

Cellular senescence in cancer treatment: friend or foe?

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Damage to DNA, the prime target of anticancer therapy, triggers programmed cellular responses. In addition to apoptosis, therapy-mediated premature senescence has been identified as another drug-responsive program that impacts the outcome of cancer therapy. Here, we discuss whether induction of senescence is a beneficial or, rather, a detrimental consequence of the therapeutic intervention.

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Achieving lasting remissions in patients suffering from nonlocalized malignancies remains the central problem of clinical oncology. Although the decades-old arsenal of classic anticancer treatment modalities such as surgery, chemotherapy, irradiation, and hormone ablation has been augmented by strategies including immunotherapy, gene therapy, inhibition of angiogenesis, hyperthermia, and a number of novel lesion-based approaches such as the administration of the Bcr-Abl kinase inhibitor STI-571, the goal to eradicate all cancer cells in a metastasized condition is rarely within reach. Anticancer treatment strategies may be insufficient for many reasons: potentially efficient therapies might not always find their way to virtually inaccessible tumor sites. Moreover, the multitude of tumor entities are known to differ remarkably in their susceptibilities to conventional DNA-damaging anticancer agents – particularly solid tumors, which often are largely refractory to chemotherapy or rapidly re-emerge from a remission. In addition, primarily susceptible tumors select for genetic defects during the course of therapy, which may render them resistant over time. While dose escalation can overcome the problem of insufficient chemosensitivity in some entities, its clinical applicability is limited by the severe toxicity codelivered to the normal cell compartment.

Traditional cytotoxic treatment strategies are driven by the assumption that quantitative execution of cell death is required to eliminate the malignant cell population.

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Nonstandard abbreviations used: senescence-associated β -galactosidase (SA- β -Gal); ataxia telangiectasia mutated kinase (ATM); cyclin-dependent kinase (CDK); retinoblastoma protein (pRB); small interfering RNA (siRNA); mouse embryonic fibroblast (MEF).

Interestingly, strategies to blunt properties of malignant growth by disabling proliferation without primarily targeting cancer viability have been less recognized, although forcing cells to exit the cell cycle by an irreversible arrest should terminate their contribution to disease progression just as effectively. In fact, recent evidence underscores the theory that premature senescence may act as an acute, drug-inducible arrest program that may contribute to the outcome of cancer therapy (1, 2). In this Perspective, we will review the role of drug-induced effector programs and discuss to what extent induction of cellular senescence may be a beneficial result of anticancer therapy.

Anticancer therapy induces programmed cellular responses

Chemotherapy remains the mainstay in the treatment of systemic or metastasized malignancies. Although these highly toxic agents interfere with a plethora of cellular functions and may damage a variety of cellular structures, their pivotal cellular target is genomic DNA. It is now a well-accepted concept that drug-mediated DNA damage is not invariably lethal per se but provokes genetically encoded cellular responses. Hence, unrelated chemotherapeutic anticancer agents – in spite of their different pharmacological features and their individual target molecules participating in DNA replication and integrity – initiate common downstream mechanisms. Upon sensing DNA damage, cellular transducers activate pathways that either temporarily halt the cell cycle to allow the DNA repair machinery to fix the damage, or execute lethal programs such as apoptosis or mitotic catastrophe to restrain the damaged cell from further expansion (see ref. 3 for review). Ultimate, i.e., irreversible responses to DNA damage do not always determine the fate of cancer cells by programmed forms of cell death but may blunt their uncontrolled proliferative capacity by provoking a terminal cell-cycle arrest termed premature senescence (4–6).

Moreover, recent reports demonstrated that tumor cell senescence is detectable following DNA-damaging treatment in vivo and significantly increases overall survival of the host (1, 2). In turn, the fact that different anticancer agents share genetic effector cascades renders the genetically encoded programs of apoptosis and senescence highly susceptible to inactivating mutations as a potential cause of chemoresistance. Hence, thorough dissection and mutational analysis of the pathways leading to cell death or cellular senescence are expected to identify specific genetic lesions that may be utilized by novel targeting therapies.

