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Biomarkers of Cellular Senescence and Aging: Current State-of-the-Art, Challenges and Future Perspectives

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Population aging has increased the global prevalence of aging-related diseases, including cancer, sarcopenia, neurological disease, arthritis, and heart disease. Understanding aging, a fundamental biological process, has led to breakthroughs in several fields. Cellular senescence, evinced by flattened cell bodies, vacuole formation, and cytoplasmic granules, ubiquitously plays crucial roles in tissue remodeling, embryogenesis, and wound repair as well as in cancer therapy and aging. The lack of universal biomarkers for detecting and quantifying senescent cells, in vitro and in vivo, constitutes a major limitation. The applications and limitations of major senescence biomarkers, including senescence-associated β-galactosidase staining, telomere shortening, cell-cycle arrest, DNA methylation, and senescence-associated secreted phenotypes are discussed. Furthermore, explore senotherapeutic approaches for aging-associated diseases and cancer. In addition to the conventional biomarkers, this review highlighted the in vitro, in vivo, and disease models used for aging studies. Further, technologies from the current decade including multi-omics and computational methods used in the fields of senescence and aging are also discussed in this review. Understanding aging-associated biological processes by using cellular senescence biomarkers can enable therapeutic innovation and interventions to improve the quality of life of older adults.

1. Introduction

In 1961, Hayflick and Moorhead first defined cellular senescence in human fibroblasts and reported that cell proliferation is arrested in embryonic tissues after a few cell divisions.[1] Senescence, a lifelong physiological mechanism for stable cell-cycle cessation, is defined as cell-cycle arrest in either the G1 or G2 phase to stop the proliferation of damaged cells.[2] In contrast to the rise in the number of senescent cells during aging, tissue remodeling, embryonic development, and wound healing, antagonistic effects regarding senescence are noted in cancer, aging-related diseases, and neurodegenerative disorders.[3] Cellular senescence is induced by several cellular impairments, including DNA damage, telomere shortening, organelle damage, oncogene activation, epigenetic changes, and the loss of tumor suppressor functions[4] (**Figure 1**). Furthermore, cellular senescence inhibits proteasomal and lysosomal pathways by altering mitochondrial, lysosomal, and endoplasmic reticular function.[5] Senescence-associated morphological changes in cells include flattened cytoplasm, enlarged and lobulated nuclei, nuclear membrane folding, and fragmented nucleoli, and senescent cancer cells exhibit morphological features similar to that of normal cells.[6]

Senescence is categorized into two major types: replicative senescence (RS) and stress-induced premature senescence (SIPS).[7] Telomere shortening induces RS, which induces

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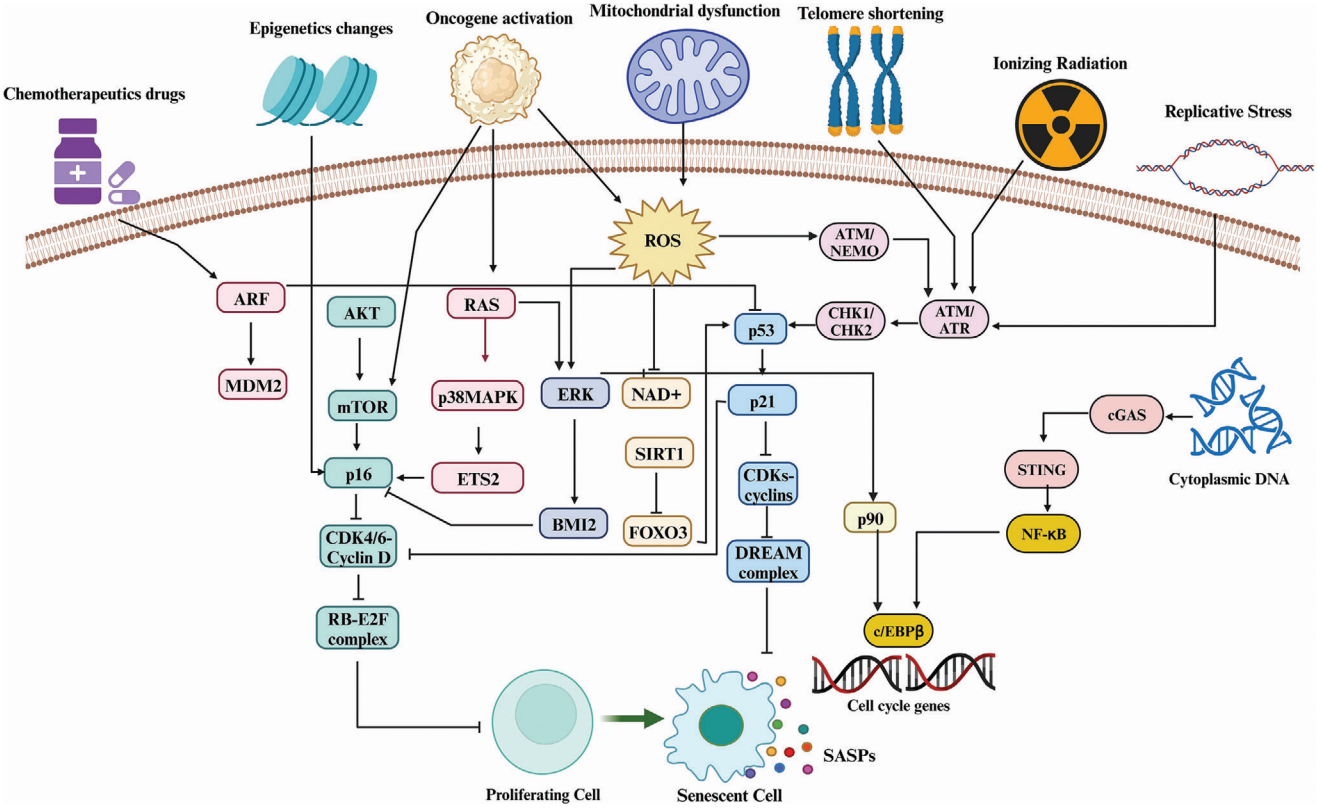

Figure 1. Inducers and signaling pathways of senescence. Senescence inducers, such as oncogene activation, mitochondrial dysfunction, epigenetic changes, oncogene activation, DNA damage, radiation, and chemotherapeutic drugs, activate senescence-associated molecular pathways and lead to the activation of tumor suppressor pathways, p53/p21$^{WAF1/CIP1}$ and p16^{INK4A}/RB, and activate the DREAM complex to induce senescence.

defects in cell proliferation and cessation of cell-cycle arrest. Several theories posited to rationalize RS frequency elucidate random changes in cellular components owing to random modifications of the cellular environment.[8] RS occurs in several cells, such as fibroblasts, glial cells, keratinocytes, endothelial cells, vascular smooth cells, lens cells, and lymphocytes; moreover, RS-inducible cells have increased sensitivity to environmental stressors, such as heat shock proteins (e.g., HSP70, HSP90, and HDP28).[9] In RS, telomeric changes or dysfunction induce a DNA damage response (DDR) that activates either cell death or cell-cycle arrest. In yeast cells, single telomeres activate RS, whereas in mammalian cells 5–10 short telomeres are required. However, the exact mechanism of telomere-induced RS is unclear. Besides telomere length, nontelomeric DNA damage, mitochondrial dysfunction, oncogene activation, and chromatin changes are responsible for RS.[10]

Furthermore, SIPS is triggered by stress inducers, including oxidative stress, mitochondrial dysfunction, DNA damage, and senescence-associated secreted phenotypes (SASPs) that are produced by premature cells and initiate the p16 or p53 pathways[11] (Figure 1). In experimental data, oxidative stress is the most frequent SIPS inducer.[12] In normal human cells, ionizing radiation induces DNA double-strand breaks, and leads to the activation of the ATM-p53-p21 pathway-mediated growth arrest. In cancer cells, because of the increased telomerase expression, RS is impossible. However, radiation therapy and anticancer agents, such as doxorubicin, camptothecin, and cisplatin, can induce SIPS. Increased oxidative stress is associated with mitochondrial dysfunction, including changes in morphology, mitochondrial mass, and membrane potential, which plays a crucial role in senescence. Particularly, the sirtuin mitochondrial proteins regulate the aging process in many species.[13] Cells that undergo RS or SIPS have similar cellular and molecular characteristics but differ only in the time of expression of those characteristics.[14] Based on the causative agent, senescence is stratified into acute and chronic senescence. Acute senescence is a programmed, natural, biological event initiated by discrete stress inducers with several beneficial effects, including improved immune responses, wound healing, and renoprotection.[15–17] Chronic senescence, induced by long-term stress or slow macromolecular damage to cells, leads to the accumulation of senescent cells, severely affects natural aging, and predisposes to aging-associated kidney diseases.[8,18]

2. Biomarkers for Senescence

Despite its description more than half a century ago, cellular senescence cannot be validated concurrently through any single method, and multiple biomarkers are required to assess the in vitro senescence phenotype.[19] The biomarkers used for the in vitro and in vivo detection of senescent cells include enzymatic detection of senescence-associated β-galactosidase staining (SA-β-gal), DNA methylation, nuclear globular actin accumulation, and cyclin-dependent kinase (CDK) inhibitors (p16INK4a and p21CIP1)[20–22] (**Figure 2**). In addition, senescence biomark-

