The Cellular and Molecular Biology of Aging

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Abstract. Aging is characterized as a complex biological process, marked by a progressive diminution in physiological capacities and an escalated susceptibility to diverse diseases. This trajectory detrimentally affects individual well-being and exerts considerable strain on healthcare frameworks and economic stability. Effective methods to prevent and reverse aging have been explored in academic research and clinical settings for a long time. However, the long-term effectiveness and safety of anti-aging measures still need to be further verified. This article provides insight into specific biological mechanisms associated with aging, such as genomic instability, telomere dysfunction, epigenetic modifications, and mitochondrial dysfunction. In addition, the complex role of autophagic activity, senescence-associated secretory phenotypes and epigenetics with aging is also discussed, emphasizing the importance of understanding these processes for aging interventions.

1 Introduction

Aging is a complicated biological phenomenon involving gradual deterioration of physiological function and increased susceptibility to various diseases. It constitutes an essential aspect of the life cycle of many organisms. At the same time, aging is a multifaceted and progressive biological phenomenon, exacerbating susceptibility to various human pathologies, including neoplastic, cardiovascular and neurodegenerative diseases[1]. This inevitable process is evident at the molecular, cellular and systemic levels and is controlled by multiple interactions between genetic, environmental and lifestyle determinants. The prevention of aging has significant social implications as the global population ages and poses significant public health challenges[2]. The evidence suggests that achieving healthy aging through the diminution of morbidity holds more significant value than extending life expectancy. Furthermore, it posits that the economic benefits of addressing the aging process may surpass those obtained by eradicating individual diseases. One investigation reveals that decelerating the aging process could enhance life expectancy by one year, an advancement equating to an economic worth of approximately $38 trillion.

Contemporary research efforts have made substantial progress in decoding the aging process and investigating potential therapeutic interventions. A groundbreaking study led by prominent anti-aging authority David Sinclair and published in the prestigious journal Cell hypothesizes that ageing in mouse models is reversible. This groundbreaking study raises the feasibility of reprogramming biological age, showing that it can restore up to 50% of an organism's actual age. These findings challenge the traditional view of aging as an irreversible consequence of genetic mutations and instead highlight the critical role of epigenetic information loss in the aging process[3]. Despite these significant advances, many aspects of aging remain to be fully elucidated. These include the long-term safety and effectiveness of anti-aging interventions in human subjects, which requires further empirical exploration. Furthermore, there remains a need to develop precise and effective anti-aging therapies and seamlessly integrate these advances into clinical applications.

This article highlights the profound significance of understanding the mechanisms of aging at the molecular and cellular levels. At the molecular level, aging is marked by cumulative changes in DNA structure, protein synthesis, and cell signalling pathways. Critical features of aging include genomic instability, telomere attrition, epigenetic changes, and perturbations in proteostasis. At the cellular level, aging is characterized by increased cellular senescence of cells, reduced stem cell viability, and changes in intercellular communication. These alterations culminate in a decrement in the functionality of tissues and organs, precipitating the onset of an array of age-associated pathologies, which includes neurodegenerative disorders, cardiovascular diseases, and oncological conditions.

2 The levels at which aging occurs

Aging represents a complex biological phenomenon, manifesting across multiple strata, from individual to molecular dimensions. This process is recognised by a temporary deterioration in physiological functioning, which is essential for survival and fertility, and is identified by a systematic decline in physical and cognitive abilities, increased vulnerability to disease, and,

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ultimately, death[4]. The universal markers of aging include phenotypic changes such as hair greying, skin wrinkling, joint stiffness, osteoporosis, muscle strength decline, cognitive deterioration, and diminished sexual response[4]. The difference in the rate of aging among individuals is attributable to genetic, environmental, cultural, nutritional, physical activity, and historical health factors[5]. At organ and tissue levels, aging is linked to attenuated repair and regeneration capacities, leading to varied aging trajectories across different organs and tissues[6]. This stage is marked by the onset of functional degeneration in vital organs and cellular alterations within tissues, characterized by increased cellular size, reduced proliferation, and the accumulation of pigments and lipids, culminating in the decline of organ and tissue function and the onset of aging-related diseases[5]. Cellular aspects of aging include increased cell variability, somatic mutations, cellular senescence and degeneration of stem cells, all of which contribute to and declining organ function and systemic aging. Research showed in pancreatic cells that aging leads to increased heterogeneity within cell populations, as evidenced by increased intercellular variability in epigenomic and transcriptomic profiles[7]. Studies have shown that the health of stem cells declines progressively with age such as haematopoietic stem cells[8]. In addition, satellite cells gradually lose their regenerative capacity with age[9]. The complex interplay between cellular senescence and aging highlights the importance of understanding the underlying mechanisms and developing interventions that target senescent cells and promote healthy aging.

