SENESCENCE

Senescence in tumours: evidence from mice and humans

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Abstract | The importance of cellular senescence, which is a stress response that stably blocks proliferation, is increasingly being recognized. Senescence is prevalent in pre-malignant tumours, and progression to malignancy requires evading senescence. Malignant tumours, however, may still undergo senescence owing to interventions that restore tumour suppressor function or inactivate oncogenes. Senescent tumour cells can be cleared by immune cells, which may result in efficient tumour regression. Standard chemotherapy also has the potential to induce senescence, which may partly underlie its therapeutic activity. Although these concepts are well supported in mouse models, translating them to clinical oncology remains a challenge.

Nevi
Benign skin lesions of melanocytes, also known as moles, which are thought to be senescent.

The initial description of cellular senescence by Hayflick and Moorhead[1] was based on the meticulous analysis of normal human cells grown *in vitro*. They found that, in contrast to cancer cells, normal cells have a finite proliferative capacity that ends in a stable and long-term cell cycle arrest. This is characterized by a lack of response to growth factors, sustained metabolic activity and changes in cell morphology[2]. The molecular basis for this response has been intensively studied and it is now considered to be triggered by a combination of at least three mechanisms: telomere shortening, upregulation of the *CDKN2A* locus (which encodes INK4A and ARF) and accumulation of DNA damage[2]. The relative contribution of these mechanisms to senescence depends on the cell type and the cell culture conditions[2].

More than a decade ago, a phenotype similar to senescence was unexpectedly observed on overexpression of an oncogenic version of HRAS ($HRAS^{G12V}$) in normal cells grown *in vitro*[3]. Normal cells forced to express high levels of the oncogene, rather than increasing their proliferation, stopped dividing and suffered morphological and molecular changes that were indistinguishable from senescence[3]. Two crucial tumour suppressors, INK4A (which activates the RB family) and ARF (which activates p53), were shown to be upregulated in oncogenically stressed cells and to be responsible for the cell cycle arrest imposed on these cells. In this manner, the concept of oncogene-induced senescence emerged as a putative tumour suppressor mechanism, similar to the better known phenomenon of oncogene-induced apoptosis[4]. The occurrence of cellular senescence in mouse and human tumours was originally reported in a series of studies describing the presence of markers of senescence (BOX 1) in pre-malignant tumours and their absence in malignant ones[5–9]. Numerous additional investigations have further refined our understanding of the role of senescence during tumorigenesis. In this Review, we discuss the current *in vivo* evidence linking senescence with tumour suppression.

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Triggers of tumour cell senescence

The oncogene used in the original description of oncogene-induced senescence *in vitro* was $HRAS^{G12V}$ (REF. 3); soon after this description, the Raf–Mek pathway downstream of Ras was revealed as the pathway that is most relevant for the induction of senescence[10,11]. These seminal *in vitro* observations were among the first to be validated *in vivo* using mouse models with inducible endogenous oncogenes (FIG. 1; TABLE 1). In particular, endogenous oncogenic *Kras* ($Kras^{G12V}$) was shown to trigger senescence during the early stages of lung and pancreatic tumorigenesis driven by this oncogene[7]. Subsequent studies by three different laboratories using similar mouse models based on endogenous $Kras^{G12D}$ have confirmed these observations in pre-malignant lesions of the lung (S. Ryeom, personal communication) and pancreas[12] (C. Carriere and M. Korc, personal communication). However, other investigations have not found evidence for senescence in $Kras^{G12D}$-driven lung lesions[13] or $Kras^{G12D}$-driven pancreatic lesions (M. Caldwell and D. Tuveson, personal communication). Understanding the basis for these discrepancies will hopefully shed additional light onto the early stages of tumorigenesis. Importantly, senescence has also been observed in lung tumours and melanocytic nevi when using

