

# Oncogene-Induced Senescence: Putting the Brakes on Tumor Development

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## Abstract

**Cellular senescence, a permanent cell cycle arrest, is considered a safeguard mechanism that may prevent aged or abnormal cells from further expansion. Although the term “replicative senescence” stands for the widely accepted model of a terminal growth arrest due to telomere attrition, the significance of “oncogene-inducible senescence” remained an issue of debate over the years. A number of recent studies now show the effect of this acute and telomere-independent form of senescence as a tumor-protective, fail-safe mechanism *in vivo* that shares conceptual and possibly therapeutic similarities with the genetically encoded apoptosis machinery.** (Cancer Res 2006; 66(6): 2881-4)

## Introduction

Decades ago, Hayflick and Moorhead (1) first described the phenomenon of “replicative senescence” as a stable cell cycle block cells may enter when a certain, finite number of divisions has been reached, leading to a mitogen-refractory growth arrest of metabolically active cells. Many years later, cellular stresses, such as enforced *in vitro* expression of activated oncogenes, were found to acutely produce an indistinguishable phenotype, termed “premature senescence,” that seemed to be independent of progressive telomere shortening (2, 3). Although reliable defining markers for the senescent state are still missing, numerous biochemical characteristics, such as elevated protein levels of p53, p16<sup>INK4a</sup>, or the promyelocytic leukemia gene product PML, as well as the senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity assayed at an acidic pH, have proven useful as indicators of cellular senescence (4). Indeed, senescent cells not only display characteristic morphologic changes, such as a flattened and vacuole-rich cytoplasmic shape, but differ in their gene expression pattern compared with reversibly arrested, quiescent cells (5, 6). In Ras-provoked senescent cells, retinoblastoma (Rb) protein-mediated senescence-associated heterochromatin foci have been identified as sites of local transcriptional repression, where heterochromatin proteins are recruited to E2F-responsive promoters (7). This observation fueled the view that cellular senescence might be an epigenetically controlled process in which S-phase-related gene activities are selectively shutdown in the vicinity of methylated lysine 9 residues at histone H3 (H3K9; ref. 8). A series of studies recently published in *Nature*

now clarify the relevance of oncogene-induced senescence as a tumor-suppressive mechanism and show the significance of an Rb pathway- and histone modification-governed senescence program *in vivo*.

## Oncogene-Induced Senescence Terminates Tumorigenesis in Premalignant Lesions

A BRAF-induced senescent growth arrest seems to limit melanomagenesis in humans. Work by Daniel Peeper and colleagues addressed the phenomenon that nevi (i.e., benign tumors of cutaneous melanocytes), frequently carrying activating mutations in the *BRAF* gene, may lack any apparent proliferative activity often for decades before lesions in a small subset of cases eventually progress into a malignant melanoma. Demonstrating the direct effect of an activated oncogene on melanocyte proliferation, sustained expression of oncogenic BRAF (i.e., BRAF<sup>V600E</sup>) in primary melanocytes provoked an SA- $\beta$ -gal-positive growth arrest virtually devoid of any DNA synthesis. BRAF<sup>V600E</sup>-senescent fibroblasts displayed focal heterochromatin accumulations, senescence-associated heterochromatin foci that overlapped with spots of trimethylated H3K9. Importantly, Michaloglou et al. (9) were able to show strong SA- $\beta$ -gal positivity and reciprocal negativity for the proliferation marker Ki67 in biopsy specimens of melanocytic nevi *in situ*, hereby confirming the senescent nature of premalignant BRAF<sup>V600E</sup>-driven hyperplastic lesions *in vivo*.

Moreover, cellular senescence may also interrupt or delay Ras-driven lung carcinogenesis. Using a conditional *K-Ras*<sup>G12V</sup> knock-in mouse model that is prone to lung tumors upon Cre recombinase-mediated activation of the endogenous oncogene (10), Manuel Serrano's group reported only sparse Ki67 reactivity, but intense SA- $\beta$ -gal staining in the upcoming lung adenomas (11). In contrast, the Ki67-positive lung adenocarcinomas that formed in this model produced little or no SA- $\beta$ -gal staining and lacked the speckled nuclear pattern of HP1- $\gamma$  expression that was observed in premalignant pulmonary lesions potentially reflecting senescence-associated heterochromatin foci-related H3K9 methylation. In addition, other mouse models of carcinogenesis (i.e., Ras-driven pancreatic and skin cancer models) also developed premalignant lesions that were positive for markers of cellular senescence. Notably, numerous novel markers for oncogene-induced senescence identified by an *in vitro* microarray analysis proved useful to distinguish senescent from nonsenescent lesions *in vivo* as well.

