Biomaterials Science

In Vitro Senescence and Senolytic Functional Assays

Journal:	*Biomaterials Science*
Manuscript ID	BM-MRV-12-2024-001684.R1
Article Type:	Minireview
Date Submitted by the Author:	29-Apr-2025
Complete List of Authors:	Ryan, Patrick; University of Massachusetts Amherst, Molecular & Cellular Biology Graduate Program Lee, Jungwoo; University of Massachusetts Amherst, Molecular & Cellular Biology Gradaute Program; University of Massachusetts Amherst, Chemical Engineering; University of Massachusetts Amherst, Biomedical Engineering

In vitro Senescence and Senolytic Functional Assays

Patrick Ryan [a], Jungwoo Lee [a,b,c*]

a. Molecular & Cellular Biology Graduate Program, University of Massachusetts, Amherst, Massachusetts, 01003, USA
b. Department of Chemical Engineering, University of Massachusetts, Amherst, Massachusetts, 01003, USA
c. Department of Biomedical Engineering, University of Massachusetts, Amherst, Massachusetts, 01003, USA

*E-mail: jungwoo@umass.edu

Abstract

A detailed understanding of aging biology and the development of anti-aging therapeutic strategies remain imperative yet inherently challenging due to the protracted nature of aging. Cellular senescence arises naturally through replicative exhaustion and is accelerated by clinical treatments or environmental stressors. The accumulation of senescent cells—defined by a loss of mitogenic potential, resistance to apoptosis, and acquisition of a pro-inflammatory secretory phenotype—has been implicated as a key driver of chronic disease, tissue degeneration, and organismal aging. Recent studies have highlighted the therapeutic promise of senolytic drugs, which selectively eliminate senescent cells. Compelling results from preclinical animal studies and ongoing clinical trials underscore this potential. However, the clinical translation of senolytics requires further pharmacological validation to refine selectivity, minimize toxicity, and determine optimal dosing. Equally important is the evaluation of senolytics' potential to restore tissue structure and function by reducing the senescent cell burden. In vitro tissue culture models offer a powerful platform to advance these efforts. This review summarizes the current landscape of in vitro systems used for inducing cellular senescence—referred to as "senescence assays"—and for screening senolytic drugs—referred to as "senolytic assays." We conclude by discussing key challenges to improving mechanistic insight, predictive accuracy, and clinical relevance in senolytic drug development, as well as emerging applications of senolytic therapies.

Keywords: In vitro, Senolytic, Senescence, Senomorphic, Aging, High Throughput Screening, Tissue Modeling.

Introduction

Aging is a complex biological process, and one hallmark is cellular senescence(1), a state in which cells lose their mitogenic ability but acquire a senescence-associated secretory phenotype (SASP)(2). While linked to aging and disease, senescence plays beneficial roles early in life by supporting tissue remodeling, regulating proliferation, and orchestrating immune responses(3). In youth, senescent cells are transient and efficiently cleared by immune surveillance(4-6), maintaining tissue homeostasis. However, with advancing age, they accumulate due to telomere attrition from repeated cell proliferation and prolonged exposure to genotoxic and oxidative stress. At the same time, declining immune function impairs their clearance. The resulting buildup of senescent cells is a major driver of age-related chronic diseases, including diabetes, osteoporosis, osteoarthritis, pulmonary fibrosis, and Alzheimer's disease(7-11). Beyond natural aging, standard cancer therapies such as radiation and chemotherapy induce substantial senescence in both malignant and normal cells(12-15). Recent studies also indicate that individuals recovering from COVID-19 often exhibit an elevated burden of senescence(16-19), further implicating senescence in post-viral pathology and chronic disease risk.

The recognition of senescent cells as key drivers of age-related pathology has led to the development of a new class of therapeutics, termed senolytics(20, 21). These agents aim to selectively eliminate senescent cells or mitigate their SASP through targeting pro-survival pathways characteristically upregulated in senescent cells(22). Several drug modalities, including small molecules, antibody-drug conjugates, and proteolysis-targeting chimeras, have been investigated(20, 23-26). However, their efficacy varies across tissues and organs(22, 27), partly due to the heterogeneous and dynamic nature of senescence. Moreover, their off-target toxicity remains largely undefined. Thus, accurate identification and robust validation of senolytic therapeutics have become critical goals in preclinical research.

A major challenge in aging research is the inherently slow pace of biological aging. While short-lived organisms like C. elegans and zebrafish offer valuable mechanistic insights, their physiological differences from humans limit translational relevance. Murine models remain a mainstay for mammalian models, but are costly to maintain when aged(28) and entail low-throughput experiments. In addition, species-specific differences constrain their clinical applicability.

In vitro tissue culture models present a promising alternative by enabling cost-effective, high-throughput, and human-relevant screening for senolytic agents(29-31). Polystyrene tissue culture plastic (TCP) has been widely used; however, its non-physiological rigidity and flat geometry, along with the absence of native extracellular matrix (ECM) components(32, 33) impose mechanical and biochemical stress on cells. This can induce premature cellular senescence and trigger cellular responses that diverge from in vivo counterparts(34). Consequently, although TCP-based 2D cultures have been instrumental in identifying candidate senolytics, most studies remain focused on measuring basic senescent biomarker changes rather than evaluating tissue-specific structural and functional restoration.

Bioengineered human tissue models, which culture human cells on substrates that mimic the structure and composition of native tissues, hold great promise for advancing senolytic drug discovery, functional assays, and safety assessment. Unlike conventional approaches that rely on a uniform TCP substrate across cell types, bioengineered models are designed to replicate tissue-specific architecture and function. Evaluating senolytic efficacy and toxicity in physiologically relevant models can improve preclinical studies' reliability and translational value. Bioengineered tissue models have been continuously developed(29-31, 35), and are expected to play a key role in expanding the landscape of senolytic targets and generating preclinical evidence of drug safety and efficacy with high predictive power(29).

Three key considerations when applying in vitro models to senolytic screening include: (i) How can cellular senescence be induced in an experimentally robust and physiologically relevant manner? (ii) What biomarkers can reliably and quantitatively identify

senescent cells with high specificity? (iii) How can senolytic efficacy be accurately evaluated, including not only the selective clearance of senescent cells but also the restoration of tissue-specific structure and function?

In this review, we first outline current senescence biomarkers, strategies to induce cellular senescence, and methods to assess senolytic drugs, with the goal of refining senescence and senolytic assays. We then highlight recent advances and applications of in vitro tissue-specific senescence models. Finally, we discuss ongoing challenges and emerging applications of bioengineered assays, including their potential to address environmental toxins and advance mechanistic understanding and therapeutic development.

A. Biomarkers to confirm senescence.

Senescent cells exhibit diverse phenotypic changes; however, no single biomarker can definitively identify cellular senescence, as many of these features are context-dependent, transient, and may also be present in quiescent or terminally differentiated cells. Therefore, accurate identification of senescent cells requires using multiple complementary biomarkers that capture the complex and heterogeneous nature of senescence **(Table 1)**.

A.1. Growth arrest. Growth arrest, a defining feature of cellular senescence, has been quantitatively assessed using three methods(36). First, nuclear staining with Hoechst or 4',6-diamidino-2-phenylindole (DAPI) quantifies nuclear number, size, and morphology(37, 38). Second, adenosine triphosphate (ATP)-based assays measure total cellular ATP levels by detecting luminescence generated through luciferase activity(39), providing an indirect readout of cell mass. Third, the 5-ethynyl-2'-deoxyuridine (Edu) incorporation assay evaluates proliferation by quantifying the incorporation of fluorescently-labeled nucleotides into newly synthesized DNA(40). Lastly, antibody staining of Ki67, a representative proliferation marker, is used to assess the mitogenic state of cells(41, 42). However, growth arrest-based assays alone are insufficient to define senescence, as quiescent cells that retain mitotic potential may be misclassified as senescent.

A.2. Expression of cycle related genes p53, p21, and p16. Senescent cells upregulate cell cycle inhibitors in response to DNA damage or oncogenic stress, measurable at both mRNA and protein levels. Common biomarkers include the tumor suppressor protein p53 and the cyclin-dependent kinase (CDK) inhibitors, p21 and $p16^{INK4A}$ (43-47). p53 plays a central role in initiating senescence(45), with its downstream effector p21(44) maintaining cell cycle arrest(48). $p16^{INK4a}$ promotes senescence by inhibiting CDK4/6, preventing phosphorylation of the retinoblastoma protein and blocking G1/S progression(47). However, elevated expression of p16, p23, and p53 alone is insufficient to define senescence, as similar profiles can be observed in quiescent cells.

A.3. Enlarged cell and nucleus size. Increased cell and nuclear size is a common feature of senescent cells(49). In vitro, senescent cells exhibit enlarged cell and nuclear size with altered morphology, which can be quantitatively measured by microscopy(50-52). However, increased size alone is insufficient to confirm senescence, as quiescent cells under certain conditions show similar features(53). Moreover, hypertrophic cells with enlarged morphology can transiently emerge during tissue repair following injury(54).

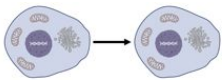
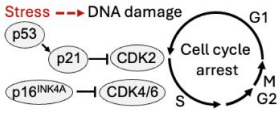
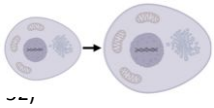
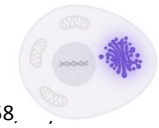
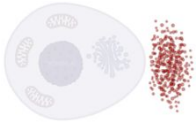
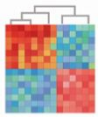
A.4. Senescence-associated-β galactose. SA-β-Gal, a lysosomal enzyme, is the most widely used biomarker for identifying senescent cells. Its increased expression is detected using two main strategies(55),(56). The first is chromogenic X-gal staining, which measures SA-β-Gal activity at pH 6.0. While simple, this method is limited by its endpoint nature. The second uses C12FDG, a fluorogenic β-galactosidase substrate that allows SA-β-Gal detection in live cells. This method is also compatible with fluorescence-activated cell sorting (FACS).(57) However, SA-β-Gal expression is influenced by assay conditions such as cell confluence, serum starvation, and medium pH(58). Moreover, cells released from quiescence can express SA-β-Gal, with levels correlating with time since release(49). Therefore, SA-β-Gal should not be used alone but in combination with additional biomarkers for accurate characterization of senescent cells.

A.5. Senescence-associated mitochondrial dysfunction. Compromised mitochondrial function is a hallmark of senescence and aging(1, 2). Senescence-associated mitochondrial dysfunction promotes the accumulation of reactive oxygen species (ROS), which exacerbate damage to both cellular and mitochondrial function(59, 60). Mitochondria function is commonly assessed by measuring the oxygen consumption rate (OCR)(61). Beyond energy production, mitochondria regulate apoptotic signaling(62) through making apoptosis-related proteins key biomarkers of senescence and targets for senolytic therapies. Notably, members of the B-cell lymphoma 2 (BCL-2) family, including BCL-xL, BCL-w, and MCL-1, are often upregulated in senescent cells, where they suppress apoptosis and mark mitochondrial-mediated senescence(63-65).

A.6. Senescence-associated secretory phenotype. Senescent cells secrete elevated levels of inflammatory cytokines, chemokines, growth factors, and matrix metalloproteinases, collectively referred to as the senescence-associated secretory phenotype (SASP)(66). A selected set of SASP factors is analyzed in conditioned medium using multiplex ELISA. More recently, omics-based approaches such as RNA-Seq and mass spectrometry have been employed for comprehensive and unbiased profiling. The Buck Institute has developed the SASP Atlas, an open-source proteomic database cataloging soluble proteins upregulated in senescent cells across diverse clinical samples and senescence triggers(67). Although core SASP components have been identified, the phenotype remains highly heterogenous, varying by cell type, time since senescence induction, and the nature of senescence-inducing stresses(68, 69). Thus, integrative efforts are needed to characterize SASP profiles across these key variables accurately.

A.7. Senescent-specific cell surface antigens. Advanced proteomic analyses have identified multiple membrane proteins selectively expressed on senescent cells, enabling their identification and isolation. Using EJp21 and EJp16 cell lines, ten senescence-associated surface markers have been identified, including DEP1, NTAL, EBP50, STX4, VAMP3, ARMX3, B2MG, LANCL1, VPS26A and PLD3(70, 71). B2MG has been further validated as a target for antibody-drug conjugates to eliminate senescent cells(24). Similarly, the membrane protein urokinase-type plasminogen activator receptor (uPAR), expressed on senescent cells, serves as a target for senolytic CAR-T cell therapies(23). The identification of surface markers represents a major advance due to their accessibility and druggability.

A.8. Transcriptomic panels. RNA-seq and meta-analyses have enabled the development of transcriptomic panels comprising core gene sets differentially expressed across diverse senescent cell types. Miossieveva et al. used a FACS-based approach to isolate senescent and non-senescent macrophages, satellite muscle cells, and fibro-adipogenic precursor cells, followed by RNA-seq analysis to identify a core senescence gene signature. Their study revealed the upregulation of genes involved in matrix remodeling, fibrosis, and inflammation, and the downregulation of genes related to cell cycle progression and protein translation(72). This study also demonstrated that muscle injury induces cellular senescence, subsequently impairing tissue regeneration. In a separate study, Saul et. al. conducted transcriptomic profiling of aged human bone biopsies and defined a novel gene set, termed “SenMayo” that reliably identified senescent cells across multiple tissues(73). While these studies provide compelling evidence for conserved senescence gene signatures, such signatures may differ by cell type and tissue context and are not universally generalizable. Continued investigation across varied biological settings is essential to define a robust senescence phenotype and discover new senolytic targets.

