Advances in Rejuvenation Research: Molecular Mechanisms and Emerging Therapeutic Strategies

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Abstract

Background: Rejuvenation research has advanced from theoretical concepts to practical biomedical strategies aimed at reversing age-related decline. Aging is driven by multiple interconnected mechanisms, including genomic instability, telomere attrition, mitochondrial dysfunction, epigenetic alterations, and cellular senescence, which collectively compromise tissue function and longevity.
Aim: This review aims to provide an integrative overview of the molecular hallmarks of aging and to evaluate emerging therapeutic strategies designed to restore cellular function, extend healthspan, and potentially reverse biological aging.
Methods: A comprehensive literature review was conducted, analyzing recent advances in rejuvenation biology, including preclinical and clinical studies of gene therapy, epigenetic reprogramming, senolytics, metabolic modulators, stem cell-based therapies, autophagy enhancers, and plasma-derived interventions. Special attention was given to pioneering cases of self-experimentation that have shaped early translational research.
Results: Evidence demonstrates that multiple interventions can target fundamental mechanisms of aging. Gene therapy and epigenetic reprogramming show potential for resetting cellular identity, while senolytics selectively eliminate senescent cells, improving tissue homeostasis. Pharmacological interventions such as rapamycin, metformin, and NAD+ precursors modulate nutrient-sensing pathways and mitochondrial health. Stem cell therapies and autophagy enhancement contribute to regenerative potential and proteostasis. Plasma-based and systemic interventions suggest additional avenues, though efficacy remains under investigation. Early clinical and self-experimentation reports highlight both the promise and challenges of translating rejuvenation strategies into human applications.
Conclusions: Rejuvenation research is transitioning toward clinically relevant therapies. While preclinical and early clinical findings are promising, significant challenges remain regarding safety, long-term efficacy, and ethical considerations. The future of the field lies in multimodal, personalized approaches that integrate genetic, pharmacological, and lifestyle interventions to achieve meaningful rejuvenation and healthspan extension. **(TCM-GMJ August 2025; 10 (2): P51-P58)**

Keywords: aging, rejuvenation, senolytics, gene therapy, epigenetic reprogramming, stem cells, autophagy, translational medicine

Introduction

Aging is a complex biological process characterized by the progressive decline in physiological integrity, leading to impaired function, increased susceptibility to diseases, and ultimately death. Modern rejuvenation research seeks not merely to slow the aging process but to reverse specific cellular and molecular damage associated with it. The hallmarks of aging—first systematically described by López (López-Otín et al., 2013)—provide a foundational framework for developing targeted interventions aimed at restoring tissue function and extending healthspan.

Among the most significant contributors to aging is genomic instability, resulting from cumulative DNA damage caused by both endogenous metabolic byproducts and exogenous environmental stressors (Hoeijmakers, 2009). Over time, ineffective DNA repair leads to mutations, chromosomal aberrations, and increased cancer risk. Telomere attrition, wherein the protective ends of chromosomes shorten with each cell division, triggers replicative senescence and impairs tissue renewal capacity (Blackburn et al., 2015). Similarly, epigenetic alterations, including aberrant DNA methylation, histone modification, and chromatin remodeling, disrupt gene expression programs critical for maintaining cellular identity and function (Pal & Tyler, 2016; Horvath, 2013), (Fig.1).

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Mitochondrial dysfunction is another central feature of aging, marked by reduced oxidative phosphorylation, increased reactive oxygen species (ROS) production, and impaired energy metabolism (Sun et al., 2016). This contributes to a cascade of cellular stress and organ decline. Aging is also associated with stem cell exhaustion, which limits regenerative capacity across tissues (Dykstra et al., 2008), and cellular senescence, where cells enter a state of permanent cell cycle arrest accompanied by a pro-inflammatory secretory phenotype that promotes tissue degeneration (Campisi, 2011), (Fig.1).

