

Life Sciences and Medicine

Potential approaches for intervening agingJianghua Shen^{1,2,3,4}, Guang-Hui Liu^{1,2,3,4,*} & Moshi Song^{1,2,3,4,*}¹State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;²Beijing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China;³Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China;⁴University of Chinese Academy of Sciences, Beijing 100049, China*Corresponding authors (emails: ghliu@ioz.ac.cn (Guang-Hui Liu); songmoshi@ioz.ac.cn (Moshi Song))

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Though immortality and regeneration were ludicrous and far-fetched in an age of ignorance and superstition, people have never stopped looking for the elixir of longevity. As a result of advancements in lifestyle and medical care, the average human life expectancy has risen in recent decades, along with the increasingly higher incidence of aging-related diseases. With the advance in biological techniques, our understanding of aging and aging-related diseases has expanded significantly. Molecular and cellular mechanisms that contribute to the aging process and age-related diseases can be classified into several categories, including genomic instability, telomere atrophy, epigenetic changes, loss of protein stability, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell depletion, and impaired intercellular communication [1].

Along with the diverse strategies that have been exploited to explain aging, our ideas of aging intervention have also grown rapidly and multiple potentially effective strategies have been investigated. Here, we summarize these approaches below, dividing them into the following categories: (1) pharmacological administration; (2) gene therapy; (3) regenerative medicine; and (4) immunogenic intervention.

Pharmacological administration

Senescent cells release enormous amounts of inflammatory cytokines, immunomodulators, growth factors and proteases, which are known as senescence-associated secretory phenotypes (SASP). Exosomes and episomes carrying enzymes, microRNAs, DNA fragments, chemokines, and other physiologically active components can also be found in the SASP [2]. Given that SASP and SASP-secreting cells have been regarded as possible therapeutic targets to ameliorate inflammatory conditions in the elderly, senolytics referred to the agents that selectively kill senescent cells are gaining traction as a way to possibly slow down the aging process and ameliorate organ malfunction. As such, the first empirical senolytic combination that extends mouse lifespan includes dasatinib, originally developed to target oncogene-addiction in leukemia, and quercetin, which inhibits the mTOR pathway, among numerous others [3]. In humans, dasatinib 100 mg

and quercetin 1000 mg given for three days decreased the number of P16- and SA- β -gal-positive cells in adipose tissues [4]. Yet, cautionary notes have also been sent as dasatinib and quercetin may exert acute tumorigenic effects [5] and exacerbate obesity- and age-dependent liver disease progression [6]. In a more recent study, a chemical screen has identified that a much lower dose (100 nmol L⁻¹) of quercetin alone functions as a geroprotector by enhancing self-renewal and restoring heterochromatin architecture in aged human MSCs [7], pointing to a new possibility of redirecting the usage of these drugs against aging and related diseases.

Geroprotective compounds, such as metformin and rapamycin, are placed high hopes for treating age-related conditions and for delaying aging. Metformin modulates the activation of AMP-activated protein kinase (AMPK), which directly regulates the activity of numerous epigenetic enzymes and restores AMPK-mediated phosphorylation and stabilizes Tet methylcytosine dioxygenase 2 (TET2), thereby preventing changes in 5-hydroxymethylcytosine levels [8]. Although data from prospective human clinical trials on metformin in aging is only now being planned or emerging, widespread use of the drug in otherwise healthy people requires a much more detailed understanding of its effects. Rapamycin treatment, on the other hand, slows the accumulation of epigenetic aging signatures in the liver cells of mice [8] but has shown a limited outcome in clinic [9]. In addition, nicotinamide adenine dinucleotide (NAD⁺) is a potent coenzyme mediating many redox reactions and NAD⁺ levels decline during the aging process, likely causing defects in nuclear and mitochondrial functions and resulting in many age-associated pathologies. Restoring NAD⁺ by supplementing NAD⁺ intermediates (such as nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR)) has been reported to ameliorate multiple age-associated functional defects in rodents [10].

Moreover, increasing evidence indicates that the rejuvenation of epigenome and transcriptional profiling may be vital for maintaining tissue function and extending the lifespan in aged animals. Oltipraz has been found to reactivate nuclear factor-erythroid factor 2-related factor 2 (NRF2) and thus decreases oxidative stress and reverses cellular HGPS (Hutchinson-Gilford progeria syndrome) dysregulated epigenome [8]. Likewise, Vitamin C has also been reported to be a geroprotector via the stabilization of heterochromatin in aged human stem cells [8].

Gene therapy

Innovative gene therapy-based techniques appear to be among the most promising for preventing and treating chronic polygenic diseases, particularly age-related disorders, at the moment. Gene-based treatment allows for both direct (e.g., gene editing) and indirect (e.g., viral or non-viral vectors) modulation of genome architecture.