Drug-inducible senescence is a p53- and p16^{INK4a}-controlled program

Premature senescence recapitulates cellular and molecular features of replicative senescence (7), which is a safeguard program that limits the growth potential, but not necessarily the viability, of a dividing cell as a consequence of the progressive shortening of its telomeres. Senescent cells, arrested in the G1 phase of the cell cycle, typically appear flattened and enlarged with increased cytoplasmic granularity. In addition to the characteristic morphology, senescent cells display enhanced activity of senescence-associated β -galactosidase (SA- β -Gal) when assessed at an acidic pH (8, 9). While refractory to mitogenic stimuli, senescent cells remain viable and metabolically active and possess a typical transcriptional profile that distinguishes them from quiescent cells (10). At the protein level, numerous regulators of cell-cycle progression, checkpoint control, and cellular integrity such as p53 or p16^{INK4a} have been found to be induced in response to various pro-senescent stimuli (4, 11). Although the molecular mechanisms underlying the senescent phenotype remain largely unknown, there is increasing evidence that formation of heterochromatin in the vicinity of promoters that control gene expression related to cell-cycle progression might be implicated in the maintenance of an irreversible growth arrest (12).

Extrinsic factors such as anticancer agents, γ -irradiation, or UV light have been shown to induce premature senescence as a DNA damage-mediated cellular stress response (4–6). DNA lesions are sensed and transduced via protein complexes involved in DNA maintenance and repair, associated with members of the PI3K superfamily that includes the ataxia telangiectasia mutated kinase (ATM) (13), the ATM-related kinase (ATR), and DNA-protein kinase, among others (14–18). Besides a network of other downstream substrates, these kinases directly or indirectly phosphorylate the gatekeeper of cellular integrity (19), the p53 protein, at certain residues. Although a cascade of posttranslational modifications has been proposed to control p53 activity in response to DNA damage (20), and different DNA-damaging stimuli such as UV light or γ -irradiation can produce distinguishable signatures of posttranslational p53 modifications (21), the actual contribution of distinct phospho-residues to p53-mediated DNA-damage

responses is still under debate (22, 23). p53 controls a plethora of effector functions (24), and the precise mechanisms by which specific downstream pathways are regulated in response to p53 activation have not been elucidated yet (25, 26). While it is now clear that p53 participates not only in apoptosis but also in the induction of DNA damage-mediated senescence (1, 5, 6), the signals that convert p53 from an apoptosis executor to a senescence inducer in response to anticancer therapy still need to be identified. Moreover, the posttranslational p53 modifications found in cells that entered replicative senescence shared only partial overlap with p53 modifications typically induced by DNA damage (27).

Comparable to p53, which functions as a fail-safe mediator, the cyclin-dependent kinase (CDK) inhibitor p16^{INK4a} has been implicated in both response to DNA damage and control of stress-induced senescence (1, 4, 28, 29). Although the molecular mechanism used by p16^{INK4a} to control not only a temporary but a permanent G1 arrest is largely unclear (30), p16^{INK4a} responds to DNA damage in a delayed manner and appears to be indispensable for the maintenance of cellular senescence (1, 2). For example, treatment of normal human foreskin and lung fibroblasts with DNA-damaging drugs such as bleomycin or actinomycin D induced an irreversible cell-cycle arrest with a senescence-like phenotype including a transient upregulation of p53 and p21 protein levels, followed by increased p16^{INK4a} protein expression and detectable SA- β -Gal activity (4). Similarly, exposure of adenocarcinoma cells to topoisomerase inhibitors produced premature senescence that was initially accompanied by overexpression of p53 and p21, whereby the subsequent overexpression of p16^{INK4a} persisted after drug withdrawal, highlighting the role of p16^{INK4a} in maintenance of the growth arrest (2). In an in vivo model of drug-senescent mouse lymphomas, repeated anticancer therapy eventually selected against senescence-controlling genes such as the p16^{INK4a}-encoding *INK4a* alleles or *p53* genes, thereby producing relapse tumors that resumed growth in an aggressive manner (1). A broader drug screen based on several tumor cell lines demonstrated inducibility of a senescence-like arrest in numerous p53-proficient cancer cell lines in response to doxorubicin and other DNA-damaging agents, but it also suggested that p53-independent pathways leading to senescence might exist when some p53-mutated cell lines were exposed to escalated doses of doxorubicin (5). Interestingly, not only DNA-damaging agents, but also compounds that primarily target microtubules or the differentiating agent retinoic acid, were found to promote an SA- β -Gal-positive arrest phenotype. Moreover, a senescence-like growth arrest was also observed after γ -irradiation of normal human diploid cells (6, 31). p53 deficiency, previously reported to disable apoptosis in response to γ -irradiation (32, 33), also accounted for impaired γ -irradiation-induced senescence in a human carcinoma cell line wherein senescence could be restored upon