At a glance

- Senescence is a stress response prevalent in the aberrant environment of tumours.
- Senescent cells are incapable of further proliferation and therefore tumour cell senescence is a brake to tumour progression.
- A large body of evidence in mouse models indicates that in pre-malignant tumours most cells are senescent, therefore explaining the slow growth and low malignancy of these tumours. There are also examples of senescence in human pre-malignant tumours.
- A class of tumour suppressors (for example, p53, INK4A and ARF) monitors stress signals, and the activation of these proteins triggers senescence. Their loss or inactivation is associated with impaired senescence, unleashing malignant progression.
- Malignant tumours, despite their impaired ability to undergo senescence, can still be forced into senescence if crucial oncogenic pathways are disabled or tumour suppressors are restored.
- Senescent tumour cells are rapidly cleared by immune cells, resulting in efficient tumour regression.
- Senescence constitutes a new end point that might be relevant for the development of new drugs or prognostic markers and the evaluation of therapeutic treatments.

mice with endogenous $Braf^{V600E}$, an oncogenic form of *Braf*[14,15]. The other two Ras family members, NRAS and HRAS, also induce senescence *in vivo*. In particular, transgenic expression of $Nras^{G12D}$ in lymphoid tissue results in lymphocytes that are highly susceptible to senescence following chemotherapy[5]. In the case of HRAS, transgenic-inducible expression of $Hras^{G12V}$ in the mammary gland leads to hyperproliferation when the oncogene is expressed at low levels, but to tumour cell senescence when the oncogene is highly expressed[16]. The observation that differences in HRAS expression levels can affect cellular phenotypes might have important implications for our understanding of the mechanism of induction of cellular senescence (discussed below). Other mouse models of oncogenic *Hras* expression, either from its endogenous promoter or targeted to the bladder epithelium, have certified the existence of tumour cell senescence[17,18]. In the case of chemically induced skin papillomas, which are associated with oncogenic activation of HRAS, senescence has also been documented[7,19] and is mediated by the downstream activation of p38 MAP kinase[20].

In addition to the Ras oncogenes and their proximal downstream kinases, distal effectors of the Ras pathway, such as the E2F family of transcription factors[21], can also induce senescence. For example, the expression of an inducible *E2f3* transgene in the intermediate lobe of the pituitary of mice causes an initial burst of proliferation, but cells subsequently stop dividing, acquire markers of senescence and do not form tumours[8].

The PI3K–Akt pathway also has a crucial role in the generation of proliferative signals. Specific genetic ablation of the gene encoding PTEN, a phosphatase that opposes PI3K activity, in mouse prostate leads to prostate intraepithelial neoplasia (PIN), with features of cellular senescence[6]. Similarly, the targeted expression of AKT1 in the prostate leads to the formation of PIN lesions, which show markers of cellular senescence[22]. Akt signalling branches out to multiple pathways but interestingly mice overexpressing RHEB, which links Akt to mTOR, also produce PIN lesions that are positive for senescence[23]. Another example of cellular senescence that is triggered by the loss of a tumour suppressor is provided by a mouse model of conditional deletion of von Hippel–Lindau (*Vhl*), a tumour suppressor frequently mutated in human renal cell carcinomas. Loss of VHL leads to senescence in the kidney that is associated with increased levels of the cell cycle inhibitor p27 (REF. 24). Finally, transgenic expression of a stabilized form of β-catenin in lymphocytes, rather than triggering lymphomagenesis, results in DNA damage, which is followed by senescence and apoptosis[25].

In summary, over the past few years senescence has moved from the realm of *in vitro* cultured cells to the complexity of mouse tumours driven by various oncogenic pathways (FIG. 1; TABLE 1). Importantly, the analysis of human tumours is starting to provide interesting examples of senescence.

