Controversy and Consensus

Cancer Research

# Tumor Cell Escape from Therapy-Induced Senescence as a Model of Disease Recurrence after Dormancy

Tareq Saleh[1], Liliya Tyutyunyk-Massey[2,3], and David A. Gewirtz[2,3]

## Abstract

Senescence, a durable form of growth arrest, represents a primary response to numerous anticancer therapies. Although the paradigm that senescence is "irreversible" has largely withstood the findings of tumor cell recovery from what has been termed "pseudo-senescence" or "senescence-like arrest," a review of the literature suggests that therapy-induced senescence in tumor cells is not obligatorily a permanent cell fate. Consequently, we propose that senescence represents one avenue whereby tumor cells evade the direct cytotoxic impact of therapy, thereby allowing for prolonged survival in a dormant state, with the potential to recover self-renewal capacity and contribute to disease recurrence.

## Introduction

Tumor cells induced into a state of senescence upon exposure to cancer chemotherapeutic drugs or radiation can recover self-renewal capacity, that is, undergo "proliferative recovery." We postulate that senescence in residual tumor cells that have survived after the bulk of the tumor cell population has been eliminated by therapy may represent one form of tumor dormancy. Consequently, senescence may represent an avenue whereby tumor cells evade the direct cytotoxic impact of therapy by entering a prolonged state of growth arrest, whereupon rare proliferating tumor cells will reemerge months or years after patients have been cured of the primary disease.

## Escape from Therapy-Induced Senescence

Proliferating cells that undergo successive duplications will eventually cease to divide as they enter a state of senescence. It has been established that senescent cells (primarily aging fibroblasts) can persist in an arrested state indefinitely, indicating that senescence represents a highly stable form of growth arrest. However, although tumor cells maintain the potential to undergo an accelerated (or premature) form of senescence in response to severe genotoxic stress, hormonal deprivation, or cell-cycle inhibition, the possibility remains that the arrest, although durable and prolonged, may not be permanent for all cells in the population.

Early studies from our laboratory demonstrated that clinically relevant concentrations of Adriamycin (doxorubicin) induce senescence in (p53 wt, $p16^{INK4a}$ null) MCF-7 breast tumor cells, from which a small population of cells evades the durable growth arrest, potentially developing resistance to senescence-inducing therapies (1). Similarly, Wu and colleagues at the University of Washington (Seattle, WA) established that H1299 lung cancer cells (deficient in $p16^{INK4a}$ as well as null in p53) can evade senescence induced by camptothecin to recover proliferative capacity (2). Both studies established an association between recovery from therapy-induced senescence (TIS) with the ability of tumor cells to express the cyclin-dependent kinase, cdc2. In the Wu and colleagues' study, the frequency of escape/recovery was 1 in $10^6$ cells, suggesting that (i) the stability of the senescent growth arrest is the more predominant phenotype, and that (ii) the escape of tumor cells from senescence is a relatively rare event. Escape from senescence has since been reported by a number of investigators including the Sikora laboratory (studies on the potential contribution of chemotherapy-induced senescent tumor cells to cancer relapse) and the Bernards group [seminal studies on the reversibility of senescence, immortalization, and escape from oncogene-induced senescence (OIS)].

Despite the accumulation of data in support of the premise that some tumor cells expressing the classical hallmarks of senescence may not be terminally arrested, investigators have generally been conservative in their conclusions, often using terminology such as "senescence-like" or "pseudo-senescence" to distinguish tumor cells that recovered proliferative capacity from tumor cells that appeared to be in a permanently arrested state. One caveat to the conclusion that tumor cells can reemerge from senescence or a senescence-like state is that studies have generally involved cells in mass culture, where the origin of the replicating cells could not be unequivocally attributed to the senescent population. In an effort to circumvent this limitation, we have recently used a flow cytometric approach to enrich and select for tumor cells induced into senescence by chemotherapy based on senescence-associated β-galactosidase (SA-β-gal) activity and cellular size (3). Live cell imaging and interferometry (3) confirmed what has been suggested by an extensive body of literature over the past decade and a half, specifically that senescent cells can undergo spontaneous cell division.

[1]Department of Basic Medical Sciences, Faculty of Medicine, The Hashemite University, Zarqa, Jordan. [2]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, Virginia. [3]Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia.

**Corresponding Author:** David A. Gewirtz, Massey Cancer Center, 401 College St., Richmond, VA 23298. Phone: 804-828-9523; Fax: 804-827-1134; E-mail: david.gewirtz@vcuhealth.org

**doi:** 10.1158/0008-5472.CAN-18-3437

## Features of Cells That Escape from Senescence

A number of characteristics that have frequently been associated with tumor cells that escape from TIS include polyploidy, stemness, and aggressiveness.