introduction of wild-type p53 (6). Thus, in a variety of different test conditions, DNA damage-induced senescence was confirmed as a p53- and p16^{INK4a}-co-controlled safeguard program.

Drug-induced senescence: substitute player or powerful reliever?

The possibility of alternative outcomes in response to drug-induced DNA damage – apoptosis, mitotic catastrophe, cellular senescence, or simply necrosis – raises the question of whether additional stimuli or specific contextual scenarios determine the ultimate cell fate. Xenotransplant and transgenic mouse models have been used to visualize premature senescence as a quantitative response to anticancer agents *in vivo* (1, 5). Importantly, lymphomas generated in the *Eμ-myc* transgenic mouse model were prone to massive apoptosis as a default response following therapy with the alkylating agent cyclophosphamide, but they uniformly displayed premature senescence when apoptosis was blocked by overexpression of the strictly anti-apoptotic mediator Bcl2 (1). Although mice harboring senescent lymphomas ultimately succumbed to their disease, they lived much longer than those bearing lymphomas with a defect in both apoptosis and senescence as a consequence of p53 loss. Given the rapid induction of apoptosis and the rather delayed detectability of an SA-β-Gal-positive long-term arrest in response to anticancer therapy *in vivo*, the data suggested that the senescence machinery may act as a back-up program to substitute for or to reinforce an insufficient apoptotic response (34). The actual impact of a fictitious population of cells that chose to primarily enter senescence is difficult to assess, since apoptotically competent tumors typically regress to a remission with no apparent residual tumor mass left. Senescent cells have also been detected in archival tumor samples from breast cancer patients who underwent neoadjuvant chemotherapy prior to surgical removal of their tumors. In contrast to untreated tumor samples, these samples revealed significant positive staining for SA-β-Gal activity associated with high expression of p16^{INK4a} (2). Hence, intrinsically rather chemosensitive entities such as hematological malignancies may use senescence as a secondary mechanism, while typically less susceptible solid tumors might rely on premature senescence as the chief drug-response program available.

According to many naturally occurring mutations in apoptosis-related genes, disruption of apoptosis is, at least in conjunction with certain oncogenic scenarios, a pivotal step in tumor development. Given the complex overlap between apoptosis and senescence as cellular fail-safe systems on one hand and tumor-suppressor mechanisms and drug-effector programs on the other hand, the capability to execute senescence might be disabled in cancer cells for numerous reasons. In fact, mutations in genes that control cellular senescence may not only be selected for during therapy but

might have been acquired already during tumor development (1). Oncogenes such as activated ras that are known to provoke premature senescence as the primary fail-safe mechanism may rely on defects in this program as a prerequisite to a fully transformed phenotype (28, 35), possibly inactivating senescence as a drug response as well. Nevertheless, tumors that preserve both an intact apoptotic and a functional senescence program may display a particularly robust drug response consisting of acutely inducible cell death in a first phase, corroborated by delayed apoptosis out of senescence at a later point (Figure 1). Although apoptosis is a fast-acting response mode, little is known about the possibility that apoptosis-competent cells might be sent into senescence following DNA-damaging therapy – and whether a senescent tumor could ever undergo apoptosis upon an additional proapoptotic signal (36). Finally, senescence could be recruited as an amplifier mechanism to lock temporarily arrested tumor cells – with their reduced susceptibility to checkpoint-licensed apoptosis – into irreversible cytostasis.