Table 1. Biomarkers to identify cellular senescence.		
Growth arrest		
DAPI/Hoechst, Ki67  Ref.: (74-76)	Function	Ki67 is expressed in proliferating cells; nuclear stains assess cell number/morphology
	Assays	IHC, western blot, qPCR, FACS, microscopy
	Advantages	IHC and DAPI retain spatial information Non-endpoint assays are possible with live DAPI stain
	Limitations	Quiescent cells also lack Ki67
p16, p21, p53  Ref.:(41, 42, 44-47, 49, 77-79)	Function	p16 and p21 are cell cycle inhibitors p53 is a tumor suppressor involved in senescence.
	Assays	IHC, western blot, qPCR
	Advantages	IHC retains spatial information.
	Limitations	Expression is also seen in quiescence and stress responses.
Morphology		
Cell & nuclear size  Ref:(50 ...,	Function	Senescent cells often exhibit enlarged and flattened morphology.
	Assays	Microscopy
	Advantages	Non-endpoint time-course microscopy imaging and measuring is possible. compatible with high-throughput imaging
	Limitations	Not senescence-specific; changes may occur in non-senescence states
Surface Antigens		
B2MG, DEP1, STX4, etc. uPAR  Ref.:(23, 70, 71	Function	Cell surface proteins upregulated on senescent cells.
	Assays	LC-MS, IHC, western blot, FACS
	Advantages	Druggable (B2MG demonstrated feasibility) Allows live cell isolation
	Limitations	Limited validation across tissues and senescence types
Intracellular cytoplasm		
SA-β-Gal  Ref.:(41, 49, 58, ...	Function	Increased lysosomal β-galactosidase activity in senescent cells
	Assays	Colorimetric (X-gal), fluorometric (C12FDG), FACS
	Advantages	C12FDG FACS allows isolation of senescent cells and supports live-cell analysis.
	Limitations	Colorimetric X-gal staining is endpoint-only, low-throughput, and limited in opaque models. SA-β-Gal expression is condition-dependent.
Mitochondria  Ref.:(63-65, 81, 82)	Function	ROS accumulation and metabolic changes are hallmarks of senescence.
	Assays	ROS: MitoSOX Red(83), dichlorodihydrofluorescein diacetate (DCFDA)(84), Microscopy & FACS Oxygen Consumption Rate, Seahorse XF Analyzer(85)
	Advantages	MitoSOX Red, DCFDA, and Seahorse XF Analyzer are compatible with live cells, MitoSOX Red is compatible with FACS sorting. Microscopy-based assays retain spatial information.
	Limitations	ROS is transient; OCR assay is costly (e.g., Seahorse XF Analyzer) and low-throughput.
Nucleus		
γ-H2AX Ref:(86, 87)	Function	Marker of DNA double-strand breaks Early senescence indicator
	Assays	IHC, western blot
	Advantages	Early detection; compatible with multiplex assays
	Limitations	May appear transiently in non-senescent cells.
Nuclear envelope: Lamin B1  Ref.:(88, 89)	Function	Lamin B1 expression decreases in senescent cells.
	Assays	IHC, western blot, qPCR
	Advantages	IHC retains spatial information; compatible with co-staining
	Limitations	Endpoint assay Weak signal in 3D or dense tissues
Secretion		
SASP  Ref.:(67, 90-92)	Function	Interleukins: IL-6, IL-8, IL-1α, IL-1β, IL-7, IL-15 Proteases: MMP1, MMP3, MMP9, PAI-1 Chemokines: MCP1, MCP2, MCP3, MCP4, MIP1α, MIP3α, HCC4, Eotaxin3, I-309, CXCL-1, CXCL-2, CXCL-4, CXCL-5, CXCL-12, GCP-2 Growth Factors: VEGF, GM-CSF, IGFBP-2, IGFBP-4, IGFBP-7
	Assays	ELISA, qPCR, RNA-seq, LC-MS
	Advantages	Omics-compatible; longitudinal tracking via conditioned media collection.
	Limitations	High cost; low throughput; transcript-protein mismatch possible
Transcriptomic Signatures		
Core gene panels (e.g. SenMayo)  Ref.:(68, 73, 93)	Function	Classify senescence subtypes, identify conserved features
	Assays	RNA-seq
	Advantages	Unbiased; support senescence classification
	Limitations	High cost; Transcript levels may not reflect function

B. Methods to induce cellular senescence. Obtaining primary senescent cells from aged animals or clinical samples is limited. Even when accessible, these cells display heterogeneous phenotypes and are impossible to expand. Thus, in vitro approaches have been exploited to expand cells, generate tissue models, and induce senescence in controlled and scalable methods. As senescence can be triggered through multiple pathways, diverse induction methods have been established—each associated with distinct mechanisms, phenotypes, and varying physiological and clinical relevance **(Table 2)**.

B.1. Radiation-induced senescence. Radiation-induced DNA damage is a well-characterized mechanism and a frontline cancer treatment(94). Since genomic instability and DNA damage are major drivers of cellular senescence, ionizing radiation has been widely used to rapidly induce cellular senescence(11, 22, 64, 81, 95-105). A common dosage is 10 Gy, although the post-irradiation culture period required to establish senescence varies from 3 days to 3 weeks, with most studies adopting a 7-10 day window. Ionizing radiation enables rapid and uniform senescence induction, making it well-studied for high-throughput applications. It also serves as a clinically relevant model for studying radiotherapy-induced senescence. However, its use is limited by the high cost and regulatory requirements associated with radiation equipment.

B.2. Drug-induced senescence. Chemotherapeutic agents such as doxorubicin, etoposide, and cisplatin are commonly used to treat various cancers and are known to induce significant cellular senescence(15, 104, 106). Chemotherapy-induced senescence is a key contributor to accelerated aging in cancer survivors. However, establishing robust chemotherapy-induced senescence models requires careful optimization and detailed characterization, as the extent of senescence and cytotoxicity varies widely depending on the specific cell type and tissue context.

B.3. Replicative stress-induced senescence. Replicative senescence, driven by repeated cell passaging, offers a physiologically relevant method for inducing senescence in vitro(107-110). This process typically involves 30-60 passages until cells cease division and enter a senescent state, primarily due to telomere attrition(111, 112). As such, replicative exhaustion is considered the most representative model of natural aging. However, this approach is time-consuming and requires prolonged culture, posing a major limitation. Moreover, the number of passages needed to reach senescence varies by cell type, species, and donor, complicating standardization across models.

B.4. Oxidative stress-induced senescence. Accumulation of reactive oxygen species (ROS) is a known driver of senescent phenotypes(113, 114). Ercc1−/− and Ercc1−/Δ mouse embryonic fibroblasts (MEFs), which lack the ERCC1-XPF DNA repair complex, are commonly used due to their rapid and cost-effective induction of oxidative stress-induced senescence under atmospheric oxygen; however, their murine origin limits translational relevance(115), (116). Hydrogen peroxide is a widely used agent for inducing oxidative stress-mediated senescence, with effects typically observed within 2 hours to 3 days(117-119). Other agents, such as ultraviolet light(120), tert-butyl hydroperoxide(121), and ethanol(122) can also induce senescence, but have not been employed in senolytic screening platforms.

B.5. Inflammatory cytokines-induced senescence. A hallmark of senescent cells is the SASP(2), characterized by a proinflammatory cytokine milieu capable of inducing senescence in neighboring healthy cells via paracrine signaling(123). This effect can be modeled by culturing healthy cells in conditioned media derived from a senescent cell population. Engineered transwell systems are particularly suited for studying paracrine-induced senescence. This approach is valuable for identifying senolytic drugs that target inflammation-driven, systemic senescence. However, it is limited by the time and resources required to produce sufficient conditioned media and the increased complexity of transwell-based assays.

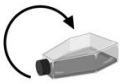
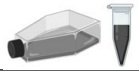
B.6. Thermal stress-induced senescence. Although limited, temperature-induced senescence presents a simple and physiologically relevant approach. The only well-established model is the ciPTEC-OAT1 kidney proximal tubule epithelial cell line, which conditionally overexpresses the organic anion transporter 1 when shifted from 33°C to 37°C(124). This temperature

increases halts cell proliferation and induces senescence-like features, including elevated expression of p53, p21, BCL-2 family proteins, and SASP components. These cells selectively respond to senolytics such as navitoclax, dasatinib, and quercetin. However, the utility of ciPTEC-OAT1 has thus far been demonstrated in 2D TCP, limiting its translational relevance for advanced bioengineered models.

B.7 Genetic manipulation-induced senescence. Senescence can be induced through overexpression of oncogenes or cell cycle inhibitors, a strategy widely applied using engineered cell lines(125). For example, the ER:Mek IMR90 human fibroblast line contains an inducible Ras construct activated by 4-hydroxy-tamoxifen, resulting in robust senescence induction(126). Similarly, bladder cancer cell lines EJp53, EJp21, and EJp16 utilize tetracycline regulated expression systems; removal of tetracycline triggers overexpression of p53, p16, or p21, leading to rapid and irreversible senescence. These models have been employed for senolytic drug screening(24, 71, 127).

Beyond engineered lines, oncogene-induced senescence can also be achieved via lentiviral or retroviral transduction. For example, Chang et al. retrovirally induced Ras into WI-38 human fibroblasts to induce senescence(96), while Amor et al. induced Ras-driven senescence in mouse hepatocytes through hydrodynamic tail vein injection in *Nras*^{G12V} mice, followed by in vitro analysis(23). Despite their utility, the availability of engineered cell lines and viral tools remains a barrier to widespread adoption.

Table 2. Strategies to induce in vitro cellular senescence.

Radiation 	**Mechanism**	Radiation-induced DNA damage activates p53-p21 or p16-Rb pathways to induce senescence.
	Conditions	2-20 Gy ionizing radiation exposure(11, 22, 64, 81, 95-105)
	Advantages	Rapid, robust, and cost-effective senescence induction. Recapitulates radiotherapy-induced senescence.
	Limitations	Does not recapitulate chronological aging; limited access to irradiation equipment.
Chemodrug 	**Mechanism**	Chemodrug-induced DNA damage activates p53-p21 or p16-Rb pathways to induce senescence.
	Conditions	Etoposide (2-50 µM), (65, 93, 128, 129) Alisertib (0.5-10µM), (93, 130) Barasertib (0.625-10µM), (130) Doxorubicin (100-200 nM), (7, 24, 101, 104, 106, 129, 131, 132) Cisplatin (15 µM), (101) Palbociclib (0.5-5 µM), (23, 101, 129) Ciprofloxacin (100 µg/mL), (133) Trametinib (25 µM), (23) Bleomycin (20 µM),(129) Gemcitabine (1 µM),(129)
	Advantages	High-throughput; Recapitulates chemotherapy-induced senescence.
	Limitations	Does not recapitulate chronological aging; Variable drug responses depending on cell type.
Replicative exhaustion 	**Mechanism**	Progressive telomere shortening leads to permanent growth arrest.
	Conditions	Extended passaging (10-60 passages) -Primary human melanocytes after 15 passages (23) -WI38 fibroblasts after 37 passages(26),[96] -Rhesus monkey retinal pigment epithelial cells after 8 passages(135) -PSC-27 prostate fibroblasts after 10 passages (134) -IMR90 fibroblasts after 40 passages(65)
	Advantages	Recapitulates chronological aging
	Limitations	Time-consuming; variable onset; heterogeneity across cell types.
Oxidative stress 	**Mechanism**	ROS-induced mitochondrial damage and DNA instability promote senescence.
	Conditions	Ercc1$^{-/-}$ mouse cells at 20% O_2(64, 128) Hydrogen peroxide (100-450 µM) (7, 117-119)
	Advantages	Rapid, scalable, and inexpensive
	Limitations	Does not recapitulate chronological aging process; ROS effects may be transient and context dependent.
SASP paracrine signalling 	**Mechanism**	Prolonged exposure to SASP induces secondary senescence.
	Conditions	Culture with conditioned media from senescent cells(7, 9, 19, 100, 135, 136)
	Advantages	Recapitulates inflammation-driven senescence; relevant for systemic aging
	Limitations	Require additional steps (conditioned medium generation); may have variable effects across cell types.
Thermal stress 	**Mechanism**	Temperature shift activates cell cycle inhibitors (e.g., p21 and p53).
	Conditions	ciPTEC-OAT1 cells cultured at 33°C from 37°C for 12 days(137)
	Advantages	Simple senescence induction.
	Limitations	Does not recapitulate chronological aging; limited to specific cell lines.
	Mechanism	Overexpression of oncogenes or cell cycle inhibitors induces senescence.
	Conditions	p16, p21, or p53 overexpression via lentivirus or tetracycline systems ER:Mek IMR90(101), EJp53(24), EJp21(24, 77), EJp16(138), FASST Cells(139), WI38(26, 96, 134), IMR-90(65, 105)

Genetic Manipulation 	**Advantages**	Controlled and rapid induction; compatible with reporter systems
	Limitations	Requires specialized cell lines or genetic tools; limited availability for all cell types

C. Assessment of senolytic drug effects.

In vitro evaluation of senolytic drugs involves three key steps: (i) establishing cell or tissue culture models, (ii) inducing and characterizing senescence, and (iii) administrating senolytics and assessing efficacy and toxicity. Accurate assessment of off-target toxicity in non-senescent cells is essential to prevent adverse side effects. First-line efficacy testing distinguishes senolytic effects, selective induction of apoptosis in senescent cells, from senomorphic effects, which suppress the SASP without inducing apoptosis. Second-line functional assays assess the extent to which senolytic restore or alleviate age-related cellular and tissue phenotypes, providing critical endpoints for determining therapeutic potential **(Fig.1)**.

C.1. Cell apoptosis. Apoptosis following senolytic treatment is commonly assessed using three different methods, First, terminal deoxynucleotidyl transferase dUTP nick-end (TUNEL) detects DNA fragmentation, a hallmark of late apoptosis(140). Second, Annexin V binds to phosphatidylserine, which is externalized on the plasma membrane during early apoptosis, making it a widely accepted marker of apoptotic initiation(141, 142). Third, caspase activation is routinely used to confirm apoptosis(62, 143). However, these markers are not specific to senescent cells, which can lead to confounded results due to cytotoxic effects on non-senescent cells. To address this, recent studies have identified dihomo-15d-PGJ_2, an oxylipin selectively released upon the apoptosis of senescent cells, as a promising senolysis-specific biomarker(105). While ELISA kits for 15d-PGJ_2 exist, they lack specificity and exhibit cross-reactivity(105). Thus, accurate detection of dihomo-15d-PGJ_2 currently relies on mass spectrometry, limiting its applicability in high-throughput senolytic screening.

C.2. Cell secretion. Reduction in SASP expression is a common method to assess senolytic efficacy. While SASP components can be measured at both the transcript and protein levels, protein-level analysis is preferred due to the limited correlation between mRNA and protein expression. Unbiased approaches such as multiplex ELISA panels or quantitative LC/MS of conditioned media enable simultaneous profiling of multiple SASP factors(144-146). For example, Mannarino et al. treated sparc -/- mice with o-vanillin and/or RG-7112, cultured intervertebral discs ex vivo, and used a Luminex multiplex assay to assess SASP protein levels(147). The main limitation of these methods is their high cost.

As a more accessible alternative, single-analysis ELISAs can be used to measure individual SASP factors. Transcript-level analysis via qPCR or RNA-seq also provides insight. For instance, Saul et al. used RNA-seq to identify a gene set upregulated in senescent bone cells and demonstrated its reduction following senolytic treatment(73).

C.3. Tissue function. Bioengineered tissue models provide a physiologically relevant and controllable platform to assess senolytic drug efficacy by replicating complex cell–matrix interactions and tissue-specific architecture. These systems enable direct evaluation of functional restoration, offering a more accurate and mechanistic measure of senolytic effectiveness in reversing age-related tissue dysfunction than surrogate markers such as senescent cell clearance alone.