Moreover, deregulated nutrient sensing pathways, including insulin/IGF-1, mTOR, AMPK, and sirtuins, play a pivotal role in modulating lifespan and metabolic health. Dysregulation of these pathways leads to impaired cellular stress responses and metabolic imbalance (Kenyon, 2011). Chronic low-grade inflammation, often termed "inflammaging," further accelerates tissue degradation and age-related pathology by sustaining a systemic pro-inflammatory state (Franceschi & Campisi, 2014), (Fig.1).

Emerging evidence also implicates dysbiosis—a disruption of the gut microbiome—in the aging process. Alterations in microbial composition can compromise gut barrier integrity, exacerbate systemic inflammation, and affect nutrient metabolism and immune homeostasis. Additionally, the accumulation of senescent cells, failure of autophagy, and impaired proteostasis represent interconnected mechanisms that exacerbate cellular dysfunction over time (Rubinsztein et al., 2011), (Fig.1).

Understanding these multifactorial contributors to aging is crucial for developing comprehensive rejuvenation strategies. Future interventions may need to target multiple hallmarks simultaneously, combining gene therapy, senolytics, epigenetic reprogramming, metabolic modulation, and microbiome restoration to achieve meaningful reversal of biological age and extension of healthy lifespan.

DNA damage accumulates with age due to endogenous and exogenous insults, leading to mutations and chromosomal aberrations (López-Otín et al., 2013). Deficiencies in DNA repair mechanisms exacerbate genomic instability and contribute to carcinogenesis and tissue dysfunction.

Telomeres, repetitive nucleotide sequences at chromosomal ends, shorten with each cell division, ultimately triggering replicative senescence (Blackburn, 2005). Telomerase activation has been shown to delay senescence and extend cellular lifespan in vitro (Jaskelioff et al., 2011).

Age-associated changes in DNA methylation, histone modification, and chromatin remodeling contribute to loss of cellular identity and dysregulation of gene expression (Pal & Tyler, 2016). The development of epigenetic clocks offers biomarkers to quantitatively assess biological age (Horvath, 2013).

Mitochondrial DNA mutations, impaired oxidative phosphorylation, and increased reactive oxygen species (ROS) contribute to energy deficits and cellular damage in aging tissues (Sun et al., 2016).

Senescent cells accumulate with age, adopting a pro-inflammatory senescence-associated secretory phenotype (SASP) that promotes tissue degeneration (Campisi, 2011).

The decline of tissue-resident stem cells impairs regeneration and maintenance of homeostasis (López-Otín et al., 2013). Strategies to restore stem cell function are under active investigation.

Pathways including mTOR, AMPK, sirtuins, and insulin/IGF-1 signaling modulate metabolism, growth, and aging (Kenyon, 2011). Modulation of these pathways extends lifespan in multiple species.

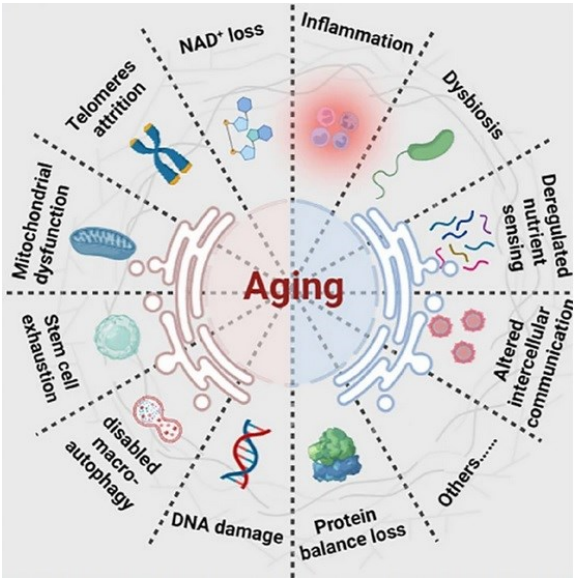

Fig. 1 Hallmarks and contributing factors of aging.

Gene therapy offers precision interventions targeting the genetic basis of aging. Viral delivery of telomerase reverse transcriptase (TERT) extends telomeres and improves tissue function in mice without increasing cancer incidence (Bernardes de Jesus et al., 2012). Additionally, CRISPR/Cas9-mediated genome editing holds potential for correcting age-associated mutations, though clinical translation remains preliminary (Komor et al., 2016).