No effect without side effects

The fate of apoptotic cells is determined by their acute disruption of metabolic processes, rapid disintegration, and engulfment by attracted macrophages. In stark contrast, induction of cellular senescence as a formally irreversible growth arrest results in the preservation of a potentially malignant cell population locked into a nondividing state, yet possessing at least some metabolic activity (8). Although apoptotic cells provoke rather little inflammatory reaction, tumor-infiltrating immune cells reportedly can recognize altered autoantigens presented by apoptotic cancer cells (37). To what extent altered senescent tumor cells that previously managed to escape immunosurveillance can now challenge an antitumor immune response requires further investigation (Figure 1). Correlative evidence points toward a link between dermal autoimmunity in the elder population and an age-dependent increase of SA-β-Gal-positive keratinocytes in human skin samples (8).

Although it is likely that senescent cells will ultimately be cleared by phagocytosis, no “eat-me” signals, as recently described for apoptotic cells, have been identified yet for the senescent state (38). While senescent neutrophils, like apoptotic granulocytes (39), might ultimately face phagocytosis through a yet unknown recognition mechanism (40), focal enrichment of lysosome-related β-galactosidase activity at autodigestive vacuoles indicated that aged human fibroblasts arrested in replicative senescence may eventually eliminate themselves by autophagy (41). As a side effect of anticancer therapy, DNA damage can also force susceptible normal cells to enter an acute SA-β-Gal-positive arrest *in vivo* (S. Lee and C.A. Schmitt, unpublished observations). Hence, it is conceivable that senescent cancer and normal bystander cells reside for some time next to

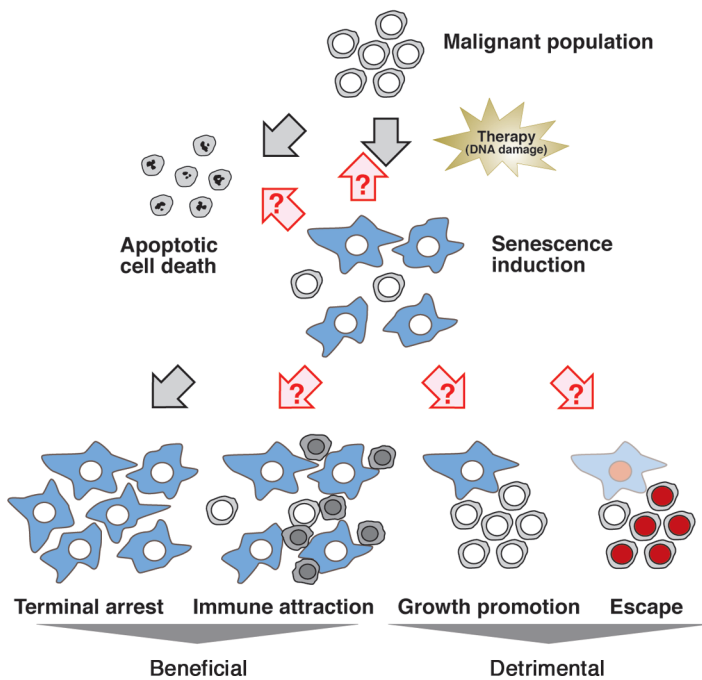


Figure 1 Drug-inducible senescence: friend or foe? In response to DNA-damaging agents, cancer cells can rapidly undergo apoptosis or may enter premature senescence as a potential back-up mechanism. Whether cells re-enter the cycle or execute apoptosis out of drug-mediated senescence remains unclear. A terminal arrest of the entire cancer cell population, possibly augmented through increased immunogenicity of senescent cells, is beneficial for the host. In contrast, feeder-like growth that reflects paracrine activity of senescent cells on their non-senescent neighbors, or escape from senescence based on acquired or preexisting mutations, is considered a detrimental outcome.