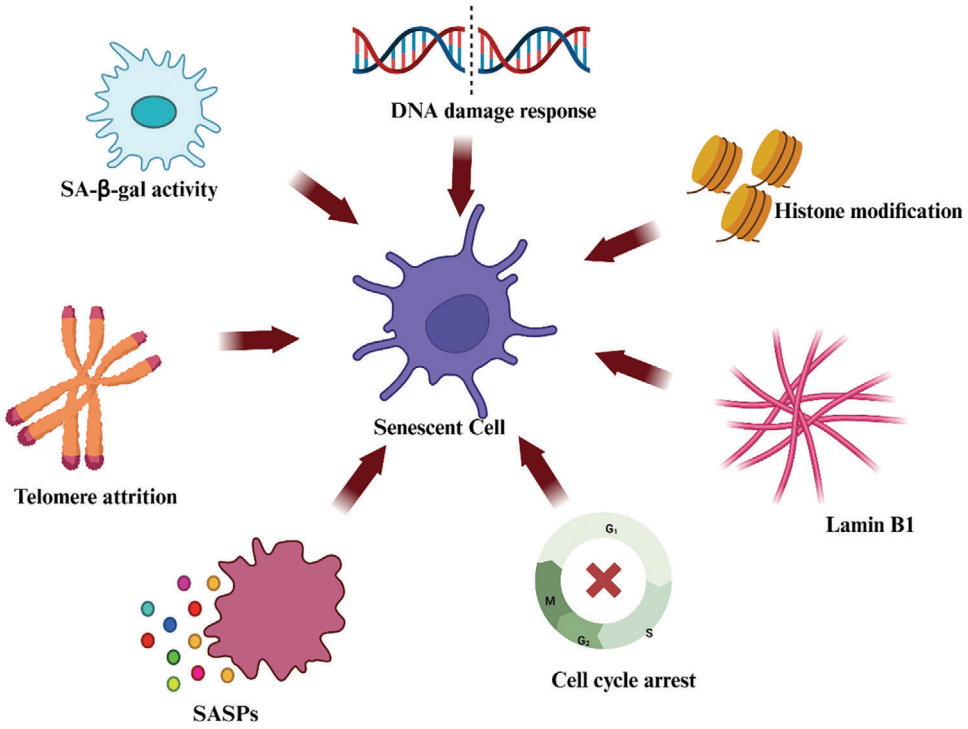

Figure 2. Hallmarks of senescence. Senescence induced by DNA damage, telomere shortening, changes in histone proteins, and p16/p21-mediated cell-cycle arrest. Senescent cells have positive SA-β-gal activity, SASP production, and reduced lamin B1 production.

ers include telomere shortening, changes in heterochromatin levels, H2AX-induced DNA damage, cytosolic double-stranded DNA, and miR-146a.[23–26] In 2013, DNA methylation-based aging clocks to determine cell and tissue ages were developed and are used as biomarkers in human developmental biology, cancer, and aging.[27] Blood and urinary biomarkers of senescence include stanniocalcin 1, growth differentiation factor 15, serine protease inhibitors, and urinary 8-oxoguanosine.[4,28] Some senescent cell types exhibit remarkable features, such as reduced cell proliferation and increased apoptosis resistance, quantified by BrdU and EdU-BCL protein expression, respectively.[29] This review discusses the reliability and limitations of conventional biomarkers of cellular senescence and describes novel senolytic drugs and senotherapeutic approaches to overcome senescence or aging-related metabolic diseases.

2.1. Senescence-Associated β-Galactosidase Staining

Among several biomarkers of senescence, the single most extensively applied enzymatic approach is SA-β-gal staining, which enables the histochemical detection of increased β-galactosidase activity due to increased lysosomal degradation in senescent cells.[20,30] In contrast to normal cells where acidic β-galactosidase activity is detected at pH 4.0, senescent cells express β-galactosidase activity at pH 6.0. Specifically, lysosomal β-galactosidase hydrolyzes the soluble chromogenic substrate 5-bromo-4-chloro-3- indoyl β-D-galactopyranoside into an insoluble product (Blue), and senescent cells have stronger SA-β-gal activity than normal cells; however, this enzymatic activity is undetectable in terminally differentiated or quiescent cells. The GLB1 gene, which encodes lysosomal β-galactosidase, is located on chromosome 3p21.33 and modulates SA-β-gal activity. Senescence-induced intra-lysosomal accretion of damaged macromolecules increases mRNA and GLB1 protein levels and thereby increases β-galactosidase activity.[31–34] Enhanced lysosomal biogenesis in senescent cells produces high levels of lysosomal β-galactosidase, which increases the SA-β-gal activity.[29,35]

Histochemical quantification of SA-β-gal activity is used to detect senescence in both cultured cell lines and tissue sections.[30,32] In this histochemical staining method, human fibroblasts that undergo senescence due to oxidative stress, oncogenic RAS, or radiation therapy are used as positive controls. Moreover, early passage cells of either cancer cell lines or human fibroblasts are used as a negative control as they exhibit no or low SA-β-gal activity. Increased cell confluence affects the SA-β-gal assay results; therefore, the assay must be conducted in sub-confluent populations. Generally, TOPRO-3 or DAPI dyes are used for nuclear staining in fluorescence microscopy. The microfluidic chip method utilizes carboxynaphthofluorescein diacetate as a cell-permeable pH indicator to detect live cells that emit fluorescence (red). Further, SPiDER-SA-β-gal was used to identify cells with doxorubicin-induced senescence (Green), and the Hoechst stain was used to counter-stain the nucleus (Blue).[32,36] Nonetheless, cytochemical and fluorescent detection methods have advantages and disadvantages, and the problems with these methods and troubleshooting guidelines are listed in **Table 1**. Comparatively, cytochemical detection is less time-consuming and is a cost-effective method for the detection of SA-β-gal activity in tissues. However, the manual counting of senescence-positive

Table 1. List of possible technical issues associated with senescence-associated β-galactosidase staining (SA-β-gal) and troubleshooting guidelines (Adapted from ref. [32]).

Method	Problem/Issue	Reason	Troubleshooting guidelines
Histochemical staining	No cells	Cells are not fixed properly and wash away during the PBS rinsing step.	Optimization of fixation time; standardization of concentration of formaldehyde and glutaraldehyde
Histochemical staining	No blue cells	Problem with preparation of staining solution	Optimization of pH of the staining solution; Use of positive control
Histochemical staining	All cells are blue	Problem with preparation of staining solution	Optimization of the pH of the staining solution; CO_2 can decrease the pH of the staining solution under 6.0; thus, a CO_2 incubator is unsuitable; Use of negative control (e.g., SV-40 WI-38 fibroblasts)
Fluorescence staining	No green fluorescence signal	Omission of C_{12}FDG or old stock solution	Repeat staining. If cells are still not fluorescent, use a fresh C_{12}FDG solution
Fluorescence staining	No green fluorescence signal	Wrong instrument setup	Adjust the acquisition parameters on the flow cytometer such that the fluorescence signal becomes detectable on microscopy
Fluorescence staining	No shift in the fluorescence pattern	No senescent cells	Use a positive control in case there are no senescent cells under the conditions

cells is difficult in large-scale experiments. Fluorescence-based detection enables better detection of senescent cells at the single-cell level but requires a flow cytometer, fluorescence microscope, or micro fluid analyzer.[32]

Since 1995, SA-β-gal activity at pH 6.0 has been extensively used as a biomarker for determining the RS of fibroblast cultures under in vitro and in vivo conditions,[34] especially to determine the impact of drugs, stress conditions, and/or genetic manipulations for activating senescence in various cell lines. Furthermore, SA-β-gal activity is used to screen for antiaging drugs or compounds, such as homocysteine, ceramide, cardiolipin, nicotinamide, carnosine, and bioactive compounds of *Astragali radix*.[37,38] In 2019 Tominaga & Suzuki[39] reported that 4 days after traumatic brain injury, SA-β-gal-positive staining indicated cells with activated cell cycle that might undergo cellular senescence. This method was used as a biomarker for detecting hepatorenal senescent cells in doxorubicin-treated mice used for the initial screening of antiaging drug candidates. Moreover, SA-β-gal staining of adipose tissues and circulatory plasminogen activator inhibitor-1 are important methods to evaluate reverse aging in nonhuman primates.[40] Additionally, the identification of species-specific SA-β-gal fluorescence staining (KSL01-KSL12 probes) constitutes a powerful tool for the identification of aging-related diseases and is used for screening anti-aging drug candidates.[41]

Despite its use as a biomarker for senescence, the recent drawbacks of using the SA-β-gal assay are listed in **Table 2** and illustrated in **Figure 3**. Although used most frequently as a marker of senescence, the SA-β-gal assay is unsuitable for paraffin-embedded tissue sections and live cells. However, despite its wide range of applications, this method lacks reliability and specificity. For instance, cytochemicals increased SA-β-gal activity in serum-starved immortalized cells,[38,42] confluent undifferentiated fibroblast cultures, after oxidative treatment with H_2O_2,[34] and in macrophages.[43] Increased lysosomal activity at pH 6.0 is a nonspecific marker of senescence as nonsenescent or young cells express SA-β-gal.[29] For example, Huang and Rivera-Pérez[44] reported increased β-gal activity in the visceral

Table 2. Recent studies reported limitations/drawbacks of using β-galactosidase staining as a biomarker for senescence.