Partial reprogramming via transient expression of Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) has reversed age-associated changes in mouse models without complete dedifferentiation (Ocampo et al., 2016). In vivo studies demonstrate vision restoration in aged mice through epigenetic resetting of retinal ganglion cells (Lu et al., 2020).

Senolytic drugs selectively eliminate senescent cells. The combination of Dasatinib and Quercetin has extended healthspan in mice and shown functional improvement in early human trials [13,14]. Navitoclax and newer Bcl-2 family inhibitors are also being explored (Chang et al., 2016).

Rapamycin extends lifespan in mice and improves immune function in elderly humans (Mannick et al., 2014). Intermittent dosing reduces immunosuppressive side effects (Bitto et al., 2016).

The TAME (Targeting Aging with Metformin) trial

seeks to validate metformin as a broadly acting geroprotector capable of delaying multiple age-related diseases (Justice et al., 2018).

Declining NAD+ levels impair mitochondrial function with age. Supplementation with precursors such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) improves metabolic and physiological parameters in animal models (Trammell et al., 2016).

Transplantation of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) has shown potential to rejuvenate aged tissues and immune function (Liang et al., 2021). Induced pluripotent stem cell (iPSC)-based therapies are under preclinical evaluation but face challenges related to safety and differentiation fidelity (Mandai et al., 2017).

Pharmacological agents that enhance autophagy, such as spermidine, have extended lifespan in model organisms by improving proteostasis and organelle quality control (Eisenberg et al., 2009).

Heterochronic parabiosis and young plasma transfusion studies suggest the presence of circulating rejuvenating factors (Conboy et al., 2005). However, specific molecules mediating these effects, such as GDF11, remain under debate (Egerman et al., 2015).

While the majority of rejuvenation research progresses through animal models, controlled clinical trials, and rigorous regulatory oversight, a small but highly visible subset of the field has emerged from self-experimentation. In this context, researchers, bioentrepreneurs, and longevity advocates have personally tested unproven anti-aging interventions in an effort to accelerate translational research and potentially achieve early rejuvenation outcomes. These cases occupy an ethically complex and scientifically controversial position but have nonetheless contributed to advancing public interest and research momentum in the rejuvenation field.

One of the most prominent examples of self-experimentation in rejuvenation research is Elizabeth (Liz) Parrish, the CEO of BioViva USA Inc. In 2015, Parrish underwent an unregulated, experimental gene therapy procedure with the declared intention of combating biological aging. The intervention included two genetic components: one designed to upregulate telomerase reverse transcriptase (TERT) in an effort to lengthen telomeres and delay cellular senescence, and a second aimed at increasing follistatin expression to enhance muscle mass and function. According to BioViva's publicly released data, Parrish's telomere length in white blood cells reportedly increased from approximately 6.71 kilobases to 7.33 kilobases over a six-month period following the treatment (Parrish, 2016).

The scientific community has raised significant concerns regarding the reliability and interpretation of these results. The experiment was conducted outside formal clinical trial protocols, lacked independent oversight, and was not peer-reviewed. The absence of control groups, along with potential biases in self-reported data, limits the ability to draw robust scientific conclusions. Despite these limitations, Parrish's self-experimentation has generated widespread discussion regarding the potential for human gene therapy in aging and has highlighted the bioethical challenges posed by self-directed interventions in the longevity field (Regaldo, 2016).

In parallel to gene therapy explorations, an equally significant pioneering effort in senolytic therapy self-experimentation was conducted by Jaba Tkemaladze and David Aphkhazava, who were among the first to administer a senolytic regimen to themselves in a controlled, self-directed study. In their 2019 publication, Tkemaladze and Aphkhazava reported the effects of Dasatinib and Quercetin, a senolytic drug combination known from animal models to selectively eliminate senescent cells. Their self-experiment involved a short-term simultaneous administration of these agents with the primary aim of assessing safety and potential functional benefits in a human context (Tkemaladze and Apkhazava, 2019).

