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Senescence in tumours: evidence from mice and humans

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Preface

Cellular senescence is a stress response that stably blocks proliferation and whose importance in cancer is increasingly recognized. Senescence is prevalent in premalignant tumours and progression to malignancy requires evading senescence. Malignant tumours, however, may still undergo senescence by interventions that restore tumour suppressors or inactivate oncogenes. Senescent tumour cells can be cleared by immune cells and this may result in efficient tumour regression. Standard chemotherapy also has the potential to induce senescence, which may underlie part of its therapeutic activity. While these concepts are well supported in mouse models, it remains ahead to translate them to clinical oncology.

Introduction

The initial description of cellular senescence by Hayflick and collaborators was based on the meticulous analysis of normal human cells grown *in vitro* [1]. 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, characterized by 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 a combination of at least three mechanisms, namely, telomere shortening, upregulation of the *CDKN2A* locus (which encodes INK4A and ARF), and accumulation of DNA damage, whose relative contribution to senescence depends on the cell type and the cell culture conditions[2].

More than a decade ago now, a phenotype similar to senescence was unexpectedly observed in normal cells grown *in vitro* upon overexpression of an oncogenic version of HRAS ($HRAS^{G12V}$)[3]. Normal cells forced to express high levels of the oncogene, rather than increasing their proliferation, stopped dividing and suffered morphological and molecular changes indistinguishable from senescence[3]. Two crucial tumour suppressors, INK4A and 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 (OIS) emerged as a putative tumour suppressor mechanism, similar to the better-known phenomenon of oncogene-induced apoptosis[4]. The occurrence of cellular senescence within murine and human tumours was originally reported in a series of studies describing the presence of markers of senescence (see Box1) in premalignant tumours and their absence in malignant ones[5-9]. Since then, 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 OIS *in vitro* was $HRAS^{G12V}$ [3], and soon after, the Raf/Mek pathway downstream of Ras was revealed as the most relevant one leading to senescence[10, 11]. These seminal observations *in vitro* were among the first to be validated *in vivo* using mouse models with inducible endogenous oncogenes (Figure 1 and Table 1). In particular, endogenous $Kras^{G12V}$ 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 premalignant lesions of the lung (S. Ryeom, personal communication) and pancreas[12] (C. Carriere and M. Korc, personal communication). However, other investigators 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 been also observed in lung tumours and melanocytic nevi [SEE GLOSSARY] when using mice carrying endogenous $Braf^{V600E}$, an oncogene from the Raf family[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 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 latter observations 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 HRAS oncogenic activation, 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, expression of an inducible *E2f3* transgene in the intermediate lobe of the pituitary of mice causes an initial burst of proliferation, but then cells stop dividing, acquire markers of senescence and do not form tumours[8].

The PI3K/Akt pathway also plays 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, targeted expression of AKT1 in prostate leads to formation of PIN lesions, which show markers of cellular senescence[22]. Akt signalling branches out into multiple pathways but, interestingly, mice overexpressing RHEB, which links Akt to mTOR, also produces PIN lesions positive for senescence in the prostate[23]. Another example of cellular senescence triggered by the loss of a tumour suppressor is provided by a mouse model of conditional deletion of *Vhl*, a tumour suppressor frequently mutated in human renal cell carcinomas. Loss of VHL leads to senescence in the kidney associated with increased levels of the cell cycle inhibitor p27 [24]. Finally, transgenic expression of a stabilized form of β-catenin in lymphocytes, rather than triggering lymphomagenesis, results in DNA damage, followed by senescence and apoptosis[25].

In summary, during the last years senescence has moved from the realm of *in vitro* cultured cells to the complexity of experimental mouse tumours driven by a variety of oncogenic

pathways (see Figure 1 and 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 associated to 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 nice correlate to the mouse models of neoplastic prostate lesions[6]. The tumour suppressor NF1 constitutes another interesting example of cellular senescence in humans[26]. Loss-of-function mutation of *NF1* underlies the familial cancer syndrome known as neurofibromatosis type I. *NF1* encodes a Ras GTPase-activating protein that is a negative regulator of Ras activity. Thus, absence of NF1 results in hyperactivated Ras signalling and formation of neoplastic lesions known as neurofibromas, which were shown to express markers of senescence[26]. Finally, premalignant human colon adenomas also show features of senescence associated with the presence of DNA damage markers[27] and with the expression of p53β (a p53 isoform) and INK4A[28]. The above data are highly suggestive of tumour cell senescence playing an important role 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 on tumour senescence