Method	Cells/animal models	Drawbacks mentioned in the study	References
Histochemical staining	*Gallus gallus* and *Tuberaria guttata* embryos	SA-β-gal activity cannot be regarded as a specific marker of senescence during retinal development	[46]
Fluorescent staining	Transgenic mice G93A	Unusual senescence profile with high mRNA and protein levels of p16 and p21 in glia, without the canonical increase in SA-β-gal activity.	[50]
Histochemical staining	Liver tissues from Wistar rats	Interexperimental variation; Changes in tissue senescence intensity between animals	[30]
Histochemical staining	Adipose-derived mesenchymal stromal cells (AdMSC), Albino C57BL/6J mice	Positive regulation of p16Ink4a and SA-β-gal activity in response to p53-independent immunomodulatory stimuli	[43]
Histochemical and immunostaining	Albino C57BL/6J mice	SA-β-gal activity during embryonic development (mid-stage)	[51]
Histochemical staining	Zebrafish embryo	Strong SA-β-gal activity was detected in the yolk, cloaca, central nervous system, intestine, liver, pronephric ducts, and lens.	[178]
Histochemical staining	Naked mole rats (NMRs)	SA-β-gal–positive cells in the nail bed, skin dermis, and dermis surrounding the hair follicles of newborn NMRs.	[52]

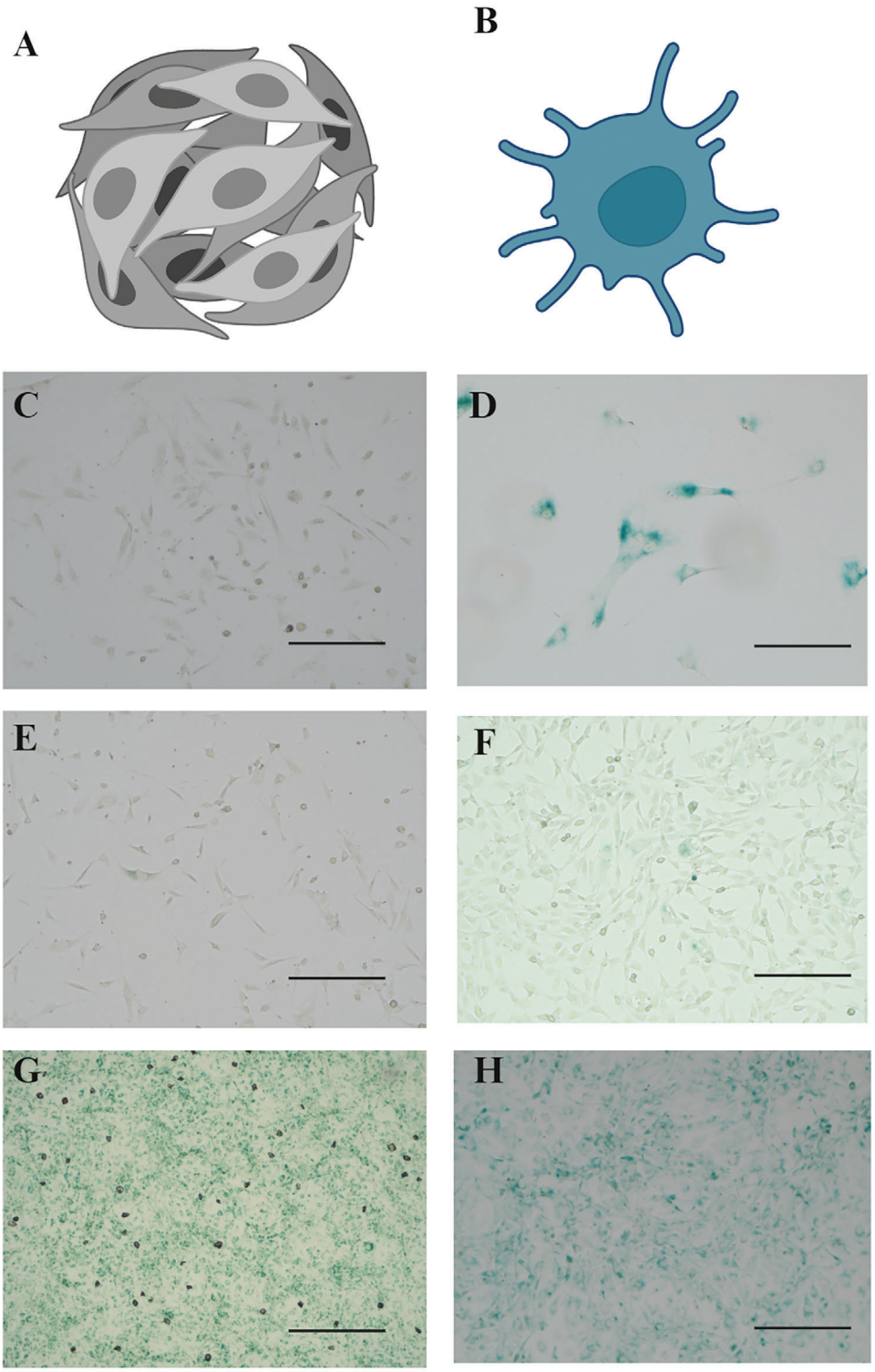

Figure 3. Experimental complications in SA-β-gal activity. Schematic representation of proliferating cells A) and senescent cells B). Negative control: 28 11 No senescent cells detected in preadipocytes 3T3L1 young passage cells C). Positive control: Blue-colored senescent-positive cells present in human diploid fibroblast D) (Second right). Poor staining of 3T3L1 senescent cells E). More than 24-h staining leads to false positives in 3T3L1 preadipocytes F). Problems in staining solution preparation result in the appearance of crystals G). The addition of more staining solution leads to all cells turning blue H). Scale bar –300 μM.

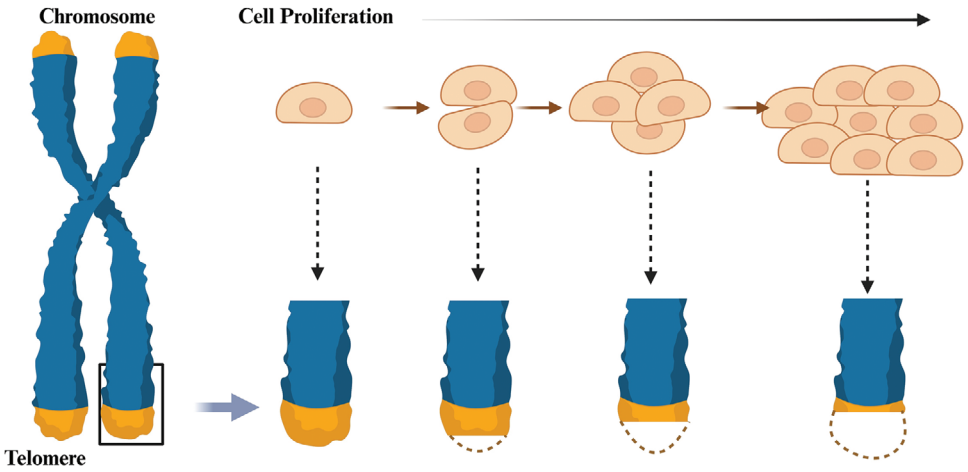

Figure 4. Telomere shortening. Schematic representation of telomere shortening with increased cell proliferation.

endoderm during early embryonic development in mice. Purkinje cells and the choroid plexus of the central nervous system of middle-aged (9-month) wild-type mice were found to be strongly positive for β-gal activity.[45] Regarding neuronal senescence, the hippocampus of younger mice (3 months old), exhibited relatively high β-gal activity, and SA-β-gal staining in the undifferentiated retina correlated with the pattern in neurons undergoing early differentiation.[46,47] Fluorescent ubiquitination-based cell cycle indicator technology is an alternative approach for detecting live premature senescent cells in human neonatal foreskin fibroblasts.[48] Accordingly, the reliability of SA-β-gal staining remains questionable, and the standalone use of this histochemical method is unsuitable for detecting senescence.[49]

2.2. Telomere Shortening

Telomere shortening is a frequently reported biomarker of cellular senescence. In eukaryotes, telomeres are comprised of nucleotides, proteins, and TTAGGG repeats at the chromosomal ends. During replication, the telomere gradually shortens during every cell division, which is known as the molecular clock[53,54] (**Figure 4**). Hayflick posited that cell division cannot exceed a set limit, wherein the telomere length reaches a certain limit and indicates cellular senescence.[55,56] Telomerases are template-independent DNA polymerases that permit the complete replication of linear DNA; thus, the lack of this enzyme induces the production of chromosomes with short telomeres.[57] Telomere shortening, owing to ineffective binding with telomere-capping proteins after p16 and p21 activation, activates DDR pathways that lead to cell-cycle arrest and prevent proliferation and thereby initiate senescence.[58] In telomeres, DDR activation induces the development of senescence markers, telomere-associated DDR foci (TAF), and telomere-induced DNA damage foci. Telomere dysfunction induces cell death, either by apoptosis or autophagy.[59,60] Several methods are available to detect telomere length and dysfunction, and these are listed below.[61]: i) Terminal restriction fragment analysis, ii) Quantitative PCR, iii) Single telomeric length analysis iv) Telomere shortest length assay, v) Fluorescence in situ hybridization (FISH), vi) including flow-FISH and q-FISH, and vii) Hybridization protection assay.

