INNOVATION

# The power and the promise of oncogene-induced senescence markers

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Abstract | Recently, it has been shown that oncogene-induced senescence (OIS) occurs during the early stages of tumorigenesis. Senescent tumour cells are abundant within premalignant neoplastic lesions, whereas they are scarce in malignant tumours. This association of senescence with the premalignant stages of tumour progression opens the possibility of using senescence markers as diagnostic and prognostic tools. Moreover, some chemotherapeutic protocols induce senescence in tumour cells and, consequently, senescence markers could help to monitor treatment response.

## Senescence is a cellular stress response

The concept of senescence was first applied to the irreversible growth arrest of cells after prolonged proliferation under *in vitro*, non-physiological conditions[1]. Now this concept is applied in general to the irreversible proliferate arrest of cells caused by various stresses[2], including oxidative damage, telomere dysfunction, DNA damage and several chemotherapeutic drugs (such as doxorubicin or cisplatin, which affect DNA structure, or taxol and vincristine, which target microtubules; reviewed in REF. 3) (FIG. 1). Tumour cells are exposed to many sources of stress, therefore the induction of senescence could constitute an important block to tumour progression. One particularly relevant source of stress is derived from the aberrant proliferative signals of oncogenes, which might eventually trigger senescence through a process known as oncogene-induced senescence (OIS)[4,5]. Ever since its original description, the possibility that OIS functioned as a potential tumour-suppressor mechanism seemed logical, but the detection of its occurrence during tumorigenesis remained elusive. This has changed after several recent reports established that OIS occurs during the early stages of tumour development both in mouse models and in humans[6–9]. These observations strongly indicate that OIS restricts the growth of oncogenically stressed cells, therefore maintaining the tumour in a non-aggressive, premalignant, state; by contrast, the absence of OIS, which is caused by the mutation of the senescence-inducing pathways, leaves the road to oncogene-driven malignant progression unimpeded[6,7]. The association of senescence with premalignant lesions (characterized by normal cell morphology and a lack of invasive growth) makes the detection of senescence an attractive biomarker that could serve as a prognostic indicator. It should be noted that the occurrence of senescence in premalignant tumours is not incompatible with the growth of the tumour. We envision a scenario in which only a fraction of the cells within a tumour are able to propagate successfully, while many undergo apoptosis or senescence triggered by the stress associated with the aberrant intracellular and extracellular conditions that are characteristically present in tumours (FIG. 2). After all, it is the balance between proliferation and apoptosis or senescence that determines the growth rate of a particular tumour.

## Oncogenic levels and senescence

Most studies of OIS have relied on the overexpression of activated oncogenes, and it might have come as a surprise to find that normal endogenous levels of an activated oncogene do not trigger senescence[10,11]. This leaves us with several questions that remain to be answered. For example, how much oncogenic activity is necessary to induce senescence? How much oncogenic activity is needed for neoplastic transformation? And finally, how do these levels compare with each other? It is important to bear in mind that the normal endogenous expression level of an activated oncogene is not only insufficient to trigger senescence, but is also extremely inefficient in the generation of tumours. For example, mice carrying endogenous activated KRAS develop premalignant lung lesions after months of latency, and few lesions progress to be fully malignant tumours[10]. This reflects, in part, the need for further stochastic mutations, but this is certainly not the whole story.

Oncogenic signalling is transmitted through complex cascades of mediators that have ample opportunities for self-attenuation through negative-feedback regulation. Consequently, the activation of an endogenous oncogene does not necessarily translate into the full activation of its downstream effectors; a more realistic scenario is one in which the influence of a particular oncogene on its downstream effectors increases progressively during tumour development, which reflects the cumulative loss of negative-feedback regulators. In this sense, the Sprouty family of genes, which are induced by Ras and inhibit Ras signalling, are frequently inactivated during tumour progression[12]. Similarly, some members of the dual-specificity family of phosphatases are induced by Ras and are negative regulators of mitogen activated protein kinase (MAPK) signalling, therefore opposing oncogenic Ras activity[13,14]. We propose that oncogenic stress increases progressively during tumour development and that senescence is triggered at a point when tumours have already been initiated, but have not reached a fully malignant phenotype.