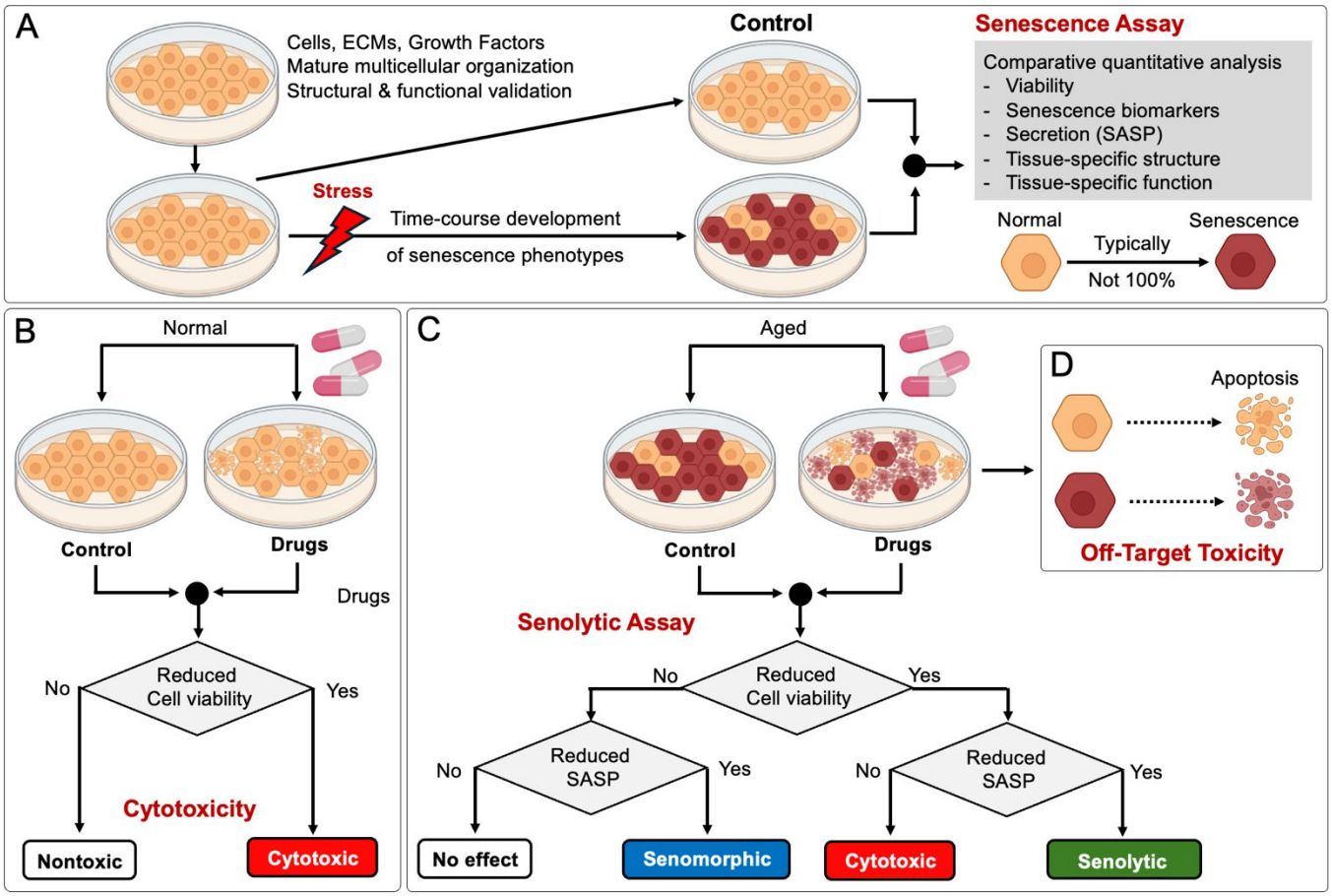


Figure 1. Workflow to Distinguish Senolytic, Senomorphic, Cytotoxic, and Off-Target Drug Effects. (A) Senescence assay: Multicellular in vitro models are stressed to induce senescence, assessed through viability, senescence biomarkers, SASP factors, tissue-specific structure, and function. Aged models typically show partial conversion to a senescent state. **(B)** Cytotoxicity assay: Candidate drugs are tested on non-senescent cells; a reduction in cell viability indicates general cytotoxicity. **(C)** Senolytic assay: Candidate drugs are tested on senescent models. Drugs that reduce both viability and SASP are classified as senolytics; those that reduce only SASP are senomorphics; drugs reducing viability without affecting SASP are classified as cytotoxic; no changes indicate no effect. **(D)** Off-target toxicity assay: Apoptosis in non-senescent cells indicates off-target toxicity. Ideal senolytics selectively eliminate senescent cells while sparing normal cells, although heterogeneous senescence complicates evaluation.

D. Tissue-specific models to assess senolytics.

Senolytic efficacy has traditionally been evaluated in 2D cell cultures by inducing senescence, applying senolytics, and measuring reductions in senescence markers. Recent advances in bioengineered models enable assessment of how senolytics restore tissue-specific structural and functional deficits associated with aging. Here, we present these tissue-specific models and highlight their utility in improving the predictive accuracy of in vitro senolytic screening.

D.1 Bone models. Osteoporosis is an age-related degenerative disease marked by declining bone density and increased fracture risk**(148-151)**. Bone homeostasis is maintained through continuous remodeling by bone-forming osteoblasts and bone-resorbing osteoclasts(152-154). Subsurface osteocytes, which constitute over 90% of bone cells, play a key role in regulating this balance through paracrine signaling(155, 156). Accumulation of senescent osteogenic cells disrupts bone remodeling, leading to net bone loss(157-159). While conventional osteoporosis treatments

remain effective, senolytic drugs offer a promising strategy to mitigate bone aging and improve skeletal health.

In vitro models have used osteoblast cell lines and primary bone marrow stromal cells (BMSCs), often in 2D monolayer cultures due to challenges in replicating the mineralized collagen-rich bone matrix. Functional assessments of senolytic efficacy have focused on enhancing osteoblast-mediated mineralization and reducing osteoclastogenesis **(Fig.2A)**. However, outcomes have been variable. For example, ABT-263 treatment of aged mouse BMSCs reduced senescence burden but impaired mineralized tissue formation(160). In contrast, another study showed that ex vivo treatment of aged mouse BMSCs with dasatinib and quercetin (DQ) improved proliferation and mineralization, evidenced by reduced SA-β-Gal, SASP factors, and cell cycle inhibitors, alongside increased calcein staining(161). Similarly, quercetin treatment of hydrogen peroxide-induced senescent rat BMSCs enhanced mineral deposition(162). Additionally, conditioned media from senescent cells impaired mineralization in osteoblastic MC3T3 cells compared to media from healthy controls (**Fig. 2B)**(9).

Beyond promoting bone anabolism, senolytics may prevent age-related bone loss by limiting osteoclastogenesis. Farr et al. demonstrated that SASP-conditioned media enhanced osteoclast precursor survival, which was reversed by JAK/STAT pathway inhibition **(Fig.2B)**(9). Samakkarnthai et al. reported that zoledronic acid exerted senolytic effects on fibroblasts and selectively reduced osteoclast precursors, as shown by single-cell proteomic analysis(163).

D.2. Cartilage models. Osteoarthritis is an age-related degenerative disease characterized by the progressive breakdown of articular cartilage, leading to joint pain, impaired mobility, and reduced quality of life(164-166). Senolytic therapies have emerged as a promising strategy to slow or reverse cartilage degeneration(165, 166). In vitro cartilage models for evaluating senolytic effects are divided into 2D monolayer and 3D pellet cultures. 2D monolayer cultures are straightforward to prepare, monitor, and scale, making them suitable for dose-response screening of senolytic compounds. In contrast, 3D pellet cultures where chondrocytes aggregate into spheroids, better replicate the ECM environment and chondrogenic differentiation, offering more physiologically relevant insights into cartilage regeneration and ECM remodeling **(Fig.2C)**.

For example, Jeon et al. used 2D cultures to identify the effective dose of UBX0101 and confirmed its regenerative effects in 3D pellets by demonstrating increased production of type II collagen and proteoglycans(8). Similarly, Yang et al. performed dose screening of ABT-263 in 2D cultures and observed enhanced expression of glycosaminoglycan, COL2A1, and ACAN in 3D human chondrocyte pellets(167). In contrast, Huang et al. reported that although the FOXO4-DRI peptide effectively eliminated senescent chondrocytes and reduced SASP markers, it did not significantly improve chondrogenesis in 3D cultures(168) **(Fig.2D)**.

D.3. Lung models. Idiopathic pulmonary fibrosis is a chronic, progressive lung disease characterized by abnormal fibroblast activation and excessive ECM deposition(169). Advanced aging is the primary risk factor, with cellular senescence playing a critical role in disease onset(170, 171). Pulmonary epithelial aging accelerates early fibrotic progression, making senolytic therapy a promising strategy to reduce senescent cell accumulation and prevent fibrosis. To date, ex vivo tissue cultures and lung organoid models have been used to study the functional impact of senescence on matrisome composition and fibrotic ECM remodeling (**Fig.2E)**.

For example, Lehmann et al. established a 3D lung explant culture model from bleomycin-induced fibrotic mice. This ex vivo model maintained tissue viability for two weeks and revealed increased senescent cell burden with fibrosis. Treatment with D+Q significantly reduced fibrotic collagen secretion during this period(172). Suezawa et al. developed human iPSC-derived lung organoids embedded in Matrigel with growth factors, generating alveolar type 2 (AT2) cells. Bleomycin treatment induced senescence in AT2 cells, while inhibition of ALK5 or blockade of integrin αVβ6 mitigated fibrogenic changes. RNA sequencing and matrisome analysis confirmed that TGF-β1 signaling is a

key driver of fibrosis mediated by bleomycin-induced senescent AT2 cells(173) (**Fig.2F).**

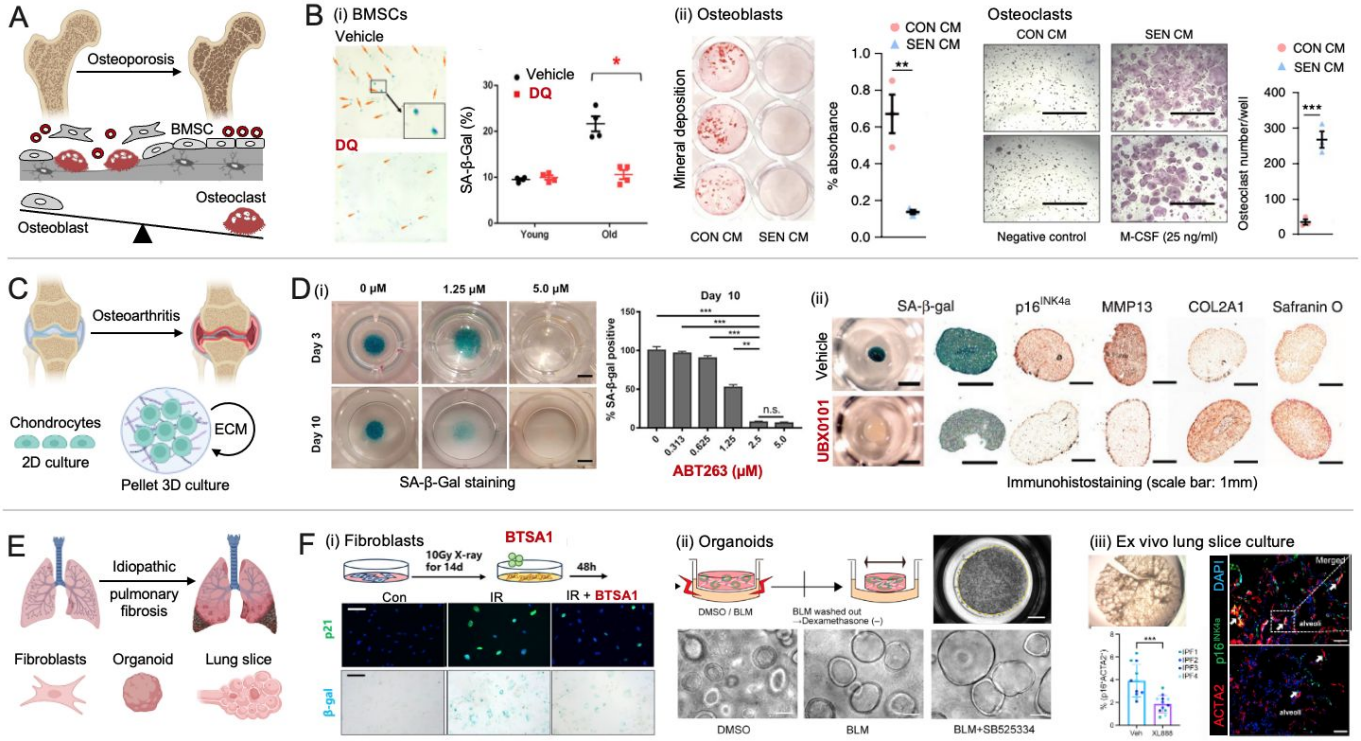


Figure 2. In vitro bone, cartilage and lung models for senescence and senolytic assays. (A) Senescence disrupts the regulation of bone remodeling, leading to osteoporosis by impairing the balance between osteoblasts and osteoclast activity. **(B)** Experimental studies demonstrate the impact of senescent bone marrow stromal cells (BMSCs): (i) DQ reduces senescent BMSCs in aged mice(161); and (ii) conditioned media from senescent cells impairs mineralization in MC3T3 osteoblasts and promotes osteoclast differentiation(9). **(C)** Senescence alters ECM deposition by chondrocytes, contributing to the development and progression of osteoarthritis which can be assessed in 3D pellet culture systems. **(D)** Senolytics have shown therapeutic potential in cartilage models: (i) ABT-263 reduces senescent chondrocytes(167); and (ii) UBX0101 modules cartilage ECM deposition in a 3D pellet culture model(8). **(E)** Senescent lung fibroblasts contribute to the pathogenesis of idiopathies of idiopathic pulmonary fibrosis by promoting a pro-fibrotic microenvironment. **(F)** Senolytic drugs reduce senescent cells in lung fibrosis models, including (i) fibroblasts in 2D culture(174), (ii) alveolar type 2 epithelial cells in lung organoids(173), and (iii) senescent cells in ex vivo human lung slice cultures(175).

D.4. Brain models. Aging is a major risk factor for neurodegenerative diseases such as Alzheimer's and Parkinson's. Accumulation of senescent brain cells contributes to disease progression by secreting pro-inflammatory cytokines, matrix-degrading enzymes, and other bioactive molecules. This persistent SASP exacerbates neuroinflammation, disrupts neuronal function, and accelerates neurodegeneration(176). Senolytic drugs hold promise for reducing neuroinflammation and slowing the progression of aging-related neurodegenerative conditions. A key advantage of engineered 3D brain models is their ability to recapitulate spatial organization, tissue architecture, and cellular complexity. Brain organoids have been widely used to study the effects of senescence and senolytic therapy on cell-type-specific gene expression, synaptic pruning, and disease progression **(Fig. 3A)**.

For example, Aguado et al. used human brain organoids to show that senolytic therapy eliminates senescent astrocytes, neurons, and neural progenitors

in physiologically aged models (**Fig.3B**). Transcriptomic profiling enabled cell-type-specific senolytic responses. They further modelled COVID-19-induced senescence and demonstrated that senolytics mitigated associated neurotoxic effects(177). Jin et al. used iPSC-derived brain organoids to demonstrate that type-I interferon signaling drives microglial over-pruning of synapses and accelerated senescence in down syndrome iPSC-derived brain organoids, and that inhibiting interferon receptor expression can partially rescue these dysfunctional phenotypes(178). Shaker et al. identified the glycosylated transmembrane protein klotho as a key regulator of cortical neuron senescence in iPSC-derived organoids(179). Lastly, brain organoids were employed to study Ataxia-telangiectasia, revealing that senescence contributes to disease progression via cGAS/STING pathway(180).

D.5. Heart models. Aging is a major risk factor for cardiovascular disease(181). Cardiomyopathy, an age-related degenerative condition, impairs cardiac tissue function(182, 183). Senescence plays a crucial role in the progression of cardiomyopathy, and emerging studies using bioengineered cardiac tissue models suggest that senolytic therapies may alleviate its detrimental effects (184). Functional characterization of cardiac organoids has focused on evaluating the ability of senolytics to restore contractile function, beating rate, and myogenic potential **(Fig.3C)**.

For example, Scalise et al. showed that doxorubicin induces senescence in human cardiac organoids, leading to cardiac progenitor exhaustion and reduced contractile function. D+Q treatment decreases oxidative stress, SASP factors, and senescence-related gene expression. D+Q treatment also restored cardiac beating rate and frequency, providing evidence for functional rejuvenation(106). Marino et al. examined cardiac tissue from patients with diabetes mellitus and observed an increased density of senescent cardiac stem cells, indicating paracrine-mediated senescence in the diabetic heart. Using a spherogenesis assay, they demonstrated that spheroids from diabetic cardiac stem cells exhibited impaired proliferation, differentiation, and function. Senolytic treatment significantly reduced the expression of SA-β-gal, $p16^{INK4A}$, and γ-H2AX, while enhancing cell proliferation and myogenic potential **(Fig.3D)**(185).