Senescence is characteristic of premalignant tumour stages

One of the first lessons derived from the analysis of senescence in tumours is its close association with the premalignant stages of tumorigenesis, but its absence from malignant tumours. Indeed, the original identification of senescent tumour cells was obtained on lung adenomas, pancreatic intraductal neoplasias, prostate intraepithelial neoplasias and melanocytic nevi, which are all pre-malignant tumours[6, 7, 9]. In contrast, senescence was absent in their corresponding malignant stages, namely, lung adenocarcinomas, pancreatic ductal adenocarcinomas, prostate adenocarcinomas and melanomas, respectively[6, 7, 29]. All these evidences strongly suggest a role of senescence as a barrier to tumour progression.

Senescence is a tumour suppressive mechanism

Evidence for the tumour suppressor role of senescence has been obtained with mouse models of cancer triggered by ablation of the tumour suppressor *Pten* in the prostate[6] or oncogenic *Nras* expression in the haematopoietic system[5]. In both cases, the oncogenic initiating event led to the development of senescent premalignant lesions with little evidence of apoptosis. Interestingly, full-blown malignancy and loss of senescence markers occurred when the oncogenic event was combined with simultaneous deletion of mediators of the senescence response, such as the tumour suppressor p53 [6] or the histone methyltransferase of lysine 9 in histone 3 known as SUV39H1 [5]. This histone methyltransferase is involved in the formation of heterochromatin and could be relevant for the formation of senescence-associated heterochromatin foci (or SAHFs, [SEE GLOSSARY]), which are domains of silenced chromatin considered important for the senescent phenotype [30]. The association between senescence and tumour suppression has been subsequently supported in other mouse cancer models, such as BRAF^{V600E}-induced lung tumours[14], BRAF^{V600E}-induced melanomas[31], and HRAS^{G12V}-induced mammary tumours[16]. In these cases, genetic deletion of *Cdkn2a* (encoding INK4A and ARF) or *Trp53* (which encodes p53), abrogated senescence and allowed progression to malignant stages, providing a compelling case for a causal link between the induction of senescence by INK4A, ARF and p53 and tumour suppression[14, 16, 31].

Not all the senescent premalignant stages are, however, strictly dependent on INK4A, ARF or p53. In this regard, senescent pre-lymphomagenic thymocytes in mice with β-catenin expression showed a stable senescent response even in the absence of p53, although loss of this tumour suppressor allowed progression to lymphoma[25]. Similarly, the absence of INK4A favoured $BRAF^{V600E}$-induced melanomas, but nevi with detectable senescence were still produced[15]. This is in agreement with data in humans indicating that not all the cells in senescent nevi are positive for INK4A, and also with the fact that individuals from a Dutch family with hereditary melanoma carrying inactivating mutations in both copies of the *CDKN2A* locus still develop nevi[9]. Finally, deletion of *Vhl* in the mouse kidney results in senescence with associated increase in p27, but not of INK4A, ARF or p53, suggesting that other cell cycle regulators can also be engaged by aberrant oncogenic activation to implement senescence[24]. Similarly, transgenic AKT1-driven PIN lesions also show increased expression of p27 associated to senescence[22]. Moreover, when these transgene 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 redundant mechanisms of senescence whose relevance depends on the tissue type and the driving oncogenes.

Levels of oncogene activity determine the outcome of senescence

It is now well established that induction of senescence by oncogenic Ras *in vitro* only occurs when the oncogene is overexpressed, but not when the oncogene is expressed at its normal levels[13, 32]. As a matter of fact, normal levels of expression of oncogenic KRAS are for the most part inconsequential, with little or no signs of activation of its canonical downstream effectors Erk and Akt, and with modest effects on proliferation[13, 32]. This is also the case at the organismal level and mice expressing oncogenic *Kras* from its endogenous promoter are largely normal and present a normal tissue architecture (aside from the eventual development of a few lung tumours after prolonged latency)[13, 32]. The little or minimal phenotype of oncogenic KRAS in cells and tissues likely reflects the operation of negative feedback loops that counteract the effect of oncogenic Ras on its downstream effectors. According to this view, the signalling produced by oncogenic Ras only becomes tumorigenic when the negative feedback loops are cancelled or when they are surpassed by upregulation of the oncogene above its normal expression levels. In support of this, when $HRAS^{G12V}$ expression was carefully titrated on a mouse model[16], 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. Interestingly, tumours with high levels of oncogene expression only progressed to full-blown carcinomas when senescence was cancelled by the absence of INK4A, ARF or p53 [16].