Telomere length decreases with age, and the telomere-shortening rate indicates the pace of aging; therefore, telomere shortening is used as a biomarker for aging. Changes in telomere length and function are intrinsic aging-associated cellular events. Telomere attrition is a common process in aging-related diseases, such as atherosclerosis, heart failure, neurological diseases, and myocardial infarction.[62] Moreover, several lifestyle factors, such as smoking, unhealthy diet, obesity, and lack of physical activity, are directly or indirectly related to telomere shortening, which is a promising biomarker for the aging clock.[63] Along with other senescence markers, such as increased p53 and p21 levels, ROS production, and DNA damage, telomere length is decreased by the highly conserved methyl transferase in mouse fibroblasts.[64] Cryopreservation (slow-freezing method) of human ovarian tissues is highly associated with telomere shortening and other markers of senescence.[56]

Telomere dysfunction plays a key role in senescence and aging. However, Arai et al.[65] reported some limitations of inflammation and telomere shortening in the assessment of cell senescence in super-aged populations. Technical limitations affect the reproducibility of telomere length measurements[66] and telomere dysfunction without telomere shortening stimulates senescence.[67] Although p16-Rb is a well-known pathway of cellular senescence, the association between p16 activation and telomere dysfunction is poorly understood.[68]

2.3. DNA Damage/Methylation

Many reports have provided compelling evidence for the use of DNA methylation-based aging clocks as biomarkers for life expectancy and mortality predictors.[69] In rats, Vanyushin et al.[70] reported aging-related changes in 5-methylcytosine levels. An epigenetic clock, based on DNA methylation, for age determination was established by using 8000 samples from publicly available DNA methylation datasets that comprised 51 types of cells and tissues. It is noteworthy that the DNA methylation age is near-zero for embryonic and pluripotent stem cells,

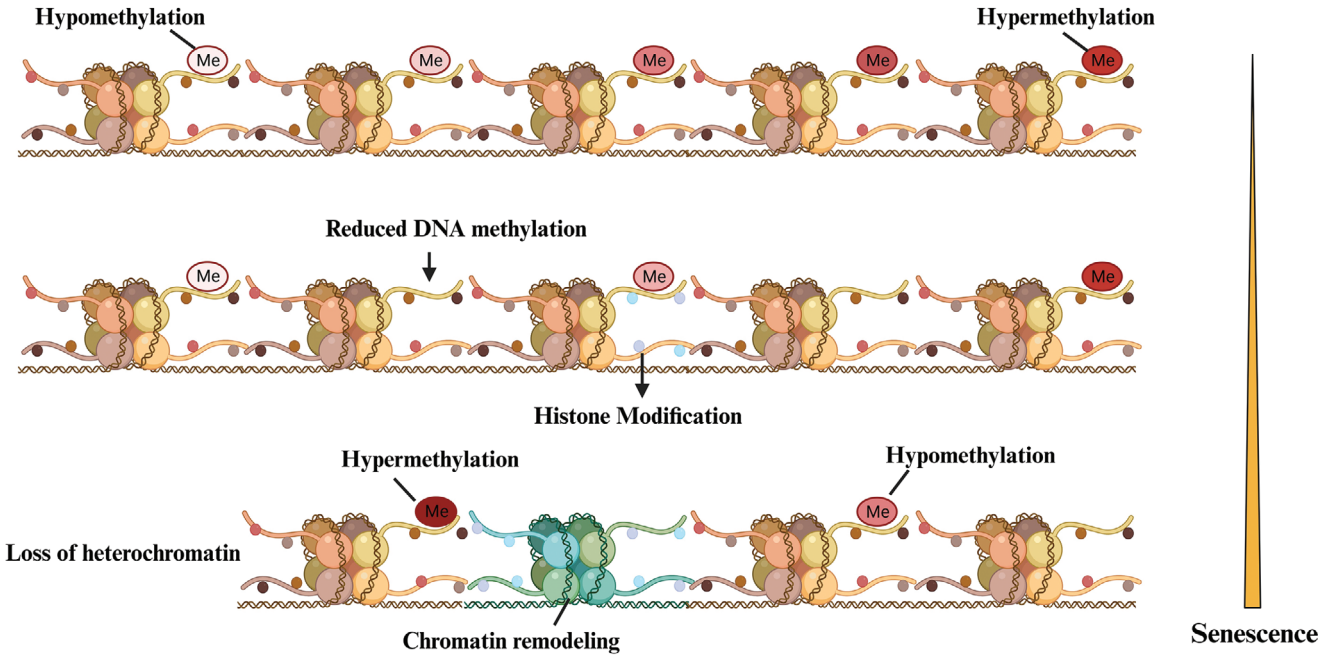

Figure 5. Overview of DNA methylation. Changes in DNA methylation during senescence involve hypomethylation, histone modification, and loss of heterochromatin.

and this age is directly related to the cell passage number. The most promising feature of DNA methylation-based age calculation is its applicability to diverse tissues and cell types, including chimpanzee tissues.[27] In general, DNA methylation (5-methylcytosine) is an epigenetic mechanism that involves the addition of a methyl group to the C-5 position of the cytosine ring to form 5-methylcytosine by methyltransferases (DNMT),[71] which are of three types: DNMT1, DNMT3A, and DNMT3B. DNMT1 expression decreased with aging and decreased DNA methylation. Conversely, increased expression of DNMT3A and DNMT3B during aging in mammalian cells is responsible for the de novo methylation of CpG islands.[72] However, DNA hydroxymethylation alters DNA through the addition of a hydroxymethyl group to cytosine, and recently, DNA methylation has been used as an epigenetic biomarker to predict chronological and biological aging[73,74] (**Figure 5**).

Both CpG hypomethylation and CpGI hypermethylation are hallmarks of RS and cancer.[75] The barcoded bisulfite amplicon sequencing (BBA-seq) method to investigate the DNA methylation profile of long-term culture-linked CpGs (CASR, CASP14, GRM7, KRTAP13.3, PRAMEF2, and SELP) identified predictors of RS that have been used to determine the passage numbers.[76,77] DNA damage, identified by the γ-H2A-X and 53BP1 foci, is an important stimulus of cellular senescence but not a standalone biomarker of senescence; thus, DNA damage in TAF is used to detect tissue aging and cellular senescence.[78]

To detect biological and chronological aging, DNA methylation profiles and DNA methyltransferase expression are used to differentiate cells at different developmental stages.[27,78] Additionally, highly reproducible DNA methylation patterns at different CpG sites were used to calculate cumulative population doubling and passage numbers.[77] Compared with normally proliferating cells, senescent cells exhibit several changes in their DNA methylation profile. Conversely, few methylation patterns are observed in senescent and cancerous cells. Cruickshanks et al.[79] reported that, by escaping senescence, the DNA methylome of premalignant senescent cells may propagate into cancer cells. Furthermore, DNA methylation-based aging clocks constitute biomarkers for the early-stage diagnosis of diseases and are predictors of life expectancy and mortality.[69] Moreover, compared with RNA or protein-based biomarkers, DNA methylation changes are very stable, easy to measure, and can be used to ascertain the influence of several lifestyle parameters and environmental factors.[80]

A limitation of this method is the age-dependent accumulation of DNA methylation in some promoters and changes in DNA methylation patterns due to environmental factors that limit its use as a biomarker for senescence.[81] Although 28 million CpG sites have been identified in the human genome, only 3% of these have been estimated using EPIC arrays. Additionally, a few studies have indicated that whole-genome bisulfite sequencing (WGBS) is possibly ineffective because many WGBS reads are noninformative and show nondynamic methylation across a large fraction of CpG sites.[82]

2.4. Cell-Cycle Arrest

The cell-cycle arrest is an alarming response initiated by abnormal cell division or stress factors that stop cell proliferation.[83] Senescence occurs at either the G1 or G2 phase of the cell cycle; in contrast, quiescence occurs when growth arrest is induced in the G0 phase. Furthermore, with appropriate stimuli, quiescent cells can resume proliferation, whereas senescent cells cannot.[84] The p53/p21WAF1/CIP1 and p16INK4A/pRB pathways mediate cell-cycle arrest during senescence (**Figure 6**). These pathways are complex, and interlinked, and maintain the senescent