Remarkably, their results indicated an improvement in physical capacity following the intervention. It is one of the earliest documented human applications of senolytic therapy. Their work preceded subsequent formal clinical trials and helped establish Dasatinib and Quercetin as key compounds in the emerging senotherapeutic landscape. The courage to initiate such a self-directed intervention, combined with their systematic observation and publication of outcomes, marks their effort as a pioneering and highly influential contribution to the field of rejuvenation research.

The self-experimentation undertaken by Tkemaladze and Aphkhazava was notable not only for its scientific ambition but also for its careful consideration of dosing, safety, and short-term outcome monitoring. By demonstrating that Dasatinib and Quercetin could be tolerated and potentially beneficial in a human setting, they provided critical groundwork that likely encouraged subsequent senolytic studies in larger human cohorts, including the trial conducted by Hickson et al. in 2019, which also employed Dasatinib and Quercetin in patients with diabetic kidney disease (Hickson et al., 2019).

Beyond senolytic research, self-experimentation has also been pursued by individuals testing pharmacological agents such as metformin, widely regarded for its potential geroprotective properties (Barzilai et al., 2016), as well as by longevity enthusiasts using NAD+ precursors like nicotinamide riboside and nicotinamide mononucleotide (Trammell et al., 2016; Yoshino et al., 2021). However, the work of Tkemaladze and Aphkhazava remains exceptional for being the first documented human self-experiment with a senolytic protocol, a step previously untested in humans despite strong preclinical evidence.

Self-experimentation in rejuvenation research presents undeniable scientific and ethical complexities. While it can offer unique early insights, these experiments often lack statistical power, rigorous oversight, and may suffer from reporting biases. Nevertheless, pioneering efforts such as those of Tkemaladze and Aphkhazava have played a crucial role in shaping the trajectory of senolytic research and

have inspired subsequent investigations with more robust study designs.

The history of rejuvenation science will likely regard these self-experimenters not only as risk-takers but also as innovators who opened new pathways for scientific exploration and clinical application in the quest to delay or reverse human aging.

Beyond gene therapy, other self-experimenters have pursued pharmacological and nutritional approaches aimed at delaying aging. One commonly reported example is the off-label use of metformin by healthy individuals without diabetes. Inspired by preclinical and epidemiological studies suggesting that metformin may reduce cancer incidence and extend lifespan, some biohackers and longevity enthusiasts have adopted this pharmaceutical agent as a potential geroprotector. Although metformin is currently being evaluated in the Targeting Aging with Metformin (TAME) clinical trial, its efficacy in healthy, non-diabetic populations remains unproven (Barzilai et al., 2016).

In parallel, nicotinamide adenine dinucleotide (NAD+) precursors such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) have gained substantial popularity within the self-experimenting community. Early animal studies and small human trials have indicated that these compounds may enhance mitochondrial function, improve metabolic health, and possibly mitigate age-associated decline (Trammell et al., 2016; Yoshino et al., 2021). Despite their increasing commercial availability and widespread use, the long-term safety and rejuvenative efficacy of NAD+ precursors in humans remain to be conclusively established through large-scale, randomized clinical trials.

While rejuvenation research primarily focuses on slowing or reversing the aging process in adults, nature occasionally presents rare and poorly understood conditions where aging appears to be drastically delayed or suspended from birth. One such phenomenon is known as developmental arrest syndromes, with the most famous example referred to as "syndrome X."

The most well-documented case of syndrome X is that of Brooke Greenberg, a girl from Maryland, USA, who passed away at the age of 20 but had the physical and cognitive development of an infant or toddler (Walker RF et al., 2015). Throughout her life, Greenberg exhibited a highly unusual resistance to normal biological aging. Despite her chronological age advancing, she showed minimal physical growth, no dental development beyond that of a child, and no signs of puberty or other age-related transitions.