## Current markers of senescence

The study of senescence has yielded several markers that are useful for the detection of this response not only *in vitro* but also *in vivo* (TABLE 1).

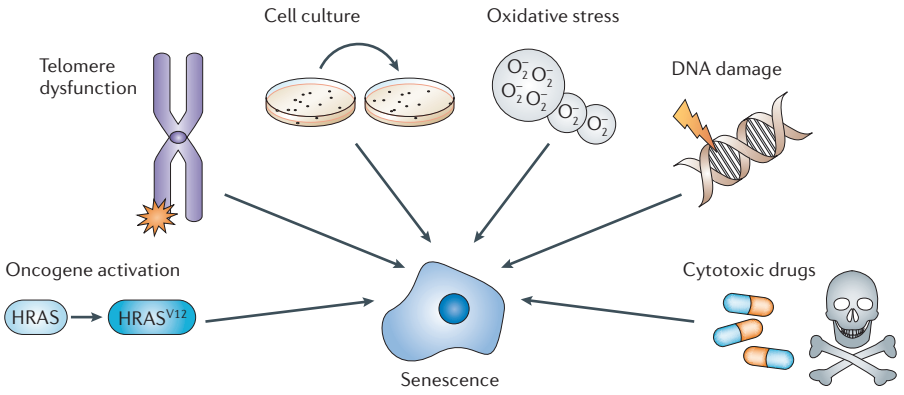


Figure 1 | **Many roads to senescence.** Diverse factors can engage a common programme the end point of which is the establishment of an irreversible proliferative arrest known as senescence. All of these stimuli represent stressful conditions for the cell, many of which are present in the tumour environment. Senescence functions as a self-defence mechanism to prevent the proliferation of potentially damaged cells. In some instances, the same stimulus might induce either senescence or apoptosis, but the mechanisms that govern the decision to engage one or the other are not known.

***Senescence-associated β-galactosidase.*** The most widely used assay for senescence is the cytochemical detection of β-galactosidase activity at pH 6.0, termed senescence-associated β-galactosidase (SA-β-Gal)[15]. β-Galactosidase activity is derived from the increased lysosomal content of senescent cells, which enables the detection of lysosomal β-galactosidase at a suboptimal pH, pH 6.0 (pH 4.0 is optimal)[16]. β-Galactosidase activity is generally accepted as a marker of senescence both *in vitro* and *in vivo*, although its biochemical basis is still unknown and there is evidence for a positive SA-β-Gal reaction in settings of cellular stress that are unrelated to senescence, such as serum withdrawal or high confluency in cell culture[17]. SA-β-Gal activity is normally assayed in cultured cells or tissue sections using the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal)[15], or the fluorescent analogue fluorescein-di-β-D-galactopyranoside (FDG)[18]. The value of histochemical SA-β-Gal staining has been further proven by its capacity to detect OIS in murine and human premalignant neoplastic lesions[6–9].

***p16INK4a–RB and ARF–p53 pathways.*** Investigation of the signalling pathways leading to OIS has shown that two main pathways are involved, p16INK4a–RB (retinoblastoma) and ARF–p53, which are responsible for the execution of the proliferative arrest that characterizes senescence[19]. These two pathways are thought to be crucial for tumour suppression and are often mutated in tumours[20–22]. Consequently, they have been the focus of intense research and many reagents for their detection have been developed in recent years. Increased expression levels of p16INK4a, ARF and p53 have been found in murine tissues with cells undergoing OIS, as well as in human premalignant neoplastic lesions[6–9,23]. These proteins can be regarded as being at the top of the senescence cascade and, precisely because of this, there are situations in which the tumour cell could have acquired downstream mutations that block senescence despite high levels of p16INK4a or ARF. For example, some malignant tumours might overexpress p16INK4a, but this overexpression could be inconsequential if the tumour lacks RB (see for example REFS 24,25). This situation highlights the convenience of markers that, in contrast to p16INK4a or ARF, are not involved in the initiation of senescence but are associated with the final endpoint of senescence.