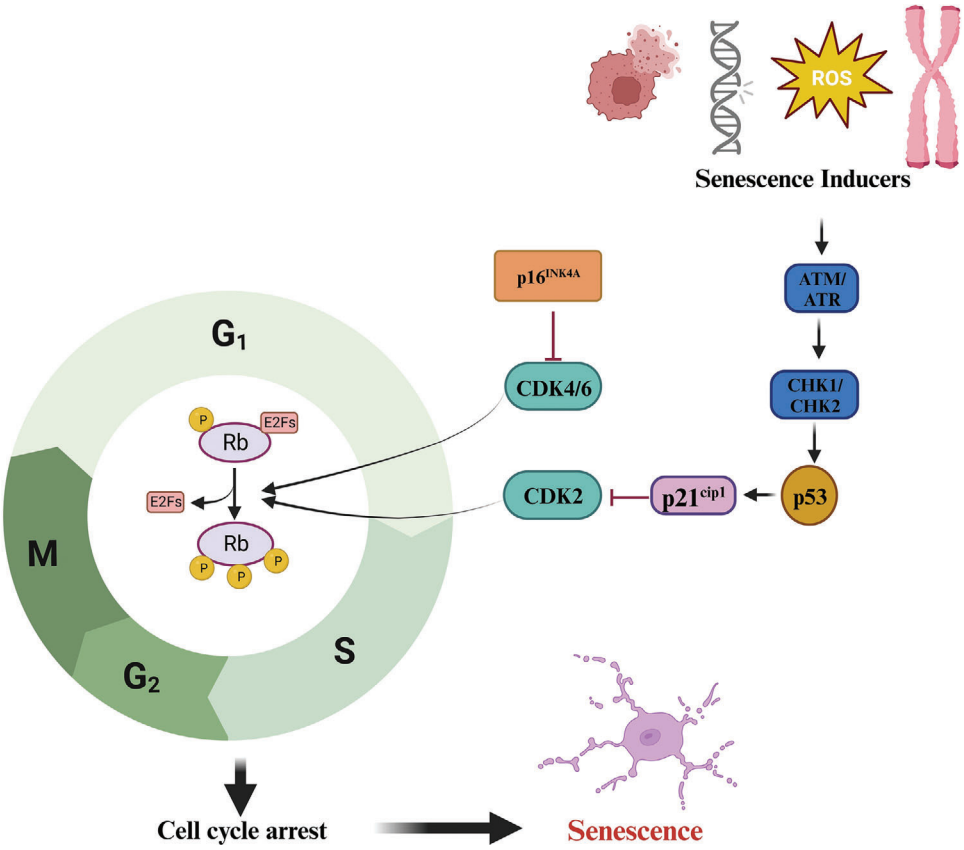

Figure 6. Schematic representation of cell-cycle arrest-mediated senescence. Senescence stimuli, such as oncogene activation, DNA damage, ROS stress, and telomere attrition, activate senescence through the regulation of the p53/p21$^{WAF1/CIP1}$ and p16^{INK4A}/RB pathways.

state by stimulating p53 and pRB, which are the main transcriptional regulators. p21WAF1/CIP1 acts downstream of p53, while p16INK4A acts upstream of pRB.[85] However, the overexpression of any of these transcriptional regulators is sufficient to induce senescence.[83] Sadasivam and DeCaprio[86] reported that the dimerization partner, RB-like, E2F and the multi-vulval class B (DREAM) complex formed by the assembly of p130 and p107, is another master regulator that mediates cell-cycle arrest during senescence.

P53 is commonly identified as the "guardian of the genome" and plays a crucial role in cellular senescence through numerous mechanisms.[87] P53 is activated in response to various cellular stresses, such as DNA damage, oxidative stress, and oncogenic signals.[88] Furthermore, P53 is activated by many post-translational modifications, including methylation, phosphorylation, ubiquitination, SUMOylation, acetylation, and neddylation. Moreover, p53 inactivation affects cellular senescence[89] as p53 plays several roles and is regulated by MDM2 which constitutes an E3 ubiquitin ligase and a positive feedback loop with p53. Also, FOXO4, a transcriptional regulator, acts as a blocker to prevent apoptosis in senescent cells. FOXO4 inhibits p53-dependent apoptosis by promoting p21 expression by binding to phosphorylated p53 and ATM. SIRT1 is derived from the sirtuin family, which plays an important role in NAD+ dependent deacetylation of proteins. SIRT1 plays an important role in cell metabolism, stress response, and senescence and the p53 negatively regulates SIRT1 transcription; SIRT1 prevents activation of the p53/p21 axis.[90] Ubiquitin-specific protease 7 (USP7) is a deubiquitinizing protease that removes ubiquitin from ubiquitinizing proteins susceptible to proteasome degradation. USP7 has an important role about cell cycle regulation through MDM2.[91] Also, inhibition of USP7 activity selectively eliminates senescent cells through recovery of p53 activity.[92]

P21, a direct target of p53, is a 21-kDa protein encoded by the CDK inhibitor gene (*CDKN1A*), which is a member of the Cip/Kip family. This protein inactivates kinase enzymatic functions in the CDK complex by interacting with two cyclin-binding motifs (Cy1 and Cy2), which stop the phosphorylation of retinoblastoma protein (RB), its connection with E2F, and the development of the DREAM complex; thus, p21 is involved in cell-cycle arrest.[93] Similarly, p16 is a 16-kDa protein that blocks cyclinD-CDK4/6 complexes by binding to CDK4/6 and thereby altering RB phosphorylation and enhancing E2F target gene expression. The inhibition or loss of p16INK4A induces escape from senescence, and thus promotes cancer progression[94] showed that DDR-induced senescence depends on p21WAF1/CIP1; conversely, epigenetically induced senescence occurs by p16INK4A induction.[83]

During stress, such as telomere dysfunction, oncogene signaling, mitochondrial dysfunction, and DDR, cells stop proliferating and enter cell-cycle arrest.[95] The CDK4/6 inhibitors, p16INK4a, and p21WAF1/Cip1, are involved in cell-cycle arrest and are

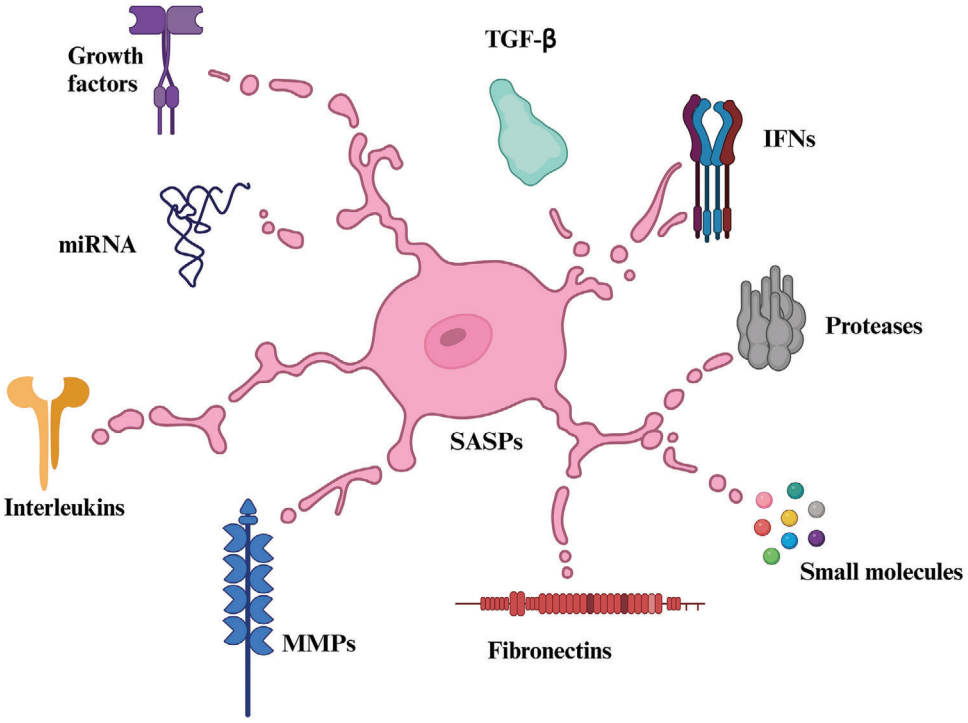

Figure 7. Components of senescence-associated secreted phenotype (SASP). SASPs act through autocrine and paracrine pathways; several types of SASPs mediate beneficial effects, such as tissue repair, immune-cell activation, and embryonic development. TGF-β and interleukins are involved in cellular senescence and aging-related diseases, inflammation, and cancer stemness.

the most frequently used biomarkers of cellular senescence. In 1993, the role of p16INK4a, an inhibitor of cell-cycle progression (from G1 to S), in senescence maintenance was discovered.[96] In 2007, Toillon et al. reported that estrogens decrease gamma ray-induced senescence in breast cancer cells by regulating the $p21^{waf1/cip1}$/Rb pathway without p53 inactivation.[97] DREAM is a transcriptional repressor with p130 or p107 proteins, the MuvB core complex, and E2F4-5/DP, which are connected to pRB. These p53–p21–DREAM–E2F/CHR pathways regulate more than 250 cell-cycle genes that are entangled in cell-cycle arrest and are alternative targets for cancer therapy.[98] Ribosomal protein S14 (RPS 14 or uS11) binds to CDK4, acts as a CDKI, and controls cell-cycle progression independent of p53. Thus, a p53 pathway-independent senescence-ribosome biogenesis program is a crucial component of anticancer therapy.[99]

Although p16 and p21 are well-known senescence biomarkers, these methods have certain limitations. In premature senescence, following senescence initiation, p16INK4a expression occurred regularly late compared to the other CDKI – p21WAF1/Cip1. Moreover, recent reports have shown p16INK4A expression in nonsenescent and transiently arrested cells. In human melanocytes, senescent developmental cells express p16 instead of p21. Furthermore, the cost and specificity of the antibodies limit the use of p16INK4A as a suitable marker for senescence studies.[4,83,95,100] These regulatory mechanisms are also critical for assessing the reliability of p53 as a senescence biomarker. In addition to p53, various biomarkers associated with p53 also regulate the cellular senescence.