D.6. Tumour models. Advanced aging is a major risk factor for both primary and metastatic tumors. Tumor cells can evade traditional therapies by entering a senescent state(12). Concurrently, non-malignant cells in cancer survivors often develop therapy-induced senescence, contributing to accelerated aging and increased risk of relapse(14, 186-188). These challenges highlight the need for pharmacological strategies to eliminate both senescent tumor cells and non-neoplastic senescent cells. Advanced organoid models offer a powerful platform to investigate the distinct roles of senescent malignant, stromal, and immune cells within the tumor microenvironment and their contributions to tumorigenesis, metastasis, and drug resistance (**Fig.3E**).

For example, Nicolas et al. used patient-derived tumor organoids to show that senescent tumor cells induce therapy-associated senescence in fibroblasts via paracrine IL-Bα signaling, leading to inflammation and therapy resistance(189). Similarly, long-term culture of high-grade serous ovarian cancer organoids in SASP-conditioned media reduced tumor cell adhesion and downregulated adhesion-related genes, suggesting senescence in metastatic dissemination(190). Organoid models have also been used to study senescence-driven chemoresistance and identify senolytic vulnerabilities in patient-derived tumors from lung(191), ovaries(192), breast(193), colon(194), and gastric cancer (195) (**Fig.3F)**.

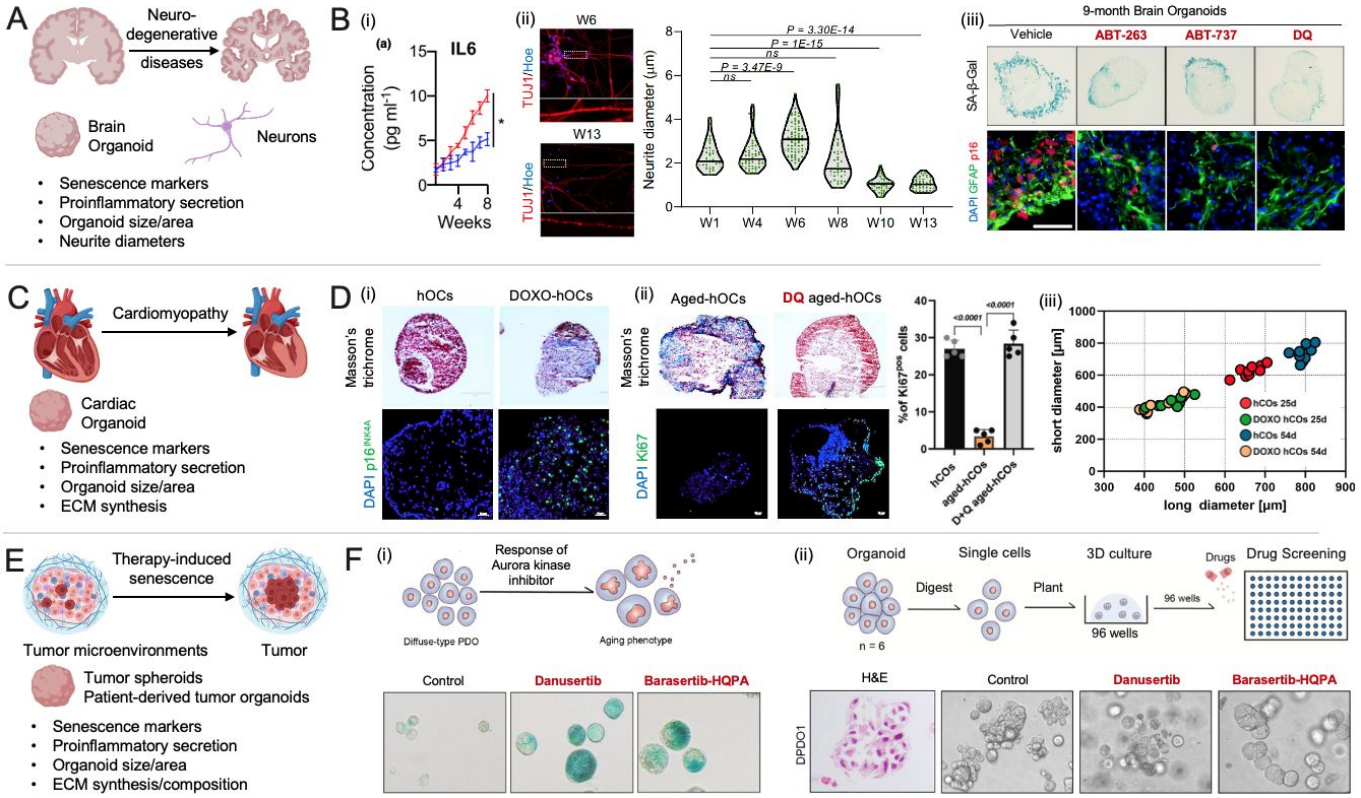


Figure 3. In vitro brain, cardiac muscle, and tumor models for senescence and senolytic assays. (A) Accumulation of senescent brain cells increases the risk of neurodegenerative diseases via persistent SASP, neuroinflammation, and neuronal dysfunction. **(B)** (i) Ataxia-telangiectasia-mimetic brain organoids exhibit increased IL-6 secretion(180), (ii) senescence reduces neurite diameter in brain organoids(179), (iii) Aged brain organoids show senolytic drug responses, decreased SA-β-gal and p16 expression(177). **(C)** Senescent cells in cardiac tissue contributes to cardiomyopathy. Cardiac organoids and cell cultures are used to study senescence and senolytic responses. **(D)** (i) Cardiac organoids exposed to doxorubicin exhibit senescence elevated p16 expression. (ii) Treatment with DQ improves myogenic cell proliferation in cardiac organoids. (iii) Organoid diameter changes serve as a quantitative measure of senescence and senolytic effects(106). **(E)** Patient-derived tumor organoids are used to study chemotherapy-induced senescence in tumor microenvironments. **(F)** (i) Gastric cancer organoids treated with aurora kinase inhibitors induce senescence and (ii) Schematic representation of senolytic drug testing using patient tumor-derived organoids(195).

E. Remaining challenges and considerations.

E.1. Advance senescence and senolytic assays.

E.1.1. Refining senolytic efficacy and toxicity. A key challenge in developing senescence assays lies in the fact that senescence is not a binary state, but rather a gradual and potentially reversible process. The emergence of senescent phenotypes is shaped by three factors: the cell or tissue types, the inducing stress, and the duration since senescence induction. For example, stress-induced senescence often involves p16/p38 activation, while replicative senescence is typically mediated by p53/p21 signaling(46). Transcriptomic analyses have revealed distinct context-dependent senescence signatures and considerable heterogeneity, particularly in replicative models(68, 69).

To capture the temporal dynamics, a three-stage model has been proposed: primary senescence (p53/p21 activation, reduced proliferation), developing senescence (p53/p21, p16 activation, SASP emergence, morphological change), and late senescence (p16

activation, SASP amplification, chromatin remodeling, and lysosomal activity)(46). While conceptually valuable, this framework lacks quantitative validation and may not be universally applicable. These complexities complicate the accurate identification of senescent cells and the quantitative assessment of senolytic efficacy and off-target toxicity. It underscores the need for comprehensive characterization across diverse models and the integration of high-content omics technologies to identify stage-specific biomarkers that can guide the development of robust, predictive senolytic assays.

E.1.2. Standardization of controls. A critical aspect of senolytic assays is the consistent inclusion of controls. Senescence controls establish baseline biomarker levels by comparing untreated cells to those exposed to senescence-inducing stressors, with commonly used markers including SA-β-gal and SASP factors. Senolytic controls consist of senescent cells that do not receive drug treatment, enabling quantification of drug-specific effects and distinguishing true senolytic activity from off-target toxicity. However, the incomplete and heterogeneous nature of senescence induction complicates accurate assessment of senolytic efficacy and toxicity. Additionally, distinguishing quiescent from senescent cells is essential, as overlapping biomarker expression can lead to misidentification. Standardized controls are especially important when validating novel senescence biomarkers and ensuring reproducibility across experimental conditions.

E.1.3. Senescence reporter cells. Senolytic effects are dynamic, yet most assays rely on static, endpoint measurements. Developing senescence reporter cell lines, which express fluorescent or luminescent signals under the control of senescence-associated promoters, can be a promising alternative strategy. For example, A375 melanoma cells engineered to express GFP or luciferase under the control of mi-RNA-146a, a marker highly active in senescent cells(196, 197), have been employed in high-throughput screening to identify potent senescence inducers(130). Similarly, iPSC-derived reporter lines driven by p21 promoter activity(198, 199) show great potential as versatile tools for senescence and senolytic research.

Several senescence reporter mouse models have also been developed, including $P16^{INK4A}$ (200, 201) and p21 reporter strains(202) **(Table 3)**. Although primary cells often undergo rapid senescence during ex vivo culture, these models remain valuable for mechanistic and quantitative analysis. For example, the p16-3MR mouse expresses fluorescence and luciferase under the p16 promoter and includes a suicide gene activated by diphtheria toxin(201). Nevertheless, careful validation of reporter fidelity and sensitivity is essential before deploying these systems in senescence and senolytic assays. For example, the p16-3MR model yielded weak endogenous fluorescence and requires endpoint antibody staining.

Table 3. Reporter cells of senescence biomarkers

mi-RNA-146a	Function	SASP regulator
	Cell line	miR146a-EGFP A375 cell line(130, 196, 197)
$p16^{INK4A}$	Function	Cell cycle inhibitor
	Mouse model	$p16^{INK4A}$-ATTAC mice(203) p16-3MR mice(201), p16-Luc mice(204) p16-tdTomato(205)
p21	Function	Cell cycle inhibitor
	Cell line	p21-mNeonGreen iPSC line(199) A549 p21V cell line(198)
	Mouse model	p21-3MR mouse model(202) p21-Luc mice(206)

E.2. Advanced in vitro human tissue models

E.2.1. Cell source. Bioengineered senolytic assays can enhance translational impact by optimizing human cell sources. First, primary human mesenchymal stem cells that readily expand and differentiate are well-suited for developing tissue-specific, high-throughput senolytic assays. Establishing cell banks from multiple donors across different ages and sexes can improve population heterogeneity. Second, induced pluripotent stem cells (iPSCs) are a promising source for developing humanized senolytic assays(173, 178, 179). Advances in iPSCs generation, expansion, and directed differentiation have enabled their broad application. Reporter iPSC lines, such as those from the Allen Institute, offer powerful tools for mechanistic studies and quantitative phenotypic assay development. Third, given cancer's heterogeneity, patient-specific senolytic assays are critical for targeting drug-resistant senescent cancer cells(207),(208). Personalized tissue models hold great

promise in advancing senolytic drug screening and clinical impact **(Fig.4A)**.

E.2.2. Extracellular matrices. Each tissue has a distinct ECM composition and structure that plays a critical role in regulating multicellular organization and supporting essential tissue functions(209). Thus, selecting an appropriate ECM is crucial for accurately recapitulating tissue-specific senescent phenotypes and senolytic drug responses. Matrigel - a mouse tumor-derived ECM gel - has been widely used in organoid culture due to its ability to support the proliferation and differentiation of various stem cells(210). However, its broad, non-organized signalling may limit its effectiveness in guiding tissue-relevant organoid development(211). To address this, ECM gels derived from target tissues via decellularization and followed mechanical or enzymatic dissociation have been used to better support organoid maturation(212-214). For example, demineralized bone paper created by thin sectioning of demineralized bovine compact bone matrix significantly improves the recapitulation of in vivo-relevant, multicellular bone remodeling processes(152, 215-217) **(Fig.4B)**.

E.2.3. Oxygen environments. Oxygen level is a key factor that significantly influences drug responses by modulating cellular metabolism(218-220). This is likely applicable to senolytic drugs as well. Mitochondrial dysfunction and ROS-induced toxicity are key features of senescence(2). Previous studies showed that low oxygen exposure can extend lifespan in mice by reducing neurological damage(221, 222), suggesting that hypoxia may attenuate senescent phenotypes. However, the expression of hypoxia-induced factors (HIFs) under low-oxygen conditions plays a context-dependent role in senescence(218). In some cases, HIFs promote senescence induction(220, 223-225), while in others, they inhibit its onset(219, 226, 227). Understanding how hypoxia regulates senescence and influences cellular responses is critical for identifying new senolytic or senomorphic targets. Recapitulating physiological oxygen gradients in senolytic assays represents great promise for elucidating hypoxia's role in senescence regulation(228, 229) (**Fig.4C)**.

E.2.4. Mechanical stimulation. Each tissue exhibits a distinct mechanical stiffness, exposing cells to dynamic physical cues that are essential for maintaining homeostasis(230-237). A decline in the ability to sense and respond to these mechanical cues is closely associated with aging and cellular senescence(238-240). Senescent cells display altered cytoskeletal architecture(241) and changes in focal adhesion protein expression(242), which disrupts mechanotransduction. Mechanosensitive ion channels, such as PIEZO1, may also become dysfunctional and contribute to senescence induction(243-245). The degradation of lamin, a key structural component of the nuclear envelope, in senescent cells further deteriorates the mechanical regulation of gene expression via nuclear deformation(246). Additionally, the inflammatory SASP can promote ECM remodeling and alter local mechanical environments(66). Recapitulating these static and dynamic changes in mechanical environments in senolytic assays is a promising strategy to accurately model normal and aged tissue phenotypes and improve the predictive power of senolytic responses (**Fig.4D)**.

E.3. Leverage advances in senescence biology.

E.3.1. Benchmark clinical data. A major challenge in developing in vitro senolytic assays is the lack of comprehensive, tissue-specific, and quantitative data on senescence phenotypes from clinical samples. Without such benchmarks, it is difficult to assess the fidelity of engineered tissue models in accurately representing senescent phenotypes. Although senescent cells are relatively rare in aged humans, efforts to collect relevant data are progressing. For example, the Buck Institute's SASP Atlas has profiled SASP expression across various cell types and senescence-inducing stressors based on clinical samples from the Baltimore Longitudinal Study of Aging(67). The Mayo Clinic's "SenMayo" gene set, derived from aged human bone samples, enables single-cell identification of senescent cells and has shown decreased expression following senolytic treatment(73). Expanding these resources to include tissue-specific data from naturally aged, young, healthy, and prematurely aged individuals across diverse demographics will be crucial for benchmarking and validating senescence and senolytic assays.

E.2.2. Integrating computational approaches. The detection and characterization of senescence have advanced significantly with the rise of computational approaches aimed at improving the precision of senescent cell identification and decoding their complex phenotypes. Early efforts focused on morphological features, particularly changes in nuclear architecture, using machine learning classifiers trained on high-content imaging data to distinguish senescent from proliferative cells(50). With the growth of transcriptomic technologies, large-scale gene expression profiling led to the development of databases such as SenoRanger, CellAge, and SenMayo, which catalog genes consistently upregulated across various senescent cell types(73, 247, 248). These resources have been instrumental in identifying conserved senescence signatures and prioritizing therapeutic targets.

Building on this foundation, tools like SenCID now integrate bulk and single-cell transcriptomic data to enhance detection sensitivity and uncover context-specific senescence identities across tissues, cell types, and stressors(249). This recognition of senescence heterogeneity represents a critical step toward understanding its biological complexity and tailoring therapeutic interventions accordingly. In parallel, machine learning models trained on known senolytics have been employed to predict senolytic potential by analyzing structural, functional, and transcriptomic features, enabling in silico screening of large chemical libraries and accelerating drug discovery(250). Collectively, these computational innovations have transformed the ability to detect, classify, and therapeutically target senescent cells, offering powerful tools to advance the next generation of senolytic strategies.