Ablation of the senescence response by p53 inactivation was now unveiled as a critical step in the development of prostate cancer. Given the frequently detected early-stage losses of the *PTEN* and late-stage mutations of the *p53* tumor suppressor genes in human prostate cancer specimens, Pier Paolo Pandolfi and colleagues addressed the collaborating role of these two genetic

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hits in a conditional *PTEN*-deficient mouse model (12). Acute inactivation of both *PTEN* alleles provoked p53-dependent senescence in mouse embryo fibroblasts and gave rise to invasive prostate cancer only after a very long latency. SA- $\beta$ -gal-positive, senescent areas were also found in early-stage human prostate cancer biopsies. However, when *PTEN* and *p53* genes were coinactivated, the mice developed a highly aggressive and lethal form of prostate cancer after short latency, indicating that cellular senescence may serve as a prime fail-safe mechanism triggered in *PTEN*-impaired prostate hyperplastic lesions that is entirely disabled on acquisition of *p53* mutations.

Studies from our own laboratory and from collaborators provided insights into the genetic mechanism of oncogene-induced senescence as an initial barrier in lymphoma development (13). Based on the attractive hypothesis that a senescent G<sub>1</sub> arrest must reflect a stable, selective silencing of S-phase genes, we investigated a putative mechanism in which the Rb-bound histone methyltransferase Suv39h1 links the Rb pathway to transcriptional repression of E2F-responsive genes via local heterochromatinization (7, 14). Using E $\mu$ -*N-Ras*<sup>G12D</sup> transgenic mice that typically succumb to late-onset neoplastic manifestations of histiocytic origin, we found that the absence of one or both *Suv39h1* alleles, like *p53* inactivation, dramatically shortened survival in this model due to early-onset invasive T-cell lymphomas. Importantly, primary lymphocytes engineered to acutely express oncogenic Ras entered an SA- $\beta$ -gal-positive growth arrest that strictly depended on Suv39h1, hereby precluding them from subsequent transforming events. Hence, a histone modification signature (i.e., Suv39h1-mediated methylation of H3K9) acts as an early safeguard against imminent lymphoma development initiated by oncogenic Ras *in vivo*.

### Oncogene-Induced Senescence: A Universal Theme with Slight Variations

Although there is little doubt that apoptotic defects not only facilitate transformation by various oncogenes *in vitro* but also contribute to tumor development *in vivo*, the physiologic role of acutely inducible, premature senescence as an oncogene-provoked, fail-safe mechanism remained an issue of debate for years (15). The recent reports on oncogene-induced senescence acting as a tumor-suppressive principle *in vivo* now establish the critical mass of evidence to no longer interpret this phenomenon as a culture artifact. Moreover, the data clearly rebut the view that oncogene-induced senescence may occur only when the driving mitogenic oncogene is expressed at supraphysiologic levels. In fact, mutant BRAF activities in patient-derived samples, titrated expression levels of exogenous BRAF<sup>V600E</sup>, transgenic N-Ras<sup>G12D</sup> expression, and activation of endogenous *K-Ras*<sup>G12V</sup> alleles equally produced cellular senescence in their respective contexts *in vitro* and *in vivo*, and cellular senescence was also observed in *PTEN*<sup>-/-</sup> prostate glands with intraepithelial neoplastic lesions without any explicit manipulation of the Ras/Raf oncogene family.