Senescence in human tumours

The observation of tumour cell senescence has not been restricted to mouse models, but has also been reported in humans (TABLE 2). In fact, melanocyte senescence that is associated with the presence of oncogenic $BRAF^{V600E}$ was part of the initial reports on cellular senescence *in vivo*[9]. Similarly, human PIN lesions express markers of senescence, providing a correlate to the mouse models of neoplastic prostate lesions[6,26]. The tumour suppressor neurofibromin 1 (NF1) is another interesting example of cellular senescence in humans[27]. Loss-of-function mutation of *NF1* underlies the familial cancer syndrome known as neurofibromatosis type 1. *NF1* encodes a Ras GTPase-activating protein that is a negative regulator of Ras activity. Therefore, the absence of NF1 results in hyperactivated Ras signalling and the formation of neoplastic lesions, known as neurofibromas, which were shown to express markers of senescence[27]. Finally, pre-malignant human colon adenomas also have features of senescence that are associated with the presence of senescence markers[28–30]. The above data are highly suggestive of tumour cell senescence playing an important part not only in mouse models of tumorigenesis but also in human cancer. Nonetheless, these studies need to be extended to more types of human cancer.

Current concepts of tumour senescence

Senescence is characteristic of pre-malignant tumour stages. One of the first lessons derived from the analysis of senescence in tumours is its close association with the pre-malignant stages of tumorigenesis, but its absence from malignant tumours. Indeed, the original identification of senescent tumour cells was obtained from lung adenomas, pancreatic intraductal neoplasias, PIN lesions and melanocytic nevi, which are all pre-malignant tumours[6,7,9]. By contrast, senescence was absent in their corresponding malignant stages, which are lung adenocarcinomas, pancreatic ductal adenocarcinomas, prostate adenocarcinomas and melanomas, respectively[6,7,31]. All this evidence strongly suggests a role for senescence as a barrier to tumour progression.

Senescence-associated heterochromatin foci
SAHF. Highly condensed chromatin regions established during senescence and thought to function as silencing domains.

Costello syndrome
A complex developmental syndrome with distinctive craniofacial features and predisposition to neoplasia development caused by activating germline mutations in *HRAS*.

Senescence is a tumour suppressive mechanism. Evidence for the tumour suppressor role of senescence was first obtained with mouse models of cancer based on the ablation of the tumour suppressor *Pten* in the prostate[6] or the expression of oncogenic *Nras* in the haematopoietic system[5]. In both cases, the initiating oncogenic event led to the development of senescent pre-malignant lesions with little evidence of apoptosis. Interestingly, loss of senescence markers and full-blown malignancy occurred when the oncogenic event was combined with simultaneous deletion of mediators of the senescence response, such as the tumour suppressor p53 (REF. 6) or the histone methyltransferase of lysine 9 in histone 3 known as SUV39H1 (REF. 5). This histone methyltransferase is involved in the formation of heterochromatin and could be relevant for the formation of senescence-associated heterochromatin foci (SAHF), which are domains of silenced chromatin considered important for the senescent phenotype[32]. The association between senescence and tumour suppression has subsequently been supported in other mouse models of cancer, such as $BRAF^{V600E}$-induced lung tumours[14], $BRAF^{V600E}$-induced melanomas[33] and $HRAS^{G12V}$-induced mammary tumours[16]. In these cases, genetic deletion of *Cdkn2a* or *Trp53* abrogated senescence and allowed progression to malignant stages, providing a compelling case for a causal link between tumour suppression and the induction of senescence by INK4A, ARF and p53 (REFS 14,16,33).

However, not all the senescent pre-malignant stages are strictly dependent on INK4A, ARF and p53. In this regard, senescent pre-lymphomagenic thymocytes produced by enforced expression of β-catenin showed a stable senescent response even in the absence of p53, although loss of p53 allowed some thymocytes to progress to lymphoma[25]. Similarly, the absence of INK4A favoured $BRAF^{V600E}$-induced melanomas, but nevi with detectable senescence were still produced[15]. This supports the data in humans indicating that not all the cells in senescent nevi are positive for INK4A. It also supports the fact that individuals from a Dutch family with hereditary melanoma still developed nevi despite carrying inactivating mutations in both copies of the *CDKN2A* locus[9]. Finally, the deletion of *Vhl* in mouse kidney results in senescence with an associated increase of p27, but not INK4A, ARF or p53. This suggests that other cell cycle regulators are also engaged by aberrant oncogenic activation to implement senescence[24]. Similarly, transgenic AKT1-driven PIN lesions also show increased expression of p27 that is associated with senescence[22]. Moreover, when AKT1 was expressed in combination with genetic deletion of *Cdkn1b* (the gene encoding p27) senescence was absent and mice developed invasive prostate cancer[22]. Together, these observations point to a causal link between loss of senescence and tumour progression to malignant stages, and indicate the existence of redundant mechanisms of senescence.