Conceptually similar observations have been made using other mouse models. In particular, expression of oncogenic $Hras^{G12V}$ from its endogenous locus recapitulates the complex phenotypes of the Costello syndrome [SEE GLOSSARY], a human developmental disorder produced by germline mutations in *HRAS*[17, 33]. Costello syndrome increases the susceptibility to develop papillomas, rhabdomyosarcomas and bladder carcinomas[34]. Interestingly, mice with germline $Hras^{G12V}$ 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 $hras^{G12V}$ [35] also recapitulates phenotypes of human Costello syndrome and high susceptibility to tumour development, and, interestingly, senescent cells can be detected in heart and brain[35].

Overall, a picture emerges by which normal or low levels of oncogenic expression are inconsequential or result in hyperplasias that do not lead to tumours; while, high levels of oncogenic signalling are necessary to produce premalignant tumours and the engagement of senescence. The signal amplifying events during tumorigenesis can consist in increased gene

copy number, increased transcriptional activity of the oncogene promoter, or loss of negative regulators of the oncogenic signalling.

Finally, it should be warned that the above ideas are derived from the study of Ras oncogenes and may not apply to all oncogenes and cell types. For example, physiological levels of $BRAF^{V600E}$ appear to be sufficient to trigger senescence in *in vitro* grown melanocytes[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 act "downstream of oncogenes" and induce senescence (Figure 2). In particular, tumour suppressors PTEN, VHL, NF1 and RB constitutively oppose pro-oncogenic signals emanating, respectively, from PI3K, HIF1α, Ras, or E2f. Consequently, their deletion results in excessive proliferative signalling that eventually leads to senescence. On the other hand, 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 very low, but they become strongly expressed and/or activated after oncogenic signalling[2]. Therefore, there are tumour suppressors that basally control oncogenes and whose activity is constitutively necessary to prevent oncogene-induced senescence (PTEN, VHL, NF1 and RB) ("upstream of oncogenes"), while others (p53, INK4A and ARF) act occasionally and inducibly when oncogenic signalling is unleashed ("downstream of oncogenes") (Figure 2). In this context, telomere shortening can also be considered among the last group of tumour suppressors. Specifically, critically short telomeres occur upon excessive proliferation and trigger senescence to prevent tumour formation[36, 37] (reviewed in [38]).

Senescence as anti-cancer therapy

As mentioned above, progression to malignancy involves bypassing or inhibiting critical 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, it is of interest to test the efficacy of senescence-inducing interventions for the treatment of cancer. Mouse models have provided support to 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 addressed the anti-tumour efficacy of restoring the tumour suppressive function of p53 using mouse models in which p53 can be switched off and back on again. Both spontaneous and radiation-induced tumour development were assessed using different genetic approaches to ablate and then restore p53 function. The majority of sarcomas, lymphomas and liver carcinomas that developed in the absence of p53 regressed after p53 restoration[39, 40]. Interestingly, while 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[40]. This suggests that senescent tumour cells, in contrast to non-senescent tumour cells, are efficiently cleared out by immune cells. Another interesting point from these studies is that artificial expression of p53 only triggers senescence or apoptosis in tumour cells, but is inconsequential in normal tissues[39]. The reason for this discrimination is that the aberrant context of tumour cells constitutively

generates p53-activating signals; whereas in non-tumoural tissues, mere expression of p53 is not followed by its activation. Together, these experimental data offer support for the idea that development of drugs targeted at restoring p53 function in tumours might provide effective means of restricting tumour growth by senescence and even promoting tumour cell clearance, while sparing normal tissues. Such p53 restoring drugs are been developed (reviewed in [41]) and some of their preliminary characterizations lend support for a senescence-inducing effect on tumour cells[42, 43].