### Polyploidy

Polyploidy, a common feature of senescent cells, is consistent with the potential to generate daughter cells, and was evident in the camptothecin-induced senescent H1299 cell population described by Wu and colleagues (2). Approximately 40% of these polyploid senescent tumor cells were able to take up EdU several days after senescence induction, suggesting that the cells retained the capacity to undergo DNA replication (4). Large polyploid cells induced into senescence by camptothecin that were sorted on the basis of nuclear content were observed to generate colonies 7–10 days after seeding, findings that were supported by live cell imaging of cells escaping from senescence. Several studies by Rajaraman and colleagues that included time-lapse microscopy suggested that senescent tumor cells replicate by budding (or neosis) from the polyploid state. Multiple follow up studies by other groups support the contention that polyploidy is a prerequisite for cells to reemerge from senescence (5).

### Stemness and aggressiveness

Sabisz and Sklandanowski determined that about 1% of cells undergoing TIS expressed the markers of cancer cell stemness (CD34 and CD117). Similarly, Was and colleagues presented evidence of cells undergoing TIS exhibiting stem cell features, specifically $CD24^+$ (about 1.5% of cells) and NANOG in the treated cell population (6). Other laboratories have provided evidence that multiple breast cancer cell lines (MCF-7, MDA MB231, and T47D) and primary tumors that escaped from TIS could be derived from the cancer stem cell population. A recent report by the Schmitt group also focused on the relationship(s) between senescence regulatory pathways and cell "stemness" (7). This work demonstrated that a single exposure of Eμ-Myc—Bcl2–overexpressing lymphoma cells to Adriamycin (0.05 μg/mL) resulted in a robust senescence induction (marked by over 80% SA-β-gal staining) and an accompanying increased expression of stem cell–related genes as well as elevated activity of aldehyde dehydrogenase and ATP-binding cassette (ABC), both associated with stem cell function (7). Importantly, enhanced stemness was not detected in cells exposed to the same concentration of Adriamycin, but which failed to undergo senescence due to the absence of Suv39h1, the enzyme responsible for the senescence-associated epigenetic signature, H3K9Me3. Using an inducible expression model for p53 and Suv39h1, the authors were able to deactivate these prosenescence proteins and facilitate resumption of S-phase activity after Adriamycin-induced senescence (marked by EdU staining and gradual decline in SA-β-gal activity). These authors argued that "senescence is, in principle, a reversible condition, which becomes evident when essential senescence maintenance genes are no longer expressed." Cells that escaped TIS and acquired stem cell properties were also more aggressive, forming rapidly growing colonies *in vitro* and more malignant tumors when implanted *in vivo* (in this study, in immunocompetent mice). Moreover, studies performed in melanoma, breast, colon, and neuroblastoma cells have shown that Adriamycin-induced senescence was accompanied by elevation of Wnt ligands associated with the epithelial–mesenchymal transition and migratory properties.

## Senescence, Tumor Dormancy, and Disease Recurrence

It is not difficult to imagine that the majority of tumor cells exposed to cytotoxic therapies undergo cell death and generate a robust immune response, leaving small and undetectable subpopulations of residual dormant cells. Although having a pivotal impact on the natural history of cancers, our understanding of the mechanisms of dormancy and how tumor cells escape from dormancy are, unfortunately, quite limited (8).

Although it has been suggested that dormant tumor cells are in a quiescent state, senescence rather than quiescence would be more likely to reflect tumor dormancy because quiescence is a short-lived process from which tumor cells escape once DNA has been repaired or favorable conditions for growth recovery have been restored. In contrast, senescent cells, by definition, do not respond to growth-promoting stimuli. Furthermore, quiescent cells will not have undergone the morphologic and genetic modifications associated with TIS. In this context, it is noteworthy that common cancer mutations involve key proteins associated with the regulation of senescence such as p53, $p16^{INK4a}$, and Rb, all of which are likely to be relevant to the escape from senescence. Finally, the aggressive nature of recurrent disease is also reflective of the aggressive phenotypes that evolve following escape from senescence, as demonstrated recently by Schmitt and colleagues in lymphoma models (7).

It can be further argued that mechanisms that facilitate the recovery of dormant cells would be more closely associated with senescence rather than quiescence. For example, dynamic alterations of the microenvironment and restoration of the blood supply, critical events contributing to the capacity of dormant tumor cells to recover, are heavily influenced by mediators such as matrix metalloproteinases and angiogenic promoters such as VEGF, both classic components of the senescence-associated secretory phenotype. Moreover, senescent cells not only interact with, but also modulate the immune system, thus possibly contributing to the evasion of immunosurveillance, which is a necessary step for cancer recurrence.

Although senescence could reflect one form of tumor dormancy, we do not presume that senescence is the only form; in fact, senescence may be only one among a number of forms of tumor dormancy, such as those represented by circulating tumor cells or cells in the perivascular niche. We postulate that a subpopulation of cancer cells that escape cell death following repeated cycles of cytotoxic therapy can undergo senescence and persist for weeks, months, or years, and, under the appropriate conditions, ultimately contribute to disease recurrence. These dormant senescent cells generate an array of soluble and nonsoluble molecules that gradually alter the surrounding tissue and slowly promote angiogenesis. Eventually, a few senescent cells that manage to escape immunosurveillance and undergo proliferative recovery would be competent to exploit the changes in their extracellular environment and the restored blood supply to reinitiate tumor formation.