***Senescence-associated heterochromatin foci.*** The basis for the irreversibility of the proliferative arrest that characterizes senescence has been the subject of intense research. Only recently, however, has a mechanism with the power to impose such a strict control over proliferation been put forward[26]. This mechanism consists of a global alteration of the chromatin initiated by RB and results in the permanent and stable repression of genes with crucial roles in proliferation, such as those regulated by the E2F family of transcription factors. This genomic alteration can be microscopically visualized by the appearance of clusters of DAPI-stained heterochromatic regions known as senescence-associated heterochromatic foci (SAHF). SAHFs bear the hallmarks of heterochromatin, such as the trimethylation of lysine 9 in histone 3 (H3K9me3) (REF. 26), or the recruitment of heterochromatin protein 1-γ (HP1γ also known as chromobox homologue 3 (CBX3)) (REF. 26) and macroH2A histone[27]. Whether SAHFs always occur in response to OIS and what the detailed mechanisms are that enable their formation are questions that remain to be answered. Despite this, SAHFs constitute a morphological feature of the nuclei of senescent cells that have been successfully exploited to identify the occurrence of OIS *in vivo*[6,8,23].

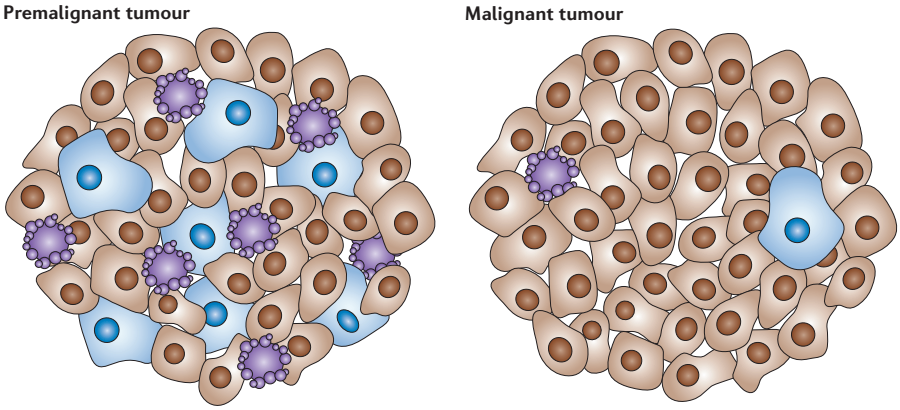


Figure 2 | **Senescence in premalignant neoplastic lesions.** A heterogeneous population of tumour cells is a characteristic of premalignant tumours. Therefore, it is possible to identify the cells that undergo apoptosis (shown in purple) or senescence (shown in blue) within premalignant lesions. These cells are probably responsible for the restriction of the growth of these lesions. By contrast, in most malignant tumours, the balance between proliferating (shown in brown) versus apoptotic and senescent cells is altered in favour of proliferation and, therefore, tumour progression.

Table 1 | **Oncogene-induced senescence (OIS) markers**

| Marker | Assay | *In vitro* | *In vivo* |
|---|---|---|---|
| SA-β-Gal | histochemistry | 15 | 6–9 |
| p16INK4a | WB, IHC | 48 | 6,8,9,23 |
| p15INK4b | WB, IHC | 28 | 8 |
| p53 | WB, IHC | 49 | 7 |
| ARF | WB, IHC | 50 | 7,8,23 |
| p21 | WB, IHC | 51 | 7 |
| SAHFs | IF | 26 | 6,8,23 |
| DEC1 | WB, IHC | 8 | 8 |
| DCR2 | WB, IHC | 8 | 8 |

A summary of the best-established markers of OIS currently available. The references in the *in vitro* column describe the use of the marker in cells cultured *in vitro*, whereas the references in the *in vivo* column report the use of these markers in studies that used either animal models or human samples. Also shown is the assay used to identify the marker. DCR2, decoy death receptor 2; DEC1, differentiated embryo-chondrocyte expressed; IF, immunofluorescence; IHC, immunohistochemistry; SA-β-Gal, senescence-associated β-galactosidase; SAHFs, senescence-associated heterochromatic foci; WB, western blot.