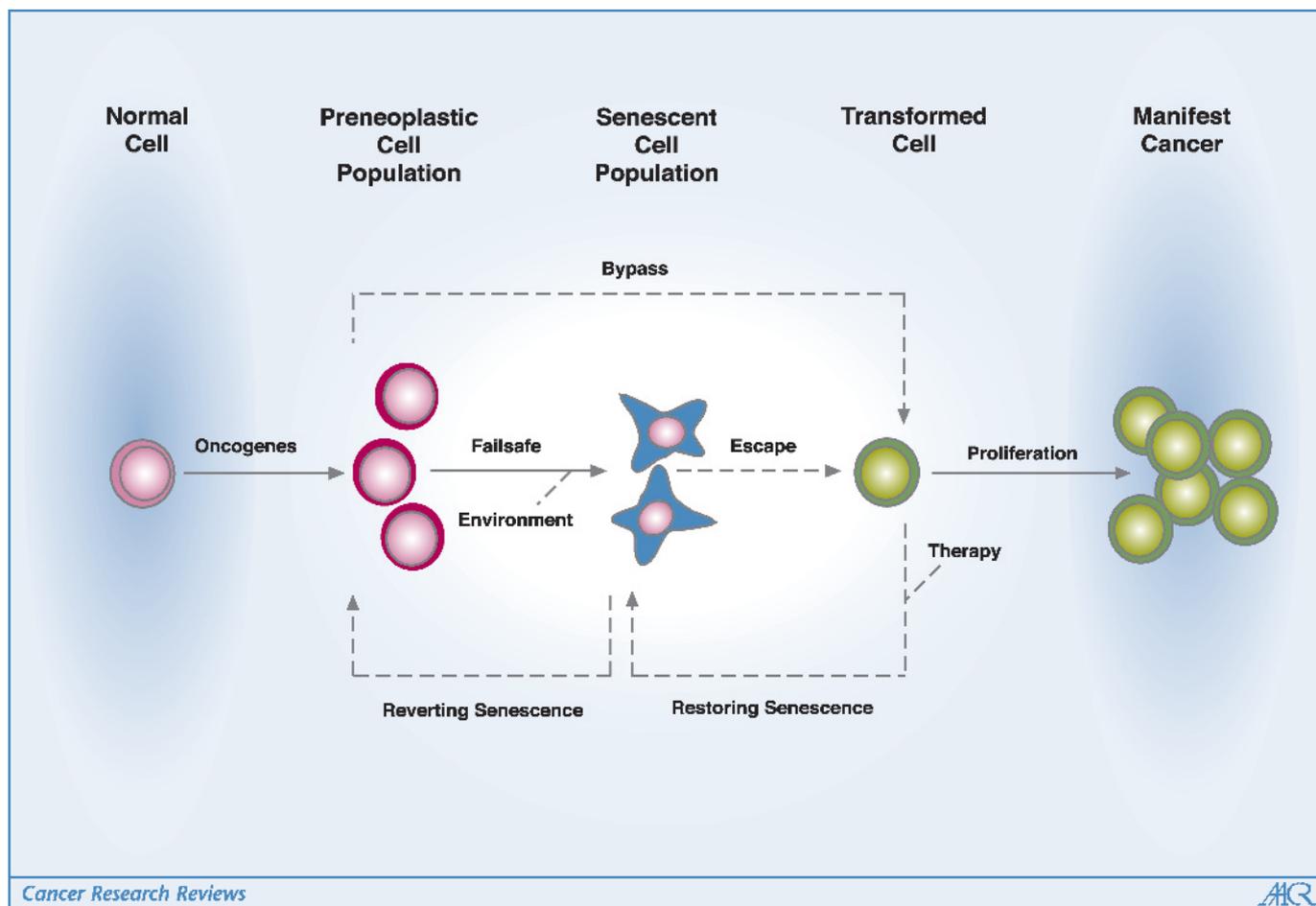
The articles underscore the importance of cellular senescence in a wide spectrum of premalignant scenarios, hereby highlighting the universal nature of this program as an antioncogenic fail-safe mechanism. Analyses using genetically tractable mouse models and human tumor biopsies, examination of solid tumors and hematologic malignancies, and exploration of mesenchymal and epithelial cancer types all accomplished to show the

significant role of oncogene-induced senescence in preventing or interrupting the progression to full-blown cancer formation *in vivo*. As suggested by a recent report on deregulated E2F activity provoking a senescence-like arrest in hyperplastic pituitary lesions (16), hyperproliferative signaling and unscheduled DNA synthesis in response to certain mitogenic oncogenes—such as the Ras/Raf prototypes—may ultimately execute Rb/E2F-related chromatin changes that lock in the arrest state. Indeed, the identification of a common epigenetic signature of histone H3K9 methylation and subsequent HP1- $\gamma$  binding in different mouse model settings as well as in human tumor material strongly supports the concept of cellular senescence as an inherited principle that irreversibly reprograms cellular capabilities via chromatin remodeling.

On the other hand, the studies leave little doubt that there is more than just a single road to senescence. In the articles, senescence is recognized as a strictly growth-arrested, SA- $\beta$ -gal-positive and Ki67-negative condition, whereas other candidate mediators, particularly those acting primarily upstream of the Rb checkpoint, seem to play a role in some scenarios of oncogene-induced senescence. The functional requirements for the cell cycle inhibitors p16<sup>INK4a</sup> and p21<sup>WAF1</sup>, and for p53 and its upstream regulator ADP ribosylation factor (ARF) in the senescent state, seemed to be heterogeneous in the examined conditions. For example, p16<sup>INK4a</sup> is induced and tolerated at high levels in Suv39h1-deficient lymphoma cells that apparently bypassed senescence, is found up-regulated in human nevi in a mosaic fashion with no clear correlation with the SA- $\beta$ -gal staining pattern, is reportedly not required for the growth arrest in these molecules, whereas, in turn, disruption of p16<sup>INK4a</sup> action at its target CDK4 has produced escape from Ras-induced senescence *in vitro* and accelerated tumorigenesis *in vivo* (17). Besides cell type- and context-dependent differences, there is increasing evidence that some players, such as p16<sup>INK4a</sup>, may rather initiate the senescence response, whereas others, like p53, might then take over to ensure maintenance of the permanent arrest (18), implying that certain crucial mediators of cellular senescence might be essential only temporarily and can be absent at other times without forcing the cell to reenter the cycle.