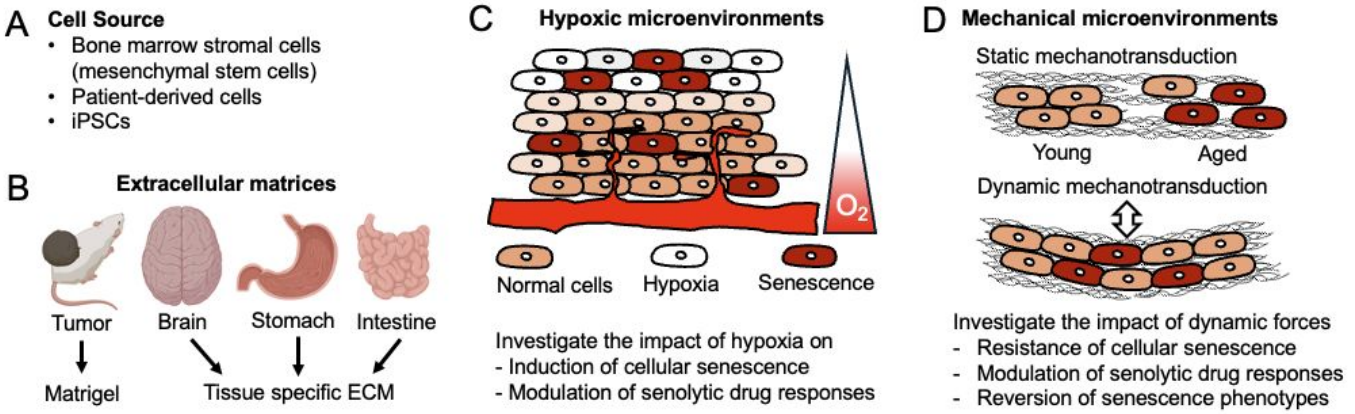


Figure 4. Emerging challenges and future directions. (A) Bioengineered models using diverse human cell sources enable personalized, tissue-relevant studies of senescence. **(B)** Tissue-specific ECMs enhance physiological relevance and support organ-specific senescent phenotypes. **(C)** Hypoxia-mimicking systems allow investigation of low-oxygen effects on senescence induction and senolytic drug efficacy in hypoxia-adapted cells. **(D)** Mechanotransduction-enabled models replicate biomechanical cues in mechanosensitive tissues to evaluate how mechanical forces influence senescence and whether senolytics restore proper signaling in aged cell.

F. Emerging applications of senolytic assays.

F.1. Senescence assays to evaluate environmental toxin-induced senescence. Modern industrialization has introduced various environmental toxins into ecosystems that may accelerate aging. Microplastics have been detected in various human tissues(251, 252). Per- and polyfluoroalkyl substances (PFAS), commonly used due to their extreme chemical stability(253), have been epidemiologically linked to osteoporosis(254), cardiovascular disease(255), and cancer(256). Heavy metals such as arsenic, mercury, lead, cadmium, chromium, and copper have also been associated with age-related pathogenesis, including neurodegenerative diseases(257), osteoporosis(258), and cancers(259). Similarly, chronic exposure to UV radiation accelerates

skin aging(260), increases the accumulation of senescent cells(261), and promotes skin cancer(262). These environmental stressors are emerging as key inducers of cellular senescence. Bioengineered human tissue-based senescence assays can be applied to assess their harmful effects, study aging-related phenotypes, and evaluate the potential of senolytic intervention (**Fig.5A)**.

F.2. Senolytic assays to expand senolytic drug repertoire. Robust senolytic assays are essential to expand senolytic drug discovery, which can be pursued through three key strategies. First, combinatorial drug approaches have shown enhanced efficacy by targeting multiple anti-apoptotic pathways that differ across senescent cell types. Core survival networks vary by cell type(22), highlighting the benefits of multi-targeted therapies for improving efficacy, tissue specificity, dose optimization, and minimizing off-target effects. A representative example is the D+Q combination, which outperforms single agents.

Second, drug repurposing is a rapid, cost-effective approach to expand the senolytic pipeline. FDA-approved drugs have well-characterized pharmacokinetic and safety profiles, enabling faster clinical transition for senolytics(194). Recent in silico analyses have identified senescence-maintaining gene networks, uncovering new targets for therapeutic intervention(250).

Third, natural compound screening, particularly of flavonoids, offers a rich source of potential senolytics. Compounds such as fisetin(128), quercetin(22), and luteolin(263) are flavonoid-derived and modulate senescence-associated pathways. With over 5,000 flavonoids identified, high-throughput functional screening could uncover novel compounds with cell-type and disease-specific senolytic activity(264-266) **(Fig.5B).**

F.3. Senescence and senolytic assays to advance adjuvant cancer therapy. Senolytic drugs are gaining traction as adjuvant therapies in cancer treatment. Chemotherapy and radiation induce widespread senescence in normal tissues, accelerating biological aging and increasing the risk of long-term complications in cancer survivors(267, 268). Incorporating senolytics into treatment regimens could mitigate therapy-induced senescence, delay aging-related sequelae, and enhance treatment outcomes. Administering senolytics post-chemotherapy or radiation may synergistically eliminate residual senescent tumor cells(93, 130) and senescent stromal cells, reducing recurrence risk(98, 186). Emerging evidence also implicates senescence in pre-metastatic niche formation and metastatic spread(139) through SASP-mediated regulation of tumor cell adhesion, invasion, and migration(190, 269, 270).

Advanced in vitro tissue models allow faithful simulation of these clinical scenarios, dissection of senescence induction in tumor versus stromal cells, and reveal new vulnerabilities(6, 12). They can also parse the roles of senescent immune and stromal cells in the tumor microenvironment in promoting cancer progression, drug resistance, and relapse. Indirect co-culture models are suited for studying long-term SASP paracrine signalling, while direct models allow analysis of ECM remodelling, cell-cell contact, and biochemical cues. Collectively, these tools will facilitate mechanistic studies and accelerate the integration of senolytic therapies into cancer treatment **(Fig.5C).**

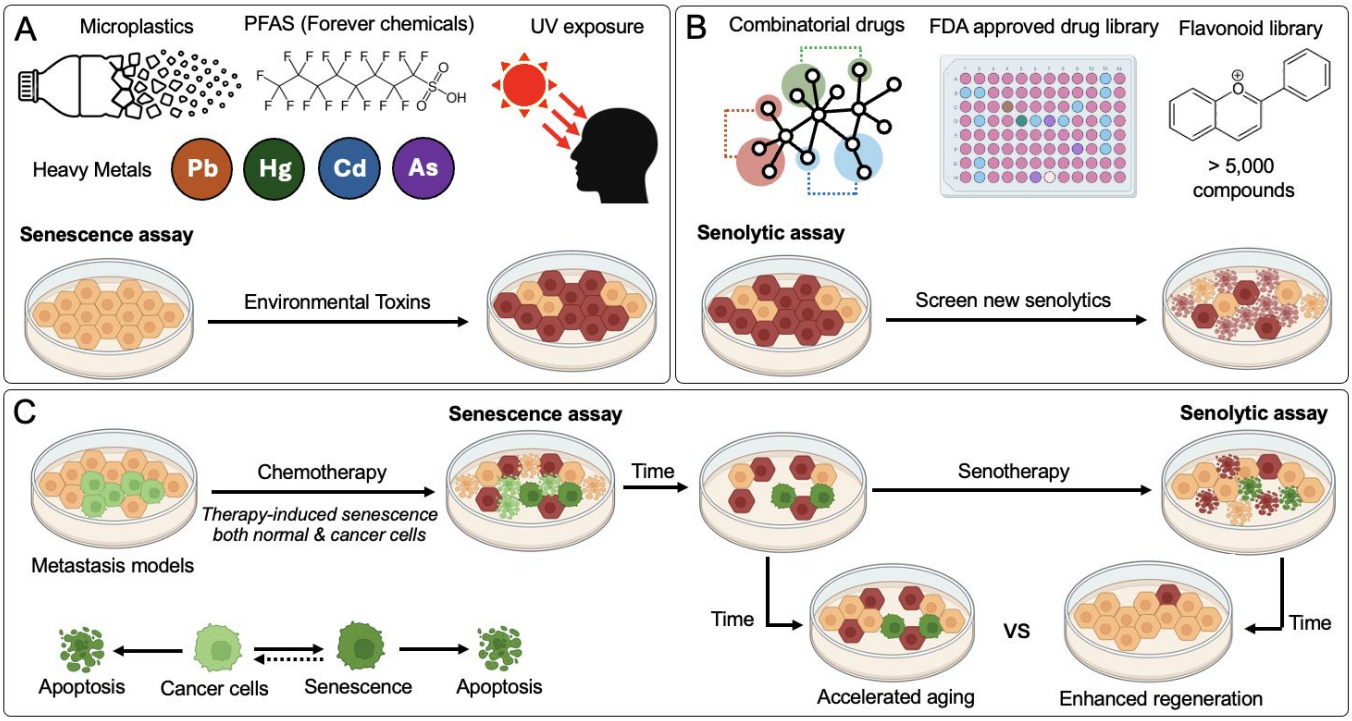


Figure 5. Emerging applications of senescence and senolytic assays. (A) Senescence assays enable the study of how environmental toxins induce cellular senescence. **(B)** Senolytic assays support the discovery of new senolytics through combinatorial strategies, repurposing FDA-approved drugs, and screening natural compounds such as flavonoids. **(C)** These assays can model therapy-induced senescence following cancer treatment and evaluate senolytic strategies to eliminate senescent tumor or stromal cells, aiming to improve treatment outcomes.

Conclusion

Human lifespan has steadily increased in recent decades, yet aging and its associated functional decline remain major contributors to chronic diseases. Senescence is traditionally considered an antagonistic pleiotropic process—beneficial during early development but detrimental later in life. Emerging evidence suggests a more nuanced view, with certain senescent cell subtypes performing beneficial roles. However, the accumulation of deleterious senescent cells poses significant health risks, underscoring the need for senolytics that selectively eliminate harmful cells while sparing beneficial ones.

Developing pharmacological strategies to slow aging remains a major scientific and medical challenge. Senolytic therapies hold great potential to delay, and possibly reverse, age-related phenotypes. The advancement of preclinical in vitro human tissue models and predictive phenotypic assays will be essential for accurately assessing senolytic efficacy and toxicity, thereby accelerating clinical translation. The FDA Modernization Act 2.0 supports this shift by authorizing validated human tissue models as alternatives to animal testing. With continued collaboration across academia, industry, and government, significant progress in aging biology and therapeutic development is expected in the next 5–10 years.

Conflicts of Interest: PR and JL are cofounders of MetaBone Inc.

Author contributions: PR and JL conceived topics. PR wrote the manuscript. JL edited the manuscript.

Funding Sources: This work was supported by NIH (R01CA237171 & R33CA291301) and NSF (1944188) grants. P.R. was supported by a NIH Biotechnology Traineeship (T32 GM13509).

References

1. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell. 2023; 186(2):243-78.
2. Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of Cellular Senescence. Trends Cell Biol. 2018; 28(6):436-53.
3. Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular Senescence: Aging, Cancer, and Injury. Physiol Rev 2019; 99(2):1047-78.
4. Hoenicke L, Zender L. Immune surveillance of senescent cells—biological significance in cancer- and non-cancer pathologies. Carcinogenesis. 2012; 33(6):1123-26.
5. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature. 2007; 445(7128):656-60.
6. Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature. 2011; 479(7374):547-51.
7. Aguayo-Mazzucato C, Andle J, Lee TB, Jr., Midha A, Talemal L, Chipashvili V, et al. Acceleration of beta Cell Aging Determines Diabetes and Senolysis Improves Disease Outcomes. Cell Metab. 2019; 30(1):129-42 e4.
8. Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP, et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med. 2017; 23(6):775-81.
9. Farr JN, Xu M, Weivoda MM, Monroe DG, Fraser DG, Onken JL, et al. Targeting cellular senescence prevents age-related bone loss in mice. Nat Med 2017; 23(9):1072-79.
10. Lehmann M, Korfei M, Mutze K, Klee S, Skronska-Wasek W, Alsafadi HN, et al. Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. Eur Respir J. 2017; 50(2):1602367.
11. Zhang P, Kishimoto Y, Grammatikakis I, Gottimukkala K, Cutler RG, Zhang S, et al. Senolytic therapy alleviates Abeta-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. Nat Neurosci. 2019; 22(5):719-28.
12. Schmitt CA, Wang B, Demaria M, Schmitt CA, Wang B, Demaria M. Senescence and cancer — role and therapeutic opportunities. Nat Rev Clin Oncol 2022; 19(10):619-36.
13. Febres-Aldana CA, Kuritzky N, Krishnamurthy K, Poppiti R, Howard L. Evaluation of the expression of P16INK4A by immunohistochemistry in post-neoadjuvant chemotherapy hormone receptor negative breast cancer specimens. Breast Dis. 2020; 39(2):51-9.
14. Sanoff HK, Deal AM, Krishnamurthy J, Torrice C, Dillon P, Sorrentino J, et al. Effect of cytotoxic chemotherapy on markers of molecular age in patients with breast cancer. J Natl Cancer Inst. 2014; 106(4):dju057.
15. Yao Z, Murali B, Ren Q, Luo X, Faget DV, Cole T, et al. Therapy-Induced Senescence Drives Bone Loss. Cancer Res. 2020;80(5):1171-82.
16. Schmitt CA, Tchkonia T, Niedernhofer LJ, Robbins PD, Kirkland JL, Lee S. COVID-19 and cellular senescence. Nat Rev Immunol. 2023; 23(4):251-63.
17. Lekva T, Ueland T, Halvorsen B, Murphy SL, Dyrhol-Riise AM, Tveita A, et al. Markers of cellular senescence is associated with persistent pulmonary pathology after COVID-19 infection. Infect Dis. 2022; 54(12):918-23.
18. D'Agnillo F, Walters KA, Xiao Y, Sheng ZM, Scherler K, Park J, et al. Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. Sci Transl Med. 2021; 13(620):eabj7790.
19. Lee S, Yu Y, Trimpert J, Benthani F, Mairhofer M, Richter-Pechanska P, et al. Virus-induced senescence is a driver and therapeutic target in COVID-19. Nature 2021; 599(7884):283-89.
20. Zhang L, Pitcher LE, Prahalad V, Niedernhofer LJ, Robbins PD. Recent advances in the discovery of senolytics. Mech Ageing Dev. 2021;200:111587.
21. Kirkland JL, Tchkonia T. Senolytic drugs: from discovery to translation. J Intern Med. 2020; 288(5):518-36.
22. Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015; 14(4):644-58.

23. Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, et al. Senolytic CAR T cells reverse senescence-associated pathologies. Nature. 2020; 583(7814):127-32.
24. Poblocka M, Bassey AL, Smith VM, Falcicchio M, Manso AS, Althubiti M, et al. Targeted clearance of senescent cells using an antibody-drug conjugate against a specific membrane marker. Sci Rep 2021; 11(1):20358.
25. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. Nat Med 2018; 24(8):1246-56.
26. He Y, Zhang X, Chang J, Kim H-N, Zhang P, Wang Y, et al. Using proteolysis-targeting chimera technology to reduce navitoclax platelet toxicity and improve its senolytic activity. Nat Commun 2020; 11(1):1996.
27. Grosse L, Wagner N, Emelyanov A, Molina C, Lacas-Gervais S, Wagner KD, Bulavin DV. Defined p16(High) Senescent Cell Types Are Indispensable for Mouse Healthspan. Cell Metab. 2020; 32(1):87-99 e6.
28. Flurkey K, Currer JM. Pitfalls of animal model systems in ageing research. Best Pract Res Clin Endocrinol Metab. 2004; 18(3):407-21.
29. Hu JL, Todhunter ME, LaBarge MA, Gartner ZJ. Opportunities for organoids as new models of aging. J Cell Biol 2018; 217(1):39-50.
30. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 2014; 345(6194):1247125.
31. Rossi G, Manfrin A, Lutolf MP, Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. Nat Rev Genet 2018; 19(11):617-87.
32. Wang H, Li S, Dai Q, Gonzalex A, Tran O, Sun H, DeFronzo R, Dean D, Yeh CK, Chen XD. Culture on a native bone marrow-derived extracellular matrix restores the pancreatic islet basement membrane, preserves islet function, and attenuates islet immunogenicity. FASEB J. 2020; 34(6):8044-56.
33. Lerman MJ, Lembong J, Muramoto S, Gillen G, Fisher JP. The Evolution of Polystyrene as a Cell Culture Material. Tissue Eng Part B: Rev. 2018; 24(5):359-72.
34. Sherr CJ, DePinho RA. Cellular senescence: mitotic clock or culture shock? Cell. 2000; 102(4):407-10.
35. Loewa A, Feng JJ, Hedtrich S, Loewa A, Feng JJ, Hedtrich S. Human disease models in drug development. Nat Rev Bioeng 2023;1:545-59.
36. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L. Cell Viability Assays. Assay Guidance Manual (Internet):2004.
37. Kapuscinski J. DAPI: a DNA-specific fluorescent probe. Biotech Histochem. 1995;70(5):220-33.
38. Atale N, Gupta S, Yadav USC, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. J Microsc. 2014; 255(1):7-19.
39. Kijanska M, Kelm J. In vitro 3D Spheroids and Microtissues: ATP-based Cell Viability and Toxicity Assays. Assay Guidance Manual (Internet), 2016.
40. Troth EV, Kyle DE. EdU Incorporation To Assess Cell Proliferation and Drug Susceptibility in Naegleria fowleri. Antimicrob Agents Chemother. 2021; 65(7):e0001721.
41. Alessio N, Aprile D, Cappabianca S, Peluso G, Dernardo GD, Galdersi U. Different Stages of Quiescence, Senescence, and Cell Stress Identified by Molecular Algorithm Based on the Expression of Ki67, RPS6, and Beta-Galactosidase Activity. Int J Mol Sci. 2021; 22(6):3102.
42. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018; 127(2):175-86.
43. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. EMBO J. 2003;22(16):4212-22.
44. Aliouat-Denis CM, Dendouga N, Wyngaer IV, Goehlmann H, Steller U, Weyer IVD, Slycken NV, Andries L, Kass S, Luyten W, Janicot M, Vialard JE, p53-independent regulation of p21Waf1/Cip1 expression and senescence by Chk2. Mol Cancer Res 2005;3(11): 627-34.
45. Fischer M, Fischer M. Census and evaluation of p53 target genes. Oncogene 2017; 36(28):3943-56.
46. Mijit M, Caracciolo V, Melillo A, Amicarelli F, Giordano A. Role of p53 in the Regulation of Cellular Senescence. Biomolecules. 2020; 10(3):420.
47. Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. Int J Cancer. 2012;130(8):1715-25.

48. Benson EK, Mungamuri SK, Attie O, Kracikova M, Sachidanandam R, Manfredi JJ, et al. p53-dependent gene repression through p21 is mediated by recruitment of E2F4 repression complexes. Oncogene 2014; 33(30):3959-69.
49. Ashraf HM, Fernandez B, Spencer SL, The intensities of canonical senescence biomarkers integrate the duration of cell-cycle withdrawal. Nat Commun. 2023;14(1):4527.
50. He L, Li M, Wang X, Wu X, Yue G, Wang T, et al. Morphology-based deep learning enables accurate detection of senescence in mesenchymal stem cell cultures. BMC Biol 2024; 22(1):1.
51. Heckenbach I, Mkrtchyan GV, Ezra MB, Bakula D, Madsen JS, Nielsen MH, et al. Nuclear morphology is a deep learning biomarker of cellular senescence. Nat Aging 2022; 2(8):742-55.
52. Wallis R, Milligan D, Hughes B, Mizen H, López-Domínguez JA, Eduputa U, et al. Senescence-associated morphological profiles (SAMPs): an image-based phenotypic profiling method for evaluating the inter and intra model heterogeneity of senescence. Aging. 2022;14(10):4220-46.
53. Wang X, Fujimaki K, Mitchell GC, Kwon JS, Della Croce K, Langsdorf C, et al. Exit from quiescence displays a memory of cell growth and division. Nat Commun. 2017; 8(1):321.
54. Burke BI, Ismaeel A, von Walden F, Murach KA, McCarthy JJ. Skeletal muscle hypertrophy: cell growth is cell growth. Am J Physiol Cell Physiol. 2024;327(3):C614-C8.
55. Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin E, Kleijer WJ, DiMaio D, Hwang ES. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell. 2006; 5(2):187-95.
56. Debacq-Chainiux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-b-gal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc. 2009; 4(12):1798-1806.
57. Goy E, Martin N, Drullion C, Saas L, Molendi-Coste O, Pineau L, et al. Flow Cytometry-based Method for Efficient Sorting of Senescent Cells. Bio-protocol. 2023; 13(7):e4612.
58. Yang NC, Hu ML. The limitations and validities of senescence associated-beta-galactosidase activity as an aging marker for human foreskin fibroblast Hs68 cells. Exp Gerontol. 2005; 40(10):813-9.
59. Vigneron A, Vousden KH. p53, ROS and senescence in the control of aging. Aging 2010;2(8):471-4.
60. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, Pramodh S, et al. Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. Cells 2022;11(3):552.
61. Gu X, Ma Y, Liu Y, Wan Q. Measurement of mitochondrial respiration in adherent cells by Seahorse XF96 Cell Mito Stress Test. STAR Protocols. 2020;2(1):100245.
62. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death - PubMed. Nat Rev Mol Cell Biol. 2020; 21(2):85-100.
63. Fan Y, Cheng J, Zeng H, Shao L. Senescent Cell Depletion Through Targeting BCL-Family Proteins and Mitochondria. Front Physiol 2020;11:593630.
64. Zhu Y, Tchonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors - PubMed. Aging Cell. 2016;15(3):428-35.
65. Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nat Commun. 2016; 7:11190.
66. Coppe JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010; 5:99-118.
67. Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. PLoS Biol. 2020; 18(1):e3000599.
68. Hernandez-Segura A, de Jong TV, Melov S, Guryev V, Campisi J, Demaria M. Unmasking Transcriptional Heterogeneity in Senescent Cells. Curr Biol. 2017; 27(17):2652-60 e4.
69. Tang H, Geng A, Zhang T, Wang C, Jiang Y, Mao Z. Single senescent cell sequencing reveals heterogeneity in senescent cells induced by telomere erosion. Protein Cell. 2018; 10(5):370-375.
70. Althubiti M, Macip S. Detection of Senescent Cells by Extracellular Markers Using a Flow Cytometry-Based Approach. Methods Mol Biol. 2017;1534:147-53.

71. Althubiti M, Lezina L, Carrera S, Jukes-Jones R, Giblett SM, Antonov A, et al. Characterization of novel markers of senescence and their prognostic potential in cancer. Cell Death Dis. 2014;5(11):e1528.
72. Moiseeva V, Cisneros A, Sica V, Deryagin O, Lai Y, Jung S, et al. Senescence atlas reveals an aged-like inflamed niche that blunts muscle regeneration. Nature 2023; 613(7942):169-78
73. Saul D, Kosinsky RL, Atkinson EJ, Doolittle ML, Zhang X, LeBrasseur NK, et al. A new gene set identifies senescent cells and predicts senescence-associated pathways across tissues. Nat Commun. 2022;13(1):4827.
74. Sauvat A, Wang Y, Segura F, Spaggiari S, Muller K, Zhou H, et al. Quantification of cellular viability by automated microscopy and flow cytometry. Oncotarget. 2015; 6(11):9467-75.
75. Alessio N, Aprile D, Cappabianca S, Peluso G, Di Bernardo G, Galderisi U. Different Stages of Quiescence, Senescence, and Cell Stress Identified by Molecular Algorithm Based on the Expression of Ki67, RPS6, and Beta-Galactosidase Activity. Int J Mol Sci. 2021;22(6):3102.
76. Ashraf HM, Fernandez B, Spencer SL. The intensities of canonical senescence biomarkers integrate the duration of cell-cycle withdrawal. Nat Commun. 2023;14(1):4527.
77. Fang L, Igarashi M, Leung J, Sugrue MM, Lee SW, Aaronson SA. p21Waf1/Cip1/Sdi1 induces permanent growth arrest with markers of replicative senescence in human tumor cells lacking functional p53. Oncogene. 1999;18(18):2789-97.
78. Yosef R, Pilpel N, Papismado N, Gal H, Ovadya Y, Vadai E, Miller S, Porat Z, Ben-Dor S, Krizhanovsky V. p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. EMBO 2017;36(15):2280-95.
79. Hsu CH, Altschuler SJ, Wu LF, Patterns of Early p21 Dynamics Determine Proliferation-Senescence Cell Fate after Chemotherapy. Cell. 2019;178(2):361-73
80. Fuhrmann-Stroissnigg H, Santiago FE, Grassi D, Ling Y, Niedernhofer LJ, Robbins PD. SA-β-Galactosidase-Based Screening Assay for the Identification of Senotherapeutic Drugs. JoVE. 2019; (148).
81. Zhu Y, Doornebal EJ, Pirtskhalava T, Giorgadze N, Wentworth M, et. al. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging. 2017;9(3):955-963.
82. Martin N, Popgeorgiev N, Ichim G, Bernard D. BCL-2 proteins in senescence: beyond a simple target for senolysis? Nat Rev Mol Cell Biol. 2023; 24(8):517-8.
83. Kauffman ME, Kauffman MK, Traore K, Zhu H, Trush MA, Jia Z, Li YR. MitoSOX-Based Flow Cytometry for Detecting Mitochondrial ROS. React Oxyg Species (Apex). 2016; 2(5):361-70.
84. Baek SH, Cho Y, Lee J, Choi BY, Choi Y, Park JS, et al. Intracellular and Mitochondrial Reactive Oxygen Species Measurement in Primary Cultured Neurons. Bio Protoc. 2018; 8(11):e2871.
85. Yoo I, Ahn I, Lee J, Lee N. Extracellular flux assay (Seahorse assay): Diverse applications in metabolic research across biological disciplines. Mol Cells. 2024;47(8):100095.
86. Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. In Vivo. 2008;22(3):305-9.
87. Siddiqui MS, Francois M, Fenech MF, Leifer WR. Persistent γH2AX: A promising molecular marker of DNA damage and aging. Mutat Res Rev Mutat Res. 2015; 766:1-9.
88. Freund A, Laberge R-M, Demaria M, Campisi J. Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell. 2012; 23(11):2066-75.
89. Lv T, Wang C, Zhou J, Feng X, Zhang L, Fan Z, et al. Mechanism and role of nuclear laminin B1 in cell senescence and malignant tumors. Cell Death Discov 2024;10(1):269.
90. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008; 6(12):2853-68.
91. Guan Y, Zhang C, Lyu G, Huang X, Zhang X, Zhuang T, et al. Senescence-activated enhancer landscape orchestrates the senescence-associated secretory phenotype in murine fibroblasts. Nucleic Acids Res. 2020; 48(19):10909-23.
92. Ozcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G, Galderisi U. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging 2016; 8(7):1316-29.

93. Jochems F, Thijssen B, De Conti G, Jansen R, Pogacar Z, Groot K, et al. The Cancer SENESCopedia: A delineation of cancer cell senescence. Cell Rep. 2021;36(4):109441.
94. Chandra RA, Keane FK, Voncken FEM, Thomas CR, Jr. Contemporary radiotherapy: present and future. Lancet. 2021; 398(10295):171-84.
95. Ogrodnik M, Miwa S, Tchkonia T, Tiniakos D, Wilson CL, Lahat A, et al. Cellular senescence drives age-dependent hepatic steatosis. Nat Commun. 2017; 8:15691.
96. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat Med. 2016; 22(1):78-83.
97. Mylonas KJ, O'Sullivan ED, Humphries D, Baird DP, Docherty MH, Neely SA, et al. Cellular senescence inhibits renal regeneration after injury in mice, with senolytic treatment promoting repair. Sci Transl Med. 2021;13(594):eabb0203.
98. Fletcher-Sananikone E, Kanji S, Tomimatsu N, Di Cristofaro LFM, Kollipara RK, Saha D, et al. Elimination of Radiation-Induced Senescence in the Brain Tumor Microenvironment Attenuates Glioblastoma Recurrence. Cancer Res. 2021; 81(23):5935-47.
99. Moaddel R, Rossi M, Rodriguez S, Munk R, Khadeer M, Abdelmohsen K, et al. Identification of gingerenone A as a novel senolytic compound. PLOS ONE. 2022; 17(3):e0266135.
100. Xu M, Palmer AK, Ding H, Weivoda MM, Pirtskhalava T, White TA, et al. Targeting senescent cells enhances adipogenesis and metabolic function in old age. Elife. 2015; 4:e12997.
101. González-Gualda E, Pàez-Ribes M, Lozano-Torres B, Macias D, Wilson JR, González-López C, et al. Galacto-conjugation of Navitoclax as an efficient strategy to increase senolytic specificity and reduce platelet toxicity. Aging Cell. 2020;19(4):e13142.
102. Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. Proc Natl Acad Sci USA. 2015; 112(46):E6301-10.
103. He Y, Zhang X, Chang J, Kim HN, Zhang P, Wang Y, et al. Using proteolysis-targeting chimera technology to reduce navitoclax platelet toxicity and improve its senolytic activity. Nat Commun. 2020;11(1):1996.
104. Baar MP, Brandt RMC, Putavet DA, Klein JDD, Derks KWJ, Bourgeois BRM, et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. Cell. 2017; 169(1):132-47 e16.
105. Wiley CD, Sharma R, Davis SS, Lopez-Dominguez JA, Mitchell KP, Wiley S, et al. Oxylipin biosynthesis reinforces cellular senescence and allows detection of senolysis. Cell Metab. 2021;33(6):1124-36 e5.
106. Scalise M, Cianflone E, Quercia C, Pagano L, Chiefalo A, Stincelli A, et al. Senolytics rejuvenate aging cardiomyopathy in human cardiac organoids. Mech Ageing Dev. 2024;223:112007.
107. Campisi J. The biology of replicative senescence. Eur J Cancer. 1997;33(5):703-9.
108. Dimri GP, Campisi J. Molecular and cell biology of replicative senescence. Cold Spring Harb Symp Quant Biol. 1994; 59:67-73.
109. Hayflick L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. Exp Cell Res. 1965; 37:614-36.
110. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961; 25:585-621.
111. Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. Nat Cell Biol. 2022; 24(2):135-47.
112. Victorelli S, Passos JF. Telomeres and Cell Senescence - Size Matters Not. EBioMedicine. 2017; 21:14-20.
113. Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct. 2012;2012:646354.
114. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, Cell Senescence, and Novel Molecular Mechanisms in Aging and Age-Related Diseases. Oxid Med Cell Longev. 2016;2016:3565127.
115. Wong A, Kieu T, Robbins PD. The Ercc1(-/Delta) mouse model of accelerated senescence and aging for identification and testing of novel senotherapeutic interventions. Aging. 2020;12(24):24481-3.
116. Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, et al. A new progeroid syndrome reveals that genotoxic