***p15INK4b, DCR2 and DEC1.*** Recently, we have reported new markers of OIS that could help to describe this process *in vivo*[8]. To this end, we generated cell lines that were either competent to undergo senescence or that carried engineered modifications that resulted in the bypass of senescence. By using DNA microarrays, we selected those changes in gene expression that only took place during senescence, but not when senescence was inhibited. In this manner, we compiled a shortlist of candidate OIS markers that were tested in several mouse models of tumorigenesis that are driven by the expression of endogenous oncogenic HRAS or KRAS. Some of these new markers — the cyclin-dependent kinase inhibitor p15INK4b, the decoy death DCR2 and the transcription factor differentiated embryo-chondrocyte expressed (DEC1) — showed an increased level of expression specifically in pre-malignant neoplastic lesions of the skin, lung and pancreas, but not in malignant tumours[8]. The high levels of these markers correlated well with the presence of SA-β-Gal activity and of SAHFs, which confirms their value in identifying the occurrence of OIS *in vivo*. In addition, p15INK4b was previously described as being involved in the growth arrest that is imposed during Ras-induced senescence and was shown to oppose transformation by Ras, at least in cultured cells[28]. The functional involvement of DCR2 (REF. 29) and of DEC1 (REF. 30) is currently unknown. *DCR2* is a p53-induced gene and is one of the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) decoy receptors. It suppresses TRAIL-induced apoptosis and has been shown to regulate chemosensitivity[31]. DEC1 is a member of the basic helix-loop-helix family of transcription factors and it has been shown to be involved in the control of circadian rhythms, but it also seems to be involved in many different signalling pathways[32]. An advantage of these markers is that, in contrast to SA-β-Gal or SAHFs, they can be readily detected in formalin-fixed paraffin-embedded sections using standard immunohistochemistry techniques.

***Other markers.*** Several reports have described the identification of additional OIS markers. In a previous effort to identify potential tumour suppressors that are involved in RAS-induced senescence, our group found that the expression of Ras-induced senescence 1 (RIS1) is increased during growth arrest triggered by the overexpression of oncogenic HRAS in human primary cells[33]. This increase was only observed after the expression of oncogenic HRAS and was not related to replicative senescence or ageing of the organism. RIS1 has been independently identified as a brain-specific membrane-receptor protein[34]. However, its role in senescence is still unknown and its value as a marker *in vivo* still needs to be proved. Similarly, other senescence markers have not yet been validated *in vivo*. This is the case with lipofuscin (an autofluorescent lysosomal pigment that is associated with certain processes of ageing[35]), which accumulates during senescence and has been related to the increased lysosomal content of senescent cells[36]. Also, plasminogen activator inhibitor 1 (PAI1), an inhibitor of the activity of the urokinase-type plasminogen activator protease, uPA, is upregulated during replicative senescence[37] and during Ras-induced senescence[4]. Other changes in extracellular-matrix components have been described during the senescence of cells in culture, such as increased levels of expression of matrix metalloproteinase 3 (MMP3) and collagenase, or decreased levels of expression of tissue inhibitor of metalloproteinase 1 (TIMP1), but neither their occurrence *in vivo* nor their potential value as OIS markers has been properly addressed. Investigation of the signalling pathways that lead to OIS has revealed several important players that, similar to p16INK4a or ARF, are involved in the initiation of senescence, such as the promyelocitic leukaemia protein (PML), or the transcription factors ID1, ETS1 and ETS2, JUN and DMP1. However, as discussed above, the value of these molecules as markers of senescence is limited because downstream alterations can cancel their effects on senescence. Finally, there are other proteins, such as those controlling the G2–M checkpoint[38], that are strongly downregulated and therefore their use as markers is less straightforward.