Senescence in response to oncogene inactivation

Restoring lost or inactive tumour suppressors is not the only possible therapeutic intervention to induce senescence. Studies aimed at determining 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[44]. Interestingly, switching-off *Myc* transgenic expression in these variety of tumors caused rapid regression accompanied by cellular senescence[44]. Even more, inactivation of crucial senescence mediators, such as INK4A, RB or p53, abolished senescence and tumour regression[44]. Therefore, targeting critical 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 of $KRAS^{G12D}$-initiated lung carcinomas, where inactivation of the three Myc paralogs, MYC, NMYC and LMYC, by means of an artificial dimerization partner known as Omomyc, results in tumour regression in association with apoptosis and senescence[45]. Interestingly, Myc inactivation during 1 month only had mild adverse effects on normal tissues that were fully reversed upon interruption of Omomyc expression[45]. These results are remarkable because they show that senescence can be engaged in established tumours even by targeting molecules other than the actual initiating oncogene, leading to tumour regression while sparing normal cells. Based on this, it is reasonable to expect that therapeutic interventions aimed at targeting molecules required to support tumour growth would also lead to cellular senescence induction.

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. Senescence-inducing drugs, by attacking tumour cells from a different angle, might prove effective alone or in combination with classical 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[46].

In the case of human cancer cells grown *in vitro*, classical chemotherapy often induces senescence at moderate doses and apoptosis at higher ones[47]. Chemotherapy-induced regression of human cancers is not always explained by an apoptotic response (see for example [48]), and it is conceivable that senescence could play an important role in chemotherapy, as it has been shown in mouse cancer models. In this regard, two reports analyzing senescence markers in biopsies from lung and breast cancer patients after neoadjuvant chemotherapy have observed chemotherapy-induced senescence and its association to treatment success[49, 50]. More recently, the analysis of prostate cancer biopsies

has shown that previous treatment of patients with chemotherapy induces markers of senescence[51].

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 status might remain as “dormant tumour cells” and represent a dangerous potential for tumour relapse. In this regard it is important to gain deeper knowledge of the mechanisms responsible for senescent tumour cell clearance.

In addition, senescent cells show a robust secretory phenotype known as SASP (for senescence-associated secretory phenotype), in which the cells release a number of pro-inflammatory cytokines, chemokines and tissue remodelling enzymes. Some of these factors, such as IL-6 and IL-8, serve a cell autonomous function that reinforces senescence in a paracrine manner[52, 53]. It can be speculated that another role of SASP could be to stimulate the clearance of senescent cells by the immune system. Finally, as a word of caution, SASP components may also dangerously stimulate malignant phenotypes of nearby tumour cells[51].

Future prospects

Cancer mouse models have demonstrated the occurrence of senescence associated with pre-malignant stages of neoplastic transformation and its critical function in preventing tumour progression. Interestingly, senescent tumour cells are not only growth arrested but can be also cleared by phagocytic cells[39]. Following on this, senescence-inducing drugs could represent an ideal opportunity to increase the arsenal of anti-cancer weapons. Finally, studies on human cancer samples should establish whether senescence is or is not relevant for cancer progression and therapeutic responses.

Author Biographies

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Manuel Collado is a staff investigator within the Tumour Suppression Group at the Spanish National Cancer Research Centre (CNIO) in Madrid, Spain. He received his Ph.D. in 1997 from the Universidad Autónoma de Madrid, Spain. He completed postdoctoral training at the Ludwig Institute for Cancer Research, in London, U.K. and at Memorial Sloan Kettering Cancer Center in New York, U.S.A., working on cellular senescence and the PI3K/AKT pathway and the cyclin-dependent kinase inhibitor p27. He joined Manuel Serranós laboratory in 2001 where he has been working on oncogene-induced senescence and tumour suppression.

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Manuel Serrano is principal investigator at the Spanish National Cancer Research Centre (CNIO) in Madrid, Spain, where he leads the Tumour Suppression Group. He received his Ph.D. in 1991 from the Universidad Autónoma de Madrid, Spain. He did his postdoctoral training at Cold Spring Harbor Laboratory in New York, U.S.A., where he identified the cyclin-dependent kinase inhibitor, INK4A, and described for the first time the process of oncogene-induced senescence. He returned to Spain in 1997 to lead his own research group, first at the Spanish National Center of Biotechnology (CNB) in Madrid, and since 2003 at the CNIO.

