The Quest to Define and Target Cellular Senescence in Cancer

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ABSTRACT

Cellular senescence represents a double-edged sword in cancer and its therapy. On one side, senescence-associated growth arrest and immunomodulatory properties exert potent antimalignant functions. On the other side, senescence bypass and secretory phenotype are associated with tumor progression and relapse. Recent studies have demonstrated the enormous potential to combine pro- to antisenescence interventions as a new anticancer approach. However, the heterogeneity of senescence-associated features makes definition and targeting of therapy-induced senescent cells a challenging task. Here, we describe these challenges and discuss how to exploit senescence-associated features to improve treatment efficacy and tolerability.

Introduction

Cellular senescence is a state of stable and generally irreversible growth arrest mediated by the CDK4/6 inhibitor p16 and the CDK4/6 and CDK2 inhibitor p21. Senescent cells also develop a multifaced secretory profile, known as the senescence-associated secretory phenotype (SASP), which includes several immunomodulatory factors (1). Because of the growth arrest and the ability to enhance immunosurveillance via the SASP, senescence represents a potent tumor-suppressive mechanism and a desired outcome of anticancer interventions. In comparison to apoptosis and other types of cell death, senescence induction can be achieved at lower dosages of conventional chemo and radiotherapy, thus potentially reducing side effects. However, recent studies have demonstrated that persistence of therapy-induced senescence can have detrimental functions for the organism and paradoxically promote tumor relapse. In agreement with this notion, the efficacy and tolerability of anticancer approaches further benefit from combining pro-senescence interventions to senolytics – compounds that selectively eliminate senescent cells. Senolytics are drugs capable of exerting selective toxicity against senescent cells by targeting senescence-associated features (1). Drugs such as ABT263 (navitoclax), the cocktail dasatinib + quercetin, and the FOXO4-DRI peptide target multiple senescent cell antiapoptotic pathways (SCAP). High activity of the lysosomal senescence-associated β-galactosidase (SA-β-Gal) enzyme, besides serving as the most commonly used senescence marker, can be exploited to activate prodrugs specifically in senescent cells. However, senescence-associated features are very heterogeneous and lack specificity and universality, especially in cancer cells. This lack of specific markers makes the definition of therapy-induced senescence challenging and often misleading, and hinders the development of broad-spectrum senolytic approaches. Here, we discuss the main challenges and controversies associated with the identification and targeting of senescent cancer cells, and potential strategies to design novel combinatorial anticancer therapeutic approaches based on modulating senescence-associated traits.

Pleiotropic Functions of Cellular Senescence in Cancer

Bypass of the senescence-associated growth arrest is a critical step in tumorigenesis. Transgenic animals lacking p53, p21, and p16 are highly predisposed to develop cancer early in life, whereas the majority of human malignant lesions carry loss-of-function mutations in the same genes. It is yet not clear whether a stable and persistent senescent state can be induced in cancer cells, particularly in the presence of mutations affecting critical senescence-associated genes. Because it is a common practice to evaluate senescence induction in malignant lesions and cells solely based on elevated SA-β-Gal activity, understanding whether SA-β-Gal activity and proliferation are mutually exclusive in cancer is of crucial importance for evaluating treatment efficacy. Milanovic and colleagues have shown that adriamycin-treated lymphoma cells increase SA-β-Gal activity, suggesting senescence induction, but later reacquire proliferative abilities and promote cancer relapse (2). Goel and colleagues have shown that exposure to the CDK4/6 inhibitor abemaciclib induced growth arrest and elevated SA-β-Gal activities in breast cancer cells, but that cell-cycle progression was resumed upon drug withdrawal (3). Duy and colleagues have shown the chemotherapy induces senescence-like and SA-β-Gal$^+$ acute myeloid leukemia cells capable of promoting recurrence (4). We have recently evaluated the possibility that certain therapy-induced SA-β-Gal$^+$ breast cancer cells might retain proliferative ability. Combining SA-β-Gal staining to EdU incorporation in the same specimen, we have observed that a significant proportion of cells exposed to the CDK4/6 inhibitor abemaciclib activated SA-β-Gal but retained proliferative capabilities (**Fig. 1**). Thus, SA-β-Gal alone as a surrogate marker for senescence is not a reliable strategy to evaluate therapy-induced senescence, suggesting the need to combine the measurement of multiple senescence-associated markers (5).