Molecularly, aging entails progressive changes in cellular and molecular processes, including alterations in DNA structure, protein homeostasis, cell signalling pathways, etc. Research in the field of aging biology is focused on elucidating these cellular and molecular foundations of age-associated changes, particularly identifying determinants that influence organismal lifespan and devising strategies to promote healthy aging and extend lifespan. The mechanistic target of the rapamycin (mTOR) signalling pathway has emerged as a pivotal regulator of lifespan and aging processes, with its inhibition demonstrating notable extensions in lifespan across various animal models[10].

Much research has elucidated that senescent cells orchestrate a complex array of inflammatory cytokines, chemokines, growth factors, and matrix remodelling factors, collectively known as the senescence-associated secretory phenotype (SASP)[11]. This array modifies the adjacent tissue milieu, leading to chronic inflammation and oncogenic processes. Notably, the SASP paradigm is evident in cultured senescent cells and contexts of aged individuals, as delineated by Watanabe[12]. The effects of SASP involve cellular and adventitious cellular senescence, tissue repair and immunity, developmental senescence, and persistent inflammation and cancer.

In the cellular response to DNA damage signals, the cyclin-dependent kinase inhibitor p21cip1/waf1 is activated via p53 signalling in the initial stages. At the same time, p16INK4a induction is mediated through the Ets family of transcription factors under sustained damage conditions. Subsequently, p21 and p16 synergistically sustain the dephosphorylated state of the RB protein, a pivotal regulator of cell cycle arrest[13]. These two CDKIs, p21 and p16, are integral to the senescence phenotype, promoting cellular senescence autocrinally. Additionally, SASP factors emitted by senescent cells can instigate a DNA damage response in adjacent cells through gap junction-mediated intercellular communication, a phenomenon recognized as a non-cell-autonomous bystander effect of senescence, culminating in the stable growth arrest of neighbouring cells.

In summary, aging is a process that unfolds from the individual to the molecular level and is characterized by intricate interactions between physiological, cellular, and molecular changes. More research is needed to promote a deeper knowledge about the aging progression and to develop strategies to promote healthy aging and longevity.

3 Biological mechanisms involved in aging

Contemporary research has identified numerous endogenous and exogenous stressors that regulate aging, such as genomic instability, compromised autophagy, telomere shortening, mitochondrial dysfunction, epigenetic change, proteostasis loss[14]. These elements contribute to declines in cellular and tissue functions and significantly increase the risk of various age-associated diseases (Fig. 1).

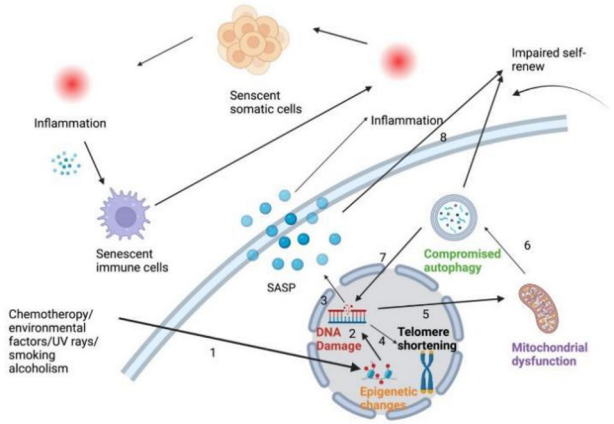

Fig. 1. The biological mechanism involved in aging.