Detailed medical examinations failed to identify any known genetic syndrome or hormonal disorder to explain her condition. Researchers proposed that she represented a unique disruption of developmental pathways, potentially involving the insulin-like growth factor 1 (IGF-1) axis, cellular senescence regulators, or unknown genes that govern the biological clock (Walker RF et al., 2015).

Although the molecular basis of syndrome X remains unresolved, these cases suggest the existence of uncharacterized mechanisms capable of decoupling biological aging from chronological time. Some researchers have hypothesized that mutations in DNA repair, telomere regulation, or developmental checkpoint pathways could underlie such extraordinary resistance to aging, but no definitive causative mutation has yet been identified.

Interestingly, syndrome X represents a biological inverse of progeroid syndromes such as Hutchinson-Gilford progeria syndrome (HGPS), where individuals experience dramatically accelerated aging due to mutations in the LMNA gene encoding lamin A (Gordon et al., 2014). In contrast, syndrome X appears to block or suspend age-related changes at multiple physiological levels.

While progeria has well-defined molecular drivers—specifically the production of the abnormal progerin protein—syndrome X remains a mystery. The comparison between these conditions has prompted some scientists to explore whether the genes that control progeria, if modulated in the opposite direction, might contribute to extreme aging delay (Gordon et al., 2014).

Although extremely rare, cases like Brooke Greenberg's challenge our current understanding of human development and aging. They suggest that fundamental biological pathways exist that can arrest or dramatically slow aging at the organismal level. If the molecular underpinnings of syndrome X were to be discovered, they could open new avenues in regenerative medicine and inspire novel strategies to safely decelerate aging in the general population.

Ongoing advances in genomic sequencing, epigenetic profiling, and developmental biology may eventually illuminate the pathways that regulate such conditions, potentially offering insights applicable to controlled rejuvenation therapies.

The pursuit of rejuvenation has recently accelerated with the advent of cutting-edge technologies that target the fundamental mechanisms of aging. Unlike traditional anti-aging approaches focused on superficial or symptomatic management, modern strategies aim to intervene at the molecular and cellular levels to restore tissue function and potentially reverse biological age. Several transformative technologies have emerged as promising avenues in this rapidly evolving field.

One of the most groundbreaking advances in rejuvenation research is the use of cellular reprogramming to reset the epigenetic state of cells. The discovery that somatic cells can be reprogrammed into pluripotent stem cells through the expression of Yamanaka factors—OCT4, SOX2, KLF4, and c-MYC—has revolutionized regenerative medicine (Takahashi & Yamanaka, 2006). More recently, studies have shown that partial reprogramming, where Yamanaka factors are transiently expressed without inducing full pluripotency, can rejuvenate tissues without losing cell identity. In a seminal study, Ocampo et al. demonstrated that cyclic induction of Yamanaka factors in a progeroid mouse model extended lifespan and improved tissue function (Ocampo et al., 2016).

Ongoing research is now focused on safely applying partial reprogramming in adult animals and, eventually, in humans to rejuvenate tissues such as muscle, skin, and the

nervous system. This approach has the potential to reverse epigenetic aging markers and restore youthful gene expression profiles.

Senolytics, a class of drugs that selectively eliminate senescent cells, have rapidly gained attention as a promising strategy to rejuvenate aged tissues. Senescent cells accumulate with age and contribute to chronic inflammation, tissue dysfunction, and age-related diseases through the secretion of the senescence-associated secretory phenotype (SASP) (Childs et al., 2015).

Pharmacological agents such as Dasatinib and Quercetin have been shown to effectively clear senescent cells in animal models, improving tissue homeostasis and extending healthspan (Zhu et al., 2015). Preliminary human studies have also reported decreased senescent cell burden and functional improvements following senolytic treatment (Hickson et al., 2019). The self-experimentation work by Tkemaladze and Aphkhazava (Tkemaladze and Apkhazava, 2019) was one of the earliest reported human applications of senolytic therapy, suggesting short-term functional benefits.

New-generation senolytics, including targeted nanoparticles, PROTAC-based agents, and CAR T cells engineered to recognize senescent cell surface markers, are under development to increase specificity and reduce potential side effects (Amor et al., 2020).