In mice, adeno-associated virus (AAV)-mediated delivery of vascular endothelial growth factor (VEGF) improved metabolic function and motor ability, and genetic overexpression of VEGF greatly lengthened mouse longevity [11]. Furthermore, researchers have recently used AAV to deliver three aging protective factors (FGF21, sTGF β 2, and α Klotho) [12], which combinatorially alleviate age-related disorders such as heart failure, renal failure, diabetes, and obesity in animal models. In addition, multiple epigenetic rejuvenation factors have also been discovered and gene therapy with such a single factor has been shown to successfully alleviate aging-related disorders in mice [8]. For example, gene therapy with lentiviruses

Aging Hallmarks and Potential Interventions

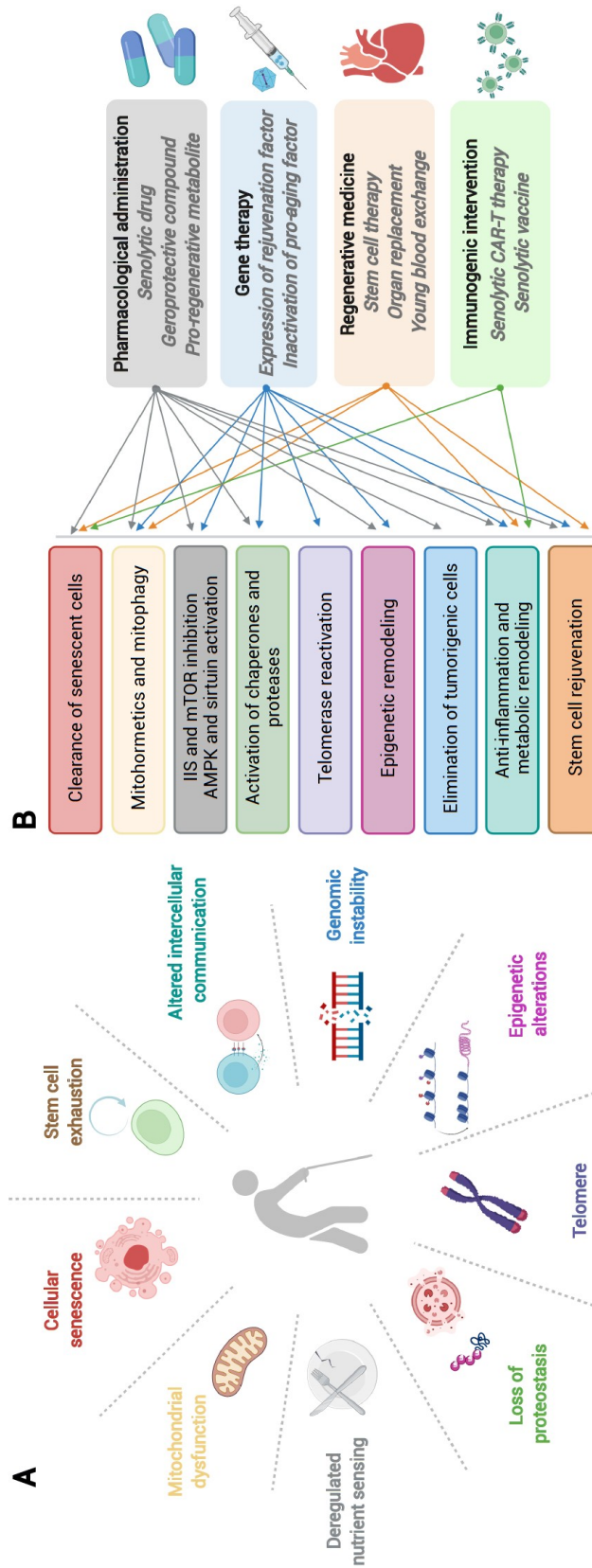


Figure 1 Aging hallmarks and potential aging interventions. (A) Aging hallmarks. Genomic instability, telomere attrition, epigenetic alterations, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication constitute nine hallmarks of the human aging process. The panel was modified from López-Otín C *et al.* [1]. (B) Potential approaches for intervening aging. Pharmacological administration, gene therapy, regenerative medicine and immunogenic intervention are four main potentially practicable strategies for aging intervention. The corresponding cellular effects include clearance of senescent cells, mitohormetics and mitophagy, IIS and mTOR inhibition, AMPK and sirtuin activation, activation of chaperones and proteases, telomerase reactivation, epigenetic remodeling, elimination of oncogenic cells, anti-inflammation and metabolic remodeling, and stem cell rejuvenation. The connecting arrows indicate the previously reported correlations between the interventions and the cellular effects. The color of the individual effect is consistent with that of the respective aging hallmark.

encoding either YAP (Yes1-associated transcriptional regulator) or FOXD1 (Forkhead box D1) injected into the joint cavity of mice with osteoarthritis has been shown to reduce the proportion of senescent cells in articular cartilage [13].

In contrast to the rejuvenation factors, dozens of potential novel human pro-aging genes have recently been discovered in a genome-wide CRISPR/CAS9 screen. KAT7, an epigenetic enzyme, stands out as one of the most powerful pro-aging factors. The use of a viral vector to partially inactivate KAT7 in the liver increased the lifespan and healthspan of mice [14]. In another study, the effect of a telomerase gene-based therapy was tested in mice with an AAV-expressing mouse telomerase reverse transcriptase (TERT). Appearing not tumorigenic, this treatment exhibits beneficial effects on insulin sensitivity, osteoporosis, neuromuscular coordination, and several aging biomarkers, resulting in a prolonged lifespan by 24% and 13% in adult and aged mice, respectively [15]. Another innovative study shows that the mouse lifespan can be extended by using the mouse cytomegalovirus (MCMV) carrying exogenous TERT or follistatin (FST) (MCMV_{TERT} or MCMV_{FST}) monthly delivered via inhalation or intraperitoneal injection [16].

