



RESEARCH HIGHLIGHT

A β -galactosidase kiss of death for senescent cells

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In a recent paper published in *Cell Research*, Cai et al. report development of the prodrug senescence-specific killing compound 1 (SSK1), which specifically clears SA- β -galactosidase-positive senescent cells both in vitro and in vivo. By activation of the p38 MAPK pathway and induction of cellular apoptosis, SSK1 functions broadly to attenuate chronic inflammation and improve physiological function.

Aging is a multifactorial progressive process that leads to dysfunction of tissue homeostasis and reduction of physiological integrity, thus being the predominant risk factor worldwide for chronic morbidities. Cellular senescence, in part by elevation of a senescence-associated secretory phenotype (SASP) mediating local and system inflammation, is known to exert a causative role in organismal aging and age-related diseases.¹ Hence, development of interventions specifically targeting senescent cells has significant therapeutic potential for ameliorating aging phenotypes, treating age-related disorders, and improving human lifespan and healthspan (healthy lifespan).

Most of the reported aging interventions fall into two categories: those that involve processes aimed at reversing cellular aging by modulating metabolic pathways or the epigenome, and those that involve the use of factors, so-called 'senolytics',^{2–4} to eliminate senescent cells by inducing apoptosis (Fig. 1a). Among the former, both heterochronic parabiosis (in which young and aged animals are surgically joined to connect their circulatory systems) and caloric restriction reverse physiological aging effects in animal models,^{5–7} but their translational potential remains uncertain. Similarly, transient reprogramming with the Yamanaka factors ameliorates phenotypic aging in progeroid mice.⁸ Other metabolic/epigenomic targeting approaches include gene therapy with rejuvenation factors,⁹ or the use of compounds such as Rapamycin, Oltipraz, Metformin, vitamin C and supplementation of NAD⁺ precursors including NR and NMN^{2,6,7} (Fig. 1a). In a different fashion, senolytics aim to extend healthspan and treat age-related chronic diseases by eliminating the senescent cells that accumulate in tissues and organs with age. Compounds with these remarkable capabilities include the Bcl-2 inhibitor Navitoclax (ABT-263), Fisetin (an antioxidant), and a Dasatinib and Quercetin combination (adding tyrosine kinase inhibitor to an antioxidant)^{4,10–12} (Fig. 1a). All these senolytics also have potential therapeutic limitations including targeting only certain types of senescent cells and off-target specificity for non-senescent cells.^{4,10,11} These data illustrate that identifying compounds with broad but simultaneously high specificity towards targeting senescent cells is challenging.

Cai et al.¹³ took advantage of a primary characteristic of senescent cells: increased activity of lysosomal β -galactosidase (β -gal), which is also a typical senescence biomarker. They designed a screen that would identify compounds with potent cytotoxicity for senescent cells as end-products and synthesized novel prodrugs uniquely converted by lysosomal β -gal into cytotoxic compounds, thereby inducing apoptosis specifically in senescent cell populations. Using this approach, they synthesized a lead candidate SSK1, which was cleaved by β -gal into cytotoxic gemcitabine in multiple mouse and human senescent cells but not in non-senescent cells. SSK1 selectively killed senescent cells under different kinds of senescence stimuli through activation of the p38 MAPK pathway and induction of apoptosis (Fig. 1b). Critically, the authors also demonstrated that SSK1 effectively ameliorated impaired physiological functions in aged mice and in mice with bleomycin-induced lung injury by attenuating SASP and injury responses.

Given the heterogeneity and complexity of aging processes, development of anti-aging interventions will need to consider specificity, efficacy, and safety aspects in order to move forward towards clinical development. The prodrug SSK1 developed by Cai et al. demonstrates functional properties that seem to offer some advantages relative to other reported senolytics. Specifically, ABT-263 eliminates senescent fibroblasts (human embryonic fibroblasts (HEFs)) and endothelial cells (human umbilical vein endothelial cells (HUVECs)), but shows little effect on human preadipocytes, whereas the Dasatinib/Quercetin combination appears to kill all of these three senescent cell types but also some non-senescent cells. On the other hand, Fisetin shows a modest effect on senescent HEFs and preadipocytes at a high dose (shown by Cai et al. and previous studies).^{10,11,13} In contrast, SSK1 overcomes these limitations by effectively eliminating senescent cells independent of cell types, species and stimuli, and by reducing toxicity to non-senescent cells. Encouragingly, the authors also show that high concentration and high frequency of SSK1 treatment causes no apparent systemic toxicities in aged mice, findings that bode well for in vivo safety in broader contexts.