2.5. Senescence-Associated Secretory Phenotype

The SASP constitutes a distinct phenotype of cellular senescence that produces various secreted proteins, cytokines, chemokines, growth factors, and proteases.[101] The major composition of this secretome is variable and primarily depends on senescence stimuli. SASPs are composed of secretory factors of senescent cells, such as chemokines, as well as pro-inflammatory and immunomodulatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), Chemokine (C-X-C motif) ligand 1(CXCL1), chemokine ligand 2 (CCL2), prosurvival molecules glial cell line-derived neurotrophic factor (GDNF), and growth modulators (Amphiregulin; AREG)[102] (**Figure 7**). Nevertheless, the true composition of SASPs has been elucidated as comprising several protein and nonprotein signaling molecules, such as proteases, extracellular matrix (ECM), ceramides, hemostatic factors, bradykinins, and damage-associated molecular patterns (DAMP).[103,104] SASPs are typically connected to the DDR, which is involved in transcriptional activation. For example, inflammasomes are a group of PRRs that can identify DAMPs and activate the IL-1 inflammatory cascade.[105] Cytosolic DNA is another key signaling molecule that induces SASPs and is detected by the enzyme cyclic GMP-AMP synthase (cGAS), which produces cyclic GMP-AMP (cGAMP) and activates stimulators of interferon genes (STING). The cGAS-mediated STING pathway is an important controller of SASP induction.[104,106] Furthermore, the activated STING pathway triggers Tank-binding kinase 1 (TBK1), induces downstream regulation of TBK1, and activates IRF3 and

NF-κB to induce Type 1 IFNs and inflammatory responses (late SASPs). SASP transcription is mainly regulated by the initiation of the two major transcription factors, C/EBP and NF-κB, in response to senescence inducers. The mechanistic target of rapamycin (mTOR) is another key regulator of protein translation in senescent cells.[104]

SASPs are most commonly known as "double-edged swords" and their major function includes the allocation of immune cells (macrophages, natural killer cells, and T-lymphocytes) to premalignant lesions and enhanced repair of damaged tissues. For instance, using the SASP factor CCL2, which stimulates TGF-β production, oncogene-induced senescent hepatocytes can transfer the senescence to adjacent cells.[107,108] In contrast, the production of proinflammatory factors (IL-6 and IL-8), macrophage inflammatory proteins, and membrane cofactor proteins may increase harmful effects, including angiogenesis, inflammation, and tumorigenesis.[109] SASPs act in autocrine and paracrine manners; senescent cells are capable of transmitting senescence to nearby normal/nonsenescent cells, and this is known as paracrine senescence.[110] Moreover, cancer therapy or other treatment methods that can induce SASPs include surgery, chemotherapy, and radiotherapy.[111]

The expression of SASP factors is considered a major characteristic of senescent tumor cells (STC), both in vivo and in vitro. Thus, SASPs are important markers for STC detection and identification of the pathophysiological mechanism of STCs.[100,112] Park et al.[113] proposed the targeting of SASP factors IL-1, IL-6, TNF-α, CSF1, and CXCL12 as an alternative approach to control STCs. Recent reports related to senescence suggest that several signaling pathways, which are not limited to cGAS-STING, JAK-STAT, TGF-β, p38MAPK, and PI3K-AKT-mTOR, might be involved in SASP signaling that pertains to the transcription, translation, and protein stability of SASP factors.[114] In preadipocytes, SASPs are responsible for tissue inflammation and induce macrophage infiltration, resulting in an increased immune response at the adipose tissue level.[115] For instance, IL-1α is a key component of SASPs and is activated during oncogene-induced, therapy-induced, and age-associated senescence; thus, IL-1α signaling contributes to immune surveillance and tissue inflammaging.[116,117] Furthermore, evolving proteomic data related to secreted proteins and SASP profiles are perfect candidates for plasma-based, senescence, and aging-related disease biomarkers.[103] In addition, SASP inhibitors or senomorphics that suppress SASPs factors without removing senescent cells are alternative approaches for improving senescence-associated diseases or phenotypes.[118]

The mechanism of action of SASP might differ with several stimuli. Therefore, identifying the specific target and SASP components in a particular cell type is essential to obtain a better understanding of SASP-related consequences.[115] Although SASPs are considered key biomarkers of senescence, their major components are involved in various pathophysiological mechanisms, including inflammation.[49]

3. Senotherapeutic Approaches

Owing to the secretion of inflammatory or tumorigenic factors, the increased accumulation of senescent cells induces detrimental effects on SASPs (**Figure 8**). Thus, the emergence of new therapeutic approaches, particularly to eradicate senescent cells, is known as "senolysis."[101] Small molecules or drugs that can preferentially target senescent cells are known as senolytic drugs, which extend the patients' lifespan.[119] In contrast, senomorphics target pathologic SASPs signaling, either by preventing the production or by antagonizing or neutralizing SASP factors.[120]

3.1. Senolytic Drugs

In recent years, senolytics have increasingly attracted interest; however, their safety and applicability as senescence control agents have mainly been assessed in clinical trials.[114] Senolytic drugs are used to prevent many pathological conditions, including osteoarthritis, osteopenia, and kidney disorders.[121] Senolytic drugs have been selected based on the hypothesis that apoptosis is triggered in senescent cells, and avoids pro-survival pathways because of apoptosis resistance in 30–70% of senescent cells.[118] At first, a combination of dasatinib (tyrosine kinase inhibitor) to inhibit ephrin-dependent survival and quercetin (flavonol), which targets the kinases, was used to eliminate senescent cells, both in vitro and in vivo. Inhibitors of the anti-apoptotic protein B-cell lymphoma 2and peptides targeting FOXO4 are used as senolytic drugs.[104] Other first-generation drugs, such as fisetin, luteolin, curcumin, navitoclax (ABT263), and A1331852, were hypothesis-driven and identified based on their mechanisms of action. Based on transcriptomics and proteomics datasets, several reports suggested that one or more senescent cell anti-apoptotic pathways prevent their removal.[122] Second-generation senolytics have been identified based on high-throughput library screening and the development of galacto-oligosaccharide-coated nanoparticles with toxic cargo engulfed by senescent cells, vaccines, and immunomodulators.[118] These senolytic drug combinations are used to remove senescent cells in many aging-related models, including aging-related hepatic steatosis, chronic atherosclerotic vascular disease, hyperoxia-induced airway dysfunction, radiation/bleomycin-induced lung fibrosis, Alzheimer's disease, arteriovenous fistulation in chronic kidney disease, obesity-associated anxiety and metabolic dysfunction, and osteoarthritis.[123] Many senolytic drugs are currently undergoing clinical trials for various age-related diseases. Moreover, first-generation senolytics have been tested in preclinical models of age-related diseases; diabetes; neurodiseases; and heart, lung, bone, and kidney disorders.[118] The combination of dasatinib +quercetin has been used in idiopathic pulmonary fibrosis (NCT028749819), Alzheimer's disease (NCT0463124), diabetes, kidney disease (NCT02848131), and age-associated osteoporosis. In addition, UBX0101 (NCT04229225; NCT04129944) and fisetin (NCT04210986) are in phase I and II clinical trials or are assigned to age-related frailty, osteoporosis, and osteoarthritis.[122]

In 2019, dasatinib and quercetin were used as senolytic drugs to remove PARP inhibitor (olaparib)-induced senescence in ovarian cancer cells; however, these drugs did not induce cell death in senescent ovarian cancer cells.[124] Moreover, combination therapy with the senolytic drugs dasatinib and quercetin showed low efficacy in reversing DOX-induced senescence in liver cancer

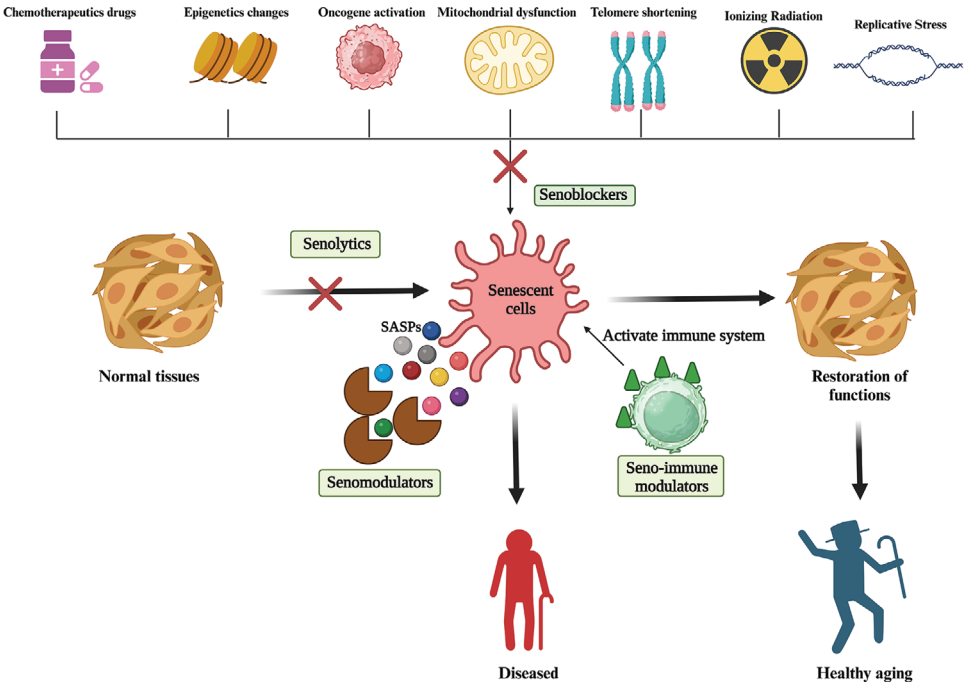

Figure 8. Different senotherapeutic approaches for the removal of senescent cells and healthy aging.

cells.[125] Ginseng has been used in traditional medicine as a natural remedy for healthy aging and promotes skin keratinocyte migration through NF-κB activation.[126,127] Although more than 20 clinical trials of senolytic drugs have been completed, these candidate drugs should not be prescribed or used to treat the general patient population without confirming their safety, tolerability, and efficacy.[4,122]

