006.06.019. PubMed.

27 Pastorino F, Brignole C, Marimpietri D, Di Paolo D, Zancolli M, Pagnan G, et al. Targeted delivery of oncogene-selective antisense oligonucleotides in neuroectodermal tumors: therapeutic implications. Ann N Y Acad Sci. 2004;1028(1):90–103. doi: http://dx.doi.org/10.1196/annals.1322.010. PubMed.

28 Civenni G, Malek A, Albino D, Garcia-Escudero R, Napoli S, Di Marco S, et al. RNAi-mediated silencing of Myc transcription inhibits stem-like cell maintenance and tumorigenicity in prostate cancer. Cancer Res. 2013;73(22):6816–27. doi: http://dx.doi.org/10.1158/0008-5472.CAN-13-0615. PubMed.

29 Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146(6):904–17. doi: http://dx.doi.org/10.1016/j.cell.2011.08.017. PubMed.

30 Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomains. Nature. 2010;468(7327):1067–73. doi: http://dx.doi.org/10.1038/nature09504. PubMed.

31 Lin HK, Chen Z, Wang G, Nardella C, Lee SW, Chan CH, et al. Skp2 targeting suppresses tumorigenesis by Arf-p53-independent cellular senescence. Nature. 2010;464(7287):374–9. doi: http://dx.doi.org/10.1038/nature08815. PubMed.

32 Campaner S, Doni M, Hydbring P, Verrecchia A, Bianchi L, Sardella D, et al. Cdk2 suppresses cellular senescence induced by the c-myc oncogene. Nat Cell Biol. 2010;12(1):54–9, 1–14. doi: http://dx.doi.org/10.1038/ncb2004. PubMed.

33 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(11):e1528. doi: http://dx.doi.org/10.1038/cddis.2014.489. PubMed.

34 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(6):1876–83. PubMed.

35 Itahana K, Campisi J, Dimri GP. Methods to detect biomarkers of cellular senescence: the senescence-associated beta-galactosidase assay. Methods Mol Biol. 2007;371:21–31. doi: http://dx.doi.org/10.1007/978-1-59745-361-5_3. PubMed.

36 Wagner J, Damaschke N, Yang B, Truong M, Guenther C, McCormick J, et al. Overexpression of the novel senescence marker β-galactosidase (GLB1) in prostate cancer predicts reduced PSA recurrence. PLoS One. 2015;10(4):e0124366. doi: http://dx.doi.org/10.1371/journal.pone.0124366. PubMed.

37 Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010;5(1):99–118. doi: http://dx.doi.org/10.1146/annurev-pathol-121808-102144. PubMed.

38 Rodier F, Campisi J. Four faces of cellular senescence. J Cell Biol. 2011;192(4):547–56. doi: http://dx.doi.org/10.1083/jcb.201009094. PubMed.

39 Pérez-Mancera PA, Young AR, Narita M. Inside and out: the activities of senescence in cancer. Nat Rev Cancer. 2014;14(8):547–58. doi: http://dx.doi.org/10.1038/nrc3773. PubMed.

40 Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, et al. Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell. 2008;133(6):1006–18. doi: http://dx.doi.org/10.1016/j.cell.2008.03.038. PubMed.

41 Acosta JC, Gil J. A role for CXCR2 in senescence, but what about in cancer? Cancer Res. 2009;69(6):2167–70. doi: http://dx.doi.org/10.1158/0008-5472.CAN-08-3772. PubMed.

42 Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell. 2008;133(6):1019–31. doi: http://dx.doi.org/10.1016/j.cell.2008.03.039. PubMed.

43 Di Mitri D, Alimonti A. Non-Cell-Autonomous Regulation of Cellular Senescence in Cancer. Trends Cell Biol. 2016;26(3):215–26. doi: http://dx.doi.org/10.1016/j.tcb.2015.10.005. PubMed.

44 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(7374):547–51. doi: http://dx.doi.org/10.1038/nature10599. PubMed.

45 Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE, et al. Non-cell-autonomous tumor suppression by p53. Cell. 2013;153(2):449–60. doi: http://dx.doi.org/10.1016/j.cell.2013.03.020. PubMed.

46 Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298–306. doi: http://dx.doi.org/10.1038/nrc3245. PubMed.

47 Reimann M, Lee S, Loddenkemper C, Dörr JR, Tabor V, Aichele P, et al. Tumor stroma-derived TGF-beta limits myc-driven lymphomagenesis via Suv39h1-dependent senescence. Cancer Cell. 2010;17(3):262–72. doi: http://dx.doi.org/10.1016/j.ccr.2009.12.043. PubMed.

48 Coppé JP, Kauser K, Campisi J, Beauséjour CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem. 2006;281(40):29568–74. doi: http://dx.doi.org/10.1074/jbc.M603307200. PubMed.

49 Rodier F, Coppé JP, Patil CK, Hoeijmakers WA, Muñoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol. 2009;11(8):973–9. doi: http://dx.doi.org/10.1038/ncb1909. PubMed.

50 Hubackova S, Krejcikova K, Bartek J, Hodny Z. IL1- and TGFβ-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. Aging (Albany NY). 2012;4(12):932–51. doi: http://dx.doi.org/10.18632/aging.100520. PubMed.

51 Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci USA. 2001;98(21):12072–7. doi: http://dx.doi.org/10.1073/pnas.211053698. PubMed.

52 Liu D, Hornsby PJ. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. Cancer Res. 2007;67(7):3117–26. doi: http://dx.doi.org/10.1158/0008-5472.CAN-06-3452. PubMed.