### Biological and Therapeutic Implications of Escape from and Return to the Senescent State

The downstream mechanisms by which Ras/Raf oncogenes, and presumably other mitogenic oncogenes as well, ultimately execute cellular senescence still remain unclear, although activation of the mitogen-activated protein kinase cascade seems to be of critical importance (19). Little is known about the dependency of the senescent state on continuous oncogenic signaling. Although low-level expression of the oncogenic moieties Ras or BRAF might be sufficient to induce senescence, it has not been reported yet whether cancellation of Ras/Raf signaling in senescent cells might revoke the arrest, leading to apoptosis or even to the resumption of regular cell divisions. Furthermore, the recent observations that acute induction of oncogenic Ras or BRAF not only provokes an immediate arrest of cultured cells but promotes the formation of premalignant, hyperplastic lesions before a senescent growth cessation *in vivo* imply that additional, non-cell-autonomous factors may serve as cotriggers of the terminal cell cycle block. Importantly, most studies agree that primary defects in regulators essential for the senescence phenotype are not sufficient to readily



**Figure 1.** Model of oncogene-induced senescence in tumor development. Oncogenic activation—particularly of Ras/Raf prototypic oncogenes—is primarily mitogenic but may provoke an early senescent growth arrest of individual cells or premalignant hyperplastic lesions (*full arrows*). Whether a fully transformed phenotype arises from oncogene-driven cells that *a priori* bypassed senescence or from those that emerged from senescence by mutating essential senescence regulators remains speculative (*hatched arrows*). Genetic defects that inactivate senescence are not necessarily sufficient to transform in cooperation with the initiating oncogene; thus, additional mutations might be required to establish cancer cells that eventually expand as a clinically relevant tumor mass. Depending on number and biological effect of senescence-compromising mutations, therapeutic approaches based on genetic manipulations or exposure to DNA-damaging agents may be applied to force proliferating cancer cells to (re-)enter senescence (*hatched arrows*).

permit transformation by the respective oncogenes, but that they tear down a barrier now allowing the Ras-driven cell to remain in cycle and to more easily acquire secondary lesions required for a fully transformed condition (Fig. 1; ref. 20). Although the cooperativity between oncogenic Ras/Raf signaling and senescence defects, on one hand, and the formal reversibility of the senescent state by external genetic manipulations of senescence mediators, on the other hand, have been shown (18, 21, 22), it remains an open question whether manifest malignant tumors emerge from a senescent cell upon acquisition of an escape mutation or expand from a clone that *a priori* bypassed senescence (Fig. 1). Alternatively, if non-cell-autonomous factors play a role in the induction of senescence, one might envision that some tumors arise and maintain their proliferative capacity without any structural defect in the senescence machinery, keeping them potentially susceptible to environmental or exogenous pro-senescent stimuli.

Hence, therapeutic utilization of the senescence response will become a central subject of future research. DNA-damaging agents have been shown to induce cellular senescence in mice and men *in vitro* and *in vivo*, particularly in the absence of an intact

apoptotic response (23–25), and might even lead to a terminal arrest in some genetic scenarios that licensed escape from oncogene-induced senescence (13). However, to avoid the genotoxicity unselectively delivered by conventional chemotherapeutics, one would like to identify drug targets that act downstream of the damaged DNA as signaling mediators in the senescence program. For example, pharmacologic stabilization of the p53 tumor suppressor in *PTEN*-deficient prostate cancer has been proposed as a novel therapeutic approach to induce senescence (12). Moreover, inducible antagonization of the senescence-suppressing moiety *Tbx2*, a T-box gene family member that represses the expression of ARF and p21<sup>WAF1</sup> in melanoma cells, was recently shown to directly restore cellular senescence in these fully malignant cells (26, 27).

In summary, oncogene-induced senescence can no longer be considered a cell culture-related artifact. Recent evidence obtained in various scenarios of tumorigenesis has now clarified that acutely available senescence may be recruited as an ultimate fail-safe mechanism to blunt oncogenic activity *in vivo*, leading to growth-arrested premalignant lesions or interrupting the transformation process at a very early stage. In addition to the

large body of data reporting on the high mutation frequency at the *INK4a/ARF* and *p53* loci in human cancers typically driven by oncogenic Ras, technically more challenging screens for genetic defects at the level of repressive histone signatures are required to uncover potentially deregulated downstream components of the senescence machinery. Given the conceptual similarity to the apoptosis program as another stress-responsive, ultimate cell cycle exit program, future studies are expected to

identify and characterize critical mediators of either program, their potential coregulatory function, and their value as novel drug targets.

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