stress suppresses the somatotroph axis. Nature. 2006;444(7122):1038-43.
117. Chen Q, Ames BN. Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. Proc Natl Acad Sci USA. 1994;91(10):4130-4.
118. Lewinska A, Adamczyk-Grochala J, Bloniarz D, Olszowka J, Kulpa-Greszta M, Litwinienko G, et al. AMPK-mediated senolytic and senostatic activity of quercetin surface functionalized Fe(3)O(4) nanoparticles during oxidant-induced senescence in human fibroblasts. Redox Biol. 2020;28:101337.
119. Zoico E, Nori N, Darra E, Tebon M, Rizzatti V, Policastro G, et al. Senolytic effects of quercetin in an in vitro model of pre-adipocytes and adipocytes induced senescence. Sci Rep. 2021;11(1):23237.
120. Debacq-Chainiaux F, Borlon C, Pascal T, Royer V, Eliaers F, Ninane N, et al. Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. J Cell Sci. 2005;118(Pt 4):743-58.
121. Wedel S, Martic I, Hrapovic N, Fabre S, Madreiter-Sokolowski CT, Haller T, et al. tBHP treatment as a model for cellular senescence and pollution-induced skin aging. Mech Ageing Dev. 2020;190:111318.
122. Dumont P, Chainiaux F, Eliaers F, Petropoulou C, Remacle J, Koch-Brandt C, et al. Overexpression of apolipoprotein J in human fibroblasts protects against cytotoxicity and premature senescence induced by ethanol and tert-butylhydroperoxide. Cell Stress Chaperones. 2002;7(1):23-35.
123. Admasu TD, Rae M, Stolzing A. Dissecting primary and secondary senescence to enable new senotherapeutic strategies. Ageing Res Rev. 2021;70:101412.
124. Yang Y, Mihajlovic M, Valentijn F, Nguyen TQ, Goldschmeding R, Masereeuw R. A Human Conditionally Immortalized Proximal Tubule Epithelial Cell Line as a Novel Model for Studying Senescence and Response to Senolytics. Front Pharmacol. 2022;13:791612.
125. Chandeck C, Mooi WJ. Oncogene-induced cellular senescence. Adv Anat Pathol. 2010;17(1):42-8.
126. Innes AJ, Gil J. IMR90 ER:RAS: A Cell Model of Oncogene-Induced Senescence. Methods Mol Biol. 2019;1896:83-92.
127. Macip S, Igarashi M, Fang L, Chen A, Pan ZQ, Lee SW, Aaronson SA. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. EMBO J. 2002;21(9):2180-8.
128. Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, et al. Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine. 2018;36:18-28.
129. Triana-Martínez F, Picallos-Rabina P, Da Silva-Álvarez S, Pietrocola F, Llanos S, Rodilla V, et al. Identification and characterization of Cardiac Glycosides as senolytic compounds. Nat Commun 2019;10(1):4731.
130. Wang L, Leite de Oliveira R, Wang C, Fernandes Neto JM, Mainardi S, Evers B, et al. High-Throughput Functional Genetic and Compound Screens Identify Targets for Senescence Induction in Cancer. Cell Rep. 2017;21(3):773-83.
131. Milanovic M, Fan DNY, Belenki D, Dabritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature. 2018;553(7686):96-100.
132. Dorr JR, Yu Y, Milanovic M, Beuster G, Zasada C, Dabritz JH, et al. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Nature. 2013;501(7467):421-5.
133. Salunkhe S, Mishra SV, Nair J, Shah S, Gardi N, Thorat R, et al. Nuclear localization of p65 reverses therapy-induced senescence. J Cell Sci. 2021;134(6):jcs253203.
134. Xu Q, Fu Q, Li Z, Liu H, Wang Y, Lin X, et al. The flavonoid procyanidin C1 has senotherapeutic activity and increases lifespan in mice. Nat Metabol 2021;3(12):1706-26.
135. Chen D-D, Peng X, Wang Y, Jiang M, Xue M, Shang G, et al. HSP90 acts as a senomorphic target in senescent retinal pigmental epithelial cells. Aging. 2021; 13(17):21547-70.
136. Shao Z, Wang B, Shi Y, Xie C, Huang C, Chen B, et al. Senolytic agent Quercetin ameliorates intervertebral disc degeneration via the Nrf2/NF-kappaB axis. Osteoarthr Cartil. 2021;29(3):413-22.
137. Ying Y, Mihajlovic M, Valentijin F, Nguyen TQ, Goldschmedig R, Masereeuw R. A Human Conditionally Immortalized Proximal Tubule Epithelial Cell Line as a Novel Model for Studying Senescence and Response to

Senolytics. Front Pharmacol. 2022;13:791612.
138. Ekpenyong-Akiba AE, Ekpenyong-Akiba AE, Canfarotta F, Canfarotta F, H. BA, H. BA, et al. Detecting and targeting senescent cells using molecularly imprinted nanoparticles. Nanoscale Horiz. 2019; 4(3):757-68.
139. Luo X, Fu Y, Loza AJ, Murali B, Leahy KM, Ruhland MK, et al. Stromal-initiated changes in the bone promote metastatic niche development. Cell Rep. 2016;14(1):82-92.
140. Darzynkiewicz Z, Galkowski D, Zhao H. Analysis of apoptosis by cytometry using TUNEL assay. Methods. 2008;44(3):250-4.
141. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods. 1995;184(1):39-51.
142. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 1994;84(5):1415-20.
143. Opdenbosch NV, Lamkanfi M. Caspases in Cell Death, Inflammation, and Disease . Immunity. 2019;50(6):1352-64.
144. Chen X, Wei S, Ji Y, Guo X, Yang F. Quantitative proteomics using SILAC: Principles, applications, and developments. Proteomics. 2015; 15(18):3175-92.
145. Zecha J, Satpathy S, Kanashova T, Avanessian SC, Kane MH, Clauser KR, et al. TMT Labeling for the Masses: A Robust and Cost-efficient, In-solution Labeling Approach. Mol Cell Proteomics. 2019; 18(7):1468-78.
146. Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. J Gerontol A Biol Sci Med Sci. 2008; 63(8):879-84.
147. Mannarino M, Cherif H, Ghazizadeh S, Martinez OW, Sheng K, Cousineau E, et al. Senolytic treatment for low back pain. Sci Adv. 2025; 11(11):eadr1719.
148. Khandelwal S, Lane NE. Osteoporosis: Review of Etiology, Mechanisms, and Approach to Management in the Aging Population. Endocrinol Metab Clin North Am 2023;52(2):259-75.
149. Cauley JA. Osteoporosis: fracture epidemiology update 2016. Curr Opin Rheumatol 2017;29(2):150-156.
150. Lo JC, Yang W, Park-Sigal JJ, Ott SM, Lo JC, Yang W, et al. Osteoporosis and Fracture Risk among Older US Asian Adults. Curr Osteoporos Rep 2023;21(5):592-608.
151. Wright NC, Loocker AC, Saag KG. Curtis JR, Delzell ES, Randall S, Dawson-Hughes B. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res 2014;29(11):2520-6.
152. Park Y, Cheong E, Kwak J-G, Carpenter R, Shim J-H, Lee J. Trabecular bone organoid model for studying the regulation of localized bone remodeling. Sci Adv. 2021;7(4):eabd6495.
153. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. J Biol Chem. 2010;285(33):25103-8.
154. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;289(5484):1504-8.
155. Schaffler DMB, Cheung DW-Y, Majeska DR, Kennedy DO. Osteocytes: Master Orchestrators of Bone. Calcif Tissue Int 2014;94(1):5-24.
156. Schaffler MB, Kennedy OD. Osteocyte Signaling in Bone. Curr Osteoporos Rep. 2012;10(2):118-25.
157. Pignolo RJ, Law SF, Chandra A. Bone Aging, Cellular Senescence, and Osteoporosis. JBMR Plus. 2021;5(4):e10488.
158. Ukon Y, Kaito T, Hirai H, Kitahara T, Bun M, Kodama J, et al. Cellular senescence by loss of Men1 in osteoblasts is critical for age-related osteoporosis. Aging Cell. 2024; 23(10):e14254.
159. Wolfel EM, Fernandez-Guerra P, Norgard MO, Jeromdesella S, Kjaer PK, Elkjaer AS, et al. Senescence of skeletal stem cells and their contribution to age-related bone loss. Mech Ageing Dev. 2024; 221:111976.
160. Sharma AK, Roberts RL, Benson RD, Jr., Pierce JL, Yu K, Hamrick MW, McGee-Lawrence ME. The Senolytic Drug Navitoclax (ABT-263) Causes Trabecular Bone Loss and Impaired Osteoprogenitor Function in Aged Mice. Front Cell Dev Biol. 2020;8:354.
161. Zhou Y, Xin X, Wang L, Wang B, Chen L, Liu O, et al. Senolytics improve bone forming potential of bone marrow mesenchymal stem

cells from aged mice. NPJ Regen Med. 2021;6(1):34.
162. Xing X, Huang H, Gao X, Yang J, Tang Q, Xu X, et al. Local Elimination of Senescent Cells Promotes Bone Defect Repair during Aging. ACS Appl Mater Interfaces. 2022;14(3):3885-99.
163. Samakkarnthai P, Saul D, Zhang L, Aversa Z, Doolittle ML, Sfeir JG, et al. In vitro and in vivo effects of zoledronic acid on senescence and senescence-associated secretory phenotype markers. Aging. 2023;15(9):3331-55.
164. Glyn-Jones S. Palmer AJR, Price AJ, Vincent TL, Weinans H, Carr AJ. Osteoarthritis. Lancet 2015;386(9991):376-87.
165. Astrike-Davis EM, Coryell P, Loeser RF. Targeting cellular senescence as a novel treatment for osteoarthritis. Curr Opin Pharmacol 2022;64:102213.
166. Coryell PR, Diekman BO, Loeser RF. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. Nat Rev Rheumatol. 2021;17(1):47-57.
167. Yang H, Chen C, Chen H, Duan X, Li J, Zhou Y, et al. Navitoclax (ABT263) reduces inflammation and promotes chondrogenic phenotype by clearing senescent osteoarthritic chondrocytes in osteoarthritis. Aging. 2020;12(13):12750-12770.
168. Huang Y, He Y, Makarcyzk MJ, Lin H. Senolytic Peptide FOXO4-DRI Selectively Removes Senescent Cells From in vitro Expanded Human Chondrocytes. Front Bioeng Biotechnol. 2021;9:677576.
169. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Primers. 2017;3:17074.
170. Han S, Lu Q, Liu X. Advances in cellular senescence in idiopathic pulmonary fibrosis (Review). Exp Ther Med. 2023;25(4):145.
171. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. 2017;8:14532.
172. Lehmann M, Korfei M, Mutze K, Klee S, Skronska-Wasek W, Alsafadi HN, et al. Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. Eur Respir J. 2017;50(2).
173. Suezawa T, Kanagaki S, Moriguchi K, Masui A, Nakao K, Toyomoto M, et al. Disease modeling of pulmonary fibrosis using human pluripotent stem cell-derived alveolar organoids. Stem Cell Rep. 2021;16(12).
174. Shen M, Fu J, Zhang Y, Chang Y, Li X, Cheng H, et al. A novel senolytic drug for pulmonary fibrosis: BTSA1 targets apoptosis of senescent myofibroblasts by activating BAX. Aging Cell. 2024;23(9):e14229.
175. Lee JY, Reyes NS, Ravishankar S, Zhou M, Krasilnikov M, Ringler C, et al. An in vivo screening platform identifies senolytic compounds that target p16INK4a+ fibroblasts in lung fibrosis. J Clin Invest. 2024;134(9):e173371.
176. Melo Dos Santos LS, Trombetta-Lima M, Eggen B, Demaria M. Cellular senescence in brain aging and neurodegeneration. Ageing Res Rev. 2024;93:102141.
177. Aguado J, Amarilla AA, Taherian Fard A, Albornoz EA, Tyshkovskiy A, Schwabenland M, et al. Senolytic therapy alleviates physiological human brain aging and COVID-19 neuropathology. Nat Aging. 2023;3(12):1561-75.
178. Jin M, Xu R, Wang L, Alam MM, Ma Z, Zhu S, et al. Type-I-interferon signaling drives microglial dysfunction and senescence in human iPSC models of Down syndrome and Alzheimer's disease. Cell Stem Cell. 2022;29(7):1135-1153.
179. Shaker MR, Aguado J, Chaggar HK, Wolvetang EJ, Shaker MR, Aguado J, et al. Klotho inhibits neuronal senescence in human brain organoids. NPJ Aging Mech Dis 2021;7(1):18.
180. Aguado J, Chaggar HK, Gómez-Inclán C, Shaker MR, Leeson HC, Mackay-Sim A, Wolvetang EJ. Inhibition of the cGAS-STING pathway ameliorates the premature senescence hallmarks of Ataxia-Telangiectasia brain organoids. Aging Cell. 2021;20(9):e13468.
181. Mensah GA, Roth GA, Fuster V. The Global Burden of Cardiovascular Diseases and Risk Factors: 2020 and Beyond. J Am Coll Cardiol. 2019;74(20):2529-32.
182. Ciarambino T, Menna G, Sansone G, Giordano M. Cardiomyopathies: An Overview. Int J Mol Sci 2021;22(14):7722.
183. Huang S, Li J, Li Q, Wang Q, Zhou X, Chen J, et al. Cardiomyopathy: pathogenesis and therapeutic interventions. MedComm. 2020;5(11):e772.
184. Cianflone E, Torella M, Biamonte F, Angelis AD, Urbanek K, Costanzo FS, et al. Targeting

Cardiac Stem Cell Senescence to Treat Cardiac Aging and Disease. Cells 2020:9(6):1558.
185. Marino F, Scalise M, Salerno N, Salerno L, Molinaro C, Cappetta D, et al. Diabetes-Induced Cellular Senescence and Senescence-Associated Secretory Phenotype Impair Cardiac Regeneration and Function Independently of Age. Diabetes. 2022;71(5):1081-1098.
186. Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, et al. Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov 2017;7(2):165-176.
187. Shafqat S, Arana Chicas E, Shafqat A, Hashmi SK. The Achilles' heel of cancer survivors: fundamentals of accelerated cellular senescence. J Clin Invest. 2022;132(13):e158452.
188. Wang S, Prizment A, Thyagarajan B, Blaes A. Cancer Treatment-Induced Accelerated Aging in Cancer Survivors: Biology and Assessment. Cancers. 2021;13(3):427.
189. Nicolas AM, Pesic M, Engel E, Ziegler PK, Diefenhardt M, Kennel KB, et al. Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. Cancer Cell. 2022;40(2):168-184.e13..
190. Cole AR, Buj R, Elhaw AT, Uboveja A, Tangudu N, Oesterreich S, et al. Paracrine effects of the senescence-associated secretory phenotype decrease cancer cell adhesion. bioRxiv. 2023.
191. Sachs N, Papaspyropoulos A, Ommen DDZv, Heo I, Böttinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. The EMBO J. 2019;38(4):e100300.
192. Cai X, Li Y, Zheng J, Liu L, Jiao Z, Lin J, et al. Modeling of senescence-related chemoresistance in ovarian cancer using data analysis and patient-derived organoids. Front Oncol 2024;13:1291559.
193. Gray GK, Li CM, Rosenbluth JM, Selfors LM, Girnius N, Lin JR, et al. A human breast atlas integrating single-cell proteomics and transcriptomics . Dev Cell 2022;57(11):1400-1420.e7..
194. Mertens S, Huismans MA, Verissimo CS, Ponsioen B, Overmeer R, Proost N, Tellingen OV, et al. Drug-repurposing screen on patient-derived organoids identifies therapy-induced vulnerability in KRAS-mutant colon cancer. Cell Rep 2023;42(4):112324.
195. Yang R, Kwan W, Du Y, Yan R, Zang L, Li C, Zhu Z, Cheong IH, Kozlakidis Z, Yu Y. Drug-induced senescence by aurora kinase inhibitors attenuates innate immune response of macrophages on gastric cancer organoids. Cancer Lett 2024;598:217106.
196. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Orjalo AV, Rodier F, Lithgow GJ, Campisi J. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging. 2009;1(4):402-11.
197. Nunes ADC, Weigl M, Schneider A, Noureddine S, Yu L, Lahde C, et al. miR-146a-5p modulates cellular senescence and apoptosis in visceral adipose tissue of long-lived Ames dwarf mice and in cultured pre-adipocytes. GeroScience. 2022;44(1):503-518.
198. Hsu CH, Altschuler SJ, Wu LF. Patterns of Early p21 Dynamics Determine Proliferation-Senescence Cell Fate after Chemotherapy. Cell. 2019;178(2):361-73 e12.
199. Sun YD, Li GH, Zhang F, Cheng T, Zhang JP, Zhang XB. A p21 reporter iPSC line for evaluating CRISPR-Cas9 and vector-induced stress responses. Stem Cells 2024;42(11):992-1005.
200. Shimada-Takayama Y, Yasuda T, Ukai T, Taguchi J, Ozawa M, Sankoda N, et al. Generation of mice for evaluating endogenous p16Ink4a protein expression. Biochem Biophys Res Commun 2022;599:43-50.
201. Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell. 2014;31(6):722-33.
202. Yi Z, Ren L, Wei Y, Chen S, Zhao J, Zhu J, Wu J. Generation of a p21 Reporter Mouse and Its Use to Identify and Eliminate $p21^{high}$ Cells In Vivo. Int J Mol Sci. 2023;24(6):5565.
203. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011;479(7372):232-6.
204. Burd CE, Sorrentino JA, Clark KS, Darr DB, Krishnamurthy J, Deal AM, et al. Monitoring tumorigenesis and senescence in vivo with a p16(INK4a)-luciferase model. Cell. 2013;152(1-2):340-51.