3.1 Compromised autophagy and aging

The aging process is intrinsically linked with a decline in autophagic activity across diverse species. Initial research has indicated a diminution in lysosomal proteolysis in aged rats, primary human cells, and *Caenorhabditis elegans* compared to their younger counterparts[15]. More contemporary studies involving *Caenorhabditis elegans* have corroborated a genuine reduction in autophagy flux in tissues persisting throughout the organism's lifespan. This decline in autophagy is age-dependent and notably occurs after the formation of autophagosomes (AP). This phenomenon is further characterized by the age-dependent accumulation of immature autophagosomes and a decrease in autophagic degradation across all examined

tissues[16]. Aging is always caused by a systematic reduction in autophagic activity, as evidenced by a decrease in lysosomal proteolysis and a decrease in the activity of specific proteases in different species.

3.2 Telomere shortening and aging

Srinivas posited that the length of telomeres might dictate the potential number of cell division cycles a cell can undergo[17]. Subsequent research by Cooke and Smith in 1986 established a direct correlation between telomere lengths and cellular senescence, as evidenced by comparative analyses of telomere lengths in various tissues[18]. It was followed by discoveries indicating that the capacity for cellular replication in human cells was enhanced by the action of telomerase in lengthening telomeres. These studies corroborated that progressive telomere shortening significantly contributes to aging[19].

A study involving cells from 31 donors, aged between 0 to 93 years, demonstrated that proliferative capacity inversely correlated with donor age, as did the relationship between telomeric DNA length and donor age[19]. Crucially, a robust correlation was observed between replicative capacity and initial telomere length, spanning the entire age spectrum of donors, which means cell lines with shorter telomeres exhibited significantly fewer doublings than those with longer telomeres. Consequently, telomere length could be a biomarker for somatic aging in humans and may play a causative role in aging process.

Telomere biology disorders (TBD) are characterized by gene mutations associated with telomere maintenance, leading to shorter telomeres and accelerated cellular senescence. However, a strict linear correlation between telomere length and the severity of the disease manifestation is only sometimes evident. In an investigation into the epigenetic aspects of aging and telomeres, Carlund conducted whole-genome DNA methylation analyses using blood samples from 35 individuals diagnosed with TBD[20]. The findings suggest that individuals with TBD have a significantly higher epigenetic age, implying that differentially methylated (DM) CpG loci may act as biomarkers of telomere shortening and contribute to the disease phenotype.

Research from Olovnikov to Carlund delineates the pivotal role of telomere dynamics in cellular senescence, establishing that telomere length not only predicts cellular replicative capacity but also implicates telomerase activity and epigenetic modifications in aging and Telomere Biology Disorders (TBD), underscoring a complex interplay between genetic and epigenetic factors in somatic aging and disease phenotypes.

3.3 Mitochondrial dysfunction and aging

Mitochondrial dysfunction can precipitate a substantial disruption in cellular energy conversion, particularly in tissues heavily dependent on the chemical energy produced by mitochondria across the lifespan[21]. Hence, the alteration of mitochondrial metabolism in response to environmental variations and the consequential elevation in reactive oxygen species (ROS) may be implicated in age-associated metabolic disorders. It is possibly through sustained alterations in mitochondrial oxidative phosphorylation (OXPHOS), culminating in mitochondrial dysfunction. Petersen revealed that the older individuals exhibited notable insulin resistance compared to the younger control group, which is attributable to decreased insulin-stimulated mitochondrial function[22].

In various eukaryotic organisms, it has been observed that mild mitochondrial stress can exert salutary effects on the organism's overall lifespan. The onset of mitochondrial dysfunction triggers the unfolded protein response (UPRmt), a stress-response signalling pathway to preserve mitochondrial homeostasis. Experimental observations in Caenorhabditis elegans have shown that mitochondrial disturbance during larval development not only retards the aging process but also sustains UPRmt signalling[23].

A pivotal study identified the conserved histone lysine demethylases jmjd-1.2/PHF8 and jmjd-3.1/JMJD3 as critical positive regulators of lifespan in the context of mitochondrial dysfunction across multiple species[23]. The results from this study indicate that a reduction in demethylase function leads to a consequent inhibition of lifespan extension and UPRmt induction. Conversely, an enhancement of demethylase function is sufficient to prolong lifespan in a manner depending on UPRmt activation.