## Senescence markers in action

Most of the chemotherapeutic agents used in the clinic are assumed to exert their anti-tumour effect through the induction of apoptosis. However, it has been known for a long time that chemotherapeutic drugs can also trigger senescence, occasionally named chemotherapy-induced premature senescence[39]. Indeed, senescence has a relevant part in the anti-tumour effect of standard chemotherapeutic drugs (reviewed in REFS 3,40). An emerging theme is that chemotherapeutic drugs trigger senescence when they are used at low doses, and trigger apoptosis when they are used at higher doses[39,41,42]. Another factor that determines the outcome of chemotherapy is the genetic makeup of the tumour cells. In this regard, it has been shown in mice that the same chemotherapeutic treatment prevents tumour progression either by inducing apoptosis or by inducing senescence, depending on the genetic alterations that are present in the tumour[40]. In the case of human tumours, it is of particular importance that senescence induced by chemotherapy has been shown to be a relevant factor in determining treatment outcome for breast and lung carcinoma[43,44]. Therefore, senescence-inducing drugs could represent an attractive alternative approach to treat tumours that are resistant to apoptosis-based therapies. In this scenario, senescence markers could be used to monitor the efficacy of these therapies.

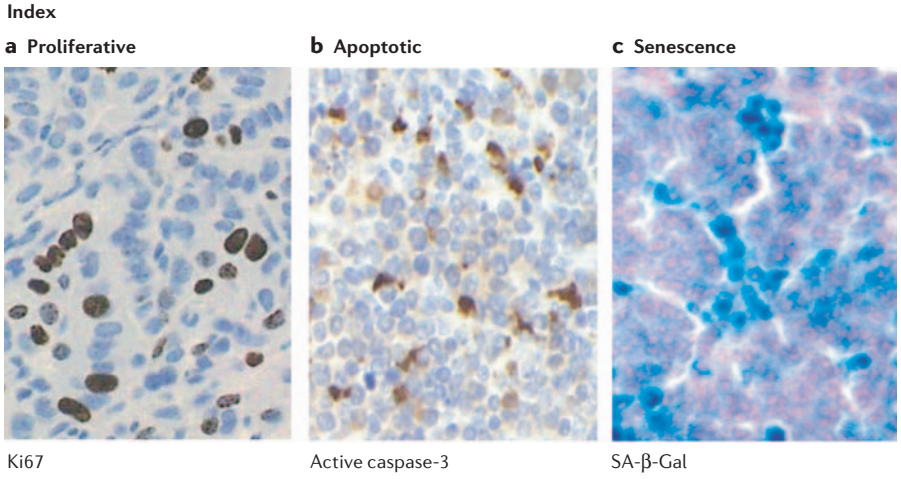


Figure 3 | **Senescence index.** Tumours can be assayed using different markers for the presence of cells that are actively proliferating (for example, using Ki67 staining), undergoing apoptosis (for example, active caspase-3 staining) or undergoing senescence (for example, using senescence-associated β-galactosidase (SA-β-Gal) staining). An 'index' could be derived from the results of these assays and would represent the percentage of cells within a tumour that are part of the pro-malignant state ('proliferative index'; brown cells (**a**)), together with the percentage of cells that restrict tumour growth, represented by apoptosis ('apoptotic index'; brown cells (**b**)) or senescence ('senescence index'; blue cells (**c**)). The images are intended only for illustrative purposes and are not from the same tumour.

**The concept of a 'senescence index'.**
The use of OIS markers in the clinic could be useful in detecting cancer at early stages, and the loss of these markers would be indicative of tumour progression to a malignant stage. We would like to propose the concept of a 'senescence index' to be applied to tumours to indicate the grade and extent to which a tumour undergoes senescence (FIG. 3). Theoretically, tumours that have a high senescence index would have a better prognosis than tumours with a low senescence index, which would be associated with more aggressive lesions that require immediate therapeutic intervention. Although the use of a similar 'apoptotic index' has not been as informative as was originally hoped, it will be interesting to see whether the induction of senescence is a better prognostic marker.