205. Omori S, Wang TW, Johmura Y, Kanai T, Nakano Y, Kido T, et al. Generation of a p16 Reporter Mouse and Its Use to Characterize and Target p16^{high} Cells In Vivo. Cell Metab. 2020;32(5):814-28 e6.
206. Wang B, Wang L, Gasek NS, Zhou Y, Kim T, Guo C, et al. An inducible p21-Cre mouse model to monitor and manipulate p21-highly-expressing senescent cells in vivo. Nat Aging. 2021;1(10):962-73.
207. Yang R, Yu Y. Patient-derived organoids in translational oncology and drug screening. Cancer Lett 2023;562:216180.
208. Gorshkov K, Chen CZ, Marshall RE, Mihatov N, Choi Y, Nguyen D-T, et al. Advancing precision medicine with personalized drug screening. Drug Discov Today. 2019;24(1):272-278.
209. Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: state of the art. Tissue Eng Part B Rev. 2008;14(1):61-86.
210. Benton G, Arnaoutova I, George J, Kleinman HK, Koblinski J. Matrigel: from discovery and ECM mimicry to assays and models for cancer research. Adv Drug Deliv Rev. 2014;79-80:3-18.
211. Kozlowski MT, Crook CJ, Ku HT. Towards organoid culture without Matrigel. Commun Biol. 2021;4(1):1387.
212. Kim S, Min S, Choi YS, Jo SH, Jung JH, Han K, et al. Tissue extracellular matrix hydrogels as alternatives to Matrigel for culturing gastrointestinal organoids. Nat Commun. 2022;13(1):1692.
213. Giobbe GG, Crowley C, Luni C, Campinoti S, Khedr M, Kretzschmar K, et al. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nat Commun. 2019;10(1):5658.
214. Song H, Jiang H, Hu W, Hai Y, Cai Y, Li H, et al. Cervical extracellular matrix hydrogel optimizes tumor heterogeneity of cervical squamous cell carcinoma organoids. Sci Adv. 2024;10(20):eadl3511.
215. Yoon H, Park Y, Kwak J-G, Lee J. Collagen structures of demineralized bone paper direct mineral metabolism. JBMR Plus. 2024;8(8):ziae080.
216. Park Y, Sato T, Lee J. Functional and analytical recapitulation of osteoclast biology on demineralized bone paper. Nat Commun 2023;14(1):8092.
217. Ryan P, Yoon H, Amin S, Chambers JJ, Lee J. AI-Assisted Label-Free Monitoring Bone Mineral Metabolism on Demineralized Bone Paper. ACS Biomater Sci Eng. 2025;11(4):2096-105.
218. Gao H, Nepovimova E, Heger Z, Valko M, Wu Q, Kuca K, Adam V. Role of hypoxia in cellular senescence. Pharmacol Res. 2023;194:106841.
219. Maity A, Koumenis C. HIF and MIF--a nifty way to delay senescence? Genes Dev. 2006;20(24):3337-41.
220. Polonis K, Becari C, Chahal CAA, Zhang Y, Allen AM, Kellogg TA, et al. Chronic Intermittent Hypoxia Triggers a Senescence-like Phenotype in Human White Preadipocytes. Sci Rep. 2020;10(1):6846.
221. Jain IH, Zazzeron L, Goli R, Alexa K, Schatzman-Bone S, Dhillon H, et al. Hypoxia as a therapy for mitochondrial disease. Science. 2016;352(6281).
222. Rogers RS, Wang H, Durham TJ, Stefely JA, Owiti NA, Markhard AL, et al. Hypoxia extends lifespan and neurological function in a mouse model of aging. PLOS Biology. 2023;21(5).
223. Wei W, Ji S. Cellular senescence: Molecular mechanisms and pathogenicity. J Cell Physiol. 2018;233(12):9121-35.
224. Tempaku PF, Mazzotti DR, Hirotsu C, Andersen ML, Xavier G, Maurya PK, et al. The effect of the severity of obstructive sleep apnea syndrome on telomere length. Oncotarget. 2016;7(43):69216-24.
225. Kim KS, Kwak JW, Lim SJ, Park YK, Yang HS, Kim HJ. Oxidative Stress-induced Telomere Length Shortening of Circulating Leukocyte in Patients with Obstructive Sleep Apnea. Aging Dis. 2016;7(5):604-13.
226. Capparelli C, Guido C, Whitaker-Menezes D, Bonuccelli G, Balliet R, Pestell TG, et al. Autophagy and senescence in cancer-associated fibroblasts metabolically supports tumor growth and metastasis, via glycolysis and ketone production. Cell Cycle. 2012;11(12):2285-302.
227. Tsai CC, Chen YJ, Yew TL, Chen LL, Wang JY, Chiu CH, Hung SC. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST. Blood. 2011;117(2):459-69.
228. Rodenhizer D, Gaude E, Cojocari D, Mahadevan R, Frezza C, Wouters BG, et al. A three-dimensional engineered tumour for spatial snapshot analysis of cell metabolism

and phenotype in hypoxic gradients. Nat Mater 2015;15(2):244.
229. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res 2016;76(8):2465-77.
230. Saraswathibhatla A, Indana D, Chaudhuri O. Cell–extracellular matrix mechanotransduction in 3D. Nat Rev Mol Cell Biol 2023;24(7):495-516.
231. Yap AS, Duszyc K, Viasnoff V. Mechanosensing and Mechanotransduction at Cell-Cell Junctions. Cold Spring Harb Perspect Biol 2018;10(8):a028761.
232. Ferrai C, Schulte C. Mechanotransduction in stem cells. Eur J Cell Biol 2024;103(2):151417.
233. Conway Daniel E, Breckenridge Mark T, Hinde E, Gratton E, Chen Christopher S, Schwartz Martin A. Fluid Shear Stress on Endothelial Cells Modulates Mechanical Tension across VE-Cadherin and PECAM-1. Curr Biol 2013;23(11):1024-30.
234. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126(4):677-89.
235. Koike M, Shimokawa H, Kanno Z, Ohya K, Soma K, Koike M, et al. Effects of mechanical strain on proliferation and differentiation of bone marrow stromal cell line ST2. J Bone Miner Metab 2005;23(3):219-25.
236. Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. J Biomech Eng. 1985;107(4):341-7.
237. Smith RL, Lindsey DP, Dhulipala L, Harris AHS, Goodman SB, Maloney WJ. Effects of intermittent hydrostatic pressure and BMP-2 on osteoarthritic human chondrocyte metabolism in vitro. J Orthop Res 2011;29(3):361-8.
238. He C, Lv X, Huang C, Hua G, Ma B, Chen X, Angeletti P, Dong J, et al. YAP1-LATS2 feedback loop dictates senescent or malignant cell fate to maintain tissue homeostasis. EMBO Rep 2019;20(3):e44948.
239. Hemmatian H, Bakker AD, Klein-Nulend J, Lenthe GHv. Aging, Osteocytes, and Mechanotransduction. Curr Osteoporos Rep 2017;15(5):401-411.
240. Pelissier FA, Garbe JC, Ananthanarayanan B, Miyano M, Lin C, Jokela T, Kumar S, et al. Age-related dysfunction in mechanotransduction impairs differentiation of human mammary epithelial progenitors. Cell Rep 2014;7(6):1926-39.
241. Li M, Peng L, Wang Z, Liu L, Cao M, Cui J, Wu F, Yang J. Roles of the cytoskeleton in human diseases. Mol Biol Rep 2023;50(3):2847-2856.
242. Mebratu YA, Soni S, Rosas L, Rojas M, Horowitz JC, Nho R. The aged extracellular matrix and the profibrotic role of senescence-associated secretory phenotype. Am J Physiol Cell Physiol. 2023;325(3):C565-C79.
243. Savadipour A, Palmer D, Ely EV, Collins KH, Garcia-Castorena JM, Harissa Z, et al. The role of PIEZO ion channels in the musculoskeletal system. Am J Physiol Cell Physiol 2023;324(3):C728-C740.
244. Wang B, Ke W, Wang K, Li G, Ma L, Lu S, et al. Mechanosensitive Ion Channel Piezo1 Activated by Matrix Stiffness Regulates Oxidative Stress-Induced Senescence and Apoptosis in Human Intervertebral Disc Degeneration. Oxid Med Cell Longev. 2021;2021:8884922.
245. Ren X, Li B, Xu C, Zhuang H, Lei T, Jiang F, Zhou P. High expression of Piezo1 induces senescence in chondrocytes through calcium ions accumulation. Biochem Biophys Res Commun 2022;607:138-145.
246. Davidson PM, Lammerding J. Broken nuclei--lamins, nuclear mechanics, and disease. Trends Cell Biol. 2014;24(4):247-56.
247. Deng EZ, Fleishman RH, Xie Z, Marino GB, Clarke DJB, Ma'ayan A. Computational screen to identify potential targets for immunotherapeutic identification and removal of senescence cells. Aging Cell. 2023;22(6):e13809.
248. Avelar RA, Ortega JG, Tacutu R, Tyler EJ, Bennett D, Binetti P, et al. A multidimensional systems biology analysis of cellular senescence in aging and disease. Genome Biol. 2020;21(1):91.
249. Tao W, Yu Z, Han JJ. Single-cell senescence identification reveals senescence heterogeneity, trajectory, and modulators. Cell Metab. 2024;36(5):1126-43 e5.
250. Smer-Barreto V, Quintanilla A, Elliott RJR, Dawson JC, Sun J, Campa VM, et al. Discovery of senolytics using machine learning. Nat Commun. 2023;14(1):3445.

251. Li Y, Tao L, Wang Q, Wang F, Li G, Song M. Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects. Environ Health. 2023;1(4):249-57.
252. Zhu L, Kang Y, Ma M, Wu Z, Zhang L, Hu R, et al. Tissue accumulation of microplastics and potential health risks in human. Sci Total Environ. 2024;915:170004.
253. Fenton SE, Ducatman A, Boobis A, DeWitt JC, Lau C, Ng C, et al. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. Environ Toxicol Chem. 2021;40(3):606-30.
254. Beglarian E, Costello E, Walker DI, Wang H, Alderete TL, Chen Z, et al. Exposure to perfluoroalkyl substances and longitudinal changes in bone mineral density in adolescents and young adults: A multi-cohort study. Environ Res. 2024;244:117611.
255. Zhu L, Liu B, Hu Y, Wang M, Furtado JD, Rimm EB, et al. Per- and polyfluoroalkyl substances, apolipoproteins and the risk of coronary heart disease in US men and women. Environ Health. 2024;23(1):108.
256. Metayer C, Morimoto LM, Vieira VM, Godri Pollitt KJ, Bartell SM, Wong L, Young TM. Exposure to per- and polyfluoroalkyl substances in residential settled dust and risk of childhood acute lymphoblastic leukemia. Int J Cancer. 2025 (early view).
257. Pyatha S, Kim H, Lee D, Kim K. Association between Heavy Metal Exposure and Parkinson's Disease: A Review of the Mechanisms Related to Oxidative Stress. Antioxidants. 2022;11(12):2467
258. Battistini B, Greggi C, Visconti VV, Albanese M, Messina A, De Filippis P, et al. Metals accumulation affects bone and muscle in osteoporotic patients: A pilot study. Environ Res. 2024;250:118514.
259. Khoshakhlagh AH, Mohammadzadeh M, Gruszecka-Kosowska A. The preventive and carcinogenic effect of metals on cancer: a systematic review. BMC Public Health. 2024;24(1):2079.
260. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med. 1997;337(20):1419-28.
261. Song S, Li F, Zhao B, Zhou M, Wang X. Ultraviolet Light Causes Skin Cell Senescence: From Mechanism to Prevention Principle. Adv Biol. 2025;9(2):e2400090.
262. Narayanan DL, Saladi RN, Fox JL. Ultraviolet radiation and skin cancer. Int J Dermatol. 2010;49(9):978-86.
263. Zhu RZ, Li BS, Gao SS, Seo JH, Choi BM. Luteolin inhibits H(2)O(2)-induced cellular senescence via modulation of SIRT1 and p53. Korean J Physiol Pharmacol. 2021;25(4):297-305.
264. Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Futur J Pharm Sci. 2021;7(1):25.
265. Hasnat H, Shompa SA, Islam MM, Alam S, Richi FT, Emon NU, et al. Flavonoids: A treasure house of prospective pharmacological potentials. Heliyon. 2024;10(6):e27533.
266. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.
267. Guida JL, Agurs-Collins T, Ahles TA, Campisi J, Dale W, Demark-Wahnefried W, et al. Strategies to Prevent or Remediate Cancer and Treatment-Related Aging. J Natl Cancer Inst. 2021;113(2):112-22.
268. JL G, TA A, D B, J C, HJ C, J D, et al. Measuring Aging and Identifying Aging Phenotypes in Cancer Survivors. J Natl Cancer Inst 2019; 111(12):1245-1254.
269. Kawaguchi K, Komoda K, Mikawa R, Asai A, Sugimoto M. Cellular senescence promotes cancer metastasis by enhancing soluble E-cadherin production. iScience. 2021;24(9):103022.
270. Guccini I, Revandkar A, D'Ambrosio M, Colucci M, Pasquini E, Mosole S, et al. Senescence Reprogramming by TIMP1 Deficiency Promotes Prostate Cancer Metastasis. Cancer Cell. 2021;39(1):68-82 e9.

Data Availability Statement

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.