their non-senescent malignant neighbors. In line with the role of irradiated fibroblasts as feeder cells, and with evidence reported by Waldman and colleagues, who described improved clonogenic growth in the presence of irradiation-arrested bystander cells (42), interspersed senescent – i.e., metabolically active – cells may support survival and growth of tumor cells in their vicinity. Campisi and coworkers demonstrated that senescent human fibroblasts stimulated proliferation of epithelial cell lines *in vitro* (43). Moreover, coimplantation of senescent fibroblasts together with preneoplastic epithelial cells in nude mice accelerated tumor formation *in vivo*, mainly via soluble factors secreted by the senescence-activated fibroblasts. Thus, the outcome of anticancer therapy is not only determined by a quantitative effect on cancer cells forced to irreversibly exit the cell cycle but may also depend on novel capabilities acquired by senescent cells that can impact their malignant neighbors in different ways (Figure 1). It is quite possible that, because of functionally compromised cell-cell interactions, senescent tumor cells can even exert a tumor-suppressive effect on their bystander cells.

Rest in peace?

Cellular senescence has become an attractive therapeutic concept because it qualitatively equals programmed cell death by excluding cells from active progression through the cell cycle. However, its therapeutic potential strongly relies on the irreversibility of this process. Unlike apoptosis, which acutely eliminates a potentially harmful cell, cellular senescence represents an operational change in a still-viable cell. Any cellular switch that could revert senescent cells into dividing cells implies the threat of a tumor relapse – at least as long

as senescent cells have not been cleared by other processes such as phagocytosis. In fact, several experiments have provided evidence that senescence is a formally reversible process if proteins involved in its maintenance are lost. For example, reversal of replicative senescence in human lung fibroblasts was achieved via functional inactivation of both p53 and the retinoblastoma protein (pRB) by the expression of simian virus 40 large T antigen protein, or, as an alternative, by a combination of p53 inactivation and knockdown of p16^{INK4a} expression using small interfering RNA (siRNA) molecules (44). In contrast, the mere suppression of p53 function proved already sufficient to revert the senescent phenotype of human foreskin fibroblasts (44). Likewise, siRNA-driven inactivation of p53 in mouse embryonic fibroblasts (MEFs) enabled the cells to resume proliferation out of replicative senescence (45). These findings are in accordance with the putative cooperativity of p53 and p16^{INK4a} – and possibly pRB – in the induction and maintenance of premature senescence *in vivo* (1). Accordingly, the acute inactivation of pRB via recombinase-mediated gene deletion in MEFs that entered senescence upon replicative exhaustion or in response to oncogenic ras allowed the cells to re-emerge from the arrested phenotype (46). However, it needs to be shown that this cell-cycle re-entry truly reflects restored proliferative capacity and will not simply promote cell death by apoptosis or mitotic catastrophe within a few additional divisions.

Importantly, these experimental scenarios have not formally tested whether drug-inducible senescence is a reversible process as well. In the p53- and pRB-deficient cell line Saos-2, premature senescence – in addition to substantial cell death – was observed in response to doxorubicin treatment, suggesting a p53-

and pRB-independent pathway to senescence (5). Furthermore, overexpression of a temperature-sensitive pRB transgene in Saos-2 cells produced a senescent phenotype. As a result of transgene inactivation, reinitiation of DNA synthesis was observed, and the cells underwent apoptotic cell death via the p53 homologue p73, indicating that active pRB is required to maintain the senescent state of these cells (47). This somewhat artificial setting underscores the complex wiring of signaling pathways into senescence, their relative responsiveness to different stimuli, their dependency on the cell type, and their sensitivity to experimental approaches based on nonphysiological overexpression of candidate regulators. Nevertheless, some of the experiments demonstrating successful reversal of cellular senescence that senescent cancer cells could actually encounter in vivo. While the acquisition of spontaneous mutations that disable p53 or pRB in a resting cell without DNA replication seems rather unlikely, epigenetic changes, for example, promoter methylation to silence p16^{INK4a} expression, might occur in senescent cells (Figure 1).