Levels of oncogene activity determine the outcome of senescence. It is now well established that induction of senescence by oncogenic Ras *in vitro* occurs only when the oncogene is overexpressed, but not when the oncogene is expressed at its normal levels[13,34]. Normal levels of oncogenic KRAS expression are mostly inconsequential, with little or no signs of activation of its canonical downstream effectors Erk and Akt and modest effects on proliferation[13,34]. This is also the case at the organismal level, and mice expressing oncogenic *Kras* from its endogenous promoter are mostly normal and present a normal tissue architecture (excepting the eventual development of a few lung tumours after prolonged latency)[13,34]. The little or minimal phenotype of oncogenic KRAS in cells and tissues probably reflects the operation of negative feedback loops that counteract the effect of oncogenic Ras on its downstream effectors. Accordingly, the signalling produced by oncogenic Ras becomes tumorigenic only when the negative feedback loops are cancelled or when they are surpassed by the upregulation of the oncogene. In support of this, when $HRAS^{G12V}$ expression was carefully titrated in a mouse model, moderate overexpression of $HRAS^{G12V}$ produced focal hyperplasias that did not lead to tumours, whereas high overexpression led to low-grade tumours with senescent markers[16]. Interestingly, tumours with high levels of oncogene expression progressed to full-blown carcinomas only when senescence was cancelled by the genetic deletion of *Cdkn2a* or *Trp53* (REF. 16).

Similar observations have been made using other mouse models. In particular, the expression of oncogenic $Hras^{G12V}$ from its endogenous locus recapitulates the complex phenotypes of Costello syndrome, a human developmental disorder produced by germline mutations in *HRAS*[17,35]. Costello syndrome increases the susceptibility to papilloma, rhabdomyosarcoma and bladder carcinoma development[36]. Interestingly, mice with germline

Box 1 | **Markers of senescence *in vivo***

Although senescent cells usually adopt a large and flat morphology *in vitro*, senescent tumour cells *in vivo* lack distinctive morphological features. Therefore, molecular markers are necessary to qualify a lesion as senescent. Despite the efforts of many laboratories, and the increasing interest in defining cellular senescence, there are still few robust markers of cellular senescence[54]. The single most accepted and widely used marker is the staining for β-galactosidase assessed at a suboptimal pH of 6.0 (known as senescence-associated β-galactosidase; SABG)[55]. Despite the existence of exceptions and limitations[17,18,56,57], this is the most consistent marker for senescent cells and it has been used to demonstrate senescence in a wide variety of cancer settings (TABLES 1,2).

Other classic markers of oncogene-induced senescence are the same proteins involved in the mechanism of cell growth arrest. The products of the *CDKN2A* locus (INK4A and ARF) have proved useful markers of senescence *in vivo*, sometimes even in the absence of a positive SABG stain[17]. For some particular models of *in vivo* senescence, other cell cycle regulators are more informative, such as p21 and p27 (REFS 22,24). Furthermore, molecules involved in the DNA damage response (such as γH2AX) or in the formation of senescence-associated heterochromatin foci (such as the heterochromatin protein HP1γ) have been used as surrogate markers of the process (TABLES 1,2).

DNA microarray analysis of senescence has also provided new markers of senescence, such as the basic helix–loop–helix transcription factor BHLHE40 (also known as DEC1) and decoy receptor 2 (DCR2; also known as TNFRSF10D)[7] that have been used successfully in the identification of *in vivo* senescence (TABLE 1).