So far, given the remarkable complexity of systems involved in aging and aging-related disorders, the efficacy of many gene therapies is frequently inadequate and constrained by side effects. Clinical adoption of such applications would undoubtedly be a lengthy process and require a substantial amount of time and cost.

Regenerative medicine

Stem cells have been shown to be a feasible remedy for a variety of health difficulties, including nerve regeneration and movement disorders and other age-related ailments. Not surprisingly, stem cells are also regarded as the white hope for aging-related disorders. In fact, recent progress in stem cell-based therapy has exhibited therapeutic effects in the amelioration of aging-related degenerative diseases. Particularly, mesenchymal stem cells (MSCs) that exert regenerative effects and possess anti-inflammatory properties have been widely tested in pre-clinical and clinical settings. For example, in addressing the pathological symptoms of aging frailty, which is characterized by a progressive decline in health and clinical symptoms of exhaustion, weight loss, a feeling of slowing down, and a decrease in functional capacity, the transplantation of MSCs has emerged as a promising therapeutic strategy [17]. In phase I and II trials in human subjects, the safety and efficacy of MSC transplantation have been demonstrated in treating aging frailty. However, stem cell therapies are generally still in their infancy in the field of aging intervention and also the potential tumorigenicity of these MSC therapies is under careful consideration, which awaits larger-scale studies to test the safety and efficacy towards different aging-related conditions.

Alternatively, heterochronic parabiosis, in which a young mouse and an aged mouse share joining the circulatory systems, is another method of rejuvenation with multiple beneficial effects, including cognition improvement and better muscle strength in mice. Recently, a single-cell RNA sequencing analysis was performed on 20 organs to reveal the cell type-specific responses to young and aged blood in heterochronic parabiosis. In particular, the most strongly responsive cell types include adipose mesenchymal cells, hematopoietic stem cells and hepatocytes. Young blood not only reverses established aging patterns, but also induces a new rescue of genes encoding subunits of the electron transport chain, suggesting the important role of mitochondrial function in mediating parabiogenic rejuvenation. Moreover, it has been found that aging

blood reduces global gene expression while young blood restores it only in certain cell types, shedding light on a systematic understanding of the cellular and molecular events underlying parabiotic rejuvenation [18]. Recently, the potential key mediators of heterochronic parabiosis-induced rejuvenating programs have been further elucidated via a systematic single-cell transcriptomic analysis across the hematopoietic and immune systems, as well as four types of solid tissues/organs affected by the hematopoietic and immune organs. Notably, adult stem cells, especially bone marrow HSPCs, are responsive to the changing milieu. Specifically, a list of factors, including CCL3 and YY1, has been identified as potent hits for HSPC rejuvenation [19].

Immunogenic intervention

Immunogenic intervention has been employed in the management of many different types of diseases. Yet, only recently, it comes to the field of aging intervention. SASP secreted by senescent cells can recruit T cells and NK (natural killer) cells and promote the clearance of senescent cells. It has been recently found that uPAR is highly expressed in senescent cells both *in vivo* and *in vitro*. Accordingly, chimeric antigen receptor (CAR) T-cell (CAR-T) therapy with the so-called “senolytic CAR-T cells” has been applied to target uPAR (Urokinase-type plasminogen activator receptor), successfully eliminating senescent premalignant and malignant cells in mouse models of liver and lung cancer. Even though, the use of senolytic CAR-T cells, although distinctive, may carry the risk of cytokine storm [20]. Therefore, more safety studies are necessary before specific application. Another new strategy for the first time put forward the concept of senolytic vaccination. Non-metastatic melanoma glycoprotein B (GPNMB) that accumulates in senescent cells has recently been identified as an aging antigen. As higher levels of GPNMB were observed in vascular endothelial cells and white blood cells in mice and patients with atherosclerosis, progeroid mice were immunized against Gpnmb and displayed a reduction in Gpnmb-positive cells, along with improved aging-related phenotypes and extended lifespan, pointing to senolytic vaccination as a potential strategy for new senolytic therapies [21].

Overall, despite that it may be argued that treating aging as a whole may be a more effective way to prevent or delay age-related illnesses than treating specific clinical problems, both targeted and general aging interventions are becoming increasingly more relevant for the pharmaceutical business and public health agencies as the population ages. As for now, the first-line strategy is still to live a healthy lifestyle that includes a balanced diet, frequent exercise, and quitting smoking and drinking. The high hopes for the future development and application of aging interventions, on the other hand, must be critically examined and evaluated in light of their economic, social, and ethical consequences.

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Conflict of interest

The authors declare that there are no conflicts of interest to disclose.

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