3.2. Senomorphic Drugs

The drug classes used to suppress the senescence markers, or their SASPs, without inducing apoptosis are known as Senomorphic drugs. Well-known examples of senomorphics include inhibitors of IkB kinaseand nuclear factor (NF)-kB, free radical scavengers, andJAK pathway inhibitors. In addition, few compounds have also been reported for the senomorphic effects; even rapamycin acts as a senomorphics to control SASPs.[128] In recent years, several senomorphic drugs have been reported to control senescence. For example, ATM kinase is a serine/threonine protein kinase triggered by DNA DSBs that also regulates cellular senescence. Therefore, KU-60019, an ATM kinase inhibitor, activated the recovery of mitochondrial functions, the autophagy mechanism, and metabolic reprogramming.[129] In another study, progerin, a truncated Lamin A protein, took part in Hutchitson-Gilford progeria syndrome accumulation during telomere shortening. JH4 is a small molecule that hinders the binding of progerin and lamin a/c is also reported as a senomorphic drug.[130] Moreover, many plant-derived natural compounds were also identified as senomorphic candidates which reduced the SA-β-gal activity and the p53 expression levels.[131,132]

4. Recent-Advanced Models for Senescence and Aging

4.1. In Vitro Models

In general, several methods were used to induce cellular senescence, including irradiation, hydrogen peroxide, and supplementation of culture medium with TNF-α and/or IL-1β. Over the past decades, to examine the molecular and cellular mechanisms responsible for senescence, several in vitro models have been proposed, which include disc, cartilage, muscle cells or tissues, and mesenchymal stem cells.[133] In addition, from the literature, there are various types of in vitro *models* employed for senescence induction: i) monolayer primary cell cultures; ii) bioreactor cultures with stimulating mechanical loading or fluid shearing; iii) 3D tissue models; iv) co-culture models with multiple cell types; and v) genetically engineered cells or tissue models.[119,134–136] In recent years, researchers have reported 22 in vitro models and three electronic databases related to senescence studies. For example, a newly developed Sudan Black-B analog, GL13-based lipofuscin staining protocol was used for the detection of senescent cells in rat models and human intervertebral disc cells.[137] Osteoarthritis is a degenerative joint disease characterized by inflammation of one or more joints and degradation of articular cartilage, which is commonly found in the aging population. Accumulation of senescent cells in joints leads to the development of OA; quantitative flow cytometry-based SA-β gal activity was used to determine the combination of DNA damage and cellular stimuli-induced senescence morphology in cartilage explants from cadaveric human ankle and equine stifle joints.[138]

Most of the aging-related in vitro models are 2D cell cultures due to being i) inexpensive, ii) easy to handle, and iii) well-

established cell culture methods that can be used for several biochemical, molecular, and microcopy analyses. For instance, the 2D culture system is reported for investigating mechanisms responsible for age-related diseases including Hutchinson-Gilford progeria syndrome, cardiovascular diseases, and other NDDs.[139] Compared to other in vitro models, the 2D culturing system is suitable for large-scale drug screening and imaging studies related to morphological changes using microscopy. However, 2D culture models have certain limitations, such as changes in apical-basal polarity, interaction with the substrate, cell migration, and the stiffness of plastic dishes, which affect cell physiology and proliferation.[140] Therefore, to assess age-related modifications in cell-cell interaction, 3D cell culture models were developed to better understand the age-related physiology of an organ or tissue. Moreover, the known fact is that senescent cells interact with young proliferative cells through SASPs such as IL-1a, IL-6, IL-8, TNF-β, lipid mediators, and extracellular vesicles. 3D culture models with defined cell types and ECM mimic the complex microenvironment used for a better understanding of intracellular communication, biochemical impact on cellular activities, and ECM remodeling.

4.2. Induced Pluripotent Stem Cell (iPSCs) Model

For studying age-related diseases in humans, iPSCs have been used as an experimental model to induce senescence in neurons, thereby reflecting disease pathological conditions in a culture dish, which creates an active platform for drug discovery.[141,142] For example, iPSC-derived cardiomyocytes show a similar structure and function to adult cardiomyocytes which can be used for investigating the mechanism of action, drug development, and pharmacological studies.[143] Notably, iPSCs derived from patients with NDDs are identified as a powerful research tool for investigating the pathology of aging-related NDDs.

4.3. Organoids

Organoid is a small, basic organ model that reconstructs physiological 3D tissue structure and cellular composition in vitro. Organoids have the potential to overcome the limitations of conventional aging models.[144] Organoid models are developed from various source materials, such as tissues, explants, reconstituted primary cell cultures, and undifferentiated stem cells which resemble the cellular and matrix organization of an in vivo environment.[145] This organoid or organ-on-a-chip model uses a broad range of tissues including heart, lung, liver, and even brain tissues. The thin section of brain tissues exhibits the cellular environment and tissue organization. Particularly, organoid models of brain tissues have been used to study aging-related NDDs, including Alzheimer's, Parkinson's. and dementia.[146] Other than brain tissues, skin, gut, and muscle tissue organoid models have been developed for studying aging and aging-related metabolic diseases.[145]

4.4. In Vivo Models

Although, the in vitro senescence model induces senescence in culture dishes through serial passaging, oxidative stress, irradiation, and genotoxic stresses, it does not depict the condition of the whole organism. As of now, techniques for the detection of cellular senescence in living animals and humans have vastly increased owing to complex metabolic diseases in the elderly population. In the aspect of senescence induction in in vivo conditions, it must be beneficial for understanding physiologically relevant environments for the development of novel therapeutic strategies and also helpful in studying the causative role of senescence in aging and metabolic diseases.[147] In the field of senescence, in recent years numerous in vivo studies have been reported related to senescence induction methods, biomarkers, and SASPs, the highly heterogeneous phenotype of senescent cells and the lack of specific biomarkers are the major challenges in in vivo detection of senescence. To detect the in vivo senescence pathways, $p16^{lnk4a}$ reporter mouse models and $p21^{CIP1}$reporter mouse models have been developed with a reporter gene. In general, firefly luciferase, renilla luciferase, or monomeric red fluorescent protein are used as the reporter genes that are expressed under the $p16^{lnk4a}$ and/or $p21^{CIP1}$ promoters in transgenic or knock-in approaches.[148] One of the important features of senescent cells is their increased lysosomal activity (SA-β gal; based on this perspective several small molecule probes targeting β gal have been developed to detect cellular senescence in vivo. For example, optical probes used for in vivo senescence imaging include NIR-BG, BOD-L-βGal, NIR-BG2, MB-βGal, and DDAOG, which selectively detect cellular senescence in either senescent cells alone or in senescent animal models[149–152] However, using in vivo models for aging studies has certain limitations. First, invertebrate models are not suitable due to their simple organ systems; second, the lifespan and immune system differ from humans; and third, age-related diseases in animal models do not completely represent disease conditions in humans; therefore, a partial estimation of the interaction between disease pathology and aging mechanisms is possible (Pitrez et al. 2024).

4.5. Disease Models for Senescence and Aging

In humans, aging is a complicated process that increases the mortality rate, weakens immunity, and causes the inability to survive. Aging is driven by major factors such as cellular senescence, genomic variability, epigenetic alterations, telomere shortening, loss of protein homeostasis, variation in nutrient sensing, mitochondrial dysfunction, stem cell enervation, and collapsed intercellular communication. Among these factors, cellular senescence is not only involved in an antitumor mechanism but also plays a key driver in aging and aging-related diseases. Accumulation of senescent cells is more common in pathological sites in major age-related diseases, including neurodegenerative diseases (NDDs), cardiovascular diseases, osteoporosis, diabetes, renal dysfunction, and liver cirrhosis.[153–155] Over the years, several in vitro methods have been developed to study the pathology and etiology of aging-related NDDs such as Alzheimer's disease, Parkinson's disease, prion disease, and multiple sclerosis. Moreover, other than these disease models, sarcopenia is an age-associated decrease in muscle mass and functionality without causing any specific diseases.[156]

NDDs are age-associated deterioration of neurons and their functions. Worldwide, millions of people are affected by these

devastating and incurable NDDs, which are considered a prime health challenge for the individual and the whole human population. Over the past decade, many in vitro, in vivo, and in silico models have been developed for the most common age-related NDDs, including Alzheimer's disease, Parkinson's disease, prion disease, and multiple sclerosis.[157,158] Several in vitro and in vivo models have been developed to understand the pathology of Parkinson's disease. For example, to understand the pathogenicity mechanism of Parkinson's disease, many in vitro cell culture systems have been developed including primary neurons from rodent brain tissues, human neuroglioma cells (H4), human neuroblastoma cells (SH-SY5Y), iPSCs derived from patients, and organoids model systems.[158] However, several models failed to provide behavioral characteristics and pharmacokinetic properties.[159] In the case of neuronal senescence, neurons experience several changes in morphology and functions, including proteostasis, imbalanced redox, and calcium ion dynamics. An ideal model for aging-induced neuronal senescence might have the properties of detecting the senescence phenotype and being helpful for the identification of responsible mechanisms.[160] The most commonly used animal models for aging include *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, Drosophila, rodents, and nonhuman primates owing to their easy handling, short generation time, and convenience for genetic engineering methods. Also, long-term cultures of animal models are a well-described model for neuronal senescence that depicts the senescence phenotype.[161] In addition, neurons differentiated from iPSCs retain age-related epigenetic modifications and allow the effect of neuronal changes in age-related NDDs.[162]