Exploiting cellular senescence for cancer therapy

The uncertainties regarding control and irreversibility of drug-induced senescence raise concerns as to what extent this effector mechanism reflects a desirable outcome of cancer therapy, particularly in light of therapy-inducible apoptosis as the alternate and possibly safer outcome in response to DNA damage. However, an intact apoptotic machinery is often unavailable in established malignancies. Since anticancer agents kill mainly by apoptotic cell death and, in turn, achieve little clinical efficacy in the presence of apoptotic defects (see, for example, refs. 48–52), promising treatment alternatives must use effector mechanisms that do not rely on an intact apoptotic machinery. Importantly, in vivo analyses of treatment responses in primary lymphomas harboring defined genetic lesions demonstrated that induction of senescence despite the presence of an apoptotic block improved the outcome of anticancer therapy (1) – regardless of a potential reversal or possible emergence of preexisting escape mutants at a later time.

Appropriate test systems are critical to elucidate the complex implications of drug-inducible senescence. Not surprisingly, drug sensitivity assays performed on primary tumor material in culture unveiled that adaptation to the nonphysiological culture conditions selected for apoptotic defects and chemoresistance (50). Paradoxically, many cell lines retain the ability to enter senescence following drug exposure in vitro (5), although inactivation of the terminal-arrest program appears to be a key prerequisite for any primary tumor during successful establishment as a continuous cell line. Irrespective of the technical inducibility of a senescence-like phenotype in culture-adapted cells, a Petri dish setting cannot mimic the complexity and interactivity of a natural tumor environment in vivo. Hence, many of the questions raised about the role of drug-induced senescence

need to be addressed in vivo using physiological model systems. Tractable mouse models of cancer, such as the transgenic *Eμ-myc* lymphoma model (1), in alliance with sophisticated genetic tools will allow researchers to dissect the pathways and the impact of drug-inducible senescence in vivo. Moreover, large-scale analyses of the transcriptome and proteome of primary human tumor samples will expand our understanding of the molecular mechanisms that underlie drug responses in sensitive and resistant conditions.

Given the impact of apoptotic defects on tumor biology and treatment outcome, it is a research priority to invent small compound- or gene therapy-based approaches that may resensitize cancer cells to death signals. Likewise, one can envision lesion-based strategies to restore a defective senescence response. Ultimately, direct activation of pro-senescent pathways without induction of deleterious DNA damage seems to be a particularly appealing concept. For instance, cDNA microarray analysis of human diploid fibroblasts revealed that cGMP synthesis was inhibited during replicative senescence. Exposure of tumor cells to a guanylate cyclase inhibitor induced, by activation of the CDK inhibitor p21, a senescent phenotype that was independent of its upstream regulator p53, indicating that induction of p21 activity might be sufficient to halt cell proliferation, even in the absence of functional p53 and without an additional DNA-damage signal (53). Furthermore, a synthetic inhibitor of CDK4 – possibly mimicking the role of p16^{INK4a} to maintain a senescent phenotype – also produced a DNA damage-independent form of premature senescence in cells lacking proper p16^{INK4a} expression and inhibited the growth of xenotransplant tumors in mice (54). Inactivation of the papilloma virus oncoproteins E6 and E7, which deregulate the p53 and Rb proteins, respectively, by siRNA molecules restored cellular senescence in cervical cancer cells (55).

Cellular senescence and its potential use as a drug-effector program remains a complex biological phenomenon with unknown significance in cancer therapy. Whether cellular senescence is rather friend or foe most likely depends on accompanying lesions, first of all in apoptotic response programs, and on the cellular context. In further preclinical investigations, it will be of particular interest to explore therapies that do not deliver devastating DNA damage to the cell, that do not rely on functional DNA-damage transducer systems, and that do not target pathways already mutated to cancel apoptosis, but that directly prompt a senescence response.

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