## Future directions

Significant advances in our understanding of the process of OIS have been achieved in recent years , but further research could still provide new molecular tools. The information that is available regarding the different pathways implicated in OIS should be exploited to obtain new senescence markers. For example, modifications to protein-degradation pathways (through the proteasome and the lysosome), the epigenetic status of the chromatin, cell-cycle arrest and others should be further investigated. An as-yet-untested possibility is the use of DNA-damage-response markers in the context of OIS *in vivo*. The accumulation of unrepaired DNA damage during ageing, as is shown by the presence of γH2AX foci, has been linked to senescence in cell cultures and in ageing organisms[45]. At the same time, DNA-damaging agents have the ability to induce senescence in normal and tumour cells[43].

Another exciting possibility is the identification of soluble factors released by senescent cells that could, for example, be measured in body fluids such us blood, urine or sputum. Thanks to the analysis of the gene-expression profile of senescent cells, it has become clear that senescent cells have characteristic alterations in secreted growth factors, inflammatory cytokines, extracellular-matrix components and matrix-degrading enzymes, some of which could be easily detected, and would facilitate the study of OIS (see, for example, REFS 39,46). This is especially attractive because it could provide a non-invasive method to monitor the onset of OIS, as well as the response of tumours to senescence-inducing therapy.

An important consideration that needs to be made in regard to the use of OIS markers to monitior the efficacy of chemotherapy is that senescent cells could favour the growth of adjacent or neighbouring tumour cells[47]. If this is proved to be the case then the induction of senescence in tumours could be a double-edged sword, both limiting the proliferation of the tumour cells, but at the same time promoting the proliferation of non-responsive tumour cells.

Finally, although senescent cells *in vitro* have been shown to be viable and metabolically active over long periods of time, the situation *in vivo* might be very different. Most cells in the body are constantly renewed and it would be very informative to know if (and the mechanism through which) oncogenically stressed senescent cells are eliminated from a tumour and if their fate is altered after chemotherapeutic treatment.

## Conclusions

The activation of OIS during tumorigenesis limits cancer progression. Accordingly, senescent cells are abundant in premalignant neoplastic lesions whereas they are almost completely absent in more advanced tumours. The specific association of senescent cells with premalignant lesions provides a unique opportunity to use senescence as an indicator of the early stages of tumorigenesis. The markers that have been identified during the study of OIS could assist in cancer staging and treatment. In addition, the finding that chemotherapeutic drugs might induce senescence in tumour cells opens the possibility of using these markers to monitor the efficacy of therapy. The identification of additional specific markers of OIS and the evaluation of their value in diagnosis and prognosis is a future prospect that could expand our ability to fight cancer.

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**Competing interests statement**
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ARF | DCR2 | DEC1 | DMP1 | ETS1 | ETS2 | HRAS | HP1γ | ID1 | JUN | KRAS | macroH2A | MMP3 | PAI1 | p15INK4b | p53 | PML | p16INK4a | RB | RIS1 | TIMP1 | TRAIL | UPA

**FURTHER INFORMATION**
**Author's homepage:** http://www.cnio.es/es/grupos/plantillas/presentacion.asp?grupo=50004260
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SCIENCE AND SOCIETY

# Genetics as a tool to improve cancer outcomes: ethics and policy

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Abstract | Genetic research is rapidly increasing the opportunities for the detection of inherited cancer risk. Clinicians and policy makers must ensure the adequate evaluation of the benefits and harms of this new area of practice, address the challenges of family-based detection of individuals at risk and develop practice guidelines and educational strategies that are responsive to rapidly changing knowledge. When the benefits of testing are well established, efforts must also be made to ensure access to genetic services for all who can benefit.

An increasing number of genetic tests provide information about cancer risk. These tests identify inherited susceptibilities to cancer, and can also guide clinical management and provide prognostic information[1–6] (TABLE 1). As with other medical innovations, clinicians and policy makers seek to identify clinical uses that will maximize benefit and minimize harm.

The evaluation of genetic tests often raises difficult questions. A particular gene variant might result in multiple cancer risks, and the effect could be modified by genetic or non-genetic risk factors. In addition, testing can only improve cancer outcomes if there are measures to reduce risk or improve treatment. As a result, the outcome of testing is highly dependent on the potential for