All of these markers, however, do not offer compelling evidence of senescence induction if not combined with the concomitant identification of lower levels of proliferation, as typically determined by low Ki67 or bromodeoxyuridine (BrdU) labelling.

Hras^{G12V} were prone to developing skin papillomas and angiosarcomas, which were invariably associated with DNA amplification of the oncogene and senescence[17]. Moreover, a zebrafish model expressing oncogenic *hras*^{G12V} (REF. 37) also recapitulates phenotypes of human Costello syndrome, including a high susceptibility to tumour development, and interestingly senescent cells can be detected in the heart and brain[37].

In summary, normal or low levels of oncogenic expression are inconsequential or result in hyperplasias that do not lead to tumours; high levels of oncogenic signalling are necessary to produce pre-malignant tumours and for the engagement of senescence. The signal amplifying events responsible for the transition from low to high oncogenic signalling can be increased gene copy number, increased transcriptional activity of the oncogene promoter or loss of negative regulators of the oncogenic signalling.

Finally, caution is necessary as the ideas expressed above are derived from the study of Ras oncogenes and might not apply to all oncogenes and cell types. For example, physiological levels of BRAF^{V600E} seem to be sufficient to trigger senescence in melanocytes grown *in vitro*[9].

Tumour suppressors inducing and preventing senescence. From work on mouse models, an interesting distinction emerges among tumour suppressors regarding their role in senescence: some of them function upstream of oncogenes and prevent senescence, while others function downstream of oncogenes and induce senescence (FIG. 2). In particular, the tumour suppressors PTEN, VHL, NF1 and RB constitutively oppose pro-oncogenic signals from PI3K, hypoxia-inducible factor 1α (HIF1α), Ras and E2F, respectively. Consequently, their deletion results in excessive proliferative signalling that eventually leads to senescence. Conversely, the tumour suppressors p53, INK4A and ARF monitor the presence of oncogenic signalling, which activates them and triggers senescence. In agreement with the sensor function of these tumour suppressors, their expression or activity under normal, non-stressful, conditions is generally low, but they become strongly expressed and/or activated after oncogenic signalling[2]. Therefore, there are tumour suppressors that basally control oncogenes and the activity of these suppressors is constitutively necessary to prevent oncogene-induced senescence (for example, PTEN, VHL, NF1 and RB, which function upstream of oncogenes). However, there are others such as p53, INK4A and ARF that function occasionally and inducibly when oncogenic signalling is unleashed (therefore functioning downstream of oncogenes) (FIG. 2). In this context, telomere shortening can also be considered as one of the inducible tumour suppressors downstream of oncogenes. Specifically, critically short telomeres occur on excessive proliferation, and they trigger senescence to prevent tumour formation[38,39] (reviewed in REF. 40).