Mitochondrial dysfunction leads to severe disruptions in cellular energetics and, ultimately, age-related metabolic disorders. However, mild stress responses may prolong the lifespan, demonstrating the complex interplay between mitochondrial function, stress responses, and ageing.

3.4 Epigenetic change and aging

Epigenetic change plays an important role in aging, with their comprehension being vital for human health and the formulation of strategies to decelerate aging and its associated pathologies. Epigenetic regulation of gene expression, characterized by modifications in histone or DNA without altering the DNA sequence, is integral to normal biological processes, influencing cellular cycles of death, renewal, and senescence[24]. Epigenetics jointly participates in the regulation of aging through DNA methylation, histone modifications, chromatin remodelling, non-coding RNA regulation and RNA modification[25]. Epigenetic regulation is fundamental in orchestrating the intricacies of the SASP. The following delineates fundamental epigenetic mechanisms implicated in SASP regulation, including alteration in histone modifications and DNA methylation, chromatin remodeling and histone Variants[26]. These epigenetic mechanisms are integral to establishing and perpetuating cellular senescence and SASP, with far-reaching implications for aging and age-associated diseases. Elucidating these epigenetic regulators and their impact on SASP offers a pathway to developing targeted approaches for modulating SASP's influence on cellular and tissue homeostasis.

4 Discussion

The complex biological mechanisms of aging, covering the molecular, cellular and systemic levels, are controlled by multiple factors such as genomic instability, telomere dysfunction and epigenetic changes, which lead to reduced cell and tissue function and increased risk of aging-related diseases. These processes, including telomere attrition, impairment of proteostasis, mitochondrial dysfunction, and cellular senescence, significantly contribute to the pathophysiological progression of aging, and this article highlights the importance of targeted research and intervention strategies to mitigate these effects.

Investigations have indicated that defects of mitochondrial respiration in Saccharomyces cerevisiae is a critical determinant in cellular aging, primarily via the accumulation of reactive oxygen species (ROS) and disruption of proteostasis[27]. However, the effects of mitochondrial respiration on aging appear to be strain-dependent, with some yeast strains exhibiting persistent or even increased replicative lifespan despite respiratory defects. This difference may be attributed to the varied extent of respiratory capacity and the different tolerance levels of different strains of ROS.

Analysis suggests that in the context of telomere biology, leukocyte telomere length (LTL) may positively determine the natural lifespan limit of an individual [28]. Steenstrup indicates that telomere lengthening increases lifespan[28]. However, potential interventions to extend telomere length may have adverse effects, such that longer LTL increases the risk of significant cancers, reflecting the evolutionary balance between cancer and aging process. This balance is thought to influence lifespan, especially after the reproductive years of contemporary humans. Subsequent investigational pursuits should concentrate on elucidating the evolutionary dynamics of telomere length and its consequential effects on human health, especially concerning the prolongation of lifespan and the potential detriment of telomere length alteration.

Senolytics are a category of life-extending medicines that selectively and aggressively remove cells that have stopped dividing, which are responsible for the diseases associated with aging. The emergence of senolytics represents a new approach to the treatment of age-related diseases with favourable outcome in preclinical models and preliminary human trials, such as dasatinib and quercetin[29]. These compounds have the potential to mitigate age-related diseases and significantly increase lifespan. Future senolytics will require a deep understanding of the specific cell types that undergo senescence and their SASP profiles, as indiscriminate elimination of senescent cells may be harmful. Furthermore, understanding the regulatory mechanisms affecting bystander cells and how these responses vary with tissue type, age, or disease state will be critical, particularly in cancer, where SASP can elicit different responses[2]. Advances in single-cell technology are expected to enhance this understanding. Although various SASP modulators have been identified, particularly those affecting pro-inflammatory and immunomodulatory aspects, the mechanisms affecting pro-fibrotic or pro-angiogenic SASP components remain unclear[2]. Birch and Gil (2020) suggest that complete suppression of SASP may not be beneficial. Instead, a nuanced understanding of its unique elements is crucial. A deeper understanding of these differences will inform applications in chemotherapy, where challenges include distinguishing SASP regulation from general inflammatory processes and identifying reliable in vivo markers of aging.

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