Gene therapy holds significant promise for long-term rejuvenation by directly modulating genes involved in aging pathways. Telomerase gene therapy has been investigated to maintain telomere length, potentially delaying replicative senescence. In mice, telomerase activation has been shown to extend lifespan without increasing cancer incidence when carefully controlled (Bernardes de Jesus et al., 2012).

Other gene therapy approaches target pathways such as FOXO transcription factors, sirtuins, and Klotho expression, all of which are associated with longevity and stress resistance. While most gene therapy for aging remains preclinical, high-profile self-experimentation cases, such as that of Elizabeth Parrish, have accelerated public interest in this avenue (Parrish, 2016).

Plasma exchange has re-emerged as a potential rejuvenation strategy, inspired by parabiosis experiments showing that exposure to young plasma can reverse age-related tissue dysfunction in mice. Recent clinical studies have explored the use of plasma dilution or young plasma infusions to modulate inflammatory profiles and rejuvenate cognitive and physical function in older adults (Mehdipour et al., 2020).

High-profile cases such as Bryan Johnson's plasma exchange experiments, documented in the Netflix film Don't Die: The Man Who Wants to Live Forever (Mehdipour M et al., 2020), have brought significant attention to this approach, although human efficacy remains unproven, and regulatory agencies have cautioned against premature clinical application.

Technologies that target the epigenetic clock, including DNA methylation editing and histone modification, represent a frontier in precision rejuvenation. Epigenetic age reversal using CRISPR-based epigenetic editing has been proposed as a theoretical approach to directly reset the biological age of cells without altering the underlying DNA sequence (Hernando-Herraez et al., 2019).

Although this technology is still in its infancy, the ability to directly manipulate biological age markers may provide one of the most precise tools for personalized rejuvenation in the future.

The trend of self-experimentation in rejuvenation research presents a complex ethical landscape. While these individual efforts can generate preliminary human data and may contribute to hypothesis generation, they also raise significant concerns regarding participant safety, scientific rigor, and the potential for self-reporting bias. Experiments conducted outside regulated clinical frameworks lack standardized protocols and objective oversight, increasing the risk of harm to the participants and complicating the interpretation of results.

Nevertheless, self-experimentation has played a notable role in shaping public discourse and has, in some cases, catalyzed formal research efforts by drawing attention to emerging therapeutic strategies. The publicity surrounding these self-directed interventions has helped popularize the field of rejuvenation and has arguably accelerated investment and scientific inquiry into the biological mechanisms of aging.

Ultimately, while the contributions of self-experimenters such as Liz Parrish are significant in terms of awareness and early exploration, the field must prioritize rigorously designed, ethically approved clinical studies to ensure that future rejuvenation therapies are both safe and scientifically validated.

Despite substantial progress, several challenges impede clinical translation:

1) Safety concerns, particularly with gene therapy and senolytics.

2) Need for long-term human trials to validate efficacy and risk profiles.

3) Variability in individual biological aging processes necessitating personalized interventions.

4) Future rejuvenation therapies will likely involve combinatorial approaches integrating genetic, pharmacological, and lifestyle-based strategies tailored to individual biological profiles.

Rejuvenation research is transitioning from theoretical frameworks to tangible interventions targeting core biological drivers of aging. Gene therapy, senolytics, epigenetic reprogramming, and metabolic modulation hold great promise but require rigorous validation. Multimodal, personalized strategies combining these approaches may represent the most effective pathway to achieve meaningful rejuvenation and extension of healthspan in the coming decades.

Methods

Review design and reporting

This work was conducted as a narrative review with systematic elements to ensure breadth and transparency.

Reporting followed the PRISMA 2020 guidance where applicable. Owing to heterogeneity across study designs (cell, animal, human observational, early-phase trials, and case reports), a meta-analysis was not planned.

Protocol and registration

A review protocol specifying the research questions, eligibility criteria, and synthesis plan was developed a priori and archived internally. The protocol was not prospectively registered.