In addition to age-related diseases, obesity or excess calorie intake causes diseases in humans with a similar mechanism of action. In fact, aging and obesity are considered the "two faces of the same coin." Both share the same phenotypical characteristics, such as genomic integrity, mitochondrial dysfunction, intracellular macromolecule accumulation, declining immunity, body composition, and increased systemic inflammation. Obesity increases disease incidence and encourages the early development of diseases. Influenza, type 2 diabetes mellitus, cardiovascular diseases, cancer, and Alzheimer's disease are the most common diseases found in obese people and older adults.[163]

5. Multi-Omics and Computational Biology Approaches

Nowadays, advancements in the fields of computational biology and in silico methods, especially in nucleic acid sequencing methods and machine learning are the reason behind the development of cell-type-specific aging clocks. These aging clocks are developed based on transcriptomic and phenotypical data from senescent cells specific to aging. In addition, with the aging clocks, the Mouse Aging Cell Atlas, or Tabula Muris Senis, has been developed based on single-cell transcriptomic data and provides molecular data related to the key players of aging specific to a broad range of tissues and cell types.[164] Furthermore, large-scale human plasmic data and machine learning models have been developed to measure organ aging in the living human population. The results of the study indicate that aging in humans originated from a single organ.[165,166]

With recent innovations in modern omics approaches and computational biology, researchers have developed novel techniques to quantify cellular senescence and also describe complex aging-related phenotypes with unparalleled molecular-level details. These multi-omics methods unravel the critical molecular level changes during the aging process and are helpful for a clear understanding of the aging mechanism.[167] In the field of aging, multi-omics approaches such as transcriptomics, proteomics, and metabolomics data are utilized to discover novel biomarkers, identify the ideal therapeutic targets, and develop anti-aging approaches (**Figure 9**). In recent years, several research papers have been published related to the application of multi-omics techniques in the field of aging. For example, Xu et al.[168] reported 94 differentially expressed genes and ten hub genes involved in cognitive brain aging by utilizing bioinformatics tools including the Gene Expression Omnibus (GEO), protein–protein interaction (PPI) network, Gene Ontology enrichment analysis, and the KEGG pathway analysis.[168] In yet another study integrated the multi-omics data used to identify the biological relationship between epigenetic age acceleration and a human longevity phenotype. In this study, they have been reported 22 and seven high-confidence genes associated with epigenetic age acceleration and multivariate longevity, respectively. This study is helpful in understanding Agong-associated biological pathways, identifying the druggable targets, and assisting the multi-omics comparison of epigenetic aging clocks and human longevity.[169] Further, in addition to the GEO, the Cancer Genome Atlas and transcriptome data were used to explore various diverse proliferation cycle phenotypes in gastric cancer patients that can be useful for targeted treatment.[170] In the case of proteomics approaches, mass spectrometry (MS)-based proteomics, multiplexed proteomic assays using modified aptamers (SOMAscan), and proximity extension assay (PEA, O-Link), are used as a biomarker for aging-related diseases. These methods have been used to identify the 232 age-associated proteins from various metabolic pathways of animal models and humans.[171] In addition, Lehallier et al. 2019, identified 2925 plasma proteins collected from 4263 people ranging from young adults to nonagenarians (18–95 years old) and developed a new bioinformatics approach to analyze dysregulated plasma proteins. From this study, the fluctuations in the plasma proteome and its association with age-related diseases might be helpful for the identification of novel therapeutic targets. In yet another study, proteogenomics, integrative analysis of genomic and mass spectrometric data has been reported as an alternative strategy for the treatment of age-related diseases.[172] Metabolomics is another recently advanced method to analyze the diversity of aging processes based on the small molecules found in the body. The human metabolome database consists of more than 200 000 metabolites of humans and the Recon3D resource has annotated over 4000 unique metabolites of humans. These computational resources are helpful in exploring the metabolic pathways related to aging and aging-related diseases.[173] Furthermore, the gut microbiome established at a young age undergoes several changes during aging which results in considerable deterioration in microbial diversity. Several reports demonstrated the influence of pathology of several age-related diseases including cancer. In particular, *Akkermansia muciniphila*, the bacteria improves the antitumor response in cancer patients and also has a

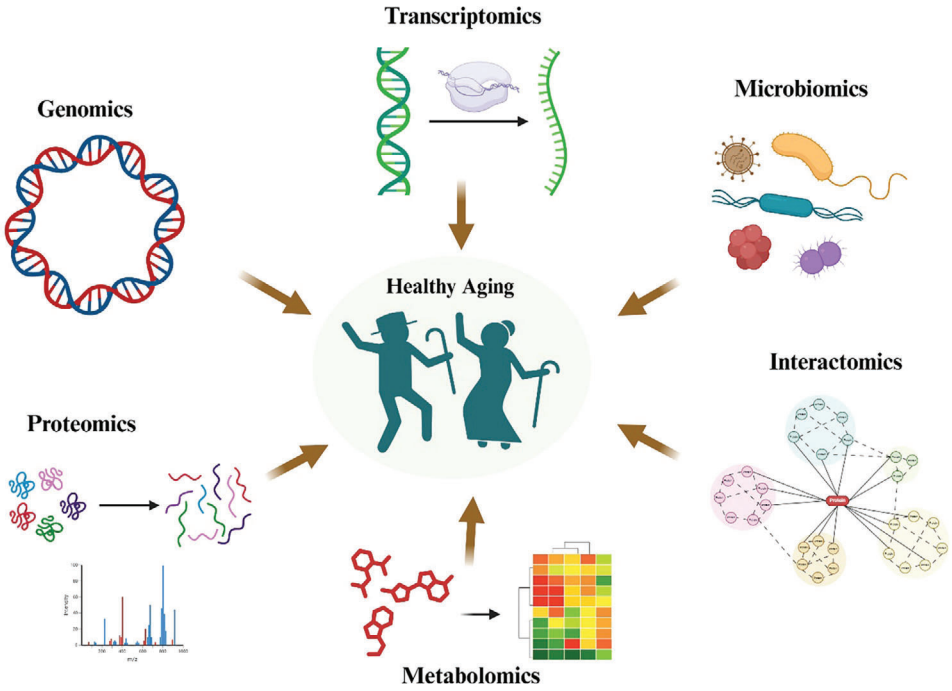

Figure 9. Schematic representation of the application of multi-omics approaches in the field of aging.

positive impact on general health.[174,175] Currently, advancements in the fields of computational biology and in silico methods, especially in nucleic acid sequencing techniques and machine learning approaches are the reason for the development of multi-omics-specific aging clocks. For example, computational models developed for Alzheimer's disease involve tau and amyloid-β-specific quantification of aggregation mechanisms which is helpful for understanding disease pathogenesis.[176] Further, SenCID is a machine-learning-based tool for the identification of senescent cells from both single-cell or bulk transcriptomes. SenCID is a collection of 52 senescence transcriptome datasets of 602 samples from 30 different cell types which discloses mainly six senescent identifies (SIDs).[177]

6. Conclusion and Perspectives

In recent years, the older population has gradually increased due to the decreased birth rate worldwide. The World Health Organization estimates that, by 2030, one in six people will be older than 60 years.[110] Owing to the consequences of an emerging aging population, there is an immediate need for candidate drugs for age-related disorders that may help older people achieve a good quality of life. Correspondingly, senescence and aging research have reached an exponential phase because of their extensive implications for aging and cancer biology studies. Although senescence largely contributes to immunosuppression, tumor evasion, and age-related diseases, senescence also plays a key role in tumorigenesis; therefore, senescence is considered a "double-edged sword." Based on this review of current senescence biomarkers and their implications and limitations, we infer that each biomarker, despite its disadvantages, can be used to detect senescence at a specific level. Senolytic drugs have become widely accepted as approaches for eliminating or reversing cell senescence. Similar to senolytics, interest in senomorphic/SASP inhibitors has increased recently. Even if senolytics or senomorphic drugs reach the preclinical level, they can be used clinically only after an assessment of their safety and efficacy. Despite these challenges, more senescence research is needed to identify accurate biomarkers of senescence and powerful senolytics/senomorphic drugs that can increase human longevity.

In addition, recent advancements in the field of aging facilitate the development of novel in vitro, in vivo, organoid, and iPSCs-based model systems for a better understanding of phenotypical changes related to aging diseases. Although in vitro 3D culture models have been developed to study several aging-related diseases, patient-level treatment is not yet economically achievable. Further, multi-omics and computational models-based novel techniques to quantify cellular senescence and explore the complex aging-related phenotypes at molecular levels. These multi-omics methods are helpful for scientifically revealing the molecular level changes during aging from a multidimensional perspective. Ultimately, we reviewed the challenges in the traditional aging biomarkers and alternative approaches currently used in the field of senescence and aging. In the future, the combined application of multi-omics approaches and computational methods with existing biomarkers might contribute to a better understanding of the senescence and aging mechanisms related to aging-related metabolic diseases.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

S.M. and J.H.P. performed conceptualization, methodology, software, validation, formal analysis, and investigation. S.M. wrote the original draft. S.M. and J.H.P. wrote reviewed, and edited the final draft. H.Y.K., H.-J.J., J.H.L., U.C.S., YH.G., S.H.P., H.G.L., and H.S. performed supervision and visualization. J.H.P. performed funding acquisition, and project administration.

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aging, SA-β-gal, multi-omics, organoids, SASPs, senescence, senotherapeutics

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