Another important but highly heterogeneous and controversial senescence-associated feature is the SASP. The SASP is enriched in proinflammatory and immunomodulatory factors that have the potential to activate and modulate various adaptive and innate immune responses. p53 restoration in liver cancer models triggered cellular senescence and contributed to the immune clearance of cancer cells (6). Moreover, the SASP triggered by trametinib/palbociclib combinatorial therapy contributed to vascular remodeling and immune cells infiltration in tumors (7). In contrast to these anticancer

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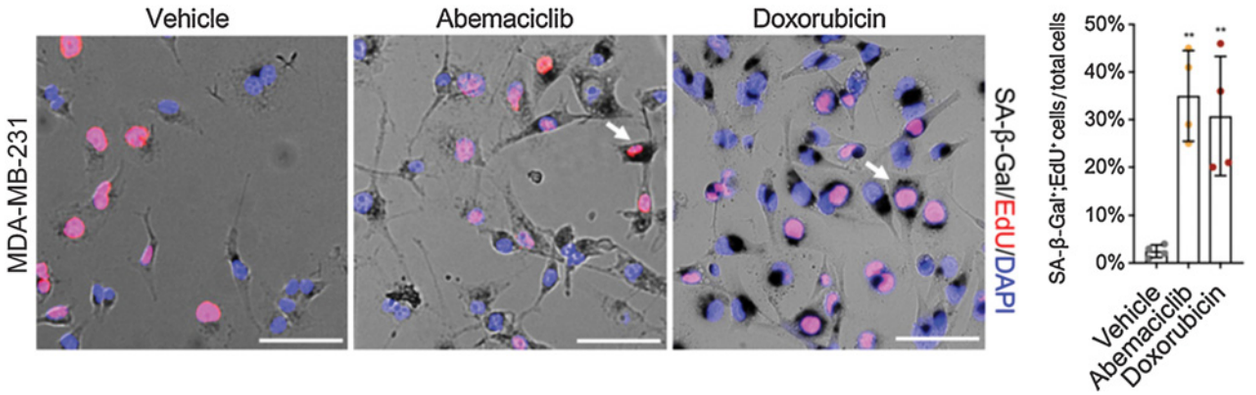

Figure 1.

Abemaciclib-treated breast cancer cells show elevated SA-β-Gal activity but retained proliferative abilities. MDA-MB-231 breast cancer cells were cultured in DMEM supplemented with 5% FBS and maintained in incubators with 5% O_2 and 5% CO_2. Cells were plated in T25 flasks at 25% density and the next day treated with vehicle (water), abemaciclib (daily treatment of 1 μmol/L for 8 consecutive days), or doxorubicin (250 nmol/L for 24 hours). At the end of treatment, cells were plated on coverslips in 24-well plates. Eight days after replating, cells were incubated with EdU for 20 hours. At the end of the EdU incubation, cells were fixed and stained for SA-β-gal (using a commercial kit) and DAPI. Images are representative of the staining and obtained by overlapping brightfield images (for SA-β-gal staining) to fluorescence imaging (for EdU and DAPI). White arrows indicate cells positive for SA-β-gal and EdU. Scale bar, 75 μm. Quantification of the number of double positive cells is provided and represent the average of four biological replicates.

functions, the SASP can also promote protumorigenic steps, including proliferation, migration, invasion, and immune evasion. Moreover, the SASP is responsible for various therapy-associated toxicities that are severely affecting quality of life of patients with cancer. Most cancer therapies inflict severe and often irreparable DNA damage, leading to a persistent DNA damage response (DDR). The DDR involves activation of NF-κB, which is responsible to transcriptionally regulate many proinflammatory SASP factors involved in the detrimental secondary effects of therapy-induced senescence.