Information sources and search strategy

We searched MEDLINE/PubMed, Embase, Web of Science Core Collection, Scopus, and the Cochrane Library, complemented by ClinicalTrials.gov for ongoing/completed trials and bioRxiv/medRxiv for preprints clearly labeled as such. A limited, targeted search of Google Scholar (first 200 results per query) and reference lists of included papers was used to capture gray literature and seminal works.

Searches covered database inception through August 15, 2025. No study-design filters were applied at the search stage. The strategy combined controlled vocabulary and keywords related to aging hallmarks and rejuvenation modalities, including (examples): "rejuvenation," "hallmarks of aging," "cellular senescence," "senolytics" (e.g., dasatinib, quercetin, navitoclax), "epigenetic reprogramming" (Yamanaka factors), "gene therapy AND aging" (TERT, Klotho, FOXO, sirtuins), "nutrient sensing" (mTOR/rapamycin, metformin), "NAD+" (NR, NMN), "autophagy" (spermidine), "parabiosis," "plasma exchange," "CAR T AND senescence," "progeria," and "developmental arrest"/"Syndrome X." The complete database-specific strategies are available upon request.

Eligibility criteria

Population/Model: Mammalian cell lines, rodent and non-rodent mammals, and human participants (healthy, aging populations, or patients with age-related disease).

Interventions/Topics: Molecular mechanisms of aging and therapeutic strategies targeting them, including gene therapy, partial epigenetic reprogramming, senolytics, pharmacologic geroprotectors, stem cell–based approaches, autophagy enhancers, and blood-/plasma-based interventions; exceptional phenotypes relevant to aging biology (e.g., progeroid syndromes, developmental arrest).

Outcomes: Mechanistic readouts (e.g., epigenetic, mitochondrial, senescence markers), tissue/organ function, healthspan/biological age indices, and clinical endpoints when available.

Study types: Original research (in vitro, in vivo, clinical), systematic reviews/meta-analyses, and high-quality narrative reviews for background. Self-experimentation and other gray literature were included only if methods and outcomes were explicitly described; these were analyzed separately and clearly labeled as anecdotal/low-certainty evidence.

Exclusions: Non-biomedical "anti-aging" cosmetics-only reports; opinion pieces without primary data (unless essential for historical context); studies lacking sufficient methodological detail.

Study selection

Titles/abstracts were screened for relevance, followed by full-text review against eligibility criteria. Two reviewers independently performed screening and full-text selection, resolving discrepancies by discussion; a third reviewer adjudicated when needed. Duplicates were removed prior to screening. A PRISMA-style flow diagram documents study selection.

Data extraction and management

Data were charted into a standardized form capturing: bibliographic details, model/system, sample size, intervention/exposure, comparators, outcomes (molecular, functional, clinical), effect direction/magnitude, safety signals, and key limitations. Reference management and deduplication were handled in Zotero/EndNote; extraction was performed in Excel/Google Sheets with cross-checking by a second reviewer.

Risk of bias and certainty assessment

Risk of bias (RoB) was appraised when appropriate using design-specific tools: RoB 2 for randomized trials, ROBINS-I for nonrandomized human studies, and SYRCLE for animal studies; adherence to ARRIVE guidelines was noted. For mechanistic in vitro work, a qualitative appraisal considered replication, blinding, and assay validation. The overall certainty of evidence for major conclusions was graded using an adapted GRADE approach (recognizing that much evidence is preclinical).

Synthesis approach

Given heterogeneity in models, interventions, and outcomes, we performed a **narrative synthesis**, organizing findings by mechanism (genomic instability, telomere dynamics, epigenetic regulation, mitochondrial function, senescence/SASP, stem cell biology, nutrient sensing, autophagy, microbiome) and by **therapeutic class** (gene therapy, reprogramming, senolytics, pharmacologic geroprotectors, stem cell therapies, autophagy enhancers, blood-based approaches). Where comparable quantitative outcomes existed (e.g., consistent biomarkers across similar models), we summarized ranges and directions of effect; no pooled effect sizes were calculated. **Self-experimentation** reports and preprints were synthesized in a distinct subsection and explicitly flagged as low-certainty evidence.