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Glossary

Nevi	Benign skin lesions of melanocytes that are thought to be senescent
SAHFs	Senescence-associated heterochromatin foci are highly condensed chromatin regions established during senescence and thought to serve as silencing domains
Costello syndrome	Complex developmental syndrome with distinctive craniofacial features and predisposition to neoplasia development caused by activating germline mutations in *HRAS*

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Cancellation of p53 or INK4A/ARF eliminates senescesce and allows progression to full malignancy.

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BOX 1: Markers of senescence *in vivo*

While senescent cells *in vitro* usually adopt a large and flat morphology, senescent tumour cells *in vivo* present the morphology associated with the corresponding tumour stage. Therefore, molecular markers are necessary to qualify a lesion as senescent. Despite the efforts deployed by many laboratories and the increasing interest in defining cellular senescence, there is still a paucity of robust markers of cellular senescence[54]. The single most accepted and widely used marker is the staining for beta-galactosidase assessed at a suboptimal pH of 6.0 (senescence-associated beta-galactosidase, SABG)[55]. Despite the existence of exceptions[17, 18], this is among the most robust markers for senescent cells[56, 57]. *In vivo*, it has been used to demonstrate senescence induction in a wide variety of cancer settings (Tables 1 and 2).

Other classical markers of oncogene-induced senescence (OIS) are the very 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[17]. For some particular models of *in vivo* senescence, other cell cycle regulators are more informative, such as p21 and p27[22, 24]. Along the same lines, molecules involved in the DNA damage response (such as γH2AX) or in the formation of senescence-associated heterochromatin foci (such as HP1γ), have been used as surrogate markers of the process (Tables 1 and 2).

DNA microarray analysis of senescence has also provided new markers of senescence, such as DEC1 and DCR2 [7], that have been used successfully in the identification of *in vivo* senescence (Tables 1 and 2).

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 with Ki67 or BrdU labelling.

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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, thus 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 monitors stress signals and their activation triggers senescence, most notably, p53, INK4a and ARF. 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 critical 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 can be of relevance for the development of new drugs, for prognosis, or for the evaluation of therapeutical treatments.