53 Jackson JG, Pant V, Li Q, Chang LL, Quintás-Cardama A, Garza D, et al. p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. Cancer Cell. 2012;21(6):793–806. doi: http://dx.doi.org/10.1016/j.ccr.2012.04.027. PubMed.

54 Guerra C, Collado M, Navas C, Schuhmacher AJ, Hernández-Porras I, Cañamero M, et al. Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. Cancer Cell. 2011;19(6):728–39. doi: http://dx.doi.org/10.1016/j.ccr.2011.05.011. PubMed.

55 Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499(7456):97–101. doi: http://dx.doi.org/10.1038/nature12347. PubMed.

56 Toso A, Di Mitri D, Alimonti A. Enhancing chemotherapy efficacy by reprogramming the senescence-associated secretory phenotype of prostate tumors: A way to reactivate the antitumor immunity. OncoImmunology. 2015;4(3):e994380. doi: http://dx.doi.org/10.4161/2162402X.2014.994380. PubMed.

57 Herranz N, Gallage S, Mellone M, Wuestefeld T, Klotz S, Hanley CJ, et al. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. Nat Cell Biol. 2015;17(9):1205–17. doi: http://dx.doi.org/10.1038/ncb3225. PubMed.

58 Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, et al. MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat Cell Biol. 2015;17(8):1049–61. doi: http://dx.doi.org/10.1038/ncb3195. PubMed.

59 Tasdemir N, Banito A, Roe JS, Alonso-Curbelo D, Camiolo M, Tschaharganeh DF, et al. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. Cancer Discov. 2016;6(6):612–29. doi: http://dx.doi.org/10.1158/2159-8290.CD-16-0217. PubMed.

60 Liu S, Uppal H, Demaria M, Desprez PY, Campisi J, Kapahi P. Simvastatin suppresses breast cancer cell proliferation induced by senescent cells. Sci Rep. 2015;5:17895. doi: http://dx.doi.org/10.1038/srep17895. PubMed.

61 Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12(4):253–68. doi: http://dx.doi.org/10.1038/nri3175. PubMed.

62 Di Mitri D, Toso A, Alimonti A. Molecular Pathways: Targeting Tumor-Infiltrating Myeloid-Derived Suppressor Cells for Cancer Therapy. Clin Cancer Res. 2015;21(14):3108–12. doi: http://dx.doi.org/10.1158/1078-0432.CCR-14-2261. PubMed.

63 Wesolowski R, Markowitz J, Carson WE, 3rd. Myeloid derived suppressor cells - a new therapeutic target in the treatment of cancer. J Immunother Cancer. 2013;1(1):10. doi: http://dx.doi.org/10.1186/2051-1426-1-10. PubMed.

64 Vuk-Pavlović S, Bulur PA, Lin Y, Qin R, Szumlanski CL, Zhao X, et al. Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate can-

cer. Prostate. 2010;70(4):443–55. doi: http://dx.doi.org/10.1002/pros.21078. PubMed.

65 Bronte V, Kasic T, Gri G, Gallana K, Borsellino G, Marigo I, et al. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. J Exp Med. 2005;201(8):1257–68. doi: http://dx.doi.org/10.1084/jem.20042028. PubMed.

66 Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009;58(1):49–59. doi: http://dx.doi.org/10.1007/s00262-008-0523-4. PubMed.

67 Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell. 2004;6(4):409–21. doi: http://dx.doi.org/10.1016/j.ccr.2004.08.031. PubMed.

68 Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008;8(8):618–31. doi: http://dx.doi.org/10.1038/nrc2444. PubMed.

69 Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature. 2007;450(7171):825–31. doi: http://dx.doi.org/10.1038/nature06348. PubMed.

70 Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, Djeu JY. A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. Clin Cancer Res. 2010;16(18):4583–94. doi: http://dx.doi.org/10.1158/1078-0432.CCR-10-0733. PubMed.

71 Sumida K, Wakita D, Narita Y, Masuko K, Terada S, Watanabe K, et al. Anti-IL-6 receptor mAb eliminates myeloid-derived suppressor cells and inhibits tumor growth by enhancing T-cell responses. Eur J Immunol. 2012;42(8):2060–72. doi: http://dx.doi.org/10.1002/eji.201142335. PubMed.

72 Crittenden MR, Savage T, Cottam B, Bahjat KS, Redmond WL, Bambina S, et al. The peripheral myeloid expansion driven by murine cancer progression is reversed by radiation therapy of the tumor. PLoS One. 2013;8(7):e69527. doi: http://dx.doi.org/10.1371/journal.pone.0069527. PubMed.

73 Vilgelm AE, Johnson DB, Richmond A. Combinatorial approach to cancer immunotherapy: strength in numbers. J Leukoc Biol. 2016;100(2):275–90. doi: http://dx.doi.org/10.1189/jlb.5RI0116-013RR. PubMed.

74 Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205–14. doi: http://dx.doi.org/10.1016/j.cell.2015.03.030. PubMed.

75 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(4):644–58. doi: http://dx.doi.org/10.1111/acel.12344. PubMed.

76 Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell. 2016;15(3):428–35. doi: http://dx.doi.org/10.1111/acel.12445. PubMed.

77 Dörr JR, Yu Y, Milanovic M, Beuster G, Zasada C, Däbritz JH, et al. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature. 2013;501(7467):421–5. doi: http://dx.doi.org/10.1038/nature12437. PubMed.