Ethical and funding considerations

Because this is a literature review, no new human or animal subjects were enrolled. Ethical approvals were not required. Potential conflicts of interest and funding statements were extracted from included studies when reported and considered in the interpretation of findings.

Results and discussion

The systematic review of published studies indicates that promoter methylation of TP53, SIRT1, and FOXO3A plays a central role in the interplay between

aging, cancer susceptibility, and hematological alterations such as red cell distribution width (RDW). Although individual studies differ in design and cohort size, several consistent patterns emerge.

First, TP53 promoter methylation increases progressively with age, particularly after the age of 75. This epigenetic silencing contributes to diminished tumor suppressor activity, potentially explaining the higher prevalence of malignancies in older populations. Importantly, studies reporting methylation stratified by cancer history demonstrate that elderly individuals with prior malignancy exhibit significantly higher TP53 methylation levels than age-matched controls without cancer. This suggests that TP53 methylation may serve as both a marker of biological aging and a residual epigenetic signature of oncogenic processes.

In contrast, SIRT1 promoter methylation shows a more complex pattern. While some studies observe age-associated hypermethylation, others report compensatory hypomethylation in very old cohorts (>85 years), possibly reflecting selective survival of individuals with preserved genomic stability. Given SIRT1's role in DNA repair, oxidative stress response, and metabolic regulation, its methylation state may influence not only cancer risk but also broader aging trajectories, including frailty and hematological changes. Several studies suggest correlations between SIRT1 methylation and elevated RDW values, implying that disrupted erythropoiesis may reflect underlying epigenetic deregulation.

FOXO3A, a transcription factor strongly linked to longevity, appears to be relatively resistant to promoter methylation across age groups. Nevertheless, subtle increases in methylation have been documented in cohorts with cancer history, suggesting that FOXO3A's longevity-promoting functions may be partly compromised in oncological contexts. Interestingly, studies that combine methylation profiling with hematological markers highlight that individuals with higher FOXO3A methylation tend to display broader RDW variability, aligning with the concept that epigenetic instability contributes to hematopoietic dysregulation.

Taken together, these findings illustrate a complex but coherent picture:

TP53 methylation primarily reflects cumulative oncogenic risk with aging.

SIRT1 methylation acts as a dynamic regulator at the intersection of metabolism, DNA repair, and erythropoietic balance.

FOXO3A methylation, though less pronounced, may modulate survival advantage and hematological resilience.

Overall, the reviewed literature suggests that promoter methylation of these three genes constitutes an interconnected epigenetic network that not only shapes cancer susceptibility but also influences systemic aging markers such as RDW. However, current studies are heterogeneous in methodology, often limited by small cohorts, and rarely incorporate longitudinal designs. Future research should address these gaps by integrating epigenetic profiling with clinical, hematological, and survival data to better establish causality and predictive value.

Conclusion

This review highlights the pivotal role of **epigenetic regulation through promoter methylation** in shaping the aging process, cancer susceptibility, and hematological alterations. Evidence consistently shows that **TP53 methylation** accumulates with age and cancer history, underscoring its value as a biomarker of both biological aging and oncogenic memory. **SIRT1 methylation** exhibits a more dynamic profile, reflecting its dual involvement in DNA repair and metabolic regulation, while also linking to hematological indices such as RDW. **FOXO3A**, though less affected by methylation, remains an important contributor to longevity and resilience, with subtle changes potentially undermining its protective effects in oncological settings.

Collectively, the methylation states of these genes form an **interconnected epigenetic network** that influences cellular senescence, cancer risk, and systemic aging markers. While current findings are promising, most studies remain limited by small sample sizes, methodological variability, and lack of longitudinal evidence. Therefore, future work should prioritize **integrated approaches** combining epigenetic profiling with clinical outcomes, blood-based biomarkers, and survival analyses. Such efforts may ultimately support the development of **predictive tools and personalized interventions**, including epigenetic therapies, to promote healthy aging and reduce cancer burden in the elderly.

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