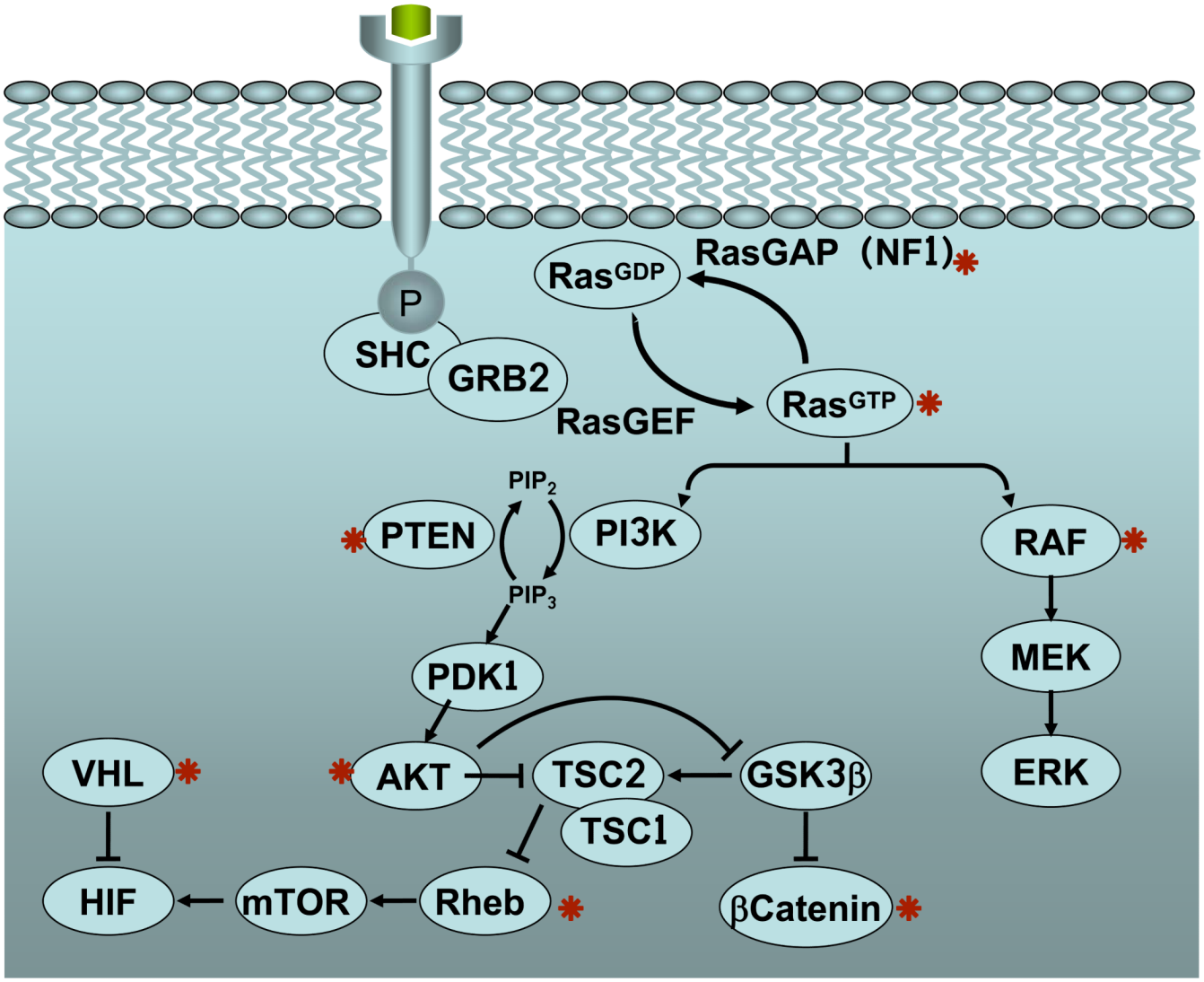

Figure 1.
Signalling pathways and oncogenes (marked by a red asterisk) whose activation leads to senescence induction *in vivo*.