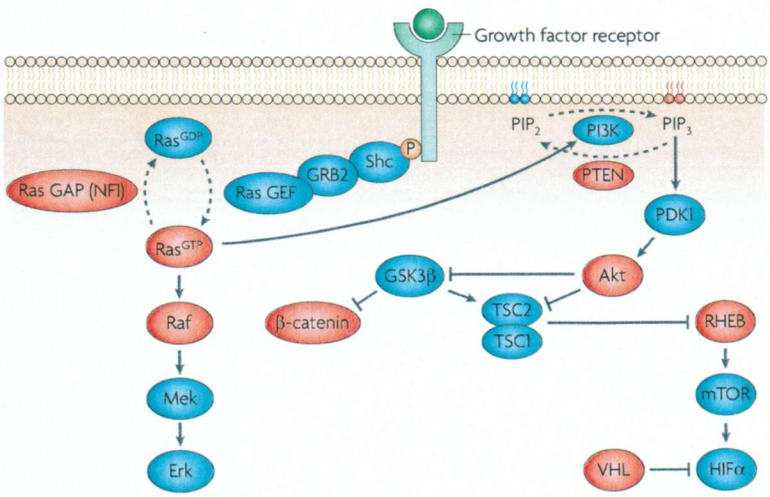

Figure 1 | **Pathways of oncogene-induced senescence.** Signalling pathways that lead to senescence induction *in vivo* when aberrantly activated. Oncogenes and tumour suppressers shown in red correspond to those that, when mutated, have been shown (using experiments) to lead to senescence *in vivo*. GRB2, growth factor receptor-bound protein 2; GSK3β, glycogen synthase kinase 3β; HIF1α, hypoxia-inducible factor 1α; NF1, neurofibromin 1; P, phosphorylation; PDK1, 3-phosphoinositide-dependent kinase 1; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; Ras GAP, Ras GTPase-activating protein; Ras GEF, Ras guanine nucleotide exchange factor; TSC, tuberous sclerosis; VHL, von Hippel–Lindau.

Senescence as anticancer therapy

As mentioned above, progression to malignancy involves bypassing or inhibiting crucial mediators of senescence, however, this does not mean that malignant cells have completely lost their capacity to undergo senescence. Given the tumour suppressive potential of senescence, testing the efficacy of senescence-inducing interventions for the treatment of cancer is of interest. Mouse models support the predicted therapeutic capacity of senescence-inducing treatments, and a few human studies are also suggestive of chemotherapeutic responses mediated by senescence.

Senescence after restoration of tumour suppression. Two recent reports have addressed the anti-tumour efficacy of restoring the tumour suppressive function of p53 using mouse models in which p53 can be switched off and on. The development of both spontaneous and radiation-induced tumours was assessed using different genetic approaches to ablate and then restore p53 function. Most of the sarcomas, lymphomas and liver carcinomas that developed in the absence of p53 regressed after p53 restoration[41,42]. Interestingly, although massive apoptosis accounted for lymphoma regression, sarcomas and liver carcinomas regressed in association with a potent senescent response. Tumour regression by senescence was accompanied by the presence of tumour-infiltrating neutrophils, macrophages and natural killer cells[42]. This suggests that senescent tumour cells, in contrast to non-senescent tumour cells, are efficiently cleared by immune cells. Another interesting observation from these studies is that artificial expression of p53 triggers senescence or apoptosis selectively in tumour cells, leaving normal tissues completely unaffected[41]. The reason for this discrimination is that the aberrant context of tumour cells

Table 1 | **Mouse models of tumour cell senescence**

Gene	Tissue or tumour	Evidence of senescence*	Refs
Oncogene activation			
Hras^{G12V}	Mammary tumours, bladder tumours, and DMBA and TPA-induced skin papillomas	SABG, γH2AX, p53, INK4A, p21, ARF and low Ki67	16–20
Kras^{G12V}	Lung adenomas and pancreatic intraductal neoplasias	SABG, INK4A, BHLHE40, DCR2, INK4B, HP1γ and low Ki67	7
Nras^{G12D}	Lymphoproliferative disorders	SABG	5
Braf^{V600E}	Nevi and lung adenomas	SABG, BHLHE40, ARF and low Ki67	14,15
Rheb	Prostate intraepithelial neoplasia	SABG and low Ki67	23
E2f3	Pituitary hyperplasia	SABG, INK4A and ARF; presence of SAHF; and low BrdU	8
Akt1	Prostate intraepithelial neoplasia	SABG, p27, HP1α, HP1γ and low BrdU	22
Ctnnb1 (which encodes β-catenin)	Thymus	SABG, INK4B, INK4A, BHLHE40, CTSF, CDH16 and low BrdU	25
Oncogene inactivation			
Myc	Lymphoma, osteosarcoma, liver carcinoma and lung carcinoma	SABG, INK4B, p21 and presence of H3K9me3	46,47
Tumour suppressor inactivation			
Pten	Prostate intraepithelial neoplasia	SABG, ARF, p53, p21 and low Ki67	6
Rb1	Thyroid C cell adenomas	SABG, HP1γ, INK4A and presence of H3K9me3	58
Vhl	Kidney	SABG, p27 and DCR2	24
Tumour suppressor activation			
Trp53	Sarcomas and liver carcinomas	SABG, INK4B, INK4A and DCR2; low histone H3 phosphorylation; and low Ki67	41,42