In addition to NF-κB, several other positive SASP regulators such as CEBPs, AP-1, NOTCH, and mTOR, and negative regulators, such as p53, have been recently identified. For this reason, a potential approach to counteract tumor senescence is the use of SASP inhibitors that are part of a class of compounds named senomorphics (i.e., compounds that modulate senescence-associated features without inducing cell death).

However, because how the various SASP modulators interact is largely unknown, therapeutic strategies aimed at interfering with such proteins and pathways remains difficult. An alternative approach to reduce detrimental effect of therapy-induced senescence, which recently gained extensive experimental support, is the use of senolytic compounds.

Two-Punch Therapeutic Approaches

Defining a cancer cell as "senescent" is not a trivial task. However, exposure to standard anticancer drugs might lead to the induction of certain senescence-associated features, which can then serve as acquired vulnerabilities, even if a full senescent state is never reached. This revisited synthetic lethality approach based on sequential exposure to prosenescence interventions and senolytics (also known as "two-punch") was first proven effective in the context of liver cancers (8). Here, it was shown that inhibition of CDC7 selectively induced cellular senescence in liver cancer cells with *TP53* mutation and led to acquisition of a new vulnerability—mTOR activation—which could be targeted using the mTOR inhibitor AZD-8055, successfully leading to tumor regression. In another study, the PARP inhibitor olaparib was used in ovarian and triple-negative breast cancer cells to induce a state of "reversible senescence" characterized by the upregulation of anti-apoptotic BCL2 proteins, which then served as target for a second hit with ABT-263 to achieve tumor regression (9). A similar strategy based on targeting antiapoptotic mechanisms was also shown to effectively clear chemotherapy- or irradiation-induced senescent breast and lung cancer cells (10). Another senescence-associated feature developed upon exposure to genotoxic stress is altered balance between sodium and potassium—a feature that can be targeted by cardiac glycosides to reduce tumor progression (11).

As described above, SA-β-Gal alone is not a reliable marker for therapy-induced senescence in cancer cells, but highlights elevated lysosomal activities and mass that could be a potentially favorable target for synthetic lethal interventions. In accordance, combining CDK4/6 inhibitors, which induce elevated lysosomal activities independent of stable growth arrest (**Fig. 1**; ref. 3), to lysosomotropic agents sensitize breast cancer cells to death (12).

Conclusions

The functions of cellular senescence in cancer therapy are complex and dynamic. On one side, the cytostatic effect of senescence can stop tumor proliferation, and the associated SASPs activate immunosurveillance. On the other side, persistence of therapy-induced senescent normal and cancer cells might predispose to toxicity and cancer relapse. Several recent studies have demonstrated the synergistic effect of combining pro- to antisenescence interventions in cancer, settling the foundations for the development of innovative therapeutic strategies. Considering the heterogeneous phenotype of fully and partially senescent cancer cells, future efforts should be dedicated to precisely characterize which senescence-associated features are developed in different tumorigenic contexts and how they can be selectively targeted. The measurement of multiple markers remains an essential step for defining tumor senescence. As we show in **Fig. 1**, SA-β-Gal staining should be combined to proliferation markers or cell-cycle inhibitors such as Ki67, p16, and/or p21. In addition, levels of the nuclear lamina protein lamin B1, which are dramatically decreased in various

senescence contexts (1), could also be combined. Moreover, the subtype of senescent cells can be further characterized by monitoring expression of certain SASP factors and SASP signaling regulators. Among them, IL6 and 8 are often upregulated in cancer cells exposed to senescence stimuli and correlate with disease progression. Nevertheless, it has also been reported that a senescence-dependent increase is not always observed, particularly for cancer cell lines where the baseline levels of IL6 and IL8 are already high (13). In alternative to ILs, recent data have suggested that matrix metalloproteases could serve as an indicator of a protumorigenic SASP and of cancer aggressiveness (14). Importantly, identification and detection of additional biomarkers associated to subtypes of (pseudo)senescent cancer cells can also help to understand the potential heterogeneity of the senescence response in the tumor environment and predict the response to certain senotherapeutics.

Authors' Disclosures

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