## Strategies to Eliminate Senescent Tumor Cells in Efforts to Delay or Prevent Disease Recurrence

It is well accepted that the senescence-associated secretory phenotype has tumor-promoting properties, although the bulk of the scientific literature on this phenomenon relates to senescence induced in normal (fibroblast) cells. Furthermore, senescence, although not formally a form of resistance, may provide a mechanism for evasion of the cytotoxic impact of various therapies by allowing the prolonged survival of tumor cells with the inherent potential to reemerge from the growth-arrested state and generate progeny that retain self-renewal properties. This premise is supported by recent work by Campisi and colleagues that demonstrated that senescent cells contribute to cancer relapse (9). Consequently, if senescence is a form of tumor dormancy, then tumor cells that escape from senescence and survive (fortunately a rare event) will, in some cases, be the source of recurrent disease, and their elimination would provide a survival advantage for patients with cancer.

Recent work, largely in the field of aging, but also in cancer, has identified senolytic agents, drugs whose cytotoxicity has a high degree of specificity against senescent cells. Among these are drugs such as navitoclax that suppress antiapoptotic proteins, Hsp90 inhibitors, and histone deacetylase inhibitors (10). It is suggested that these drugs could be used as "clearing" agents to eliminate residual senescent tumor cells surviving after chemotherapy or radiation, with the goal of delaying or ideally preventing disease recurrence. Drug efficacy could be maximized and patient toxicity reduced by treatment with senolytics after the standard therapy has been completed.

## Summary and Conclusions

It is important to note that we do not propose that senescence is actually reversible in the manner of a reversible chemical reaction. Instead, we propose that although the bulk of a "senescent" population is likely to be indefinitely arrested, there will be subpopulation(s) of cells capable of recovering self-renewal capacity, particularly in the context of TIS in tumor cells that inherently harbor genetic derangements (3, 7). The results of our own recent studies confirm that only a subpopulation of tumor cells is capable of escaping the growth arrest (3), which likely reflects the heterogeneity of the senescent phenotype that has been established by Demaria and colleagues. Furthermore, we show that both lung cancer and breast cancer cells selected for senescence can form tumors when implanted in mice.

Certain caveats to these findings must be acknowledged. Many of the senescence markers, such as the induction of p21, expression of the cytokines and chemokines associated with the SASP, and even the classical SA-β-gal enzyme, are not exclusively specific to senescence. Furthermore, escape of tumor cells induced into senescence by chemotherapy or radiation in tumor-bearing animals remains to be conclusively demonstrated.

Despite these reservations, the possibility that TIS results in the survival of a residual tumor cell population from which cells with self-renewal capacity can emerge suggests that senescent tumor cells may represent one form of cancer dormancy. Given the tumor-promoting properties of the senescence-associated secretory phenotype coupled with the potential for regrowth and disease recurrence, efforts to eliminate this small but significant tumor population may represent a clinically relevant strategy for prolonging the life of patients with cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received November 12, 2018; revised January 9, 2019; accepted January 16, 2019; published first February 25, 2019.

### References


1. Elmore LW, Di X, Dumur C, Holt SE, Gewirtz DA. Evasion of a single-step, chemotherapy-induced senescence in breast cancer cells: implications for treatment response. Cancer Ther Clin 2005;11:2637–43.
2. Roberson RS, Kussick SJ, Vallieres E, Chen SJ, Wu DY. Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. Cancer Res 2005;65:2795–803.
3. Saleh T, Tyutyunyk-Massey L, Murray GF, Alotaibi MR, Kawale AS, Elsayed Z, et al. Tumor cell escape from therapy-induced senescence. Biochem Pharmacol 2018 Dec 19 [Epub ahead of print].
4. Wang Q, Wu PC, Dong DZ, Ivanova I, Chu E, Zeliadt S, et al. Polyploidy road to therapy-induced cellular senescence and escape. Int J Cancer 2013; 132:1505–15.
5. Rajaraman R, Guensey DL, Rajaraman MM, Rajaraman SR. Stem cells, senescence, neosis and self-renewal in cancer. Cancer Cell Int 2006;6:25.
6. Was H, Barszcz K, Czarnecka J, Kowalczyk A, Uzarowska E, Koza P, et al. Bafilomycin A1 triggers proliferative potential of senescent cancer cells *in vitro* and in NOD/SCID mice. Oncotarget 2017;8:9303–22.
7. Milanovic M, Fan DNY, Belenki D, Däbritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature 2018;553:96–100.
8. Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. Nat Rev Cancer 2014;14: 611–22.
9. Demaria M, Leary MNO, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov 2017;7:165–76.
10. Sieben CJ, Sturmlechner I, van de Sluis B, van Deursen JM. Two-step senescence-focused cancer therapies. Trends Cell Biol 2018;28:723–37.