BHLHE40, basic helix–loop–helix family, member e40 (also known as DEC1); BrdU, bromodeoxyuridine; CDH16, cadherin 16; CTSF, cathepsin F; DCR2, decoy receptor 2 (also known as TNFRSF10D); H3K9me3, histone H3 lysine 9 trimethylation; HP1, heterochromatin protein 1; SABG, senescence-associated β-galactosidase; SAHF, senescence-associated heterochromatin foci; *Vhl*, von Hippel–Lindau. *Evidence of senescence based on staining and immunohistochemistry.

constitutively generates p53-activating signals, whereas in non-tumoural tissues, mere expression of p53 is not followed by its activation. Together, these experimental data support the idea that the development of drugs targeted at restoring p53 function in tumours might provide an effective means of restricting tumour growth by senescence and promoting tumour cell clearance, while sparing normal tissues. Such p53-restoring drugs are currently being developed (reviewed in REF. 43) and some of their preliminary characterizations lend support for a senescence-inducing effect on tumour cells[44,45].

Senescence in response to oncogene inactivation. Restoring lost or inactive tumour suppressors is not the only possible therapeutic intervention to induce senescence. Studies to determine the necessity of oncogenic signalling for the maintenance of the malignant phenotype have also shown the potency of cellular senescence in controlling tumour growth. Mouse models of *Myc* transgenic expression under inducible tissue-specific promoters showed that the maintenance of MYC-initiated hepatocellular carcinomas, lymphomas or osteosarcomas depends on the continuous expression of the oncogene[46]. Interestingly, switching off *Myc* transgenic expression in these tumours caused rapid regression accompanied by cellular senescence[46]. Furthermore, inactivation of crucial senescence mediators, such as INK4A, RB and p53, abolished senescence and tumour regression[46]. Therefore, targeting crucial oncogenes can reactivate senescence and induce tumour regression even in full-blown malignancies.

Oncogene inactivation may also induce cellular senescence when the targeted oncogene is not the tumour-initiating event. This is the case for KRAS^{G12D}-initiated lung carcinomas, in which inactivation of the three Myc paralogues, MYC, MYCN and MYCL1, by an artificial dimerization partner known as Omomyc, results in tumour regression in association with apoptosis and senescence[47]. Interestingly, Myc inactivation for 1 month had only mild adverse effects on normal tissues, which were fully reversed on interruption of Omomyc expression[47]. These results are remarkable because they show that senescence can be engaged in established tumours by targeting molecules other than the actual initiating oncogene, leading to tumour regression while sparing normal cells. On this basis, it is reasonable to expect that therapeutic interventions aimed at targeting molecules required to support tumour growth would also lead to cellular senescence induction.

Table 2 | **Human tumours showing cell senescence**

Associated oncogenic event	Tumour	Evidence of senescence*	Refs
NF1 inactivation	Dermal neurofibromas	SABG and INK4A	27
$BRAF^{V600E}$ mutation	Nevi	SABG, INK4A and low Ki67	31
Not determined	Prostate intraepithelial neoplasia	SABG and CXCR2	6,26
Not determined	Colon adenomas	SABG, INK4A and IL-8	28–30

CXCR2, chemokine (C-X-C) receptor 2; IL-8, interleukin-8; *NF1*, neurofibromin 1; SABG, senescence-associated β-galactosidase.
*Evidence of senescence based on staining and immunohistochemistry.