Although SSK1 demonstrates promising advantages relative to other senolytics, challenges to be considered for clinical development remain. Firstly, senescence in and of itself provides physiological benefits to the body during development, wound healing, regeneration, and resistance to tumorigenesis.^{1,4} Hence, potential physiological side effects of SSK1, as well as other senolytics, need to be investigated. Secondly, senolytics as a class have been comprehensively investigated in a range of preclinical disease and aging models (including cardiovascular diseases,

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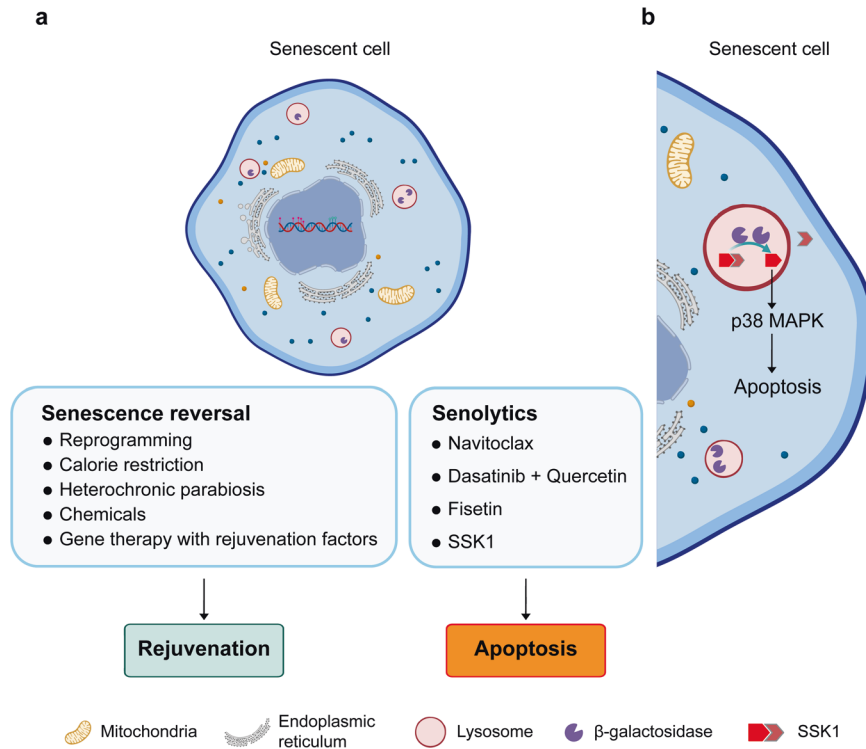


Fig. 1 Strategies for rejuvenation or elimination of senescent cells. **a** Schematic diagram of strategies that rejuvenate or eliminate senescent cells via modulation of metabolic pathways and epigenetic reprogramming to a younger state, or induction of apoptosis, respectively. **b** Processing of the prodrug SSK1 by lysosomal β -gal into a cytotoxic compound elicits activation of the p38 MAPK apoptotic pathway and clearance of senescent cells.

idiopathic pulmonary fibrosis, neurodegeneration disorders, diabetes, stress-induced mobility and frailty), with Dasatinib/Quercetin underway in preclinical trials (phase I in diabetic kidney disease and Alzheimer's disease) and fisetin already in clinical trials (phase IIb to reduce senescent phenotypes in older women).^{2,4} Relative to these exciting advances, the *in vivo* effects of SSK1 in such settings are currently unknown. Thirdly, there is an overall lack of knowledge on how senolytics including SSK1, impact on aging in higher-order mammals whose lifespan is closer to that of humans.

In conclusion, by targeting a senescence biomarker, the novel prodrug developed by Cai et al. successfully achieves selective deletion of senescent cells across a broad range of cell types. These findings align with previous work that has demonstrated the potential of senolytics as a class for extending lifespan and healthspan in experimental disease models. Future efforts focused on the safety and efficacy of SSK1 in more clinically relevant *in vivo* models will help inform feasibility of SSK1 therapy in human aging. Taking into account the complexity of human aging, it is possible that regimens that combine senolytics with

interventions targeting other fundamental processes may be needed to improve the health of the elderly.

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