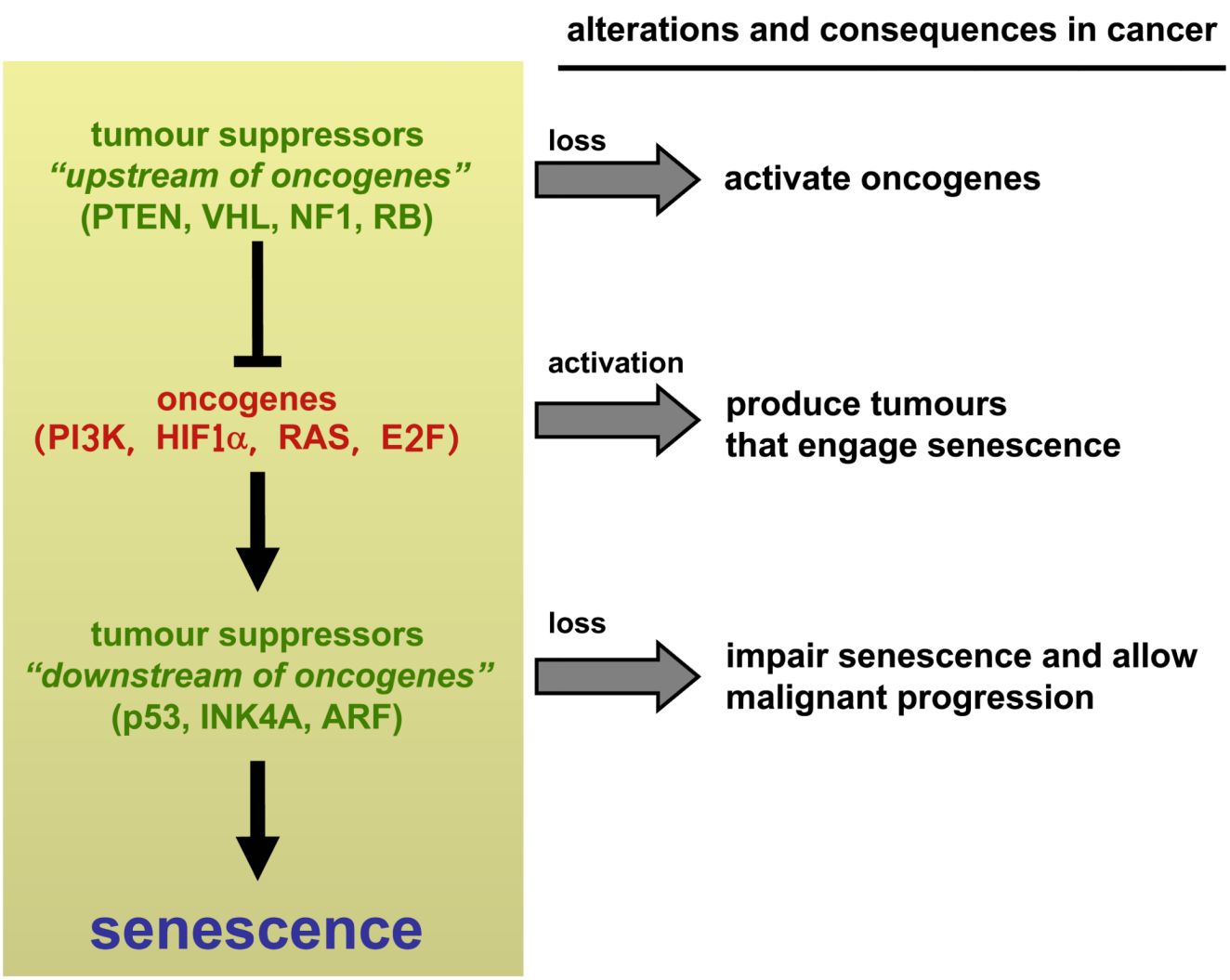

Figure 2.
Tumour suppressors can be grouped into two categories depending on their effect on senescence. Some tumour suppressors (at the top of the cascade, *i.e.* "upstream of oncogenes") prevent excessive oncogenic signalling and their deletion in normal cells triggers senescence. Other tumour suppressors ("downstream of oncogenes") sense excessive oncogenic signalling and induce senescence, and their absence in tumour cells allows progression to malignancy.

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Table 1

Mouse models of tumour cell senescence

Senescence inducing event		*Tissue/Tumour*	*Evidence of senescence*	*Refs.*
Oncogene activation	*Hras^{G12V}*	Mammary tumours, bladder tumours, DMBA/TPA skin papillomas	SABG, γH2AX, p53, INK4A, p21, ARF, low Ki67	16-20
	Kras^{G12V}	Lung adenomas, Pancreatic intraductal neoplasias	SABG, INK4A, DEC1, DCR2, INK4B, HP1γ, low Ki67	7
	Nras^{G12D}	Lymphoproliferative disorders	SABG	5
	Braf^{V600E}	Nevi, Lung adenomas,	SABG, DEC1, ARF, low Ki67	14, 15
	Rheb	Prostate PIN	SABG, low Ki67	23
	E2f3	Pituitary hyperplasia	SABG, INK4A, ARF, SAHFs, low BrdU	8
	Akt1	Prostate PIN	SABG, p27, HP1α, HP1γ, low BrdU	22
	β-catenin	Thymus	SABG, INK4B, INK4A, DEC1, CTSF, CDH16, low BrdU	25
Oncogene inactivation	*Myc*	Lymphoma, osteosarcoma, liver carcinoma, lung carcinoma	SABG, INK4B, p21, H3K9me3	44, 45
Tumour suppressor inactivation	*Pten*	Prostate PIN	ARF, p53, p21, SABG, low Ki67	6
	Rb	Thyroid C cell adenomas	SABG, H3K9me3, HP1γ, INK4A	58
	Vhl	Kidney	SABG, p27, DCR2	24
Tumour suppressor activation	p53	Sarcomas, liver carcinomas	SABG, INK4B, INK4A DCR2, low P-histone H3, low Ki67	39, 40

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Table 2

Human tumours showing cell senescence

Associated oncogenic event	*Tissue/Tumour*	*Evidence of senescence*	*Refs.*
NF1 inactivation	Dermal neurofibromas	SABG, INK4A	26
$BRAF^{V600E}$	Nevi	SABG, INK4A, low Ki67	29
not determined	Prostate PIN	SABG, CXCR2	6, 52
not determined	Colon adenomas	SABG, INK4A, IL8	27, 28, 53