Senescence-inducing chemotherapy. Current anti-tumour strategies are designed to kill cancer cells, although they are often limited by pro-survival alterations present in cancer cells and by their toxicity to normal cells. Senescence-inducing drugs, by attacking tumour cells from a different angle, might prove effective alone or in combination with classic therapeutic approaches, and might offer an opportunity to reduce the toxicity of chemotherapy. Mouse models of chemotherapy have shown that MYC-initiated lymphomas respond to cyclophosphamide by inducing tumour cell senescence mediated by INK4A and p53, and this correlated with a better prognosis following chemotherapy[48].

In the case of human cancer cells grown *in vitro*, classic chemotherapy often induces senescence at moderate doses and apoptosis at higher ones[49].

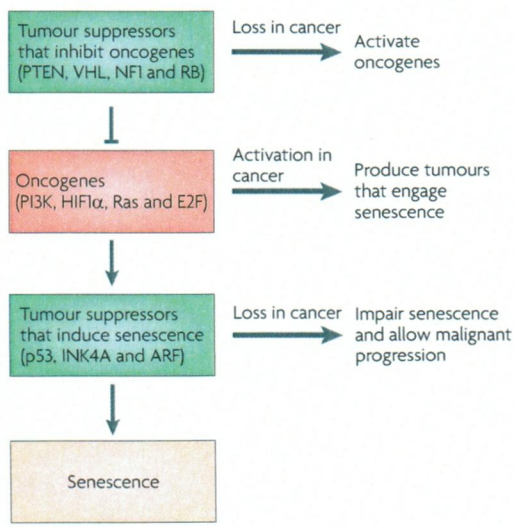

Figure 2 | **Tumour suppressors can be grouped into two categories depending on their effect on senescence.** Some tumour suppressors (at the top of the cascade, that is upstream of oncogenes) prevent excessive oncogenic signalling in a constitutive manner. Loss of these tumour suppressors results in activation of oncogenes, and subsequently the induction of senescence. Other tumour suppressors (downstream of oncogenes) are normally inactive except when they sense excessive oncogenic signalling and induce senescence. Loss of these tumour suppressors impairs senescence and allows progression to malignancy. HIF1α, hypoxia-inducible factor 1α; NF1, neurofibromin 1; VHL, von Hippel–Lindau.

Chemotherapy-induced regression of human cancers is not always explained by an apoptotic response[50], and it is conceivable that senescence could have an important role in chemotherapy, as has been shown in mouse models of cancer. In this regard, two reports analysing senescence markers in biopsies from patients with lung or breast cancer after neoadjuvant chemotherapy have observed chemotherapy-induced senescence and its association with treatment success[51,52]. More recently, the analysis of biopsy material from patients with prostate cancer has shown that chemotherapy induces markers of senescence[53].

A note of caution for senescence-inducing therapies. As a note of caution, we must bear in mind the potential problems that might arise from senescence-inducing therapies. It is conceivable that cancer cells in a senescence-like state might remain as 'dormant' tumour cells and therefore represent a dangerous potential for tumour relapse. In this regard it is important to gain a deeper knowledge of the mechanisms responsible for senescent tumour cell clearance.

In addition, senescent cells show a robust secretory phenotype, known as a senescence-associated secretory phenotype (SASP), in which cells release several pro-inflammatory cytokines, chemokines and tissue-remodelling enzymes. Some of these factors, such as interleukin-6 (IL-6) and IL-8, have a cell autonomous function that reinforces senescence in a paracrine manner[26,30]. It can be speculated that another role of SASP could be to stimulate the clearance of senescent cells by the immune system. However, SASP components might also stimulate the malignant phenotypes of nearby tumour cells[53].

Future prospects

Mouse models of cancer have demonstrated that senescence is associated with pre-malignant stages of neoplastic transformation and has a crucial function in preventing tumour progression. Interestingly, senescent tumour cells are not only growth arrested but can be also cleared by phagocytic cells[41]. Therefore, senescence-inducing drugs could represent an ideal opportunity to increase the arsenal of anticancer weapons. Finally, studies on human cancer samples should establish whether senescence is or is not relevant for cancer progression and therapeutic responses.

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Competing interests statement
The authors declare no competing financial interests.

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